CHARLES B. BECK

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An Introduction to Plant Structure and Development

Plant Anatomy for the Twenty-First Century
Second Edition

This is a plant anatomy textbook unlike any other on the market today. As suggested by the subtitle, it is plant anatomy for the twenty-first century. Whereas traditional plant anatomy texts include primarily descriptive aspects of structure with some emphasis on patterns of development, this book not only provides a comprehensive coverage of plant structure, but also introduces, in some detail, aspects of the mechanisms of development, especially the genetic and hormonal controls, and the roles of the cytoskeleton. The evolution of plant structure and the relationship between structure and function are also discussed throughout the book. Consequently, it provides students and, perhaps, some teachers as well, with an introduction to many of the exciting, contemporary areas at the forefront of research, especially those areas concerning development of plant structure. Those who wish to delve more deeply into areas of plant development will find the extensive bibliographies at the end of each chapter indispensible. If this book stimulates a few students to become leaders in teaching and research in plant anatomy of the future, the goal of the author will have been accomplished.

CHARLES B. BECK, Professor Emeritus of Botany at the University of Michigan, received his PhD degree from Cornell University where he developed an intense interest in the structure of fossil and living plants under the influence of Professor Harlan Banks and Professor Arthur Eames. Following post-doctoral study with Professor John Walton at Glasgow University in Scotland, he joined the faculty of the University of Michigan. At Michigan he served one term each as Chairman of the Department of Botany and Director of the Museum of Paleontology. His graduate students pursued research in either plant structure and development or paleobotany. He taught courses in plant anatomy, plant morphology and paleobotany over a period of 35 years.

An Introduction to Plant Structure and Development

Plant Anatomy for the Twenty-First Century

Second Edition

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To
My wife, Janice,
and our daughters, Ann and Sara
for their love, encouragement,
and enduring support,

and

to my students,

David Benzing
Robert Chau
Crispin Devadas
Margaret Knaus
G. Kadambari Kumari
Rudolf Schmid
William Stein
Garland Upchurch
Richard White
David Wight

who are a continuing inspiration and
from whom I have learned much.

It is important that students bring
a certain ragamuffin barefoot
irreverence to their studies; they
are not here to worship what is
known but to question it.

Jacob Bronowski
The Ascent of Man (1975)

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Preface to the second edition

Although it has been only five years since this book was first published, research activity during this period in many areas of plant development has resulted in much new and important information. The basic information on plant structure is quite stable. As a result, inclusion of new information about various aspects of development comprise the major changes in this 2nd edition. In addition, a new section on the evolution of tracheary elements has been added.

The areas expanded and/or upgraded include the structure and function of the cytoskeleton, and its roles in cell wall formation and pollen tube tip growth; the role of auxin and other hormones in development, especially in the development of tracheary elements, as well as in cambial activity and tissue patterning, and the role of PIN proteins in the movement of auxin from cell to cell by auxin efflux transporters. The discussion on the mechanism of movement of stomatal guard cells has been expanded and improved. Sections on long-distance transport in the secondary xylem and phloem have been modified to emphasize widely accepted mechanisms of transport, and the discussion of bidirectional transport in the phloem has been expanded. The discussion of gravitropism has been brought up to date. Finally, throughout the book, discussions of the role of genetics in plant development have been expanded.

I believe the changes listed above have made the book more useful to advanced students and researchers without adversely affecting its usefulness as an introductory plant anatomy textbook. It is not designed to be used as the only source of information in a course in plant anatomy, i.e., to take the place of the teacher, but rather, to be a supplement to the teacher's lectures and a means for the student to reinforce information from the teacher and the laboratory exercises. The book can, of course, also be an original source of information for students beyond that provided by the teacher. When used in a course that emphasizes development, the student will have the opportunity to expand his or her knowledge of plant structure, and in a course that emphasizes plant structure, the student can expand his or her knowledge of plant development. Ideally, however, a twenty-first century course in plant anatomy should consist of an integration of structure and development. It is this ideal that I have tried to promote in the design and preparation of this book.

For granting me permission to use photographs in this 2nd edition of the book, I express my appreciation to university and commercial publishers and all other copyright holders. I am especially grateful to Professor Philip Gingerich for allowing me to use the facilities and services of the University of Michigan Museum of Paleontology, and to three very talented persons in this unit, Bonnie Miljour, senior scientific

illustrator, Cindy Stauch, business administrator, and Christina Minor, research secretary who were so helpful in many ways.

I express my gratitude to Dominic Lewis, commissioning editor, life sciences, Rachel Eley, assistant editor, life sciences, Caroline Brown, production editor, and Lesley Bennun, copy-editor, and acknowledge their important roles in the preparation and production of this book.

Finally, I owe a special debt of gratitude to my wife, Janice, whose patience and encouragement have contributed so importantly to the completion of this project.

Charles B. Beck
Ann Arbor, 2009

Preface

Since my introduction to plant anatomy by William Strickland at the University of Richmond and my interaction with Arthur Eames and Harlan Banks at Cornell University during graduate study, I have been entranced by the elegant beauty of plant structure. At the University of Michigan I taught both paleobotany and plant anatomy for many years, and served as committee chair for graduate students, some of whom studied fossil plants and others of whom worked on the structure and development of extant taxa. During the past several decades during which the introduction of new techniques of study at the subcellular and molecular levels has resulted in a resurgence of research throughout the world, my interest in the development of plant structure has grown steadily.

Many books on plant structure, some highly technical, have appeared since the publication of the seminal textbooks of Katherine Esau during the 1950s and 1960s, but no single book that, in my opinion, incorporates both the basic knowledge of plant anatomy and contemporary information and ideas about the development of structure and form that could be used as an effective introductory textbook. Consequently, I have tried to meet the challenge of preparing such a book. In each chapter I have presented what I consider to be the fundamental knowledge essential for an understanding of basic plant structure and development and have integrated with this the results of some of the most significant recent research on plant development. Whereas emphasis throughout the book is on structure and development, I have also included sections on evolution and function where it seemed essential and appropriate to do so. The application of cellular and molecular biological approaches and techniques in the study of plant development has revolutionized the field. Understanding of the integrative significance of plasmodesmata and the concept of the symplast have led to an appreciation and widespread acceptance of the organismal theory of plant multicellularity which in turn has influenced research on plant development. Exciting and significant areas of research such as the role of the cytoskeleton in development, signal transduction, genetic control of development, among others have greatly advanced our understanding. I have not treated the very important subject of the genetic control of development in any depth because it requires a much deeper knowledge of genetics than the undergraduate for which this book is written is likely to have attained. I have, however, included references to important genetic studies in the bibliographies of several chapters. Other subjects may not be as fully covered as some teachers and researchers would desire, but they are very likely to find pertinent references to literature on those subjects in the extensive bibliographies to which they can direct their students who have the necessary backgrounds.

Diverging from the approach in many textbooks, I have included in this book tentative conclusions that are essentially still hypotheses, and discussions of research that is controversial, often providing opposing viewpoints. I believe that, in addition to providing well-established information on a subject, a textbook should also provide the student with an understanding of the nature of ongoing scientific research.

In order to make this book more readable for the undergraduate, I have omitted most literature citations in sections of the text in which the basic, widely accepted knowledge in the field is presented, but have included some references of historical importance in the references at the end of each chapter. On the other hand, when presenting new information, ideas, and conclusions that are not yet widely accepted, I have cited in the text and included in the references the sources of this information. Thus, students as well as researchers who wish to consult the original papers may find the reference sections useful.

My objective has been to prepare a new plant anatomy textbook for a new century, incorporating the best research in the most active and significant areas with the widely accepted common knowledge that provides the foundation of the field. Only you the readers can decide whether or not I have succeeded.

Charles B. Beck
Ann Arbor, 2004

Acknowledgements

One's knowledge comes from many sources. Not least are the research and writings of many predecessors in the field. Men and women such as Nägeli, De Bary, Strasburger, Haberlandt, Van Tieghem, Solereder, Jeffrey, Eames, Bailey, Metcalfe and Chalk, Esau and countless others have provided the foundation upon which current-day researchers are building. To these, whom sometimes we forget, we owe a debt of gratitude. I acknowledge a profound debt to my college and university teachers, William Strickland and Robert Smart who introduced me to plant structure in the first place, Arthur Eames and Harlan Banks who widened my horizons and reinforced my understanding of the fundamentals of plant anatomy, and to John Walton who encouraged me to take risks and taught me how to write. I acknowledge, as well, the significant contributions to my knowledge of the many researchers who are currently active in the field.

Direct assistance during the preparation of this book has come from many sources. I feel particularly indebted to colleagues who have critically read chapters in manuscript and made important suggestions for change and improvement. These are Professor William Stein of the State University of New York, Binghamton who read several chapters, Professor Shirley Tucker of the University of California at Santa Barbara, Professor Nancy Dengler of the University of Toronto, and Professor Darleen DeMason of the University of California, Riverside. Other colleagues have provided information on special topics. Professor Peter Ray of Stanford University provided information on the functional significance of the optical qualities of epidermal cells in leaves, Professor Judy Jernstedt of the University of California at Davis provided information on contractile roots, Professor Larry Nooden of the University of Michigan was a source of important information on several aspects of plant physiology, Professor Robert Fogel of the University of Michigan provided information on mycorrhizae, and Professor Edward Voss and Dr. Christiane Anderson of the University of Michigan were valuable sources of information on plant taxonomy. To all of these I express my sincere appreciation.

Professor Philip Gingerich, Director of the Museum of Paleontology at the University of Michigan, made available to me the resources and services of the Museum. Preparation of this book would not have been possible without this assistance, and to Phil I express my sincere gratitude. The illustrations are nearly as important as the text in a plant anatomy book. In this book all original line drawings were finished by Bonnie Miljour, artist *par excellence* of the Museum of Paleontology. Ms. Miljour also grouped and placed all illustrations in electronic files. The importance to this project of her great expertise cannot be overemphasized. Thank you, Bonnie, for the beauty of your work and for your very important contribution to this book. Two members of the Museums

office staff, Cindy Stauch and Meegan Novara, were also of inestimable assistance in many ways. I express my sincere appreciation to them.

The original photographs were taken primarily by two University of Michigan photographers, Louis Martonyi, now deceased, who was photographer for the Department of Biology during the 1980s, and David Bay, current photographer for the Department of Ecology and Evolutionary Biology. Thank you, David, for your excellent work. A few photographs were taken by the author in the facilities of the Microscopy and Image-analysis Laboratory of the University of Michigan Medical School. This was made possible by the kindness of the Laboratory Manager, Chris Edwards and with the technical assistance of Shelley Almburg, to both of whom I express my appreciation. I express my sincere gratitude to colleagues who provided photographs: Professor Pedro J. Casero of Universidad de Extremadura, Badajoz, Spain; Professor P. Dayanandan of Madras Christian College, India; Dr. Elisabeth de Faÿ of Université Henri Poincaré, Nancy, France; Professor Nancy Dengler of the University of Toronto, Canada; Dr. Katrin Ehlers of the Justus-Liebig-Universität, Giessen, Germany; Dr. Irene Lichtscheidl of Universität Wien, Austria; Dr. E. Panteris of the University of Athens, Greece; and Dr. Koichi Uehara of Chiba University, Japan. Professor P. Maheshwari of the University of New Delhi sent me many excellent slides during his lifetime, many of which have been photographed for use in this book. I have also used many illustrations from published sources, and I express my gratitude to the individuals, commercial publishers, university presses, and professional societies that have granted permission for the use of their copyrighted materials.

Although every effort has been made to secure necessary permissions to reproduce copyrighted material in this work, it has proved impossible in two cases to trace the copyright holders. The copyright holder of the original illustration from Lehninger (1961), which I have used as my Fig. 3.7a, is Dr. A. E. Vatter. The copyright holder of the original illustrations from Eames and MacDaniels (1925), which I have used as my Figs 13.3, 13.4, 14.5, 16.15c, and 16.21 is David Eames. Appropriate acknowledgements will be included in any reprinting or in any subsequent edition of this book if the copyright holders are located.

In order to understand copyright law, which varies somewhat throughout the world, I called on my friend, Professor John Reed of the University of Michigan Law School, for advice. He directed me to Professor Molley Van Houweling, a specialist in copyright law, who gave me valuable information. I am grateful to these colleagues.

Without the resources of the University of Michigan Library this book could not have been written, and the excellent assistance of the reference librarians in the Shapiro Science Library is acknowledged with gratitude.

To the editors of Cambridge University Press I express my gratitude, and acknowledge their important roles in the preparation and production of this book. In particular, I wish to thank Ward Cooper, commissioning editor, life sciences; Clare Georgy, assistant editor, life

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Chapter 1

Problems of adaptation to a terrestrial environment

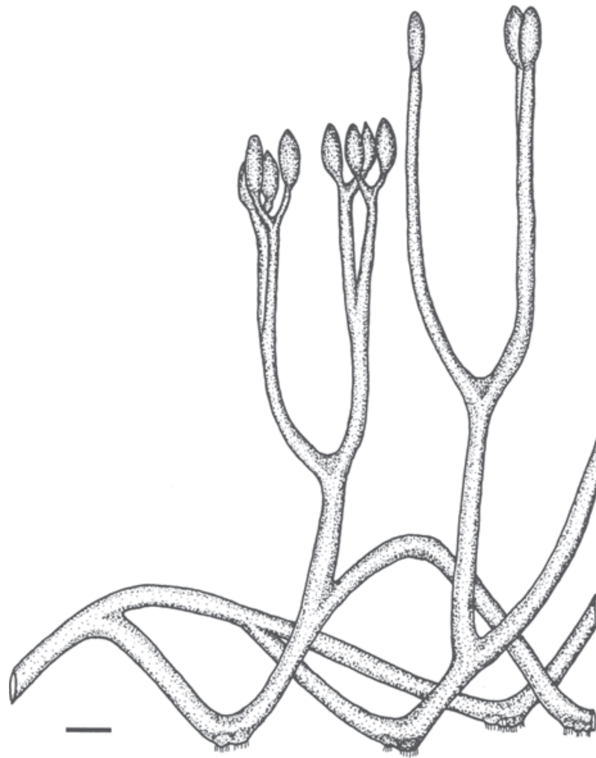
Perspective: the origin of vascular plants

Land plants, plants that complete their life cycle entirely in a terrestrial environment, are represented largely by bryophytes and vascular plants. In all taxa except seed plants, however, at least a thin film of water is required for fertilization; and even in two primitive groups of seed plants, the cycads and *Ginkgo*, fertilization is by free-swimming spermatozoids released into a liquid medium in the archegonial chamber. A few angiosperms, although terrestrial in origin, have reverted to an aquatic existence.

Vascular plants are by far the dominant groups on the Earth comprising over 255 000 species in contrast to about 22 000 species of bryophytes and approximately 20 000 species of algae. The first vascular plants appear in the fossil record in the late Silurian, about 420 million years ago, but their green algal ancestors are thought to have appeared nearly 400 million years earlier! Shared features comprise the major evidence that vascular plants, possibly also bryophytes, evolved from green algae: both synthesize chlorophylls a and b, both store true starch in plastids; both have motile cells with whiplash flagella, and both (but only a few green algae) are characterized by phragmoplast and cell plate formation following mitosis. A green alga, with these and other significant characteristics, that may provide a model of an algal ancestor of vascular plants is *Coleochaete*, a member of the Charophyceae. Features in addition to those listed above that lead to this conclusion are the development in *Coleochaete* of a zygote in which cell division begins while embedded in the gametophyte thallus, the presence of sporopollenin in the wall of the zygote, and the presence of lignin in the gametophyte. It is widely believed that the Embryophyta (bryophytes and vascular plants) and the Charophyceae evolved from a common aquatic ancestor. Detailed presentations of the evidence for this viewpoint and the nature of the presumed common ancestor can be found in major works by Graham (1993) and Niklas (1997, 2000).

The first, indisputable vascular plants were characterized by a conducting system containing xylem and phloem, a waxy cuticle,

Figure 1.1 Reconstruction of *Aglaophyton major*. Bar = 10 mm. From Edwards (1986). Used by permission of The Linnean Society of London.



epidermal stomata, and a reproductive system that produced **trilete spores** (spores with a triradiate scar resulting from their development in spherical tetrads) and probably containing sporopollenin in the walls. Such plants appear first in the late Silurian, but *Aglaophyton major* (see Edwards, 1986) from the Lower Devonian, which has morphologic and structural features of both some bryophytes and primitive vascular plants, provides perhaps the best available model of a vascular plant precursor. *Aglaophyton* was a small plant, probably no more than 180 mm (about 7 inches) high, composed of dichotomous, upright axes that branched from rhizomes on the surface of the substrate (Fig. 1.1). The epidermis of all axes was covered by a cuticle and contained stomata. Some upright axes were terminated in pairs of sporangia, containing small spores of one size only. Edwards suggested that the plant probably formed extensive mats, consisting largely of vegetative axes, but produced fertile axes, bearing sporangia, "at irregular intervals." The rhizomes were probably vegetative axes that formed clusters of **rhizoids** (absorbing structures) where some axes arched over and contacted the substrate. One of the most interesting structural features of the axes of *Aglaophyton* was the central conducting strand. Although appearing superficially as a vascular strand consisting of xylem and phloem, and described that way by earlier workers, Edwards was unable to detect characteristic structural features of tracheary elements (that is, cells with secondary wall material deposited in the form of rings, helices, or a reticulum) or of sieve elements. Instead, he found three

regions of cells, an inner column of thin-walled cells surrounded by thick-walled cells, the walls of both of which were dark in color. These were enclosed by an outer zone of thin-walled cells with light-colored walls. He concluded that the two inner regions of cells with dark cell walls were probably analogous to tracheids but most similar to the **hydroids** (water-conducting cells) of some mosses and that the outermost cells with light-colored walls were analogous to sieve elements and very similar to the **leptoids** (photosynthate-conducting cells) of mosses. *Aglaophyton* was, therefore, a non-vascular plant sporophyte in which the sporophyte was the dominant phase in a system of pteridophytic (free-sporing) reproduction. In gross morphology and branching pattern, and the presence of an epidermis covered by a cuticle and containing stomata, it was very similar to primitive vascular plants that lived during Upper Silurian and Lower Devonian times. In its water- and photosynthate-conducting cells closely resembling, respectively, hydroids and leptoids, as well as in its small size and free-sporing reproduction, it closely resembled mosses. It is reasonable, therefore, to hypothesize that vascular plants evolved from this or plants of similar morphology and anatomy. (For detailed information on the earliest vascular plants, see Stewart and Rothwell (1993) and Taylor and Taylor (1993).)

Structural adaptations

During the past 350–400 million years many structural and physiological changes occurred as vascular plants evolved. Evolution on land posed many problems for plants such as *Aglaophyton* and its descendants not shared with their marine algal ancestors. In an aquatic environment, conditions are equable, and problems of water loss, support, absorption of water and minerals, and transport of water and minerals, photosynthate, and hormones, are either minimal or non-existent. This is true also, in large part, for very small plants such as most mosses. For example, the absence of efficient water-conducting cells in mosses apparently does not pose a problem for them since in many taxa water and minerals are absorbed through the external surfaces of the sporophytes and gametophytes. This is not unlike the situation in aquatic plants in which water and minerals are absorbed by all parts of the plant directly through the epidermis, which lacks a cuticle. Consequently, there is no need for a highly efficient system of transport of water and minerals. Likewise, with few exceptions, the transport of hormones and photosynthate is also not a problem since these substances are produced in all cells. On land, however, solar radiation, wind, and temperature extremes result in a much harsher environment. As *Aglaophyton* and its descendants evolved on land, structural features evolved as adaptations to both this harsher environment and to their increase in size.

Adaptations reducing water loss were the evolution of a three-dimensional, rod-like plant body which decreased the surface/volume

ratio, and an epidermis covered by a waxy cuticle largely impermeable to the passage of water. Although the evolution of a rod-like form was advantageous in restricting the surface area from which water could be lost, an optimal surface area in relation to volume was required through which transpiration as well as gaseous exchange could occur. The evolution of stomata in the epidermis allowed the exchange of O_2 and CO_2, essential in respiration and photosynthesis, and by their ability to control the size of pores through which water vapor diffused, stomata also contributed to a restriction of water loss from the plant. Adequate surface area was also required, however, through which the plant could receive signals from the environment – signals such as light, temperature, or the presence of other organisms such as pathogens or symbionts as well as chemical signals from the atmosphere or from other organisms. We now know that chemicals produced by plants living today are also released through the surface and may elicit responses from other organisms such as moths and hummingbirds that function as pollinators. The response of plants to environmental signals, referred to as **signal transduction**, is a new and active area of research in plant biology.

Protection of spores and gametes, so very important in a terrestrial environment, was accomplished through the evolution of sporangia and gametangia enclosed in sterile jackets of cells. The spores themselves became encased in walls containing **sporopollenin**, a substance which restricts water loss and is highly resistant to decay.

Absorption of water and minerals from the soil was facilitated by the evolution of rhizoids and roots, the latter often containing symbiotic fungi forming mycorrhizae which, as we shall see in detail later, enhanced their absorptive function. Roots, in particular, also served to anchor the plant in its substrate and to prevent its displacement by wind and flowing water.

The effective transport of water and minerals as well as hormones, photosynthate, and other substances became increasingly important with increase in size of the descendants of *Aglaophyton* or other vascular plant precursors. This was accomplished by the evolution of complex vascular tissues containing tracheids and vessel members in the xylem and sieve cells and sieve tube members in the phloem, conducting cells especially adapted structurally for the transport of these materials. Associated with the evolution of cellular transport systems, specialized mechanisms evolved which facilitated the efficient transport of water and minerals from the roots to and out of the leaves of tall trees. Concurrently, mechanisms for the translocation of photosynthate and other assimilates throughout the plant evolved.

The problem of support of the plant body also became increasingly severe with increase in size and was solved by structural adaptations. In plants, or parts of plants, consisting largely of living tissues, support was provided by their enclosure by an epidermis as well as by turgor pressure within the cells. Ultimately, some of the functions of the epidermis were taken over by **periderm** (a major component of bark) consisting

largely of non-living cells, the walls of which are impregnated with **suberin** that restricted the passage of water through them. Support was also provided by the production of tissues consisting largely of non-living, longitudinally elongate cells with thick, lignified, cellulosic walls. The major supporting tissue in large plants is the xylem, consisting in pteridophytes and their ancestors as well as in gymnosperms primarily of tracheids, and in angiosperms of fibers and vessel members. **Lignin** in the cell walls increased the tensile strength of elongate cells comprising the xylem, thus endowing vascular plants with both strength and flexibility, so very important in conditions of high wind velocity.

The above-ground parts of the plant bodies of primitive vascular plants consisted primarily of radially symmetrical branching axes, all of which were photosynthetic. With the evolution of larger vascular plants consisting of stems and branches covered with bark, an adaptation that facilitated the process of photosynthesis was necessary. This was accomplished by the evolution of leaves which increased the surface/volume ratio of photosynthetic tissues in the plant. Structural adaptations in the leaves, such as the orientation of thin-walled elongate cells at right angles to the upper surface which channeled light at relatively high intensity into the leaves, and the complex system of intercellular channels which provided extensive wet surface area for the absorption of CO_2 facilitated efficient photosynthesis. For further information on adaptations by early plants to a terrestrial environment, and the evolution of plant body plans, please see Niklas (2000).

Preview of subsequent chapters

As we proceed through this book we shall encounter progressively detailed information on the structure and development as well as some aspects of evolution and function of the descendants of primitive plants such as *Aglaophyton*. We shall consider many members of the Embryophyta, including the Lycophyta (lycopods and their relatives), Sphenophyta (sphenophytes), and Pterophyta (ferns), but the major emphasis will be on the seed plants (gymnosperms and angiosperms). In order to provide an orientation to all who have had little or no training in plant anatomy, and to introduce some important concepts, the following chapter will be an overview of plant structure and development. If you have had a good course in introductory botany or biology, you may wish to proceed to later chapters. Chapters 3 and 4 present, respectively, basic information on the cell protoplast and the cell wall. The cell protoplast is usually covered in some detail in introductory courses, but the cell wall is often neglected. Consequently this book provides a fairly comprehensive discussion of its structure and development.

Chapter 5 presents very important information on apical meristems of the shoot, apical regions from which other cells and tissues in the

shoot system are derived, and which provide to vascular plants their distinctive characteristic of indeterminate growth. Appendages such as leaves and lateral branches also are ultimately derived from the apical meristems. In Chapters 6, 7, and 8 we consider the structure and development of the various tissues and tissue systems that result from the activity of apical meristems. These chapters include, in sequence, discussions of the primary vascular tissues (xylem and phloem) that are embedded in the parenchyma of the pith and cortex; the architecture of the primary vascular system and its relationship to the arrangement of leaves; and the epidermis, the single layer of tissue that bounds all of these other primary tissues and tissue systems, and which forms an outer protective and supportive layer of the plant prior to the development of secondary tissues.

The second part of the book, Chapters 9 through 12, consists of discussions of the vascular cambium, a lateral meristem, the activity of which results in the formation of secondary vascular tissues, and the effects of their formation on the tissues and tissue systems produced by the apical meristems early in the development of the plant body. It also includes detailed presentations of the structure, development, and to a lesser extent evolution and function of the secondary xylem and the secondary phloem.

Chapters 13 through 18 deal with secretory structures and functions; anomalous stem and root structure; the outer protective tissues and tissue regions of stems that produce secondary tissues (the periderm and rhytidome) that comprise the bark; the structure, development, and function of leaves as well as a brief discussion of their evolution; a presentation of the structure, development, and function of roots, with some comments on their evolution; and finally, a chapter on reproduction which includes some basic life cycles, discussions of the structure and morphology of flowers, the structure and development of fruits and seeds, and some aspects of the ecology of reproduction in angiosperms.

The author hopes that you will enjoy this book and that by the end of your course in plant anatomy you will be as enthusiastic about this exciting field as he is.

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Chapter 2

An overview of plant structure and development

Perspective: origin of multicellularity

Since early in the study of plants botanists have been interested in the structure, function, development, and evolution of cells, tissues, and organs. Because some green plants are very small and unicellular, but others are large and multicellular, the origin of multicellularity in plants also has been of great interest to botanists. Among the green algae from which higher plants are thought to have evolved, some colonial taxa such as *Pandorina*, *Volvox*, and relatives consist of aggregations of motile cells that individually appear identical to apparently related unicellular forms (Fig. 2.1). Consequently, it was concluded early in the history of botany, and widely accepted, that multicellular plants evolved by the aggregation of unicellular organisms. This viewpoint led to the establishment of the **cell theory of multicellularity** in plants which proposes that cells are the building blocks of multicellular plants (Fig. 2.2). As early as 1867, however, Hoffmeister proposed that cells are simply subdivisions within an organism. This viewpoint, supported and expanded upon in 1906 by Lester Sharp at Cornell University, has been elucidated and clarified more recently by Hagemann (1982), Kaplan (1992), and Wojtaszek (2000) among others. These workers conclude on the basis of abundant evidence that a unicellular alga and a large vascular plant are organisms that differ primarily in size and in the degree to which they have been *subdivided* by cells (Fig. 2.2). This **organismal theory of multicellularity** has gained many adherents within the past several decades (see Kaplan, 1992), and is of great importance because of ways in which it has influenced the thinking of botanists about the processes of development.

A primary and convincing basis for the organismal theory is the nature and result of cell division in plants. Following mitosis and cell plate formation, the protoplasts of the two resulting daughter cells maintain continuity through highly specialized cytoplasmic strands called **plasmodesmata** (Fig. 2.3) (see Chapter 4 for a detailed discussion of plasmodesmata). Thus, although the plant is blocked off in regions called cells, the plasmodesmata provide for an interconnected system

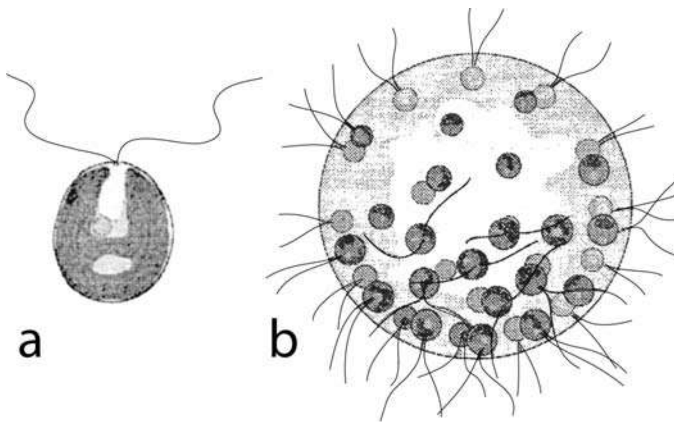


Figure 2.1 Unicellular and colonial body plans among green algae. (a) *Chlamydomonas* sp. (b) *Pandorina morum*. From Niklas (2000). Used by permission of Oxford University Press.

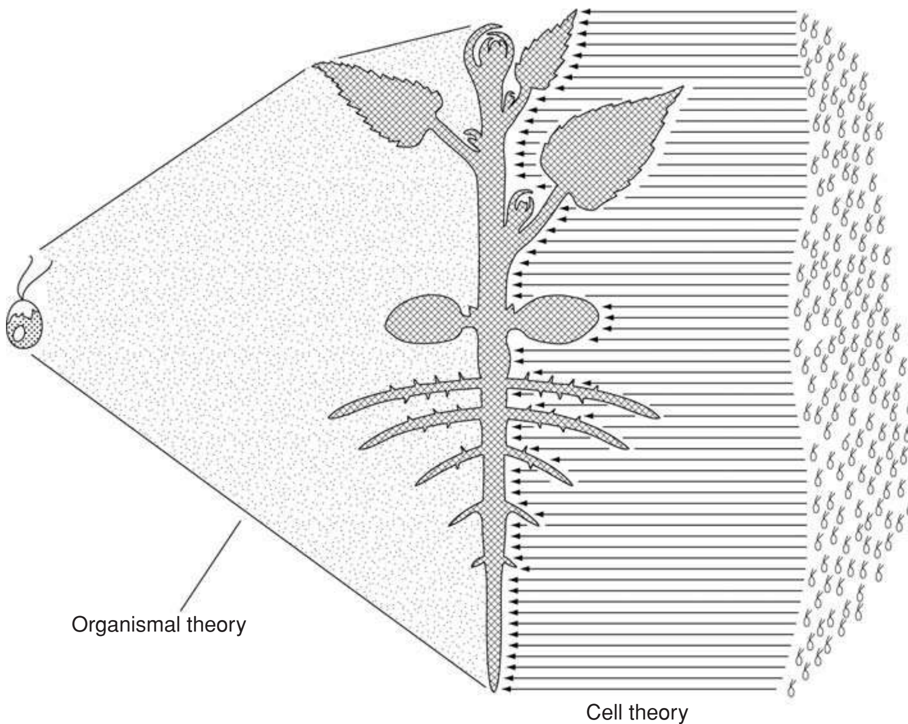


Figure 2.2 Diagram showing the relationship between a unicellular and a multicellular organism according to the organismal theory of multicellularity and the cell theory. From Kaplan (1992). Used by permission of the University of Chicago Press. © 1992 The University of Chicago. All rights reserved.

of protoplasts called the **symplast**. The plasmodesmata function as passageways for communication between living cells, that is, for the transmission between cells of molecules of varying size including even large molecules such as proteins and nucleic acids (e.g., Lucas *et al*., 1993; Kragler *et al*., 1998; Ehlers and Kollmann, 2001). It has become

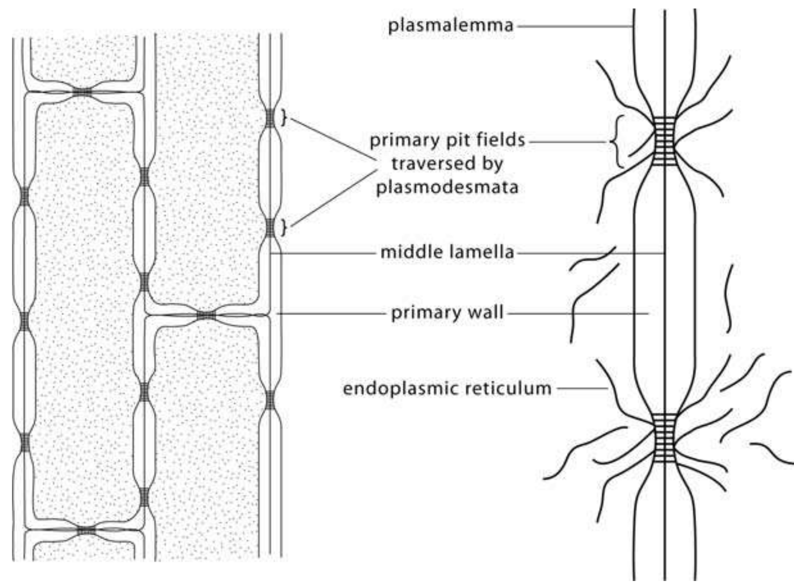


Figure 2.3 Diagrams of primary pit fields traversed by plasmodesmata in primary cell walls. The plasmodesmata connect the protoplasts of adjacent parenchyma cells.

clear in recent years that this communication between cells has a profound influence in plant development (e.g., Verbeke, 1992). These and other workers believe that plasmodesmata may exert a "controlling influence" on cell differentiation, tissue formation, organogenesis, and specialized physiological functions.

For more detailed discussions of evidence in support of the organismal theory of multicellularity in plants and the significance of this theory in understanding plant development, see Kaplan and Hagemann (1991), Niklas and Kaplan (1991), Kaplan (1992), and Wojtaszek (2000).

Let us now look at the vascular plant body in general terms and obtain an overview of its structure and development. In subsequent chapters we shall consider in more detail many aspects of plant structure and development as well as of function and evolution.

Some aspects of the shoot system of the vascular plant

The vascular plant consists of an aerial shoot system and, typically, a subterranean root system (Fig. 2.4). The **shoot system** consists of a main axis that bears lateral branches. Leaves may be borne on both the main axis and lateral branches in plants that complete their life cycle in one growing season, but in those that live for several to many years, leaves are usually found only on the parts of lateral branches that have developed in the past year or the last several years. For example, in deciduous plants (e.g., many woody angiosperms), leaves develop only on the most distal segments of the laterals, i.e., the parts produced during the most recent growing season (Fig. 2.5) and will fall from the plant at the end of the same growing season. In most conifers and other

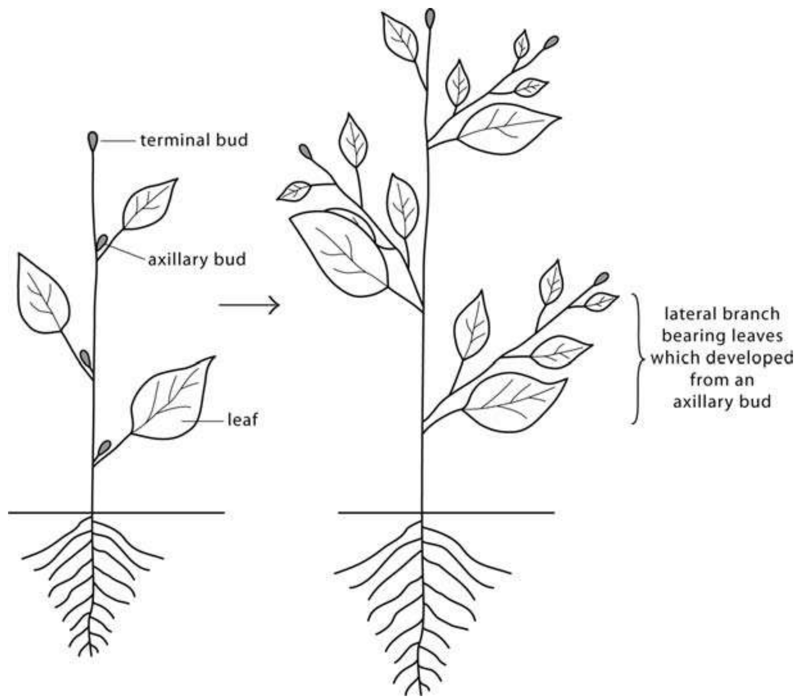


Figure 2.4 Diagrams of two stages in the development of an annual vascular plant.

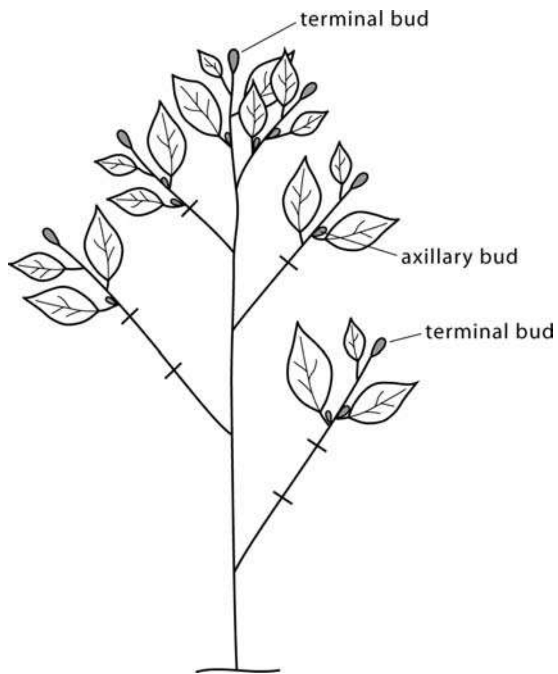
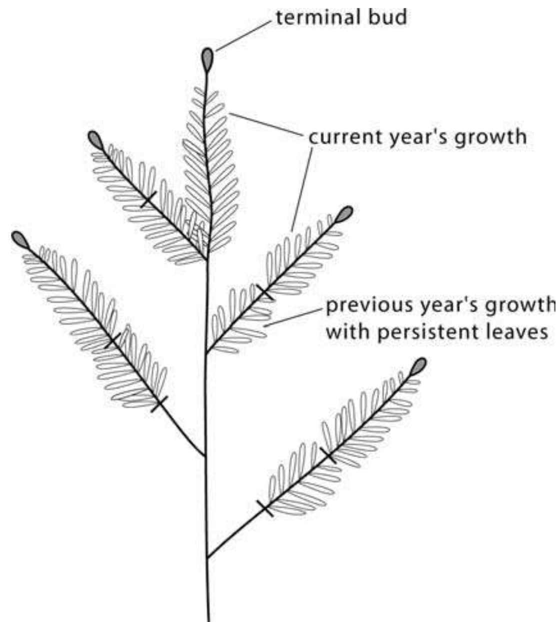


Figure 2.5 Diagram of the shoot of a deciduous, woody perennial with leaves and axillary buds, on terminal twigs only, produced during the current growing season. Each bar (at right angles to a lateral branch) indicates the previous site of a terminal bud.

Figure 2.6 Diagram of an "evergreen" conifer shoot with leaves on the current and previous year's growth. The bars at right angles to the lateral branches indicate previous positions of terminal buds.



evergreens, however, leaves may stay on the plant for several years, but the youngest, i.e., the most recently developed leaves, are borne on twigs produced in the current or most recent past growing season (Fig. 2.6).

A unique feature of vascular plants is the presence of buds which occur at the tips of the main and lateral branches and, in gymnosperms and angiosperms, commonly (but not always in gymnosperms) in the **axils of leaves** (the angle between leaves and the stem to which they are attached) (Fig. 2.5). A **bud** consists of an apical meristem enclothed by protective bud scales (modified leaves) (Fig. 2.7). A **meristem** is a localized region of cells that is characterized by active cell division, the ultimate result of which is the addition of new cells, tissues, and organs (such as leaves) to the plant body. It is, thus, the structural feature that imparts to plants their distinctive serial mode of development – so different from that of animals.

In contrast to animals whose development can be characterized as determinate, plant development, by virtue of the presence of apical meristems, is **indeterminate**. That is, plants have the ability to add new cells and tissues to the plant body during each growing season as long as the plant lives. This makes possible the enormous size of very old trees such as the redwoods of California or the very large deciduous trees of virgin hardwood forests of northeastern USA. The development of some parts of plants such as leaves and components of flowers, however, is **determinate** in that their form, and to some extent, size are genetically predetermined. Once these plant parts have completed their development, they do not grow further no matter how long they remain on the plant as functional entities.

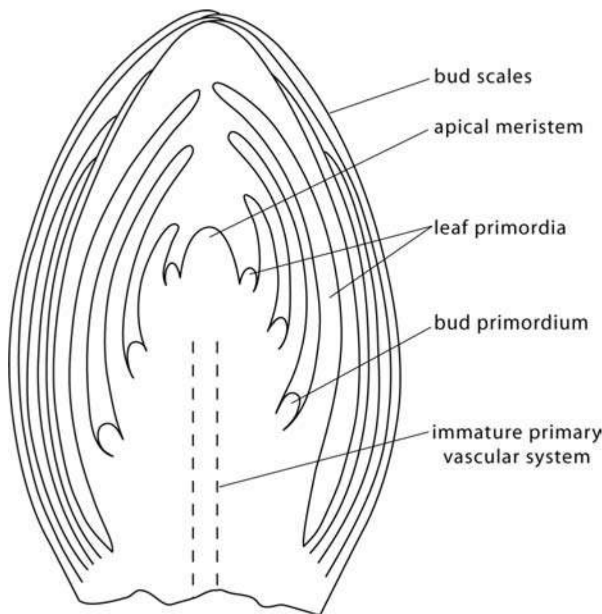


Figure 2.7 Diagram of a terminal bud as viewed in a median longitudinal section. Space is left between foliar components for clarity.

Apical meristems

We shall now look at the gross internal structure of buds and, in particular, consider the activity of apical meristems and some aspects of the differentiation of cells and tissues resulting from this activity. The apical meristem of the shoot is a dome-shaped structure that comprises the apical-most region of the main and lateral axes. In the root, the apical meristem is covered by the root cap. In this chapter we shall consider only the apical meristem of the shoot, deferring discussion of the apical meristem of the root to Chapter 16. In the shoot, small protuberances, the **leaf primordia**, from which leaves will develop, form around the base on the periphery of the apical meristem (Fig. 2.8). **Bud primordia** develop in the axils of older leaf primordia. Because of its permanently apical position, cells, tissues, and structures such as leaf and bud primordia close to the apical meristem are younger, and consequently, less mature than those farther away. It is apparent, therefore, that the direction in which differentiation proceeds is **acropetal**, that is, from the more mature, proximal part of the shoot toward the less mature, distal region near the apical meristem. In other words, cells, tissues, and lateral appendages (e.g., leaves) become progressively more mature in the direction of the apical meristem which is actively producing new cells that are added on to those produced earlier. If this concept of acropetal development is difficult for you, consider this analogy. In building a wall a bricklayer starts at the base, adding layer after layer of bricks until the wall is completed. The mortar between the first two layers of bricks at the base of the wall sets (i.e., hardens or "differentiates") first. That between subsequent layers sets later and later until finally that between the last two layers at the top of the walls has set.

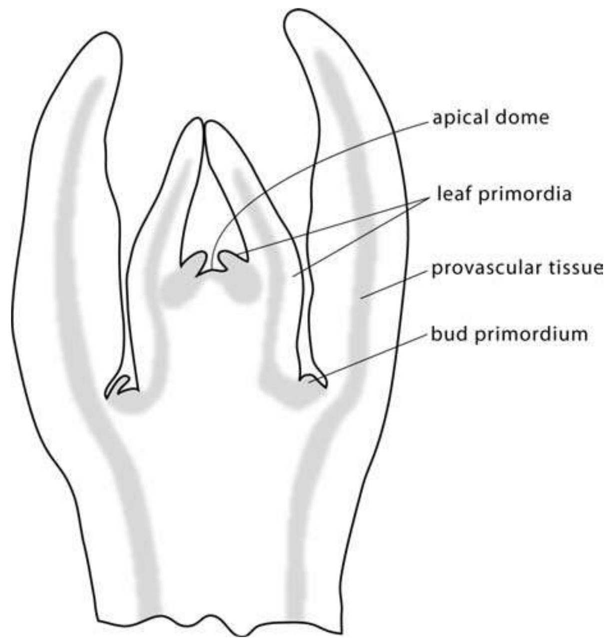


Figure 2.8 Diagram of a shoot apex as viewed in a median longitudinal section.

In other words, the mortar in the wall can be thought of as having differentiated sequentially from bottom to top, that is, acropetally.

Cell derivatives of the apical meristem, as well as of other meristems, initially resemble closely cells of the meristem. Plant tissues are composed of many different types of cells, and the cells produced by meristems must, therefore, grow and differentiate during development, ultimately attaining the specific characteristics of particular, mature, functional cells (Fig. 2.9).

Before proceeding further, we must distinguish between the processes of development and differentiation. **Development** consists of the integrated processes of growth, differentiation, and morphogenesis. Growth results in increase in size, and during growth, differentiation occurs. **Differentiation** is the process whereby cells, tissues, or organs achieve their distinctive, mature morphological and physiological characteristics during gradual change from their state as single or groups of meristematic derivatives. **Morphogenesis** is the achievement of form during development. Although commonly applied to the whole plant it, like differentiation, can also be applied to the various components of the plant body. Morphogenesis and differentiation are highly integrated processes, but they do not necessarily have a controlling influence on each other. They are probably controlled separately by different genes or gene complexes.

Now in order to more fully understand the process of differentiation, let us consider the differentiation through time of a single cell derivative of an apical meristem. From the time it is first produced it begins to grow, to some degree in diameter, but primarily in length, with its long axis paralleling the long axis of the stem, root, or branch in which it resides. In other words, the derivative grows longitudinally.

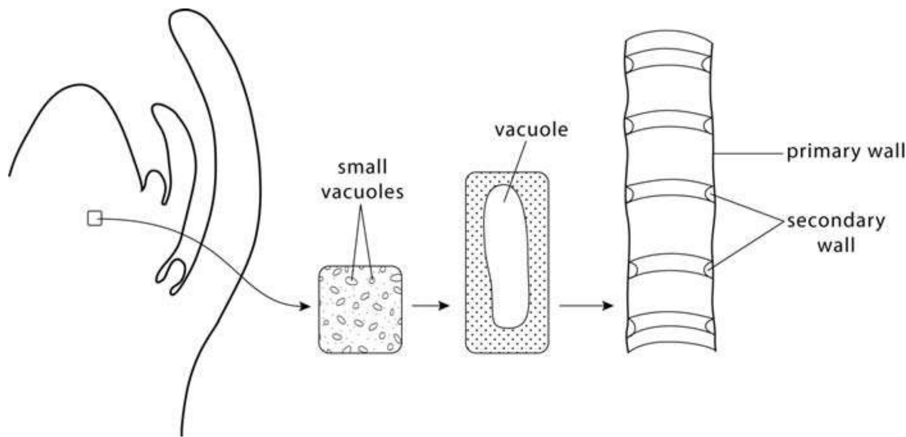


Figure 2.9 Diagrammatic representation of growth and differentiation of a type of water-conducting cell produced by the apical meristem. Note that growth consists primarily of increase in length. Very small vacuoles fuse to form a large central vacuole resulting in the peripheral location of the cytoplasm. Prior to death of the protoplast, secondary wall is deposited in the form of rings.

(Although this is a common pattern of cell growth in plants, some cells grow almost equally in diameter and length.) It is generally agreed that the motive force for cell growth is turgor pressure within the cell. In order for the protoplast to grow, the cell wall must also grow, concurrently, by increasing in surface area. (Growth of the cell wall, which includes synthesis of the chemical and structural components of which it is composed, is an area of great interest and active research, and will be considered in detail later.) Changes that occur in the cell protoplast during growth and differentiation include the fusion of small vacuoles to form larger vacuoles, and increase in the volume of cytoplasm within which develop various cell organelles such as mitochondria, plastids, and Golgi bodies as well as endoplasmic reticulum, microtubules, and microfilaments. Eventually, the total complement of originally small vacuoles will have fused into a single central vacuole forcing the cytoplasm with its organelles, filamentous structures and nucleus into a peripheral position (Fig. 2.9). Other dramatic changes in cells during differentiation are exemplified by conducting cells in the phloem and xylem. In the phloem the organelles and even the nucleus of a differentiating conducting cell become modified or may even degenerate prior to achievement of functional maturity by the cell. Concurrently, the end walls become highly perforate, which facilitates the transport of photosynthate, hormones, and other chemical substances from cell to cell. The protoplasts in conducting cells of the xylem actually die, but prior to death the cell walls become highly modified. The walls increase in thickness, often differentially, resulting in structural features that strengthen the cells, and the walls may become pitted. They may also become heavily impregnated with lignin, a compound that increases their tensile strength. The absence of a protoplast and the

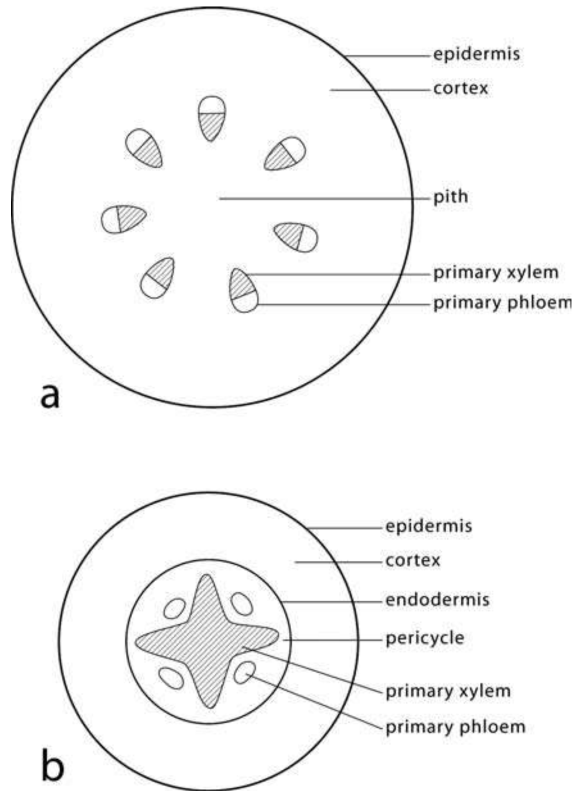


Figure 2.10 (a) Primary tissue regions of a dicotyledon stem shown in transverse section. (b) Primary tissue regions of a seed plant root. Note the ribbed primary xylem column, and the separate bundles of primary phloem.

modifications of the cell walls facilitate the transport of water and dissolved mineral nutrients. In later chapters we shall consider in detail the structural changes of conducting cells in the phloem and xylem and the relationship of these to the processes of transport.

A major activity of apical meristems is the production of **primary tissues** in stems and roots, resulting in an increase in the length and, to a lesser extent, an increase in thickness (diameter) of these axes. Among the primary tissues produced are the primary phloem and primary xylem which, in seed plants, comprise vascular bundles, and the parenchyma, collenchyma, and sclerenchyma of which the pith and cortex are composed (Fig. 2.10). Meristematic activity in shoot systems also results in the production of leaf primordia that define **nodes**, sites of attachments of leaves, and **internodes**, segments of the stem between nodes. Close to the apical meristem, internodes are very short, but increase in length with distance from it. In most vascular plants internodal elongation results primarily from the growth of cells produced earlier by the apical meristem and subjacent tissues. As meristematic activity continues, the internodal tissues differentiate largely acropetally. In some plants, e.g., *Equisetum* (horsetail) and grasses, however, differentiation in internodes is primarily **basipetal** (i.e., tissues differentiate progressively toward the base). We shall consider development in these plants in some detail in Chapter 5.

Primary tissue regions of the stem and root

As a result of the activity of apical meristems, and subsequent differentiation of cells and tissues, axes of the shoot and root systems are composed of several distinct **tissue regions** (Fig. 2.10). In the stem and the root there may be a central pith surrounded by vascular tissue (xylem and phloem) variously arranged. The vascular tissue may be continuous, or comprised of vascular bundles that may or may not be interconnected. The central region in some stems and most roots is a solid column of vascular tissue and, thus, there is no pith (Fig. 2.10b). To the exterior of the region of vascular tissue are the cortex and epidermis. In some stems a single-layered region called the endodermis comprises the innermost layer of the cortex. It bounds the pericycle, a tissue region between itself and the vascular tissue. The pericycle is usually recognized only in axes that also have an endodermis which defines its outer limit. The pericycle and endodermis are common in roots (Fig. 2.10b) as well as in the stems of plants that live in aquatic environments.

Each tissue region may be composed of several **simple tissues**, containing a single type of cell, or a **complex tissue**, comprising several types of cells. As we now know, tissues derived from the apical meristems are **primary tissues**, whereas those derived from lateral meristems are **secondary tissues**. In order to better understand the nature of these tissues we must be able to visualize their component cells in three dimensions. Different types of cells have evolved as adaptations to the terrestrial environment in which most vascular plants live and have become specialized for the performance of one or more functions. Some cells function largely in the synthesis of important compounds or in other metabolic processes such as respiration, digestion, etc. Others function solely or largely in transport (or conduction) of substances whereas some function mainly in providing mechanical support. Thus there are many different types of cells with different morphologies that we must understand. Since all of these types often occur together in complex tissues this poses a difficult, but not insoluble problem. There are two ways in which we can comprehend the morphology of a particular cell in three dimensions. For example, we can macerate a piece of tissue by immersing it in an acid solution that dissolves the intercellular cementing material which holds cells together in tissues. Thus separated, we can observe the individual cells. We may, however, wish to comprehend the three-dimensional form of cells in intact tissues such as sections. To do this we must learn to think in three dimensions on the basis of sections of cells in two dimensions. If we observe the cell in question in both transverse and longitudinal views, we can then combine these, mentally, to obtain a three-dimensional conception of the cell (Fig. 2.11a). To understand the detailed morphology of a cell type we will usually have to observe the cell in transverse view as well as in two longitudinal views: radial and tangential (Fig. 2.11b). Transverse, radial, and tangential views of cells can be

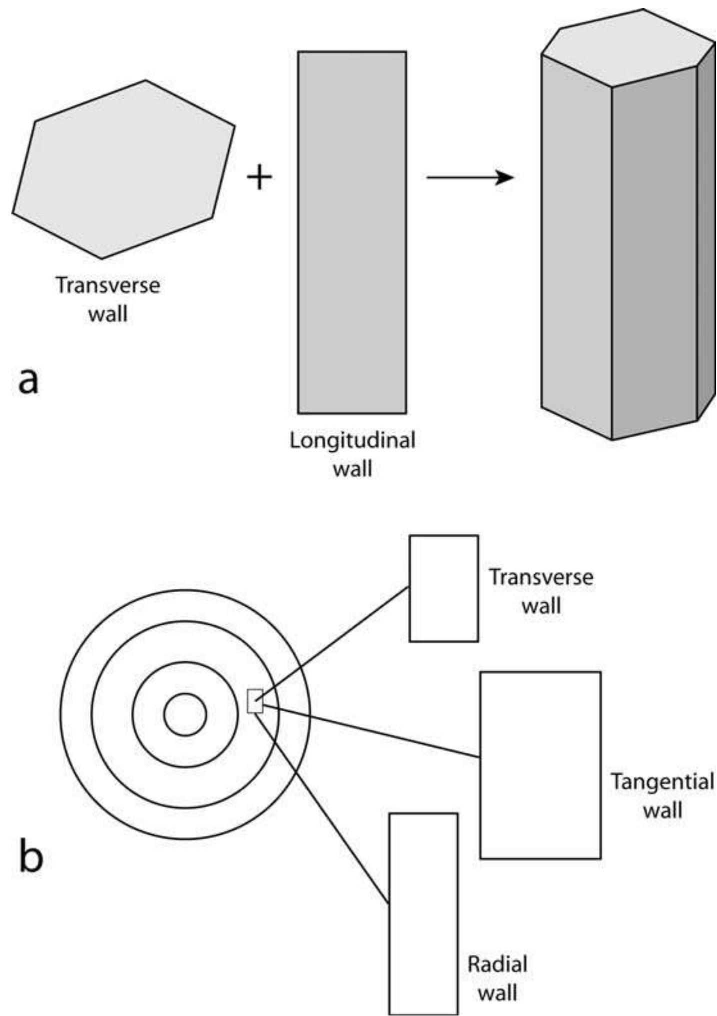


Figure 2.11 (a) With knowledge of the shape of a transverse wall and a longitudinal wall of a cell, one can envision its three-dimensional form. (b) If a wall of a cell is parallel to a transverse plane it is called a transverse wall; if parallel to a radial plane it is called a radial wall; if parallel to a tangential plane, it is called a tangential wall.

observed in transverse, radial, and tangential sections (Fig. 2.12). A **transverse section** is a thin sheet of tissue cut at right angles to the long axis of a stem or root, a **radial section** is one cut along a radius of the circle formed by the exterior boundary of the axis, whereas a **tangential section** is one cut on a tangent perpendicular to the radius (Fig. 2.12). One can refer to the walls of cells that lie in the same plane as radial or tangential sections as radial or tangential walls. Likewise, end walls of cells may be called transverse walls when they occur in a plane parallel to a transverse section.

Let us now consider the tissues of the pith and cortex. Three simple tissues, parenchyma, collenchyma, and sclerenchyma, singly, in combinations of two, or all three, may comprise either or both of these tissue regions. **Parenchyma** is thought of as the ground tissue of an axis since it occurs in greatest abundance and is the tissue in which the vascular tissues are embedded. Parenchyma cells (Fig. 2.13a, b) may be variably isodiametric in both the pith and the cortex, but

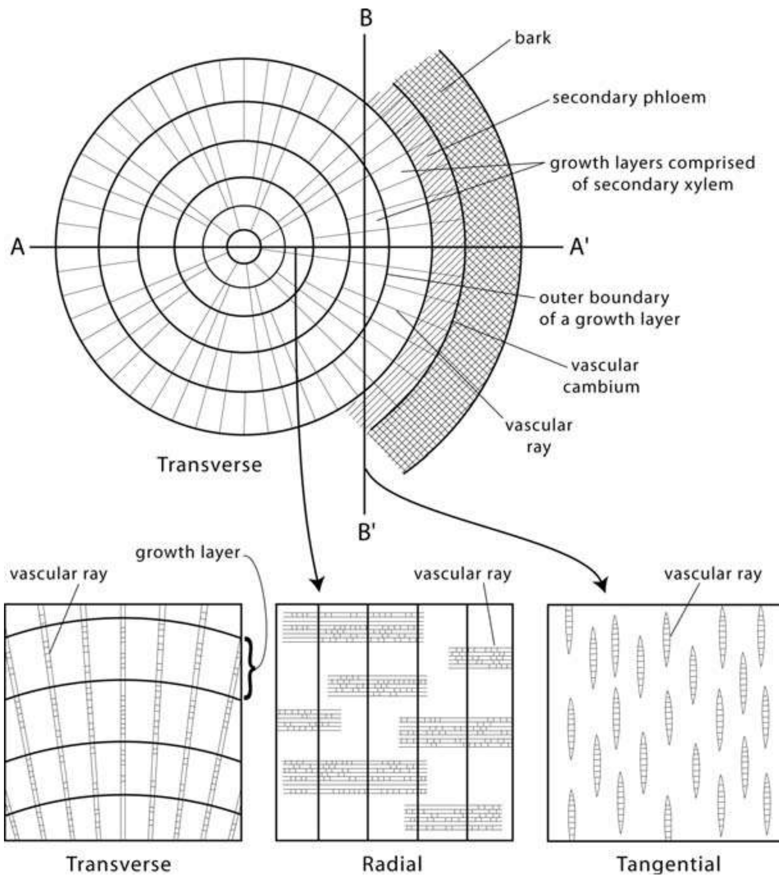


Figure 2.12 Planes of section through secondary xylem. Any section cut parallel to the plane of the page is a transverse section. Note that vascular rays cross growth layers at right angles. Any section cut parallel to a radius of the circle formed by any growth layer is a radial section as, for example, a section cut parallel to plane A–A′. In a radial section, rays appear in side view, crossing growth layers at right angles. Any section cut parallel to a tangent to any imaginary circle parallel to the boundary of a growth layer is a tangential section as, for example, a section cut parallel to plane B–B′. In a tangential section vascular rays appear in end view.

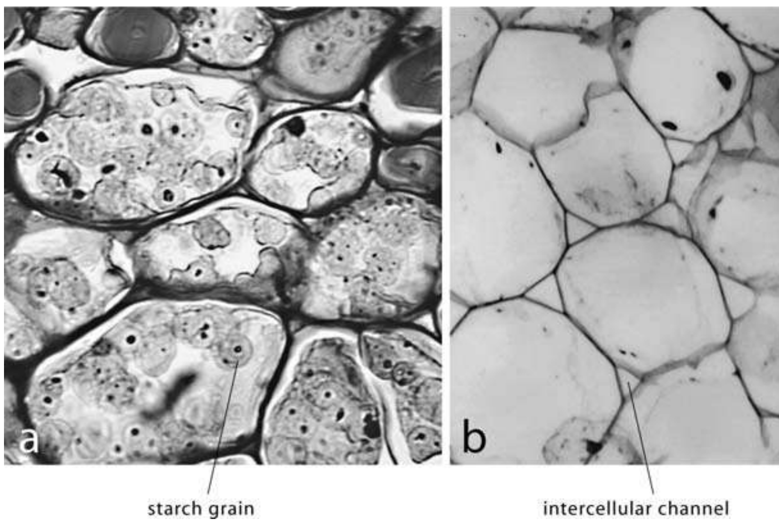


Figure 2.13 (a) Starch grains in parenchyma cells of the cortex. (b) Parenchyma tissue in the pith containing conspicuous intercellular channels. Magnification $\times$ 408.

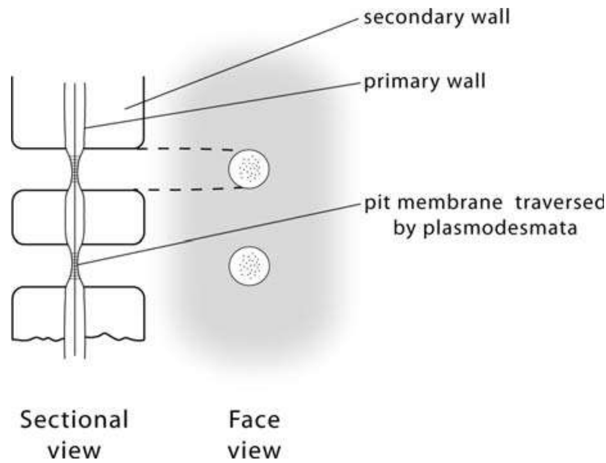


Figure 2.14 Simple pit-pairs in sectional and face views with plasmodesmata connecting the protoplasts of contiguous cells.

are more commonly longitudinally elongate in the cortex. They have relatively thin walls that almost always consist of both primary and secondary wall layers. Wall layers are continuous except in the regions of **simple pits**, circular to irregularly shaped regions lacking secondary wall material. These simple pits usually occur opposite each other, forming **pit-pairs** in the walls of contiguous cells (Fig. 2.14). Multiple **plasmodesmata** (specialized strands of endoplasmic reticulum) form interconnections with the protoplasts of adjacent living cells through the primary walls of simple pit-pairs. Plasmodesmata are not restricted to pit-pairs, however, and may traverse other regions of the wall as well. These interconnections make possible communication (i.e., the transport of chemical compounds) between cell protoplasts which, collectively, comprise the **symplast**. The transport of water and inorganic solvents can occur solely through the cell walls which, collectively, comprise the **apoplast**. Long-distance transport, however, occurs primarily through the **lumina** (the cavities enclosed by cell walls) of non-living cells such as tracheids and vessel members. Some workers include the lumina of these cells as components of the apoplast.

Various metabolic processes occur in parenchyma tissues including the synthesis of hormones, enzymes, pigments, essential oils, toxic substances, etc. One of the processes of synthesis most important to both the plant and to animals including humans, is photosynthesis which produces glucose, the basic food substance for all living beings. Another equally important metabolic process is respiration which provides the source of energy utilized by the plant in carrying out its various activities. Other important processes are the conversion of glucose to starch, the form in which it is stored in parenchyma tissues, and the reverse process of digestion of starch which makes glucose available for use by the plant. Another highly important compound composed of glucose molecules is **cellulose** which is the primary structural material of cell walls.

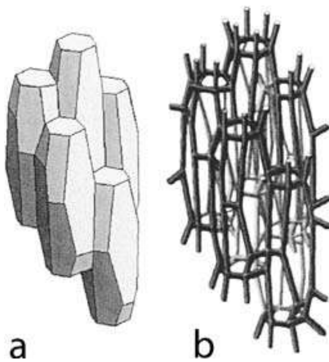


Figure 2.15 Diagram of the system of intercellular channels (b) between parenchyma cells (a).

A system of **intercellular channels** (Figs 2.13b, 2.15), often referred to as gas spaces, characterizes the parenchyma tissue of plants. The intercellular channels, commonly connected to the outside atmosphere

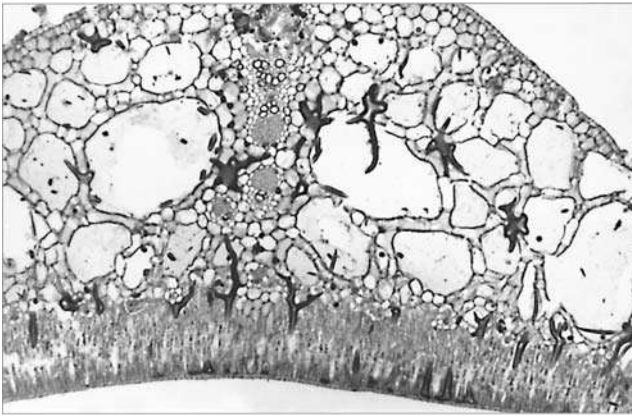


Figure 2.16 Large gas-filled spaces in the leaf of *Nymphaea* (water lily), an aquatic dicotyledon. Note, also, the large astrosclereids. Magnification × 65.

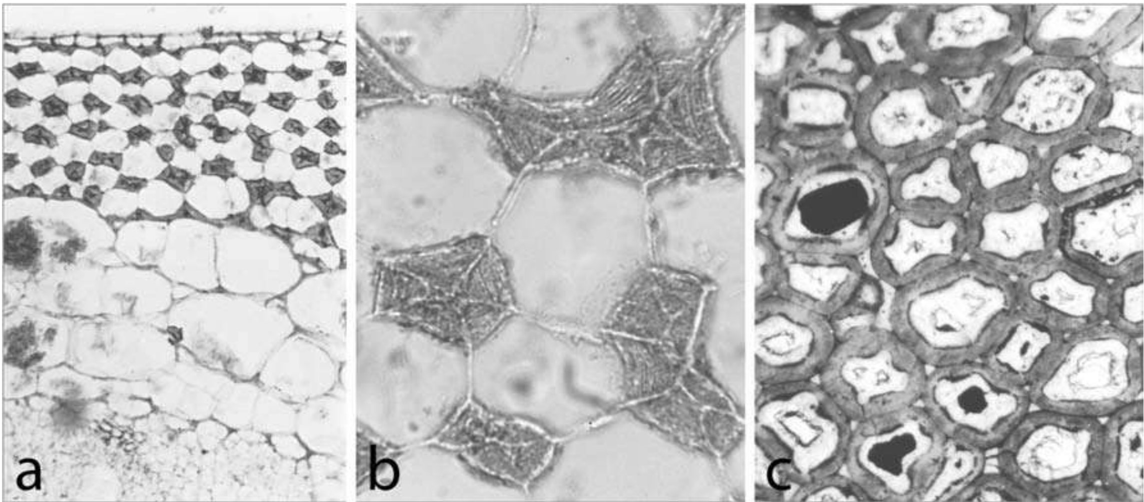


Figure 2.17 Collenchyma. (a) Angular collenchyma in the outer cortex just beneath the epidermis with thickened walls in the corners of the cells. Magnification × 18. (b) Enlargement of a part of (a) showing the lamellate nature of the thickened regions of the cell walls. Magnification × 1150. (c) Lamellar collenchyma with regions of thickened walls between cell corners. Magnification × 220.

through stomata (or lenticels in older stems), comprise an extensive system in the pith and cortex in stems, roots, and some rhizomes as well as in leaves and some fruits. They are especially well developed in roots and rhizomes in aquatic plants or plants that live in wet soils. The gas-filled spaces vary from the narrow channels between cells to the large, irregular spaces in the mesophyll of leaves and in the aerenchyma of aquatic plants (Fig. 2.16) (Raven, 1996; Prat *et al.*, 1997). Intercellular channels are significant in providing aeration, i.e., facilitating an interchange of O_2 and CO_2 in this tissue which is the site of both photosynthesis (which utilizes CO_2 and produces O_2) and respiration (which utilizes O_2 and produces CO_2). In aquatic plants the system of gas-filled intercellular channels is also important in providing buoyancy.

Collenchyma, like parenchyma, is a tissue in which the various metabolic processes mentioned above also occur. Unlike parenchyma, however, collenchyma forms only a small part of the pith or cortex. In fact, it is only rarely found in the pith, but occurs commonly in the cortex, especially in the outer cortex of herbaceous stems (Fig. 2.17). It is also an important component of leaves and some flower parts. In the stem it often occurs as a continuous layer or as longitudinal ribs in

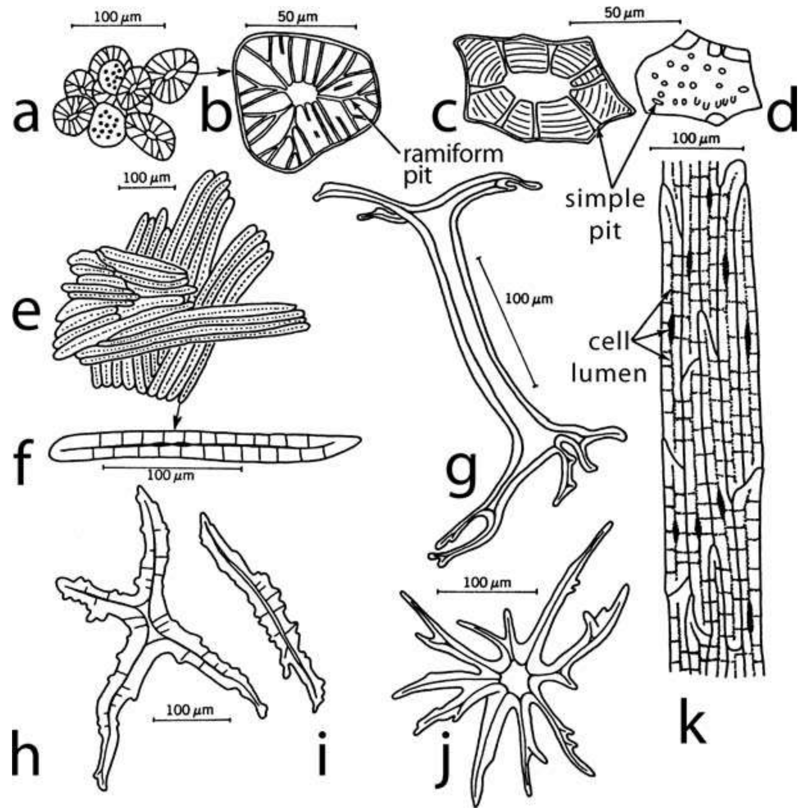


Figure 2.18 Sclereids of diverse forms. (a, b) Brachysclereids (stone cells) from the fruit of *Pyrus* (pear). (c, d) Sclereids from the stem cortex of *Hoya* (wax plant), in sectional (c) and surface (d) views. (e, f) Macrosclereids from the endocarp of *Malus* (apple). (g) Columnar sclereid with branched ends from the palisade mesophyll of *Hakea*. (h, i) Sclereids of variable form from the petiole of *Camellia*. (j) An astrosclereid from the stem cortex of *Trochodendron*. (k) An extensive layer of macrosclereids from the epidermis of a clove scale of *Allium sativum* (garlic). From Esau (1977). Used by permission of John Wiley and Sons, Inc.

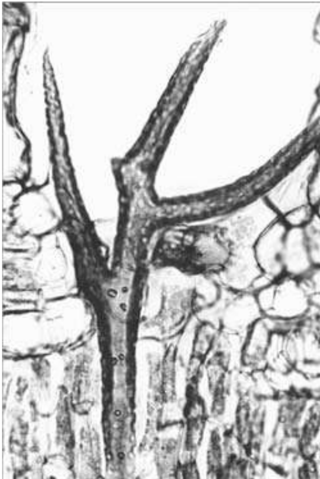


Figure 2.19 A large sclereid from the leaf of *Nymphaea*. Note the thin cell wall containing numerous simple pits. Sclereids of this type often retain a living protoplast. Magnification × 370.

the outermost cortex. In leaves and flower parts it often accompanies major vascular bundles. Wherever it occurs it provides a supporting function.

Collenchyma cells are characterized by relatively thick, but unevenly thickened and unlignified primary walls. The walls are usually highly hydrated, and often conspicuously lamellate (Fig. 2.17b). In the most common type of collenchyma the greatest wall thickening occurs in the corners of the longitudinally elongate cells which are polygonal in transverse shape. Collenchyma of this type is called **angular collenchyma** (Fig. 2.17a, b) if there are no, or only very small, intercellular channels in the tissue, or **lacunar collenchyma** if the tissue contains conspicuous intercellular channels. In a third type of collenchyma, however, the regions of greatest wall thickening occur between the corners. This type is called **lamellar collenchyma** (Fig. 2.17c).

Sclerenchyma is the major supporting tissue in primary tissue regions and consists of either fibers or sclereids. **Sclereids** are more or less isodiametric, but of highly variable form (Fig. 2.18), and have relatively thin to very thick lignified walls containing, in very thick walls, simple pits that are canal-like and often branched (ramiform). Many sclereids, especially those with relatively thin walls, have living protoplasts (Fig. 2.19). Sclereids with different shapes are given different

names (Fig. 2.18). **Brachysclereids**, a common type of ovoid to somewhat irregular shape, and often called "stone cells," are common in the flesh of fruits occurring singly or in clusters, as in pear. In fruits with stony endocarps, e.g., peaches, almonds, cherries, etc., they may be the sole cell type comprising the stony layer. They also occur singly or clustered in the pith and/or cortex of some stems. **Macrosclereids** are columnar or rod-shaped and are common components of the epidermis of seeds and the endocarp of fruits. **Osteosclereids** are bone-shaped and are common in leaves and seed coats. **Astrosclereids** are irregularly star-shaped with elongate, relatively thin-walled processes extending from a central region. They are common in the leaves of many tropical dicotyledons and in the stems and leaves of some aquatic plants (Fig. 2.16). **Trichosclereids** are very slender, hair-like, and sparsely branched. They may reach lengths of several millimeters and are common in the stems and roots of aquatic plants.

Fibers that occur in primary tissues of roots and stems are commonly very elongate cells with relatively thick, lignified walls containing simple pits (Fig. 2.20). They may comprise separate bundles or bands, often in the peripheral regions of stems, sometimes in the inner cortex (Fig. 2.21a) or, very commonly, may be associated with vascular bundles as bundle sheaths or bundle caps (Fig. 2.21b, c). Fibers serve a largely supporting function in the plant. They tend to be flexible and have great tensile strength and, thus, allow bending without breaking of plant axes. Fibers in primary tissue regions, especially those obtained from some monocots (Fig. 2.21c), are utilized commercially in the production of twine, rope, doormats, burlap, etc. Somewhat similar fibers occur in the secondary phloem, and fibers that are shorter, narrower and ofter thinner-walled are very common in the secondary xylem. In this book we shall restrict the term sclerenchyma fibers to those that occur in primary tissues.

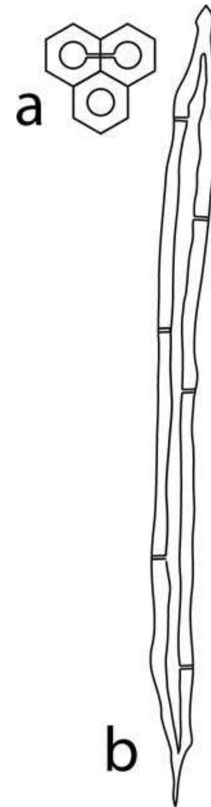


Figure 2.20 (a) Sclerenchyma fibers in transverse view, showing a simple pit-pair. (b) Sclerenchyma fiber in longitudinal view.

We shall defer a discussion of the pericycle and endodermis until a later chapter on the root (Chapter 16). The very important single-layered tissue region, the epidermis, which comprises the outer boundary of stems, roots, and other plant organs in regions in which periderm has not formed will receive detailed treatment in Chapter 8, devoted solely to it. All other important tissues and tissue regions will also be discussed in detail in later chapters.

Vascular bundle types

Primary xylem and primary phloem, are complex tissues, comprising several distinctive cell types. In later chapters we shall consider in detail the composition, development, and functions of these tissues, but shall now consider the morphology of the different types of vascular bundles in which they occur in seed plants.

Vascular bundles vary in morphology largely on the basis of the topographic arrangement of their constituent primary xylem and primary phloem. The vascular tissues are, typically, though not always,

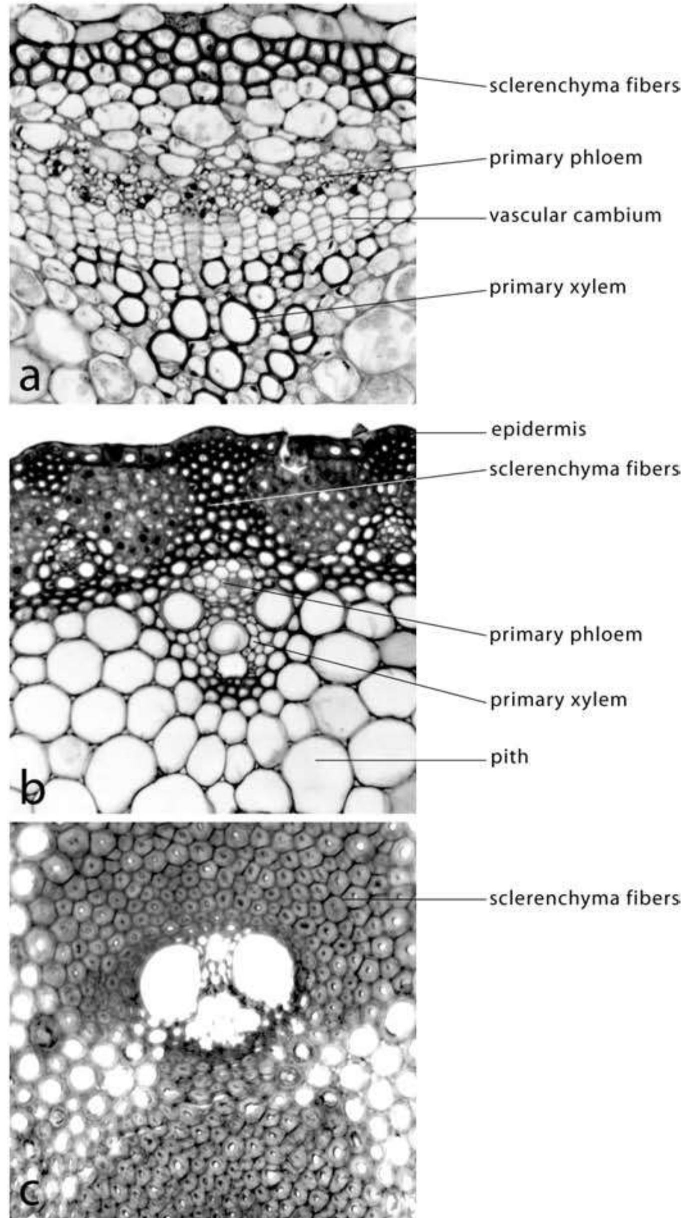


Figure 2.21 (a) Sclerenchyma fibers in the inner cortex of *Pelargonium*. (b) Fibers forming the bundle caps of vascular bundles of *Triticum* (wheat) and extending to the epidermis. (c) Very thick-walled fibers comprising the bundle cap, and enclosing the conducting cells, of a vascular bundle of *Agave*. Magnification × 255.

enclosed by one or more layers of either parenchyma or sclerenchyma cells which comprise the bundle sheath (Fig. 2.21b). Many vascular bundles are also characterized by a bundle cap of fibers (Fig. 2.21b, c).

Four bundle types are widely recognized. The most common type is the **collateral bundle** in which the primary xylem is innermost and primary phloem comprises the outer part (Fig. 2.22a). The collateral bundle is of frequent occurrence in both gymnosperms and angiosperms. In a **bicollateral bundle** the primary xylem is bounded both to the inside and outside by strands of primary phloem (Fig. 2.22b). These two strands of phloem are referred to, respectively, as internal and

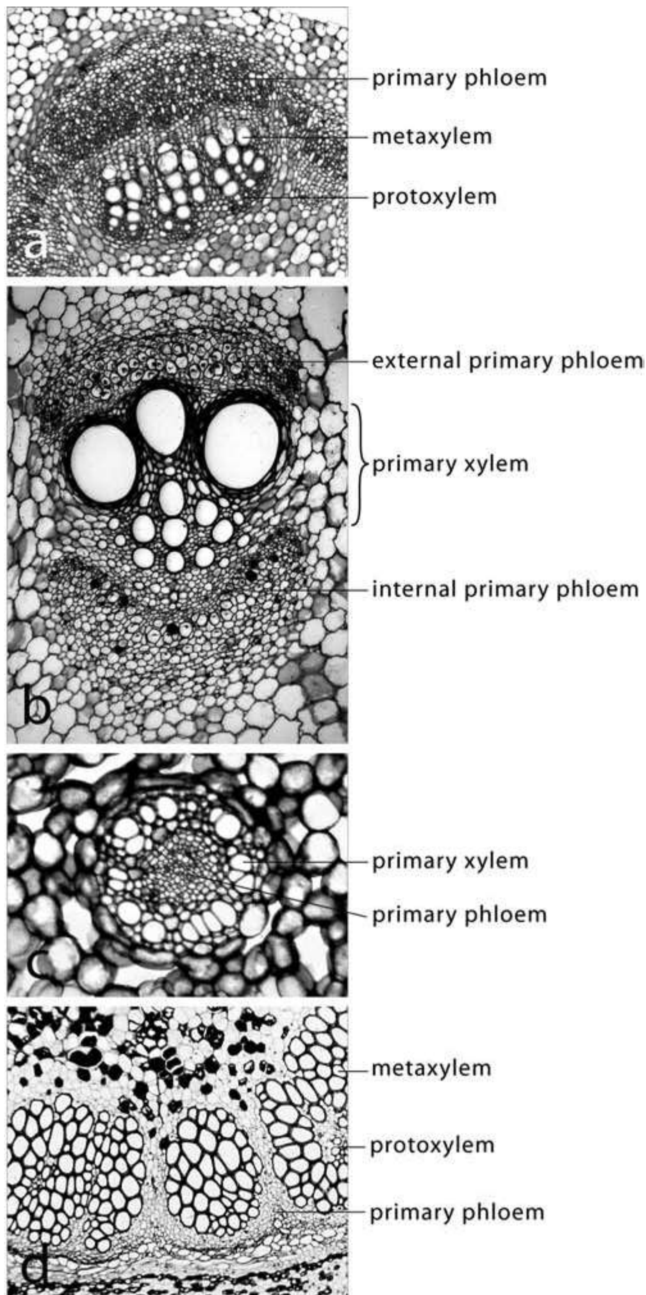


Figure 2.22 Types of primary vascular bundles. (a) A collateral bundle of *Cassia*. Magnification × 120. (b) A bicollateral bundle of *Cucurbita* (squash). Magnification × 70. (c) An amphivasal bundle of *Acorus calamus*. Magnification × 157. (d) An amphicribral bundle in the rhizome of the fern *Osmunda*. Magnification × 47.

external phloem. Bicollateral bundles occur only in some angiosperms. There are two types of **concentric bundles** in which one vascular tissue completely encloses the other. In **amphivasal bundles** (Fig. 2.22c), of frequent occurrence in some families of monocotyledons and a few dicotyledons, the primary xylem surrounds the primary phloem. In **amphicribral bundles** (Fig. 2.22d), common in some ferns, but also

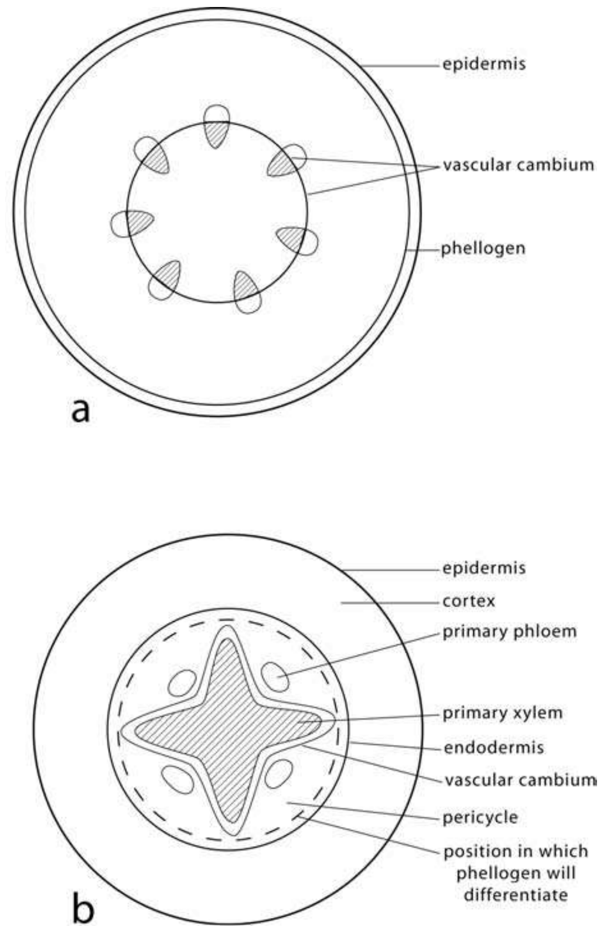


Figure 2.23 Sites of differentiation of the vascular cambium and the phellogen (cork cambium) lateral meristems in (a) a dicotyledon stem and (b) a root, both shown in transverse section.

found in a few aquatic angiosperms, the primary phloem surrounds the primary xylem.

Secondary growth

Activity of the apical meristems results in the development of the primary tissues and tissue regions and the lateral appendages of the shoot and root, all of which comprise what is often termed the **primary plant body**. As growth continues, in many plants, additional tissues are added laterally to stems and roots which add substantially to their thickness. These tissues, called **secondary tissues**, are produced by lateral meristems, the vascular cambium and the phellogen or cork cambium. In seed plant stems, the **vascular cambium** differentiates from immature tissue between the primary xylem and phloem in the vascular bundles as well as from tissue between the bundles, forming a continuous circle as viewed in transverse section (Fig. 2.23a). In roots it differentiates between the central primary xylem and bundles of primary phloem as well as around the ends of the ribs of primary xylem (Fig. 2.23b). This

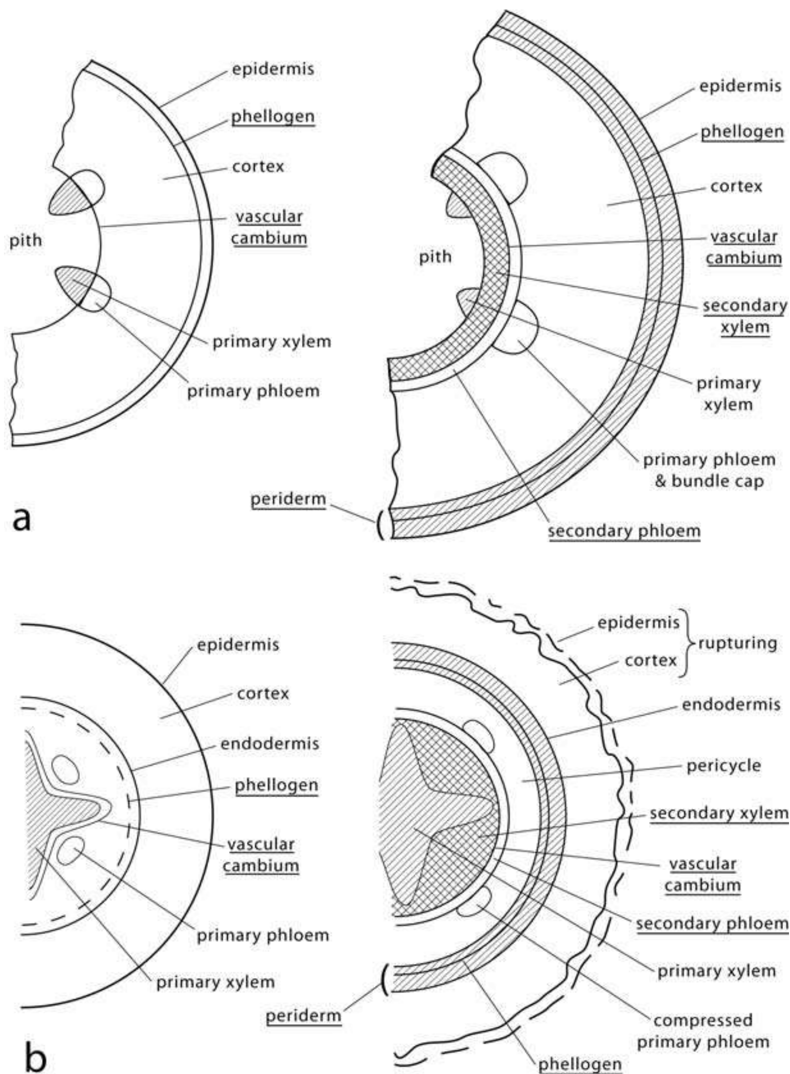


Figure 2.24 Diagrams showing the lateral meristems (vascular cambium and phellogen) and the secondary tissues which are formed by their cell divisional activity. (a) Production of secondary xylem and phloem, and periderm in a dicotyledon stem. (b) Production of secondary vascular tissues and periderm in a root. Note the position of secondary vascular tissues and periderm in relation to primary tissue regions. Because, in the root, the phellogen develops to the inside of the endodermis, the epidermis, cortex, and endodermis eventually disintegrate and slough off. The labels indicating the meristems and the secondary tissues they produce are underlined.

meristem, one cell layer in thickness, which produces several layers of immature derivatives called the cambial zone, extends from near the stem and root tips to the base of the plant. In three dimensions, the vascular cambium in stems is essentially conical. In roots it is of irregular form initially, becoming more regularly conical as secondary xylem is produced. During periods of growth, cells of the vascular cambium divide in a manner that results in the production of new cells to both the interior and to the exterior of the meristem (Fig. 2.24). This cambial activity results in layers of secondary tissue called **growth layers** and leads to an increase in diameter of the stem or root (see also Fig. 2.12). Although these layers are often called cylinders, they are, in reality, elongate cones (Fig. 2.25). The vascular cambium produces, to the exterior, secondary phloem in which photosynthate and hormones are transported, primarily from the leaves, downward into the roots

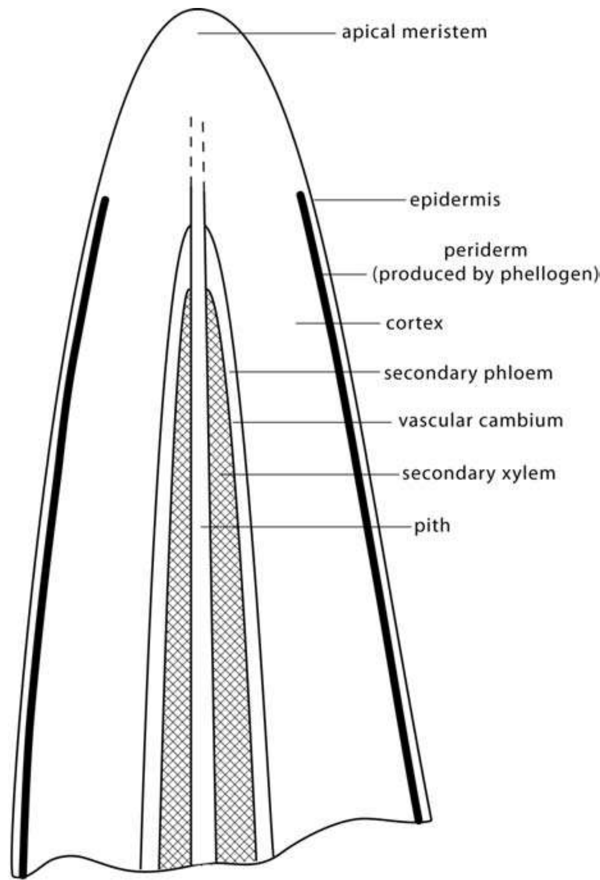


Figure 2.25 Stem tip of a woody dicotyledon shown diagrammatically in longitudinal section, illustrating the secondary tissues produced by the vascular cambium and the phellogen. Primary xylem, primary phloem, and lateral appendages are omitted to simplify the diagram.

and, to the interior, secondary xylem in which water and inorganic solvents are transported, primarily from the roots, upward into the stem. In temperate climatic zones the layers of secondary xylem are usually called annual rings since each layer usually represents the result of cambial activity during one year. The boundaries of these layers are recognizable because the cells produced near the end of a growing season are typically much smaller than those produced earlier. The phellogen, also a single-layered meristem, located in the outer cortex, often differentiates in the layer just inside the epidermis. It produces **phellem** to the exterior and **phelloderm** to the interior, tissues which comprise the **periderm**, a major component of the bark (Fig. 2.24).

The secondary xylem consists largely of **tracheary elements** (tracheids and/or vessel members) and associated fibers and parenchyma cells, all of which are elongate cells that parallel the long axis of stems and roots. These cells comprise the **axial system. Rays**, thin ribbons of, largely, parenchyma cells traverse the secondary xylem at right angles to the elongate cells, that is, along radii of the circles formed by the growth layers of secondary tissues (Fig. 2.22a–d). They comprise the **radial system**, extending from the secondary xylem across the vascular cambium and through the secondary phloem. The axial system in the

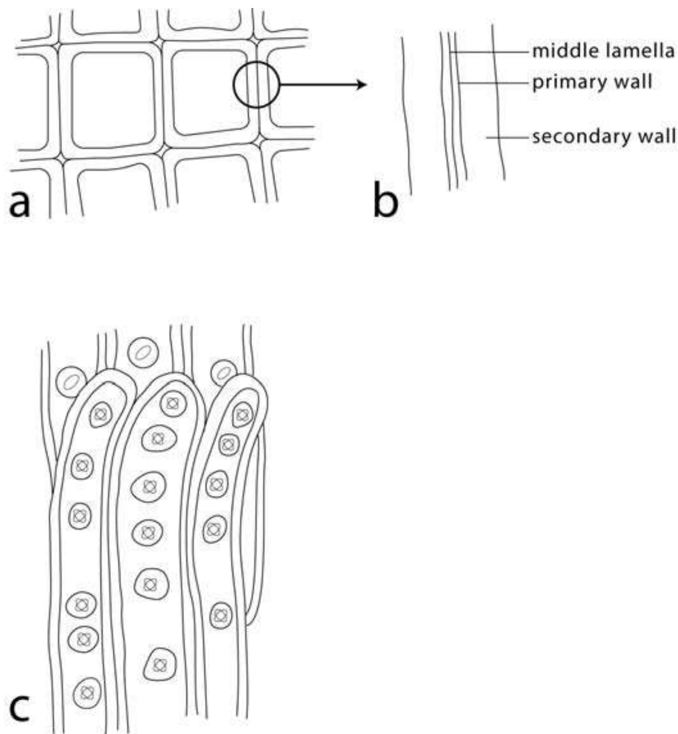


Figure 2.26 Conifer tracheids.
(a) Transverse view.
(b) Enlargement of a section of the cell walls of contiguous tracheids showing wall layers.
(c) Longitudinal section illustrating bordered pits on radial walls and overlapping ends.

secondary phloem consists of elongate conducting cells and associated companion cells, fibers, and phloem parenchyma cells.

Cells of the xylem

Both primary and secondary xylem may contain tracheids, vessels (consisting of vessel members), fibers, and parenchyma cells. The xylem of the earliest vascular plants in the fossil record as well as the most primitive living vascular plants contain only tracheids. **Tracheids** serve two important functions: support, and transport of water and minerals. Vessel members and wood fibers appear in the xylem of plants that evolved later in geologic time. The tracheid is, therefore, considered the basic conducting cell in the xylem and the type from which vessel members and fibers evolved. During its evolution the vessel member became highly specialized for transport and the wood fiber for support (for more detail, see Bailey (1953) and references therein).

The tracheid (Fig. 2.26), at functional maturity, is a non-living, longitudinally elongate cell usually with tapered ends, although the ends may be rather blunt in some taxa (Fig. 2.26c), especially some conifers. In sectional view the tracheid may be circular or polygonal, the latter being characteristic of tracheids when compactly arranged. Tracheids in secondary xylem are characterized, typically, by several to many wall facets, and in conifers they often appear rectangular in sectional view (Fig. 2.26a). The walls of tracheids, as in most other mature cells

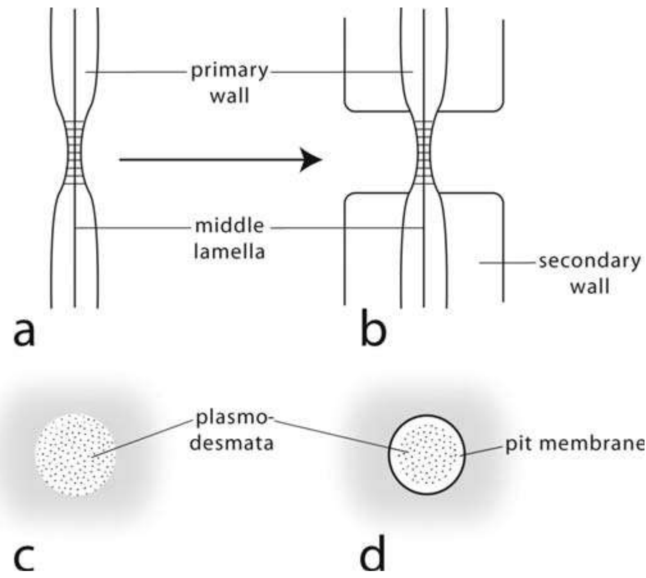


Figure 2.27 (a, c) Primary pit fields in (a) sectional and (c) face views, showing plasmodesmata. (b, d) Simple pit-pairs in (b) sectional and (d) face views. Note that the simple pit-pair develops in relation to the primary pit field by the production of secondary walls in contiguous cells.

in vascular plants, are comprised of long strands of cellulose, called **microfibrils**, embedded in an amorphous matrix of lignin and other substances. Cell walls are usually composed of two layers, an outer **primary wall layer** and an inner, thicker **secondary wall layer** (Fig. 2.26b). The thin primary wall is produced following mitosis and cell plate formation, and the secondary wall is added on to the inner surface of the primary wall as development of the cell proceeds. The secondary wall layer may be largely discontinuous, with secondary wall material deposited on the primary wall in helical, reticulate, or scalariform patterns, or largely continuous, but containing pits, well-defined areas lacking secondary wall (see below). More detail, and aspects of development of these variations in the morphology of tracheid walls will be presented in Chapters 4 and 11.

In the secondary xylem the tracheid ends overlap those of other tracheids (Fig. 2.26c). In a living tree the tracheid lumina are filled with water which, during transpiration, moves in a helical path from one tracheid end into that of the adjacent, overlapping tracheid. Transport is facilitated by the lack of a protoplast in the tracheids, by the permeability of their cellulosic cell walls, and by the presence of pits which occur, in abundance, in pairs in the walls of the contiguous, overlapping tracheid ends.

Before describing vessel members and wood fibers, let us consider in more detail the nature of pits. **Pits** (Figs 2.27, 2.28) are canals in the secondary wall layer, but these canals do not extend through the primary wall. They develop because of the lack of deposition, or the deposition of very little, secondary wall material at the sites of pit formation during wall development. Pits commonly develop opposite sites of **primary pit fields** (Fig. 2.27a), thin regions in the primary wall that are traversed by **plasmodesmata**, specialized strands of endoplasmic reticulum that connect and provide avenues for molecular transport

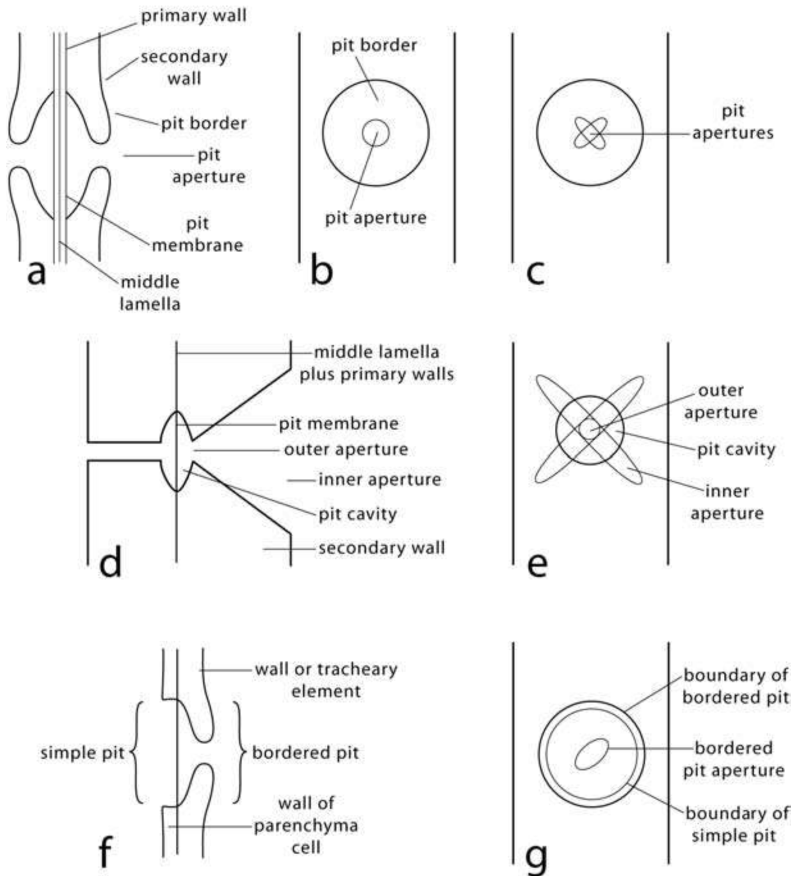


Figure 2.28 Bordered and half-bordered pit-pairs. (a) Sectional view of a bordered pit-pair. (b) Face view of a bordered pit-pair with circular apertures. (c) Face view of a bordered pit-pair with crossed, elliptical apertures. (d, e) Sectional and face views of a bordered pit-pair in thick secondary walls. The pit canal, in the form of a flattened cone, has inner and outer apertures. The inner, crossed apertures extend beyond the boundary of the pit cavity. (f, g) A half-bordered pit-pair in sectional and face views.

between the protoplasts of adjacent cells. The failure of secondary wall to be synthesized at these sites of pit formation may be controlled by informational molecules that are transported by the plasmodesmata (see Chapter 4 on the cell wall).

Typically pits occur in pairs (Figs 2.27b, 2.28a, d, f) with each pit of a pit-pair occurring opposite the other in a contiguous cell wall of adjacent cells. The primary walls of the two cells and the middle lamella lie between the two pits of a pair and form the pit-pair membrane, usually called simply the **pit membrane**. There are two types of pits, simple and bordered. A **simple pit** is a canal in a secondary wall with straight sides, which in face view may be circular, oval, or somewhat irregular (Fig. 2.27b, d). A **face view** is obtained when one looks directly into the pit from the inside or the outside of the cell. In **sectional view**, obtained when the wall in which the pit-pair occurs is cut perpendicular to the plane in which the wall lies, the pit-pair may be seen as two opposing canals separated by the pit membrane (Fig. 2.27b). A **bordered pit** differs from a simple pit primarily in the presence of a **pit border**, composed of secondary wall, that overhangs and partially encloses the **pit cavity** thus formed (Fig. 2.28a, d). The opening into the pit cavity (the **pit aperture**) varies in size and shape with the degree of overhang

of the border. When a pit is viewed in face view, the aperture may appear circular (Fig. 2.28b), elliptical, or lenticular. When elliptical or lenticular, the apertures of the two pits of the pit-pair will be crossed (Fig. 2.28c), the angle of each aperture reflecting the predominant angle of the cellulose microfibrils in the respective secondary cell walls. In pit-pairs in very thick walls, the pit canal is often shaped like a greatly flattened cone with two apertures, an outer, circular aperture adjacent to the pit cavity and an inner elliptical aperture bordering the cell lumen (Fig. 2.28d). In such pits the crossed, inner apertures of the pit-pair often extend beyond the boundary of the pit cavity (Fig. 2.28e). If a tracheid or vessel member is adjacent to a parenchyma cell a **half-bordered pit-pair** (Fig. 2.28f, g) may develop consisting of a simple pit in the wall of the living parenchyma cell and a bordered pit in the wall of the non-living, water-conducting cell. From this observation you might conclude that simple pits occur commonly in living cells and that bordered pits occur in cells that, when functionally mature, are non-living. You should note, however, that whereas simple pits are not exclusive to living cells, bordered pits occur only in cells that when functionally mature are non-living.

The pit membrane may be highly specialized and, in the bordered pits of water-conducting cells, is highly porous which facilitates the transport of water and solutes from one cell to an adjacent one. In some conifers, pit membranes are characterized by a central, thickened region called the torus which functions as a valve that, under certain conditions, closes the aperture, preventing the entry of air into the xylem. (We shall consider the structure and function of these pit membranes in detail in Chapter 11 on the secondary xylem.)

Vessel members (Fig. 2.29a–c), characteristic of the xylem of most angiosperms, differ from tracheids in several fundamental ways. On average they are shorter and of greater diameter, and have perforate end walls. In the xylem they are superposed, end to end, forming long tubes, the vessels (Fig. 2.29d). Transport of water and inorganic solutes may thus follow a longitudinal rather than the helical course characteristic of plants that contain only tracheids. Transport efficiency may be enhanced thereby and by the perforate structure of the end walls.

Since vessel members evolved from tracheids, it is not surprising that those in the most primitive angiosperms are similar to tracheids, being relatively long, of small diameter, angular in section, and with oblique end walls containing numerous, usually elliptical, perforations (Fig. 2.29a). In more advanced angiosperms, vessel members are shorter, of greater diameter, circular in section, and with transverse end walls with a single (simple) perforation (Fig. 2.29c). Variation in these characteristics is continuous between these extremes. Pits on the lateral walls of vessel members are generally small and vary from elliptical- to circular-bordered.

Wood fibers, unlike tracheids and vessel members, often retain their protoplasts at functional maturity – some for a relatively short time, others apparently for many years, possibly for the life of the plant. They are usually longer than the longest vessel members in the same

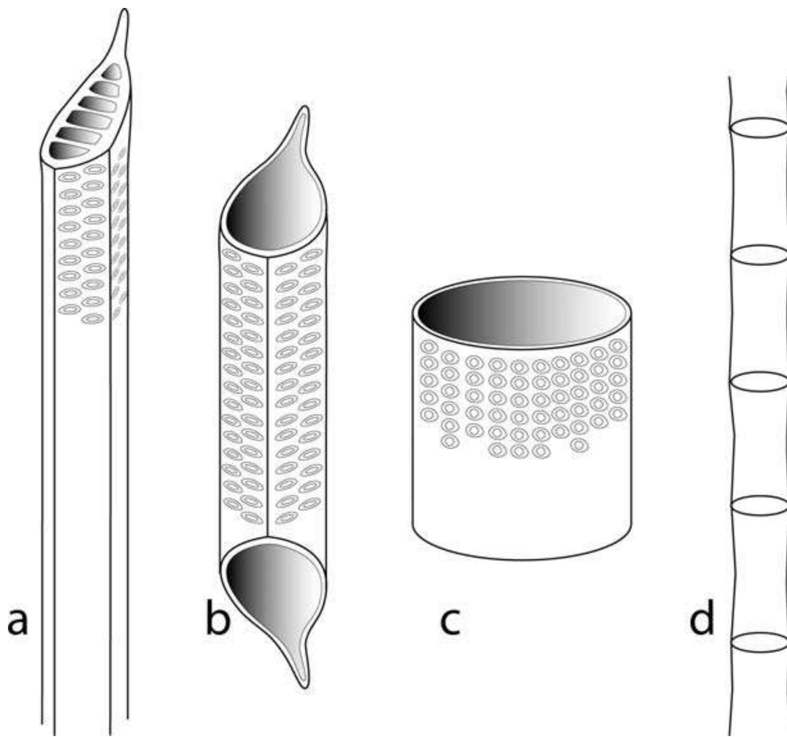


Figure 2.29 Diagrams of vessel members. (a) A vessel member with an oblique end wall and a compound perforation plate, representative of a relatively primitive angiosperm. (b, c) Vessel members with simple perforation plates, representative of more evolutionarily advanced angiosperms. Note the elliptical- to circular-bordered pits in the lateral walls. (d) A series of superposed vessel members comprising a vessel.

wood, of relatively small diameter, are circular to polygonal in section, have relatively thick secondary walls (although in fibers in the wood of different species the wall thickness may be highly variable), and have pointed ends. Pits on the lateral walls are bordered, but are commonly greatly reduced in size, and may appear to be simple. Fibers provide the major support in secondary wood of angiosperms and, in volume occupied, comprise the major component of many woods.

In some woods, especially those of relatively primitive species, the supporting cells are intermediate in morphology between tracheids and the fibers of woods in more advanced species. Such cells are called **fiber-tracheids**. Some fiber-tracheids possess living protoplasts and are characterized by septa of secondary wall material that divide the cells into compartments that apparently function much like **wood parenchyma** as sites of storage of starch and other metabolites. Parenchyma in the secondary xylem is distributed in axial columns and in the vascular rays, ribbons of cells that run radially through the tissue (see Chapter 11 on the secondary xylem).

Cells of the phloem

There are two types of conducting cells in the phloem, sieve cells and sieve tube members. Both are living cells, but both at functional maturity have highly modified protoplasts lacking vacuolar membranes, an adaptation to their function in conduction. **Sieve cells** (Fig. 2.30a),

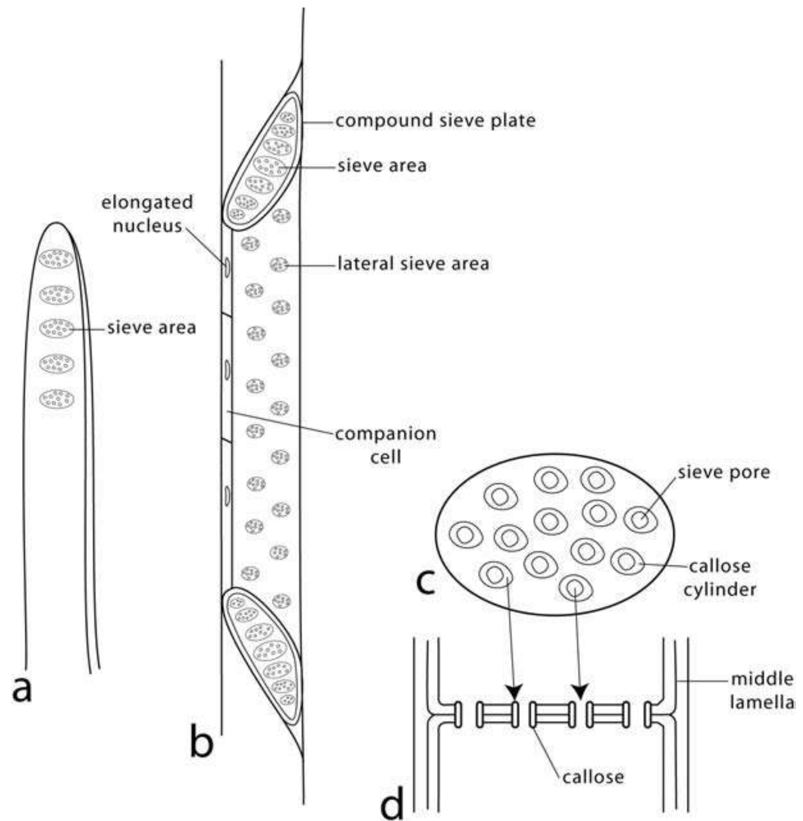


Figure 2.30 Diagrams of sieve elements. (a) A sieve cell. Although sieve areas occur over the entire length of the cell they are shown here only at one end. (b) A sieve tube member with associated companion cells. Note the compound sieve plate and the lateral sieve areas. (c) A sieve area in face view with callose cylinders enclosing the sieve pores. (d) The sieve area in sectional view.

characteristic of primitive vascular plants and gymnosperms, are longitudinally elongate, and have tapered to blunt ends that overlap the ends of other sieve cells. Specialized perforate regions called **sieve areas** (Fig. 2.30a, c) occur in the lateral walls, and are the sites of transport of photosynthate and other organic substances (enzymes, hormones, etc.) from one cell to another. Sieve areas are abundant in the walls of the overlapping ends of sieve cells. **Sieve tube members** (Fig. 2.30b), which occur in angiosperms, have sieve areas on their end walls, and are arranged in superposed columns called **sieve tubes**. Although small, indistinct sieve areas may occur in the lateral walls of sieve tube members, well-developed sieve areas are usually restricted to the end walls and provide the predominant pathway for the passage of photosynthate and other organic solutes from one cell to the next. Sieve tube members in primitive angiosperms are commonly long and have oblique end walls containing **compound sieve plates** consisting of several to many sieve areas (Fig. 2.30b) whereas those in the most advanced angiosperms are relatively short and have transverse ends and **simple sieve plates** consisting of single sieve areas. The sieve areas are specialized regions which consist of the walls and middle lamella of contiguous cells (Fig. 2.30d). These regions contain numerous **sieve pores**, often lined with **callose** (Fig. 2.30c, d), through which the protoplasts of contiguous sieve tube members are interconnected. As we shall see in the chapter

on the phloem, the callose cylinders may not be present in the functioning phloem, developing in response to the trauma of cutting the plant for sectioning.

In intimate contact with sieve tube members are **companion cells** (Fig. 2.30b), which in some taxa are **transfer cells**. Companion cells and transfer cells facilitate the transfer of photosynthate from sites of its production in leaves into the sieve tube members. Transfer cells have distinctive secondary walls with extensive ingrowths that increase the inner wall area and, thus, the area of the plasma membrane which lines the wall. Increase in the area of the plasma membrane increases the efficiency of active transfer of photosynthate into the cell protoplast. We shall look at companion cells and transfer cells in more detail in Chapter 12. In conifers, specialized marginal ray cells, called **Strasburger cells**, serve the same function as companion cells in angiosperms.

Fibers are also characteristic of the phloem. In primary vascular bundles, strands of phloem fibers develop along the outer edge forming what appear in transverse section to be bundle caps. **Phloem fibers** are especially abundant in secondary phloem, occurring in dense tissues between, and often enclosing, strands of conducting cells. Phloem fibers, which have important supporting and protective functions, are very long with sharply tapered ends and often very thick secondary walls containing simple pits. **Axial parenchyma cells**, which typically occur in longitudinal columns, as well as parenchyma cells of the rays are present in the phloem and probably function with those in the secondary xylem as a system in which photosynthate and hormones are transported both longitudinally and laterally in secondary vascular tissues.

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Chapter 3

The protoplast of the eukaryotic cell

Perspective

The eukaryotic cell is composed, with a few exceptions, of both a living protoplast, the site of cellular metabolism, and an enclosing cellulosic wall of one or more layers (Fig. 3.1). While not alive as a structural unit, the wall is commonly traversed by living components, **plasmodesmata**, which connect adjacent protoplasts and thus facilitate communication between, and the integration of, cells within a tissue. All plant cells possess a protoplast during development, and in many it persists throughout the life of the plant. Some cells, however, do not achieve their ultimate functional state until the protoplast dies as, for example, a specialized water-conducting cell such as a vessel member.

The protoplasts of all plant cells are basically similar, but may differ in relation to the function of the mature cells. For example, the protoplast of a parenchyma cell in the outer cortex or in a leaf will contain many chloroplasts since a major function of these cells is photosynthesis. In contrast, a cell of the pith (a storage region) in the center of the stem may lack chloroplasts but will contain unpigmented plastids in which starch is synthesized (amyloplasts). The protoplast of an immature vessel member, however, destined to die, may contain no plastids at all, or plastids of a highly modified type.

Each cell protoplast is characterized by the potential for the development of an entire organism (see Steward *et al.*, 1964). This total potentiality is, however, rarely achieved under normal conditions. It is, indeed, primarily the evolution of mechanisms that control (restrict) the expression of this potentiality that accounts for the differences in the morphology and function of cells.

The unity of individual cell protoplasts notwithstanding, cells are influenced during development by the environment resulting from their association in tissues. They may function in concert with other cells during the differentiation of tissues and the development of organs. This coordination of development is facilitated by the plasmodesmata which connect the protoplasts of adjacent cells and which we shall consider in detail in the following chapter on the cell wall.

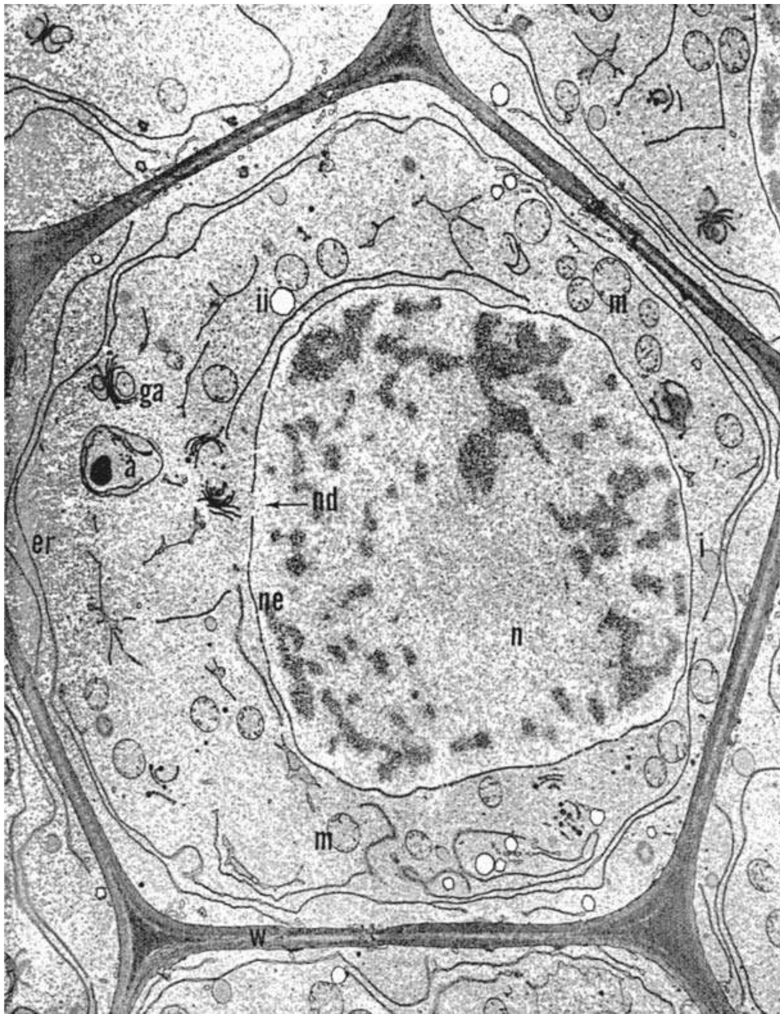

Figure 3.1 Transmission electron micrograph of a meristematic cell from the root cap of *Zea mays* (maize). The protoplast, dominated by the nucleus (n), contains mitochondria (m), Golgi bodies (ga), and endoplasmic reticulum (er). The protoplast is enclosed by a cellulosic primary wall (W). Note that in this meristematic cell only a few small vacuoles (white) are visible. The dark structures in the nucleus are chromosomes. nd, nuclear membrane discontinuity; ne, nuclear envelope. Magnification × 5973. From Whaley *et al.* (1960). Used by permission of the Botanical Society of America.

Morphology of the protoplast

The protoplast consists of a relatively liquid, colloidal phase, the cytoplasm, in which reside a number of morphologically distinct membrane systems such as the endoplasmic reticulum and Golgi bodies, and membrane-bound organelles among which are included the nucleus, mitochondria, and plastids (Figs 3.1, 3.2). In addition, microtubules and actin microfilaments also reside within the cytoplasm. The protoplast, itself, is enclosed by a membrane.

The cytoplasm

The cytoplasm is the relatively liquid matrix of the protoplast, often referred to as the **hyaloplasm**, in which the organelles and membrane systems are suspended. Its viscosity may vary in different regions of the cell, and may fluctuate during different stages of development. It

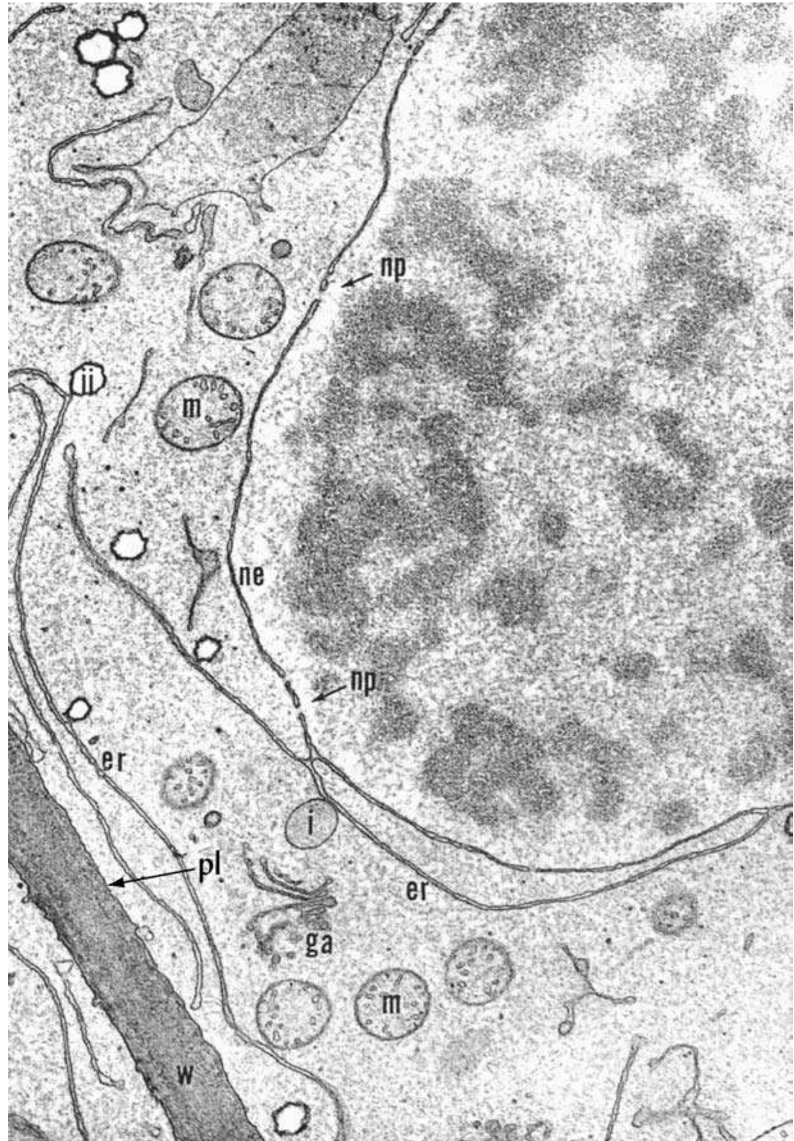

Figure 3.2 Enlargement of part of a cell protoplast of a meristematic cell similar to that shown in Fig. 3.1. The double membrane comprising the nuclear envelope (ne) is continuous with endoplasmic reticulum (er). Note the nuclear pores (np) and the plasmalemma (pl) bounding the protoplast and lining the primary cell wall (W). ga, Golgi body; m, mitochondrion. Magnification × 13 248. From Whaley *et al.* (1960). Used by permission of the Botanical Society of America.

is composed, primarily, of protein macromolecules in colloidal suspension.

The plasma membrane

Enclosing the protoplast is a bounding membrane, the **plasma membrane** (Fig. 3.2), or plasmalemma, sometimes also called the ectoplast. As observed in section, the plasma membrane is three-layered consisting of two electron-dense (dark) layers separated by an electron-transparent (clear) layer. Such a membrane is referred to as a **unit membrane**. Although the outer and inner layers commonly appear of similar thickness and density, it has been observed that in some plants the inner layer may be denser than the outer, suggesting that the layers

vary in chemical composition. Several models of membrane structure have been proposed. Most are characterized by globular proteins that comprise both the inner and outer layers between which is a lipid bilayer. It is important to emphasize that membrane structure is not static but, rather, dynamic, possibly constantly changing depending on the particular function the membrane serves. Unit membranes function not only as structural units but also as foundations for enzymes as well as in the control of the passage of various substances into and out of the protoplast and the various membrane-bound organelles of the protoplast. Membrane function may also vary during different stages of development. For example, during cell growth when new cell wall is being formed, clusters of globules called **rosettes** form in the plasma membrane. These are clusters of enzymes which mediate the synthesis of cellulose microfibrils (see Chapter 4 for detail).

The plasma membrane (Fig. 3.2) usually lies in contact with or closely parallel to the cell wall. In certain specialized absorptive and secretory cells (**transfer cells**) which accumulate and transfer substances into other cells of the plant body or to the exterior, the wall is characterized by the development of numerous ingrowths. Consequently, the plasma membrane becomes highly invaginated, resulting in an increase in surface area which facilitates transfer of materials. In some other types of secretory cells, the plasma membrane may be highly convoluted even though the wall is of uniform thickness. This may result from the continued incorporation into the plasma membrane of membrane from Golgi vesicles after the cells have ceased to increase in size. It is well established that the plasma membrane controls the entry into and exit from the protoplast of various substances. Furthermore, the establishment of turgor pressure within the cell, a condition essential to growth as well as to maintenance of normal function in living cells, is dependent upon the presence of this selectively permeable surface membrane.

The nucleus and ribosomes

As the control center of the cell, the **nucleus** is the dominant organelle, often in size, always in influence (Figs 3.1, 3.2). Within its chromosomes is the DNA (deoxyribonucleic acid), the repository of information encoded in the genes throughout the evolution of the group. The nucleus is also the major site of RNA (ribonucleic acid) synthesis in the cell. The information of DNA is transferred to RNA during its synthesis. RNA (of which there are several kinds) is transferred to the exterior of the nucleus where it acts as an intermediary in protein synthesis. The actual sites of protein synthesis are the **ribosomes** (Fig. 3.3a, b), small spherical bodies about 15–20 nm in diameter which consist of protein and RNA. During protein synthesis the ribosomes aggregate along tubules of endoplasmic reticulum forming **polyribosomes** (Fig. 3.3b). Ribosomes may be dispersed within the cytoplasm or bound to the surface of the internal membrane system (Fig. 3.3a). They also occur in mitochondria and plastids.

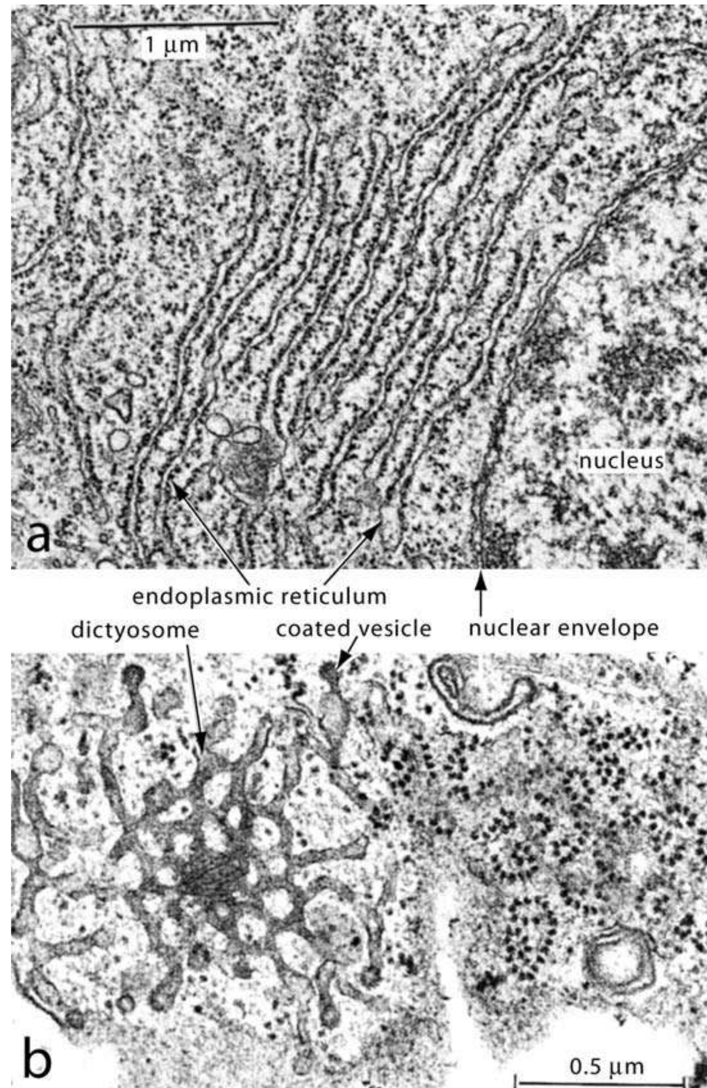

Figure 3.3 (a) Rough endoplasmic reticulum (ER) in a mesophyll cell of *Nicotiana tabacum* (tobacco). Note the numerous ribosomes on the surface of the ER. (b) Polyribosomes on the surface of an ER cisterna in a leaf of tobacco. To the left is a highly fenestrated Golgi body seen in surface view. Compare this with that in Fig. 3.5a. From Esau (1977). Used by permission of John Wiley and Sons, Inc.

The internal membrane system

One of the most interesting morphological features of the protoplast is the intricate membrane system which consists of the nuclear envelope and the endoplasmic reticulum. The **nuclear envelope** consists of two closely parallel membranes perforated by nuclear pores (Fig. 3.2). The pores are apertures through which molecules of RNA may leave the nucleus and enter the cytoplasm.

In continuity with the nuclear envelope is the extensive system of membranes called the **endoplasmic reticulum** (Fig. 3.2) (Porter and Machado, 1960; Porter, 1961) and commonly indicated simply as ER. This reticulate system consists of tubules, vesicles, and cisternae (regions enclosed by parallel membranes). Recent studies have demonstrated that, in general, the ER in mature cells forms a well-defined, polygonal network closely associated with the plasma membrane

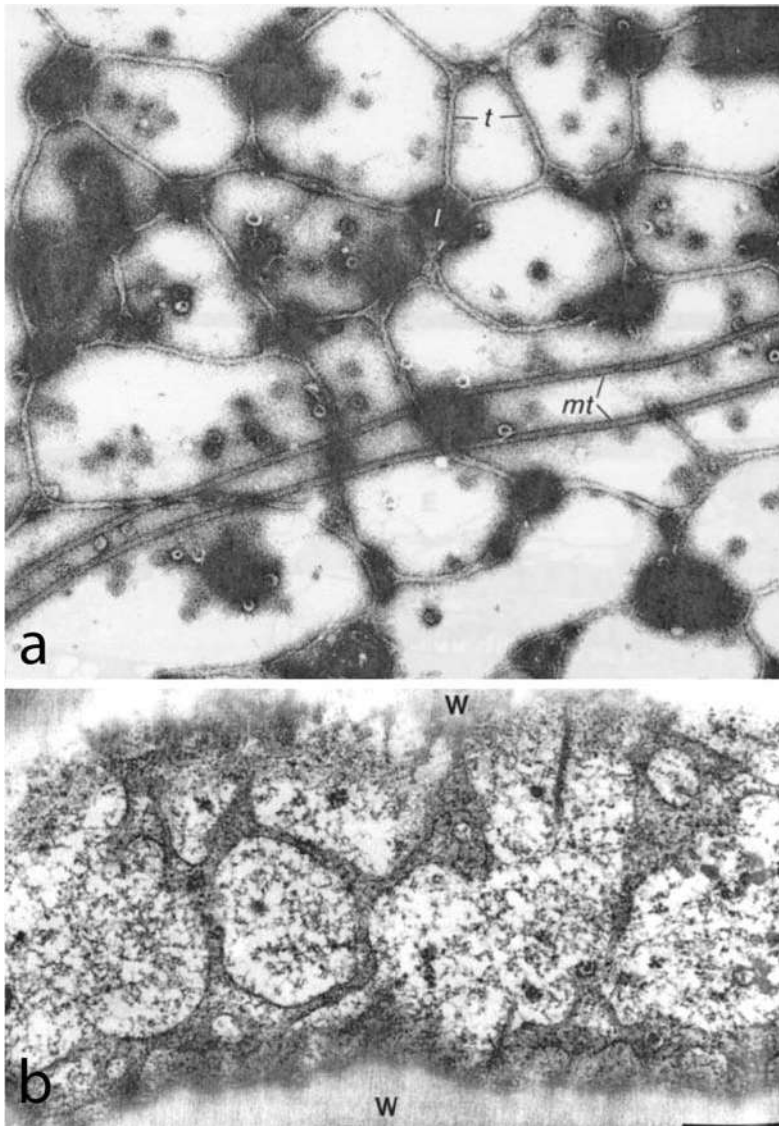

Figure 3.4 (a) Smooth, tubular endoplasmic reticulum from the protoplast of a guard cell of *Vicia faba* (broad bean). The ER has a predominantly reticulate form, but contains some largely obscured lamellar regions. This peripheral (cortical) ER was stationary, and adhered to the plasmalemma but was not fused to it. Note the associated microtubules (mt). t, tubular ER; l, lamellar ER. Magnification × 22 000. (b) ER in the peripheral cytoplasm of cells of *Drosera*. Note the flattened, fenestrated regions. W, cell wall. Bar = 0.5 μm. (a) From a thesis by Wiedenhoeft (1985) reproduced in Hepler *et al.* (1990). Used by permission of The Company of Biologists, Ltd. (b) From Lichtscheidl *et al.* (1990). Used by permission of Springer-Verlag Wien.

(Fig. 3.4a, b) (see e.g., Hepler *et al.*, 1990). This peripheral ER is stationary and may contact the plasma membrane, but whether it actually fuses with it is unclear. Although connected to the peripheral ER, the interior part of the system is more labile and, because of cytoplasmic streaming, its reticulate morphology may be obscured.

The endoplasmic reticulum is characterized as smooth or rough on the basis of the presence or absence on its surface of ribosomes. Because of its extensive surface area and vesiculate nature, it is generally thought to provide a system through, or along which, various cellular substances, especially precursor compounds of large molecules, are transported within the cell. Smooth ER is common in cells in which metabolic activity is reduced whereas rough ER is characteristic of cells with high rates of metabolism such as actively dividing cambial cells. It

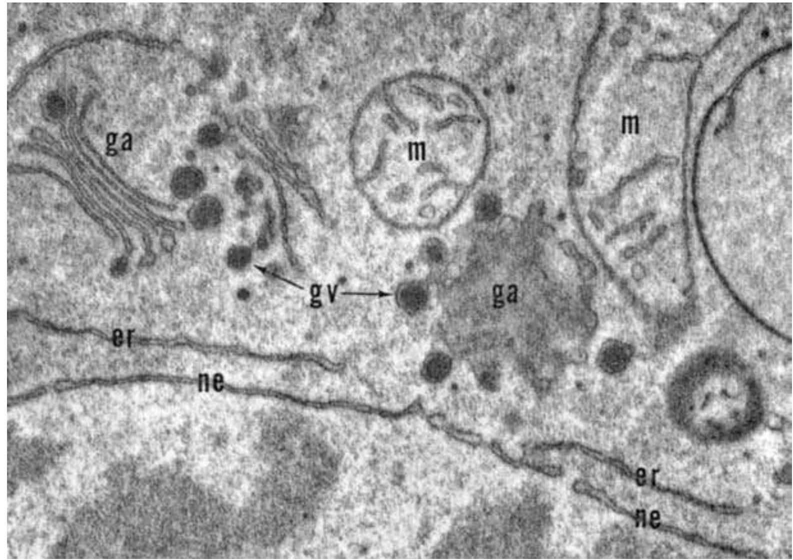

Figure 3.5 Golgi bodies (ga) in a meristematic cell of *Zea mays* (maize), on the left in sectional view showing the stack of membrane cisternae, and on the right in surface view. Note the vesicles produced at the edges of the cisternae. Compare these images with that of the Golgi body in Fig. 3.3b. m, mitochondrion; er, endoplasmic reticulum; ne, nuclear envelope; gv, Golgi vesicles. Magnification × 32 860. From Whaley *et al.* (1960). Used by permission of the Botanical Society of America.

is probable that the products of protein synthesis in the ribosomes on the surface of rough ER are deposited within the ER cisternae and then transported to various regions of the cell. Pectic compounds and simple carbohydrates essential to formation of the middle lamella and the cell wall may also be transported through the endoplasmic reticulum and transferred in ER or Golgi vesicles to the immediate sites of biosynthesis. The peripheral network of endoplasmic reticulum, in association with a system of actin microfilaments, also functions as a pathway along which move organelles such as Golgi bodies and mitochondria (Boevink *et al*., 1998; Nebenführ *et al*., 1999; Hawes and Satiat-Jeunemaitre, 2001).

Golgi bodies

Golgi bodies (Figs 3.3b, 3.5) (also called dictyosomes, Golgi stacks, Golgi apparatus, or simply Golgi) consist of stacks of **cisternae**, essentially circular in surface view and appearing as flattened sacs in sectional view (Mollenhauer and Morré, 1966). Interconnected tubules from which small vesicles originate comprise the border of each cisterna (Figs 3.3b, 3.5) which may be highly fenestrated. Golgi cisternae range in diameter from about 0.5 to 1.0 μm (Robards, 1970). The **Golgi vesicles**, apparently produced in succession from the margins of the cisternae, contain a variety of compounds, some probably absorbed from the surrounding cytoplasm, some synthesized within the Golgi body, others transferred through the endoplasmic reticulum for deposition at sites of synthesis. During the production of new cells, Golgi bodies play important roles in the development of the middle lamella as well as the plasma membranes and cell walls of contiguous cells (see Chapter 4 for details). During cell wall formation, precursor compounds of cell wall components are transferred in Golgi vesicles to the sites of wall synthesis (Northcote and Pickett-Heaps, 1966). The method of Golgi movement within the protoplast has been a mystery until recently (see pp. 51–53

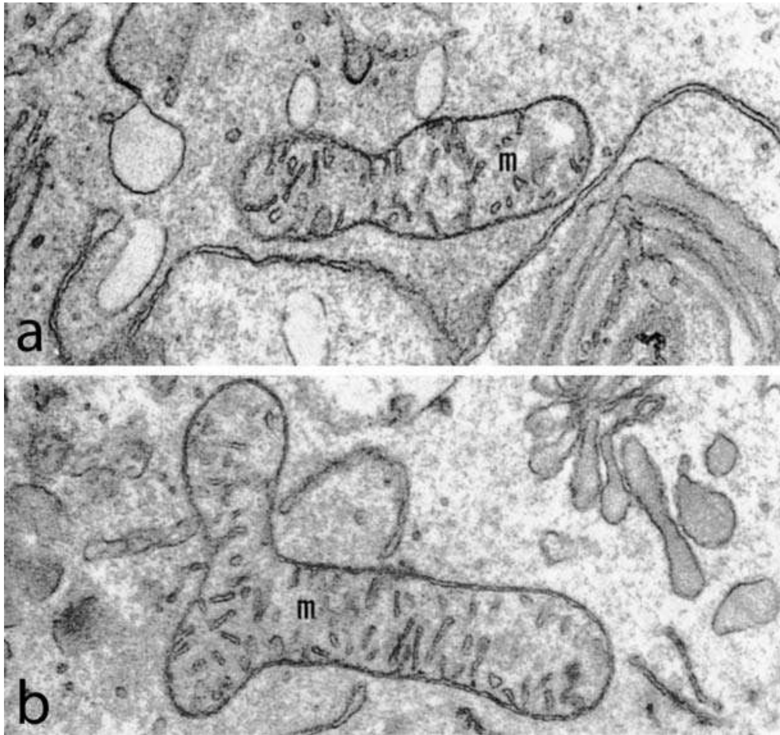

Figure 3.6 (a, b) Mitochondria. Note the two bounding unit membranes and the cristae which are invaginations of the inner membrane; also the variation in form. Magnification × 22 000. From Whaley *et al.* (1960). Used by permission of the Botanical Society of America.

for a new hypothesis). Earlier workers assumed that they were formed anew in the regions in which they aggregated.

Mitochondria

Mitochondria (Figs 3.2, 3.6) are sites of respiration and synthesis of adenosine triphosphate (ATP) which supplies the energy required for the numerous metabolic activities of the cell (see Lehninger, 1964). Mitochondria, usually less than 1.0 μm in diameter and no more than 5.0 μm long, are commonly rod-shaped, but may be spherical, dumb-bell shaped or sometimes branched; and they may vary from one form to another. Each mitochondrion is composed of two unit membranes, an outer bounding one and an inner one that is invaginated in varying degrees (Fig. 3.6a, b). The invaginations, called **cristae**, often oriented at approximately right angles to the surface, extend in some cases nearly across the organelle. It is difficult to determine whether these invaginations are tubules or flattened vesicles although the latter structure is probably the more common in higher plants. The enzymes involved in the complex series of reactions which culminate in the production of ATP are thought to be localized in the membranes, especially the inner membrane. Apparently, small densely staining granules called **osmiophilic granules** that occur to the interior of the two membranes are a constant feature of mitochondria. These granules are thought to be the sites of deposition of calcium phosphate during oxidative phosphorylation (Peachy, 1964). Mitochondria both divide and fuse, and are

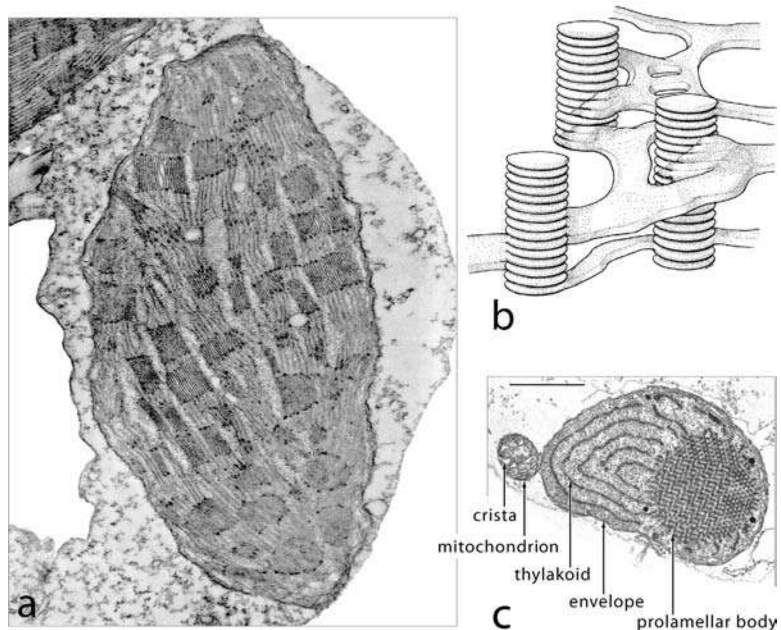

Figure 3.7 (a) Chloroplast from a mesophyll cell of *Zea mays* (maize) showing the extensive and intricate membrane system within a matrix termed the stroma. Compact stacks of specialized membranes called thylakoids comprise grana. Since chlorophyll is restricted to the grana, they are the sites of photosynthesis in the chloroplast. Note also the numerous ribosomes in the stroma. Magnification × 16 636. (b) Diagram illustrating the relationship between the grana and the stroma lamellae. (c) Etiolated chloroplast containing a paracrystalline, prolamellar body in a mesophyll cell of *Saccharum officinarum* (sugar cane). (a) From Lehninger (1961). (b) From Weier *et al.* (1967). Used by permission of Brookhaven National Laboratory. (c) From Esau (1977). Used by permission of John Wiley and Sons, Inc.

highly mobile within the cell, moving to and aggregating near sites requiring sources of energy.

Plastids

Whereas most mitochondria are too small to be easily observed with the light microscope, plastids are much larger and one of the distinctive and conspicuous features of plant cell protoplasts (Fig. 3.7). They may be unpigmented and, thus, called **leucoplasts**, or pigmented and called **chromoplasts**. Chromoplasts are commonly grouped in two categories, one characterized by orange and yellow carotenoid pigments only, the other by a predominance of chlorophylls. The latter type, the **chloroplast**, also contains the orange and yellow pigments which, during most of a growing season, are masked by chlorophyll. The cessation of chlorophyll synthesis in late summer and early fall, and the resultant visibility of the **carotenoid pigments**, is largely responsible for the appearance of orange and yellow autumnal coloration in the leaves of trees and shrubs. The deep purplish-red leaf color as well as

that of flower petals results from increased synthesis of **anthocyanins**, water-soluble pigments, in the cell vacuoles.

Some chloroplasts, such as those of green tomatoes, or immature red peppers, change during development into chromoplasts of the orange type. Whether this is the result primarily of a cessation of chlorophyll synthesis, or an increase in the synthesis of carotenoid pigments, or a combination of these, is not clear. Some carotenoid chromoplasts have an angular, crystalline appearance, apparently the result of crystallization of the carotene within them (Ledbetter and Porter, 1970). In such cases it is not certain whether they retain an enclosing plastid membrane.

It was not until the advent of the electron microscope that plastids were observed to be of membranous construction. The chloroplast (Fig. 3.7), extensively studied because of its role in photosynthesis, is by far the best understood of plastids (see Gunning, 1965a, 1965b; Laetsch and Price, 1969). It is bounded by a double membrane which encloses an intricate membrane system supported within a matrix termed the **stroma**. The membrane system (Fig. 3.7a, b) consists of parallel **stroma lamellae** which connect compact stacks of specialized membrane, each component of which is called a **thylakoid**. Stacks of thylakoids comprise **grana**. In the chloroplasts of vascular plants, chlorophyll is restricted to the grana. Chromoplasts that lack chlorophyll also lack a complex internal membrane system. When plants are grown in the dark, the development of typical chloroplasts is inhibited, chlorophyll is not synthesized in the plastids that develop, and they are, thus, called **etioplasts**. They are characterized by **prolamellar bodies** (Fig. 3.7c) composed of tubular membranes which comprise a paracrystalline lattice. When exposed to light thylakoids develop from the components of the prolamellar body. It should not be surprising, therefore, that prior to seed germination, cells of the suspensor and embryo in some taxa are characterized by leucoplasts that contain prolamellar bodies (see e.g., Johansson and Walles, 1993).

Chloroplasts, like mitochondria, contain ribosomes and, thus, have the ability to manufacture ATP. Both contain DNA and RNA, and are capable of protein synthesis. Both, furthermore, can replicate by simple fission. For these and other reasons it has been suggested that these organelles might have evolved from bacterial symbionts (e.g., Cohen, 1970; Raven, 1970).

Spherosomes and microbodies

Spherosomes are small bodies (about 1.0 μm in diameter) bounded by a single membrane and contain enzymes that synthesize oils and fats. They are thought to be derived from endoplasmic reticulum (Robards, 1970). **Microbodies** (Fig. 3.8) like spherosomes are bounded by a single membrane. They have a granular appearance and sometimes contain a conspicuous crystal. Some microbodies play an important role in photorespiration whereas others contain enzymes required for the conversion of fats to carbohydrates during seed germination.

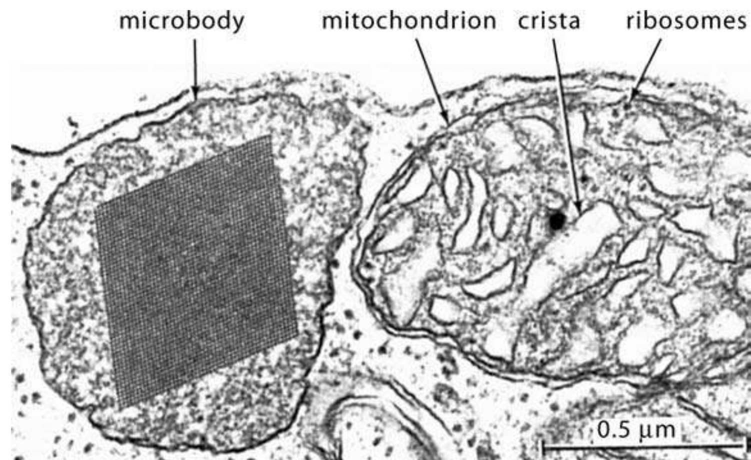

Figure 3.8 A microbody from a mesophyll cell of *Nicotiana tabacum* containing a large crystal. The microbody contrasts with plastids in having a single bounding unit membrane. From Esau (1977). Used by permission of John Wiley and Sons, Inc.

Microtubules

Plant **microtubules** are slender tubes of indeterminate length, usually straight, with a sectional diameter of about 25 nm. These common constituents of eukaryotic cells were discovered in 1963 by Ledbetter and Porter who demonstrated that they are composed of spherical protein subunits (dimers of alpha and beta tubulin), forming a circle of 13 when observed in transverse section. Microtubules grow at each end by polymerization of tubulin dimers, and may become up to 1000 times as long as they are thick. Microtubules are commonly observed in the peripheral regions of cell protoplasts (Fig. 3.9a, b). They also comprise the spindle in dividing cells. The microtubules of the nuclear spindle are, however, somewhat smaller in diameter than others having a diameter of about 20 nm (Robards, 1970). In regions of cell wall growth and cellulose microfibril synthesis, microtubules, just below the plasma membrane, are routinely observed in an orientation parallel to that of the newly synthesized microfibrils (Fig. 3.9b). This correlation suggests that they may exert some control over the orientation of the microfibrils. (For more detail, see Chapter 4.)

Actin microfilaments

These long, thin, protein (actin) filaments, about 8 nm in diameter, are frequently found in association with microtubules as well as with tubules of endoplasmic reticulum in the peripheral region of the protoplast. Interaction of actin with the myosin of cell organelles results in movement of the organelles along actin microfilaments or bundles of actin microfilaments (Williamson, 1993). In cells of the Characean alga *Nitella*, just to the inside of the stationary, peripheral endoplasmic reticulum where cytoplasmic streaming occurs, the ER itself has been shown to move along bundles of actin microfilaments (actin cables) (Fig. 3.10a, b), exerting a shear force that seems to result in cytoplasmic streaming throughout the interior of the cells (Kachar and Reese, 1988). For more detail on the role of actin microfilaments in the transport of

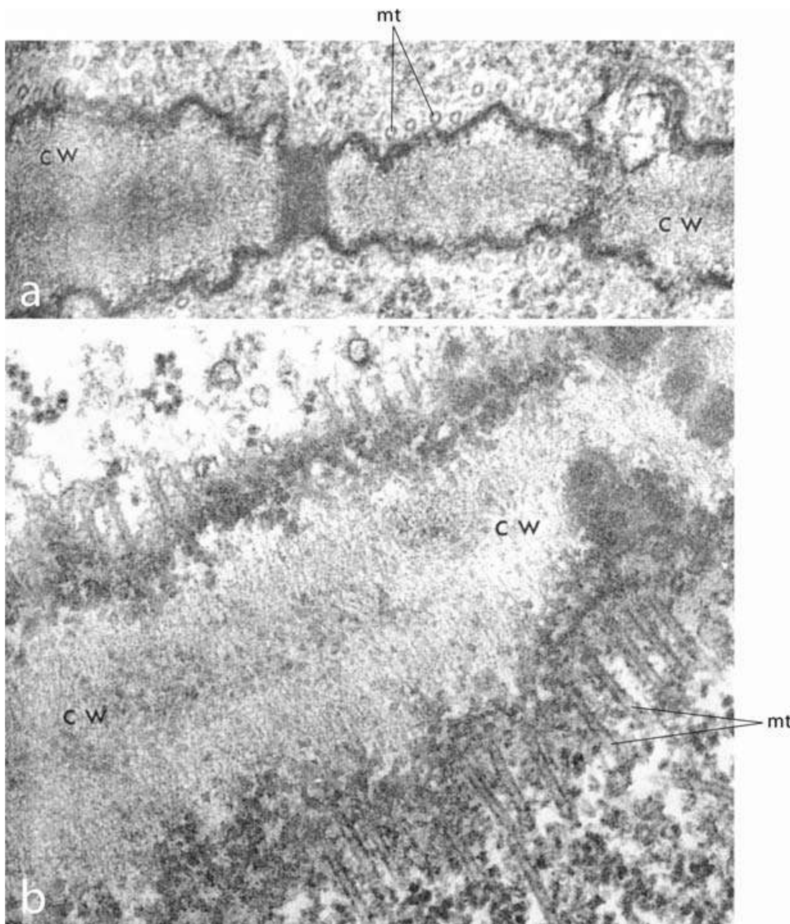

Figure 3.9 (a) Microtubules (mt), in sectional view, just inside the plasmalemma (and cell wall, CW) of contiguous cells of *Juniperus*. Magnification × 64 150. (b) Microtubules in an oblique section showing their parallel relationship to cellulose microfibrils in the cell wall. Magnification × 74 600. From Ledbetter and Porter (1963). Used by copyright permission of the Rockefeller University Press.

cell organelles and Golgi vesicles, please see the section on the role of the cytoskeleton in pollen tube growth in Chapter 18.

The cytoskeleton

Microtubules and actin microfilaments comprise a complex, often interacting, and dynamic living network called the **cytoskeleton**. Components of the cytoskeleton were studied separately initially, but are now known to interact in several, perhaps many, important cell functions. One of the most widely studied functions is the movement of cell organelles as, for example, the movement of mitochondria and Golgi bodies within tip-growing cells such as root hairs and pollen tubes. Another area of great interest is the movement of Golgi vesicles containing **cellulose synthase complexes**, clusters of enzymes from which cellulose microfibrils are generated, to sites of wall synthesis. This intra-cell movement, called **organelle (or vesicle) trafficking**, is dependent on actin microfibrils and myosin motors which provide the motive force. At the plasma membrane the cellulose

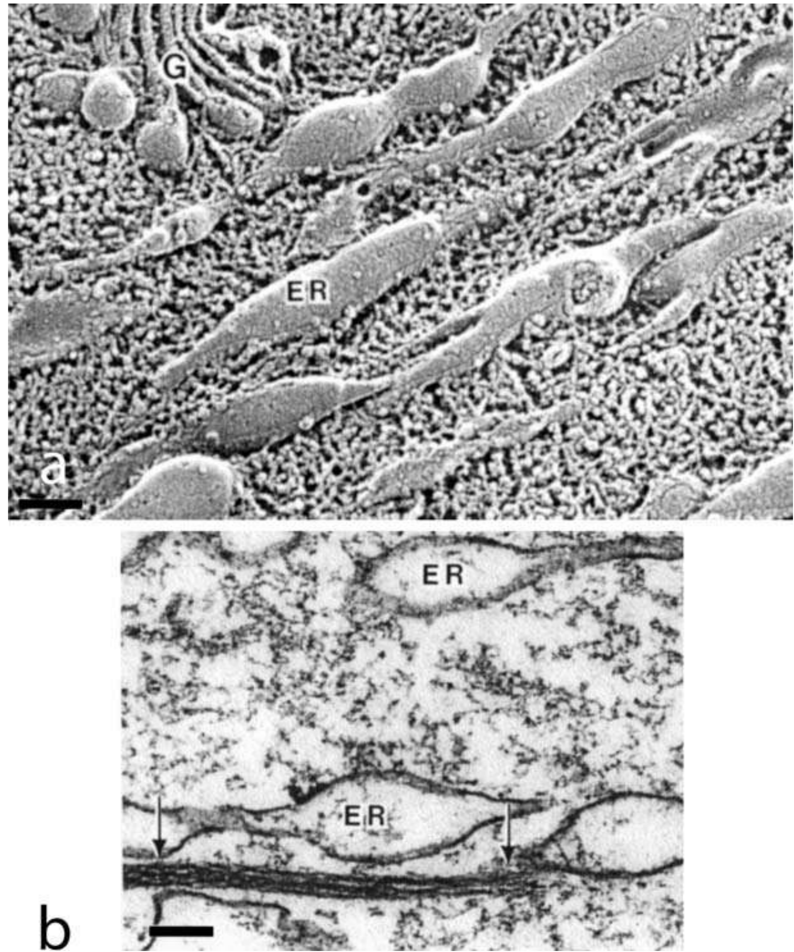

Figure 3.10 (a) Freeze-etch views of tubules of endoplasmic reticulum in the alga *Nitella*. The reticulate form of the ER has been distorted by cytoplasmic streaming. G, Golgi body. Bar = 0.17 μm. (b) The ER is underlain by cables of actin microfilaments (arrows) along which it moves. Bar = 0.17 μm. (a, b) From Kachar and Reese (1998). Used by copyright permission of the Rockefeller University Press.

synthase complexes are intimately associated with microtubules, and are considered to be guided by the microtubules to the positions of cellulose microfibril formation (Paredez *et al*., 2006; for more detail, see Chapter 4). The microtubules, therefore, can be considered to exert control of the orientation of the cellulose microfibrils (e.g., Wightman and Turner, 2008).

Although past research has centered on the roles of the cytoskeleton in organelle movement, cellulose synthesis and cell wall formation, new areas of great interest are its roles in gravity perception, in regulating **signaling pathways** through which plants respond to hormones and to sensory inputs from the external environment (low temperature, the effect of pathogens, osmotic stress, etc.), and in controlling cell morphogenesis (the development of cell form) among others.

Considering the large number of important processes controlled or regulated by the cytoskeleton, it is not surprising that the organization and activity of microtubules and actin microfilaments themselves

are regulated by hormonal and environmental signals (Wasteneys and Yang, 2004).

Vacuoles

A **vacuole** is a region within the cell bound by a differentially permeable membrane called the **tonoplast**. An immature cell usually contains many small vacuoles which fuse to form a single large central vacuole as the cell matures. As a consequence, the cytoplasm with its cell organelles, endoplasmic reticulum and cytoskeletal components becomes restricted to the periphery of the cell, a region comprising only about 10% of the total cell volume. Vacuoles contain water and dissolved substances such as pigments, salts, sugars, and compounds such as calcium oxylate which often crystallizes. Vacuoles may also contain tannins, organic acids, and amino acids. One of the most significant functions of the vacuole is its role in the water balance of the cell. Active absorption of ions in excess of the concentration to the exterior results in increase in turgor pressure in the cell and its expansion and growth. (For a discussion of cell growth, please see Chapter 5.) The vacuole also has a hydrolytic function. In some taxa, for example, in the cotyledons of some embryos, protein grains develop within small vacuoles. Upon digestion of the protein grains, the vacuoles fuse to form a larger vacuole. An especially interesting aspect of some vacuoles is the incorporation within them of organelles such as mitochondria, ribosomes, and plastids. This occurs when the tonoplast becomes invaginated (i.e., extended inwardly) and organelles in the adjacent cytoplasm become enclosed by a vesicle composed of vacuolar membrane which pinches off within the vacuole. After lysis of the organelles the enclosing membrane disappears. The molecular components of the organelles can then be recycled within the cell. This is a function comparable to that of the lysosomes of animal cells.

Movement of organelles in the protoplast

It has been known for many years that organelles such as Golgi, mitochondria, and plastids move within the protoplast in what has been described as cytoplasmic streaming. Golgi have been observed to aggregate in regions of synthesis, such as sites of new cell wall formation, and mitochondria in regions requiring energy such as regions of active transport through the plasma membrane or at the ends of elongating cells. It is clear, therefore, that such movement is not simply random as might be implied from the term cytoplasmic streaming. Recent research has demonstrated that organelle movements are related to tubules of endoplasmic reticulum (or in some cases to microtubules), closely associated actin microfilaments and myosin "motors" (Williamson, 1993). A study by Boevink *et al*. (1998) shows conclusively that Golgi move along the ER tubules of the polygonal network just

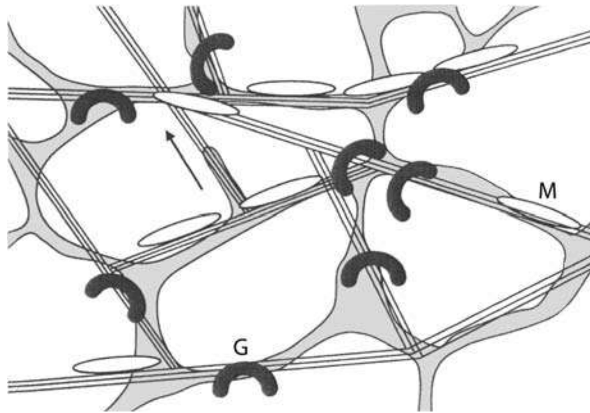

Figure 3.11 Diagram of a model of the organization of the peripheral (cortical) cytoplasm of a cell. The reticulate ER network (light shading) overlies a cytoskeleton of actin cables over which move Golgi bodies (G) and mitochondria (M). ER tubules can grow along actin cables and fuse with other tubules. The microtubule cytoskeleton has been omitted from the diagram. From Hawes and Satiat-Jeunemaitre (2001). Used by permission of the American Society of Plant Biologists.

beneath the plasma membrane, and that a system of actin microfilaments precisely parallels the ER network. These results are consonant with reported observations of ER–actin complexes in several taxa by other researchers (e.g., Lichtscheidl *et al.*, 1990; Nebenführ *et al.*, 1999). It is now widely accepted that the motility of organelles such as Golgi, mitochondria, plastids, etc. results from an interaction of myosin motors with the stationary actin microfilaments, utilizing energy from ATP (Fig. 3.11). It is known that Golgi accumulate various precursor compounds which are transported in Golgi vesicles to sites of synthesis as, for example, a region of cell wall in which wall thickening is occurring. The presumed function of Golgi as they move along the actin network is the absorption of these compounds from the stationary peripheral endoplasmic reticulum (Boevink *et al.*, 1998).

It seems unlikely that the release and transport of Golgi vesicles to the plasma membrane or vacuoles, or from the ER to Golgi, are random. In fact, Golgi have been observed to exhibit rapid stop and go movements. An interesting hypothesis of Nebenführ *et al.* (1999) (Fig. 3.12) describes a possible regulated stop and go system as follows: Golgi bodies with attached myosin motors move along actin microfilaments. At an ER exit site, a local "stop" signal disconnects the Golgi body from the actin track which allows the transfer of ER vesicles to the Golgi body. At a site of cell wall thickening, for example, another local stop signal results in uncoupling the Golgi from the actin track and the transfer of vesicles containing precursor compounds of cellulose or cell wall matrix to the plasma membrane where the contents are expelled by exocytosis into the sites of cellulose and/or matrix synthesis. A candidate for the stop signal is calcium (Nebenführ *et al.*, 1999) which, in high concentrations, is known to block cytoplasmic streaming

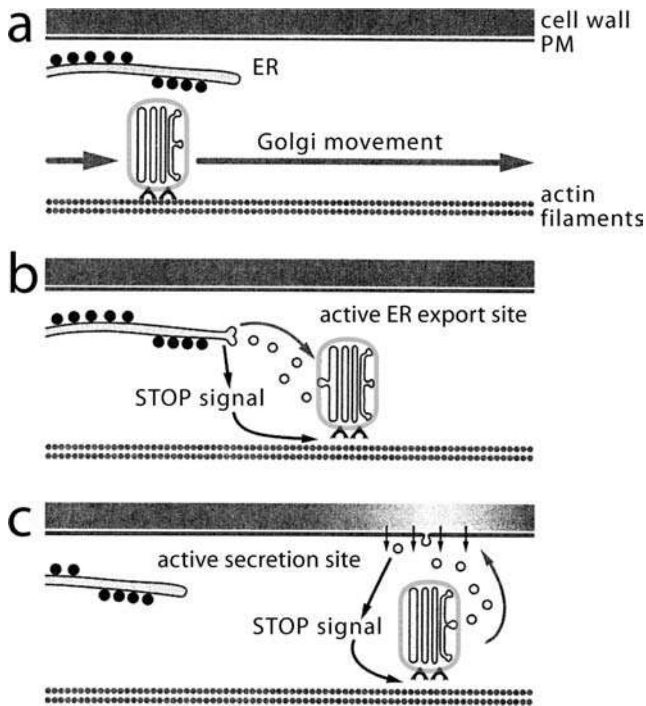

Figure 3.12 A diagrammatic representation of a hypothesis of regulated stop and go movement of Golgi bodies and its relationship to the transport of products through the secretory pathway. (a) A Golgi body with myosin motors attached moves along actin filaments. PM, plasma membrane. (b) At an ER exit site a local signal is produced that inhibits movement of the Golgi by releasing it from the actin tracks and allowing uptake from ER to Golgi transport vesicles. (c) At cell wall expansion sites, or sites of secondary wall thickening, a signal is produced that stops the Golgi movement and leads to the release of Golgi secretory vesicles. For more detail, see the text. From Nebenführ *et al.* (1999). Used by permission of the American Society of Plant Biologists.

(Shimmen and Yokota, 1994). (For more detail, see Nebenführ *et al*., 1999, and Shimmen and Yokota, 1994.)

Movement of structures within pollen tubes has been shown to result from the association of actin microfilaments and microtubules. In *Nicotiana alata* (tobacco), for example, Lancelle *et al*. (1987) demonstrated actin microfilaments oriented in parallel with and crossbridged to microtubules in the peripheral region of pollen tubes. It is generally believed that myosin is chemically bound to the tube nucleus and the generative cell in pollen tubes and that interaction between myosin and actin results in the movement of these structures (Williamson, 1993). The mechanism of nuclear movement is, however, complex and unclear at present. For an in-depth discussion of nuclear movement and extensive reference to the literature, see Williamson (1993).

Ergastic substances

Certain products of cell metabolism are appropriately referred to as **ergastic substances**, that is, substances resulting from the "work" of the cell. These substances are categorized as either **storage products** or **waste products**. Among the storage products are starch which accumulates in bodies of characteristic morphology termed **starch grains**, protein which forms **aleurone grains**, and oils often contained in plastids termed **elaioplasts** or in **spherosomes**, oil droplets sometimes enclosed in single membranes, but often lacking membranes.

Starch grains begin their development in either chloroplasts or leucoplasts. A leucoplast in which starch accumulates is termed an **amyloplast**. Starch grains vary in size from 12 to 100 μm in greatest dimension. They are variable in form as well, but are often ovoid, occurring singly or in aggregates. Each grain is composed of eccentric or concentric layers of starch around a **hilum**, the site around which starch synthesis is initiated. In the light microscope the hilum often appears dark and highly refractive. Although it has sometimes been considered a structure, its true nature is unclear. Since there is no evidence of the hilum in electron microscope photographs, it is probable that its appearance under the light microscope is an optical artifact. The layering of starch grains, often visible with the light microscope, is also not apparent in electron micrographs. The visible layering might be caused by imbibition of water and differential swelling of alternating layers of different composition (Esau, 1965). Starch, like cellulose, is composed of chains of glucose residues in orderly array. Consequently it is optically anisotropic and doubly refractive when viewed with polarized light.

Storage protein may occur in several forms, but it is best known as aleurone grains which occur in seeds. Fats and oils are of common occurrence in plant cells and take the form of solid, spheroidal particles or liquid droplets. Fats and oils may form either within the cytoplasm, where they are known as spherosomes, or within leucoplasts termed elaioplasts. Certain aromatic and highly volatile essential oils provide the distinctive odors associated with plants such as mint (*Mentha*), cedar (*Juniperus*), nutmeg (*Myristica*), etc. (see Chapter 15 on secretory structures).

Waste metabolites of the protoplast fall into two major categories, calcium oxylate crystals and tanniniferous substances. **Calcium oxylate crystals** (see Arnott and Pautard, 1970) often occur as very small, prismatic crystals in the cytoplasm. Since such crystals are sometimes so small that their edges and angles could not be resolved by nineteenth-century microscopes, botanists of that period often referred to them as crystal dust. Prismatic crystals may, however, also occur as single, large crystals which nearly fill a cell. Such cells which apparently function primarily as repositories for crystals are called **crystal idioblasts**. Aggregations of needle-like crystals, termed **raphides**, and compact, spherical aggregates of angular crystals, called **druses**, also occur in crystal idioblasts. Idioblasts containing solitary prismatic crystals are of common occurrence in the phloem of certain plants, especially in the Pomoideae. Raphide and druse idioblasts occur in many diverse groups, but the former are especially common in the monocotyledons, the latter possibly more common in dicotyledons. Crystal idioblasts may occur singly or in longitudinal rows. Although calcium oxylate crystals generally have been considered waste products, Arnott and Pautard (1970) suggest that some crystalline deposits may be a form of stored calcium. The crystal or crystal cluster apparently develops in the cell vacuole, and it is known that, in many cases, the protoplast dies following crystal formation. There is, however, good evidence that it may

remain alive in some plants and retain a relatively normal appearance (Price, 1970).

Solitary protein crystals occur in some microbodies, organelles bounded by a single membrane. Crystalline calcium carbonate is rare in plants, and is best known in **cystoliths** that occur in specialized epidermal cells of the leaves of *Ficus elastica* (rubber plant). **Silica** is a common constituent of epidermal cell wall of grasses and also occurs as **silica bodies**, masses of silica that fill some epidermal cells.

Tannins and **tanniniferous substances** are phenolic by-products and are usually considered to be waste products. Although generally toxic to the protoplast in certain concentrations, they may be converted to non-toxic substances and stored or transferred to other regions of the plant. Some, such as cinnamic acid, are converted to lignans, and subsequently to lignin, an important component of the cell wall matrix (see Chapter 4). Tannin often appears microscopically as fine to coarsely granular material, but sometimes may occur in large, compact masses. It can occur in almost any tissue, and is characteristic of all major groups of plants. It is translocated into both the bark and the central secondary xylem of trees where its accumulation apparently contributes importantly to the death of these regions. It is very conspicuous in tissues of the cortex and pith of pteridophytes where it occurs in non-toxic form.

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Chapter 4

Structure and development of the cell wall

Perspective

Nearly all plant cells are characterized by an enclosing, cellulosic wall. Those that are not, such as gametes, are either very short-lived or are protected by enclosure within a sheath or tissue of walled cells. Cell walls were first observed, in cork, by Robert Hooke in 1663 and considered to be "dead" structures. Furthermore, the cell wall, produced by and to the exterior of the protoplast, has been considered by some biologists to be an extracellular structure. Most botanists, however, have persisted in considering the wall to be the outer part of the cell, a view based largely on the integration of cytokinesis and cell wall formation. Strong justification for this viewpoint has been provided by research during the past several decades which has shown that the wall is a dynamic structure that receives biochemical information from the protoplast and sends information to it. Recent studies suggest that the wall is an integral component of a cell wall–plasma membrane–cytoskeleton continuum which provides a pathway for molecular and mechanical signals between cells in a tissue, or between cells and the external environment (Wyatt and Carpita, 1993; Reuzeau and Pont-Lezica, 1995; see also Wojtaszek, 2000). Major components of this continuum are plasmodesmata, highly specialized strands of endoplasmic reticulum which traverse the walls and connect the protoplasts of adjacent cells, microtubules, thought to play important roles in determining the orientation of cellulose microfibrils in the cell wall (Baskin, 2001), and actin microfilaments which have been implicated in cytoplasmic streaming and in the transport of vesicles containing precursor compounds to the sites of wall synthesis (Chaffey *et al*., 2000). In addition to its vital role in communication between cells, the wall provides support and protection for the protoplast and shape for the cell. However, during growth it is highly flexible. Wall loosening at specific sites allows for directional growth which results in diverse cell shapes (Sugimoto-Shirasu *et al*., 2004).

The realization that the protoplasts of living cells of plants are interconnected by plasmodesmata has led to the concept of the **symplast**, a system that encompasses the totality of living protoplasts in a plant.

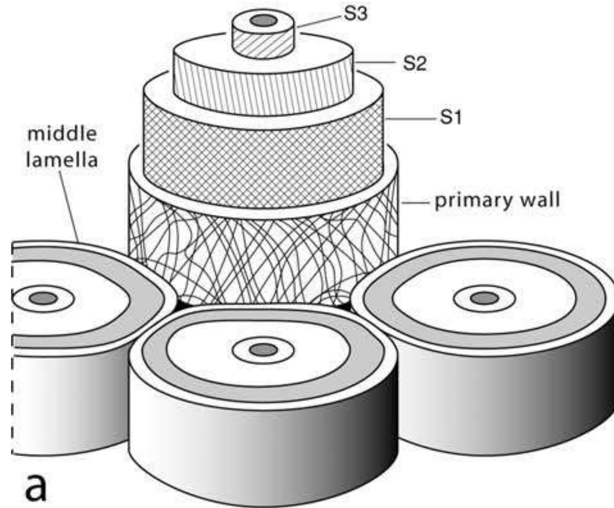
The concept of the symplast can be traced back to Eduard Tangl who in 1897 observed fine strands connecting the protoplasts of adjacent cells which, he noted, “unite them to an entity of higher order” (Oparka and Roberts, 2001). Similarly, the interconnected system of plant cell walls comprises the **apoplast** through which water and small molecules can be transported within the plant as well as to and from the external environment. The presence of the symplast is one of several major bases for the organismal theory of multicellularity in plants which views cells as compartments of the organism rather than as the building blocks as suggested by the cell theory (Kaplan and Hagemann, 1991; Kaplan, 1992; see also Wojtaszek, 2000; for more detail, see Chapter 2).

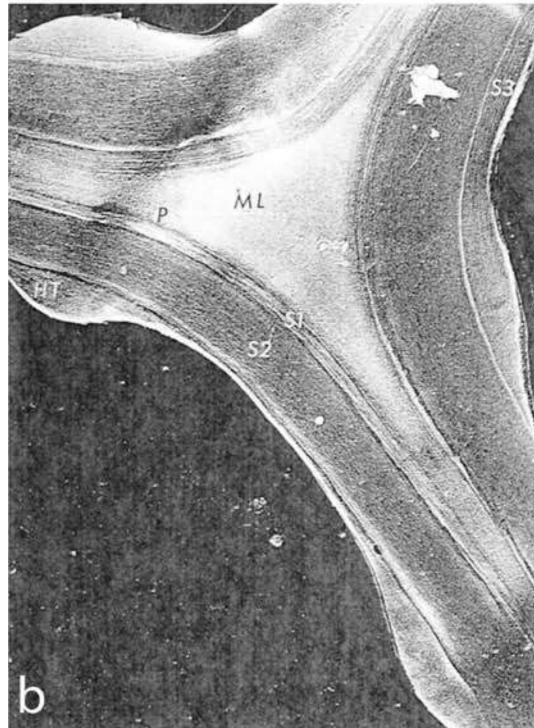
Structure and composition of the cell wall

As we have learned in Chapter 2, most cell walls are layered, consisting of both a **primary wall**, produced by the protoplast following mitosis and formation of the middle lamella, and a **secondary wall**, deposited subsequently on the inner surface of the primary wall. The secondary wall is commonly composed of three distinct layers, S1, S2, and S3 (Figs 4.1a, b, 4.2). The microfibrils in the S1 layer, the first and outermost layer to be formed, are usually arranged in a shallow helix and, thus, the angle with the long axis of the cell is great. The S2 layer, typically the thickest layer of the secondary wall, is characterized by a steep helix of microfibrils, and the angle with the long axis of the cell is small. The S3 layer, the innermost layer of the secondary wall, is characterized by a shallow helix of microfibrils, thus the angle with the long axis of the cell, like that in the S1 layer, is great. Each of these layers is often composed of several to many thin lamellae (Fig. 4.2) (see Preston, 1974 and references therein for early studies) in which parallel microfibrils are oriented at slightly and progressively different angles, thus forming a helicoid pattern (Roland, 1981; Roland *et al*., 1987; see also Neville and Levy, 1985). It has been shown, further, that the change in direction of microfibrils between S1, S2, and S3 layers in secondary walls also results from a helicoidal rotation of microfibrils between these layers (Roland, 1981; Roland *et al*., 1987).

The microfibrillar structure of primary and secondary walls differs in part because the primary walls are synthesized during expansion growth (primarily elongation) of a cell whereas secondary walls develop after most cell growth has ceased. In primary walls, microfibrils appear to be randomly arranged (Figs 4.1a, 4.3a), but with a generally transverse orientation in the inner part of the wall and a generally longitudinal orientation in the outer part. In contrast, microfibrils in the secondary wall are arranged in parallel within wall lamellae, but at different angles in different lamellae (Figs 4.2, 4.3b). The structure of certain macroscopic features, for example the shape and orientation of pit borders and apertures, is related to the orientation of cellulose microfibrils in the middle (S2) layer of the secondary wall.

Figure 4.1 (a) Diagram illustrating the orientation of cellulose microfibrils in the primary cell wall, and the S1, S2, and S3 secondary wall layers and their relative thicknesses. (b) Transmission electron micrograph of a transverse section of parts of the walls of three tracheids in the secondary xylem of *Pseudotsuga* (Douglas fir) showing the middle lamella (ML), primary walls (P), and the three secondary wall layers (S1, S2, S3). Note also the sectional views of helical thickenings (HT) that form part of the S3 layer. Magnification × 6424. (b) From Côté (1967). Used by permission of the University of Washington Press.





The cellulose microfibrils, the basic units of wall structure, are embedded in a matrix of carbohydrates and other compounds (see below). If, however, we exclude, for the time being, consideration of the matrix, primary and secondary wall will be seen to contain cellulose microfibrils (Fig. 4.3a, b), often bound together into **macrofibrils** (Fig. 4.4). A **cellulose microfibril** consists of a core of glucose residues comprising cellulose molecules enclosed in hemicelluloses and pectins. As viewed in transverse section, microfibrils are oval to circular in section, and vary in diameter from about 5 to 35 nm. In most plants

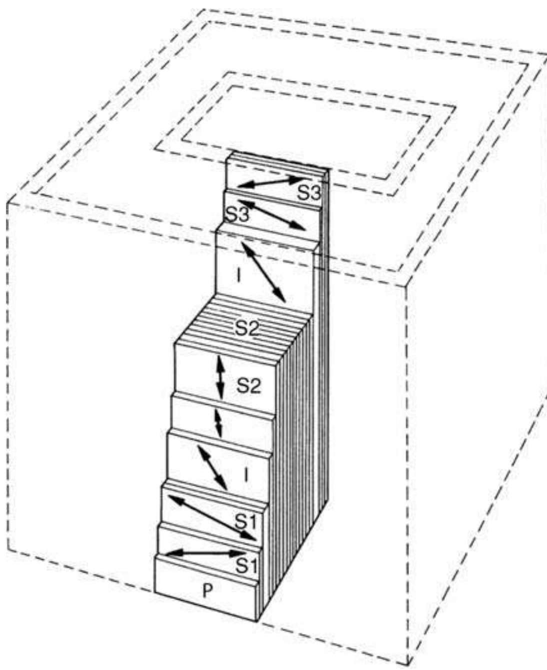


Figure 4.2 Diagrammatic representation of the wall layers of a tracheid. Arrows indicate the general orientation of the cellulose microfibrils in the several layers. Note the lamellae in the S1, S2, and S3 layers. The intermediate layers (I) and the S3 layer are absent in some species. From Preston (1974). Used by permission of Springer-Verlag New York, Inc.

microfibrils average about 10 nm in diameter, but in some algae they are much larger, averaging about 25 nm in diameter. Microfibrils are composed of groups of cellulose molecules arranged in crystalline lattices called **micelles** (Fig. 4.4) (Robards, 1970) which, together, form what is often referred to as the **crystallite**. The concept of the micelle as a distinct and separable entity, consisting of strands of cellulose molecules, was established in the nineteenth century by the German botanist Carl von Nägeli. The Swiss researcher Frey-Wyssling (1954) considered micelles to be subunits of strands of cellulose molecules that comprised what he called "elementary fibrils." Preston (1974) concluded that "the elementary fibril is an illusion based in part upon a confusion between microfibril size and crystallite size and in part upon a misinterpretation of electron microscope images," a viewpoint that has been widely accepted. Today, therefore, the basic cell wall unit is considered to be the microfibril, the central crystalline cellulose core of which contains micellar strands (Fig. 4.4). Whether or not the strands of cellulose of the crystallite are "distinct and separable entities" is still controversial.

Cellulose microfibrils are separated by **interfibrillar capillaries** (Fig. 4.4) containing a **matrix** composed of various mixtures of some, or all, of the following compounds: hemicelluloses, lignins, pectic compounds, suberin, cutin, simple sugars, proteins containing the amino acids, proline and hydroxyproline, and water. The molecules of the hemicelluloses (e.g., xylan, mannan, and glucan), as well as the pectin residues (galacturonic acid), are linked to the cellulose molecules; and according to Preston (1974) the molecular chains of the matrix are probably oriented parallel to the length of the microfibrils. Several models of the relationship between the cellulose microfibrils and the

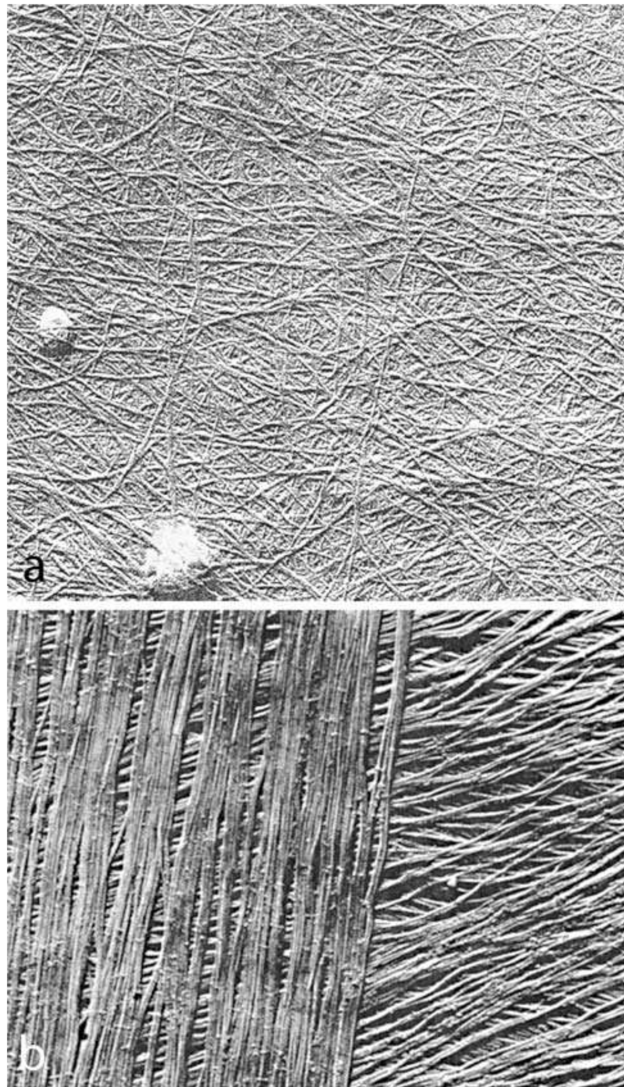


Figure 4.3 (a) Primary wall of a fiber from the secondary xylem of an angiosperm showing the arrangement of cellulose microfibrils. Magnification × 21 900. (b) Cell wall from the alga *Cladophora prolifera* showing different orientations of cellulose microfibrils in adjacent wall lamellae. Magnification × 15 230. (b) From Preston (1974). Used by permission of Springer-Verlag New York, Inc.

enclosing hemicelluloses and other compounds have been proposed. It was early suggested that the matrix polymers and the cellulose were bound together by covalent or hydrogen bonding (Keegstra *et al*., 1973). In a more recent model (Fig. 4.5) the microfibrils are tethered by xyloglucan chains with the pectins and structural proteins filling the space between. The strength of a wall of this type would result from non-covalent bonding of the xyloglucan chains to the microfibril surfaces and the fact that some xyloglucan chains are embedded within the microfibrils (Fry, 1989; Hayashi, 1989; Cosgrove, 2001). A model of the developing primary wall proposed by Talbot and Ray (1992) envisions the microfibrils enclosed by a layer of tightly bound hemicelluloses such as xyloglucan ensheathed by a layer of loosely bound hemicelluloses (Fig. 4.6). The microfibrils and associated hemicelluloses are

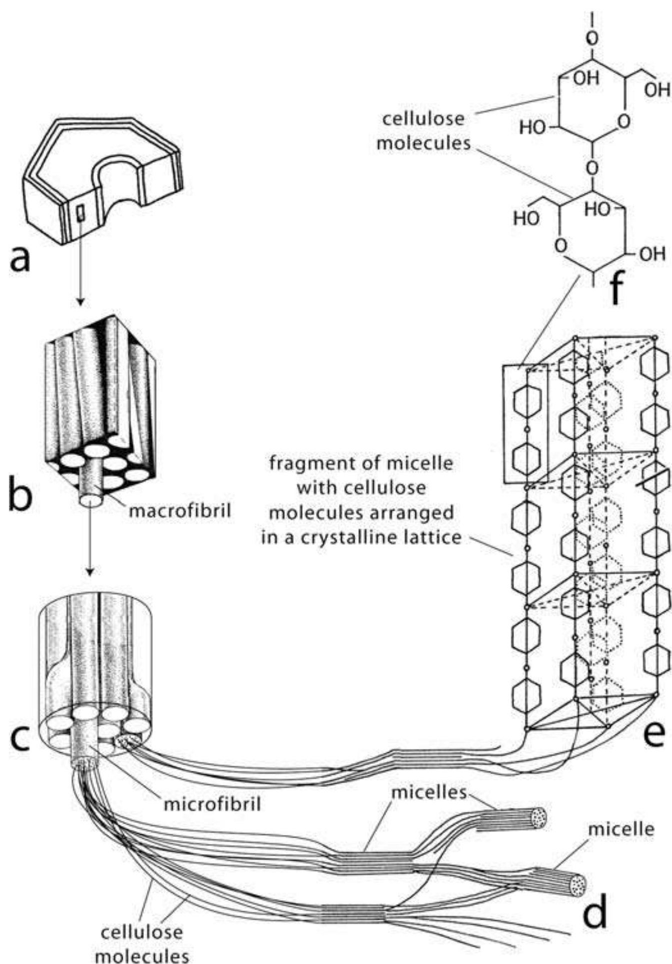


Figure 4.4 Diagrammatic representation of cell wall structure. (a) Part of a fiber. (b) A small fragment of the S2 layer of the wall of one cell consists of macrofibrils of cellulose between which are interfibrillar spaces filled with components of the wall matrix, shown in black. (c) Each macrofibril is composed of cellulose microfibrils embedded in non-cellulosic wall matrix. (d) Each microfribil consists of cellulose molecules. (e) Groups of cellulose molecules called micelles are arranged in a crystalline lattice. (f) A fragment of a cellulose molecule consisting of two glucose residues connected by an oxygen atom. From Esau (1977), slightly modified. Used by permission of John Wiley and Sons, Inc.

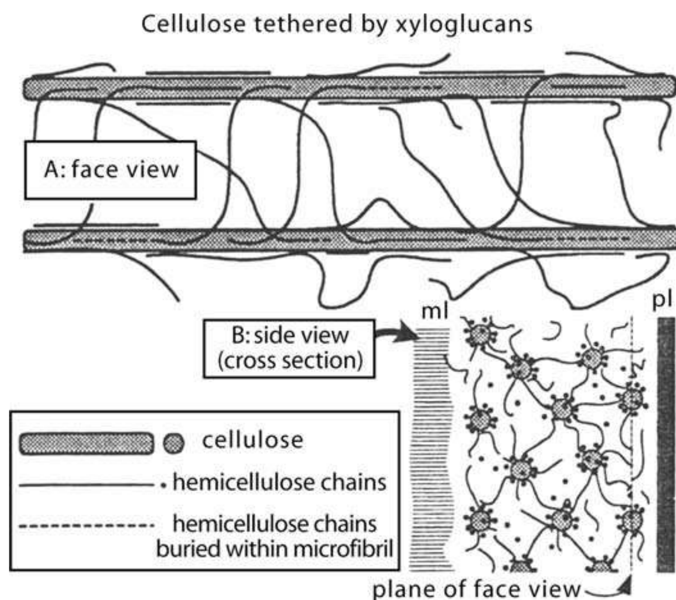


Figure 4.5 A model of cell wall structure in which the cellulose microfibrils are tethered to each other by chains of xyloglucans (hemicelluloses). Pectins and structural proteins fill the space in between. A is a view in the plane of the wall, i.e., parallel to the plasmalemma. B is a sectional view, at right angles to that in A in which one is looking into the ends of the microfibrils of cellulose. ml, middle lamella; pl, plasma membrane. From Cosgrove (1999). Reprinted with permission from the *Annual Review of Plant Physiology and Plant Molecular Biology*, Volume 50, © 1999 Annual Reviews, http://www.annualreviews.org.

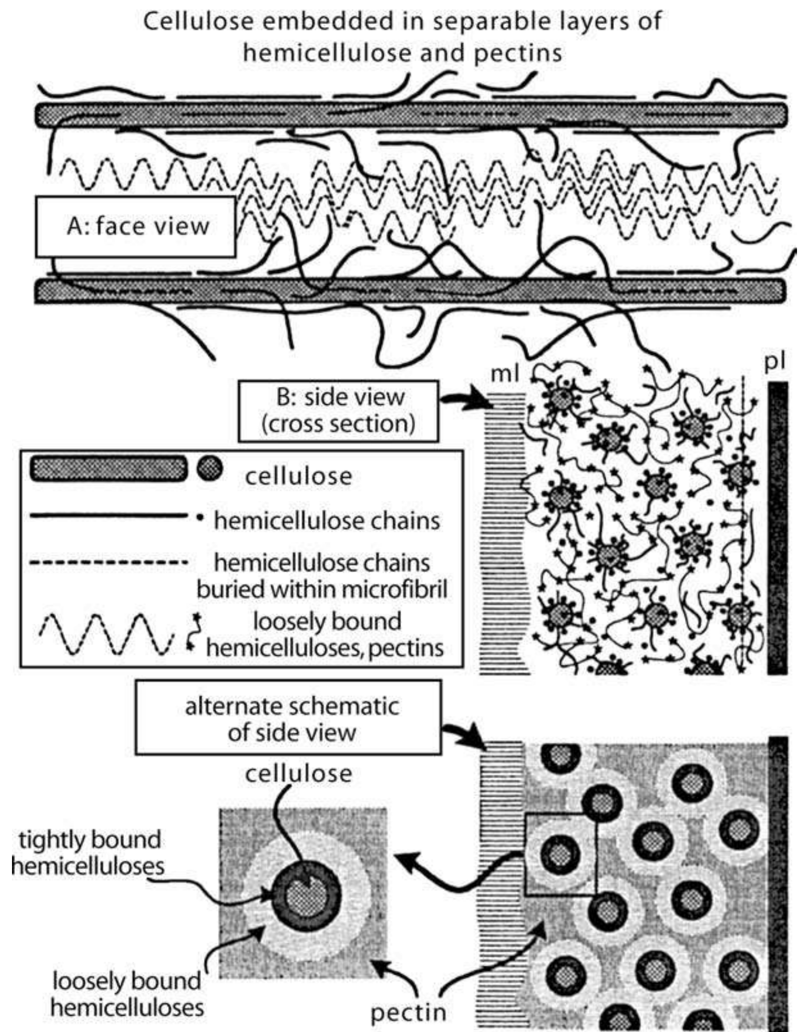


Figure 4.6 A model of cell wall structure, adapted from Talbot and Ray (1992), in which cellulose microfibrils are enclosed by a layer of tightly bound hemicelluloses. The microfibrils and associated hemicelluloses are embedded in a matrix of pectin. ml, middle lamella; pl, plasma membrane. From Cosgrove (1999). Reprinted with permission from the *Annual Review of Plant Physiology and Plant Molecular Biology*, Volume 50, © 1999 Annual Reviews, http://www.annualreviews.org.

embedded in a matrix of pectin. Whereas the xyloglucan chains may be embedded in the microfibrils, they are not linked with other microfibrils. The strength of the walls is thought to result from non-covalent bonding between matrix polymers (Talbot and Ray, 1992; Cosgrove, 1999, 2001).

The relative proportions of the compounds in the matrix vary in accordance with the nature of the cell and its function. For example, the wall matrix in fibers that function in mechanical support contain large amounts of lignin which imparts both strength and flexibility to the cells. On the other hand, the wall matrix of epidermal parenchyma cells that prevent water loss from the plant contains large quantities of cutin, a compound impermeable to the passage of water. Except in walls containing suberin or cutin, walls are relatively porous. The interfibrillar capillaries in such walls are composed primarily of hydrated pectins that comprise the hydrophilic domain of the cell wall through

which pass solutes of low molecular weight and which allow the functioning of enzymes that facilitate the synthesis of wall components (Canny, 1995). The hydrophobic domain is formed by the crystalline cellulose/hemicellulose network where bonding between molecules of cellulose and hemicellulose lead to exclusion of water molecules from between the microfibrils (Wojtaszek, 2000).

Lignin, one of the most important compounds in the cell wall matrix, is a complex polymer of phenylpropane with *p*-coumarylic and synapylic acids. It is added to the wall in various quantities, and at varying stages during development, and is especially strongly concentrated in the primary wall and the S1 layer of the secondary wall. Its presence imparts strength to the wall, especially important in cells such as tracheids and vessel members through which water is transported. Were the walls of these cells unlignified they would probably collapse upon death of the protoplasts and the consequent loss of turgor pressure, thus impeding the transport of water through them. Another matrix compound of potentially great significance is the glycoprotein **expansin**, a component of the primary wall, which is thought to control wall extension during growth (Romberger *et al.*, 1993; Cosgrove, 2000; Cosgrove *et al.*, 2002).

Growth of the cell wall

Historically, two types of wall growth have been postulated, growth by intussusception and growth by apposition. Growth by **intussusception** as first proposed in 1858 by von Nägeli would require the synthesis of new wall substance *within* the primary wall during wall expansion. This concept, which would require the transport of precursor compounds of wall components into the developing wall, would, therefore, seem to necessitate a loose, reticulate meshwork within which new matrix and cellulose microfibrils could be synthesized. In the past, some workers have cited the fact that microfibrils in the primary wall *appear* to be intertwined as evidence for growth by intussusception. Although others have found it difficult to apply the concept of intussusception to the synthesis of new microfibrils within the wall, they have noted that it could easily be applied to the synthesis of new matrix compounds.

The concept of wall growth by **apposition** was suggested by Strasburger in 1882. Apposition of wall material would require the production of new layers of wall material, one upon another, like the application of plaster to the wall of a house. Strasburger as well as more recent workers considered this to be the pattern of secondary wall synthesis after the cell had stopped expanding. However, the Dutch botanists Roelofson and Houwink (1953) applied the concept of apposition to primary cell wall growth as expounded in their **multinet hypothesis** of wall growth. It has been observed that the outermost (oldest, and first-formed) primary wall layers contain longitudinally oriented microfibrils and that those in the most recently synthesized part of the wall (the youngest part) are more nearly transversely oriented. On

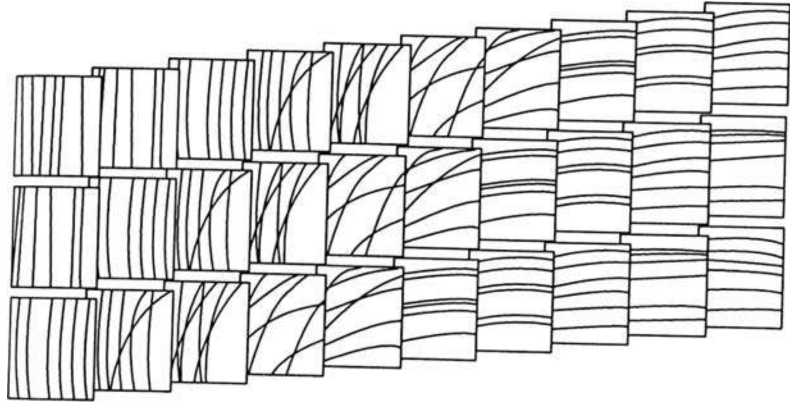


Figure 4.7 A diagram showing change of about 90 degrees in the orientation of cellulose microfibrils in the primary walls of meristematic and isodiametric parenchyma cells as wall synthesis occurs over time. The squares showing orientation of microfibrils (lines) represent a succession of inner wall sites. Note the gradual change in orientation from horizontal (transverse) on the right to vertical on the left. The changes in orientation of microfibrils do not occur simultaneously at all sites, but follow the same pattern. For more detail, please see the text. From Wolters-Arts *et al.* (1993). Used by permission of Springer-Verlag Wien.

this basis Roelofson and Houwink concluded that as layers of cellulose microfibrils (and associated matrix) are deposited one upon the other there is a passive change in orientation of microfibrils from nearly transverse (or sometimes random) to longitudinal resulting from elongation of the wall (see also Preston, 1982). Although this hypothesis has gained widespread acceptance, evidence of a helicoidal pattern of microfibrils in primary walls (Neville and Levy, 1984; Vian *et al.*, 1993; Wolters-Arts *et al.*, 1993) has suggested a different interpretation of the observations upon which it was based. For example, Wolters-Arts *et al.* (1993) observed a helicoidal change, over time, of about 90 degrees in orientation of newly deposited microfibrils in primary walls. They observed such changes in cells of several types including cylindrical cells obtained from shoot apices, isodiametric cells obtained from mature bulbs and cells in suspension obtained from tobacco. They concluded that the change in orientation occurred gradually as successive layers of microfibrils were deposited at different angles (Fig. 4.7) and emphasize that the change in direction of the microfibrils "could not have been due to passive reorientation" as proposed by the multinet hypothesis since the microfibrils of changed orientation at each site were newly synthesized and deposited. Romberger *et al.* (1993) believe that a helicoidal pattern of wall growth is "not necessarily irreconcilable with the multi-net growth hypothesis." They suggest further that "[this type of growth] allows the primary wall to be an active rather than a merely passive, mechanical participant in determining the direction and extent of growth."

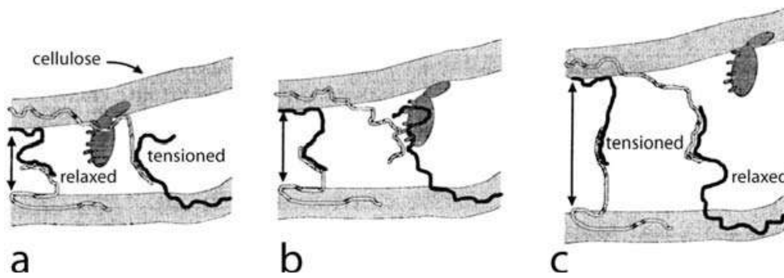


Figure 4.8 A diagrammatic illustration of wall loosening by expansin. It is hypothesized that the cellulose microfibrils become more widely separated when the expansin protein disrupts the bonding of the glycans (hemicellulose chains) to either the microfibril (a) or to each other (b) resulting in wall loosening (c). From Cosgrove (2000). Used with permission of Macmillan Magazines Limited.

Whereas growth in thickness of the secondary wall occurs largely after cell elongation has ceased, the primary wall which is deposited during cell elongation must expand in area predominantly in the direction of cell growth, and at the same time maintain its continuity. Several decades ago, resulting from the research of many workers, it was concluded that plant cells increase in size more rapidly in conditions of low pH, especially when it is below 5.5. It was hypothesized that in the early stages of wall growth auxin influences its acidification and stimulates the synthesis of one or more wall-loosening enzymes (see Rayle and Cleland, 1992; Cosgrove, 2001). Subsequent efforts to identify wall-loosening enzymes resulted in the discovery of two proteins called **expansins** that mediate acid-induced wall extension (see Cosgrove, 2000, 2001). Consequently, it is widely accepted today that expansion of the primary wall involves wall loosening mediated by hormones such as auxin and the protein expansin, and the constant addition of new cell wall constituents required to maintain the continuity of the wall (see Lyndon, 1994; Fleming *et al.*, 1999; Cosgrove, 2000). Although the mechanism of wall loosening is unknown, expansin is thought to "unlock" the system of wall polysaccharides, possibly through weakening glucan–glucan bonding, thus allowing a separation of microfibrils (Fig. 4.8). With the continuing synthesis of new cellulose and under the influence of turgor pressure, cell walls grow (Cosgrove, 2000; Cosgrove *et al.*, 2002). As noted above, cellulose microfibrils (and associated microtubules lying just beneath the plasma membrane) are oriented more or less transversely to the long axis of the elongating cells. This orientation of microfibrils is believed to facilitate longitudinal expansion of the primary wall following wall loosening whereas a longitudinal orientation apparently restricts longitudinal wall growth (Seagull, 1986; Sauter *et al.*, 1993; Paolillo, 1995). It should be noted, however, that Paollilo (2000) has observed that in the outer epidermal walls in several taxa cell elongation continues after microfibrils become oriented longitudinally.

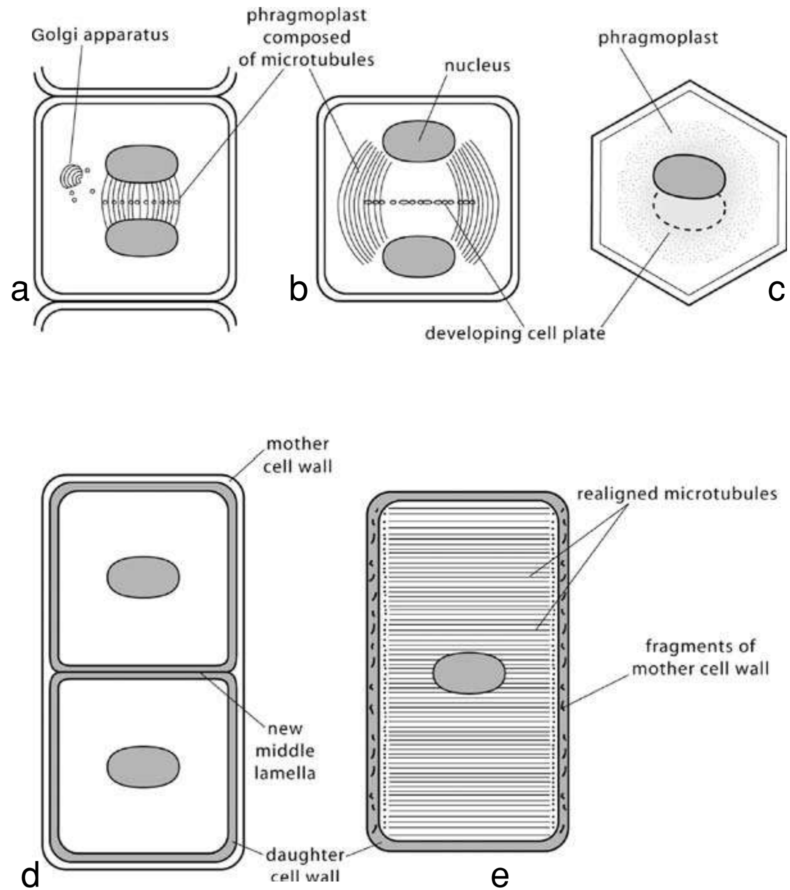


Figure 4.9 Formation of a new cell wall following mitosis. (a) Upon separation of the daughter nuclei, vesicles derived from Golgi bodies accumulate in a plane among the microtubules of the phragmoplast. (b) The phragmoplast begins to migrate toward the lateral walls of the cell. The Golgi vesicles begin to fuse, forming the cell plate. (c) The phragmoplast and the developing cell plate between the daughter nuclei, as seen from above. (d) The two new cells are separated by a new middle lamella, and each cell has a newly formed cell wall. (e) As each new cell begins to elongate, the microtubules become realigned transversely just beneath the plasma membrane. The mother cell wall disintegrates.

Cell wall development

The precursor compounds of both cellulose and matrix materials are synthesized in the protoplast and transported to the developing wall where synthesis is completed. Let us consider how this transfer of materials is accomplished. During the latter stages of mitosis, microtubules become arranged between the daughter nuclei, forming the phragmoplast, and Golgi vesicles aggregate in the "equatorial" plane between incipient daughter cells (Figs 4.9a–c, 4.10). This disk-like aggregation of vesicles, called the cell plate and the surrounding phragmoplast, extend toward the original wall of the mother cell (Fig. 4.9b, c). Upon fusion, the vesicle membranes become the plasma membranes of contiguous protoplasts of the new cells, and the contents of the vesicles the new middle lamella that separates them (Fig. 4.9d). Golgi vesicles containing precursor compounds of wall components fuse with the plasma membrane of each new cell (Figs 4.11, 4.12), adding to its area and at the same time ejecting their contents into the **periplast** immediately outside the plasma membrane where wall formation takes place (see Cosgrove, 1997, 1999). A new primary wall is synthesized in both

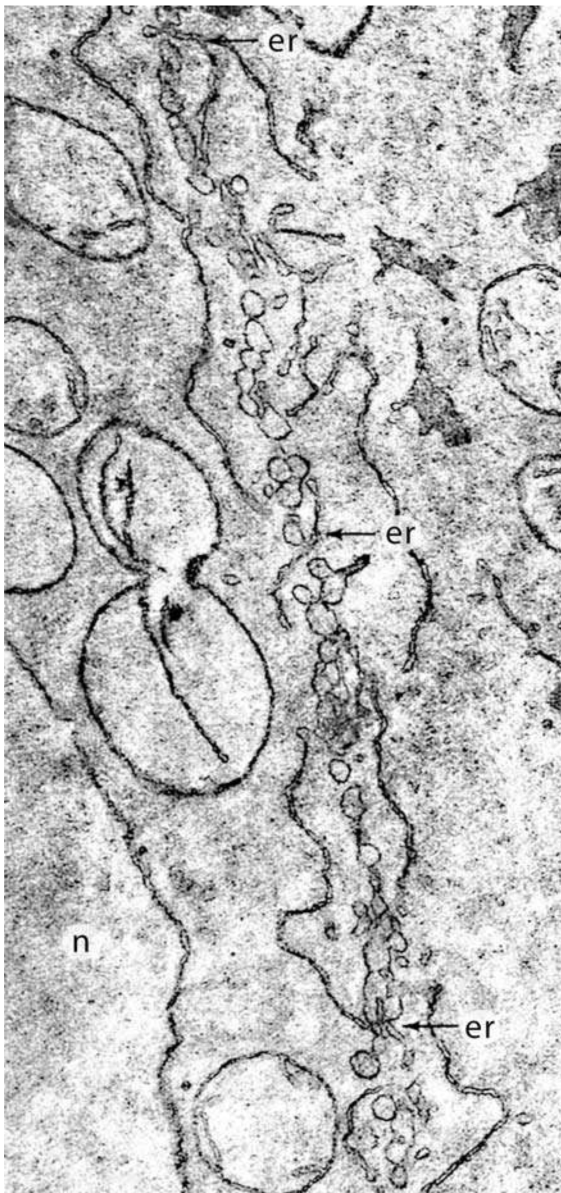


Figure 4.10 A developing cell plate consisting of numerous Golgi vesicles, some of which have fused with others. The vesicle membranes become the plasma membranes of the new cells, the vesicle contents the new middle lamella. er, endoplasmic reticulum; n, nucleus. Magnification × 31 930. From Whaley *et al.* (1960). Used by permission of the Botanical Society of America.

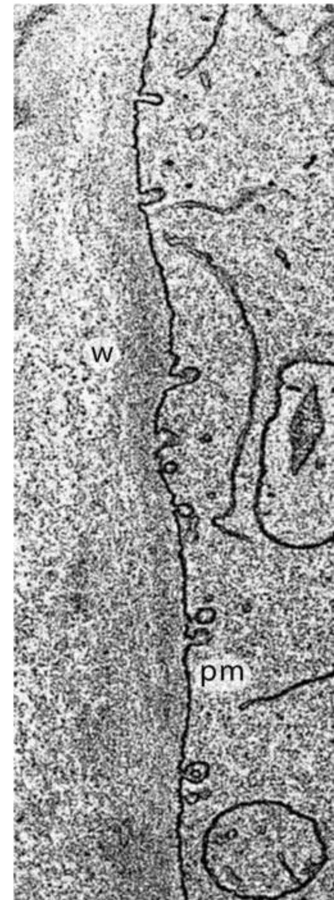


Figure 4.11 Fusion of Golgi vesicles with the plasma membrane during wall formation. Wall matrix compounds such as hemicelluloses and pectins contained within the vesicles are, thus, emptied into the region of wall formation. pm, plasma membrane; w, wall. Magnification × 23 520. From Whaley *et al.* (1960). Used by permission of the Botanical Society of America.

daughter cells. The original primary wall of the mother cell disintegrates (Fig. 4.9e), and the middle lamella between the daughter cells becomes continuous with that between the mother cell and contiguous cells. In preparation for elongation of a new cell, microtubules become transversely arranged just under the plasma membrane (Fig. 4.9e).

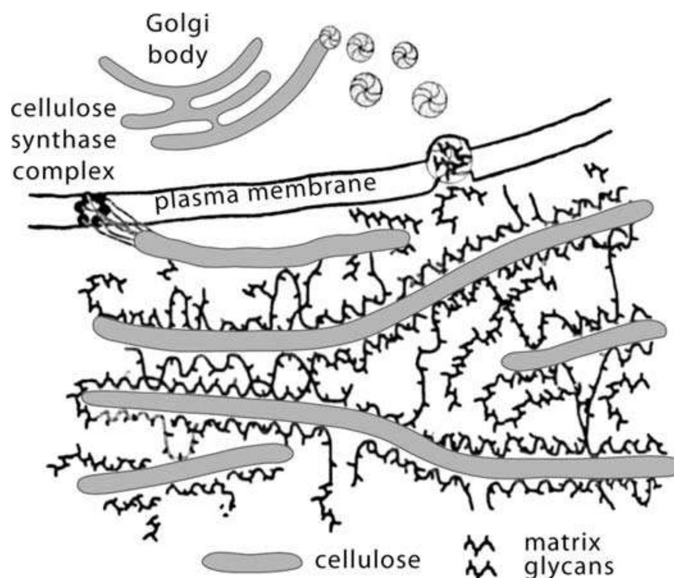


Figure 4.12 Diagram illustrating the biosynthesis of the cell wall. Cellulose microfibrils are generated by cellulose synthase complexes (large complexes of enzymes) in the plasma membrane, and hemicelluloses and pectins (glycans) which comprise the wall matrix are synthesized in Golgi bodies and delivered to the wall by secretory vesicles. Within the developing wall, pectins form ionic gels and the hemicelluloses bind to the cellulose microfibrils. From Cosgrove (2000). Used by permission of Macmillan Magazines Limited.

The processes of cellulose microfibril formation and orientation during wall development have been the subjects of extensive research by many scientists during the past 30 years or so. Roelofson (1958) and Heath (1974) hypothesized that enzymes, or enzyme complexes, located at the ends of microfibrils might be responsible for the polymerization of glucose and its crystallization into microfibrils (see Brown, 1985). In 1976 Brown and Montezinos actually discovered cellulose microfibril synthesizing complexes in the alga *Oocystis*. With continuing research in many laboratories it is now well established that, in algae, pteridophytes and seed plants, microfibrils are generated by terminal **cellulose synthase complexes** in the plasma membrane (Fig. 4.12). These enzyme complexes are probably delivered to the plasma membrane in Golgi vesicles (Giddings and Staehelin, 1991). Two types of microfibril-generating complexes have been recognized, **linear complexes** of particles, commonly found in algae, and **rosette-shaped complexes** (Fig. 4.13) found in some algae and in higher plants. The rosette-shaped complexes consist of a cluster of six particles and sometimes contain a centrally located globular component (Fig. 4.14) (Brown, 1985; Delmer, 1987). These particles are the enzymes responsible for the synthesis of β-1,4-glucan chains of which cellulose is comprised (Giddings and Staehelin, 1988). The glucan chains "self-associate" through intra- and interchain hydrogen bonding forming insoluble, crystalline microfibrils (Delmer, 1987).

Extensive research during the past 20 years has demonstrated that there is frequently, but not always, a correlation between the orientation of microtubules and microfibrils. Consequently, it is believed that microtubules play an important role, perhaps assert some control, in aligning microfibrils (e.g., Lloyd, 1984; Seagull, 1990, 1991; Wymer and Lloyd, 1996; Baskin, 2001; Baskin *et al.*, 2004). Correlation between

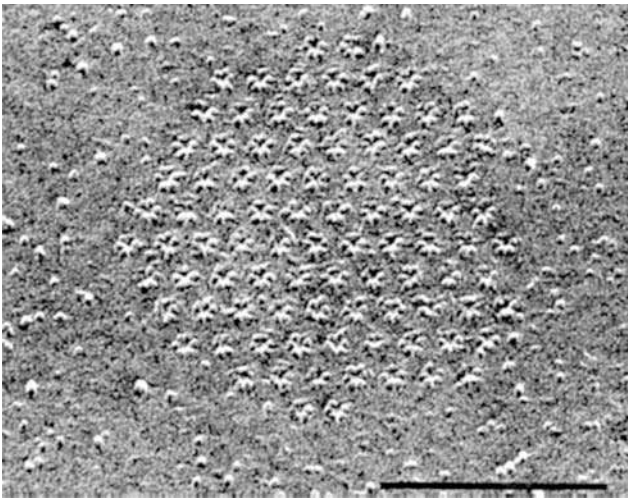


Figure 4.13 Cellulose synthase complexes, also called rosettes, in the plasmalemma of a cell of the alga *Microsterias denticulata*, as seen in a freeze-fracture electron micrograph. Bar = 0.1 μm. From Giddings *et al.* (1980). Used by copyright permission of the Rockefeller University Press.

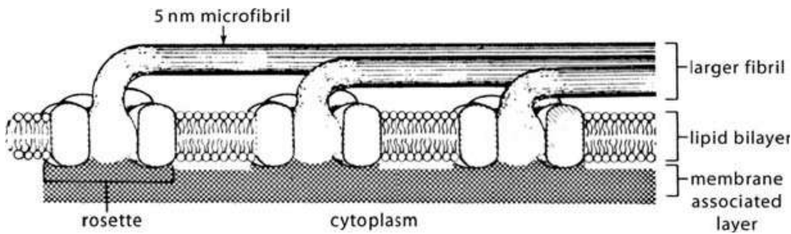


Figure 4.14 Diagram of a model of cellulose synthase complexes (rosettes) in the plasmalemma of *Microsterias*, and the microfibrils they have formed. From Giddings *et al.* (1980). Used by copyright permission of the Rockefeller University Press.

microtubules and microfibrils is especially apparent in regions of synthesis of the borders of circular bordered pits as well as the annular and helical thickenings in the primary walls of tracheary elements (Hogetsu, 1991). Immediately below the developing borders and wall thickenings lie microtubules, in contact with the plasma membrane, oriented in patterns identical to those of the developing pit borders and wall thickenings.

In recent research of great significance, Paredez *et al.* (2006) were able, *visually*, to confirm a close, *functional* relationship between cellulose synthase complexes and microtubules in developing primary xylem cells in the hypocotyls of *Arabidopsis*. Using citrine yellow fluorescent protein as a label, and spinning disk confocal microscopy, these workers were able to observe fluorescence in subcellular, motile, molecular structures in the plasma membrane which they determined to be cellulose synthase complexes. They also observed labeled Golgi bodies probaby containing cellulose synthase complexes. It is thought that the cellulose synthase complexes are organized within the Golgi bodies and transferred in Golgi vesicles to the plasma membrane where cellulose microfibrils are synthesized (e.g., Haigler and Brown, 1986).

The movement of the cellulose synthase complexes within the plasma membrane was observed to be directly related to their intimate association with microtubules. Cellulose synthase complexes were observed to move along paired, linear trajectories, one on either side of the microtubules (Paredez *et al*., 2006). Even when the arrangement of microtubules was distorted by application of the drug, oryzalin, the co-orientation of microtubules and the trajectories of cellulose synthase complexes persisted. This indicates that the microtubules might actually guide the cellulose synthase complexes, with the result that newly synthesized cellulose microfibrils will be arranged parallel to the microtubules (Paradez *et al*., 2006; Mutwil *et al*., 2008).

Ten cellulose synthase proteins (CESAs) are known. At least three are required to form a functional rosette in *Arabidopsis*, and probably in other vascular plants as well (Desprez *et al*., 2007). Synthesis of cellulose in the primary wall requires the presence of CESA1, CESA3 and CESA6 (which may be replaced by CESA2 or CESA5) (Desprez *et al*., 2007) whereas CESA4, CESA7 and CESA9 are required for the synthesis of cellulose in the secondary wall (Tanaka *et al*., 2003).

Because there is not always a parallelism between microtubules and cellulose microfibrils, several authors have concluded that microtubules are not essential for the alignment of cellulose microfibrils (Roland and Vian, 1979; Boyd, 1985; Emons and Kieft, 1994; Emons and Mulder, 1998). This viewpoint is supported by more recent studies (e.g., Sugimoto *et al*., 2003; Wasteneys, 2004) which do not support the concept of a causal relationship between microtubules and the movement of cellulose synthase complexes. At present, there seems to be no clear explanation for this discrepancy.

An interesting, early hypothesis to explain the movement of cellulose synthase complexes where strong evidence indicates a close relationship between microtubule and cellulose microfibril orientation was proposed by Giddings and Staehelin (1988). They hypothesized that the microtubules were restricted to “channels” between adjacent microtubules, and were pushed along these channels by the polymerization and crystallization of cellulose microfibrils. This concept of passive movement differs significantly from that of Paredez *et al*. (2006) wherein the cellulose synthase complexes exhibit a “tight spatial and temporal” coupling to microtubules. This implies an active participation by microtubules in guidance of the cellulose synthase complexes and, thus, in the deposition of cellulose.

Plasmodesmata

Although plasmodesmata pass through cell walls they are not, strictly speaking, parts of the walls. Because, however, they play such an important role in development it is appropriate to provide here a detailed discussion of their structure and function. **Plasmodesmata** are highly specialized structures that connect the protoplasts of adjacent cells

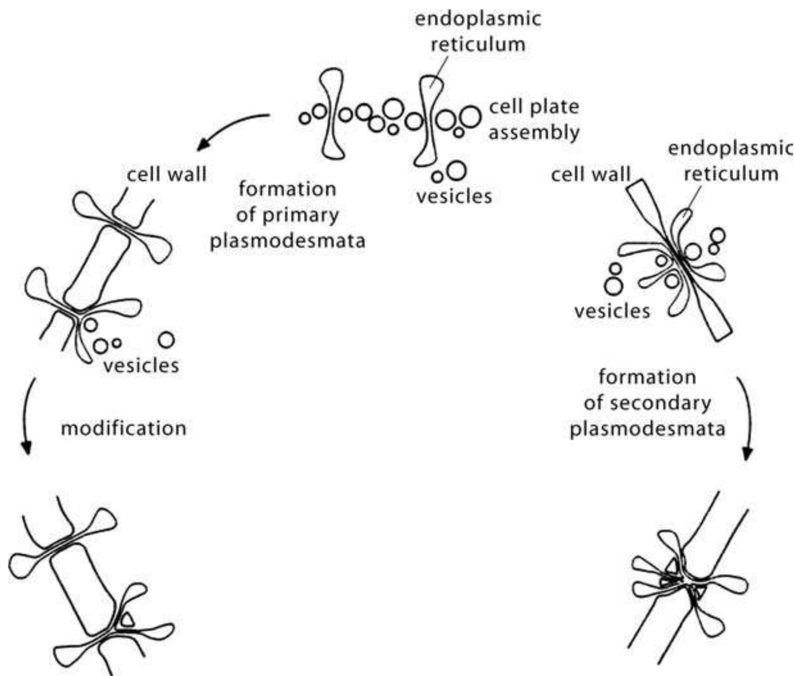


Figure 4.15 Diagram illustrating the formation of primary and secondary plasmodesmata. Primary plasmodesmata develop from tubules of endoplasmic reticulum that are trapped between Golgi vesicles during cell plate formation just prior to the completion of cytokinesis. Secondary plasmodesmata develop in previously formed walls. Endoplasmic reticulum and Golgi vesicles aggregate on opposite sides of the wall which thins greatly, the endoplasmic reticulum tubules penetrate the wall, fuse, and differentiate, usually forming branched plasmodesmata. New wall is synthesized around the plasmodesmata and the wall regains its original thickness. From Kragler *et al.* (1998). Used by permission of Oxford University Press.

through the cell walls. They provide passageways for intercellular communication through the transport between cells of chemical substances ranging from small, soluble molecules to macromolecules such as proteins and nucleic acids (Ehlers and Kollmann, 2001). Although it is theoretically possible that all protoplasts may be connected by plasmodesmata, it is not certain that in large multicellular organisms there is ever any coordinated control throughout the entire organism. Recent research has shown, instead, that specific regions may be isolated from others by the absence of plasmodesmata, or even the modification of plasmodesmatal function (Ehlers *et al*., 1999), resulting in the formation of **symplastic domains**, localized sites of function, and/or development of certain tissue regions, tissues, or cell types.

Plasmodesmata originate following mitosis during cell plate formation. During cell division, Golgi and ER vesicles accumulate in the plane of the developing cell plate (Figs 4.10, 4.15), and tubules of ER become trapped between them. As the vesicles fuse, forming the middle lamella, and primary wall is synthesized, these tubules of ER and associated cytoplasm (Fig. 4.16) become adpressed and differentiate into mature plasmodesmata (Figs 4.17, 4.18). A plasmodesma consists of a **central cylinder** to which helically arranged proteinaceous particles are fused, comprising the **desmotubule** enclosed by plasma membrane (derived from vesicle membrane) which is continuous between adjacent cells (Figs 4.17a, b, 4.18a, b). Centrally located within the desmotubule is a row of particles, embedded in the lipid of the ER, referred to as the **central column** (Fig. 4.18a, b). Embedded within its inner surface is a cylinder of protein particles (Fig. 4.18a, b). With expansion of the outer cylinder of plasma membrane and formation of the

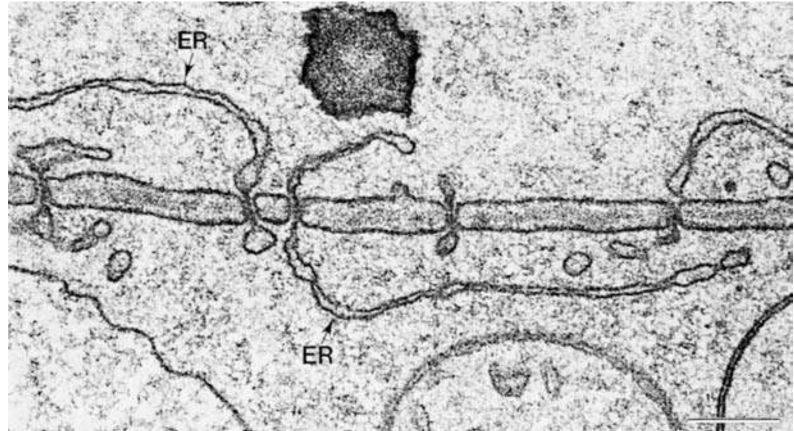


Figure 4.16 Primary plasmodesmata in the young walls of cells in a root tip of *Zea mays*, showing their relationship with endosplasmic reticulum (ER). Bar = 0.1 μm. From Lucas *et al.* (1993). Used by permission of Blackwell Publishing, Ltd.

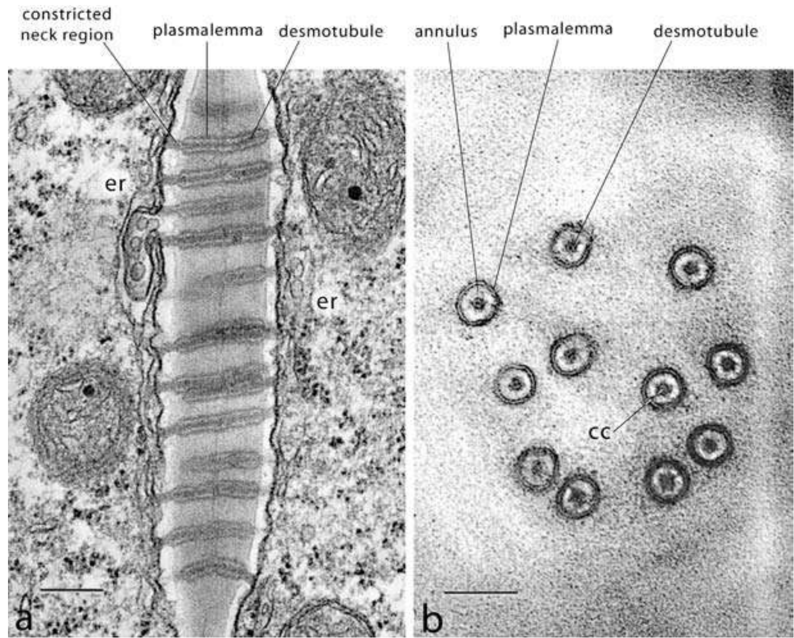


Figure 4.17 Plasmodesmata traversing the walls of adjacent phloem parenchyma cells in a leaf of *Saccharum officinarum*. (a) Plasmodesmata as seen in longitudinal view. er, endoplasmic reticulum. Bar = 200 nm. (b) Plasmodesmata in sectional view. cc, central column. Bar = 100 nm. From Robinson-Beers and Evert (1991). Used by permission of Springer-Verlag GmbH and Co. KG. © Springer-Verlag Berlin Heidelberg.

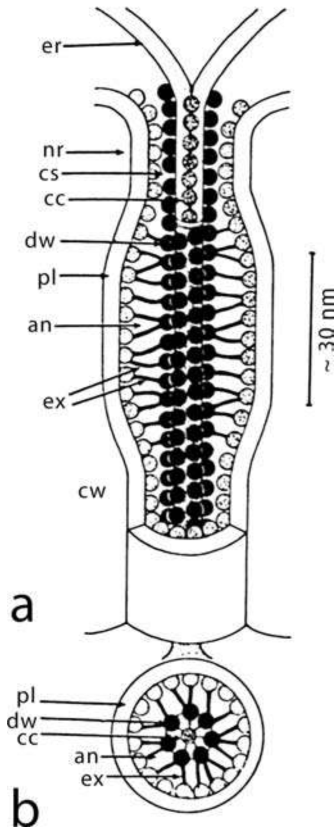


Figure 4.18 A model of a plasmodesma. (a) Longitudinal view. (b) Transverse (sectional) view. an, annulus; cc, central column; cs, cytoplasmic sleeve; cw, cell wall; dw, desmotubule wall; er, endoplasmic reticulum; ex, spoke-like extensions; nr, neck region; pl, plasma membrane. From Ding *et al.* (1992) (labeling slightly modified). Used by permission of Springer-Verlag Wien.

cytoplasmic annulus, some of these particles become connected with those of the desmotubule by "spoke-like extensions," resulting in a system within the annulus of "microchannels" through which molecules are thought to pass (Fig. 4.18a, b). The narrow region at each end of the plasma membrane sheath is called the **orifice**, the region in which molecular transport is believed to be regulated. For more detail, see

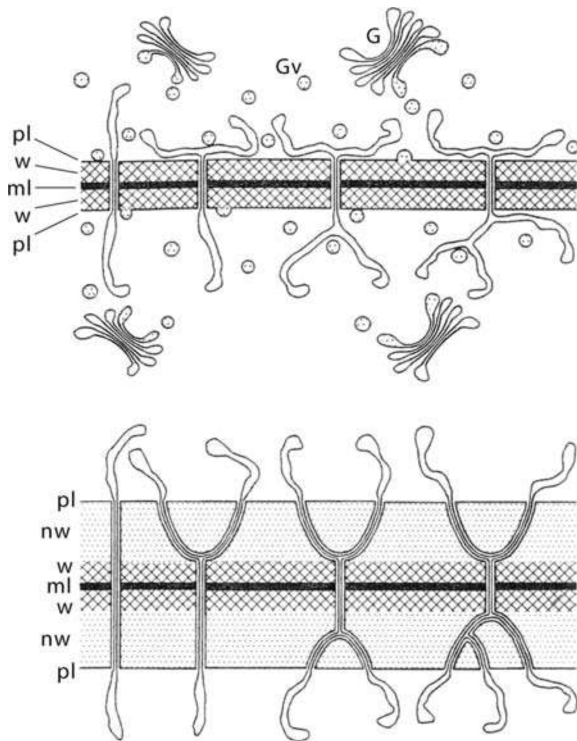


Figure 4.19 Modification of primary plasmodesmata during increase in cell wall thickness. Branches develop by the enclosure of branched tubules of endoplasmic reticulum. pl, plasma membrane; w, original cell wall; ml, middle lamella; nw, new cell wall; G, Golgi body; Gv, Golgi vesicle. From Ehlers and Kollmann (2001). Used by permission of Springer-Verlag Wien.

Ding *et al*. (1992) and Lucas *et al*. (1993) from which the foregoing description is taken.

Plasmodesmata that are formed during the process of cytokinesis are called **primary plasmodesmata** (Fig. 4.15). With increase in wall thickness, primary plasmodesmata may branch (Figs 4.15, 4.19) (Kragler *et al*., 1998; Ehlers and Kollmann, 2001). **Secondary plasmodesmata** form, *de novo*, in developing tissues across previously formed walls. At the sites of new secondary plasmodesmata the wall thins and ER cisternae and Golgi vesicles aggregate on either side of these regions (Fig. 4.15). The ER cisternae penetrate the thin wall, fuse, and differentiate, resulting in plasmodesmata, often branched, with a structure apparently identical to that of primary plasmodesmata. At these sites, following synthesis of new wall components (matrix from precursor compounds provided by the Golgi vesicles, and new cellulose microfibrils) the wall thickens around the secondary plasmodesmata (Monzer, 1991; see also Kragler *et al*., 1998; Ehlers and Kollmann, 2001). Formation of secondary plasmodesmata is common in the walls of cells that elongate greatly during development, thus compensating for the decrease in frequency of primary plasmodesmata. Secondary plasmodesmata are also thought commonly to occur in "non-division" walls as, for example the walls of cells that form the bounding layers between tissue regions of different ontogenetic origin such as the ground tissue (incipient mesophyll) and provascular tissue in developing leaves (Ehlers and Kollmann, 2001; see also Dengler *et al*., 1985). Another good

example is their presence in the periclinal walls between protodermal and underlying cells. Since, during development of the epidermis, protodermal cells divide only anticlinally, the walls between them and underlying cells are non-division walls. The non-division walls of cells in the carpel margins of some taxa which fuse only during gynoecium development also contain secondary plasmodesmata (van der Schoot *et al.*, 1995). The importance of secondary plasmodesmata is emphasized by the fact that they comprise a significant proportion of plasmodesmata that connect living cells in the plant (Ding *et al.*, 1992; see also Kragler *et al.*, 1998).

A major function of plasmodesmata is the interchange of information through the transmission of molecules from one protoplast into another, a process generally referred to as "trafficking." **Molecular trafficking** is essential to the regulation of physiological and developmental processes in the plant. Small molecules can diffuse freely through plasmodesmata, but large molecules may be restricted from passage by the molecular **size exclusion limit** of the plasmodesmata. Some large molecules bind with certain proteins, called **movement proteins**, which facilitate the passage through the plasmodesma by increasing its size exclusion limit (see e.g., Kragler *et al.*, 2000; Oparka and Roberts, 2001; Aoki *et al.*, 2002). On the other hand, some plasmodesmata, such as those between companion cells and sieve tube members, have sufficiently large size exclusion limits which allow the direct passage of proteins that are essential to maintain the viability of the enucleate sieve tube members. Since the nutrients, hormones, and proteins essential to support the regulation of development and other physiological processes which characterize a particular developmental or functional domain vary, it is probable that specific mechanisms for the control of molecular trafficking in domains with different requirements must have evolved (Ehlers and Kollmann, 2001).

Recent studies (see Evert *et al.*, 1996) have demonstrated that the function of plasmodesmata may change during development. During early stages of development, leaves function as sinks since they utilize more photosynthate than they produce. Upon maturity, however, they become nutrient sources. During this transition from sink to source, plasmodesmata undergo a change in structure and function. Studies on tobacco by Oparka *et al.* (1999) indicate that during the stage in which developing leaves are nutrient sinks, the simple, unbranched plasmodesmata have a high size exclusion limit and photosynthate is transmitted from the phloem throughout the immature tissues of the leaf where it is utilized in the various processes of metabolism during development. As the leaf becomes a source and exporter of photosynthate, the plasmodesmata become highly branched which increases their cross-sectional area, and which may enhance their capacity in phloem loading (Ehlers and Kollmann, 2001; see also Beebe and Russin, 1999; Oparka *et al.*, 1999). These changes in structure and function involve both primary and secondary plasmodesmata (Ehlers and Kollmann, 2001).

New plasmodesmata are not only formed during development, but also may become non-functional, or even eliminated, another way in which development can be controlled in the plant. For example, the protoplasts of developing tracheary cells are connected to adjacent parenchyma cells by primary pit fields that contain numerous plasmodesmata. With approaching maturity of the tracheary cells and just prior to autolysis of their protoplasts, both ends of the plasmodesmata are covered by newly synthesized layers of cell wall material, thus eliminating their functional ability (Lachaud and Maurosset, 1996; Kragler *et al*., 1998).

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Chapter 5

Meristems of the shoot and their role in plant growth and development

Perspective

Among the unusually interesting and unique aspects of plants is their indeterminate mode of growth. This results from the presence of apical meristems by which new cells and tissues are added to the plant body during every period of growth. As a consequence plants have the potential to increase in size at regular intervals throughout their lives, which accounts for the large size of some plants such as the redwoods of California as well as many hardwood tree species of temperate and tropical forests.

A **meristem** is a localized region of tissue which, by cell division, adds new cells to a plant or plant part. In the shoots of vascular plants the activity of meristems results in an increase in length and/or diameter, and following cell growth and differentiation, formation of the various mature tissue regions of the axes as well as the formation of organs such as leaves, cone scales, sporophylls, stipules, flower parts, etc. Some meristems are **self-perpetuating**, and thus can be considered to be "permanent" meristems. Most apical meristems and the vascular cambium are meristems of this type and, as a result of their activity, provide vascular plants with their mode of **indeterminate growth**. Others, such as the meristems that contribute to the formation of the petiole and blade of leaves, flower parts, and the various other lateral appendages of non-seed plants, cease functioning when these organs, characterized by **determinate growth**, reach their genetically predetermined size and form. For recent, in-depth discussions of the structure and function of apical and other meristems that cover material beyond the scope of this book, see Steeves and Sussex (1989) and Lyndon (1998).

Apical meristems

Apical meristems are borne at the tip of the **shoot apex**, the most distal region of a stem or lateral branch, and near the tip of roots (just behind

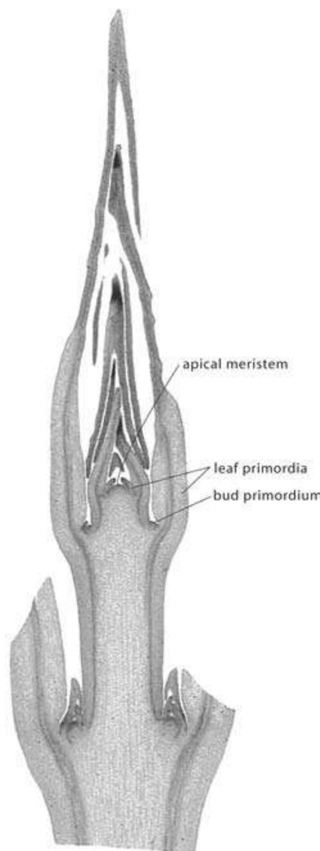


Figure 5.1 The shoot apex of a stem of *Syringa vulgaris* (lilac).

the root cap). We shall defer consideration of apical meristems of roots until the chapter on the root (Chapter 16). The shoot apex (Fig. 5.1) is comprised not only of the apical meristem but also of the subjacent transitional tissue regions in the axis, protoderm, ground meristem and provascular tissue (which we shall consider later in this chapter), and the leaf primordia near the stem tip. The term apical meristem, as used in this book, refers to that part of the shoot apex immediately distal to the first leaf primordium (Fig. 5.1), often referred to as the **apical dome**. The concept **promeristem** is essentially equivalent to apical meristem, as applied to the stems of seed plants. As generally defined it is considered to be the "self-perpetuating group of cells which does not undergo tissue differentiation but continually produces the cells which do differentiate" (Sussex and Steeves, 1967; see also Steeves and Sussex, 1989). Whereas apical meristem and promeristem are approximately equivalent terms when applied to seed plants, they differ strikingly when applied to some ferns. Ma and Steeves (1994, 1995a, 1995b) provide evidence that in *Matteuccia struthiopteris* and *Osmunda cinnammomea* the first stages of differentiation of provascular tissue and parenchyma of the pith begin in tissues subjacent to the outer cell layer and distal to the first leaf primordia. Consequently, they conclude that in these taxa the apical cell and the single peripheral layer of cells produced by its cell divisional activity comprise the promeristem. Apical meristems vary in morphology and activity in the several major taxa of vascular plants, but all are characterized by some type of histological zonation. Whereas all cells in the apical meristem are meristematic, not all are **apical initials**, i.e., the cells that are the source, ultimately, of all other cells in the shoot system. Among extant vascular plants several of the more primitive taxa, Psilotaceae, *Equisetum*, some species of *Selaginella*, and most ferns are characterized by a single apical cell considered to be an **apical initial** (Fig. 5.2a, b) which resides at the tip of the apical meristem and from which all other cells therein are ultimately derived (see Bierhorst, 1971, 1977). In general, the apical cell of these plants is a four-sided tetrahedral cell with three triangular cutting faces (wall facets parallel to which new cells are produced by mitosis, cytokinesis, and cell wall deposition) and an outer, curved, triangular face (Fig. 5.3a). During some phases of development, however, the apical cell may be more irregular with more than three cutting faces. Typically, new cells are formed in a helical succession in three ranks (Fig. 5.3a). In a few ferns, the apical cell produces new cells from only two faces and, consequently, the histology of the stem has a bilateral symmetry as, for example, in *Pteridium aquilinum* (Bierhorst, 1971). The apical meristem of extant lycophytes (except *Selaginella*) differs from that of ferns in the presence of a small cluster of apical initials at the tip of the apical dome.

In contrast to the concept of the promeristem proposed by Ma and Steeves (1994, 1995a, 1995b), McAlpin and White (1974) suggest that the promeristem of many ferns consists of both the peripheral layer of the apical meristem between the most distal leaf primordia, and a subjacent group of cells. They also recognize a rib meristem from which the pith is derived, and an internal peripheral zone (Fig. 5.3b).

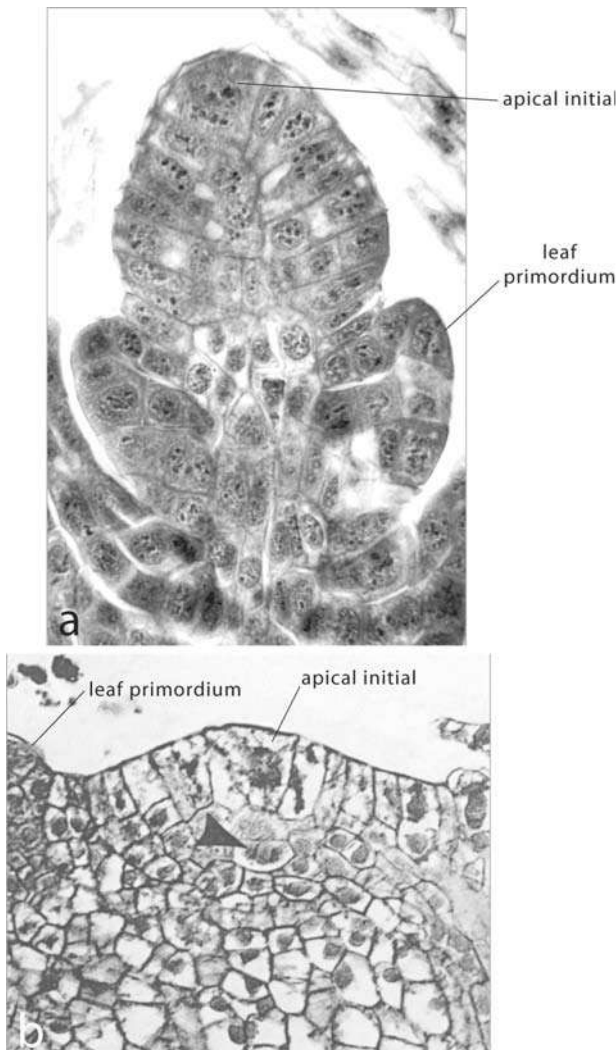


Figure 5.2 (a) Median longitudinal section of the shoot apex of *Equisetum*, showing the apical meristem containing a solitary apical initial. Magnification × 380. (b) Median longitudinal section of a shoot apex of *Quercifilix zeilanica*, a leptosporangiate fern. Note the broad apical meristem and the centrally located apical cell. Magnification × 158. (b) From McAlpin and White (1974). Used by permission of the Botanical Society of America.

They note that this pattern of zonation is similar to that of seed plants. McAlpin and White (1974) precipitated a controversy by suggesting that ferns with apical meristems characterized by patterns of histological zonation do not contain a solitary apical initial in the classical sense, that is, one from which all other cells in the shoot are derived. Bierhorst (1977) strongly disagreed with this viewpoint and demonstrated the presence in 50 fern species of a solitary apical initial which he believed to be the source of all other cells in the shoot. For a detailed discussion of these two interpretations of the fern apical meristem, see Lyndon (1998: ch. 1).

The apical meristem of many gymnosperms, including cycads and *Ginkgo*, is characterized by distinctive cytohistological zonation (Fig. 5.4a, b). Typically, the apical meristem is bounded by a single surface layer below which is a small cluster of relatively large, vacuolate cells comprising the **central mother cell zone**. This zone is flanked

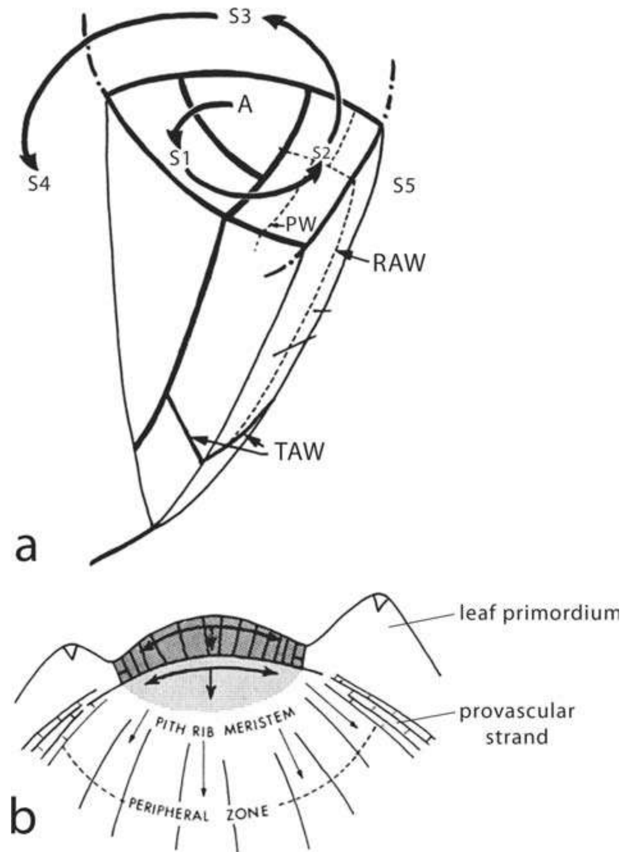


Figure 5.3 (a) Drawing of the apical cell (A) of a leptosporangiate fern and its derivatives. Note the helical succession of new cells (S1–S5). TAW, transverse anticlinal wall; RAW, radial anticlinal wall; PW, periclinal wall. (b) Diagram illustrating zonation in the apical meristem of a fern during active growth. Densely shaded surface cells and the less densely shaded subsurface zone comprise the promeristem according to McAlpin and White (1974). (a) Modified from Hébant-Mauri (1993). Used by permission of the National Research Council of Canada. (b) From McAlpin and White (1974). Used by permission of the Botanical Society of America.

by a **peripheral zone** comprised of small, conspicuously nucleate and densely staining cells characterized by active cell division. The peripheral zone encloses a central region called the **transition zone** below which is a **rib meristem** consisting predominantly of longitudinal files of cells.

Many (possibly all) angiosperms are also characterized by apical meristems with patterns of cytohistological zonation similar to those of gymnosperms. Unlike most gymnosperms, however, superimposed on this pattern of zonation is a pattern of tunica–corpus organization, based on the orientation of new cell walls as seen in median longitudinal sections (Fig. 5.5). The **tunica** is the outermost one to several (commonly two) layers of cells of the apical meristem distal to the first visible leaf primordium. During cell division in the tunica, new cell walls occur in **anticlinal planes**, i.e., in planes perpendicular to the surface of the apical dome. The **corpus**, the tissue internal to the tunica, comprises the major part of the apical meristem. During mitosis within the corpus new walls may be **periclinal**, i.e., in planes parallel to the surface, or in numerous other planes. In plants with two tunica layers, the designations, L1, L2, and L3, often used by researchers, correspond to the outer and inner tunica layers and the corpus (Lyndon, 1998). Cells of the tunica and corpus are connected by plasmodesmata which,

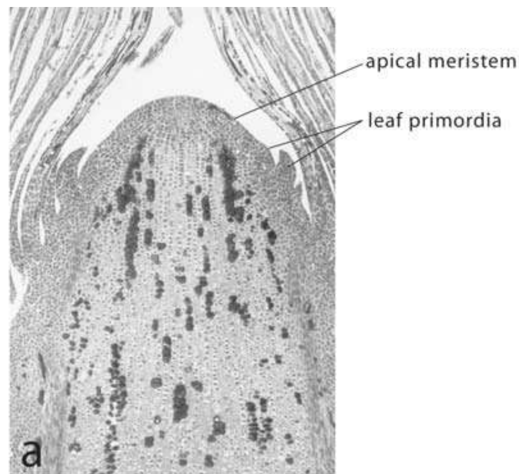


Figure 5.4 (a) Median longitudinal section of the shoot apex of *Pinus strobus* (white pine). Magnification × 38. (b) The apical meristem of *Pinus strobus* showing histological zonation. Magnification × 107.

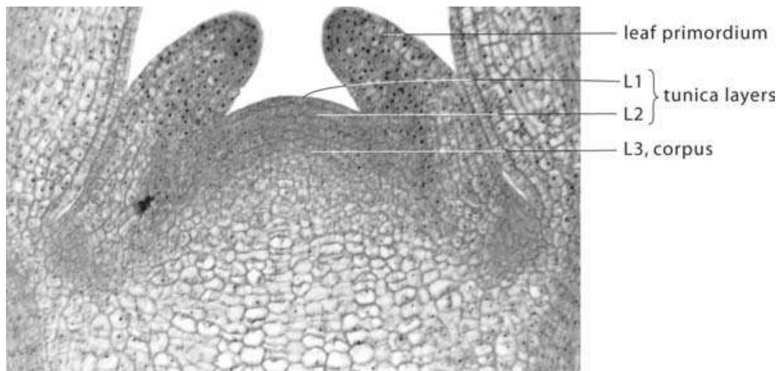


Figure 5.5 Near-median longitudinal section of the apical meristem of *Syringa vulgaris*. Magnification × 170.

as in other parts of the plant, are thought to control the formation of **symplastic domains** in which gene expression leads to the formation of protoderm, and ultimately to organs such as leaves and flower parts, peripherally, and in the more central part of the stem to production of cells that comprise the transitional tissue regions, ground meristem and provascular tissue (e.g., Clark, 2001; Ehlers and Kollmann,

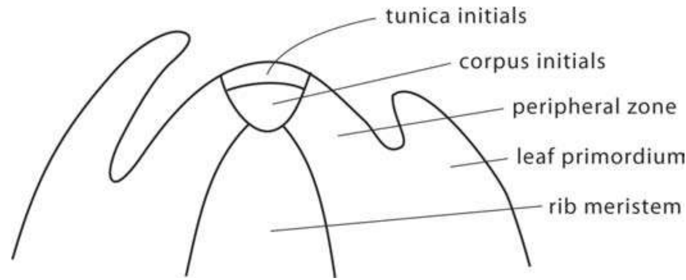


Figure 5.6 Diagram of a median longitudinal section of an apical meristem characteristic of many angiosperms, showing cytohistological zonation. Note the similarity of this apical meristem to that of *Pinus strobus* (Fig. 5.4b). Based on data and illustrations in Gifford (1950).

2001). Such developmental domains are established by the differentiation of plasmodesmata with different size exclusion limits whereby molecules of certain sizes are allowed to be transported through them while passage of others is restricted. Domains may also be defined by an absence or very low frequency of plasmodesmata between certain tissue interfaces, or by the plugging of plasmodesmata by callose. Both the degree and mechanism of restriction of molecular transport through plasmodesmata can be modified by environmental conditions such as photoperiod (see Chapter 4 for more detail).

As indicated above, shoot apices of angiosperms exhibit, in addition to tunica–corpus zonation, patterns of **cytohistological zonation** similar to those of gymnosperms (e.g., Plantefol, 1947; Gifford, 1950; Popham and Chan, 1950; Buvat, 1952, 1955). Gifford (1950) recognized four zones in the shoot apices of a group of woody ranalean taxa (Fig. 5.6): an apical group of "tunica initials," a subtending zone of cells (which he called corpus initials) similar to the zone of central mother cells of gymnosperms, a peripheral zone, and a rib meristem. Similar patterns have been observed in many other angiosperms. In seed plants the apical initials and the central mother cells divide more slowly than those in the peripheral zone. Because of the conical nature of the apical meristem in most plants, and the presence of these cells in its summit, they can divide at a slow rate and still add cells to the more proximal zones in sufficient quantity to maintain the form and viability of the meristem. Even in plants with relatively flat or even concave apical meristems, the variation in frequency of cell division in the various zones is similar.

During periods of cytokinesis in angiosperms, the apical (tunica) initials contribute cells to the central mother cell zone and to the peripheral meristem. The central mother cell zone is the direct source of the cells of the rib meristem and the pith. The proximal region of the peripheral meristem is highly meristematic and is the site of formation of new leaf primordia and associated immature internodal tissue (Fig. 5.6). This region, often referred to as the organogenic region of the apical meristem, is approximately equivalent to that called the ring initial ("anneau initiale") in the French literature. (For detailed information on the anneau initiale and other concepts and terminology applied by French anatomists to apical meristems, see Plantefol (1947) and Buvat (1952, 1955).)

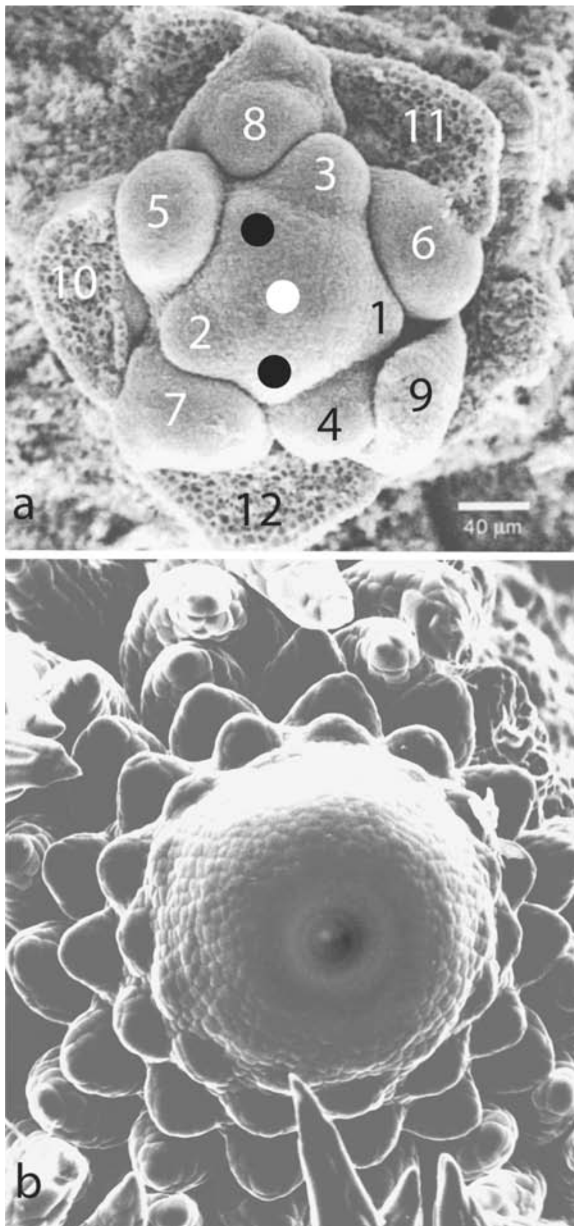


Figure 5.7 (a) Apical meristem and leaf primordia of *Arabidopsis thaliana* as seen from above. The primordia, arranged in a helix, are numbered from youngest to oldest. Positions of the next two primordia are indicated by dark dots, the apical meristem by a light dot. (b) The apical meristem of *Lycopodium* sp. and the numerous, helically arranged leaf primordia and young leaves it has produced. Magnification × 303. (a) From Clark (2001). Used by permission of Elsevier Science Ltd. (b) Photograph by P. Dayanandan.

As viewed in a median longitudinal section, only a few leaf primordia are visible in the shoot apex (Figs 5.1, 5.5a), thus imparting a misleading impression of its three-dimensional form and the actual number of leaf primordia. In fact, there are many leaf primordia, often tightly packed, in contact with one another (Fig. 5.7a, b), that vary in size in relation to their distance from the apical meristem, those farther away being larger (and more mature) than the younger primordia more recently produced. They are arranged in decussate, distichous, verticillate (whorled), or helical patterns, as will be the mature leaves

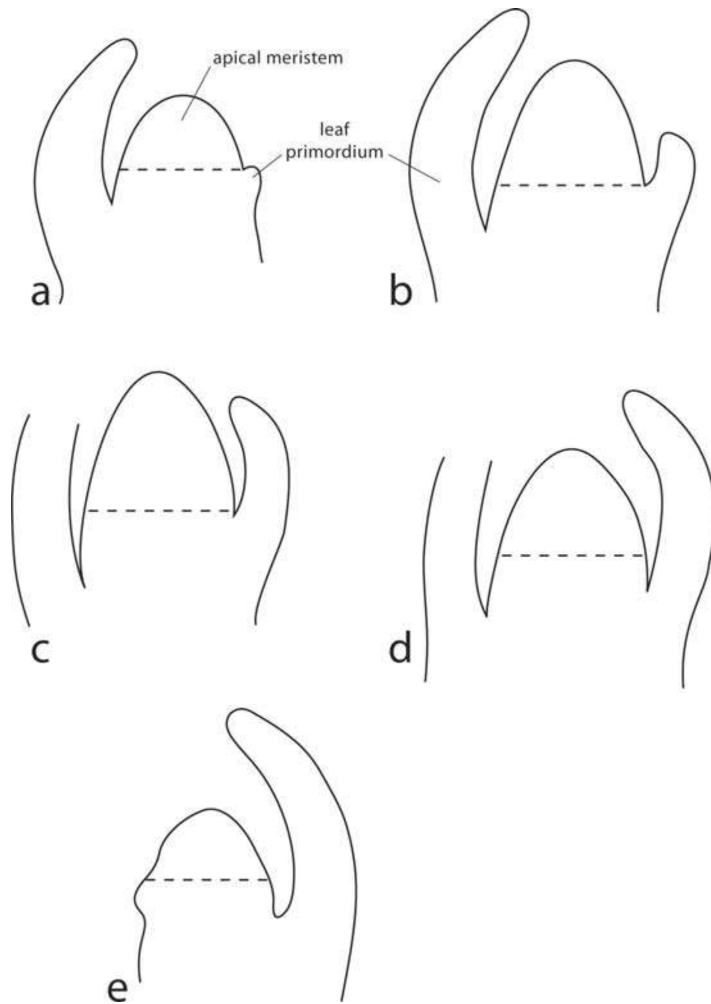


Figure 5.8 Diagrams illustrating the variation in size of the apical meristem during a plastochron. Note the increase in length of the apical meristem following production of a leaf primordium (a–c) and its decrease in length prior to production of the next primordium (d–e). Based on data and diagrams in Abbe *et al.* (1951).

that develop from them. Over time, as the shoot elongates, the leaf primordia and the developing young leaves will become progressively more widely separated, and nodes, marked by the position of leaves, and the internodes will become more well defined (Fig. 5.1).

Formation of leaf primordia

During the formation of leaf primordia, the apical meristem fluctuates in size and form. Just prior to the formation of a leaf primordium (or a pair of primordia if leaf arrangement is decussate), the apical meristem reaches its maximum size and longitudinal extent. As a leaf primordium develops, the meristem diminishes in size. Prior to the formation of the next leaf primordium it increases again to its maximum size (Fig. 5.8). The time between the formation of successive leaf primordia, and during which these changes in size and form occur, is

called a **plastochron**. The degree to which size and form of the apical meristem vary during a plastochron differs greatly in different taxa, and is related both to the size of the primordia and to phyllotaxy.

In recent years, progress has been made in understanding factors that influence or control the formation of leaf primordia and their arrangement in phyllotactic patterns (for a discussion of phyllotaxy, see Chapter 7). The hormones auxin and gibberellin have long been thought to influence the initiation of leaf primordia, and more recently, expansin, a protein that seems to enhance the extensibility of the cell wall (Fleming *et al*., 1999), has also been implicated in the initiation of leaf primordia (see also Berleth and Sachs, 2001). Among other related factors are the plane of division of cells in the tunica and subjacent layers at the site of primordium initiation, the frequency of cell division, the rate and direction of cell growth, and the arrangement of cellulose microfibrils in the walls of these cells. A primordium becomes apparent as the result of periclinal divisions in one or more cell layers in a local site on the flank of the apical meristem. The resulting cells grow at a rate faster than those that contribute to the re-establishment of the size and form of the apical meristem, and in a direction controlled, at least in part, by the orientation of microfibrils in the anticlinal primary walls (see Green, 1984). As we have seen in Chapter 4 (see also the later sections on cell growth and development in this chapter) a transverse orientation of microfibrils is thought to facilitate cell elongation whereas a longitudinal orientation restricts it.

Whereas all of these aspects of development probably contribute to the formation of leaf primordia, Lyndon (1994) suggests that extensibility through wall loosening in cells that form the surface of the apical meristem is especially important. As noted above, auxin is known to stimulate primordium initiation. This conclusion is based in part on evidence that auxin stimulates the plastic (i.e., irreversible) extensibility of cell walls of epidermal and subepidermal tissues (Masuda, 1990; Kutschera, 1992; Edelmann and Kutschera, 1993). Thus, the enhancement of the extensibility of cell walls by auxin, in concert with expansin, and the alteration of the pattern of cellulose microfibrils, possibly caused by stresses in the apical meristem resulting from the growth of adjacent primordia (Jesuthasan and Green, 1989) characterize local sites on the flanks of the meristem. It is at these sites that new leaf primordia are initiated (Fig. 5.7a). These sites, directly related to the position of previously formed primordia, are apparently equivalent to the "first available space" (on the apical dome) proposed by Snow and Snow (1947) as sites of initiation of new leaf primordia. It is significant that, in some species, these sites are just distal to acropetally differentiating provascular strands (see Larson, 1983; Nelson and Dengler, 1997) and it is possible, therefore, that the auxin signal which controls the initiation of leaf primordia is transported to the sites from a distant source through the provascular tissue (Dengler and Kang, 2001; see also Berleth and Sachs, 2001).

Research by Benková *et al*. (2003) indicates that, in *Arabidopsis*, auxin enters the leaf primordia through the protoderm and accumulates at

the primordia tips. From there it moves into the interior of the primordia where it controls the basipetal development of the leaves, and from which it is transported downward through provascular strands (Aloni *et al*., 2003). Leaf traces differentiate along these pathways of auxin transport and ultimately connect to the acropetally differentiating vascular strands in the stem below, supporting the viewpoint of earlier workers (e.g., Sachs 1969, 1984; also Chapter 6).

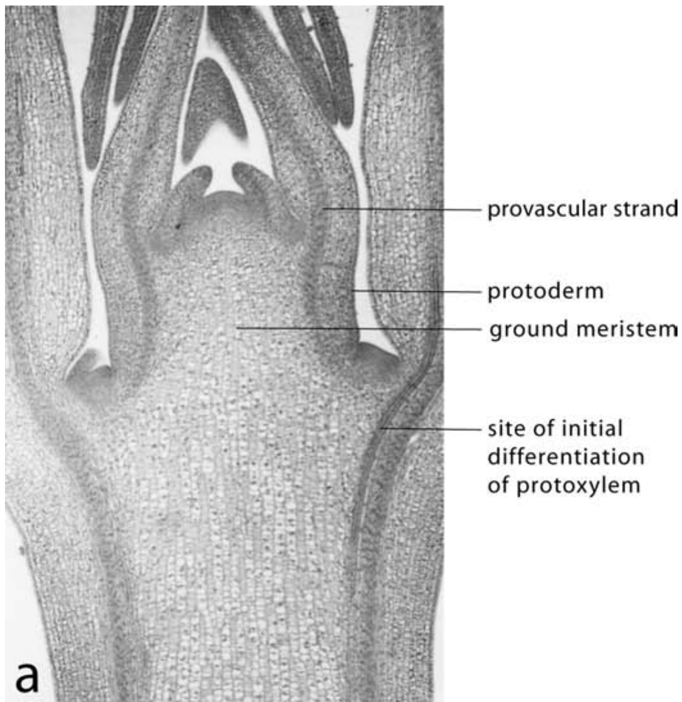
Transitional tissue regions

As new cells are being added to the more proximal regions of the apical meristem by the apical initials and central mother cells, the derivatives of these new cells are being added to the subjacent axial region of the shoot apex. The resulting transitional **tissue regions** (also called tissue domains), protoderm, ground meristem, and provascular tissue, are located between the apical meristem and mature tissues (Fig. 5.9). Cell division continues in upper parts of these regions and growth and differentiation take place more proximally. As a result of both cell division and growth, the shoot apex undergoes extensive elongation (Fig. 5.1). Steeves and Sussex (1989) characterize these activities as comprising the "expansion phase" of shoot development. Protoderm, ground meristem, and provascular tissue will ultimately develop into epidermis, cortex and pith, and the primary vascular system of the mature stem. Because, as development in these transitional tissue regions is proceeding, new cells are being added to them by the apical meristem, they are constant features of the shoot apex. Unlike the apical meristem upon which their presence is dependent, however, they are not self-perpetuating.

Cells closest to the apical meristem in these regions have been produced most recently and, are, of course, younger and thus, less mature, than those farther from it. As time passes, these cells and their derivatives elongate and differentiate (see a later section in this chapter on "Cell growth and development"). Since the cells more proximal to these have passed through the same stages of growth and differention even earlier, they are more mature. It is apparent, therefore, that the direction of differentiation in this region is **acropetal**, that is, in the direction of the shoot tip. Although this is generally true, as we shall see shortly, some tissues also differentiate **basipetally**, i.e., in the direction of the base of the plant.

In most vascular plants the **protoderm** is a single layer of cells which bounds the axis and its immature lateral appendages (primarily leaf primordia) in the region between the apical meristem and the proximal, mature part of the vegetative shoot (Fig. 5.9a, b). Over time, the various components of the epidermis (e.g., tabular cells, trichomes, guard cells, etc.) (see Chapter 8) develop concurrently with the addition to the shoot, distally, of new protoderm by the apical meristem. Differentiation within the protoderm is acropetal.

The protoderm encloses the **ground meristem** in which the provascular strands are embedded (Fig. 5.9). In general, cells of the ground



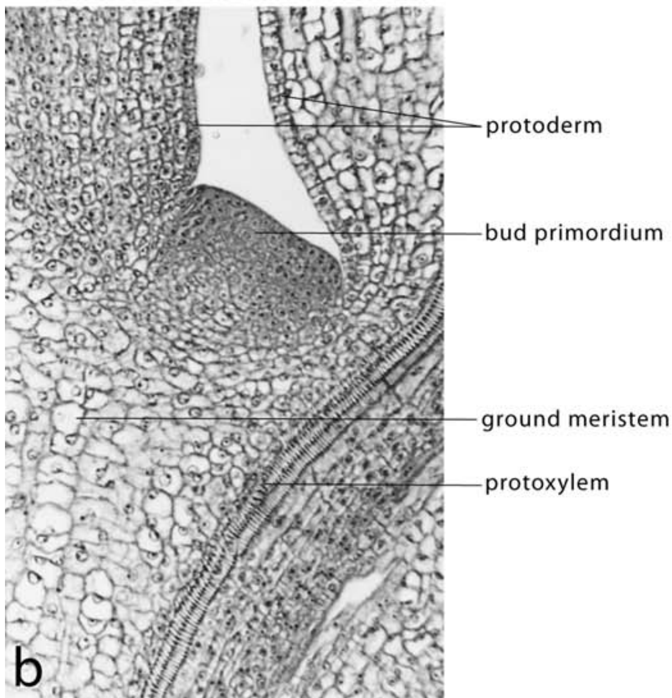
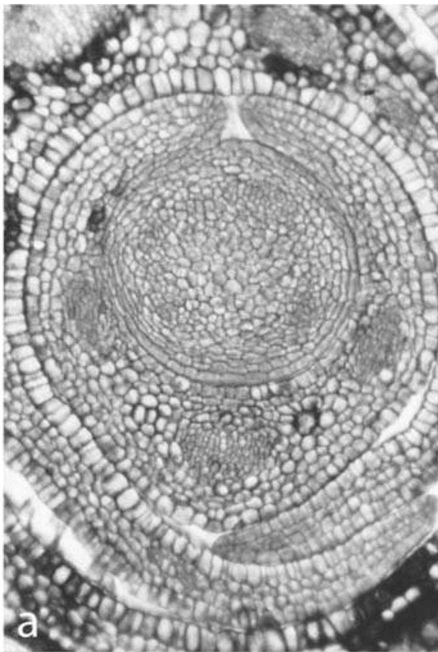


Figure 5.9 (a) Longitudinal section of the shoot apex of *Syringa vulgaris* illustrating provascular strands, ground meristem, and protoderm. The first evidence of differentiation of primary xylem is seen in the developing vascular supply to the third oldest leaf primordium. Magnification × 53. (b) Enlargement of part of (a) showing detail of the developing vascular bundle which enters a leaf primordium. Note also the bud primordium in the axil of the leaf primordium. Magnification × 210.

meristem increase in length. However, in many plants, cells in the region that will differentiate into the tissue of the pith, although elongating longitudinally to the same extent as those in the immature cortex, also increase in girth to the same or even greater extent. In fact most cells in the differentiating ground meristem, including those in the immature cortex, increase in girth to some extent with a consequent increase in diameter of the axis. It is in the ground meristem that the several tissue types such as parenchyma, collenchyma, and sclerenchyma differentiate, as well as a variety of idioblasts, cells which accumulate waste metabolites (e.g., calcium oxylate crystals), pigments, aromatic oils, poisons such as alkaloids, etc. Specialized latex-producing structures called laticifers, which originate, in different taxa, in the embryo or the apical meristem, may also undergo a major part of their development in the ground meristem and its derivatives although they also occur in the secondary phloem of some taxa. (For more detail, see Chapter 12.)

The **provascular strands** (provascular column or provascular cylinder in pteridophytes), often called procambial strands or procambium, surround the immature pith (Figs 5.9, 5.10). Provascular strands in many taxa differentiate in a cylindrical zone of vacuolate meristematic tissue immediately below the apical meristem called **residual meristem** (Fig. 5.10). It is not clear whether this zone is derived directly from the apical meristem or from the tissues associated with developing leaf primordia. The first strands of provascular tissue to differentiate within the residual meristem, however, become leaf traces. As development proceeds, additional provascular strands, some of which will become leaf traces, others of which will become axial bundles, differentiate within the residual meristem. Upon formation of the total complement of provascular bundles at a particular level in the developing shoot apex, any remaining residual meristem differentiates into **interfascicular parenchyma** (parenchyma between the bundles). The cells of provascular strands are characterized by very narrow diameters (as seen in transverse section). Although their longitudinal dimensions may be no greater than those of adjacent cells of ground meristem, their length/width ratio gives the impression of considerable length. Furthermore, because the cell nuclei are large and frequently visible, these cells are easily recognized in stained sections. Thus, even in regions in which no differentiation of primary vascular tissues is apparent, provascular tissue can be distinguished from the ground tissue on the basis of cytohistological characteristics.

The basic pattern of the primary vascular system in the stem can be recognized in the arrangement of the provascular tissue. Thus, in a mature stem with a protostele (a central, solid column of vascular tissue) the provascular tissue comprises a central column. Likewise in plants with siphonosteles (a cylindrical or tubular arrangement of primary vascular tissue) and those with eusteles (vascular tissue arranged in well-defined bundles which may or may not be interconnected) the provascular tissue from which these systems differentiate is similarly arranged (for detail, see Chapter 7).



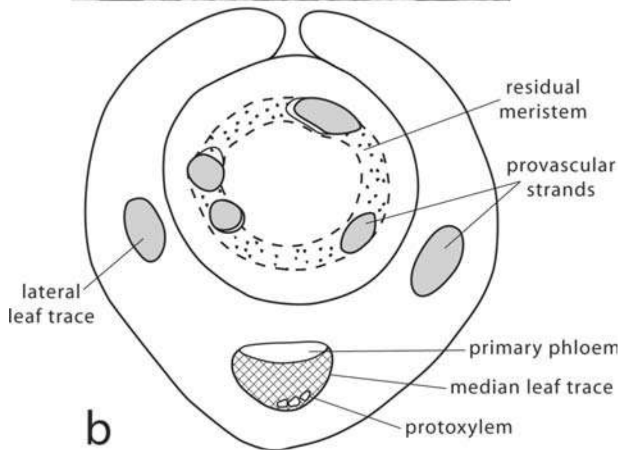
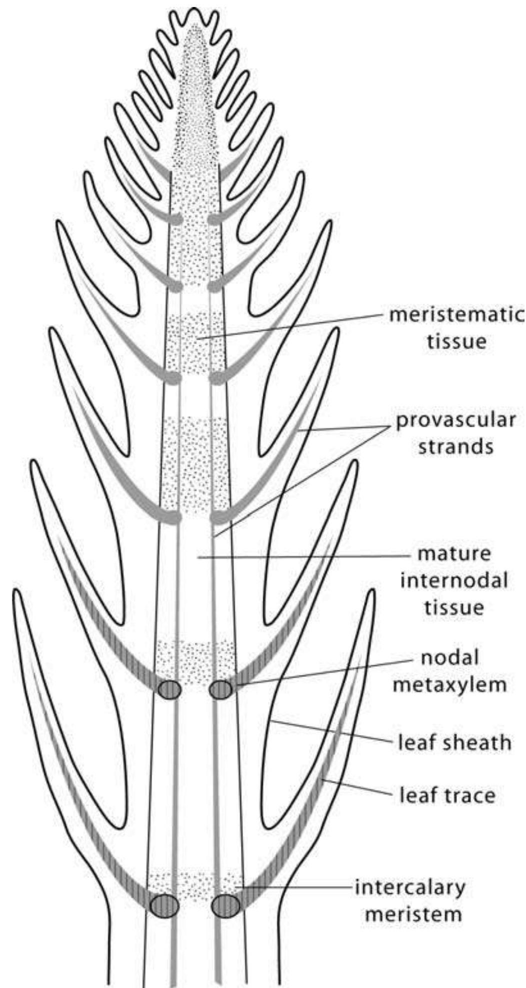


Figure 5.10 (a) A transverse section through the shoot apex of *Cassia didymobotrya* (Leguminosae). This immature apical region of the stem, enclosed by immature leaves, contains several provascular strands within residual meristem. Note the developing median and lateral leaf traces. The lateral traces consist almost entirely of provascular tissue whereas primary phloem and several primary xylem tracheary elements have differentiated in the median trace. Magnification $\times$ 230. (b) A drawing of part of (a) more clearly showing residual meristem. From Devadas and Beck (1971).

Intercalary meristems

Intercalary meristems are regions of meristematic tissue between regions of more mature tissues. They have been extensively studied in the stems of grasses and are also well known in the stem of *Equisetum* (Fig. 5.11). In both grasses and *Equisetum*, the first internodes in the developing shoot are very short and entirely meristematic. As development proceeds, tissue differentiates basipetally, resulting in more mature tissues in the distal regions with meristematic tissue becoming progressively restricted to the basal regions of the internodes. With production of new cells by the intercalary meristems and their subsequent differentiation and growth, the internodes elongate and the

Figure 5.11 Diagrammatic representation of the terminal region of an *Equisetum* shoot as seen in median longitudinal section showing the basipetal differentiation of internodal tissue, the intercalary meristems, and the nodal metaxylem.



leaves attached at the nodes become more widely separated longitudinally. The vascular tissue that has differentiated within the elongating internodes is stretched, and in some species disrupted. New xylem and phloem elements differentiate from adjacent parenchyma cells, replacing those that have been destroyed, thus maintaining vascular tissue continuity (Evans, 1965). The basal meristems of many developing leaves, especially those with prominent petioles or that are needle-like, such as those of pines, are also considered by many workers to be intercalary meristems.

The primary peripheral thickening meristem of monocotyledons

Whereas increase in diameter of axes in the primary shoot system results partly from the diametric growth of individual cells, the major cause of diametric growth is the production of additional cells in the

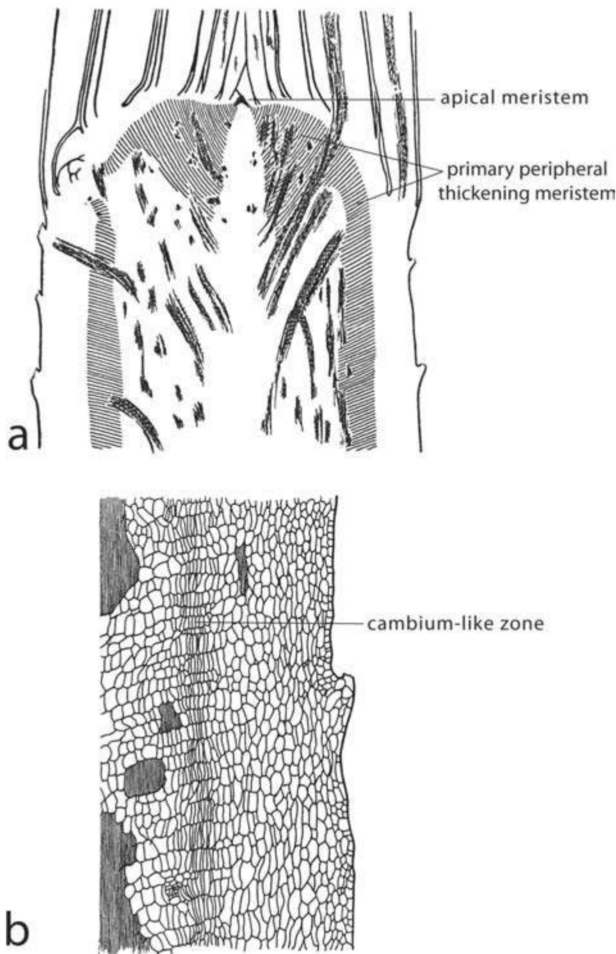


Figure 5.12 (a) Diagram of a median longitudinal section of the rhizome apex of *Ophiopogon japonicus* (Liliaceae) illustrating the primary peripheral thickening meristem (lined). (b) The cambium-like meristematic zone which is an extension down the axis of the peripheral region of the primary thickening meristem. This cambium-like meristem produces secondary tissues in some large monocotyledons, e.g., *Dracaena* (for more detail and illustrations, see Chapter 14). From Eckardt (1941).

ground tissue. In particular, in large plants consisting solely of primary growth, such as palms and some other monocotyledons that have very broad shoot apices, new cells are added below the apical meristem by a **primary peripheral thickening meristem** (Fig. 5.12a) (Eckardt, 1941). This meristem is a broadly obconical region in the shoulders of the shoot apex. Anticlinal files of cells are produced toward the center as well as toward the surface of the axis, resulting in a peripherally located region of curved files of cells surrounding the axis just proximal to the apical meristem, and more proximally, narrowing into a cylinder of radial files of cells in the immature internodes (Fig. 5.12a, b). In monocotyledons that produce secondary tissues, this cylinder of meristematic cells becomes the initiating region of this tissue (see Chapter 14).

Cell growth and development

Following cytokinesis, new cells begin a period of growth and differentiation. In other words, they increase in size and develop distinctive characteristics related to their function. As the protoplast

enlarges, small vacuoles fuse, often forming a single large centrally located vacuole, and cell organelles such as ER, Golgi bodies, plastids, microtubules, etc. increase in number. ER and Golgi vesicles fuse with the plasma membrane allowing it to increase in area, thus keeping pace with the enlarging cell protoplast. As they fuse with the plasma membrane they also expel precursor compounds of wall matrix and structural (cellulosic) components as well as enzymes into the area of wall synthesis (for details of wall synthesis see Chapter 4).

Most new cells grow predominantly in length, and cell wall growth occurs concurrently with growth of the protoplast. It is widely accepted that cell growth is directly related to turgor pressure. It has also been suggested that wall growth might be the motive force in cell growth and that the protoplast simply keeps pace as the cell becomes larger. However, the mechanism(s) whereby the protoplast and the cell wall grow in an integrated manner is still not clear, although there has been extensive research in this area during the past several decades.

The effect of hormones on cell growth and development

Two plant hormones, indole-3-acetic acid (IAA; commonly called auxin) and gibberellic acid (GA; commonly called gibberellin) have long been implicated in plant growth processes. It is widely accepted that auxin is important in the coordination of differentiation of diverse cell types, tissues, and organs (Berleth and Sachs, 2001). In fact, these authors believe that auxins may function as intercellular messengers, and that "auxin-mediated long-distance signaling could simultaneously integrate morphogenesis throughout the plant." Auxin is synthesized in apical meristems, leaf primordia, and young leaves of the shoot from which it is transported to other regions of the plant body. **Polar auxin transport**, that is, the basipetal flow of auxin toward the base of the plant, occurs through meristematic regions such as provascular strands and the vascular cambial zone. Non-directional auxin transport takes place through the phloem and can be either acropetal or basipetal. Whereas auxin can move into cells by diffusion, it can move out of cells only by auxin transport proteins called **PIN efflux transporters** (see Muday and DeLong, 2001). PIN genes (named for the pin-like phenotype of mutant forms) encode the auxin efflux transporters. Within cells, auxin efflux transporters may be transferred in Golgi vesicles along actin microfilament tracks to the plasma membrane where the actin may also function in fixing the auxin efflux transporters to specific locations on the surface of the plasma membrane (see Steinmann *et al*., 1999; Geldner *et al*., 2001). A model for auxin-mediated development has been proposed by Benková *et al*. (2003). They conclude that during development of an organ such as a leaf primordium or lateral root, auxin accumulates at the site of organ initiation. Cytokinesis begins and the direction of PIN-mediated auxin transport determines

the axis of growth and establishes an efflux-dependent auxin gradient which becomes maximal at its tip. Although their research was based on *Arabidopsis*, Benková *et al*. (2003) suggest that "local, efflux-dependent auxin gradients as a common module for organ development may also apply to other [higher] plants." More recent studies confirm and expand this viewpoint, (e.g., Scarpella *et al*., 2006; Scheres and Xu, 2006).

Following initiation of organs and tissues, cytokinesis, cell growth and differentiation occur. Recent studies indicate that auxin, and especially the protein expansin, play a role in wall loosening that facilitates wall surface expansion during cell growth (e.g., Cosgrove, 1993, 1999, 2000; Sauter *et al*., 1993; Thimann and Biradivolu, 1994; Cho and Cosgrove, 2000). Wall loosening implies changes that allow slippage of cellulose molecules or even cellulose microfibrils with a resultant decrease in wall stress. This would result, initially, in reduced turgor pressure and a consequent uptake of water which would extend the cell (Cosgrove, 1993; see also Ray *et al*., 1972; Hohl and Schopfer, 1992; and Chapter 4 for more detail).

Genetic control of cell growth and development

It has long been understood that cell growth and differentiation are under genetic control. Only recently, however, have the techniques been perfected that allow biologists to recognize genes that control various aspects of development (e.g., Pyke, 1994; Jones *et al*., 1997). It has rarely been possible to directly identify such genes, and the most useful information has come from studies of mutant forms. By comparing wild-type characteristics with morphologic mutants in several plants, e.g., *Arabidopsis*, *Antirrhinum*, *Zea mays*, and others, researchers have been able to identify many genes and to determine their roles in the differentiation and morphogenesis of plant structure. As noted above, a group of genes called PIN genes are responsible for the transport of auxin from cell to cell and, thereby, can control the development of auxin concentration gradients which regulate cell differentiation. Auxin signaling and transport is of crucial importance in many aspects of plant development. (See the preceding section in this chapter, and Chapters 16 and 17 for more detail about the importance of PIN genes in development.)

The activity of genes is referred to as **gene expression**. A gene is expressed through the translation of encoded information into a protein which, either singly or through interaction with the products of other genes, modulates the initiation and differentiation of cells, tissues, and organs. Some genes are expressed constantly in all living cells. Others are expressed only in some cells and at certain stages in development. Hormones such as auxin can act as signals that activate or inhibit gene action. Through these regulatory mechanisms, cells are able to control the timing and location of gene expression.

A good example of comprehensive research on the genetic control of development has involved studies of the initiation and growth of root

hairs in *Arabidopsis thaliana* (see Chapter 16 on the root, and Schiefelbein (2000) and references therein). Schiefelbein discusses the roles of several cell-specification genes, the expression of which is required for the differentiation of epidermal cell types. He notes that several of these genes also specify trichomes in the shoot epidermis, and that one is required for cell expansion of most cells throughout the plant (also see Wang *et al*., 1997). Several workers have emphasized that the control by individual genes is related to the environment in which they function, and gene expression may vary depending on the location or the developmental context of differentiating cells and tissues (Verbeke, 1992; Wojtaszek, 2000; Kaplan, 2001). Wojtaszek has emphasized that the development of cells, even cell walls, may be under local, structural, physiological, or organismal genetic controls. For example, he observes that transfer cells routinely differentiate adjacent to tracheary elements which, at maturity, are non-living. He notes that, although they share a common middle lamella, the walls on either side are very different in both construction and physicochemical properties. The secondary walls of the tracheary elements are highly lignified whereas the adjacent primary walls of the transfer cells are characterized by complex ingrowths. This illustrates the requirement for positional genetic control of cell differentiation as well as wall synthesis in cells of dramatically different function. Consider further the genetic control of variation in differentiation of the outer and inner walls of an epidermal cell. Wojtaszek (2000) notes that as an epidermal cell differentiates, the anticlinal walls reach their maximum extent quickly whereas the outer periclinal wall must continue to grow over an extended period during elongation of the cell. Consequently the periclinal wall must be provided with building elements (precursor compounds) for wall synthesis over a much longer period than the anticlinal walls. Furthermore, because it comprises a component of the external surface layer of the plant it differs from the anticlinal walls in chemical composition, containing within the wall and on its surface water-impermeable substances such as cutin and waxes. There must, therefore, be gene-controlled differential biosynthesis of the several different chemical components required during differentiation of epidermal cells, and the secretory mechanisms to deliver these components to the precise locations of the developing wall where they are required. All of these functions and the development of diverse structures and substructures are examples of the control by specific genes or groups of genes.

Genetic control of leaf development is, at present, an area of intense interest and active research. For detailed information about this subject, please see Sinha (1999), Tsukaya (2006), Wang and Li (2008), and Chapter 17.

Role of the cytoskeleton in cell growth and development

Following cell division in meristematic tissues of the primary body, new cells generally divide further, followed ultimately by growth. In

primary tissues the predominant cell growth is axial, that is, elongate, usually parallel to the long axis of the organ in which growth is occurring. In such **anisotropic growth** the lateral walls of the cells grow, and thus, elongate more extensively than the end walls. One would, therefore, expect end walls and lateral walls to differ, not only in growth potential, but also in polysaccharide structure and chemical composition, especially the presence of growth-promoting compounds such as **expansin**, a wall-loosening protein. Unlike end walls which would contain minimal amounts of wall-loosening substance, or none at all, the lateral walls would require larger amounts of such compounds, and also a cellulose structure that would allow for wall extensibility. These requirements are facilitated by the cytoskeleton.

In cells that are growing predominantly in length, cellulose microfibrils in the primary cell walls are oriented more or less transversely, and microtubules just beneath the plasma membrane are parallel to the microfibrils (Baskin, 2001; Baskin *et al*., 2004). It has been widely accepted that microtubules are somehow responsible for this orientation of cellulose microfibrils. Recently, Paredez *et al*. (2006) have visually observed the process of cellulose deposition in living cells of *Arabidopsis* by labeling cellulose synthase complexes (rosettes) and microtubules with a yellow, fluorescent protein. Trajectories along which cellulose synthase complexes moved in the plasma membrane were observed to precisely parallel the microtubules in contact with the membrane. This intimate association of cellulose synthase complexes and microtubules was maintained even when the array of microtubules was disorganized by the drug, oryzalin, strongly suggesting, with other evidence, that microtubules play a direct and active role in guiding their movement and orientation and, ultimately, with the deposition of cellulose and the structure of the primary cell wall. For more detailed discussion of the work of Paredez *et al*. (2006), see Chapter 4.

As cells grow there is a (possibly) passive change in cellulose microfibril orientation (but see Wolters-Arts *et al*., 1993), becoming random in cells that, at maturity, are isodiametric, and more or less parallel to the long axis in cells that are longitudinally elongate at maturity (e.g., Seagull, 1986; Sauter *et al*., 1993; Morrison *et al*., 1993; Emons and Kieft, 1994; Paolillo, 1995). Whereas a transverse orientation of microfibrils allows cell wall extension in primary cell walls, and an elongate orientation restricts it, recent studies have shown that even when microtubules are disturbed or removed, the transverse orientation of cellulose microfibrils in the walls of elongating cells is retained in some taxa (see Himmelspach *et al*., 2003; Sugimoto *et al*., 2003). This suggests that the transverse orientation of microtubules, although essential for growth in cell length, might not be the *sole* regulator of microfibril orientation.

Associated with and parallel to microtubules at the plasma membrane is a network of actin microfilaments. Studies by Thimann *et al*. (1992) and Thimann and Biradivolu (1994) demonstrated that growth in elongating cells of *Avena* (oats) was inhibited when the continuity of the actin microfilaments was disrupted by cytochalasin D, a compound

that restricts actin polymerization. It has been shown further that disruption of microfilaments by cytochalasins also inhibits pollen tube growth (Anderhag *et al*., 2000). This inhibition of growth following disruption of actin microfilaments is probably related directly to the fact that in growing pollen tubes, as well as in immature cells of many types, Golgi vesicles containing the precursor compounds of cellulose and other cell wall components are transported along actin microfilaments to sites of wall synthesis (see Miller *et al*., 1996; Vidali *et al*., 2001). It is now well established that the presence of functional actin microfilaments is essential for cell growth, but their role in cell growth is only beginning to be understood.

As in most developmental events, it is highly probable that cell growth results from a series of complex chemical and physical interactions. Among the conspicuous controlling factors in cell growth are turgor pressure, which may be the primary motive force, correlated with wall loosening (mediated by hormones such as auxin, and the protein expansin), transverse orientation of cellulose microfibrils, polymerization of new actin microfilaments, wall synthesis, and wall extension (see also Chapter 4 on the cell wall).

Cell shaping by microtubules

The mechanism whereby cells of complex form acquire their particular shapes, and cell walls their unique patterns of thickening during development has long been a mystery to botanists. Excellent recent evidence suggests that microtubules of the cytoskeleton play an important role in these processes. For example, Jung and Wernicke (1990) observed, in developing walls of mesophyll cells of wheat, a system of interconnected rings of cellulosic wall thickenings that correlated exactly with the position of microtubules in the outer cytoplasm. As cell growth proceeded, cell expansion was apparently restricted in the regions of the rings, but the intervening thinner regions of the walls bulged out resulting in elongate cells with fairly regularly spaced constrictions. The multilobed mesophyll cells of the fern *Adiantum capillis-veneris* seem to be formed in a similar manner. A network of wall thickenings consisting of parallel cellulose microfibrils develops external to a network of bundles of microtubules in the same position beneath the plasma membrane. The areas of thinner wall between the thicker regions of the reticulum expand laterally, presumably under the influence of turgor pressure in the cell, forming arm-like extensions and resulting in the multilobed form of these cells (Panteris *et al*., 1993). There are also striking examples of the correlation between the orientation of microtubules and microfibrils in developing pit borders and annular and helical wall thickenings in tracheary elements. (For a detailed discussion of the development of cell wall ornamentation please see Chapter 11 on the secondary xylem.)

Following the formation of thickened wall regions, the alignment of the microtubules changes and they seem to spread out in a more

uniform pattern. The mechanism of this realignment is unknown although it has been suggested that it might be facilitated by actin microfilaments (see Seagull, 1989).

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Chapter 6

Morphology and development of the primary vascular system of the stem

Perspective

The primary vascular system extends throughout the root system, the stem and its lateral branches, and appendages of the stem such as leaves, flowers, and fruits. The basic pattern of the primary vascular system is established initially by the arrangement of **provascular tissue** in the embryo. As development of the young plant proceeds, the provascular tissue becomes restricted to the shoot apex and to the root tip proximal to the root cap. Differentiation in the provascular tissue leads to the development of mature, functional primary xylem and primary phloem (Fig. 6.1). In primitive plants with central columns of primary vascular tissue (**protosteles**) (many pteridophytes as well as the roots of most plants), phloem surrounds the xylem (Fig. 6.1a). In those with tubular vascular systems (**siphonosteles**) this is usually also true, but in some taxa phloem may bound the xylem on the interior as well as on the exterior (Fig. 6.1b). In seed plants in which the primary vascular systems consist of discrete, or relatively discrete, vascular bundles (**eusteles**) (Fig. 6.1c, d), the spatial relationship of primary xylem and primary phloem varies according to the bundle type, i.e., whether collateral, bicollateral, amphicribral, or amphivasal. In **collateral bundles**, the primary xylem comprises the part of the bundle toward the inside of the stem and the primary phloem comprises the outer part (Figs 6.1, 6.2, 6.4) whereas in **bicollateral bundles** phloem occurs both to the inside and to the outside of the primary xylem. In **amphicribral bundles** primary xylem is enclosed by primary phloem, and in **amphivasal bundles** primary phloem is enclosed by primary xylem (for illustrations, see Chapter 2). In some dicotyledons, in which vascular bundles are very close together, primary phloem may appear to form a nearly continuous cylinder (Fig. 6.2). In many monocotyledons, the primary phloem occurs in compact, well-defined strands enclosed by primary xylem to the inside and sclerenchyma fibers to the outside (Fig. 6.4).

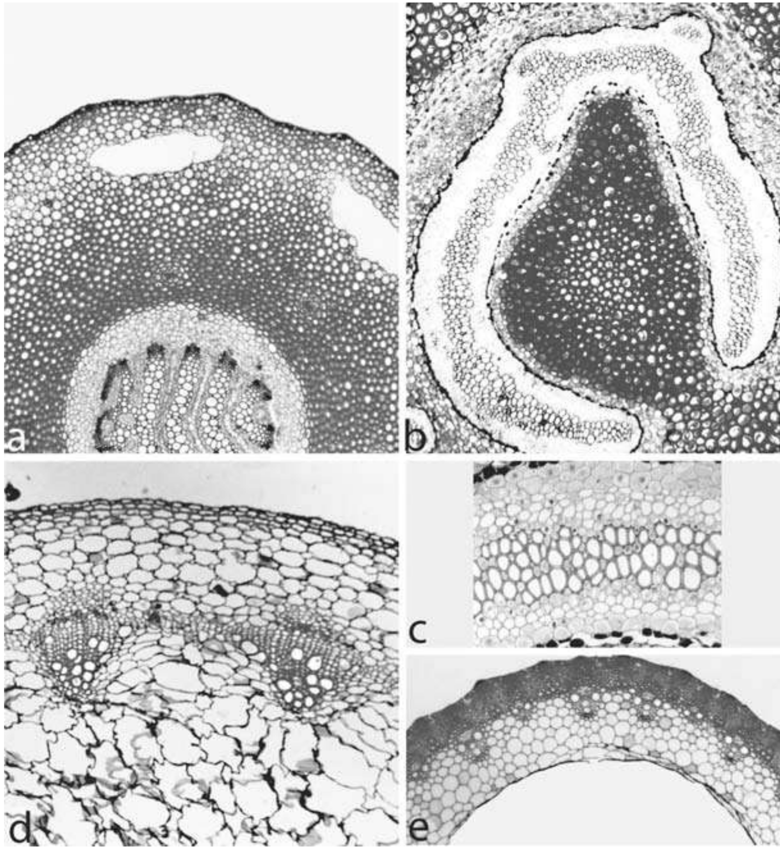


Figure 6.1 Some patterns of primary vascular tissues in pteridophytes (a, b, c) and seed plants (d, e) as seen in transverse sections. (a) A stem of *Lycopodium flabelliforme* with primary xylem and primary phloem arranged in a central column (a protostele). A protostele of this type in which primary phloem is interspersed in a system of interconnected plates of primary xylem is called a plectostele. Magnification × 44. (b) Primary xylem in the form of a cylinder is enclosed by primary phloem in the rhizome of the fern *Adiantum pedatum*. Magnification × 44. (c) Enlargement of a segment of (b) showing detail of the primary xylem and primary phloem (on both sides of the primary xylem). Magnification × 125. (d) Part of a cylinder of collateral vascular bundles composed of primary xylem and primary phloem in a stem of *Helianthus* (sunflower), a dicotyledon. Magnification × 48. (e) Peripheral collateral vascular bundles in the stem of *Triticum*, a monocotyledon. In many monocotyledons, vascular bundles are distributed throughout a parenchymatous ground tissue. Magnification × 43.

Cellular composition and patterns of development of primary xylem

Primary xylem is composed of both protoxylem and metaxylem; and primary phloem, likewise, is composed of protophloem and metaphloem (Fig. 6.2). In pteridophytes and gymnosperms, primary xylem consists of tracheids and parenchyma. In angiosperms it consists

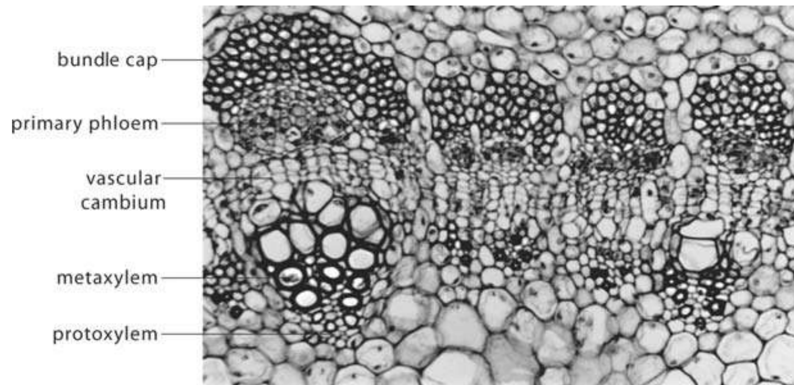


Figure 6.2 Collateral vascular bundles of *Quercus* (oak), a dicotyledon. The vascular cambium had begun actively dividing prior to the preparation of this section. Note also the large bundle caps of sclerenchyma fibers. Magnification × 244.

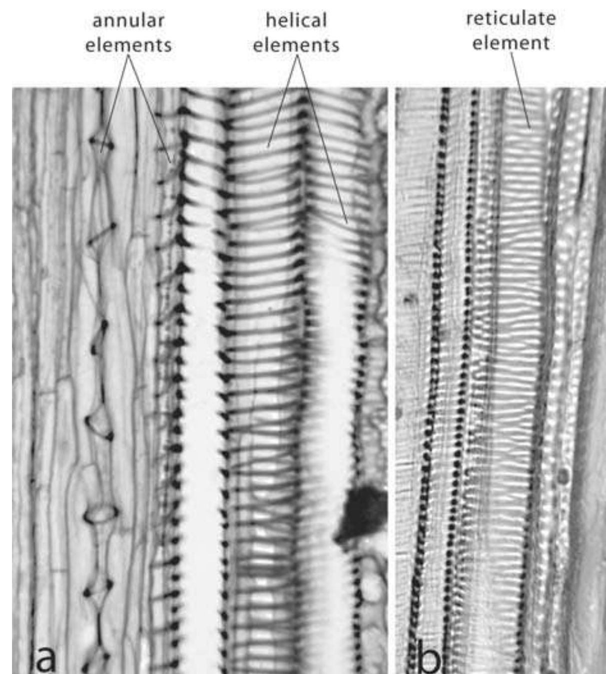


Figure 6.3 Tracheary elements in the primary xylem. Magnification × 275. (a) Annular and helical elements. (b) A reticulate element.

of vessel members, fibers, and parenchyma. The quantity and distribution of these cell types in the primary xylem varies greatly among different species. Conducting cells of the protoxylem and metaxylem are categorized on the basis of the morphology of their secondary walls. In the **protoxylem**, conducting cells called **annular elements** are characterized by secondary wall in the form of rings, whereas the **helical elements** have a secondary wall deposited in the form of helices (Fig. 6.3a). The first tracheary elements of protoxylem to develop, the annular elements, usually have very small diameters, as viewed in transverse section, whereas those that develop subsequently are often progressively larger in diameter. In some monocots, especially grasses (Fig. 6.4) both annular and helical elements may have very large diameters.

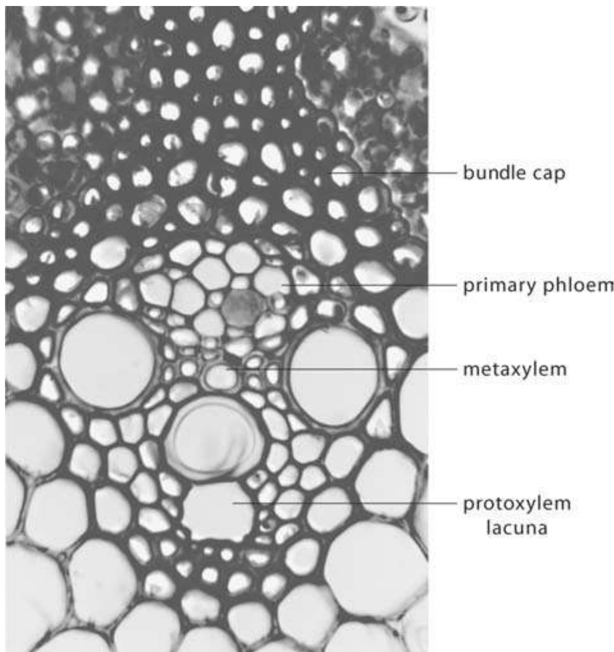


Figure 6.4 A collateral vascular bundle of *Triticum*. Note the protoxylem lacuna which resulted from the break-up of one or more protoxylem elements during extension growth of the region. Magnification × 515.

Scalariform, reticulate, and pitted tracheary elements, which comprise the metaxylem, vary widely in distribution among different taxa. **Scalariform elements** are characteristic of many pteridophytes, and are relatively less common in seed plants. Their secondary walls are deposited in the form of closely spaced, slightly inclined to nearly transverse bars which, in some taxa, seem to alternate in position on adjacent wall facets. It is often difficult to distinguish between some helical elements with secondary walls consisting of tight helices of low pitch, and scalariform elements. The secondary wall of **reticulate elements** is deposited in the form of a reticulum (Fig. 6.3b), and that of **pitted elements** is continuous except for the presence of bordered pits.

During development, annular elements differentiate first followed sequentially by helical, scalariform, reticulate, and pitted elements. Intergradation of wall characteristics in tracheary elements of primary xylem is common in some taxa resulting in cells that are appropriately labeled annular–helical, helical–scalariform, or scalariform–reticulate.

Although protoxylem and metaxylem elements are usually distinguished by their secondary wall characteristics, a more accurate basis for distinction of protoxylem and metaxylem is their time of development relative to that of other tissues, and their positions in the shoot or root. **Protoxylem** develops and becomes functional in shoot and root systems in apical regions undergoing elongation whereas **metaxylem** develops and becomes functional in more proximal regions that have ceased, or nearly ceased, to elongate. Consequently, the morphology of cells of the protoxylem and metaxylem reflect the milieu of their

development. Since vessel members in protoxylem mature (i.e., die and become functional) prior to cessation of elongation of surrounding tissues, they have evolved with a structure that allows them to remain functional while undergoing longitudinal (extension) growth. Their cell walls are largely thin, unlignified primary walls which are prevented from collapsing during rapid growth in early stages of development by the presence in annular elements of the closely spaced rings of secondary wall material or, in helical elements, the helical secondary wall thickenings. As the tracheary elements continue to elongate, the rings of secondary wall become more widely separated, and the pitch of secondary wall helices steeper, but, at the same time, they continue to provide support for the cells which thus maintain their function of transport. Ultimately, however, in many species, after death of the protoplast, continued elongation of the region and/or compression from the growth of surrounding cells results in the destruction of the protoxylem (Figs 6.4, 6.10c) and, in some species, the formation of **protoxylem lacunae**. Lacunae formed in this way are especially prominent in many taxa of monocotyledons (Fig. 6.4). The tracheary elements of the metaxylem that differentiate in regions in which elongation has ceased are, on average, shorter than those of the protoxylem and have more continuous secondary walls, as we have seen in scalariform, reticulate, or pitted patterns.

Cellular composition and patterns of development of primary phloem

In pteridophytes and gymnosperms, the primary phloem consists of sieve cells and parenchyma. In angiosperms it consists of sieve tube members, companion cells, parenchyma, and fibers (Figs 6.2, 6.4, 6.5). As in the primary xylem, different cell types in the phloem vary greatly in quantity and distribution in different species. Whereas recognition of protophloem and metaphloem on the basis of structural features is difficult if not impossible, they can be distinguished on the basis of time of development. Sieve elements in the protophloem, which differentiate early in regions of elongation, are subjected to stretching and ultimately are obliterated whereas the differentiation of sieve elements in the metaphloem occurs later in regions in which elongation has ceased.

In many angiosperms, the sieve tube members in protophloem are typically associated with phloem parenchyma cells (fiber primordia) that differentiate into fibers which comprise bundle caps (Fig. 6.2). Companion cells typically accompany sieve elements in the metaphloem (Figs 6.4, 6.5), but may be absent in protophloem. As in secondary phloem, the sieve elements are characterized by protoplasts that lack nuclei, but which retain functional plasma membranes; and as we shall see in Chapter 12, they are similar in many other ways. Sieve tube members have end walls that usually consist of simple sieve plates (Fig. 6.5)

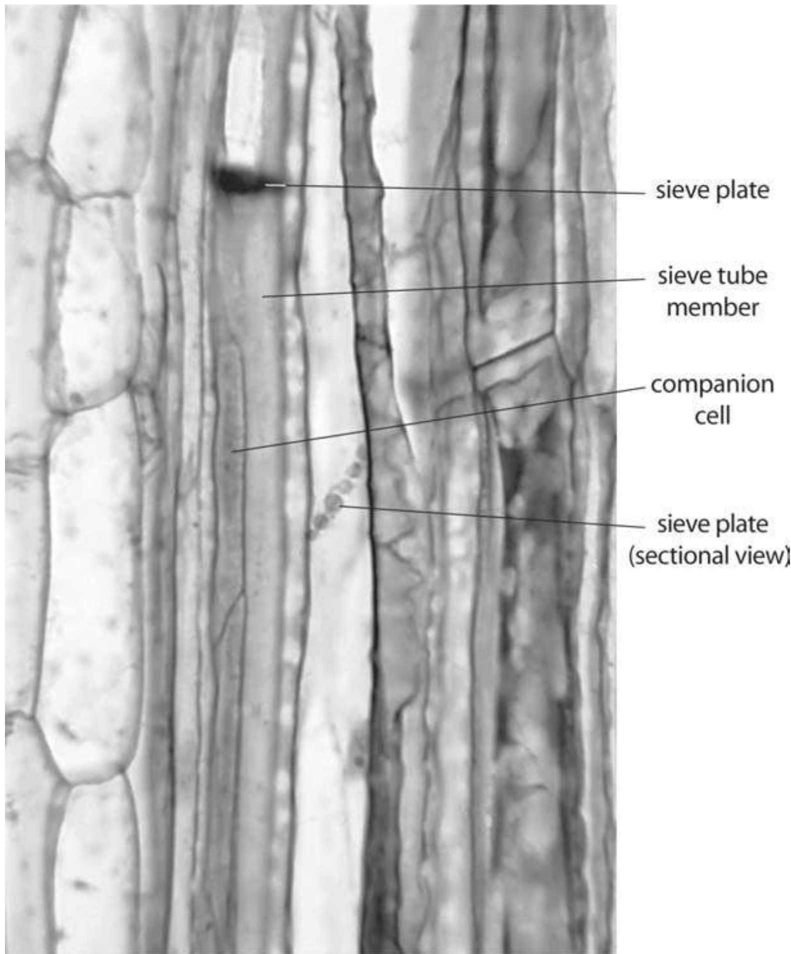


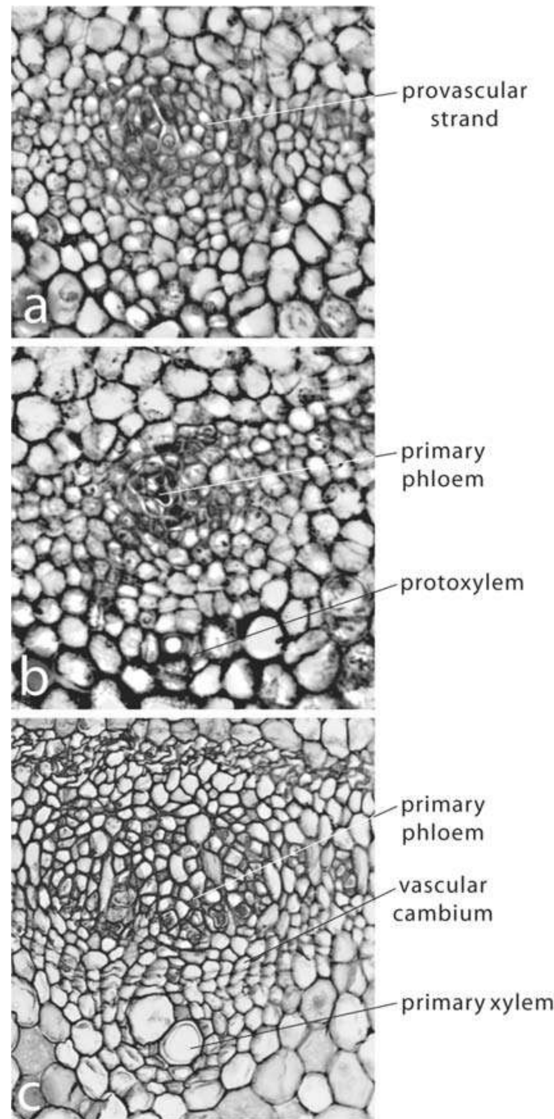
Figure 6.5 Longitudinal section of primary phloem of *Cucurbita*. Note the companion cells associated with the sieve tube members, and the oblique and sectional views of simple sieve plates. Magnification × 389.

containing sieve pores which, during development, become enclosed by callose cylinders. The plasma membrane lines the pores and extends from cell to cell in the sieve tubes.

Differentiation of primary vascular tissues

In general, differentiation within provascular strands proceeds acropetally (i.e., toward the shoot apex) from sites of mature vascular tissues. Primary phloem which is continuous, and which differentiates acropetally, begins its development earlier than primary xylem and, thus, protophloem, the first primary phloem to differentiate (Fig. 6.6a), occurs closer than protoxylem to the apical meristem. The presence of functional protophloem in close proximity to the apical meristem is highly adaptive, providing to a region of very active metabolism and growth photosynthate, an essential energy source as well as the raw material from which protoplast and cell wall components are synthesized.

Figure 6.6 Transverse sections of *Cassia didymobotrya* illustrating relative levels of differentiation of primary xylem and phloem in a developing vascular bundle. (a) A provascular strand in the stem with some mature primary phloem. Magnification × 439. (b) A developing vascular bundle with mature primary phloem and a single, recognizable protoxylem element. Magnification × 439. (c) A nearly mature vascular bundle. Note the protoxylem and an immature metaxylem element, lacking evidence of a secondary wall. Magnification × 244. From Devadas and Beck (1971).



Primary xylem (Fig. 6.6b, c) differentiates later than primary phloem and at more proximal levels in the same provascular strand. Unlike primary phloem the direction of its differentiation is acropetal in some regions and basipetal in others. Furthermore, it usually differentiates in several isolated regions. Initially, therefore, the most distal primary xylem to differentiate is commonly discontinuous with that in more proximal regions (see Jacobs and Morrow, 1957; Larson, 1975).

Differentiation of primary vascular tissues in *Coleus blumei*, described by Jacobs and Morrow (1957), provides an excellent example which is generally applicable to other taxa of dicotyledons with collateral vascular bundles (Fig. 6.7). In *Coleus*, characterized by opposite and alternate pairs of leaves (decussate phyllotaxy), the initial site of differentiation of the first mature protoxylem in the shoot apex is usually in the base

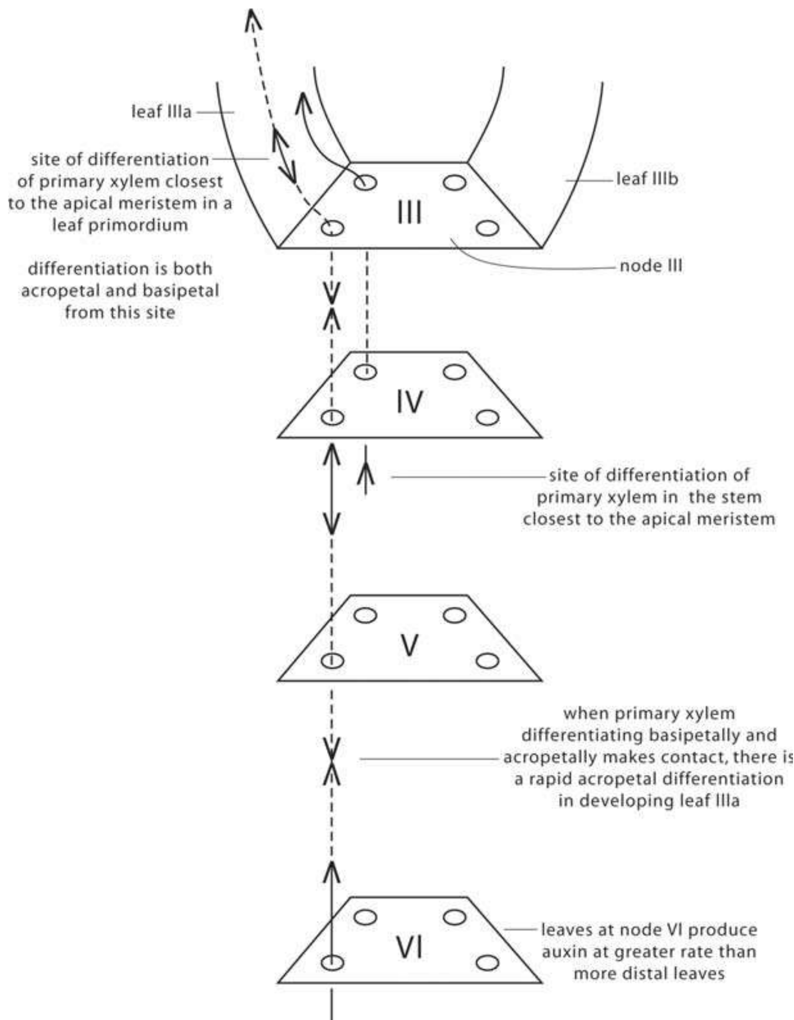


Figure 6.7 Diagrammatic representation of the sites of differentiation of primary xylem in *Coleus blumei*. Modified from Jacobs and Morrow (1957). Used by permission of the Botanical Society of America.

of a third visible leaf primordium below the apical meristem. From this site protoxylem differentiates acropetally into the leaf primordium and basipetally into the shoot axis, eventually fusing with the acropetally differentiating protoxylem in the axis (see also Fig. 6.8 which shows a similar site of first initiation of protoxylem in a different taxon). In the shoot axis, the site of initial differentiation of protoxylem is just below node four. From this site, differentiation of primary xylem is also both acropetal and basipetal. The basipetally differentiating protoxylem will eventually contact that differentiating acropetally from the region of mature primary xylem, and that differentiating acropetally will contact that differentiating basipetally between nodes three and four. If the strands of basipetally and acropetally differentiating protoxylem are offset (i.e., fail to make direct contact), a bridge of protoxylem cells will differentiate between them (Jacobs and Morrow, 1957). Considering the fact that leaf arrangement in *Coleus* is decussate, one might expect the pattern of protoxylem differentiation in pairs of leaves to

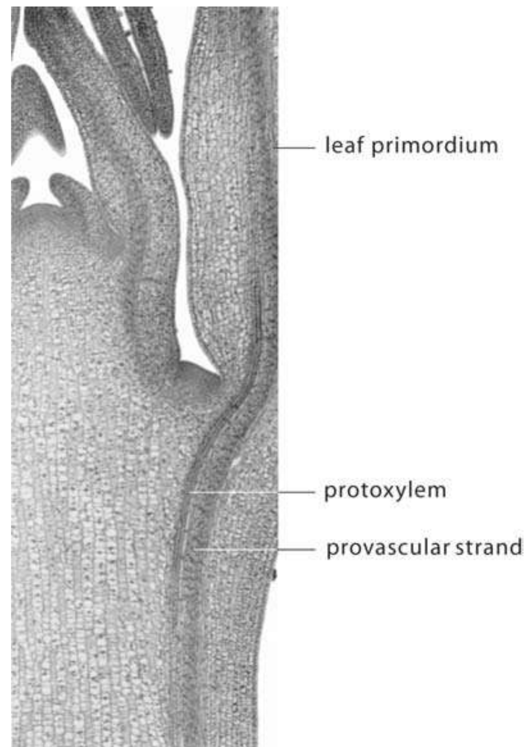


Figure 6.8 Longitudinal section of part of the shoot apex of *Syringa vulgaris* showing a site of protoxylem differentiation in the base of a leaf primordium. From this site differentiation progresses both acropetally and basipetally. Magnification × 62.

be very similar but, in fact, it is often quite different. It differs even in the two vascular bundles (leaf traces) that enter the leaf primordia (Jacobs and Morrow, 1957) (Fig. 6.7). For a description of the pattern of differentiation of primary vascular tissues in *Arabidopsis* which differs in some ways from that of *Coleus*, see Busse and Evert (1999).

During the development of the primary vascular system, tissues not only differentiate longitudinally, but also latitudinally. Figure 6.9a–c represents an apical region of a seed plant stem containing developing, collateral vascular bundles in which primary xylem and phloem are differentiating acropetally. The primary phloem which develops closer to the apical meristem than the primary xylem (Fig. 6.9c, d) differentiates within the outer part of the provascular strand (i.e., the part adjacent to the ground meristem, or immature cortex) in regions of the shoot apex that are still elongating. Because of this elongation as well as compression resulting from the growth of surrounding cells, the sieve cells of the protophloem are ultimately obliterated, but not before sieve elements in the metaphloem have differentiated at levels adjacent to newly differentiated protophloem. Consequently, there is no discontinuity in the column of primary phloem. In the region of the protophloem, during, and to some degree, following obliteration of sieve elements, primary phloem fibers develop from parenchyma cells (fiber primordia) that comprise part of the protophloem, forming bundle caps. The fiber primordia often increase in number by division of parenchyma cells, resulting in large, conspicuous bundle caps

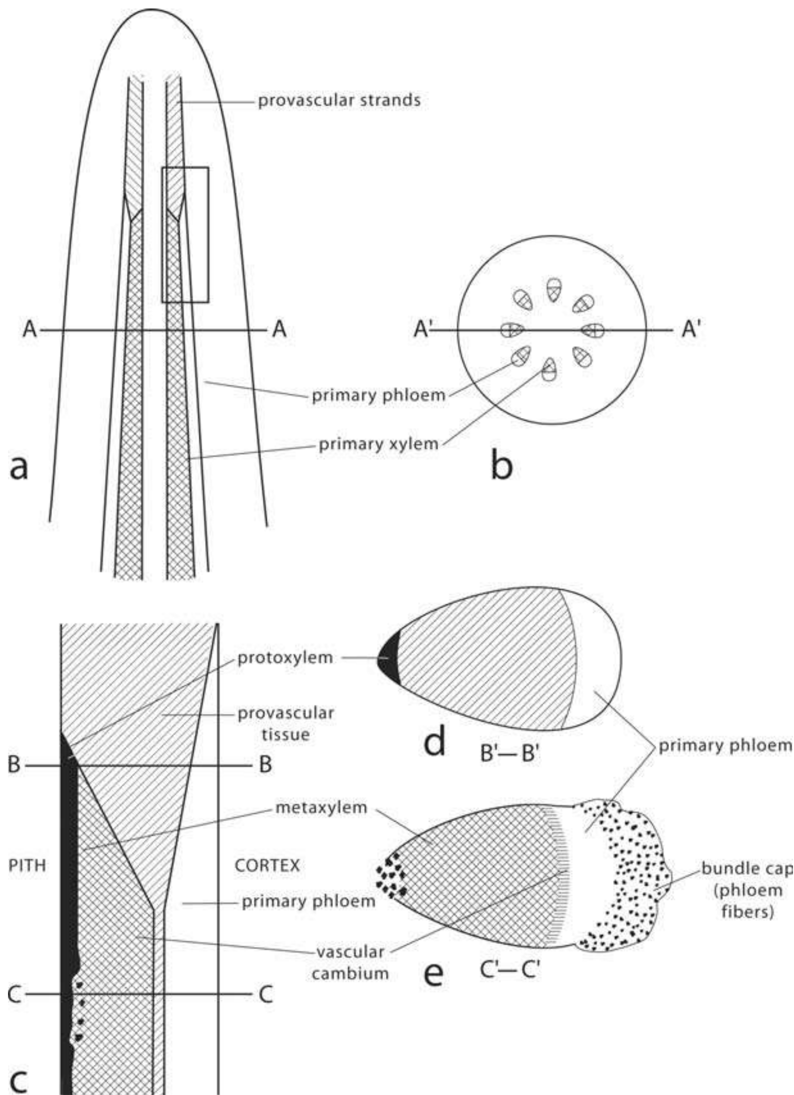


Figure 6.9 Diagrammatic representation of patterns of longitudinal and latitudinal (transverse) differentiation of primary xylem and primary phloem in a developing vascular bundle of a seed plant. Note that the bundle cap illustrated in (e) is not shown in (c). The distal, mature region of the vascular bundle in (a) and (c) are greatly foreshortened in relation to their width. See the text for descriptions.

(Fig. 6.2). Bundle cap fibers increase greatly in length during development and consequently intrude between surrounding cells. Since metaphloem develops to the inside of the protophloem after elongation has ceased in the region, its unity is maintained and it may remain functional for long periods of time, in herbaceous plants for as long as the plants live.

In most vascular plants primary xylem begins its differentiation later than primary phloem, that is, in more proximal regions of the provascular strands (Fig. 6.9c). Protoxylem differentiates within the inner part of the strands next to the immature pith and, as development proceeds, protoxylem and metaxylem gradually extend toward the exterior of the axis (Figs 6.6b, c, 6.9d, e). Because, as noted above, protoxylem differentiates in a region of the stem still elongating, it is stretched and often fractured (Figs 6.9e, 6.10c). In contrast, metaxylem

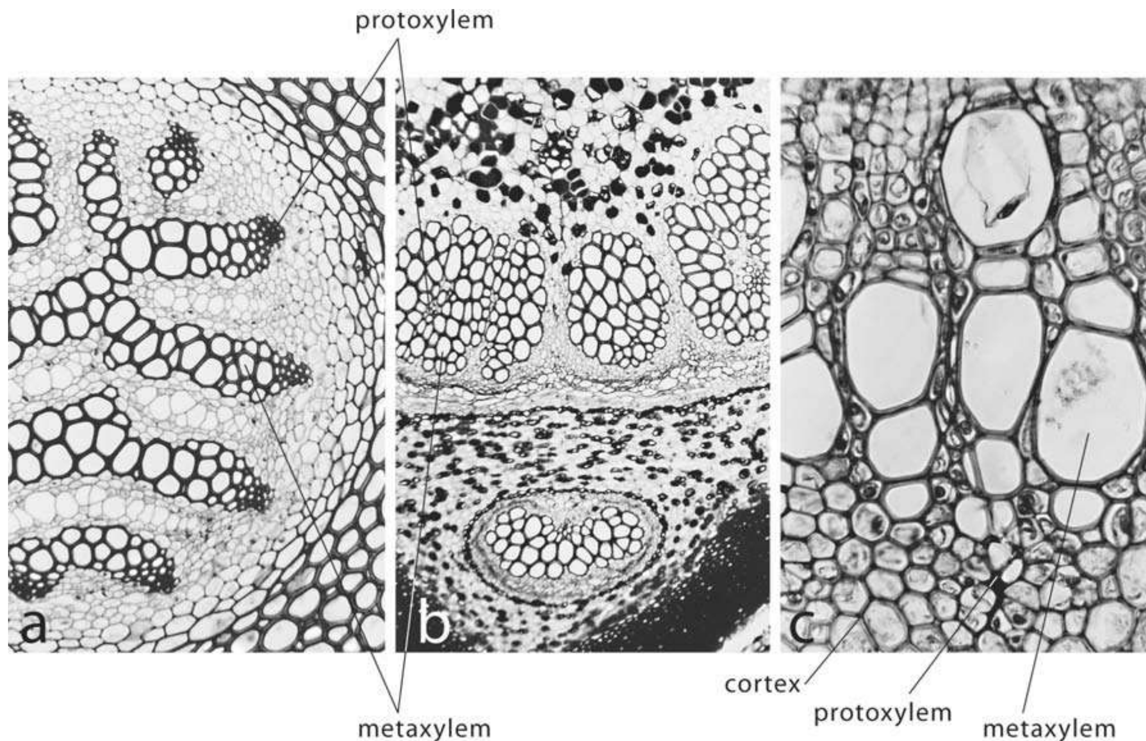


Figure 6.10 Transverse sections of stems showing order of maturation (latitudinal direction of development) of primary xylem. (a) Exarch (centripetal) development in the rhizome of *Lycopodium flabelliforme*. Magnification × 184. (b) Mesarch development in the fern *Osmunda*. Magnification × 45. (c) Endarch (centrifugal) development in the stem of *Quercus*. Magnification × 465.

differentiates after most, or all, elongation in the region has ceased and thus may maintain its function for some time, in plants in which secondary xylem is not produced, for the life of the plant.

The patterns of latitudinal differentiation of primary vascular tissues are useful in plant taxonomy. The differentiation of primary phloem from the outer surface toward the inner surface of a provascular bundle (Fig. 6.9c) is referred to, especially in the older literature, as **centripetal development** whereas differentiation of the primary xylem from the inner toward the outer part of a bundle is referred to as **centrifugal development** (Figs 6.6b, c, 6.9c, e, 6.10c). This pattern of development of protoxylem in relation to metaxylem is also called **endarch order of maturation** of primary xylem. If the sequence of development of primary xylem tracheary elements is reversed, that is, with annular elements to the exterior and helical, scalariform, and/or reticulate and pitted elements developing sequentially toward the center of the axis, the pattern of development, or order of maturation, is described as **exarch** (or centripetal) (Fig. 6.10a). This pattern is common in roots of vascular plants as well as in the roots and stems of pteridophytes which often, but do not always, contain central columns of primary vascular tissue (protosteles). If protoxylem elements differentiate more deeply within vascular bundles or central vascular columns and metaxylem elements develop around them, often in radiating files, the pattern of development or order of maturation is described as **mesarch** (Fig. 6.10b).

The role of auxin in the development of the primary vascular system

It is widely accepted that differentiation of primary xylem and primary phloem from provascular tissue is directly related to the controlling influence of the hormone auxin (e.g., Sachs, 1981, 1991; Aloni, 1987; Roberts, 1988; Steeves and Sussex, 1989; Lyndon, 1990; Ma and Steeves, 1992; Stein, 1993). Auxin concentration and "flux" (= flow rate) (see Sachs, 1991; Stein, 1993) are thought to be primary factors influencing the type of cell or tissue that differentiates. For example, Wetmore and Rier (1963) showed that, in *Syringa* callus, primary xylem differentiated in high concentrations of auxin whereas primary phloem differentiated under lower concentrations. In the intact plant axis, auxin, synthesized in the shoot apex and/or leaf primordia, is transmitted through the symplast via plasmodesmata to the derivatives of meristems where it has a controlling influence on differentiation of protoderm, ground meristem, and provascular tissue, and subsequently, on differentiation of the mature tissue regions of the axis (i.e., epidermis, cortex, pith, primary vascular system, etc.).

Extensive evidence (see Sachs, 1981; Steeves and Sussex, 1989) indicates that developing leaf primordia are the primary source of auxin in angiosperms although the apical dome has been suggested as a source in some taxa (Sachs, 1981). Several workers have concluded that, in some ferns, auxin produced in the apical meristem controls the basic form of the primary vascular system (Wardlaw, 1946; Soe, 1959; Ma and Steeves, 1992). For example, Ma and Steeves (1992, 1995) demonstrated that, in *Matteuccia struthiopteris*, leaf primordia control leaf gap formation as well as the differentiation of the pith. When, in their studies, leaf primordia were punctured (and, thereby, suppressed), the primary vascular system developed as a continuous cylinder without leaf gaps. If leaf primordia were prevented from forming over a period of several weeks, no pith was formed and the primary vascular system attained the form of a protostele. They observed, further, that when all leaf primordia were suppressed, no protoxylem or protophloem differentiated; but when a single primordium was left intact, protoxylem and protophloem differentiated in relation to that primordium.

Although extensive evidence indicates that auxin has a controlling influence on the development of leaf traces in seed plants, the mechanism of this control is unclear and obviously complex. We know that the primary phloem in leaf traces differentiates acropetally whereas the primary xylem differentiates both acropetally and basipetally in different sites in the shoot apex. These different patterns of development are thought to be related to differences in auxin concentration and flow rate (see Benková *et al.*, 2003; see also Chapter 5).

Early in the history of plant anatomy the primary vascular system of the plant axis (stem plus root) and certain associated tissues were recognized by the French botanists van Tieghem and Duliot as a unified system which they called the stele. Various types of steles were

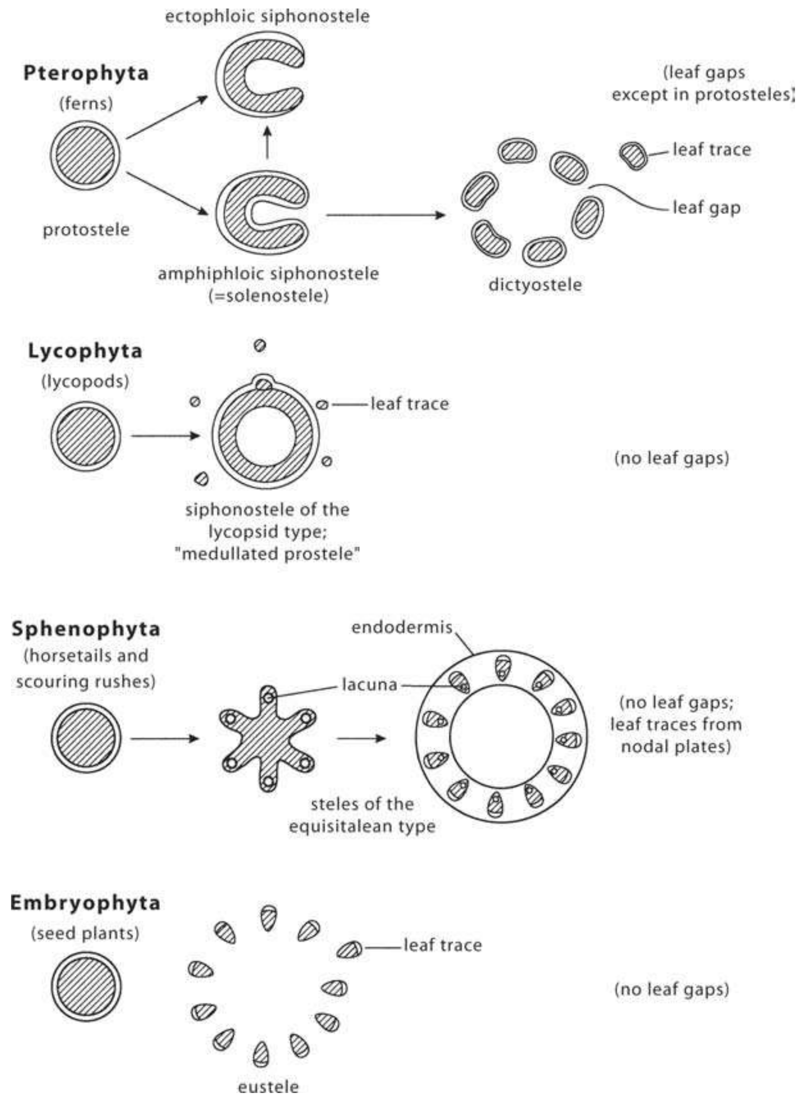


Figure 6.11 Diagrams illustrating some aspects of the structure and evolution of the steles in pterophytes, lycophytes, sphenophytes, and embryophytes.

described by these and later workers (see Beck *et al*., 1983). Many recent workers use the term "stele" as a synonym for the primary xylem and phloem in stems and roots. Since reference to stelar types is common in the anatomical and morphological literature, a summary of the most widely recognized types is provided in Fig. 6.11. The British botanist F. O. Bower (1930) proposed that the variation in stelar form was directly related to the size of the plant and its ability to provide, through the primary vascular system, the necessary nutrients and photosynthate to support the growth and development of the plant. A modern viewpoint, proposed by Stein (1993), suggests that these stelar types evolved not only in relation to the increasing size of the plant, but also, and perhaps more importantly, in relation to the size and complexity of its lateral branch systems and leaves, and the available auxin sources. Stein developed a computer model based on an initial hypothesis of

Wight (1987) who proposed that the ribbed steles of some Devonian Aneurophytalean taxa developed under the influence of hormones produced by the lateral appendages. He suggested that hormones stimulated the differentiation of vascular tissue in the direction of the leaf traces which in these taxa diverged directly from the tips of the stelar ribs. Stein's model incorporates a detailed analysis of current knowledge of the induction of primary vascular tissues during development, and the application of this knowledge to stelar structure in both fossil and living plants, the size and structure of lateral branch systems and leaves, phyllotaxy, and the sources and sites of auxin production and transport as represented in mature axes by protoxylem and metaxylem strands. Predictions in the model of hormone availability and concentrations during development often conform to the actual structure of steles in members of the fossil Aneurophytales. Parameters of the model are also applied with equal success to other fossil taxa and to living seed plants. Stein's approach makes possible for the first time the interpretation of stelar evolution not only through a comparison of mature stelar patterns, but also through the developmental changes that accompanied, through time, the evolution of these patterns. His work also strongly supports the viewpoint that there is a causal relationship in several lines of evolution between the increasing size of plants during the Devonian and the increasing size and complexity of their lateral appendages (see also Niklas, 1984).

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Chapter 7

Sympodial systems and patterns of nodal anatomy

Perspective: leaf traces

Vegetative shoots consist of stems bearing leaves. In order to develop, and to synthesize various necessary compounds required by the plant, leaves must have access to a source of water and essential minerals which are transported into them from the stem through the primary xylem. Photosynthate and other compounds synthesized in the leaves are, in turn, transported through the primary phloem into the stem and root system for storage and/or use. This transport of substances takes place in primary vascular connections between the stem vascular system and the base of leaves called **leaf traces**. Traces may diverge from the stem vascular system some distance below, or very near, the **nodes** (sites of attachment of leaves to stems) at which they enter the leaves. Leaf traces are composed of protoxylem, metaxylem, protophloem, and metaphloem, and typically contain **transfer cells** in both primary xylem and primary phloem. In seed plants, leaf traces are often larger and contain more tracheary cells than the vascular bundles from which they diverge, and they may increase in size distally. A leaf may be vascularized by only one or by several to many leaf traces.

In order to understand the morphology of nodal regions of shoots we must observe both transverse and median longitudinal sections through these regions. Remember that stems bear many leaves in various spatial distributions. Nevertheless in sections, depending on the distance between leaves and the size of the sections, only a single leaf and its vascular connection to the stem (leaf trace) may be visible.

Nodal structure of pteridophytes

The simplest nodal structure occurs in pteridophytes (Psilophyta, Lycophyta, Sphenophyta, and Pterophyta). In taxa with **protosteles** leaf traces simply diverge from the surface of the central vascular column near the level of the leaf and enter the leaf base. Commonly leaves of plants with protosteles are supplied by only one leaf trace (Fig. 7.1). In

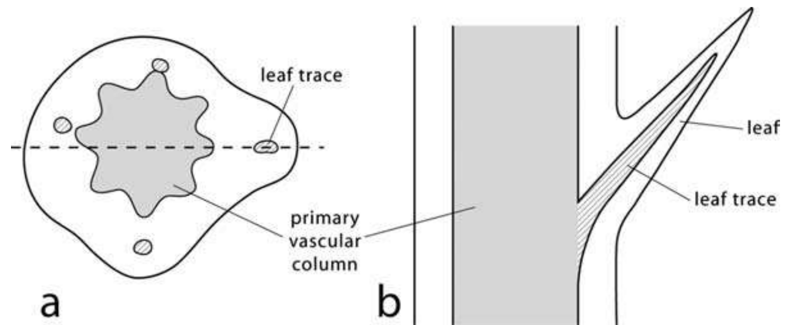


Figure 7.1 Nodal structure of a stem containing a protostele. (a) Transverse view showing a solid column of primary vascular tissue from which leaf traces diverge. (b) Longitudinal section along the plane indicated by the dashed line in (a).

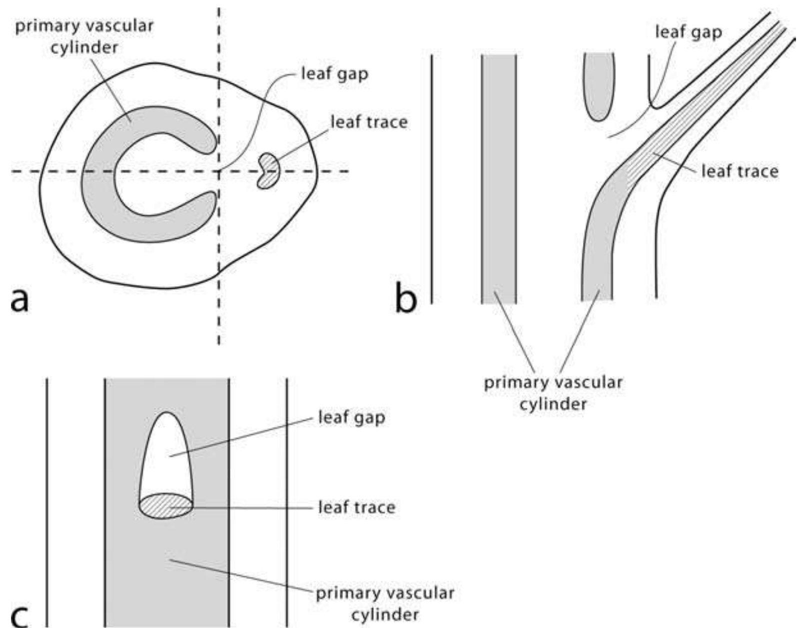


Figure 7.2 Nodal structure of a stem containing a siphonostele. (a) Transverse section. Primary xylem and primary phloem comprise a cylinder in which develop discontinuities called leaf gaps. (b) Longitudinal view of nodal structure as seen in a section along the plane indicated by the horizontal dashed line in (a). Note the leaf gap and its positional relationship to the leaf trace. (c) Longitudinal view of nodal structure along the plane indicated by the vertical dashed line in (a). Note the form of the leaf gap.

stems with **siphonosteles**, such as those of many ferns, the continuity of the cylinder of primary vascular tissue is broken immediately above the position of outward divergence of the leaf trace, leaving a discontinuity called a **leaf gap**, a region through which the parenchyma of the pith and cortex are continuous (Fig. 7.2). If the leaves of a particular species are closely spaced, the cylinder will be dissected by many such leaf gaps, and depending on their longitudinal extent (that is, their height as observed in face view), several to many of these gaps will be visible between regions of primary vascular tissue, giving, in transverse sections, the false impression of a system made up of separate vascular bundles. Such a system is termed a **dictyostele** (dissected stele) (Fig. 7.3). Whereas in many plants with dictyosteles, all discontinuites are in fact leaf gaps, there are some plants in which some discontinuities in the dictyostele are not associated with leaves and, thus, are not leaf gaps. Studies by White and Weidlich (1995) demonstrate that the dictyosteles in the filicalean ferns *Diplazium* and *Blechnum* show a striking resemblence to the eusteles of some gymnosperms and dicotyledons

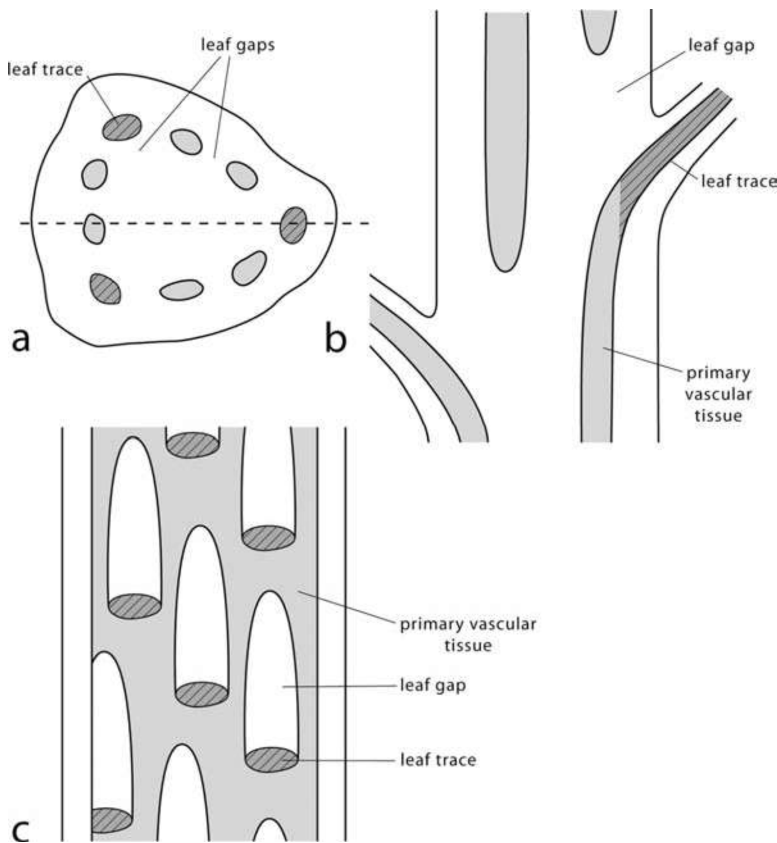


Figure 7.3 Nodal structure of a stem containing a dictyostele. (a) Transverse section. (b) Longitudinal section along the dashed line in (a). (c) Longitudinal view of the stelar surface showing dissection of the stele by numerous leaf gaps. Compare (b) and (c).

with helically arranged leaves. Considering the wealth of information which suggests that ferns and seed plants are unrelated or, at best, only very distantly related (see Stewart and Rothwell, 1993), it seems likely that this similarity is the result of homoplasy (parallel evolution).

Sympodial systems of seed plants

The **eustele**, the primary vascular system in the stem of seed plants, consists of a system of vascular bundles plus the leaf traces that diverge from them. The stem vascular bundles, or **axial bundles**, and associated leaf traces comprise **sympodia**. The eustele, therefore, is a cylindrical system of sympodia. There are no leaf gaps in a eustele. The sympodia are considered to be discrete entities although there may be vascular connections between them. This is especially true among angiosperms, but in the more primitive angiosperms and in many gymnosperms the sympodia are not interconnected (see Beck *et al.*, 1983). As viewed in transverse section, a eustele appears as a cylinder of separate vascular bundles, some of which are axial bundles, others of which are leaf traces (Fig. 7.4).

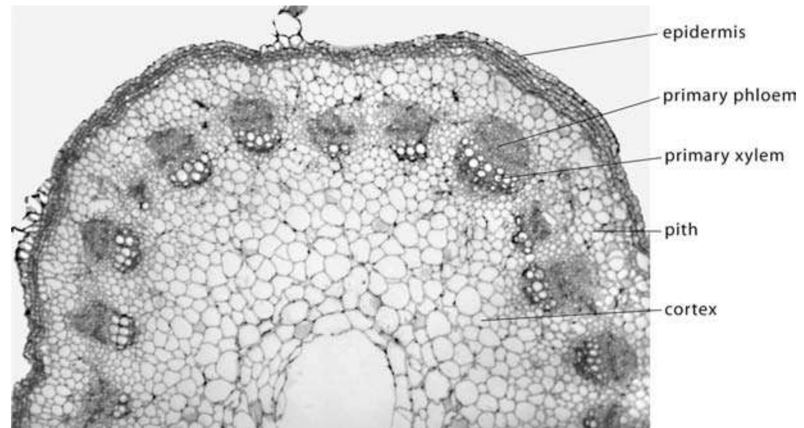


Figure 7.4 Transverse section of a eustele of a young stem of *Helianthus*. Magnification × 90.

The primary vascular systems of seed plants are often illustrated spread out in one plane. In order to thoroughly understand the eustele and some of its many manifestations, we shall illustrate it with a very simple system similar to that of a primitive gymnosperm (Fig. 7.5a). The system consists of five sympodia, labeled 1 through 5. Leaf traces diverge from every other axial bundle in a helical pattern. This helix, which represents the sequential development of leaf primordia by the apical meristem is called the **generative** (or **ontogenetic**) **spiral**. The levels at which traces enter the bases of leaves are indicated by triangles. Note that the oldest leaf trace visible in the diagram, that is, the lowest one (the one farthest from the apical meristem) diverged from axial bundle 4. During development, the next trace in the chronological sequence diverged from axial bundle 1, followed by a trace from axial bundle 3. The next youngest trace diverged from axial bundle 5, followed by traces from axial bundles 2 and 4. The last that we shall consider, and the most apical trace, diverged from axial bundle 1. You will note that in this region of the eustele, axial bundles 1 and 4 have each contributed a leaf trace to two different leaves. A helix connecting the two traces that diverged from one axial bundle would encircle the stem twice. A stem with a leaf arrangement in this pattern is referred to as having a **phyllotaxy** of 2/5, the numerator referring to the number of turns around the stem between two traces in the same sympodium (or, as seen on the stem surface, leaves in the same orthostichy) and the denominator indicating the number of sympodia in the system.

Figure 7.5b–e represents transverse sections at levels A–A through C–C in the primary vascular system. Axial bundles 1–5 are illustrated in all sections. Note that traces diverge laterally from axial bundles and then follow radial courses through the cortex. In section A the leaf trace that diverged from axial bundle 4 is shown within the cortex because the section was taken from a level below its entrance into the leaf. The next trace to diverge during development is shown in contact with the axial bundle 1 because section A represents a level at which the separation of the trace from the axial bundle is not complete. In section B the trace that diverged from axial bundle 4 is in a leaf that

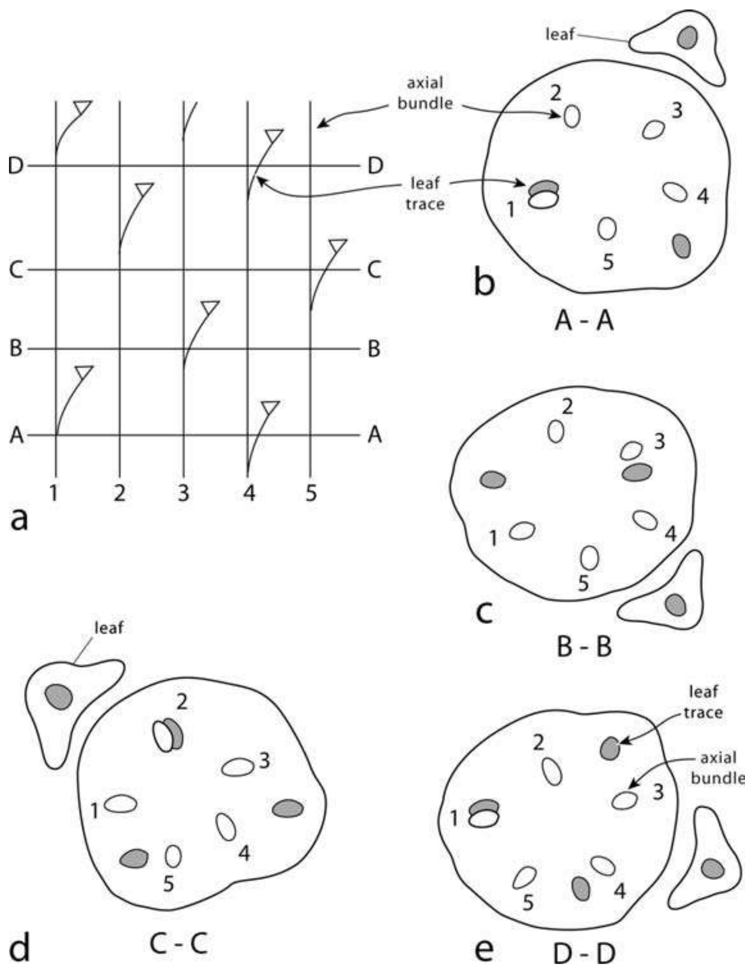


Figure 7.5 Diagrams illustrating the architecture of a simple eustele consisting of five sympodia. (a) A longitudinal view shown as if the cylinder of primary vascular bundles (1–5) had been spread out in one plane. (b–e) Transverse sections taken at the levels indicated by horizontal lines in (a). Please see the text for a detailed explanation.

has separated from the stem whereas the trace that diverged from axial bundle 1 is still in the stem cortex at this level. Note also the trace that diverged from axial bundle 3 just below the level of section B. It appears in section C in the outer cortex, and in section D in a leaf. Trace divergence from other axial bundles as shown at levels C and D follow similar patterns.

In summary, it is clear from these diagrams that, in the hypothetical plant represented, there was a eustele consisting of five sympodia, that during development leaf traces diverged in sequence along the generative spiral from every other axial bundle, that leaf traces (and, thus, leaves) were arranged in a helical pattern comprising a 2/5 phyllotaxy, and that each leaf was supplied by a single leaf trace that originated close to the node at which it entered the leaf. All eusteles are similar to the one just described, but may differ in being composed of fewer or more sympodia, and some, as we shall see, in being more complex in several other ways.

The eustele of fir (*Abies concolor*) is basically similar to the system we have described above, but differs in consisting of 13 sympodia and

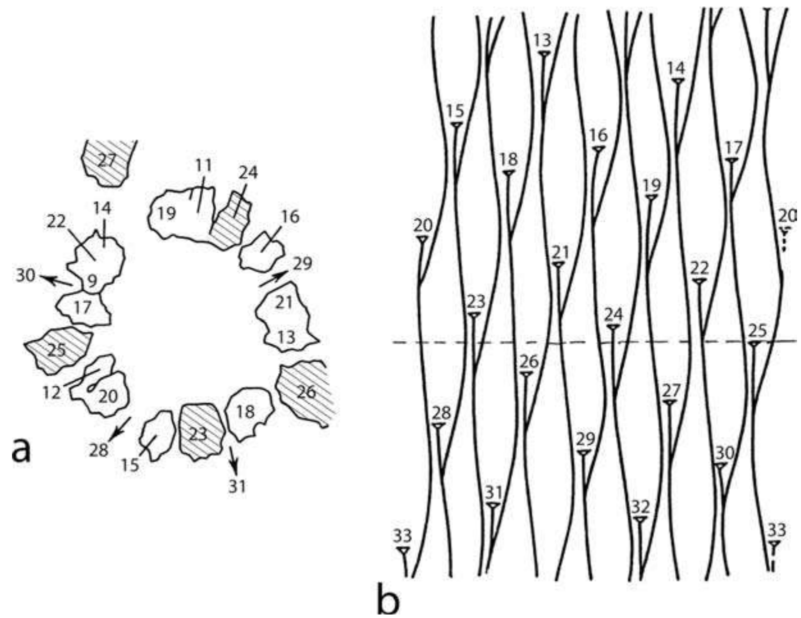


Figure 7.6 (a) The eustele of *Abies concolor* (fir) as seen in transverse section. An axial bundle is given the number of the leaf trace that will next diverge from it. Leaf traces are shaded. The approximate level of this section in the stele is indicated by the horizontal dashed line in (b). (b) Longitudinal diagrammatic representation of the pattern of the primary vascular system illustrated in (a). The triangles represent leaf traces near the levels of their entry into leaves. The traces are numbered in the order of their development, with the older traces indicated by higher numbers, the younger by lower numbers. This eustele consists of 13 sympodia (axial bundles bearing leaf traces). Note that the leaf traces follow a shallow helix called the ontogenetic spiral. From Namboodiri and Beck (1968a).

having a phyllotaxy of 5/13 (Fig. 7.6). The eustele of the ancient gymnosperm, *Ginkgo biloba* (Fig. 7.7) also consists of 13 sympodia and has a phyllotaxy of 5/13. It is distinctive, however, in that two leaf traces, each derived from a different, adjacent axial bundle, enter each leaf. Note also that the traces are quite long and vary greatly in length. Because some of the traces traverse, longitudinally, four or five nodes, a transverse section from any level of the mature region of a young stem will contain not just 13 vascular bundles (the number of axial bundles), but commonly 26, 13 axial bundles and 13 leaf traces.

Angiosperms have more complex eusteles, related primarily to the large size of their leaves. The leaves of many dicotyledons are supplied by three or five (occasionally seven) leaf traces. In such cases there will be a central trace, often larger than others, called the **median trace** flanked on either side by one or more smaller **lateral traces**, all of which enter the same leaf (Fig. 7.8). It is customary in diagrams of primary vascular systems, illustrated in one plane, to portray at the same level all traces that supply one leaf. These traces will usually be assigned the same number (indicating their position in the

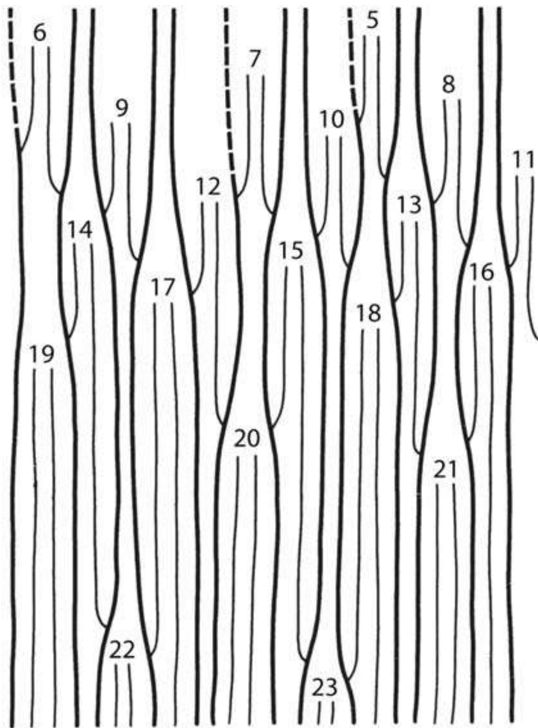


Figure 7.7 Diagram of the eustele of *Ginkgo biloba*. Axial bundles are indicated by bold lines. Pairs of leaf traces are indicated by numbers in descending order along the ontogenetic spiral. See the text for more detail. Redrawn from Gunckel and Wetmore (1946). Used by permission of the Botanical Society of America.

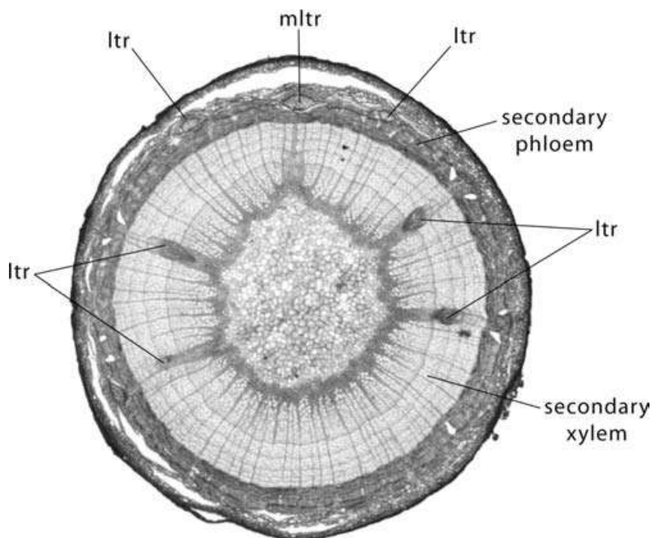


Figure 7.8 Transverse section of a seven-trace, multilacunate node of *Quercus* (oak). mltr, median leaf trace; ltr, lateral leaf traces. Magnification × 86.

developmental sequence). This is clearly illustrated in the primary vascular systems of *Drimys* and *Potentilla* (Fig. 7.9a, b). Each leaf of these plants is provided with a median, and a right and a left lateral, trace. In *Potentilla* (Fig. 7.9b), the lateral and median traces have been assigned the same number as, for example M6 (median), L6 (left lateral), and R6 (right lateral). Note also that each of these three traces diverges from the axial bundle of a different sympodium, and that the median trace

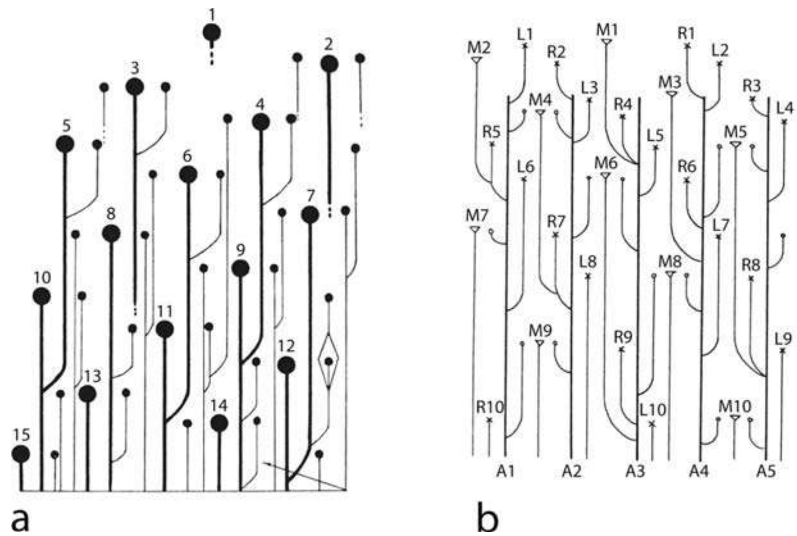


Figure 7.9 (a) Diagram of the eustele of *Drimys winteri* consisting of five sympodia. Note that each leaf receives three leaf traces, a median trace (large black dot) and right and left lateral traces (small black dots). The median and lateral traces branch from axial bundles of different sympodia. (b) Diagram of the eustele of *Potentilla fruticosa*. Median traces are indicated by triangles and laterals by Xs. This primary vascular system resembles that of *Drimys*, but the diagram differs in showing pairs of branch traces (small circles) flanking each median trace. Each pair of branch traces enters a lateral bud in the axil of the associated leaf. (a) From Benzing (1967b). Used by permission of the Botanical Society of America. (b) From Devadas and Beck (1972).

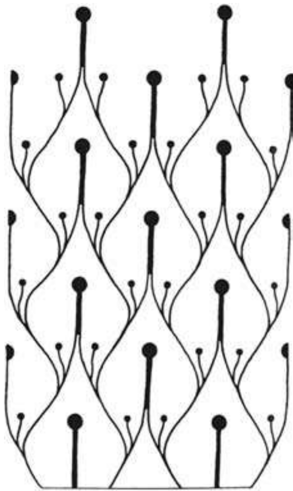


Figure 7.10 Diagram of the primary vascular system (eustele) of *Cercidiphyllum japonicum*. The large dots indicate median leaf traces, the small, laterals. Compare this closed system, consisting of four sympodia, with the open systems of *Drimys* and *Potentilla* (Fig. 7.9). For details please see the text. From Benzing (1967b). Used by permission of the Botanical Society of America.

is much longer than the laterals. A final feature of importance in this diagram are the two traces, of variable length, on either side of the median that terminate (in the diagram) in two small circles. These are branch traces that enter the axillary bud just above the level of entry of the median trace into the leaf base. Note that in both *Drimys* (Fig. 7.9a) and *Potentilla* (Fig. 7.9b) there are five sympodia, that the phyllotaxy is 2/5, and that the large number of vascular bundles in the systems is related not only to the number of axial bundles, but also to the number of traces per leaf and to the fact that the traces extend longitudinally through several internodes and, thus, overlap those to other leaves.

The primary vascular systems described above are **open systems** in that the sympodia are not interconnected, a feature of many plants with helical phyllotaxy. **Closed systems**, characterized usually by an even number of sympodia, are common in plants with **verticillate** (leaves whorled), **distichous** (leaves two-ranked), or **decussate** (leaves opposite with alternating pairs at right angles) phyllotaxy. *Cercidiphyllum* provides a good example of a closed primary vascular system in a plant with decussate phyllotaxy (Fig. 7.10). The four axial bundles are interconnected by pairs of leaf traces derived from adjacent axial bundles that fuse to form each median trace prior to its entrance into a leaf base. It is particularly interesting to note in this system, in which a

median and two lateral traces enter each leaf, that the laterals branch from the traces that fuse to form the median traces of the next more distal pair of leaves. The eusteles of plants with distichous and decussate phyllotaxy are commonly composed of four sympodia; those with verticillate phyllotaxy, of six. For more information on the many architectural variations of eusteles in dicotyledons, please refer to Beck *et al.* (1983).

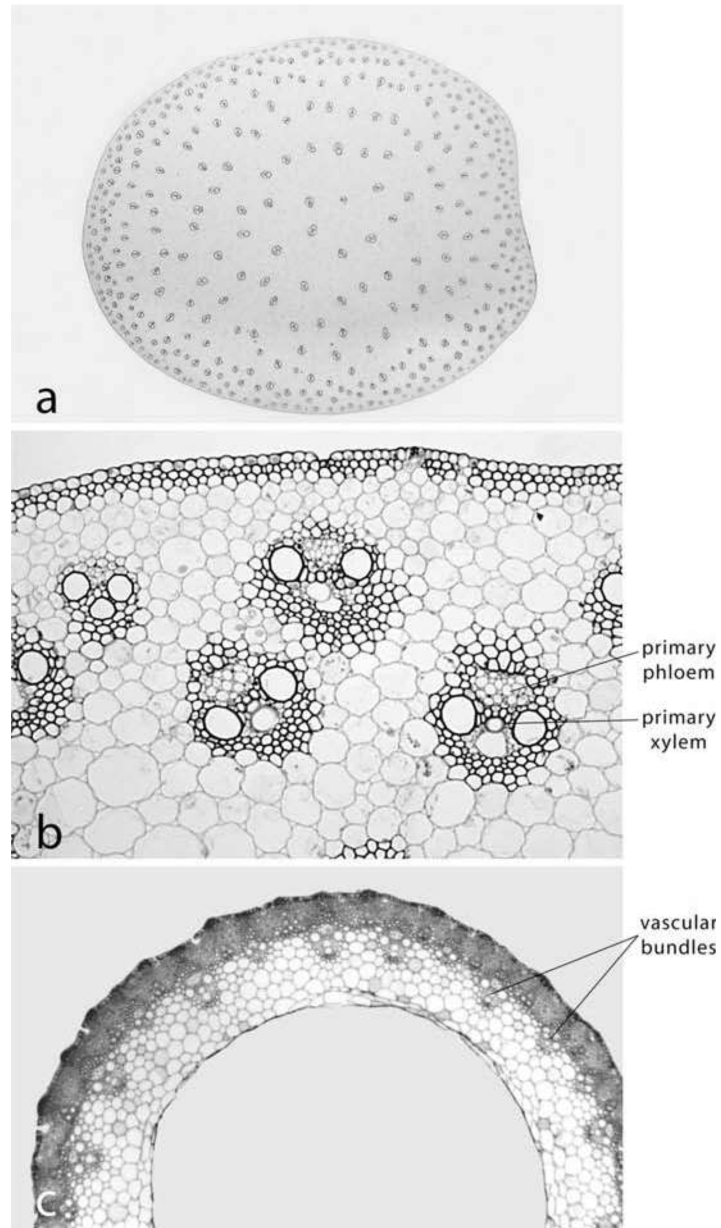
The primary vascular systems in the stems of monocotyledons, termed atactosteles, are considered by some to be fundamentally different from those of dicotyledons (Zimmermann and Tomlinson, 1972). The monocotyledon primary vascular system, however, may be simply a highly modified eustele (Beck *et al.*, 1983). These authors believe that the distinctive structural features of the primary vascular systems of monocotyledons are related primarily to the nature of the large leaves with broad leaf bases that, in some groups (e.g., grasses), overlap and encircle the stem, and which in many taxa are supplied by a very large number of leaf traces.

As viewed in transverse section the primary vascular system of many monocotyledons appears to consist of numerous, randomly scattered bundles (Fig. 7.11a, b). Those in a more peripheral position are often smaller and comprise a more compact zone than the larger, more central ones. Because some monocotyledons contain so many vascular bundles in their stems (hundreds or even thousands), development of an accurate understanding of the architecture of the system has been difficult. Major progress in understanding the system has resulted from the work of Zimmermann and Tomlinson (1972) and many other researchers (see Beck *et al.* (1983) for more literature and a review). The description of the system below is based primarily on the work of Zimmermann and Tomlinson.

In monocotyledon seedlings the vascular system begins as a cylinder of relatively few bundles similar to that of dicotyledons. Furthermore, some mature monocotyledons (some members of the Liliaceae that do not have sheathing leaf bases) and several grasses such as *Avena* (oats) and *Triticum* (wheat) have a central pith surrounded by a cylinder of vascular bundles (Fig. 7.11c). In many monocotyledons, especially large ones, as development proceeds and the stem increases in diameter, the original axial bundles gradually increase in number by branching tangentially. Two types of axial bundles are recognized: **major bundles** which traverse, longitudinally, relatively great distances between the levels of divergence of consecutive leaf traces, and **minor bundles** which traverse much shorter distances between the levels of divergence of consecutive leaf traces (Fig. 7.12a). A unique feature of some monocotyledons is the presence of discontinuous cortical bundles which may accompany leaf traces as they enter leaf bases.

Following the divergence of a leaf trace an axial bundle follows an oblique (and often helical) course from a peripheral position toward the center of the stem, eventually turning sharply outward toward the periphery. After the divergence of another leaf trace, the course toward the center of the stem, and then outward, is repeated. Each axial bundle,

Figure 7.11 (a, b) Transverse sections of a stem of *Zea mays* illustrating the numerous vascular bundles of which the primary vascular system is comprised. Magnification (a) × 4.4, (b) × 182. (c) Transverse section of a stem of *Triticum* sp. Magnification × 49. characterized by an irregular cylinder of peripheral vascular bundles.



consequently, follows an undulating, helical course through the stem with successive leaf traces diverging at regular intervals (Fig. 7.12a, b). Those axial bundles characterized by the greatest distances between divergence of successive leaf traces (major bundles) approach most closely the center of the stem during their longitudinal, undulating course and give rise to median and near-median traces. Those characterized by shorter distances between levels of divergence of successive leaf traces (minor bundles) follow a similar course, but do not approach

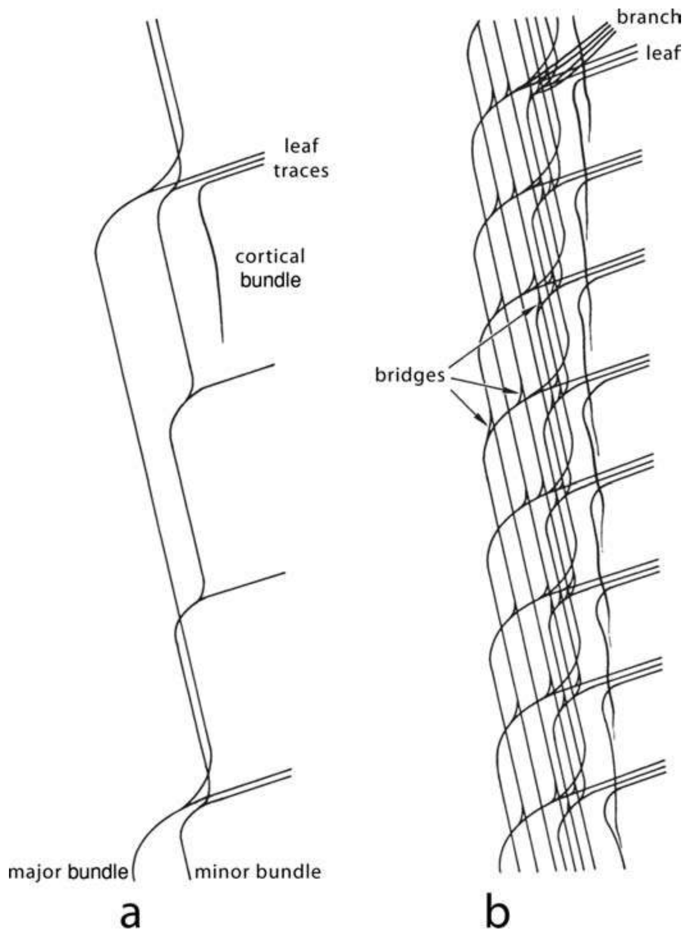


Figure 7.12 Diagrams illustrating the longitudinal course of vascular bundles in the primary vascular system of monocotyledons. (a) The basic components of the system are major bundles, minor bundles, and cortical bundles. Major and minor bundles are axial bundles from which leaf traces diverge. A leaf trace may also contain an outward extension of a cortical bundle. Following divergence of a leaf trace, both major and minor axial bundles follow oblique courses toward the center of the stem and then turn outward until another leaf trace diverges. (b) A part of the primary vascular system illustrating its complexity. For more detail, please refer to the text. (a, b) From Zimmermann and Tomlinson (1972). Used by permission of the University of Chicago Press. © 1972 University of Chicago. All rights reserved.

as close to the stem center (Fig. 7.12b). These give rise to lateral traces. Adjacent axial bundles are often connected by bridge bundles which facilitate lateral transport in the vascular system. Branch traces that supply lateral appendages branch from axial bundles and leaf traces (Fig. 7.12b).

With the information at hand, we can now interpret the pattern of zonation in a transverse section of a monocotyledon stem (Fig. 7.11a). The larger, more central bundles are major and minor axial bundles from which leaf and branch traces diverge. The peripheral zone of smaller bundles is a mixture of leaf and branch traces and in some taxa, cortical traces and bundles of fibers. Crowding in the peripheral zone is increased by the presence in it of segments of axial bundles distal to the levels of divergence of leaf traces (reduced in size in consequence of their branching in the formation of traces). The presence of these segments of axial bundles is related to the fact that some parts of all axial bundles are peripheral by virtue of their undulating, longitudinal course through the stem.

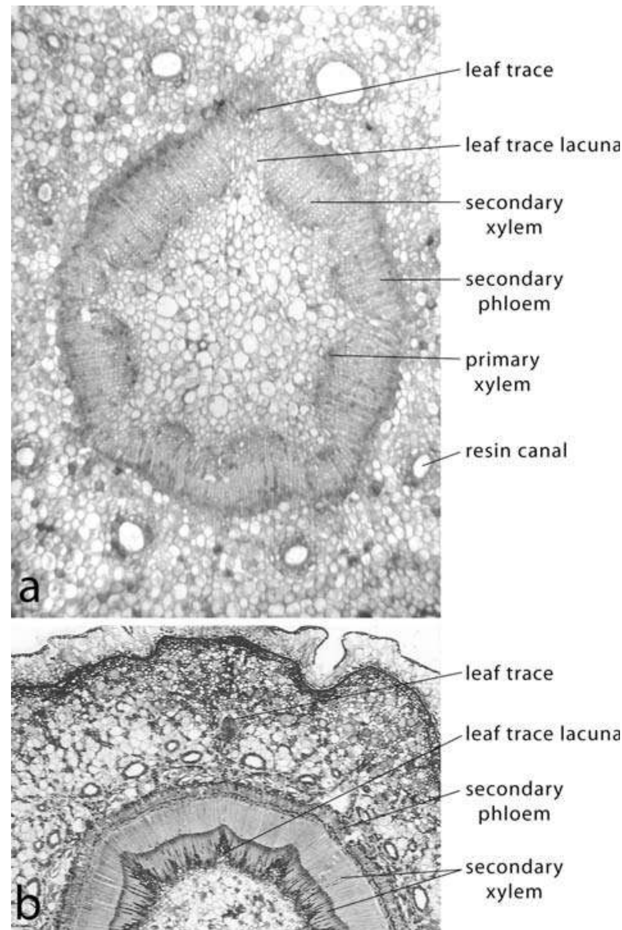


Figure 7.13 (a) Transverse section of a young stem of *Pinus* sp. (pine) showing a leaf trace lacuna across which the vascular cambium has not differentiated. Magnification × 47. (b) Transverse section of a 2-year-old stem of *Abies concolor*. The vascular cambium became continuous across the leaf trace lacuna toward the end of the first growing season. Magnification × 12.

Leaf trace lacunae

In contrast to most monocotyledons which produce only primary vascular tissues, most dicotyledons and all gymnosperms produce secondary vascular tissues as well which, as development proceeds, enclose the primary xylem in the stem and often make its analysis difficult. Typically, in these plants, however, the regions through which leaf traces pass are devoid of secondary vascular tissues (Fig. 7.13a) because, at this stage of development, the cambium has not differentiated across this region. These parenchymatous regions are called **leaf trace lacunae**. Unfortunately, in much of the older, and some of the recent botanical literature, these lacunae are confused with, and incorrectly labeled, leaf gaps. As we know, leaf gaps occur only in primary vascular cylinders, and among living plants are characteristic only of many ferns. Upon differentiation of vascular cambium across the lacunae, often toward the end of the first season of growth, secondary xylem becomes continuous (Fig. 7.13b).

A widely used terminology applied to the nodal structure of woody dicotyledons and gymnosperms describes the relationship of leaf traces that supply a leaf and the lacunae in the secondary vascular cylinder through which they pass. If each leaf is supplied by only one leaf trace, the nodal structure is described as **one trace, unilacunar** or, simply, **unilacunar** (Fig. 7.13a). This relationship can also usually be accurately determined by examining spread out diagrams of the primary vascular system as, for example in Fig. 7.6b which illustrates the nodal structure of *Abies concolor* which is also unilacunar. If each leaf is supplied by two traces, as in *Ginkgo biloba*, the nodal structure is described as **two trace, unilacunar** (Fig. 7.7). In situations in which a single leaf is supplied by three traces, each traversing a separate lacuna, as in *Drimys winteri* (Fig. 7.9a) and *Potentilla fruticosa* (Fig. 7.9b), the nodal structure is described as **trilacunar**. In some taxa the median trace of a trilacunar node is comprised of several closely placed small bundles that may fuse in the leaf base. If a single leaf is supplied by five or more leaf traces, the nodal structure is described as **multilacunar** (Fig. 7.8).

The cauline vs. foliar nature of vascular bundles in the eustele

As emphasized by Stein (1993) the evolution of the various stelar architectures in plants is considered to be related to, and correlated with, the evolution of leaf and branch size and complexity, and to hormonal control of differentiation of primary xylem and phloem. Stein proposed a model of stelar evolution based on control of differentiation as it is presumed to be related to auxin sources (lateral branches and leaves and, in the most primitive, leafless vascular plants, the apical meristems of branches), the size of the apical meristem, longitudinal spacing of primordia, phyllotaxis, the rates of auxin synthesis in the sources, the concentration and rates of flow of the hormone, and the reaction to the hormone by the target tissue.

Stein's analysis throws interesting light on the controversy regarding the nature and evolution of the eustele. As he notes, "the currently popular evolutionary perspective" interprets the eustele as a system of discrete sympodia, each consisting of an axial bundle from which leaf traces diverge (see Namboodiri and Beck, 1968a, 1968b, 1968c; Beck *et al*., 1983). The sympodia are considered to be cauline, that is, derived, evolutionarily, from stem vascular tissue. This viewpoint is based on comparisons of stelar morphology and leaf evolution of plants through time as demonstrated by the fossil record. The competing hypothesis considers the vascular bundles of the eustele to be composed solely of leaf traces and thus to be of foliar origin (see Esau, 1965). It is based on studies that show leaf primordia and young leaves to be primary sources of auxin that influence the differentiation of the primary vascular system. These two viewpoints may differ only in perspective, however. If one accepts that the most primitive vascular plants were leafless,

and that leaves evolved from lateral branch systems (see Chapter 1), it follows that the hormonal source in leafless plants as well as those exhibiting intermediate stages in the evolution of the leaf was the apical meristems of individual lateral branches. One may speculate that upon evolution of leaf blades (according to the telome hypothesis, by a process of "webbing"of branch systems; see Stewart and Rothwell, 1993) the source of auxin became established in the leaf primordia, which accordingly would be, from an evolutionary standpoint, immature, reduced, and highly modified lateral branch systems. At various stages in the evolution of the stele, therefore, the source of the hormone controlling differentiation of the vascular tissues of the stem and its branches, whether apical meristems of lateral branch systems or leaf primordia and young leaves, probably varied with the level of evolutionary specialization of the plant.

Phyllotaxy

Let us now consider in more detail the nature of vegetative **phyllotaxy**, or leaf arrangement. Phyllotaxy in the mature plant is directly related to the position and size of leaf primordia produced on apical meristems of varying size and shape. During growth of the shoot apex, the leaf primordia produced nearest the tip of the apical meristem are gradually shifted laterally toward the periphery, and new primordia develop in sites above the older primordia. Auxin or some other morphogen, such as expansin, is thought to influence the extensibility of cell walls in these sites on the surface of apical meristems destined to become leaf primordia. Masuda (1990) and Kutschera (1992) have shown that auxin increases the extensibility of epidermal cells, and Lyndon (1994) believes that auxin is the hormone most likely to cause epidermal wall extensibility in the surface layers of the apical meristem. Green (1985) has shown that in sites of incipient leaf primordia, cellulose microfibrils are oriented more or less tangential to the surface which, with wall loosening mediated by expansin (Fleming et al., 1999), apparently facilitates the outward development of the primordium as a bulge from the surface of the meristem (see also Lyndon, 1994). However, the mechanism by which the microfibrils attain their tangential orientation is not clear at present. Jesuthasan and Green (1989) proposed that the microfibrils in the surface layer of the apical meristem of *Vinca* (periwinkle) are reoriented by being stretched by the growth of older, adjacent leaf bases. This, however, is at odds with the hypothesis that microfibril orientation is directly related to the orientation of microtubules in the outer protoplast (see Baskin, 2001). Marc and Hackett (1991, 1992) provide evidence that in *Hedera helix* (ivy) gibberellin influences rearrangement of cortical microtubules in cells of the surface layer in regions destined to become leaf primordia with the result that cellulose microfibrils become oriented tangentially in the cell walls. It has been suggested, also, that recently formed primordia may produce inhibitors that prevent new primordia from developing until the field

of inhibition has diminished by virtue of the continued growth of the shoot apex and the lateral displacement of older primordia (Lyndon, 1994).

Factors such as hormonal influence on the extensibility of cell walls, orientation of microtubules and microfibrils that are directly related to the plane of cell division, and inhibition of new by older primordia all have a controlling influence on the position of leaf primordia in the shoot apex. Therefore, as suggested by Lyndon (1994), control of the sites of initiation of leaf primordia (and thus, control of phyllotaxy, the pattern of leaf arrangement) rests with "two cooperating systems, a chemical mechanism determining whether a primordium would form, and a bio-physical system determining precisely where it would form." The ultimate genetic control and details of the mechanism whereby genes influence the various factors involved in the establishment of phyllotaxy must await much additional research. (For more detail on the initiation and development of leaf primordia, please see Chapter 5.)

It is clear that auxin (and/or other plant hormones, e.g., gibberellin, cytokinin) produced by leaf primordia and young leaves has a controlling influence on the differentiation of the primary vascular system, and that there is a direct relationship between phyllotaxy and the arrangement of vascular sympodia. We know, also, that phyllotaxy may change within a plant as, for example, the apical meristem enlarges during growth of a seedling. It may also differ in the main stem and lateral branches.

In plants with helical phyllotaxy, leaf primordia are produced in sequence along a helical pathway called the **generative spiral**. It is useful to number the primordia indicating their sequence of development. Many workers assign a high number to the oldest primordium on the apical dome and progressively lower numbers to younger primordia along the generative spiral. Others prefer to number the primordia in reverse order. As we have seen in the spread-out diagrams of primary vascular systems, leaf traces can be similarly numbered. It is also apparent that the leaf trace pattern in the diagrams of primary vascular systems we have studied earlier in this chapter directly reflects the pattern of leaf traces and leaves on the stem surface.

The angle between radii drawn to the center of successively produced primordia is called the **angle of divergence**. On average, in plants with only one generative spiral (**monojugate plants**), the angle of divergence is approximately 137.5 degrees. In **bijugate plants**, with two generative spirals, one clockwise, the other counter-clockwise, the angle of divergence is 68.7 degrees.

A line connecting the primordia in the generative spiral is called a **parastichy**. In fact this term is applied to any helical line connecting leaf primordia. If the primordia in a helical series are in physical contact in a bud the parastichy is referred to as a **contact parastichy**. The generative spiral is a non-contact parastichy, and the parastichy characterized by the shallowest helix on an apical dome.

The leaf primordia in a shoot apex, as viewed from above, comprise two conspicuous sets of contact parastichies, one set spiraling to the

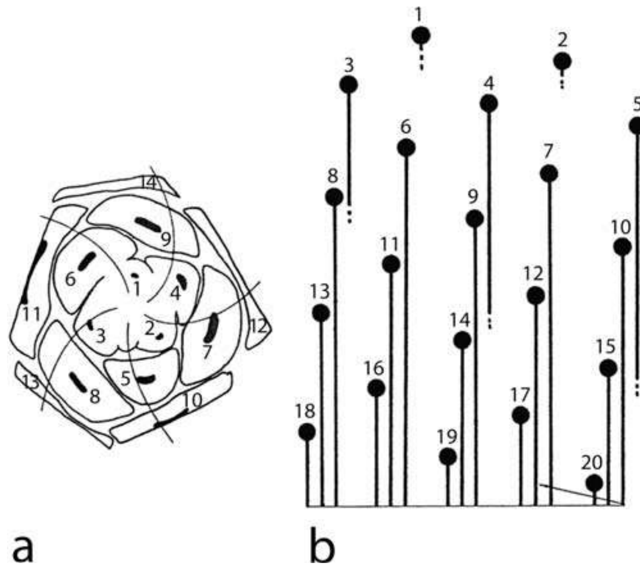


Figure 7.14 Diagrams of *Illicium parviflorum*. (a) A transverse view of a shoot apex showing leaf primordia arranged in two sets of contact parastichies, one set containing five parastichies spiraling to the left and the other containing three parastichies, spiraling to the right. (b) Diagram of the primary vascular system in longitudinal view. Please refer to the text for a detailed description. (a, b) From Benzing (1967a). Used by permission of the Botanical Society of America.

right, the other to the left, and one steeper than the other (Fig. 7.14a). The numerical difference between the primordia in these two sets is used to characterize the phyllotaxy of particular plants. The steeper parastichies (Fig. 7.14b), which include leaf primordia 1, 6, 11; 4, 9, 14; 2, 7, 12, etc. (indicated by curved lines in Fig. 7.14a), are characterized by primordia with numbers that differ by 5. The other set of parastichies which include leaf primordia 1, 4, 7, 10; 2, 5, 8, 11, etc., are characterized by numbers that differ by 3 (Fig. 7.14a, b). The phyllotaxy of this plant can, therefore, be indicated as 3 : 5 which tells us that the leaf primordia in each of the steep parastichies were initiated by the apical meristem along the generative spiral five plastochrons apart (a plastochron is the time between the initiation of successive primordia in a parastichy) and that leaf primordia in the other, shallower, parastichy were initiated three plastochrons apart. We can also conclude that every leaf primordium produced along the generative spiral occurs in one of the steep parastichies. The same logic can be applied to the shallower parastichies.

One can often recognize more than two sets of parastichies in the shoot apices of most plants with helical phyllotaxy. The steeper the parastichy, the higher the characterizing number. The steepest parastichies, in which mature leaves would be nearly directly above each other, are called **orthostichies**. If in the example above, the steep parastichies were, in fact, orthostichies, we would observe that the leaves in each would be separated by five internodes (Fig. 7.14b).

Most commonly, leaf primordia occur in 1, 2, 3, 5, 8, or 13 parastichies. These numbers comprise the mathematical series known as the **primary Fibonacci series**, named after the Italian mathematician Leonardo Fibonacci. Addition, in sequence, of any two numbers of this series will give the number following the preceding pair. The denominator of common phyllotactic fractions, 5, 8, or 13, indicates the number

of orthostichies and, as we know, also the number of vascular sympodia in the primary vascular system. The numerator represents the number of turns around the stem and along the generative spiral between successive leaves in an orthostichy, usually 2 as in 2/5, 3 as in 3/8 and 5 as in 5/13.

In plants with decussate phyllotaxy, the leaf primordia develop in pairs 180 degrees apart, and successive pairs occur perpendicular to each other. In plants with distichous phyllotaxy, primordia are also 180 degrees apart, but occur in only one plane. The concept "angle of divergence" is usually not applied to primordia of plants with decussate or distichous phyllotaxy since, during development, the leaf primordia are not clearly arranged in a helical pattern.

The concepts of the generative spiral, parastichies, and orthostichies that have been applied to phyllotactic systems are important because they not only have a mathematical, but also a biological basis. For more detail, and in-depth discussions of the mathematical and biological bases for phyllotaxy, see Richards (1951) and Romberger *et al.* (1993).

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Chapter 8

The epidermis

Perspective

Most terrestrial plants live in a highly evaporative environment and one in which they are constantly exposed to toxic substances, to attack and invasion by various small insects and pathogens, to the potentially damaging effects of solar radiation, and to potential damage from high winds. Consequently, several protective tissues have evolved that reduce water loss from the plant, restrict the entry of organisms and toxic substances into the plant body, mitigate the effects of radiation, and strengthen and support the plant thereby reducing its susceptibility to damage from rapid air movement. These include the epidermis of shoot and root systems (sometimes called rhizodermis in the root), the periderm and the rhytidome. These tissues, while providing these functions, must also under certain conditions allow oxygen used in respiration to enter the plant and carbon dioxide utilized in photosynthesis to exit the plant. Consequently, the epidermis and other superficial, protective tissues represent both structural and functional compromises. As the bounding tissue of all young parts of a plant, and of the aerial parts of plants that are comprised solely or largely of primary tissues, the epidermis also provides an important supporting function. In the stem of *Tulipa* (tulip), for example, the epidermis plus a layer of subepidermal collenchyma can contribute as much as 50% to overall stem stiffness (Niklas and Paolillo, 1997). We shall consider the epidermis in some detail in this chapter, and periderm and rhytidome in Chapter 13.

Epidermis of the shoot

In the shoot, the **epidermis** develops directly from the protoderm (Fig. 8.1). In most plants it is composed of a single layer of specialized cells that comprises the outer layer of all parts of the shoot system in regions distal to the development of periderm. The epidermis consists largely of living parenchyma cells of various shapes, usually tabular (i.e., with a narrow radial dimension and comparatively large

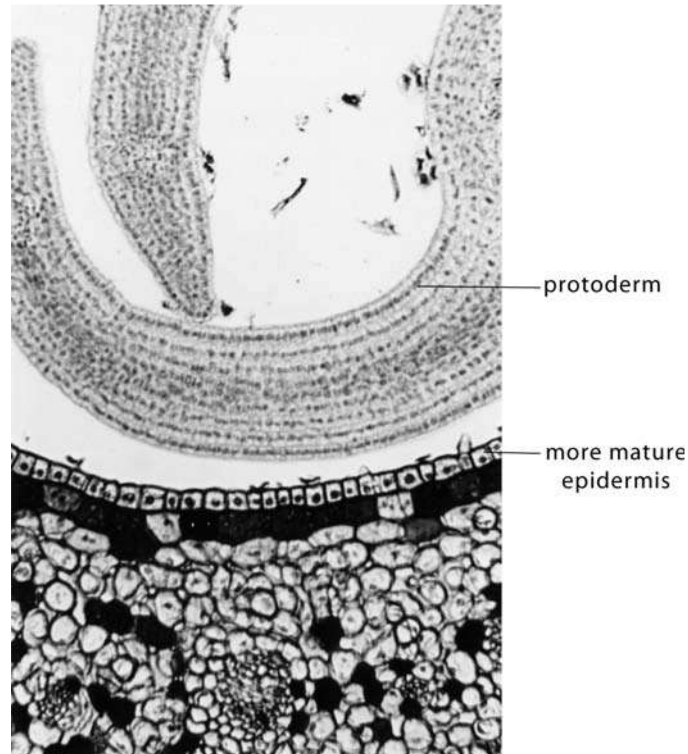


Figure 8.1 Two stages in the development of the epidermis of a leaf. Magnification × 234.

inner and outer surface areas), and frequently with wavy anticlinal walls (especially in leaves and petals). Interspersed in the parenchymatous groundmass of leaves are stomata (Fig. 8.2) (most commonly in the lower epidermis) and associated subsidiary cells. In grasses and many other monocotyledons, and on elongate parts (e.g., stems, petioles, midribs, etc.) of most vascular plants, epidermal cells are usually elongate (Fig. 8.3) with the long axis parallel to the long axis of the plant structures. The sinuous nature of the walls in some gymnosperms (Fig. 8.3a) and most grasses (Figs 8.3b, 8.4) results from the synthesis during development of thickened bands of cellulose microfibrils that extend across the anticlinal walls between inner and outer periclinal walls. During growth, the epidermal cell walls expand between the bands (Panteris *et al*., 1994). Epidermal cells of grasses may contain **idioblasts** (single or small groups of specialized cells) such as fibers, as well as cork and silica cells (Fig. 8.4) which often occur in pairs. The function of the non-living **cork cells**, characterized by suberized walls, is unknown. The SiO_2 in the **silica cells**, often deposited in laminae, is the basis of the abrasive nature of many grasses. Specialized epidermal cells may also contain pigments, oils, crystals, tannins, etc. Grasses are also characterized by the presence of **bulliform cells** (Fig. 8.5), large thin-walled cells which, during expansion growth, contribute to the unrolling of young leaves. In mature leaves during periods of drought bulliform cells seem to participate in **involution** (the rolling up of leaves) by losing water, becoming flaccid, and thereby facilitating the process.

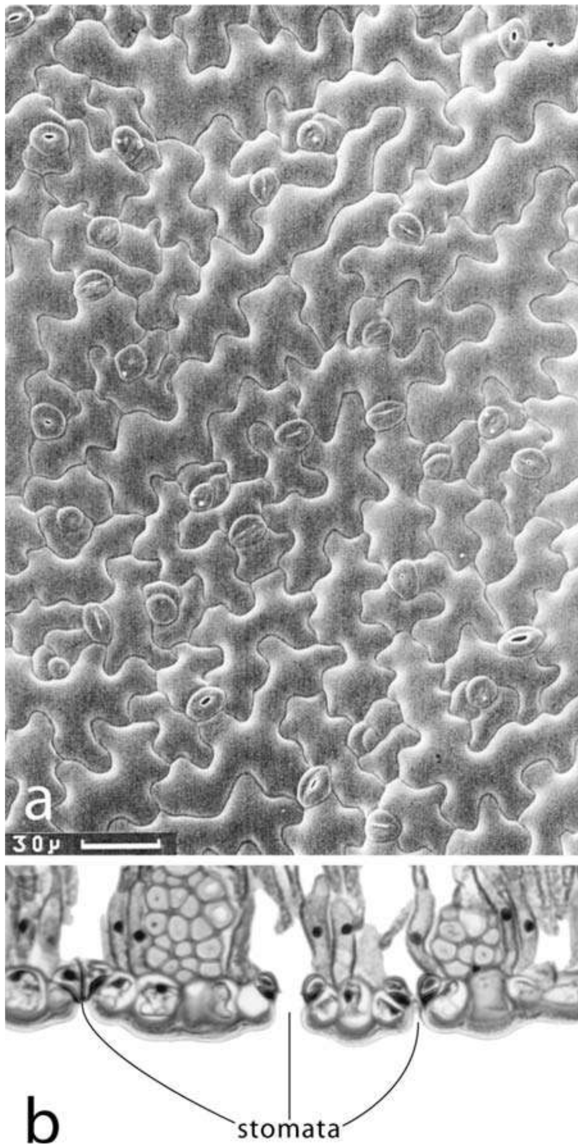


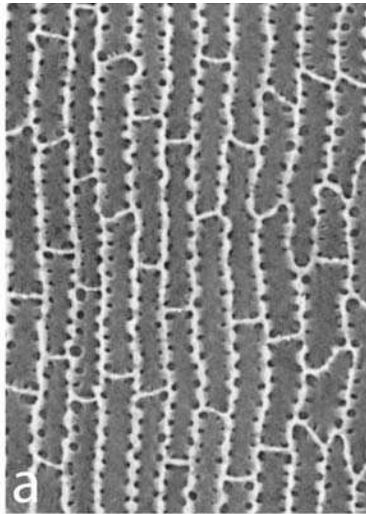
Figure 8.2 (a) Surface view of the epidermis of a leaf of *Arabidopsis thaliana*. Note the stomata, the undulating anticlinal walls of the epidermal cells, and the absence of intercellular spaces (except between guard cells). (b) Section of the leaf of *Welwitschia mirabilis* illustrating stomata in sectional view in the lower epidermis. Magnification × 260. (a) From Bowman (1994). Used by permission of Springer-Verlag Heidelberg. © Springer-Verlag Berlin Heidelberg.

Extending from the epidermis are trichomes, or hairs, varying in form, cell composition, and function, which we shall discuss in a later part of this chapter.

Some plants possess a multilayered epidermis (Fig. 8.6), called a **multiple epidermis** (as, for example, in *Ficus*, members of the Moraceae, Pittosporaceae, Piperaceae, etc.). The **velamen** of the aerial roots of orchids is also a multiple epidermis. A multiple epidermis develops by the production in the protoderm of periclinal and oblique cell walls during cytokinesis. In addition to the usual functions of an epidermis, the multiple epidermis is adapted for water storage.

As one might expect in a surface layer, there are no intercellular spaces in the epidermis except the stomatal apertures (the openings

Figure 8.3 (a) Inner surface of the cuticle of a leaf of *Dacrydium guillauminii*, a gymnosperm, showing the elongated form of the epidermal cells and the sinuous nature of the cell walls. Magnification × 247. (b) Scanning electron micrograph of the surface of a leaf of *Sorghastrum balansae*, a grass, illustrating the sinuous nature of the cell walls, and the parallel rows of stomata. Bar = 20 μm. (a) From Stockey and Ko (1990). Used by permission of the University of Chicago Press. © 1990 The University of Chicago. All rights reserved. (b) From Dávilla and Clark (1990). Used by permission of the Botanical Society of America.



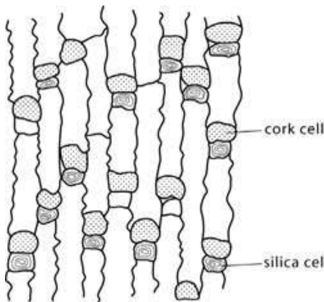


Figure 8.4 Upper epidermis of *Saccharum* consisting of elongate cells interspersed with pairs of cork cells and silica cells.

between guard cells), an adaptation that results in the restriction of water loss. Other adaptations that inhibit water loss are the highly specialized outer walls of the epidermal cells, and the presence of the **cuticle** that covers the outer surface of the epidermis (Fig. 8.6a, b). The outer cell walls consist usually of several distinct and heavily lignified layers which may contain cutin, waxes, soluble cuticular lipids, proteins, and mineral incrustations (Fig. 8.6b) (Tenberge, 1991). The cuticle consists of **cutin**, an insoluble polymer, and soluble waxes (aliphatic compounds), and it is largely but not entirely impermeable to the passage of water and other small molecules. It varies in thickness from less than 1 μm to 15 μm or more, and its thickness seems to be related to the environment in which the plant lives. For example, plants living in moist conditions usually have thin cuticles whereas those that live in arid conditions often have thick cuticles.

In some plants the innermost layer of the cuticle has a high content of **pectin** (easily detected by staining with ruthenium red) which

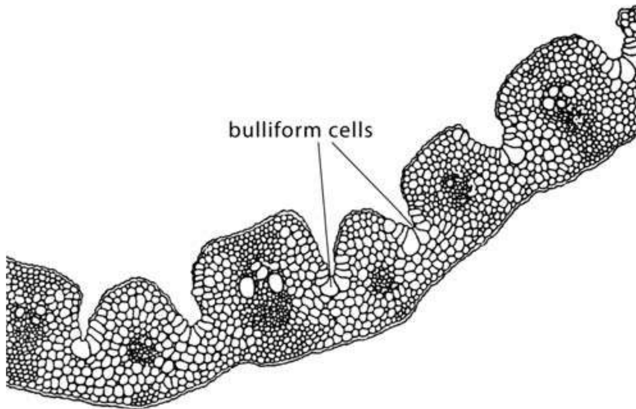


Figure 8.5 Section of a leaf of *Stipa robusta*, a grass, containing bulliform cells in grooves in the adaxial surface.

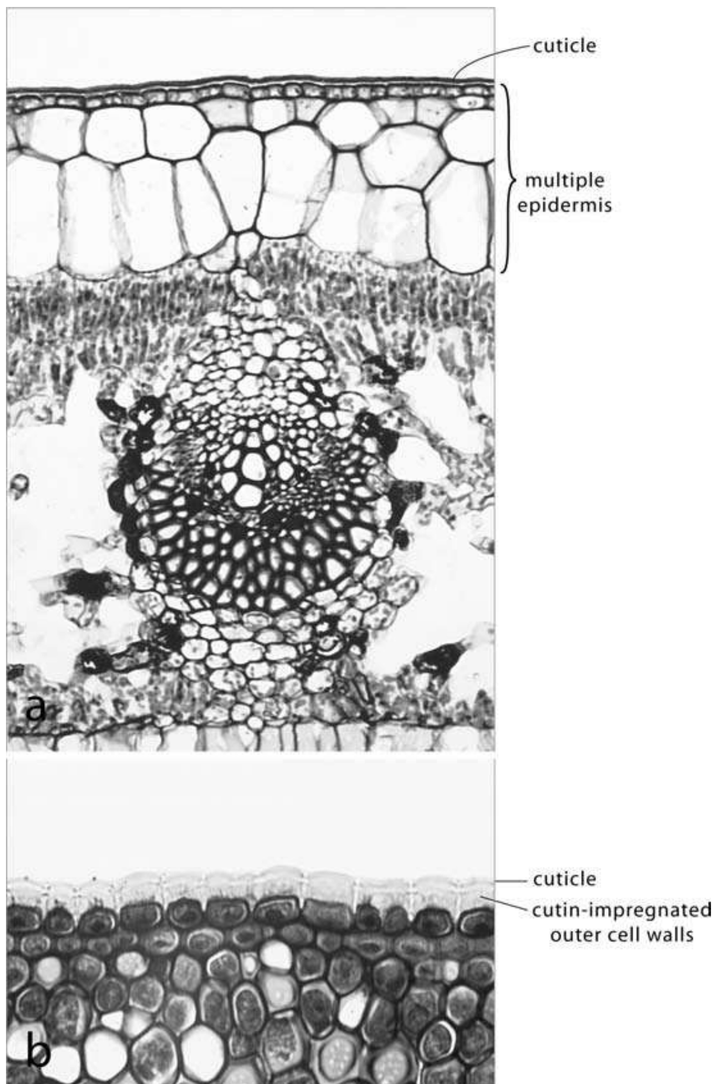


Figure 8.6 (a) A multiple epidermis of the leaf of *Ficus*. Note the thin cuticle. The large cells below the outer layer, lacking chloroplasts, are considered to function in water storage. Magnification × 193. (b) The epidermis of a stem of *Smilax*. The outer periclinal walls of the epidermal cells are impregnated with lignin and cutin, and have become greatly expanded. Overlying the expanded cell walls is a thick cuticle. Magnification × 167.

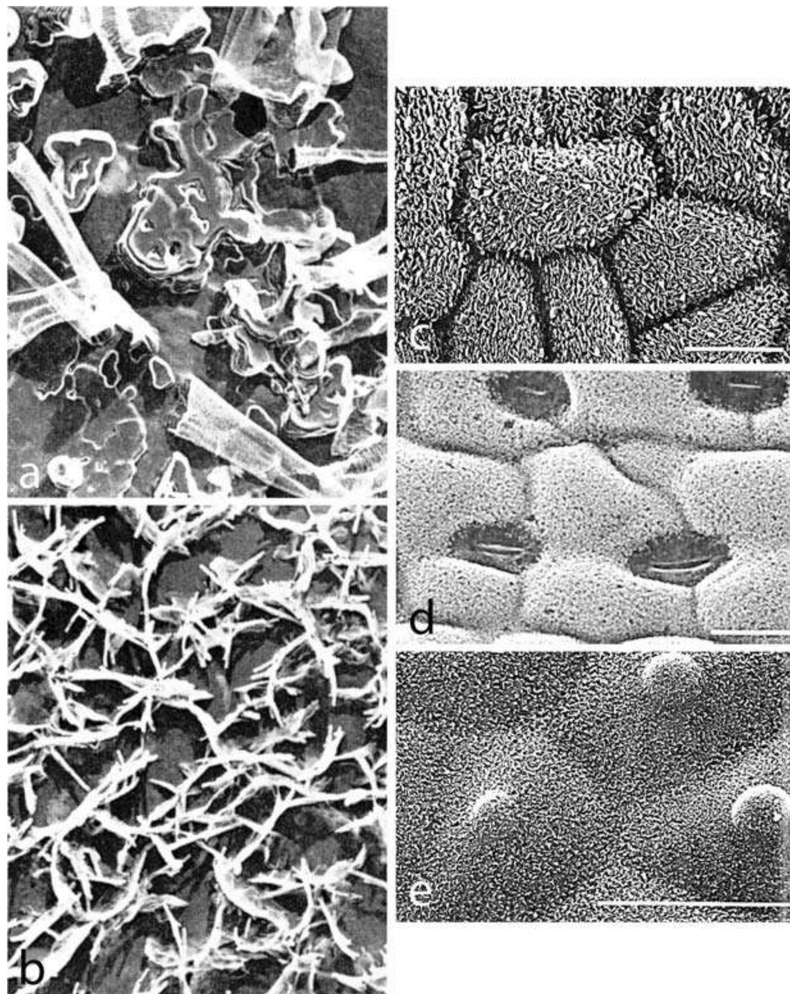


Figure 8.7 (a, b) Scale-like wax platelets on the adaxial surface of the leaf of *Brassica oleracea* (a), and *Lupinus albus* (b). Magnification × 9206. (c–e) Characteristics of water-repellent leaf surfaces. (c, d) Epidermal cells with convex outer walls covered by a dense layer of epicuticular waxes in *Hypericum aegypticum* (c) and *Marsilea mutica* (d). Note the stomata in *Hypericum*. Bars = 10 μm. (e) Epidermal cells with convex outer walls with conspicuous papillae covered by epicuticular waxes in *Lupinus polyphyllos*. Bar = 50 μm. The covering of fine wax platelets makes the leaf surfaces hydrophobic. Consequently, water and contaminating particles flow off because of the reduction in adhesion to the leaf surfaces. (a, b) From Juniper (1959). Used by permission of Elsevier. (c–e) From Neinhuis and Barthlott (1997). Used by permission of Oxford University Press.

often impregnates the outer laminae of the epidermal cell walls. In the cuticle, pectin decreases in concentration outwardly whereas the wax content increases outwardly, and the outer surface layer consists solely of **epicuticular wax**. Highly magnified, the wax can be seen to consist of scale-like platelets (Fig. 8.7a, b) of various forms, but without magnification it may appear smooth and glossy. How the precursor compounds of cutin and wax move from the plasmalemma through the cell wall and to the exterior is not clearly understood. It has been suggested that, during development, minute **cuticular pores** (often called microchannels) facilitate the transfer of wax to the surface of the cuticle, but Neinhuis *et al.* (2001) provide indirect evidence that molecules of wax and water simply move together through the cuticle under the influence of cuticular transpiration.

Whereas the major role of the cuticle and intracuticular waxes is restriction of water loss from the plant, the epicuticular waxes play a significant additional role in reflecting light, thus reducing the possibility of overheating and excessive water loss through transpiration

(see Barnes and Cadoso-Vilhena, 1996; Neinhuis *et al.*, 2001). The epicuticular wax also "waterproofs" the plant, i.e., makes it water-repellent (Fig. 8.7c–e). Consequently, leaves are "self-cleaning." According to Neinhuis and Barthlott (1997), during rainfall, water and contaminating particles flow off the leaf because of the consequent reduction in adhesion to the hydrophobic surfaces. Variations in the structure of epicuticular wax are thought to correlate with specific epicuticular wax alleles in *Sorghum bicolor* (Jenks *et al.*, 1992) and therefore could be useful taxonomic characters. Epidermal cell patterns, imprinted in great detail on the inner surface of the cuticle, are also used as taxonomic characters in some gymnosperms as, for example, in *Dacrydium* (Podocarpaceae) (Fig. 8.8a, b) (Stockey *et al.*, 1998).

In some ferns (e.g., members of the Polypodiaceae which thrive in conditions of low light intensity) as well as in a few aquatic angiosperms epidermal cells contain chloroplasts and thus function in photosynthesis.

Since the epidermis comprises the outer covering of plants and plant parts in contact with the atmosphere, cell development is more complex than that of internal parenchymatous cells. During differentiation, because of their generally tabular form the anticlinal walls reach their greatest extent sooner than the periclinal walls which have a considerably greater surface area and grow over a longer period of time. Furthermore, the outer periclinal walls, containing cutin and waxes, differ in chemical composition, and are often thicker than inner walls. Consequently, genetic and other control mechanisms must not only direct the differential synthesis of precursor compounds as well as the production of secretory vesicles but must also control the transfer of the correct compounds to the appropriate sites of wall synthesis (see Wojtaszek, 2000).

Trichomes in the shoot system are appendages of the epidermis. They may remain alive or may die following development. Unlike root hairs, which are extensions of single epidermal cells, trichomes of the shoot consist of one or more cells. They may be simple, consisting of only a single non-epidermal cell, or remarkably complex and multicellular. Some are glandular, others non-glandular. We shall defer consideration of glandular hairs to Chapter 15 on secretion in plants and emphasize non-glandular hairs in this chapter. Some non-glandular trichomes develop from cells of the protoderm as in *Gossypium* (cotton), others are initiated by cell division in a mature epidermal cell. They vary in both function and morphology, and may be branched, unbranched, or peltate. **Unbranched hairs** are either unicellular (Fig. 8.9a) or multicellular (Fig. 8.9b) and sometimes spine-like. **Branched trichomes** are often digitate or stellate (Fig. 8.9c). **Peltate hairs** are scale-like consisting of a group of cells forming a plate oriented parallel to the epidermal surface and attached by a very short stalk, often consisting of a single cell. Hairs occur on all parts of the plant including floral organs. The function of non-glandular trichomes is poorly understood. Dense coverings of non-living, air-filled, branched hairs such as those of *Verbascum* (mullein) tend to hold a layer of vapor-filled air on the surface of the

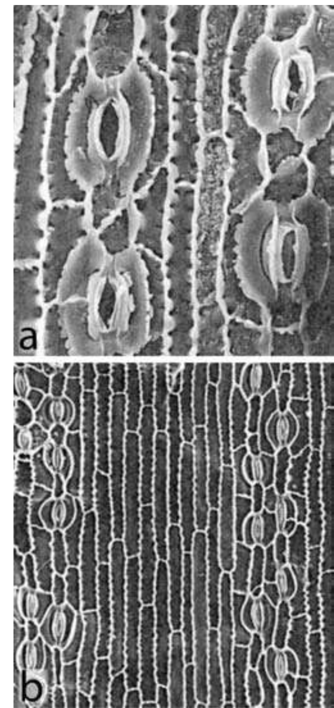


Figure 8.8 Variation in epidermal cell patterns on the inner surface of the cuticle of species of *Dacrydium*, a gymnosperm (Podocarpaceae), considered useful taxonomic characters in this family. (a) *D. guillauminii*. Magnification × 278. (b) *D. lycopodioides*. Magnification × 118. From Stockey and Ko (1990). Used by permission of the University of Chicago Press. © 1990 The University of Chicago. All rights reserved.

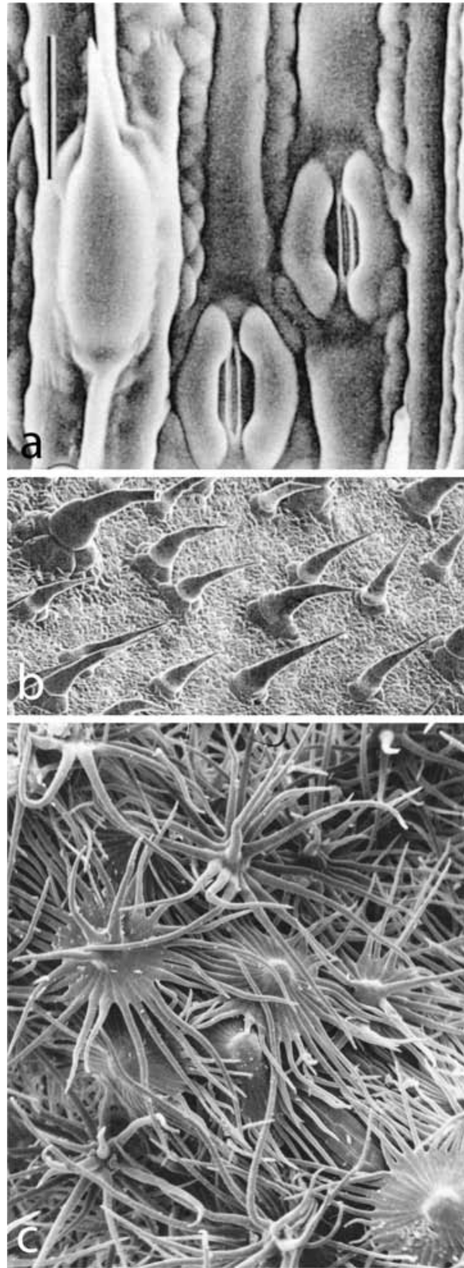


Figure 8.9 Non-glandular epidermal trichomes. (a) A unicellular trichome of *Sorghastrum contractum*. Bar = 20 μm. (b) Multicellular, unbranched trichomes on the abaxial surface of a leaf of *Cucumis sativus*. Magnification × 136. (c) Multicellular, branched trichomes on a leaf of *Verbascum* (mullein). Magnification × 50. (a) From Dávilla and Clark (1990). Used by permission of the Botanical Society of America. (b) From Troughton and Donaldson (1972). Used by permission of the New Zealand Ministry of Research, Science and Technology. (c) Photograph by P. Dayanandan.

epidermis that is thought to reduce transpiration, and thus desiccation, in dry and/or windy conditions. The presence of such pubescent layers, especially on leaf surfaces may, under certain conditions, be a very important supplement to the cuticle in inhibiting water loss from the plant although there is little experimental evidence to support this viewpoint. It seems very likely, however, that a dense pubescence would reflect light and thus, prevent overheating of the plant. It has been suggested that trichomes may also prevent or restrict insect predation.

The vesiculate hairs of saltmarsh plants such as *Atriplex* (saltbush) are repositories of salt which prevent the build-up of toxic levels of salt in the plant. Epidermal trichomes are also useful in taxonomy (e.g., Amarasinghe *et al*., 1991).

Epidermis of the root

The **root epidermis**, often called the rhizodermis, differs from that of the shoot in development as well as in structure and function. In monocotyledons the epidermis originates from a tier of meristematic cells at the root tip which also gives rise to the cortex. In dicotyledons the epidermis originates from an apical tier of cells which also gives rise to the root cap. In each case, the outermost layer of cells derived from these meristematic regions comprises a protoderm from which the epidermis ultimately develops.

In the absence of a cuticle, the epidermis of the root in most plants differs significantly from that of the shoot. The presence of a very thin, cuticle-like layer, however, has been observed in a few taxa. Furthermore, there are usually no, or only a very few, stomata in the root epidermis. These structural differences are, of course, directly related to the absorptive function of the root, and to the fact that roots of most plants are subterranean, non-photosynthetic, and not subject to the desiccating effects of the aerial environment. Root hairs, unlike the trichomes of the shoot, are simple extensions from single epidermal cells. Root hairs develop from specialized cells in the epidermis called **trichoblasts**, and occupy a position between the root tip and the level of the root at which periderm develops. Absorption occurs through the root hairs which greatly expand the absorptive surface of the root, but absorption also occurs through all outer surfaces of the epidermal cells. The percentage of absorption that occurs directly through root hairs and other root surface areas is unknown. In most plants, however, it is clear that a great part of all absorption occurs through the fungal hyphae of mycorrhizae that may extend out from the root in all directions over a large area. As the root elongates, the more proximal root hairs cease to function and new root hairs develop acropetally in the distal part of the root hair zone. We shall discuss in more detail the development of root hairs as well as the role of mycorrhizae in root absorption in the chapter on the root (Chapter 16).

Stomata

Stomata (Figs 8.2a, b, 8.8a, b, 8.10a, b) occur in the epidermis of all parts of the shoot system, even in flower parts such as stamens and pistils. They occur in great frequency in leaves where they are most abundant in the lower epidermis. A stoma consists of the **stomatal aperture** and two enclosing **guard cells**. Some workers prefer to use the term “stoma” for the aperture or pore only, and “stomatal apparatus” for the stoma

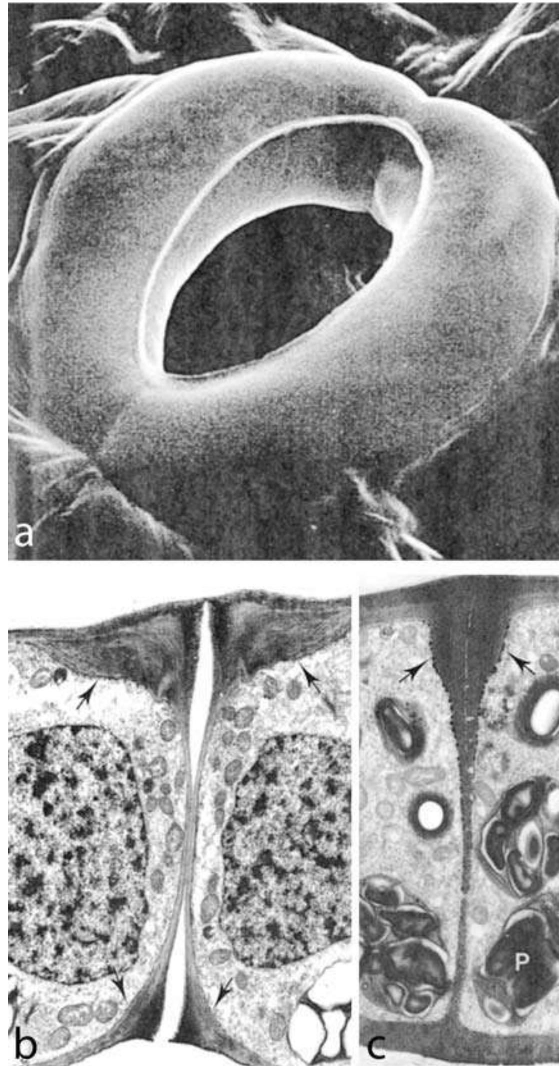


Figure 8.10 (a) Scanning electron micrograph of a stoma from the epidermis of *Cucumis sativus* showing two expanded, kidney-shaped guard cells enclosing an open stomatal aperture. Magnification × 224. (b) Transverse section of a stoma of the fern *Asplenium nidus*, passing through the closed stomatal aperture. Note the thickened cell wall regions (arrows), the large nuclei, and the numerous mitochondria. Magnification × 4260. (c) Section through one of the ends of a stoma of *Asplenium nidus* where the guard cells are connected to each other. Note the wall thickenings (arrows) and the large plastids (p) containing starch grains. Magnification × 4714. (a) From Troughton and Donaldson (1972). Used by permission of the New Zealand Ministry of Research, Science and Technology. (b, c) From Apostolakos and Galatis (1999). Used by permission of Blackwell Publishing, Ltd.

plus the guard cells. Because stoma (singular) and stomata (plural) are used so extensively in the plant physiology and plant development literature to mean aperture plus guard cells, they will be used that way in this book. The stomatal aperture, when open, allows CO_2 to enter the leaf and results in water loss through transpiration.

With the exception of those in grasses and sedges, the stomata of all plants are very similar. The guard cells are kidney-shaped, and the cell walls in the polar regions (where the ends of the pair of cells are attached) are thicker than other parts of the cell wall (Fig. 8.10b). Unlike other cells in the epidermis of most plants, the guard cells contain chloroplasts in which starch grains may develop (Fig. 8.10c). At maturity the guard cells may be at the same level in the epidermis as the surrounding subsidiary cells, raised above them (Fig. 8.10a), or sunken

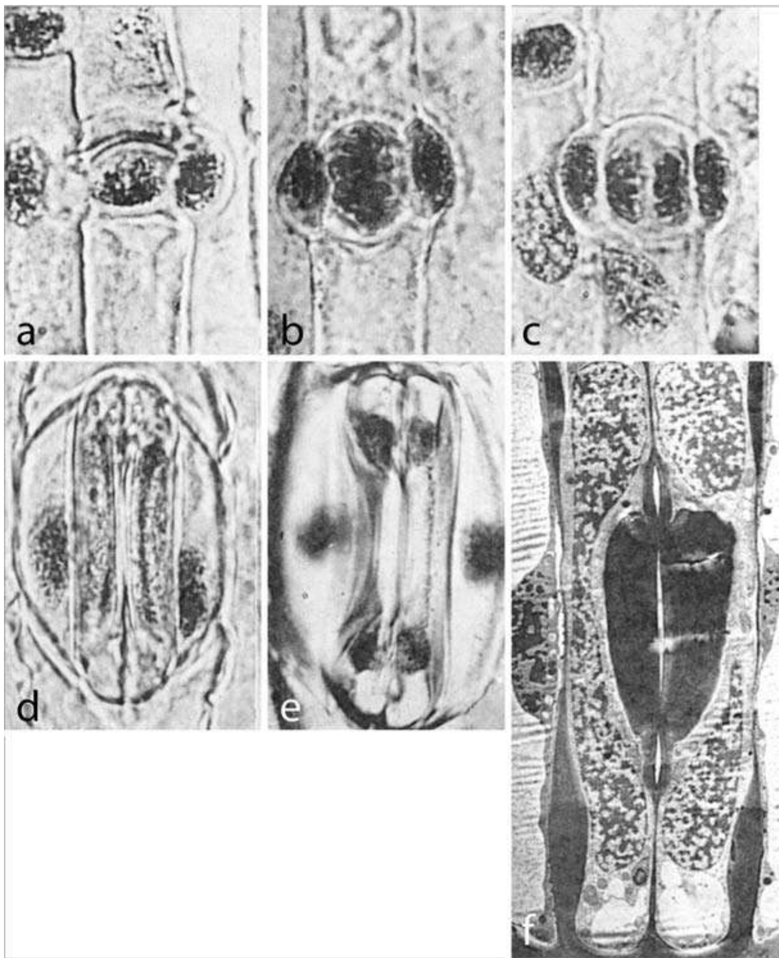


Figure 8.11 Perigenous development of a stoma in the epidermis of the internode of *Avena*, a grass. (a–c) Guard cells and subsidiary cells originate independently from different mother cells. (d) Nearly mature stoma and associated subsidiary cells. (e) Mature stoma. On the basis of the arrangement of guard cells and subsidiary cells in the mature state, and without knowledge of early developmental stages, one would probably conclude, incorrectly, that development of the grass stoma was mesogenous. (f) Enlargement of a mature stoma of *Avena*. Note the elongate nucleus and the thick walls opposite the stomatal aperture. Magnification (a–e) × 1333, (f) × 4425. From Kaufman *et al.* (1970). Used by permission of the Botanical Society of America.

below them (Fig. 8.2b). Commonly, immediately below the stoma there is a large air space in the leaf mesophyll called the **substomatal chamber** (Fig. 8.2b).

In contrast to the kidney-shaped guard cells of most plants, grasses and sedges have guard cells of very different morphology. The ends of the guard cells are thin-walled and bulbous whereas the central region is narrow and thicker-walled (Fig. 8.11e, f). Consequently, the cell resembles somewhat a dumb-bell. When the bulbous ends are turgid and expanded, the aperture is open; when not expanded, the aperture is closed. The shape of the nucleus conforms to that of the cell with the ends enlarged and connected by a slender strand (Fig. 8.11f). An especially interesting aspect of the grass stoma is the fact that the protoplasts of the guard cells are connected through a pore in the common walls of the bulbous ends (polar regions). Consequently changes in turgor pressure occur nearly simultaneously in the two guard cells.

Signal transduction and movement in guard cells

Because plants are immobile, they must have the ability to adapt to the variously changing environmental conditions where they live. Extensive studies (e.g., Schroeder *et al.*, 2001) have demonstrated that **signal transduction** (transmission of, and response to, environmental stimuli) in guard cells regulates the opening and closing of the stomatal aperture by controlling the increase or decrease in turgor pressure in the pair of enclosing guard cells. As turgor increases the guard cells expand away from each other, opening the aperture, allowing the intake of CO_2 and resulting in a consequent loss of water. Upon loss of turgor pressure, the aperture closes. Mechanisms of signal transduction integrate the many stimuli that control stomatal opening and closing. Among these are CO_2, blue light, hormones such as abscisic acid, auxin, cytokinin and gibberellins, water availability, and plant pathogens.

Until relatively recently, the direct cause of increase in turgor pressure in guard cells had been thought to be the result of an increase in concentration of photosynthate in the protoplast. It is now known, however, that photosynthate is utilized primarily as a source of energy for the guard cells; and excellent evidence indicates that the primary solute that controls turgor in the guard cells is potassium ions (K^+). In the presence of blue light, as anions of malic acid and chloride accumulate in the guard cell vacuoles, potassium ions migrate into the vacuoles, neutralizing the anions, and with an increase in concentration of K^+ in the cells osmosis occurs, resulting in increase in turgor pressure. Consequently, the cells swell; but since the ends of the guard cells are attached they bulge away from each other resulting in opening of the aperture. Stomata close upon decrease in turgor pressure caused by an efflux of K^+, mediated by the hormone abscisic acid.

Subsidiary cells, surrounding the guard cells, are the source of water and ions during the process of stomatal opening. Interestingly, there are very few plasmodesmata, in some cases none at all, between the subsidiary cells and the guard cells. Consequently, the movement of water and ions into and out of the guard cells is through the cell wall apoplast and the ion channels in the plasma membrane and tonoplast. As in the case of transfer cells, the walls of the subsidiary cells contiguous with those of the guard cells often have extensive infoldings which results in great increase in the area of the plasma membrane, thus facilitating the process.

Whether the stoma is open or closed is correlated with CO_2 concentration in the substomatal chamber. During periods of photosynthesis when CO_2 is utilized and the concentration in the substomatal chamber is low, the stomata are open, but during periods of very low light intensity when the CO_2 concentration is high, the stomata close. They also close under conditions of water stress which usually occurs when the temperature is high and air movement is great, thus reducing water loss through the stomatal apertures. During such conditions, although

photosynthesis may be occurring and CO_2 is being utilized, the concentration in the substomatal chambers may also be high since the air movement increases the available supply of CO_2 thus steepening the gradient of CO_2 into the leaf.

Development of stomata

As a leaf primordium grows, stomata develop in one of three ways. Following an unequal cell division in the protoderm, the smaller cell may divide followed by another division in each daughter cell to form four cells. The two inner cells will become guard cells, the two outer, subsidiary cells. This type of development, in which cells of the stoma and subsidiary cells are derived from the same mother cell, is referred to as **mesogenous development** (for some variations, see Carr and Carr, 1991). It is characteristic of many gymnosperms, in which this pattern of development is also termed **syndetocheilic**. If the guard cells and the subsidiary cells are derived from different protodermal initials, development is referred to as **perigenous**. This pattern is common in many dicotyledons. In gymnosperms this pattern of development is also called **haplocheilic**. In some plants in which one of two subsidiary cells has the same origin as the guard cells, development is termed **mesoperigenous**.

One is not always able accurately to determine these patterns of development by observing mature stomata. In grasses, for example, the guard cells and the two adjacent subsidiary cells appear to have had a common origin and to have developed mesogenously. In fact, the guard cells and subsidiary cells originate from separate initials and, thus, the development of stomata is perigenous (Fig. 8.11a–e) (Kaufman *et al*., 1970).

The form of guard cells is directly related to the presence, during development, of microtubules (often associated with actin microfilaments) immediately below the plasma membrane that mirror the synthesis of cellulose microfibrils in the cell walls. Both shape and function are related to variable thickness of the cell walls and the effect this has on the expansion of the cells during growth and during fluctuations in turgor pressure. This association of microtubules with cell wall synthesis adjacent to the developing pore in the stomata of the fern *Asplenium nidus* (Fig. 8.12a, b) has been beautifully demonstrated by Apostolakos and Galatis (1999). An association of microtubules with wall synthesis in developing stomata of *Avena sativa* (oats) was demonstrated as early as 1970 by Kaufman and co-workers.

Several different patterns of guard cells and subsidiary cells have been recognized, and used for taxonomic purposes (see Metcalfe and Chalk, 1950: vol. I, p. xiv). We shall consider the four most common types (Fig. 8.13), referred to as anomocytic (irregular), also called "ranunculaceous" (because this type is of frequent occurrence in the Ranunculaceae); paracytic (parallel), also "rubiaceous"; anisocytic (unequal), also "cruciferous"; and diacytic (cross-celled), also "caryophyllaceous".

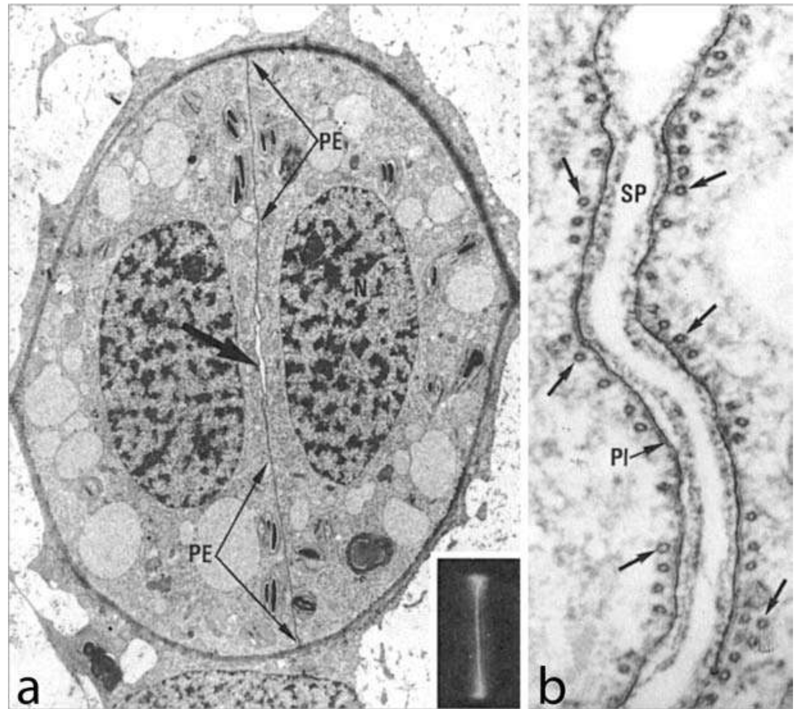
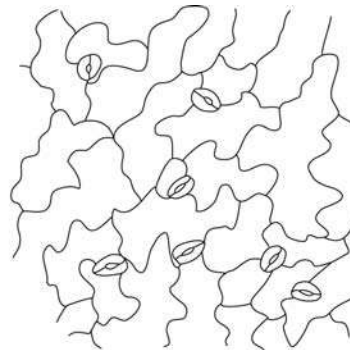
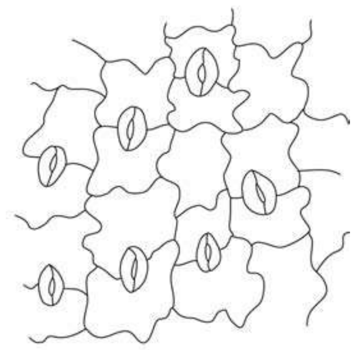
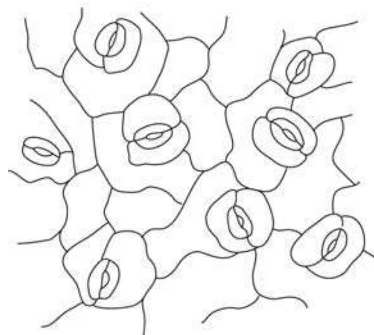


Figure 8.12 (a) Paradermal section of an immature stoma of the fern *Asplenium nidus*. Thin arrows delimit polar ends (PE) between which is the developing aperture (thick arrow). Magnification × 743. (b) Enlargement showing stomatal aperture at approximately the same developmental stage as that shown in (a) and the associated microtubules (arrows). Pl, plasma membrane; Magnification × 66 340. SP, developing aperture. From Apostolakos and Galatis (1999). Used by permission of Blackwell Publishing, Ltd.







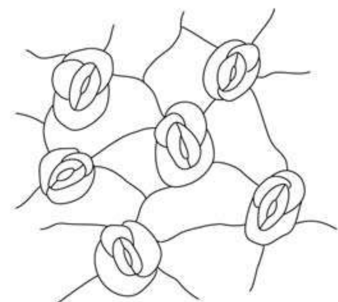


Figure 8.13 Diagrams illustrating the relationship between guard cells and associated subsidiary cells. Please see the text for a detailed explanation.

In the **anomocytic** type the guard cells and subsidiary cells have no well-defined pattern; or, from a developmental standpoint, there may be no subsidiary cells. The **paracytic** type is characterized by subsidiary cells and guard cells having parallel long axes. The **anisocytic** type is distinctive in having three subsidiary cells each of a different size. In the **diacytic** type, there are two subsidiary cells that have contiguous walls that are at right angles to the long axis of the guard cells, and which enclose the guard cells.

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Chapter 9

The origin of secondary tissue systems and the effect of their formation on the primary body in seed plants

Perspective: role of the vascular cambium

As the vascular cambium becomes active and secondary tissues are formed, the consequent increase in diameter of the stem may have profound effects on the primary body. This is especially true in woody, arborescent taxa among conifers and dicotyledons. The vascular cambium is an extensive, permanent secondary meristem, one cell thick, conical in form, often described as cylindrical, that begins its development between primary xylem and primary phloem. In most gymnosperms and dicotyledons it is present in all main stems and roots and their branches, extending from near their tips to the bases of stems and roots. In some woody plants it even extends into leaf petioles. In most woody taxa it differentiates first in developing vascular bundles at about the same time as metaxylem begins its development (Figs 9.1a, b, 9.2), that is, after elongation in the provascular strands has ceased. This **fascicular cambium** may become active, producing some secondary xylem and phloem before cambial differentiation occurs between the bundles (Fig. 9.1b), that is, in the **interfascicular regions**. In many woody, arborescent taxa, additional provascular strands differentiate between the initial vascular bundles, often so close together that they may contact each other laterally (Fig. 9.1b, c). The vascular cambium then becomes continuous across the vascular bundles (Fig. 9.1c, d).

If the **interfascicular cambium** differentiates relatively early, as in many woody herbaceous taxa, it may develop from cells of ground meristem (Fig. 9.3a); if later, its interfascicular development may actually result from dedifferentiation of mature interfascicular parenchyma. Cambial activity begins initially in the vascular bundles (Fig. 9.4a). Upon the establishment of continuity of the cambium, its cell divisional activity results in continuous increments of secondary xylem and secondary phloem (Figs 9.3b, 9.4b). In many woody vines, only

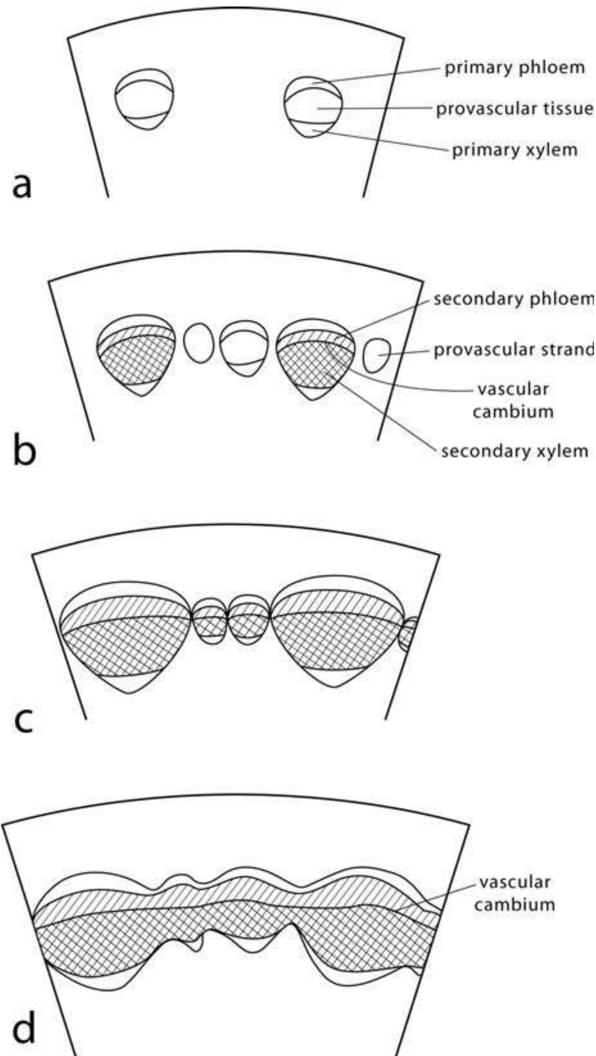

Figure 9.1 Diagrams illustrating the sites of development of the vascular cambium and its formation as a continuous cylinder in woody, arborescent seed plants. Please see the text for a detailed explanation.

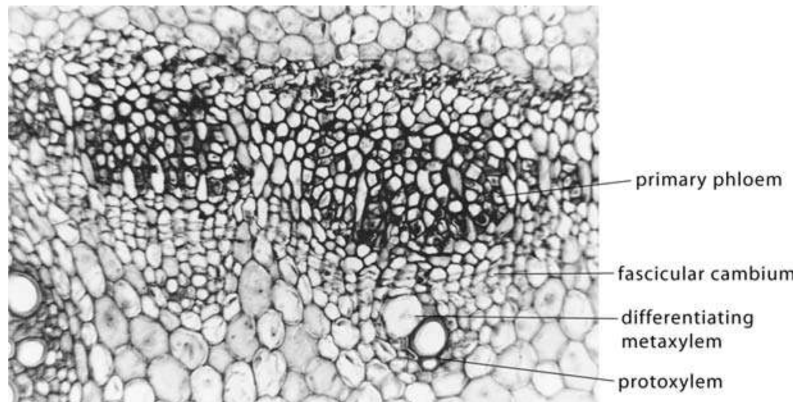

Figure 9.2 Transverse section of vascular bundles in *Cassia didymobotrya* (Leguminosae) showing the presence of fascicular cambium within individual bundles. Fascicular cambium becomes functional at the approximate level at which metaxylem elements are differentiating. Magnification × 275. From Devadas and Beck (1971).

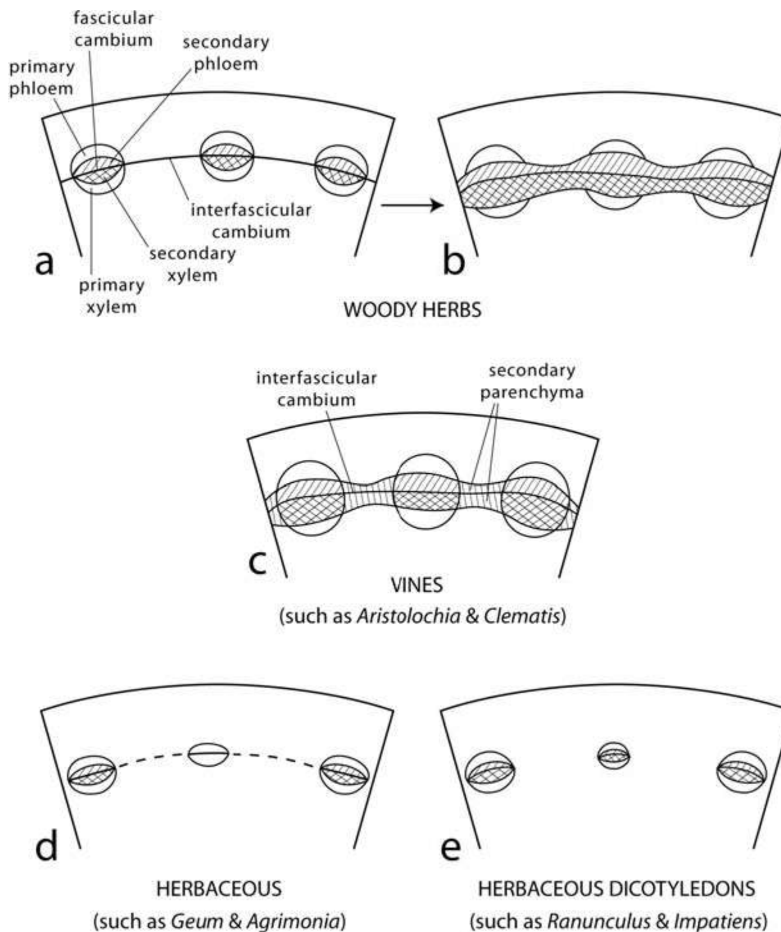

Figure 9.3 Diagrams showing origin of the vascular cambium in woody, herbaceous dicotyledons (a, b), vines (c), and herbaceous dicotyledons (d, e). Please see the text for descriptions.

the fascicular cambium produces typical secondary xylem and phloem whereas the interfascicular cambium produces secondary parenchyma (Fig. 9.3c) (see Chapter 14 for more information on the structure of woody vines).

In herbaceous taxa that produce only meager amounts of secondary tissues, the fascicular cambium is the most, or in some plants, the only, active region (Fig. 9.3d, e). Thus secondary vascular tissues are restricted to the sites of the original primary vascular bundles. In non-woody herbs, the cambium, of course, does not develop, or if it develops will not become very active (Fig. 9.3e). As you might expect, the degree of development and activity of the vascular cambium intergrades between these several conditions. In plants characterized by a residual meristem, fascicular cambium develops from provascular tissue (which has developed from residual meristem) and interfascicular cambium develops from any intervening residual meristem; or if intervening residual meristem differentiates into interfascicular parenchyma, the interfascicular cambium will develop by dedifferentiation of cells in this tissue. (For more detail, see Chapter 5 on meristems of the shoot).

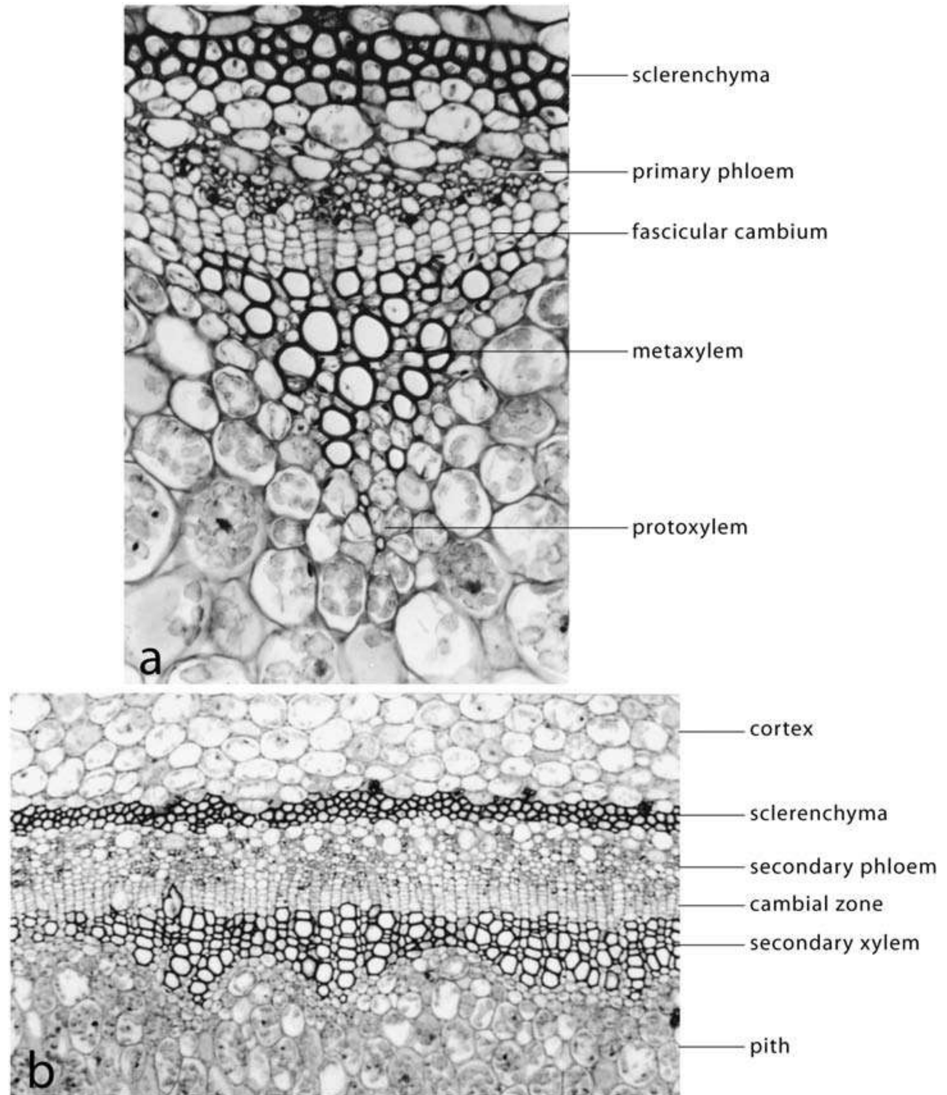

Figure 9.4 Transverse sections of *Pelargonium*. (a) An active fascicular vascular cambium has produced some secondary xylem. Magnification × 263. (b) Fascicular and interfascicular cambia have formed a continuous cylinder, and have produced continuous increments of secondary xylem and secondary phloem. Magnification × 100.

In arborescent forms and woody perennials the vascular cambium functions throughout the life of the plants, in some for hundreds of years. In annuals, the cambium persists only while the plant is actively growing. Before the plant dies it may cease its activity and develop into xylem and/or phloem. There is no vascular cambium in some very herbaceous dicotyledons, most monocotyledons, and most pteridophytes.

The effect of secondary growth on the primary body

Once the cambium has differentiated as a continuous layer in the most distal regions of the stem and in lateral branches, its activity results, as we now know, in the production of a layer of secondary xylem to the

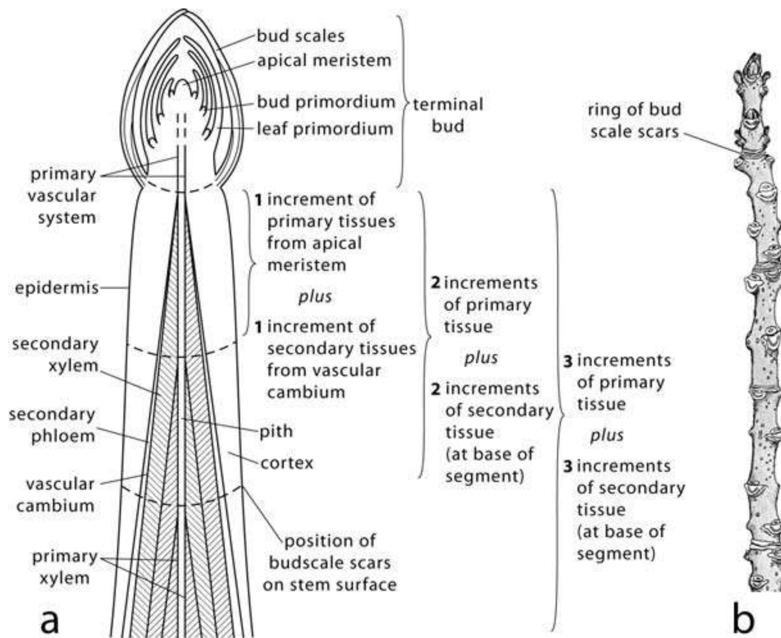

Figure 9.5 (a) Diagram illustrating the relationship between primary and secondary tissue regions in a dicotyledon stem. (b) A woody twig showing rings of bud scale scars.

inside and a layer of secondary phloem to the outside of itself. Since, of course, the newly formed cambium is simply the most distal part of the vascular cambium that extends to the base of the stem, these new layers of vascular tissue also extend to the base of the stem (Fig. 9.5). Each year, therefore, in any one region of the stem, an additional increment of secondary xylem and an additional increment of secondary phloem will be produced by activity of the vascular cambium.

The annual increments of secondary xylem are quite conspicuous, but the increments of secondary phloem, produced in lesser quantity, and subjected to compression by virtue of their position to the exterior of the xylem, are relatively less conspicuous. The number of annual increments (rings) of secondary xylem provide a relatively accurate basis for determining the age of the plant at any level in which the cambium has been active (Fig. 9.5a). The segment of a stem produced in a particular year can be determined by rings of bud scale scars on its surface (Fig. 9.5a, b). The segment produced in the current year will, of course, have only one increment of secondary xylem and phloem, the segment immediately below, produced during the previous growing season will have two, the one below that three, etc. (Fig. 9.5a). It is apparent therefore that, because of the indeterminate nature of growth, the different regions of most gymnosperms and dicotyledons vary in age from one year old or less near the apical meristem to from several to many years at the base of the stem. The length of the plant's lifespan, therefore, can only be determined by counting the growth (usually annual) layers of secondary wood at the base of its main stem. Similarly, the age of lateral branches can be determined by counting the growth layers at their bases.

The effect on the plant of cambial activity is profound. The production of successive increments of secondary tissues results in diametric growth, that is, increase in the diameter or thickness of the stem. The force exerted by this diametric expansion results in outward displacement of the primary and secondary phloem, with the concomitant compression of these tissues, as well as the endodermis and pericyle where present, and the cortex. As a result, in most woody plants, only the inner one or two increments of secondary phloem are functional, and the primary phloem is often completely obliterated. In some plants, however, the cortical parenchyma is stimulated to dedifferentiate and to produce new tissue through cell division. The cortex may, thus, maintain or even increase its original thickness at the same time that it is increasing in circumference. In many plants, however, the phellogen (cork cambium) differentiates in the outer cortex at about the same level in the stem as the vascular cambium. In woody plants, following cessation of activity of the original phellogen, additional cork cambia differentiate deeper within the cortex and outer secondary phloem with the result that these tissues are included in a complex, non-living tissue called rhytidome. (We shall discuss rhytidome structure and formation in detail in Chapter 13 on the bark.) Shortly after the phellogen becomes active, the epidermis dies because it is isolated from inner living tissue by the formation of a layer of non-living, relatively water-impermeable cork.

Whereas cambial activity has a profound effect on the living tissue regions to the exterior of the secondary xylem, in most plants it has little or no effect on those to its interior, the primary xylem and pith. Following the production of the first increment of secondary xylem, this curved barrier of largely non-living cells with lignified, thick, secondary walls effectively resists the forces exerted against it resulting from subsequent cambial activity. Consequently, the tissues to the inside are protected from compression. It should be noted, however, that in vines in which secondary parenchyma is produced by the cambium between regions of typical secondary vascular tissues there is often some inward compression of the primary xylem and pith.

The effect of secondary growth on leaf and branch traces

In seed plants a leaf trace diverges laterally from an axial bundle and follows an upward radial course into a leaf (Fig. 9.6a–c). In the innermost (first) increments of secondary vascular tissues there are regions lacking secondary vascular tissues, called **lacunae**, in the cylinder just above the positions of leaf traces (Fig. 9.6d–f). The lacunae form because the vascular cambium does not differentiate during the first year across the regions outlined by the lacunae. Usually, by the end of the second year of cambial activity, however, the cambium has differentiated across these regions (Fig. 9.6e), often even within the trace itself by dedifferentiation of parenchyma in the trace. Consequently, evidence of the lacunae

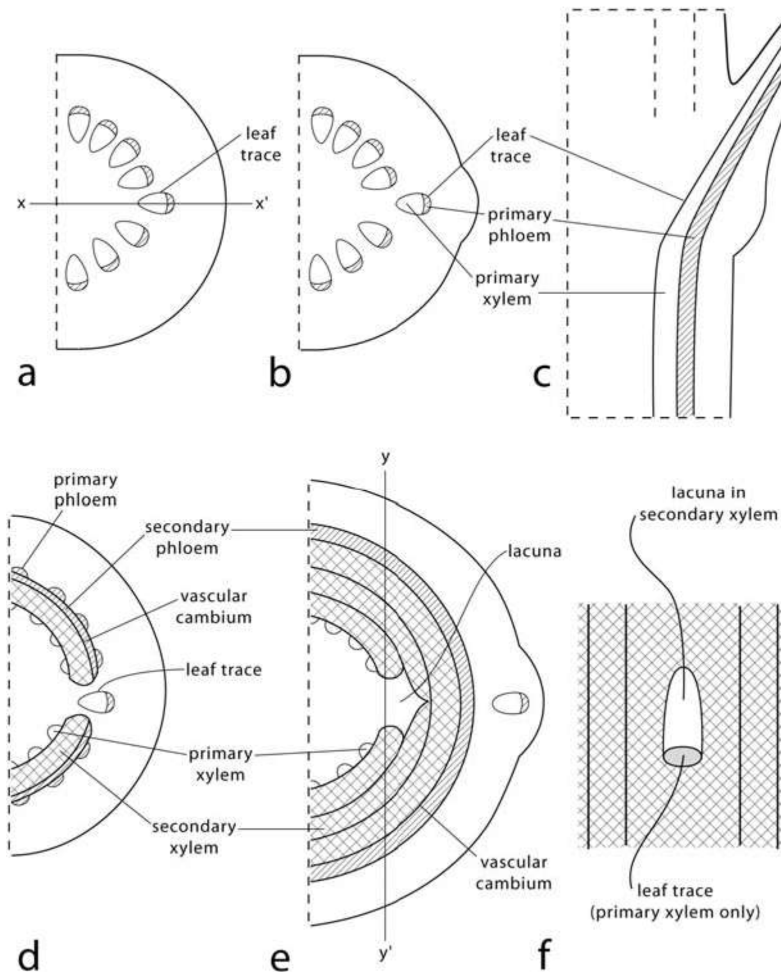

Figure 9.6 (a–c) Diagrams illustrating the divergence of a leaf trace from the cylinder of primary vascular bundles and its entrance into a leaf. (a, b) Transverse sections. (c) Radial section along the plane *x–x′* in (a). (d, e) Transverse sections illustrating the divergence of a leaf trace at levels of one (a) and three (e) increments of secondary vascular tissues, and illustrating the presence of a lacuna (a discontinuity in the secondary xylem) just above the leaf trace. (f) Tangential section along the plane *y–y′* in (e).

is obscured by the production of secondary xylem to the exterior of these structures. In deciduous, woody dicotyledons differentiation of vascular cambium through the trace and the subsequent production of secondary xylem also results in a severing of the leaf trace (Fig. 9.7a). In conifers and non-deciduous dicotyledons (evergreens) that retain their leaves for several years, leaf traces are not severed until after leaf fall (Fig. 9.7b). Such traces are described as being persistent. In a few plants, for example, species in the Araucariaceae, leaf traces may increase in length for many years after the leaves have abscissed. **Persistent traces** are maintained by the activity of a small, specialized meristem within the vascular cambium in contact with the ends of the traces. These, small, generally circular regions are called **armpit cambia**. They produce new conducting tissues that are composed of cells that simulate those of the primary xylem, and that add to the length of the traces (Fig. 9.7b).

As a bud primordium develops, commonly in the axil of a leaf primordium, two incipient branch traces consisting of provascular tissue extend into the base of the young bud (Fig. 9.8a, b). Each branch trace

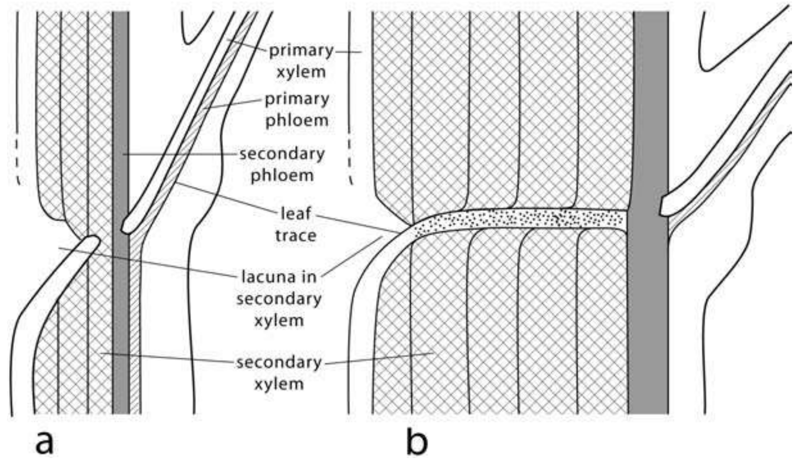

Figure 9.7 Diagrams of radial sections showing (a) a severing of the leaf trace following differentiation of the vascular cambium through the trace, a common occurrence in deciduous taxa, and (b) a persistent trace which increases in length over a period of several years by production of additional conducting tissue by the "armpit" cambium. Please see the text for more detail.

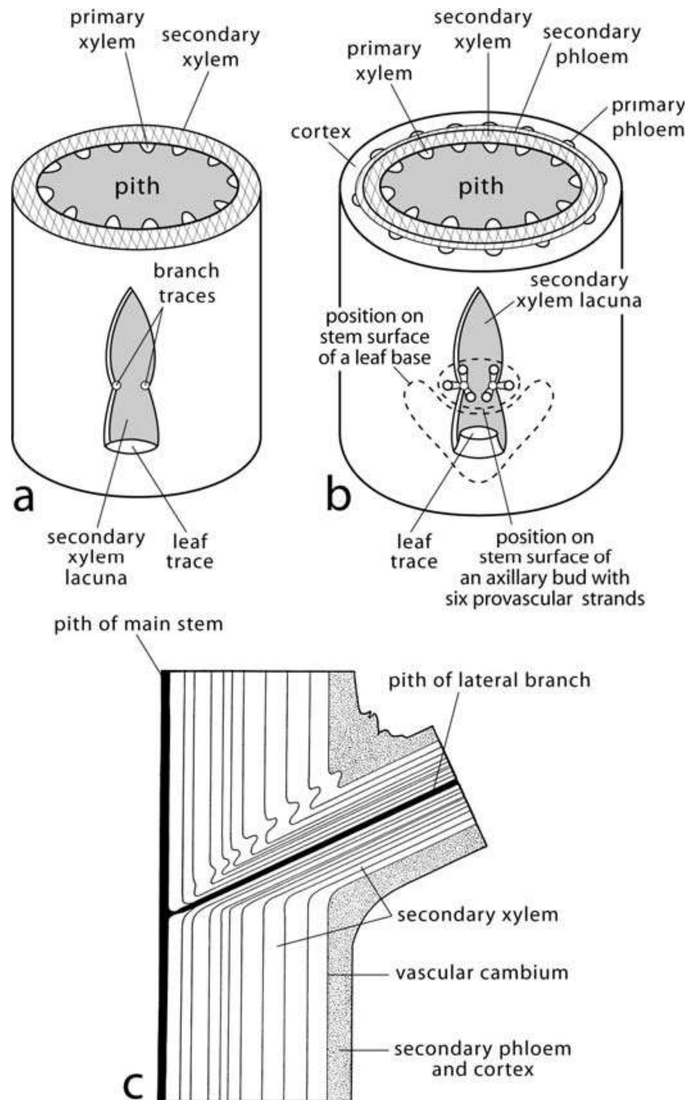

Figure 9.8 (a) Diagram showing the positional relationship of branch traces and a leaf trace in a woody dicotyledon stem with one increment of secondary xylem. (b) Diagram showing the positional relationship of a leaf base and an axial bud on the surface of the stem; also the origin of the cylinder of provascular strands in the bud. (c) Diagram of a radial section illustrating the enclosure by secondary xylem of the base of a lateral branch. Note the continuity of the vascular cambium in the main stem and lateral branch; also the continuation of the pith and primary xylem (black) from the stem into the lateral branch.

divides several times forming a new, cylindrical, system of provascular strands (Fig. 9.8b). In a subsequent year, as the bud begins to grow, this will differentiate into the primary vascular system of a lateral branch. As development continues, transitional meristems, including provascular tissue, are produced by activity of the apical meristem in the bud; the vascular cambium differentiates within these provascular strands, as described above, which produces concentric increments of secondary xylem and phloem in the lateral branch (Fig. 9.8c). A lacuna, usually in the first increment of secondary xylem in the lateral branch, is associated with each pair of branch traces (Fig. 9.8a, b). Since the vascular cambium of the main axis becomes continuous with that of the lateral branch, the pith and primary xylem of the main axis are continuous through the lacuna, extending into the lateral (Fig. 9.8c). Thus, we see that the innermost primary tissue regions (pith and primary xylem) and secondary tissues are continuous in all branches of the plant body.

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Chapter 10

The vascular cambium: structure and function

Perspective

It is difficult to overemphasize the importance of the vascular cambium which produces secondary xylem and secondary phloem. In the following two chapters we shall discuss in detail the structure, functions, and the importance to the plant of these tissues which also have great significance for mankind. Wood (i.e., secondary xylem) is a material of which the buildings in which we live and work are constructed. It is the source of the paper on which we write, on which newspapers, magazines, and books are printed, and of many synthetic fabrics such as rayon and nylon of which our clothes are made, to name only a few of its many uses. The phloem is of the utmost importance as the tissue through which photosynthate is transported from the leaves to sites of utilization or storage in the plant. It is the availability of photosynthate which makes possible the development of nutritious, edible parts of plants, such as fruits, nuts and grains, bulbs, tubers, other edible roots, and leaves, etc., the source of so much of the food supply of humans and other organisms. It is important, therefore, that we know more about the detailed structure and activity of the vascular cambium, a lateral meristem of such great significance.

Structure of the vascular cambium

It is generally agreed that the vascular cambium is composed of a layer of cells only one cell thick, and that all of these cells are meristematic cambial initials from which cells of the secondary xylem and secondary phloem are derived. Cambial initials may be displaced in relation to one another, however, because of the differential growth of immature cambial derivatives and the resultant forces generated. Furthermore, in most plants, the derivatives of cambial initials divide further, forming a zone of immature cells, the **cambial zone** (Fig. 10.1a). The cells of this zone to the inside of the cambium will ultimately differentiate into cells of the secondary xylem, and those to the outside into cells of

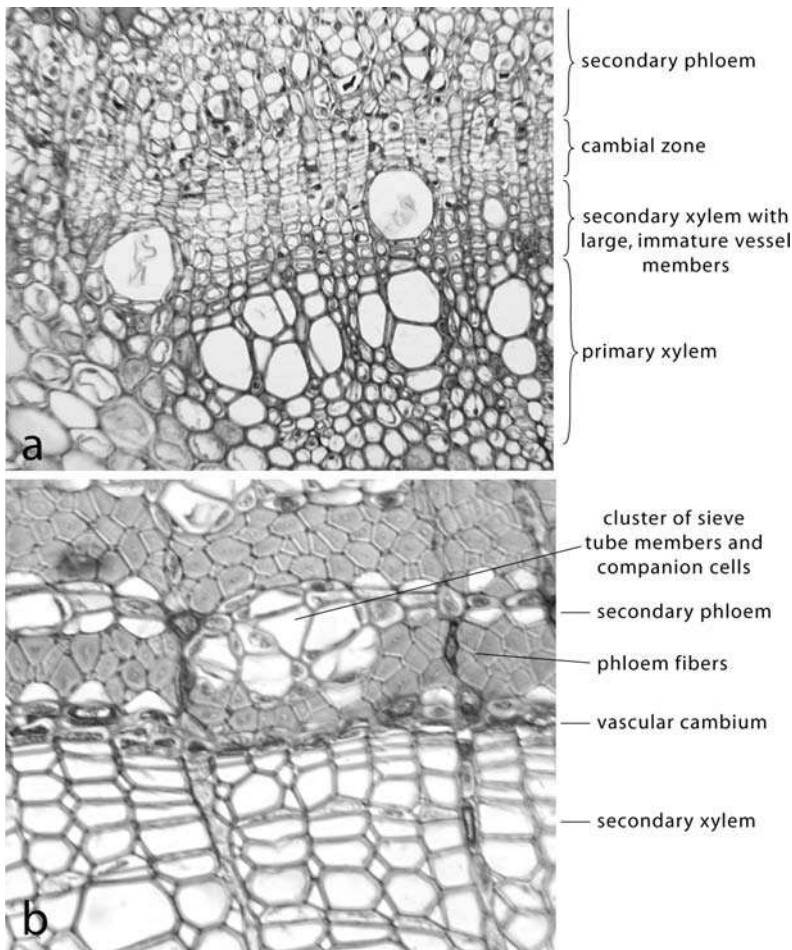

Figure 10.1 (a) Transverse section of part of a young stem of *Quercus* sp. (oak) showing an active cambial zone and its derivatives. Magnification × 238.
(b) Transverse section showing an inactive vascular cambium of *Tilia americana* (basswood) bounded by secondary xylem and secondary phloem produced in the previous growing season. Magnification × 476.

the secondary phloem. The width of the cambial zone reflects the ratio of the rate of production of cambial derivatives and the rate of their differentiation into mature cells of the xylem and phloem. When the rate of cell division exceeds that of differentiation (at the beginning of growth) the cambial zone is broad (Fig. 10.1a), whereas when the rate of differentiation exceeds that of cell division, the zone becomes narrow (Fig. 10.1b). During periods of active growth the cambial initials are difficult, often impossible, to distinguish from their recently formed derivatives. During periods of dormancy, however, at least in trees of temperate zones, the cambium can often be recognized as the layer of cells immediately adjacent to the boundary layer of the secondary xylem (Fig. 10.1b), i.e., the last layer of cells to differentiate prior to cessation of cambial activity (see, e.g., Barnett, 1992). (See Larson (1994) for a detailed, historical review of the nature and activity of the vascular cambium, and Lachaud *et al.* (1999) for a comprehensive review of the structure and function of the cambium.)

Two classes of cambial initials are recognized: **fusiform initials** from which the longitudinally elongate conducting, and other

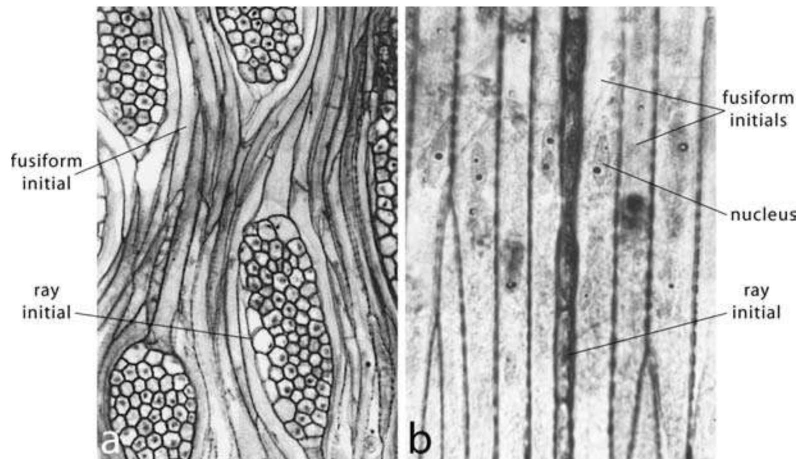

Figure 10.2 Tangential sections showing fusiform and ray initials in the fascicular cambium. (a) *Kalopanax pictus*. The broad ray initials, comprising numerous ray cell initials, will give rise to multiseriate rays. Magnification × 132. (b) *Tilia americana*. Note the uniseriate ray initial, the large nuclei, the tapered ends of the fusiform initials, and the beaded appearance of the walls of contiguous cells which results from the presence of primary pit fields. Magnification × 371. (a) From Kitin *et al*. (1999). Used by permission of Oxford University Press.

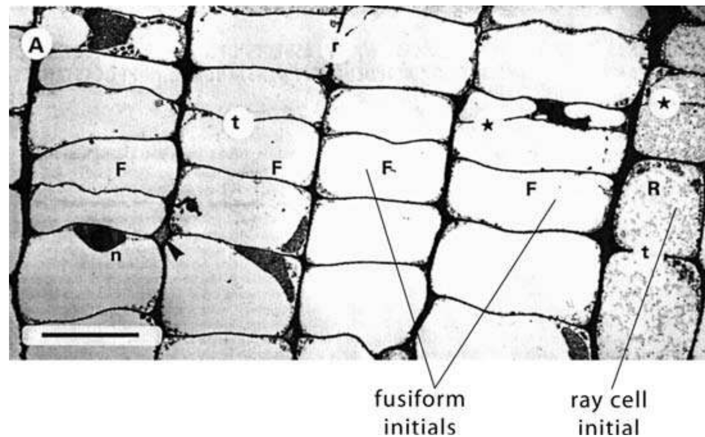

Figure 10.3 Transverse section of the cambial zone of the root of *Aesculus hippocastanum* (horse chestnut) showing the short radial and longer tangential dimensions of the fusiform initials (F). Note how the dimensions of the ray cell initial differ. Bar = 10 μm. From Chaffey *et al*. (1997). Used by permission of the University of Chicago Press. © 1997 The University of Chicago. All rights reserved.

associated cells of the secondary vascular tissues are derived, and **ray initials** from which vascular rays are derived (Fig. 10.2). Cells derived from fusiform initials comprise the **axial system** of secondary vascular tissues and those derived from the ray initials, the **radial system** of vascular rays. Fusiform initials are longitudinally elongate and, typically, characterized by tapered ends (Fig. 10.2). They vary in length from less than 0.2 mm (about 170 μm in *Robinia pseudoacacia* (black locust)) to nearly 7 mm in the gymnosperm *Agathis robusta*. They are uninucleate and have unlignified primary walls, are quite narrow radially and much wider in tangential dimension (Fig. 10.3). Although basically tabular, these cells often have many wall facets. Within the cambium

they may be storied or non-storied. If **storied**, the initials are usually short, all of about the same length and arranged in horizontal files as observed in tangential view. If **non-storied** the tapered ends of fusiform initials overlap each other in a random arrangement when observed in tangential view. During periods of cell division, the tangential walls of fusiform initials are conspicuously thin, but become thicker during periods of dormancy. The radial walls also become thicker and develop a distinctly beaded appearance (Fig. 10.2b) resulting from the presence of numerous **primary pit fields**, thin areas through which pass plasmodesmata.

The term **ray initial**, unlike fusiform initial (a single cell), refers to a group of cells from which a vascular ray is derived. As viewed tangentially, a ray initial is characterized by the same number and arrangement of cells as the adjacent part of its derivative ray (Fig. 10.2a, b). It may be uniseriate, biseriate, or multiseriate, that is, consisting of one or more longitudinal rows of cells, and it may vary in height from one to many cells. We shall call a single cell of a ray initial a **ray cell initial**. A ray cell initial is roughly cuboidal, but during periods of cambial activity, its radial dimension is usually greater than its longitudinal dimension, and greater than the width of fusiform initials (Fig. 10.3).

General overview of cambial activity

Cambial activity is seasonal and generally related to temperature in temperate regions and to rainfall in tropical regions. However, the cambium of some species in tropical zones is continually active (Lachaud *et al.*, 1999). During a growing season, cambial activity in trees begins in or just proximal to buds from which it gradually extends, basipetally, to other parts of the plant. Whereas this is generally true in young trees and in the young branches of older trees, resumption of cambial activity at the beginning of a growing season in older branches and in the trunk may be nearly simultaneous. It has been noted, furthermore, that simultaneous reactivation of cambial activity, following a period of dormancy, occurs at an earlier age in ring porous trees, such as *Quercus* (oak), than in diffuse porous trees, such as *Fagus* (beech) (Lachaud *et al.*, 1999). Toward the end of a growing season, cambial activity ceases first in buds followed sequentially in small branches, stems, and roots. Two types of mitotic divisions characterize the active cambium: **periclinal divisions** in which the new cell walls between daughter cells are essentially parallel to the surface of the axis, and **anticlinal divisions** in which the new cell walls are perpendicular to the surface of the axis. New cells of the secondary xylem and secondary phloem are produced by periclinal divisions of cambial initials and their derivatives (Figs 10.1a, 10.4) whereas new cambial initials are produced by anticlinal divisions. Following a periclinal division of a fusiform initial, one daughter cell, an immature cambial derivative, is added to the cambial zone, whereas the other remains a cambial initial. Immature

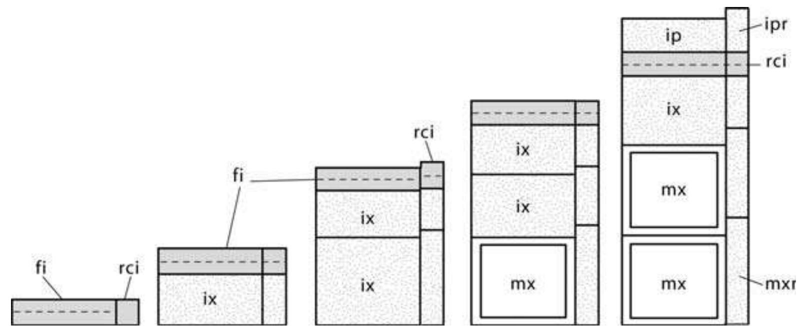

Figure 10.4 Diagram illustrating the production by cambial initials of cells of the secondary xylem and secondary phloem. fi, fusiform initial; ip, immature phloem cell; ipr, immature phloem ray cell; ix, immature xylem cell; mx, mature xylem cell; mxr, mature xylem ray cell; rci, ray cell initial. Dashed line indicates a dividing initial. See the text for further explanation.

cambial derivatives are cut off both to the interior and to the exterior of the cambial initial. These may differentiate directly into cells of the secondary xylem or secondary phloem, but more commonly divide further. If the former, the cambial zone will be relatively narrow, if the latter, it will be relatively wide. A series of periclinal divisions in a fusiform cambial initial and its immature derivatives results in a radial file of cells of similar shape. Radial files of mature cambial derivatives can be observed in many secondary vascular tissues, especially in the secondary xylem of conifers which are composed largely of tracheids (Fig. 10.5a). Such files are usually less conspicuous in angiosperms in which the secondary xylem consists of both fibers and vessel members, the latter of which often increase greatly in diameter during development with consequent distortion of the files (Fig. 10.5b).

The production of new ray cell derivatives (which comprise new vascular rays) results from periclinal divisions of ray cell initials. Following a periclinal division in a ray cell initial the daughter cells will be of unequal radial extent, the shorter of the two usually remaining as the initial, the longer becoming the derivative. Prior to another division, the ray cell initial will enlongate radially. Consequently, at any one time, the ray cell initials in a ray initial vary greatly in radial dimension (Fig. 10.6).

During cell divisional activity, the cambial initials produce incipient secondary xylem cells and incipient secondary phloem cells in a ratio, on average, of about 3 to 1 in conifers. In dicotyledons this ratio is highly variable, but may be as great as 10 to 1. This fact, and the compression which the secondary phloem undergoes as a result of the outward expansion of developing secondary vascular tissues, largely explain the much greater thickness of the secondary xylem as compared with that of the secondary phloem.

As the circumference of the axis increases with cambial activity, the circumference of the vascular cambium must also increase in order to

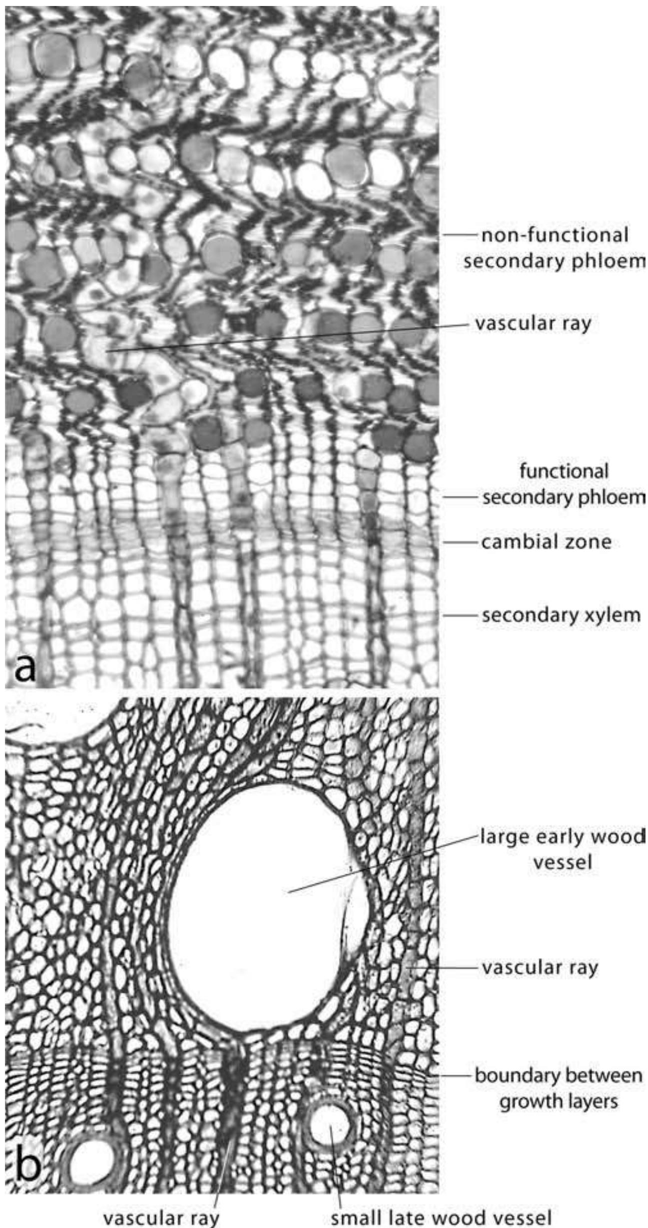

Figure 10.5 Arrangement of cambial derivatives. (a) Transverse section of *Pinus strobus* (white pine) illustrating radial files of tracheids in the secondary xylem, and sieve cells in the functional secondary phloem. Magnification $\times$ 218. (b) Transverse section of secondary xylem of *Quercus rubra* (red oak) illustrating distortion of radial files of cambial derivatives which resulted from great increase in size of vessel members. Magnification $\times$ 160.

prevent its fragmentation. This is accomplished by anticlinal divisions in fusiform initials which result in the addition of new initials to the cambium. In relatively short fusiform initials these divisions are radial anticlinal divisions in which the resulting cell walls between daughter cells are parallel to their long axes (Fig. 10.7a). The production of new initials from long fusiform initials is by oblique anticlinal divisions, followed by intrusive growth of the overlapping ends of the daughter cells (Fig. 10.7b). New fusiform initials can also be formed by elongation

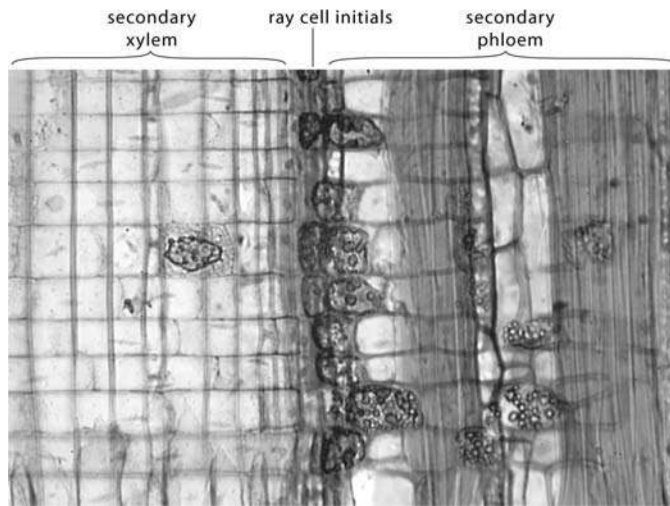

Figure 10.6 Radial longitudinal section of *Tilia americana* showing ray cell initials. Note variation in the radial dimensions of ray cell initials and derivatives. Magnification × 253.

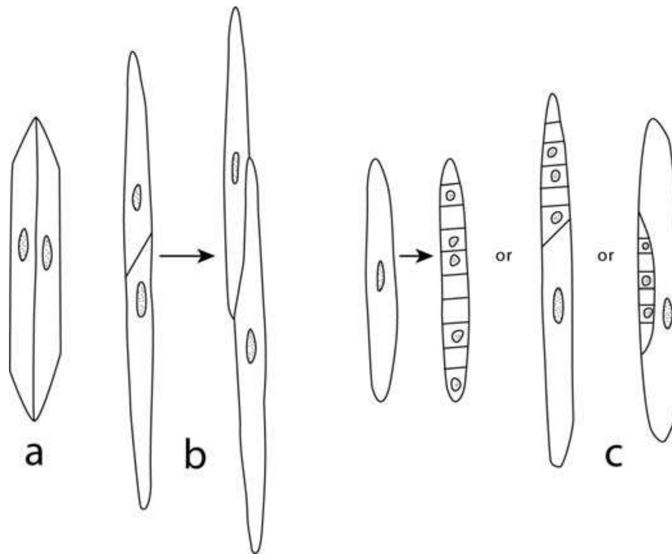

Figure 10.7 Diagrams illustrating increase in circumference of the cambium through production of new cambial initials by anticlinal divisions in fusiform initials. (a) A short fusiform initial may divide by a radial anticlinal division. (b) Long fusiform initials usually divide by oblique anticlinal divisions followed by intrusive tip growth of the daughter initials. (c) A new ray initial may be formed by subdivision of an entire short fusiform initial by a series of transverse anticlinal divisions, by segmentation of the end of a fusiform initial, or by subdivision of a short fusiform initial formed, following mitosis, by the development of an arcuate wall in a longer initial (right).

(parallel to the long axis of the stem) and intrusive growth of ray cell initials (Lachaud *et al*., 1999).

New ray initials are formed from fusiform initials in three ways (Fig. 10.7c). In plants with relatively short fusiform initials, the entire initial becomes subdivided into ray cell initials by a series of transverse

anticlinal divisions. In longer fusiform initials, new ray initials may be formed by the development of an oblique anticlinal wall near one end followed by a series of transverse anticlinal divisions in the part of the initial thus cut off. The formation in a fusiform initial of a more centrally located arcuate wall followed by a series of transverse anticlinal divisions will also result in the formation of a new ray initial. The result in each case is the formation of a uniseriate ray initial. A multiseriate ray initial is formed by a series of longitudinal, anticlinal divisions in its constituent cells which increases its tangential width.

As the cambium increases in circumference during secondary growth, a balance must be maintained between the number and distribution of fusiform and ray cell initials. It is especially important to maintain the ratio of ray initials to fusiform initials because rays provide passageways for the transport of both nutrients and messenger molecules to the cambium and the actively differentiating cambial derivatives, incipient secondary xylem and phloem cells (Chaffey and Barlow, 2001; Van Bel, 1990; Van Bel and Ehlers, 2000).

Although divisions that result in the production of new fusiform and ray cell initials and that contribute toward an increase in the girth of the vascular cambium occur throughout a period of cambial activity, the greatest number occur toward the end of a period of growth. Thus, on average, fusiform cambial initials are shorter at the end of a growth period and gradually increase in length as growth begins in the next season. New ray initials are usually shorter (longitudinally) than the average height of ray initials, and this is reflected in the height of the rays that result from their cytokinetic activity. During development, however, the initials and the derivative rays will increase in height. Over the life of the plant there is also an increase in the length of fusiform initials and in the height of ray initials from the first year of cambial activity through successive years until stabilization occurs. Consequently, the longest tracheids, fibers, and vessel members as well as the tallest rays are usually found in the most recently formed secondary wood.

An interesting characteristic of cambial activity, especially common in trees of both gymnosperms and angiosperms, is the loss of initials from the cambium. This loss of both fusiform and ray cell initials occurs during the production of new initials which increase the girth of the cambium during secondary growth of stems and roots. If more new cambial initials are produced than required to maintain the continuity of the cambium, some are eliminated and mature into cells of the secondary xylem or phloem. (For more detail, see Bannan (1956, 1968), Evert (1961), and Cheadle and Esau (1964).)

Plant hormones and cambial activity

The provascular strands and the vascular cambium and its immature derivatives comprise the major pathways for the **polar transport** of the hormone, auxin (indole-3-acetic acid). As auxin, produced in the

apical meristem and young leaves, moves basipetally, auxin concentration gradients are formed along these pathways. The movement of auxin from cell to cell is mediated by **carrier proteins** which facilitate the movement into and out of cells across the plasma membranes (see Schrader *et al*., 2003). AUX-1 carriers control influx of auxin and PIN-1 carriers control auxin efflux. At the beginning of a growing season, the basipetal flux of auxin is correlated with the basipetal reactivation of the dormant cambium. This and subsequent activity of the cambium are regulated by plant hormones, in particular, auxins and gibberellins, both of which are known to stimulate cell division in cambial initials and young cambial derivatives, and may have a significant role in regulating the frequency and distribution of fusiform and ray cell initials (Little and Savidge, 1987; Lev-Yadun and Aloni, 1991; Björklund *et al*., 2007). Although the production of radial files of cambial derivatives seems to indicate a significant role for cell lineage, Lachaud *et al*. (1999) conclude, on the basis of studies of auxin concentrations in the cambium, that the identity of immature cambial derivatives (i.e., whether they will become xylem or phloem cells) is also regulated through positional information and intercellular communication. A good example of the role of positional information has been provided by Uggla *et al*. (1996, 1998). In *Pinus sylvestris* (Scots pine) there is a *radial* transport of auxin (derived from the polar flow) across the cambial zone. The highest concentration of auxin occurs in the region of greatest frequency of cell division, and decreases toward both the mature xylem and mature phloem. Uggla *et al*. (1998) conclude, therefore, that variations in the concentration of auxin along the concentration gradient provide positional information that controls both the rate of cell division and the identity of the differentiating cambial derivatives as either xylem or phloem cell precursors. Other hormones also have significant roles in the regulation of cambial activity. For example, cytokinin increases the sensitivity of cambial cells to auxin and stimulates cell division (Ye, 2002; Aloni *et al*., 2006), whereas abscisic acid tends to inhibit periclinal divisions (see Lachaud *et al*., 1999). Some workers believe that sucrose in cambial cells is important in the initiation of growth during reactivation of the dormant cambium (Iqbal, 1995; see also Krabel *et al*., 1994). Physical factors such as short day length and water stress induce dormancy by stimulating the synthesis of inhibitors of cytokinesis. Whereas abscisic acid synthesis is known to result from water stress, some evidence indicates that there is no correlation between the advent of short days and its synthesis (e.g., Alvim *et al*., 1979).

Submicroscopic structure of cambial initials

In a general sense, the cells of the cambium resemble those of other meristematic cells in their submicroscopic structure (e.g., Rao, 1985; Catesson, 1994; Chaffey *et al*., 1997; Lachaud *et al*., 1999). Each contains a nucleus, mitochondria, endoplasmic reticulum, ribosomes, Golgi bodies, microtubules, microfilaments, storage products including starch

grains, and spherosomes (membrane-bound oil globules). Variations in cell organelles can be seasonal or taxonomic. For example, plastids containing starch grains are abundant in some species during the early phases of dormancy, and occur rarely or not at all in some other taxa. During periods of cytokinesis, cyclosis (streaming of cytoplasm) is conspicuous, especially in fusiform initials, somewhat less so in ray cell initials.

Cambial initials are characterized by thin, unlignified, primary walls, containing numerous primary pit fields traversed by plasmodesmata which connect the protoplasts of adjacent cambial cells or cambial derivatives. It is through these symplastic connections that hormone signals are transmitted to the sites of developing xylem and phloem cells. One of the most intriguing questions is how this essential symplastic continuity between cells is maintained during growth and elongation of fusiform initials and cambial derivatives such as incipient fibers or phloem parenchyma cells. We shall discuss this problem in a later section of this chapter.

The onset of dormancy and the reactivation of dormant cambium

During the period leading up to the cessation of cell division (in temperate zones, usually in mid-autumn), many changes occur in cambial initials and immature derivatives. Perhaps the most conspicuous is an increase in thickness of the cell walls, especially the radial walls (Fig. 10.8). Increase in wall thickening is correlated with the presence of a helical array of microtubules beneath the plasma membrane which are thought to influence the synthesis of additional cellulose microfibrils, thus increasing the wall thickness (Chaffey *et al*., 1998). Other dramatic changes include the division of the large central vacuole into many small ones (Fig. 10.8), cessation of cyclosis, and the synthesis of starch grains and spherosomes (spherical bodies containing lipid) which, according to Catesson (1994), serve as nutrient sources necessary during the resumption of cell divisional activity. Hydrolysis of starch during the cold-hardening phase of dormancy is of great importance since it increases the osmotic potential in the cell, thus increasing frost resistance (Krabel *et al*., 1994). Additional changes include an increase in the number of mitochondria, reduction in the amount of rough ER, an increase in the number of free ribosomes, deposition in the vacuoles of sugars and amino acids as well as (in some taxa) tannins, and cessation of Golgi activity (see Rao and Dave, 1983; Rao, 1985; Catesson, 1994; Farrar and Evert, 1997).

The period of dormancy in *Abies balsamea* has been characterized as consisting of phases of “rest” and “quiescence” (Little and Bonga, 1974) followed by transition to meristematic activity. Some workers have applied this terminology generally (e.g., Catesson, 1994). It is clear, however, that although cytokinesis ceases during the early, “rest” phase

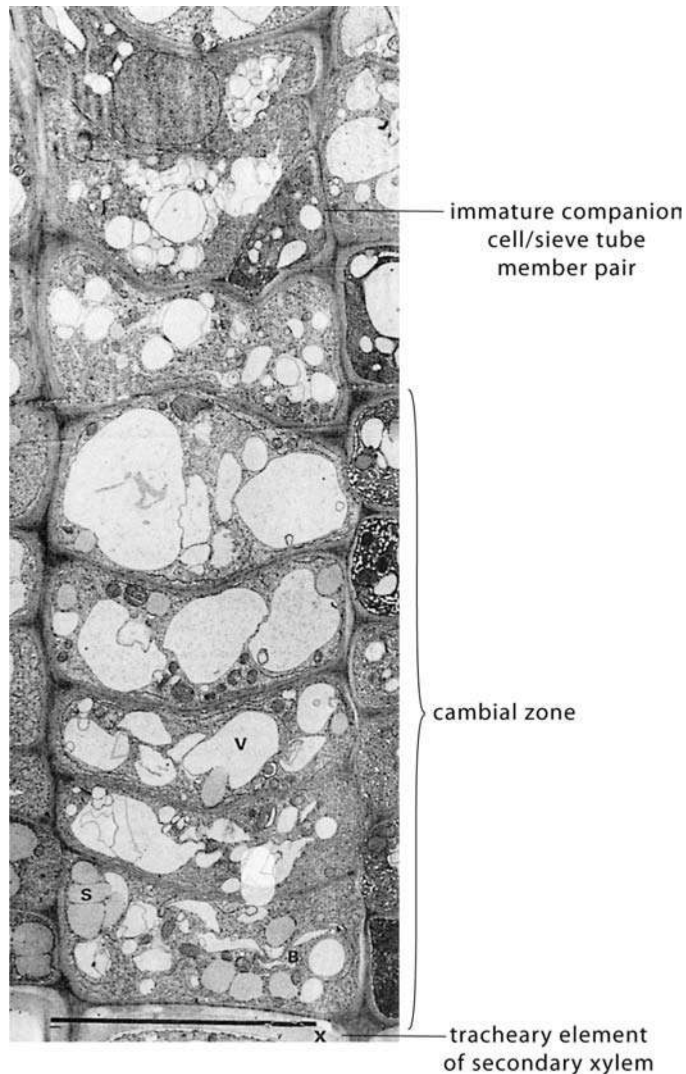

Figure 10.8 A transverse section through the dormant cambial zone of *Aesculus hippocastanum*. The fusiform initials are characterized by thickened radial walls and numerous vacuoles (V). Note also the spherosome (S) in the layer adjacent to the mature secondary xylem, and the immature companion cell–sieve tube member pair produced during the previous year's growth. Bar = 10 μm. From Barnett (1992). Used by permission of Oxford University Press.

of dormancy and does not resume until late winter or early spring, metabolic activity in fusiform initials may continue at a relatively high level. Catesson (1994) suggests that this metabolic activity might be related to the development of frost hardiness, but Lachaud *et al*. (1999) warn that distinguishing between changes related to cold hardening and those linked to cell division could be difficult.

In late winter or early spring, dramatic changes occur in cambial initials that lead to a resumption of cell division by the cambium. The cells swell and their walls undergo a process of thinning. Chaffey *et al*. (1998) suggest that this reduction in cell wall thickness results from a process of enzymatic dissolution of the inner cell wall produced prior to dormancy, but leaving intact the original, outer primary cell wall. They and others have observed that as cambial activity is initiated cortical (i.e., peripheral) microtubules are randomly arranged in the cambial

initials and their immature derivatives. During differentiation of cambial derivatives, the orientation of microtubules typically changes from random to helical, as secondary wall material is synthesized and the cells increase in length. This change in orientation of microtubules characterizes differentiating cells of both the secondary xylem and secondary phloem (e.g., Fukuda, 1997; Chaffey *et al*., 2000, 2002). Other changes include the resumption of cyclosis, fusion of small vacuoles into a single, large central vacuole, hydrolysis of metabolic products such as starch and lipids, increase of Golgi bodies and resumption of their activity, and an increase of rough ER (Farrar and Evert, 1997).

As cell division begins in the cambial zone, several different patterns have been observed in different species. For example, Evert (1963) and Derr and Evert (1967) observed cell divisions throughout the cambial zone beginning at about the same time in *Pyrus* and *Robinia*. However, Grillos and Smith (1959) concluded that in *Pseudotsuga taxifolia* (Douglas fir) the first divisions occur adjacent to the xylem. In a study of reactivation of the cambium of roots of *Aesculus* (horse chestnut), Barnett (1992) demonstrated that new cells are first formed in a zone of incipient phloem cells immediately outside the cambium, produced prior to dormancy. These cells complete their development upon resumption of growth in early spring. (Similar observations were made by Catesson (1964) and Tucker and Evert (1969) in *Acer* (maple).) In *Aesculus* the cambial initials did not begin to divide for 2 weeks after the begining of cytokinesis and cell differentiation in the phloem region; and not until 4 weeks later were cambial derivatives produced that developed into xylem cells. It is not clear whether or not this pattern is characteristic of other temperate, hardwood species.

Cytokinesis in fusiform initials

Cytokinesis in fusiform initials in which the new cell wall is parallel to the long axis of the cell is of considerable interest because of the great distance the phragmoplast must traverse following nuclear division (Fig. 10.9a, b). Prior to mitosis a pre-prophase band composed of cortical microtubules (see Lambert *et al*., 1991) and associated actin microfilaments appears, which marks the site of the future cell plate (Lloyd, 1991; Wick, 1991). In early prophase the nucleus migrates to the center of the cell supported by an aggregation of microtubules and microfilaments which become arranged in the plane of division. This microskeletal sheet comprises the **phragmosome**. The phragmosomal microtubules then disappear through depolymerization leaving only microfilaments which, with others, comprise the mitotic spindle. Upon completion of mitosis the **phragmoplast** consisting of newly formed microtubules and microfilaments begins its expansion in the division plane toward the walls of the parent cell (Fig. 10.9b) (see Lloyd, 1989, 1991). In radial view (Fig. 10.9a), the cell plate, consisting of numerous Golgi and/or ER vesicles gradually extends along the division plane behind the phragmoplast as it migrates toward the cell wall.

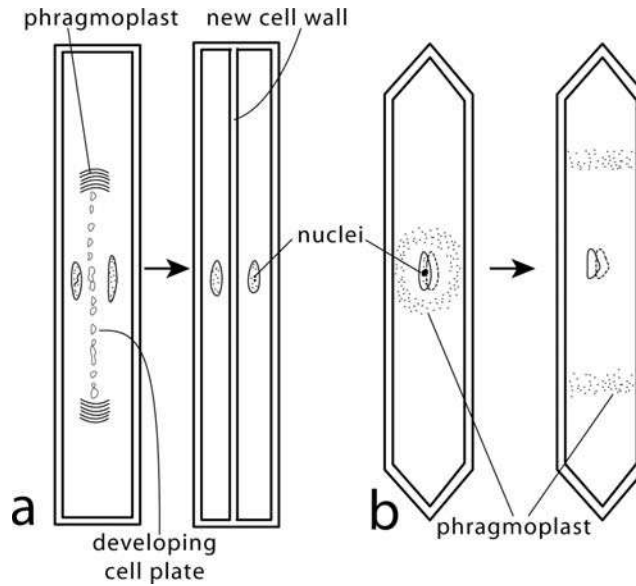

Figure 10.9 (a) Diagrams of radial views of fusiform initials illustrating the developing cell plate as the phragmoplast moves toward each end of the cell followed by formation of the middle lamella and cell walls. (b) Tangential views illustrating the early circular form of the phragmoplast and its subsequent bar-like appearance.

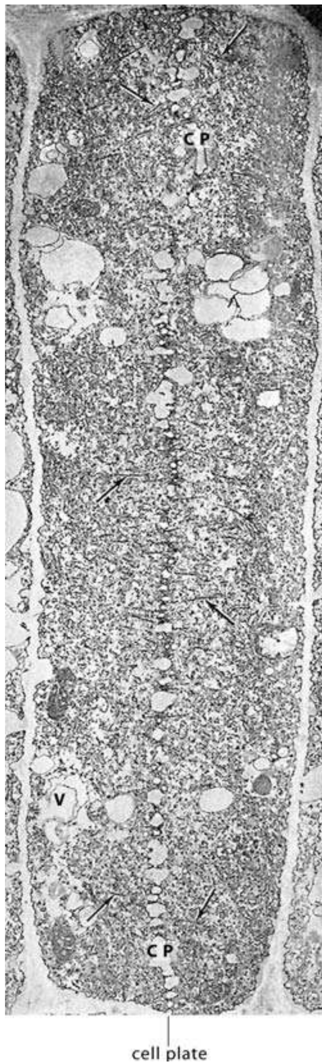

Figure 10.10 Transmission electron micrograph of a transverse section of the developing cell plate (CP) of a dividing fusiform initial of *Ulmus americana* (elm). The phragmoplast and cell plate have reached the radial walls prior to completion of vesicle fusion. Note the phragmoplast microtubules (arrows). Magnification × 4749. From Evert and Deshpande (1970). Used by permission of the Botanical Society of America.

Initially the phragmoplast, as seen in tangential view, appears as a circle of microtubules and microfilaments (Fig. 10.9b). Subsequently, however, as it proceeds toward the ends of the cell it appears as two transverse bands. By this stage, the cell plate has expanded to such an extent that it is in contact with both radial walls, as can be seen in a transverse section (Fig. 10.10). It is interesting to note that as the results of some unknown control system, the phragmosome and, as a result, the cell plate do not become aligned with the walls in adjacent cells. Consequently, the daughter cells resulting from cytokinesis overlap the ends of other cells in the developing tissue like bricks in a wall (see Flanders *et al*., 1990; Lloyd, 1991). For an extensive bibliography and more detailed discussions of the role of the cytoskeleton in cell division including the origin and role of the pre-prophase band, the architecture of the phragmoplast, origin of the microtubules, their role and the role of actin microfilaments in cytokinesis, see Lloyd (1988, 1989), Seagull (1989), Baskin and Cande (1990), Flanders *et al*. (1990), Lambert *et al*. (1991), and Wick (1991).

The problem of differential growth of cambial cells and immature cambial derivatives

A long-standing problem of cell growth in the cambium and its immature derivatives involves the method whereby two adjacent cells, or

parts of cells, can grow differentially, i.e., at different rates, and maintain their symplastic continuity. It is well established that when a new cambial initial is formed following an oblique, anticlinal division, the cell elongates by growth of its tips. A cambial derivative such as an incipient fiber may elongate greatly during its development adjacent to an incipient vessel member that may elongate only slightly, or not at all. It has been assumed that elongation of contiguous cells at different rates would, of necessity, disrupt both developing pit pairs and plasmodesmata. Several explanations have been proposed by which differential growth of contiguous cells could occur without such disruptions (see Larson (1994) for a detailed, historical account). Earlier workers strongly supported the view that cambial fusiform initials and certain cells in the cambial zone such as incipient fibers elongated primarily by symplastic growth. Although symplastic growth in the main body of immature cells may play a contributing role in their elongation, several studies (e.g., Evert, 1960; Barnett and Harris, 1975; Wenham and Cusick, 1975; Barnett, 1981; Robards and Lucas, 1990) have shown that new cambial initials and immature cambial derivatives increase in length primarily by intrusive growth of the cell tips which extend between other cells in the tissue. This process may be facilitated by digestion of the middle lamella by enzymes produced by the immature growing tip which allows it more effectively to push its way between contiguous cells (Hejnowicz, 1980). Evidence of tip growth comes from the thin-walled nature of the tips, the presence in them of numerous Golgi bodies, mitochondria, and ribosomes, and the progressive increase in thickness of the cell walls toward the cell tips during development. It has been shown that cambial fusiform initials in several conifers and angiosperms seem to have no plasmodesmata in their radial walls (Barnett and Harris, 1975; Rao and Dave, 1983). Others have observed plasmodesmata in both radial and tangential walls (e.g., Kidwai and Robards, 1969). It is widely accepted, however, that early derivatives of fusiform initials lack both pits and plasmodesmata in the walls of cell tips that are undergoing intrusive growth (Wardrop, 1954; Bannan, 1956; Barnett and Harris, 1975). If this were true also of fusiform cambial initials as suggested by Larson (1994), the potential problem of disruption of symplasmic connections would become moot. Prior to completion of the development of cambial initials and their derivatives, new pits pairs and plasmodesmatal connections (secondary plasmodesmata; see Chapter 4) would form between contiguous cells.

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Chapter 11

Secondary xylem

Perspective

Most of the major taxa of vascular plants produce secondary xylem derived from the vascular cambium. Pteridophytes (except some extinct taxa), most monocotyledons, and a few species of largely aquatic dicotyledons, however, produce only primary vascular tissues. In woody plants secondary xylem comprises the bulk of the tissue in the stems and roots. It is the most important supporting tissue in arborescent dicotyledons and most gymnosperms, and the major tissue for the transport of water and essential minerals in woody plants. Secondary xylem is a complex tissue that consists not only of non-living supporting and conducting cells but also of important living components (rays and axial wood parenchyma) which, with those in the secondary phloem, comprise a three-dimensional symplastic pathway through which photosynthate and other essential molecular substances are transported thoughout the secondary tissues of the plant (Chaffey and Barlow, 2001; see pp. 206–207 for more detail). Additional increments of this tissue are added during each growing season (usually annually), but in older regions of most woody species only the outer increments are functional in transport although the number of increments that remain functional varies greatly among different species. Older increments gradually become plugged by the deposition in them of waste metabolites such as resins, tannins, and in some species by the formation of tyloses (balloon-like extensions of axial or ray parenchyma cells into adjacent conducting cells). The inner non-functional secondary xylem is called heartwood, the outer functional secondary xylem, sapwood. We shall discuss the characteristics and formation of heartwood in more detail later in this chapter.

Overview of the structure of secondary xylem

Secondary xylem is made up of an **axial system** of longitudinally oriented cells, derived from the fusiform initials of the vascular cambium,

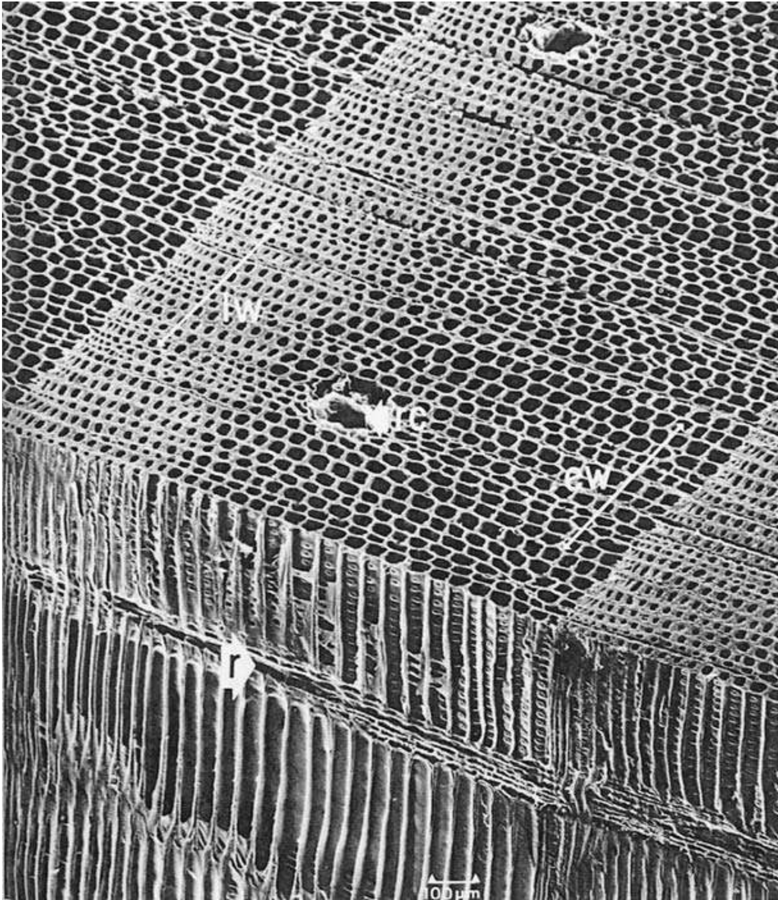


Figure 11.1 Scanning electron micrograph of a block of secondary xylem of *Pinus resinosa* (red pine) showing transverse and radial surfaces. This conifer wood consists primarily of tracheids and vascular rays. The sharp distinction between growth layers results from the great difference in size and wall thickness of tracheids in the late wood (lw) and early wood (ew). Note also the resin canals (rc), the vascular ray (r) exposed on the radial surface, and the large circular bordered pits in the radial walls of tracheids. From Core *et al.* (1979). Used by permission of Syracuse University Press.

and a **radial system** consisting of rays derived from the ray initials of the vascular cambium. In gymnosperms the axial system consists of tracheids and axial parenchyma (Fig. 11.1), and in many conifers resin ducts also develop as part of both the axial and radial systems. In angiosperms the axial system consists of tracheids, fibers, vessel members, and axial parenchyma. Secretory ducts as well as secretory cavities also occur in some angiosperms and contain, in different species, a variety of substances (see Chapter 15 on secretory structures).

The successive increments of xylem cells produced by the cambium comprise **growth layers**, often called annual rings (Fig. 11.2a, b). The cells produced in the early part of a growing season (in the inner part of the growth layers), especially the tracheary cells, are typically larger in transverse dimensions than those produced later, and may have thinner cell walls. These cells comprise the **early wood** (sometimes called spring wood). **Late wood** (sometimes called summer wood), produced later in the growing season, especially that produced just prior to cessation of activity of the cambium, is composed of smaller cells that often have much thicker walls than cells of the early wood. Consequently,

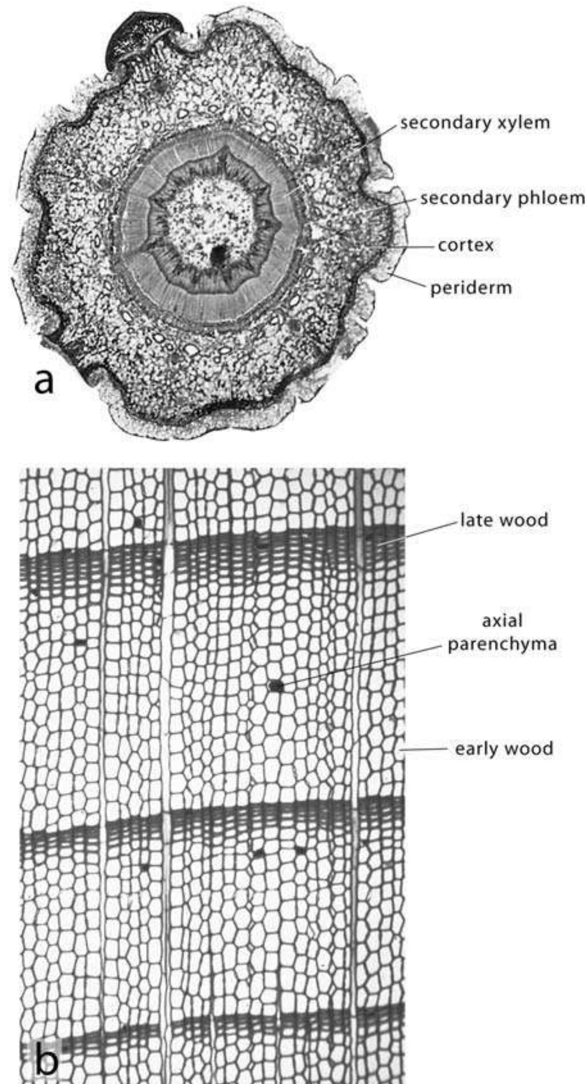


Figure 11.2 (a) Transverse section of a 2-year-old stem of *Abies*. Magnification × 7.3. (b) Transverse section of secondary xylem of *Sequoia sempervirens* (redwood) illustrating growth layers consisting of early wood containing large, thin-walled tracheids and late wood containing thick-walled tracheids. The late wood tracheids have much narrower radial dimensions than the early wood tracheids. Note also the uniseriate and biseriate vascular rays and the axial wood parenchyma containing dark contents. Magnification × 44.

there is usually a well-defined line of demarcation between successively produced growth layers (Fig. 11.2).

Whereas in temperate climatic zones growth layers are typically produced annually, in tropical regions growth layers often reflect periodicity in rainfall rather than temperature and photoperiod, and thus may not be annual increments of secondary xylem. Under some circumstances, even in temperate zones, more than one growth layer may develop during a growing season. Such **false growth layers** can result from environmental factors such as defoliation by insects, forest fire, or severe drought.

Rays, which comprise the radial system of the secondary xylem, function in storage as well as in lateral transport of various materials. They are composed primarily of **ray parenchyma**, but in many conifers, some other gymnosperms, and a few angiosperms, they also contain **ray**

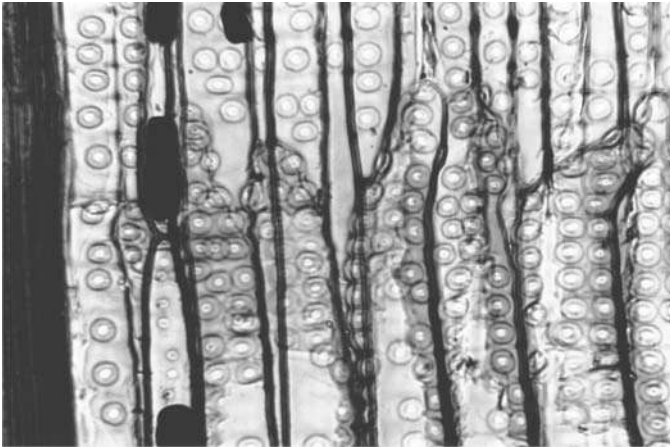


Figure 11.3 Radial section of secondary xylem of *Sequoia sempervirens* showing overlapping, blunt tracheid ends. Circular-bordered pits are especially abundant in the walls of the tracheid ends. Note the row of axial parenchyma cells on the left. Magnification × 178.

tracheids, non-living cells, the walls of which contain bordered pits. As we shall see later, in different species rays are variable in height, width, and arrangement of cells as well as in distribution in the wood.

Secondary xylem of gymnosperms

Gymnosperm wood which consists primarily of tracheids and rays (Fig. 11.2b) is less complex than the wood of angiosperms which is characterized by a greater diversity of cell types and cell sizes. We shall now consider in some detail the secondary xylem of conifers. The conifer **tracheid** has dual functions, providing both support and conduction, and its structure is an adaptation to those functions. It may be very long, reaching 8 mm in some members of the Araucariaceae and, on average, is longer than the average length of tracheids in secondary wood of angiosperms. The tracheids of conifers have tapered to relatively blunt ends that overlap those of other tracheids and are characterized by large, usually **circular-bordered pits** that are very abundant on the overlapping ends (Fig. 11.3). The pits have highly specialized pit membranes that both facilitate the lateral transport of water and minerals from one tracheid into another, and, under certain conditions, function as valves that restrict the movement of air within the xylem.

Bordered pits occur only in the radial walls of conifer tracheids except in those in the late wood in which they may occur in the tangential walls (Fig. 11.4). They form **bordered pit-pairs** with pits of contiguous tracheids (Fig. 11.5a–c) and **half-bordered pit-pairs** with simple pits of contiguous axial or ray parenchyma cells (Fig. 11.6). In conifers, bordered pits are typically uniseriate or biseriate (Fig. 11.5b, c), but may also be multiseriate. It is very interesting that the Devonian plant *Archaeopteris*, a pteridophyte of arborescent stature which lived over 350 million years ago, produced secondary xylem remarkably similar to that of conifers (Fig. 11.7a, b). Its wood is distinctive, however, in that the circular-bordered pits occur in groups arranged in radial bands. The

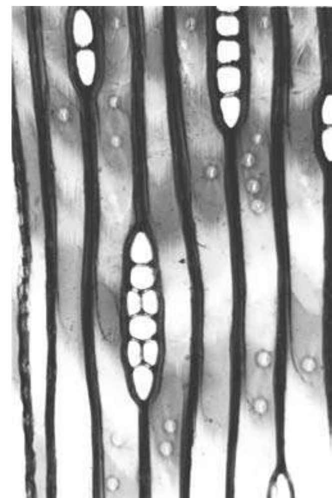


Figure 11.4 Tangential section of late wood of *Sequoia sempervirens* showing the low uniseriate and biseriate vascular rays, and the bordered pits in the tangential walls of tracheids. Magnification × 124.

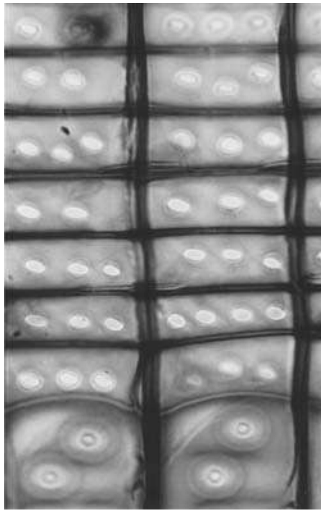


Figure 11.6 Radial section of secondary xylem of *Sequoia sempervirens* showing half-bordered pit-pairs between ray parenchyma cells and tracheids, as seen in face view. Magnification × 355.

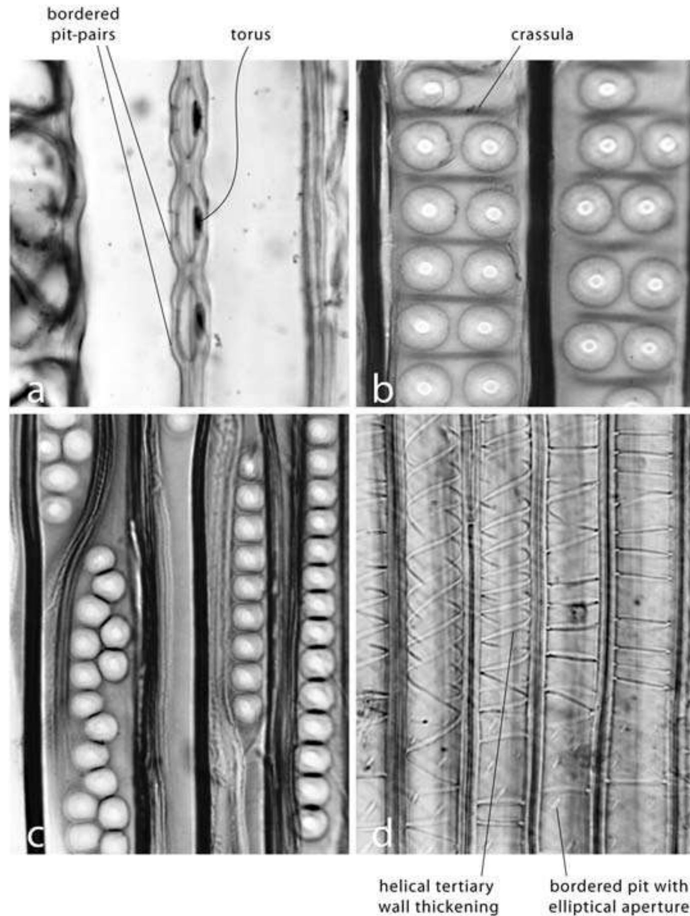


Figure 11.5 Secondary xylem in conifers. (a) Tangential section of *Pinus jeffreyi* illustrating bordered pit-pairs in sectional view. Note the aspirated pit membranes, each containing a thick, centrally located torus. Magnification × 700. (b) Radial section of *Sequoia sempervirens* showing circular-bordered pit-pairs in face view. The faint rim around the pit apertures represents the lateral extent of the torus. Radiating and anastomosing strands extend from the torus and comprise the margo, the peripheral region of the pit membrane. Compare Figs 11.5a and b with Fig. 11.9. The dark, horizontal bands between pits are crassulae, regions of primary wall heavily impregnated with lignin that separate primary pit fields prior to the deposition of secondary wall layers. Magnification × 400. (c) Radial section showing bordered pits of angular outline in *Araucaria bidwillii*. The occurrence of pits in groups is common in this species. Magnification × 400. (d) Radial section of *Picea rubra* (spruce). Note the small, circular-bordered pits with elliptical apertures. Superimposed on the secondary wall are tertiary thickenings which form one or two helices. Magnification × 373.

bordered pits of conifers are predominantly circular in shape as seen in face view, but when crowded, may be angular (Fig. 11.5c). The highly lignified, slightly thickened regions of the primary walls and middle lamella between pit-pairs, often visible in radial sections as heavily stained bands (Fig. 11.5b), are **crassulae**. In older literature these are

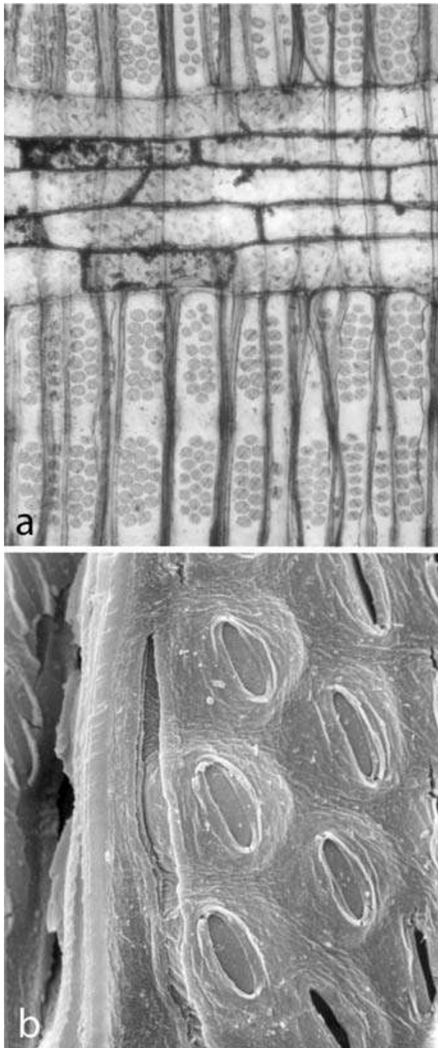


Figure 11.7 Radial sections of *Callixylon newberryi*, the secondary wood of *Archaeopteris*, an extinct Devonian plant. (a) Groups of circular-bordered pit-pairs in radial rows. Note also the vascular ray. Magnification × 136. (b) Scanning electron micrograph of circular-bordered pits showing the elliptical apertures. Magnification × 1943. From Beck *et al.* (1982).

called bars of Sanio after the Italian botanist who first described them in the late nineteenth century. In some conifers helical, **tertiary wall thickenings** are synthesized between bordered pits on the inner surfaces of the secondary wall (Fig. 11.5d).

Bordered pit-pairs vary in structure depending on the thickness of the walls in which they occur. If the secondary walls are thin the pit border of each pit is usually described as having a single **aperture** and no pit canal (Fig. 11.5a). If the walls are thick, however, there will be a **pit canal**, typically in the shape of a flattened cone, with inner and outer apertures (Fig. 11.8b). The inner aperture is commonly oval to elliptical, the outer circular. In all cases, the long axis of the inner aperture is parallel to the microfibrils of the S2 layer of the secondary cell wall, and apertures of the opposing bordered pits of a pit-pair are always crossed (Fig. 11.8a).

Figure 11.8 (a) Bordered pit-pairs in a thick-walled cell such as a fiber, in face view. The angle of the long axis of the inner apertures reflects the orientation of the cellulose microfibrils in the S2 wall layers of the contiguous cells. (b) Sectional view of a pit-pair such as that seen in (a). The pit canal has the form of a flattened cone.

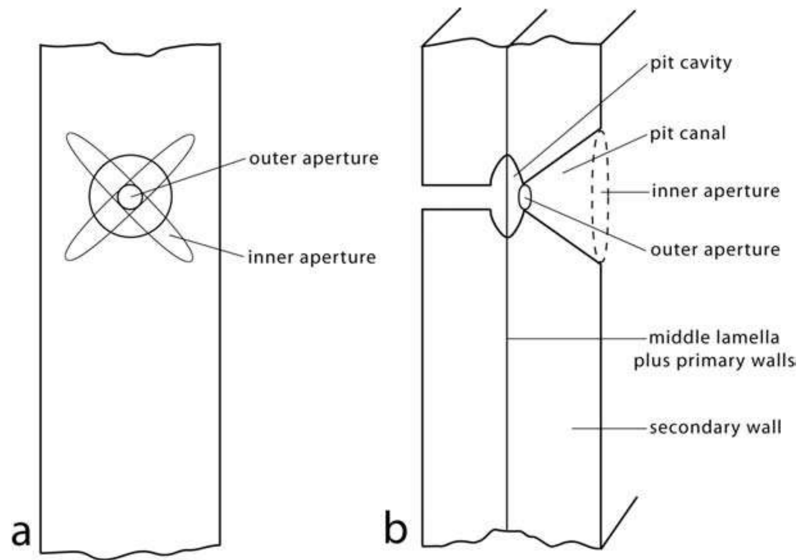
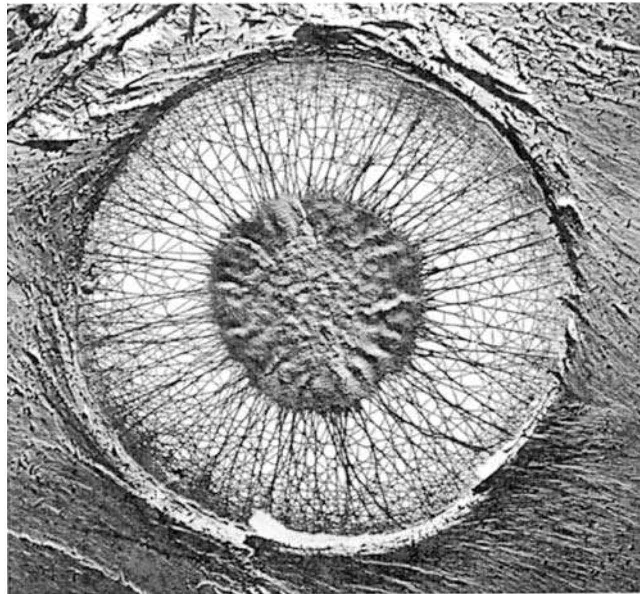


Figure 11.9 Scanning electron micrograph of the pit membrane of a bordered pit-pair of *Abies grandis* (fir). The dense central structure is the torus; the peripheral region of radiating and anastomosing strands of cellulose microfibrils is the margo. Magnification × 4192. From Preston (1974). Used by permission of Springer-Verlag New York, Inc.



In most conifers, the **pit membrane** (a highly modified region of the primary cell walls and intervening middle lamella) which separates the two **pit cavities** of a bordered pit-pair consists of a central, thickened, lignified, circular region called the **torus** and a thin surrounding region called the **margo** (Figs 11.5a, 11.9). In the functional tracheid, the torus is dense, heavily impregnated with pectic compounds, and impermeable to the passage of water. In contrast, the margo is highly porous and consists of radiating and anastomosing bundles of lignified microfibrils (Fig. 11.9) that are exposed by hydrolysis of the encrusting pectic compounds just prior to the death of the cell protoplast. The margo provides the major passageway for the transport of water and

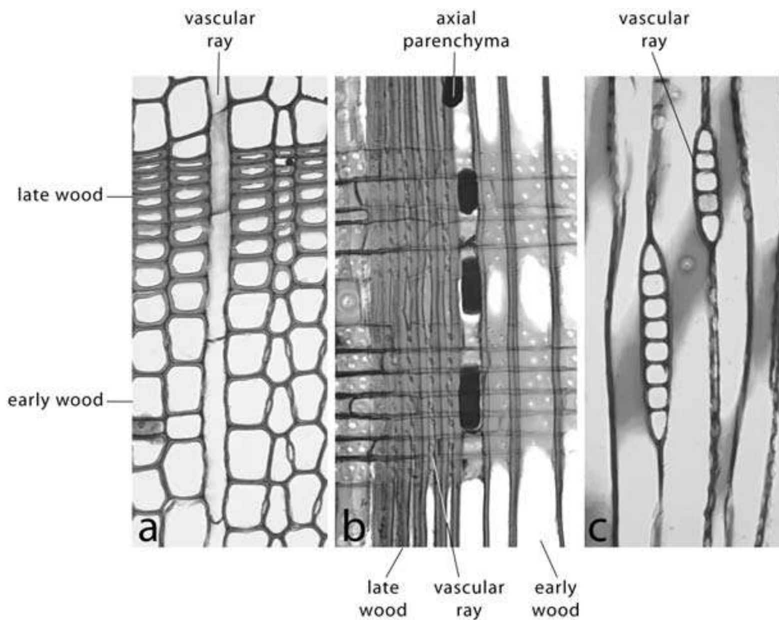


Figure 11.10 Uniseriate vascular rays in the secondary xylem of *Sequoia sempervirens*. (a) Transverse section. (b) Radial section. (c) Tangential section. Note the axial parenchyma in (b). Magnification (a), (b), and (c) × 145.

minerals from one tracheid to an adjacent one, and facilitates the efficient transport through the apoplast. The torus, the diameter of which exceeds that of the pit aperture, functions like a valve, closing (i.e., pressing against) a pit aperture (Fig. 11.5a) when the pressure differential between two adjacent cells is great, a condition that can occur during periods of rapid transpiration and resulting negative water pressures. During such conditions air bubbles (embolisms) may form in tracheid lumina. Closure of the pit apertures by the tori effectively confines air to limited areas which if more widespread in the wood could seriously disrupt water flow.

In addition to tracheids, axial wood parenchyma and the epithelial cells of longitudinally oriented resin ducts are also derived from the fusiform initials in conifers. **Axial wood parenchyma** (Fig. 11.10b) develops from immature derivatives of fusiform initials by transverse divisions in these derivatives resulting in longitudinal strands of axial parenchyma cells. These strands of xylem parenchyma are scattered throughout the wood (Fig. 11.2b), often in well-defined patterns. The dark contents of axial parenchyma cells of conifers were thought by early botanists to be resin. In fact, the nature of this material is unknown, but it may be tannin.

The **vascular rays** of conifers, relatively low and uniseriate to biseriate in most species (Fig. 11.10b, c), are commonly composed of two types of cells, ray parenchyma cells and ray tracheids. In species containing resin ducts the rays also contain epithelial cells lining the ducts which are large intercellular spaces.

Ray parenchyma cells are typically (but not without exception) radially elongate, that is, their radial dimension is greater than their height (Fig. 11.10a–c). Within a ray, cells are arranged like bricks in a wall

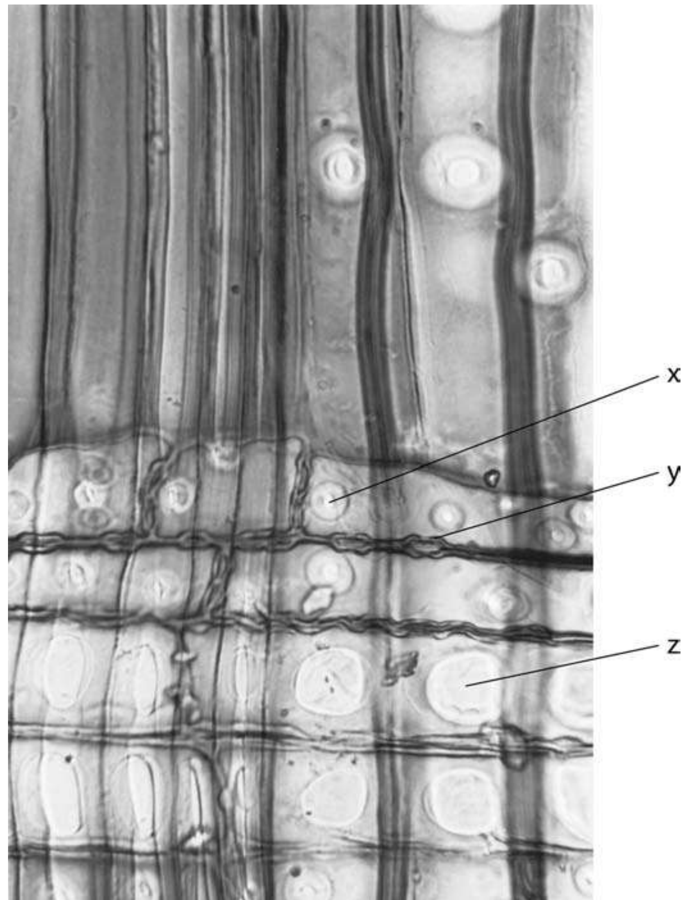


Figure 11.11 Radial section of secondary xylem of *Pinus strobus* showing bordered pit-pairs in the walls of ray tracheids and contiguous axial tracheids (*x*) (face views), and in the walls of adjacent ray tracheids (*y*) (sectional views). Note also the large fenestriform (window-like) half-bordered pit-pairs in the walls of contiguous ray parenchyma cells and axial tracheids (*z*) (face views). Magnification × 500.

overlapping the ends of cells in contiguous files. Contact zones between axial tracheids and ray parenchyma cells are called **cross-fields** and are categorized by the characteristics of the half-bordered pit-pairs in these zones (Figs 11.6, 11.11). Cross-fields are useful in determining the source of isolated secondary wood of conifers since those of different species may have different characteristics. Compare the small cross-field pits of *Sequoia sempervirens* (Fig. 11.6) with the large fenestriform (window-like) pits of *Pinus strobus* (Fig. 11.11).

Ray tracheids are non-living ray cells that occur in either marginal (Fig. 11.11) or marginal and interspersed rows. They are often more radially elongate, and their secondary walls, which contain bordered pits, thicker than those of ray parenchyma cells. As one would expect, bordered pit-pairs occur in the walls of contiguous ray tracheids as well as between ray tracheids and axial tracheids, and half-bordered pit-pairs occur in the contiguous walls of ray tracheids and ray parenchyma cells (Fig. 11.11). Ray tracheids are thought to facilitate more rapid transport of water and minerals through the rays than would be possible through ray parenchyma cells, but there is no experimental evidence to support this viewpoint.

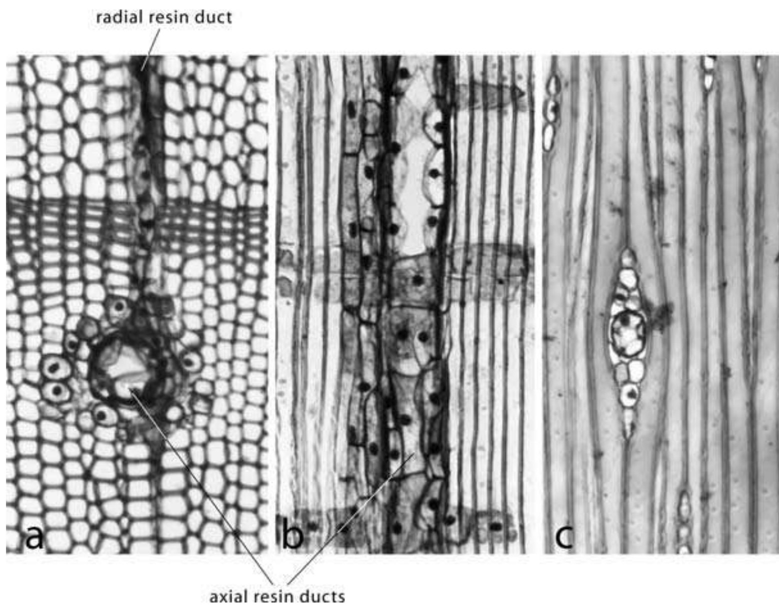


Figure 11.12 Resin ducts in the secondary xylem of *Pinus strobus*. (a) Intersection of an axial and a radial resin duct as viewed in transverse section. (b) An axial resin duct viewed in radial section. (c) A radial resin duct within a vascular ray viewed in tangential section. Note the epithelial (secretory) cells lining the ducts, and in (a) and (b) the surrounding axial parenchyma cells. Magnification (a), (b), and (c) × 174.

Resin ducts

Resin ducts (Fig. 11.12) are a distinctive feature in the secondary xylem of many conifers. They consist of long channels enclosed by **epithelial cells** which secrete resin into them granulocrinously. In length they range up to 40 mm, and in diameter to 150 μm. They are a constant and, presumably, genetically controlled feature of *Pinus*, *Pseudotsuga*, *Larix*, and *Picea*, and have the potential to develop following wounding in *Sequoia*, *Cedrus*, and *Abies*. They are unknown in *Cupressus*. The frequency and distribution of resin ducts in genera in which they normally develop can be severely modified by the response to injury (see Romberger *et al*., 1993). Resin ducts also develop in the primary xylem of roots and shoots of some conifers, and the ducts in the cortex of young shoots may extend into the mesophyll of leaves (Werker and Fahn, 1969). In the secondary xylem resin ducts are both axial, oriented longitudinally among tracheary cells (Fig. 11.12a, b), and radial, extending through vascular rays (Fig. 11.12a, c). The axial and radial ducts are connected to and continuous with each other (Fig. 11.12a), thus comprising an extensive, three-dimensional system. In some taxa the radial ducts extend into the secondary phloem, but are probably not open in the immature region across the cambial zone (see Werker and Fahn, 1969).

The epithelial cells of axial ducts (Fig. 11.12a, b) are derived from the derivatives of fusiform cambial initials whereas those of the radial ducts (Fig. 11.12c) are derived from derivatives of ray cell initials. By a series of anticlinal and periclinal divisions in fusiform cambial derivatives, columns of incipient epithelial cells are formed. As development proceeds, the epithelial cells separate from each other schizogenously, forming channels which they enclose. Radial resin ducts originate in

developing uniseriate rays following anticlinal and periclinal divisions in ray cell derivatives, and schizogenous separation of incipient epithelial cells. As the resin duct develops, the ray expands and becomes multiseriate (Fig. 11.12c). The complex mechanisms of the genetic control of resin duct development are unknown, but signals (probably hormonal) distinguish between the specific sites of axial and radial duct development.

The function of resin in conifers is not clearly understood, but is thought to protect the plant from invasion by fungi and insects following injury. According to Romberger *et al*. (1993) the resin in the ducts is under positive pressure, the result of turgor pressure in the epithelial cells. Upon rupture of the ducts resin flows out slowly due in part to its viscosity and in part to the local reduction in pressure. Osmotic uptake of water in adjacent epithelial cells leads to their expansion and consequent constriction of the rupture (Romberger *et al*., 1993).

Secondary xylem of dicotyledons

The secondary xylem of dicotyledons differs from that of gymnosperms primarily in the greater complexity of the tissue (Fig. 11.13a, b) and the greater structural diversity among different taxa. The most significant difference is the functional separation in dicotyledons of cells providing mechanical support and those which provide transport of water and minerals. Whereas in gymnosperms both of these functions are served by tracheids, in dicotyledons support is provided largely by fibers and longitudinal transport by vessel members except in the most primitive angiosperms (e.g., members of the Winteraceae of the Magnoliidae) which lack vessel members. The secondary xylem of these primitive taxa closely resembles that of gymnosperms, the axial system being composed primarily of tracheids. Another difference between some gymnosperms and angiosperms is the nature of the pit membranes in the pits of tracheary elements. Whereas in conifers the pit membranes have a central, thickened torus and a surrounding, highly porous margo of intertwined cellulosic strands, the pit membrane of tracheary elements in angiosperms is a much simpler sheet of primary wall of uniform structure (Fig. 11.14a).

Whereas one thinks of the axial system of angiosperm secondary xylem as being composed largely of fibers, vessel members, and axial parenchyma, the diversity of cell types in this wood is somewhat greater than that. The following types of tracheary cells characterize the axial system.

Tracheids

In the vesselless angiosperms of the Winteraceae the tracheids are very long (up to 4.5 mm), relatively thin walled, and bear circular-bordered or scalariform-bordered pits. They have relatively blunt ends that overlap others in the tissue. Some taxa in more advanced families of dicotyledons (e.g., *Quercus*, of the Fagaceae) also contain tracheids as well as fibers

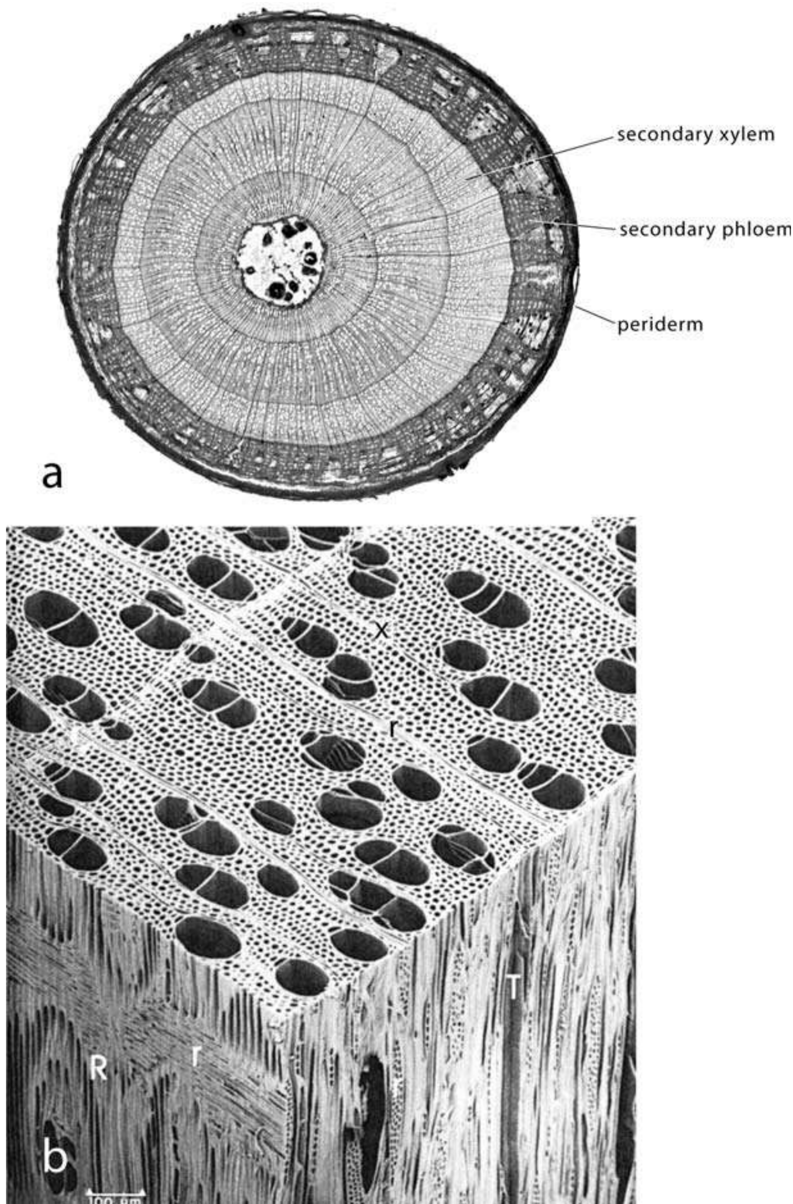


Figure 11.13 (a) Transverse section of a 3-year-old stem of *Tilia americana*. Magnification × 1.7. (b) Scanning electron micrograph of a block of secondary xylem of *Betula allegheniensis* (yellow birch) showing transverse (X), radial (R), and tangential (T) surfaces. Note the vascular rays (r) on all surfaces. The axial system of the xylem of this dicotyledon is composed predominantly of fibers and clusters of large vessels. The fibers abruptly diminish in size at the end of a growing season, marking the boundary of the growth layers. (b) From Core *et al.* (1979). Used by permission of Syracuse University Press.

and vessel members. These tracheids are typically relatively short, often somewhat distorted in form, bear circular-bordered pits, and have blunt to sharply tapered ends.

Fiber-tracheids

These cells are of frequent occurrence in primitive dicotyledons, the secondary xylem of which is also characterized by long, slender vessel members (see p. 194). As their name suggests they are intermediate in structure between typical fibers and tracheids although they are usually longer than tracheids in the same wood. Their bordered pits are

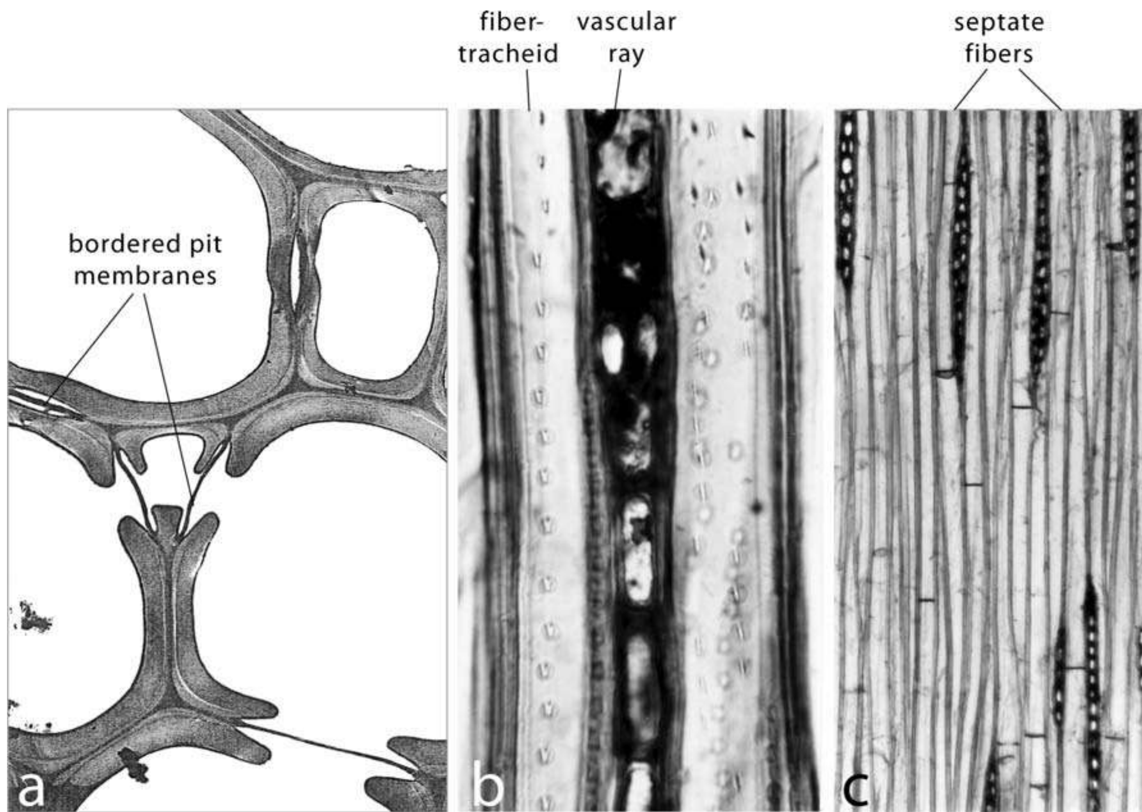


Figure 11.14 (a) Transverse section of tracheids in the secondary xylem of *Quercus rubra* illustrating the homogeneous membranes of bordered pit-pairs. Magnification × 3000. (b) Fiber-tracheids in secondary xylem of *Cercidiphyllum japonicum* bearing circular-bordered pit-pairs with elliptical apertures extending to the boundary of the pit borders. Magnification × 600. (c) Septate fibers in *Albizzia saponaria*. Note that septa extend only to the inner surface of the secondary walls. Magnification × 210. (a) From Côté (1967). Used by permission of the University of Washington Press.

relatively small with apertures that extend to or beyond the borders as in *Cercidiphyllum japonicum* (Fig. 11.14b). In some genera (e.g., *Albizzia*) of a few families they may be **septate** (Fig. 11.14c) and retain a functional protoplast containing a nucleus between each pair of septa. These living septate fiber-tracheids usually store photosynthate in the form of starch grains and may remain alive for several to many years. Thus they function in both support and storage of photosynthate. Their cell walls are typically much thinner than those of libriform fibers, and their bordered pits less reduced. Typically, the septa between protoplasts consist solely of primary wall. Since the septa extend only to the inner surface of the secondary wall of the fiber-tracheid (Fig. 11.14c), it is apparent that they were deposited by the protoplasts following each nuclear division late in the development of the cell.

Libriform fibers

As the most highly specialized wood fibers, libriform fibers (Fig. 11.15) are the most abundant cell type in many woods and are the major elements of structural support. In many taxa their protoplasts are maintained for several years. They are characterized by great length, relatively small, transverse diameters, thick secondary walls (Fig. 11.15a) and sharply tapered ends (Fig. 11.15b). Typically they are several times the length of the fusiform initials from which they were derived, their

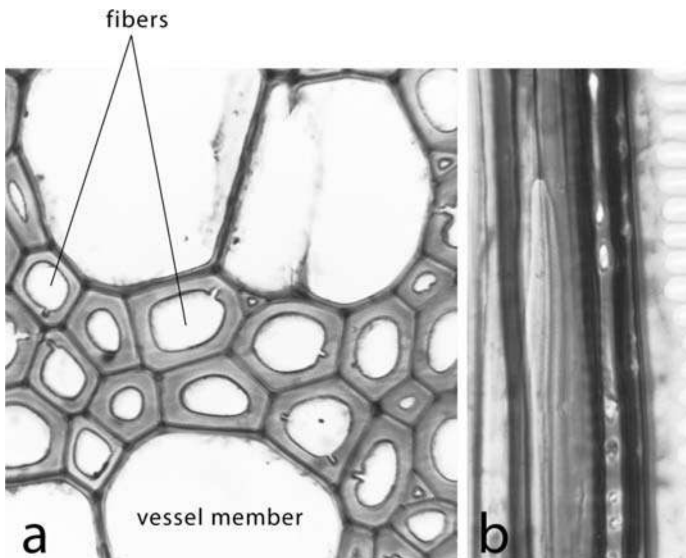


Figure 11.15 (a) Transverse section of secondary xylem of *Liriodendron tulipifera* (tulip tree) illustrating libriform fibers. The discontinuities in the walls are cracks resulting from improper drying prior to sectioning, not simple pits. (b) A sharply tapered fiber end. Magnification (a) and (b) × 528.

greater length resulting from intrusive tip growth during development. Their pits are highly reduced with very small borders and slit-like apertures that extend beyond the pit borders. In some species the pits are so greatly reduced that they have the appearance of simple pits. It is interesting to note that the most highly specialized fibers occur in the same wood as structurally specialized vessel members which suggests that they evolved concurrently.

Gelatinous fibers

Fibers in **reaction wood** (wood of modified structure by virtue of its location in sites of tension or compression) frequently have secondary wall layers that are hygroscopic, under certain conditions absorbing water and becoming swollen. These so-called "gelatinous fibers" are common in the secondary xylem on the upper sides of tree limbs (tension wood).

Substitute fibers

In reality not tracheary elements, substitute fibers form a large part of the secondary xylem of some suffrutescent (shrubby) plants with fleshy stems such as *Pelargonium*. These cells are longitudinally elongate parenchyma cells with tapered ends that have the appearance of short tracheids or fibers, but are usually of greater diameter than fibers. They provide both support and storage functions for the plants.

Vessel members

Vessel members, characteristic of all angiosperms except the most primitive taxa, function primarily in the transport of water and minerals. Unlike tracheids and fibers, vessel members have end walls, and occur in superposed columns of cells comprising **vessels** (Fig. 11.16a–c). During development, the end walls between vessel members become perforated. Consequently, vessels are elongate tubes well adapted for the

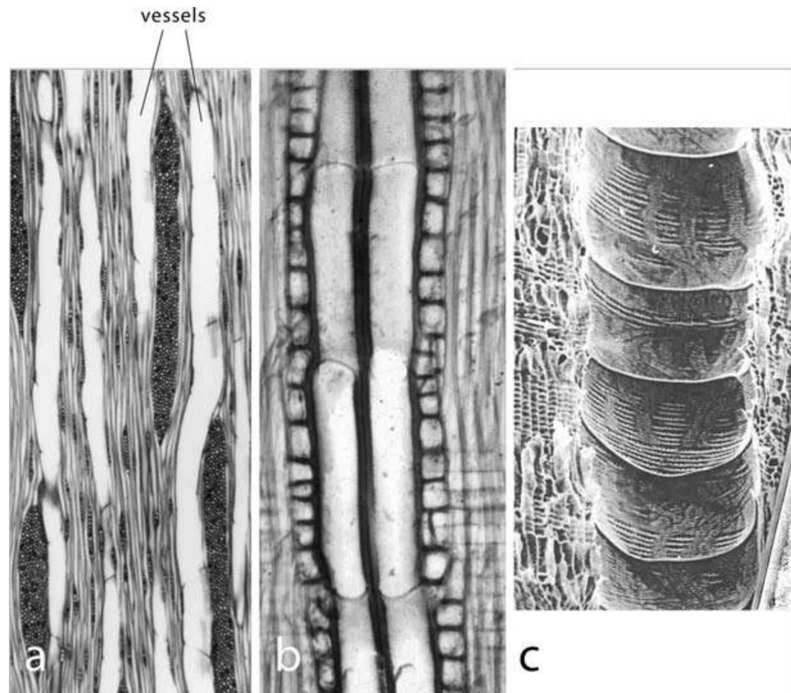


Figure 11.16 Superposed vessel members comprising vessels. (a) Tangential section of *Acer saccharum* (sugar maple). Note also the small uniseriate and very large multiseriate vascular rays. Magnification × 50. (b) Radial section of *Fraxinus americana* (ash). Axial parenchyma cells are associated with the cluster of vessels. Magnification × 150. (c) Scanning electron micrograph of a vessel of *Quercus*. Magnification × 113. (c) From Core *et al.* (1979). Used by permission of Syracuse University Press.

movement of water and solutes in the plant. Vessel members are, typically, similar in length to the cambial fusiform initials from which they were derived. As they develop there is little or no growth in length, but in many species there is considerable lateral growth resulting in increase in diameter. This lateral growth results in displacement of developing fibers, axial parenchyma, and rays.

Vessel members as well as libriform fibers evolved from tracheids (see Bailey, 1953, 1954, 1957). It is not surprising, therefore, that vessel members in primitive taxa (and the fusiform cambial initials from which they were derived) are quite long. Such vessel members typically have oblique, **compound perforation plates** containing several to many perforations (Figs 11.17a–c, 11.18c). They are often angular in transverse section, of relatively small diameter, and characterized by scalariform bordered pits on their lateral walls (Fig. 11.17d). All of these characters are considered to represent primitive states. In contrast, vessel members of more highly specialized (evolutionarily advanced) taxa are short, often very broad (Fig. 11.16c), have **simple perforation plates** with one large opening in the end wall (Fig. 11.18g) and circular-bordered pits on the lateral walls. Structurally intermediate vessel members occur between these extremes (Fig. 11.18e, f). The vessel members of some taxa in diverse families are characterized by helical wall thickenings which comprise the inner part of the S3 layer as in *Tilia* (basswood) (Fig. 11.17e). Because genera are usually characterized by vessel members of a single structural type, vessel members are often used, with other evidence, to suggest their level of evolutionary specialization.

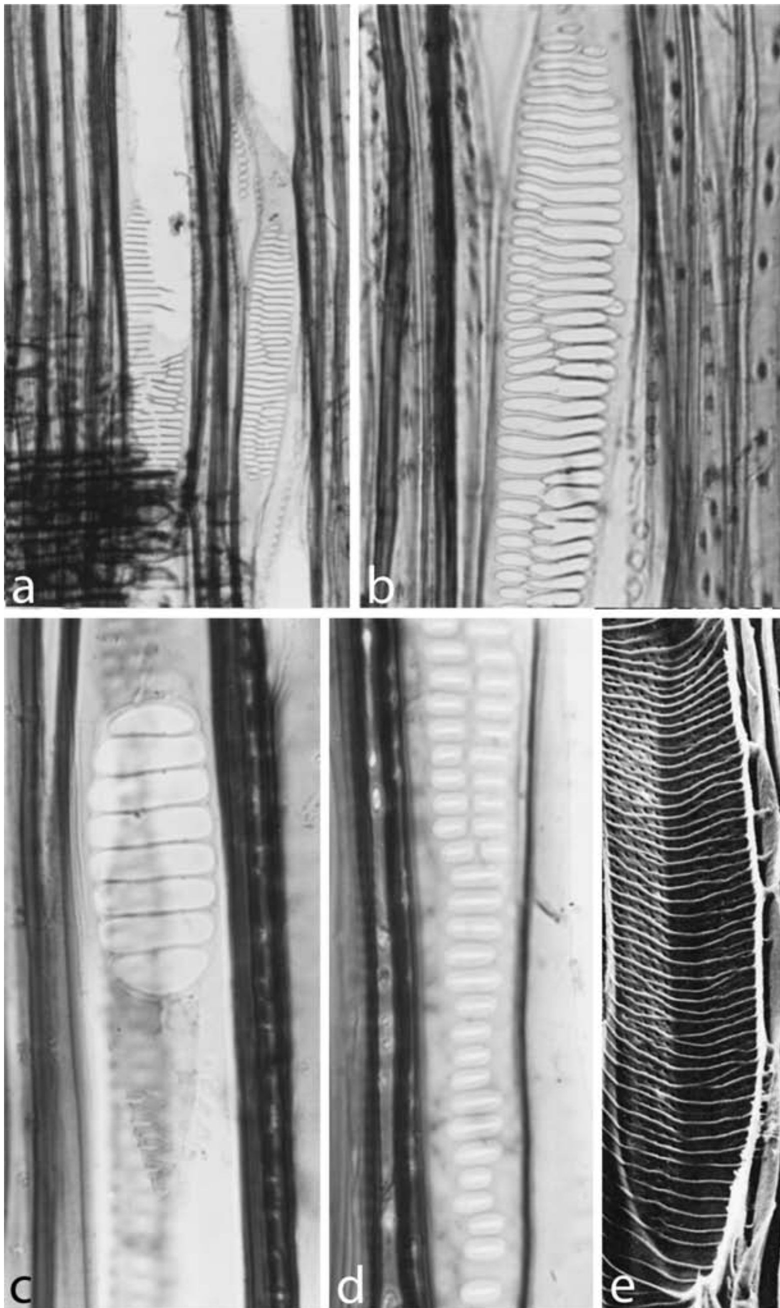


Figure 11.17 (a, b) Vessel members with very oblique scalariform to reticulate perforation plates in the secondary xylem of *Cercidiphyllum japonicum*. Note the fiber-tracheids associated with the vessel members. Magnification (a) × 159, (b) × 397. (c) Vessel member of *Liriodendron tulipifera* with a scalariform perforation plate. Magnification × 209. (d) Scalariform-bordered pits in the wall of a vessel member in the same wood. Magnification × 412. (e) Scanning electron micrograph of a vessel member from the secondary xylem of *Tilia americana* with helical thickenings lining the secondary wall. Magnification × 408. (e) From Core *et al.* (1979). Used by permission of Syracuse University Press.

Differentiation of tracheary elements

Tracheary elements resemble the fusiform cambial initials from which they are derived, especially in length. Although there may be some intrusive growth of the *cambial derivatives* during differentiation of tracheids in gymnosperms, and extensive intrusive tip growth during the differentiation of fibers in the wood of dicotyledons, there is essentially

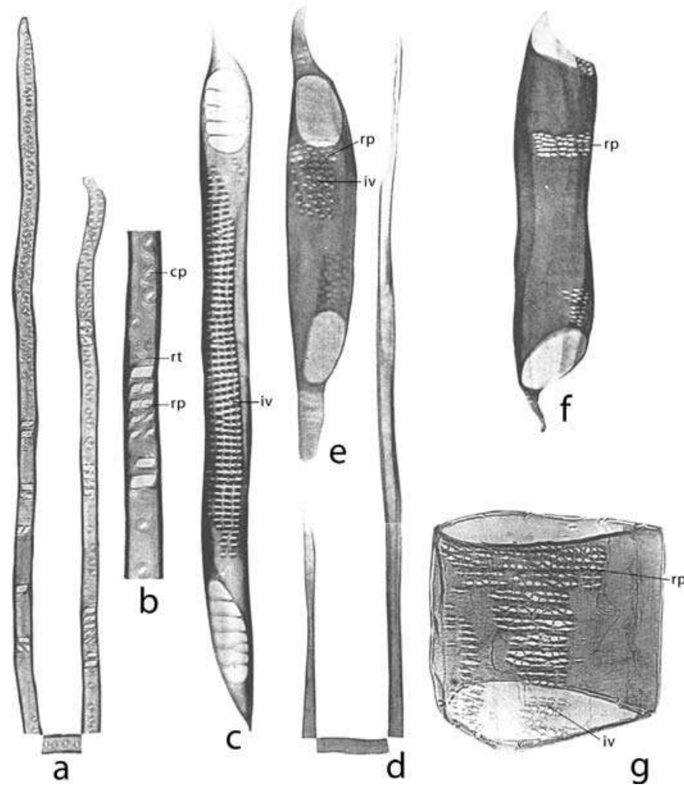


Figure 11.18 Tracheary elements from secondary xylem. (a) A tracheid from *Pinus resinosa*. Magnification × 54. (b) Enlarged segment of the tracheid shown in (a). (c) A vessel member with scalariform perforation plates from *Liriodendron tulipifera*. (d) A libriform fiber from *Populus grandidentata* (big-tooth aspen). (e) A vessel member from *P. grandidentata* with simple perforation plates. (f) A vessel member from *Salix nigra* (black willow) with simple perforation plates. (g) A vessel member from *Sassafras albidum* with simple perforation plates. All vessel members, the libriform fiber, and the enlarged part of the tracheid are shown at the same magnification. The tracheid in (a) is shown at half the magnification of the vessel members. cp, circular-bordered pits; iv, intervessel pits (bordered pits that comprise bordered pit-pairs with pits in the walls of contiguous vessel members); rp, pits of tracheids or vessel members that comprise half-bordered pit-pairs with simple pits in contiguous ray parenchyma cells; rt, small bordered pits that comprise bordered pit-pairs with pits in contiguous tracheids or vessel members. Magnification (b–g) × 109. From Carpenter and Leney (1952). Used by permission of the SUNY College of Environmental Sciences and Forestry at Syracuse University.

no increase in length of the derivatives that differentiate into the vessel members. This is not surprising considering the fact that vessel members are longitudinally stacked one upon another, at maturity forming long tubes (Fig. 11.16a, b). Most of the growth of vessel members is, therefore, diametric, resulting in lateral increase in size. As differentiating vessel members increase in size, diametrically, the inclination of their end walls decreases.

During differentiation, tracheary elements progress through several well-defined stages, including initiation or specification (i.e., the determination of the identity of the cell type into which a cambial derivative will differentiate), cell growth of the protoplast and primary cell wall, deposition of secondary wall and wall patterning, cell wall removal (as, for example, during the formation of the perforations in vessel member end walls), and programmed cell death.

It is widely accepted that tracheary cell differentiation and growth are influenced by several different plant hormones. Research of Sachs (1981) and Aloni (1992, 2001), among many others, has convincingly demonstrated the presence of a polar flow of auxin through the cambial zone from the leaves to the roots. During periods of growth this basipetal flow of auxin plays a significant role in the continuing, generally acropetal, differentiation of vascular tissues. In developing secondary xylem, hormone function is under the control of specific genes, the expression of which is thought to be regulated by variation in auxin concentrations across the cambial zone (Uggla *et al*., 1998; Schrader *et al*., 2003). High auxin concentrations maintain a population of actively dividing cambial initials, whereas different lower concentrations facilitate the expression of genes controlling the differentiation from the cambial derivatives of the various types of tracheary elements (Turner *et al*., 2007). Whereas differentiation of xylem occurs at high levels of auxin, the differentiation of vessel members is dependent, in addition, on cytokinin originating in root apices (Aloni, 1992; see also Turner *et al*., 2007). It has been suggested that cytokinin increases the sensitivity of cambial derivatives to auxin, thus stimulating them to differentiate into tracheary elements (Baum *et al*., 1991; Fukuda, 1997). High levels of auxin stimulate the rapid differentiation of fibers with thick secondary walls whereas high levels of gibberellic acid influence the differentiation of long fibers with thin walls (Roberts *et al*., 1988).

As incipient tracheary elements differentiate, they grow, primarily in length (tracheids and fibers) or in diameter (vessel members). Enlargement of the protoplast and expansion of the enclosing primary cell wall must occur synchronously. Primary wall expansion is facilitated by an enzymatic process called **wall loosening**. According to Cosgrove (1993), enzymatic loosening of the cell wall results initially in a reduction of turgor pressure with the result that water is drawn into the cell which physically extends it, a viewpoint promoted earlier by Lockhart (1965) and Ray *et al*. (1972). Thus, wall loosening is considered by some researchers to be the primary determinant in cell expansion (Sugimoto-Shirasu *et al*., 2004). Recent research indicates that the protein **expansin** controls wall loosening by uncoupling the molecular strands comprising the polysaccharide network of the wall (Cosgrove, 2000; Cosgrove *et al*., 2002). Of the several described families of expansins, two, the α- and β-expansins are known to mediate cell wall loosening. The several members of these expansin gene families are expressed in different tracheary cell types, or during different developmental stages of cell wall differentiation (Gray-Mitsumune *et al*., 2004).

Some cells of the secondary xylem, for example, tracheids and, especially, libriform fibers, become much longer during differentiation than the fusiform initials from which they were derived. Whereas the central region of such developing cells may grow symplastically with surrounding cells in the tissue, their growth beyond the longitudinal extent of their cambial precursors results from **intrusive tip growth**. The primary wall of the growing tips of these cells is very thin, and as the tip is extended, new wall is synthesized. Evidence for intrusive tip growth is the presence of the nucleus and dense cytoplasm in the growing tips, and during development, the gradual addition of secondary wall toward the cell tips (see Larson, 1994). Chaffey *et al*. (2000) have suggested that the movement of Golgi and ER vesicles, carrying precursor compounds of wall synthesis into the cell tip, is facilitated by actin microfilaments which are thought to control cyclosis (cytoplasmic streaming).

The means whereby growing cell tips intrude between adjacent cells is unclear. Early workers apparently thought that the growing tips simply pushed apart the cells and, in the process, disrupted the middle lamella. More recently, the possibility of enzymatic digestion of the middle lamella (Hejnowicz, 1980) and wall loosening by expansins in the walls of cells between which intrusive growth takes place (Barnett, 1981; Gray-Mitsumune *et al*., 2004) have been suggested as means which facilitate the intrusion of cell tips.

Just prior to the cessation of growth in cells of the secondary xylem, the cell wall becomes radically modified by the addition of secondary wall layers in various patterns. Components of the cytoskeleton, especially microtubules, are intimately associated with cellulose synthesis, the orientation of cellulose microfibrils, and with wall sculpturing (e.g., Baskin, 2001). During the processes associated with this phase of differentiation the nucleus enlarges, Golgi bodies and endoplasmic reticulum become associated with sites of wall synthesis, and microtubules commonly become aligned beneath the plasma membrane in patterns that will reflect the ultimate patterns of secondary wall deposition (e.g., Hirakawa, 1984; Uehara and Hogetsu, 1993; Abe *et al*., 1994; Turner *et al*., 2007). Transport of secondary wall components into the region of wall synthesis is accomplished by fusion of Golgi and/or ER vesicles with the plasma membrane, thus expelling into the sites of wall synthesis precursor compounds of cellulose and wall matrix substances. **Cellulose synthase complexes** from which cellulose microfibrils are generated (see Chapter 4 on the cell wall) are also transported to the plasma membrane in Golgi vesicles.

There are many examples of the correlation between the position of microtubules and the orientation of microfibrils. During the formation of annular and helical secondary wall thickenings of tracheary elements in the primary xylem, helical wall thickenings in tracheids of the secondary xylem, and in developing pit borders, cellulose microfibrils are synthesized in patterns parallel to the microtubules underlying the plasma membrane (Hogetsu, 1991; Uehara and Hogetsu, 1993; Chaffey *et al*., 1997; Funada *et al*., 2000; Funada, 2002). It is interesting

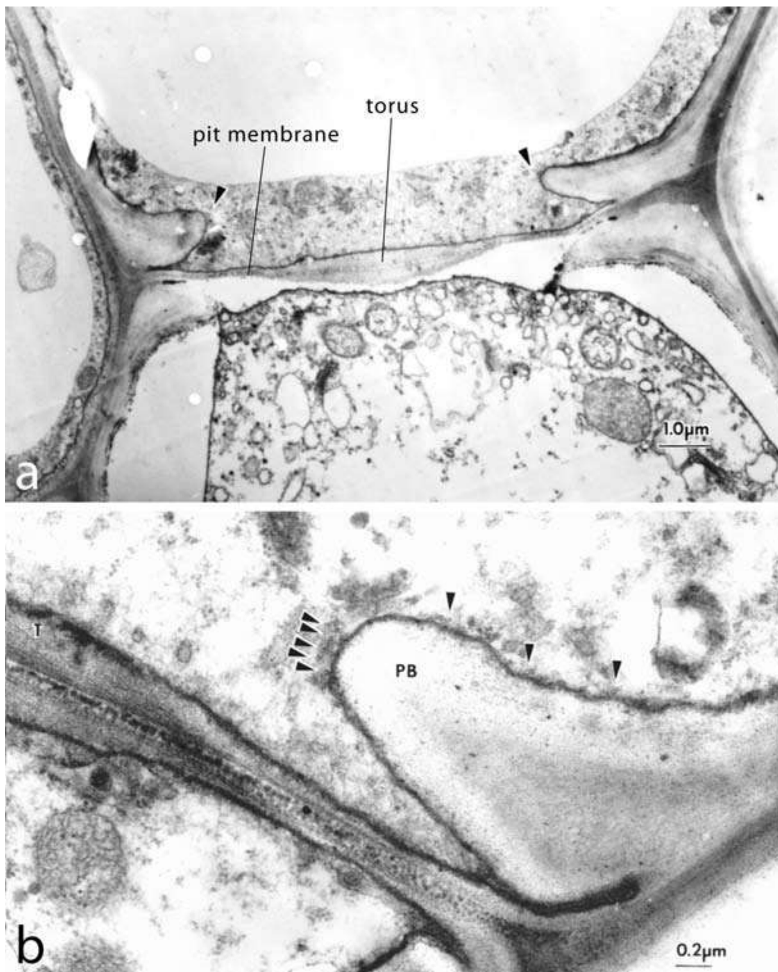


Figure 11.19 Bordered pit development in *Taxus* (yew). (a) Sectional view of a developing bordered pit-pair showing extension of the pit borders (arrowheads) over the pit membrane. (b) Enlargement showing a cluster of microtubules in sectional view (cluster of arrowheads) just inside the plasma membrane. These microtubules form a ring around the inner, developing edge of the pit border (PB). Microtubules also occur over the surface of the pit border, some of which are indicated by arrow heads. From Uehara and Hogetsu (1993). Used by permission of Springer-Verlag Wien.

to note, however, that the fusiform cambial initials, from which tracheary elements and associated cells are ultimately derived, typically contain randomly arranged, peripheral microtubules. As cambial derivatives begin their differentiation, the microtubules become arranged in specific patterns in relation to the ultimate orientation of cellulose microfibrils. In the conifer *Taxus* (yew), for example, very early in tracheid development, regions which become free of microtubules are thought to indicate the sites in which pits will form (Funada, 2002). These incipient pits are quite large, up to 8–9 µm in diameter (Uehara and Hogetsu, 1993). They become surrounded on their inner margins by rings of microtubules (Fig. 11.19a, b). As seen in face view in confocal immunofluorescence images they appear as bright rings around wide apertures (Fig. 11.20). As differentiation proceeds and additional wall material is added to the border, the ring of microtubules decreases in diameter as the border expands over the pit cavity and the aperture becomes smaller (Fig. 11.21) (Uehara and Hogetsu, 1993; Funada, 2002). Concurrently, microtubules between adjacent pits become

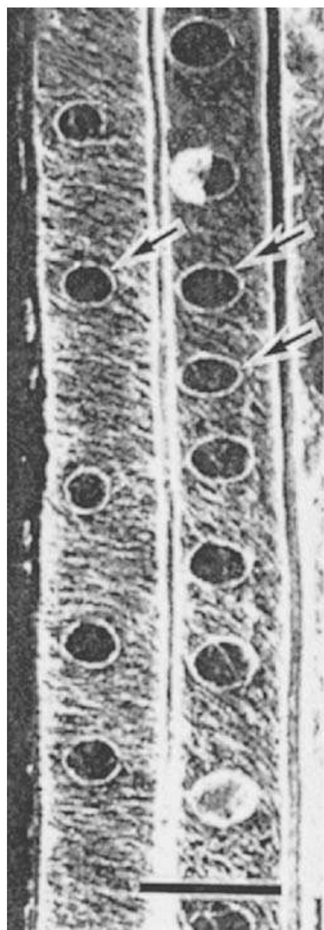


Figure 11.20 Rings of microtubules (arrows) around the inner edges of developing pit borders. Note also the helical bands of microtubules, associated with helical wall thickening, between the developing pits. Bar = 25 μm. From Funada (2002). Used by permission of Taylor and Francis Books Ltd.

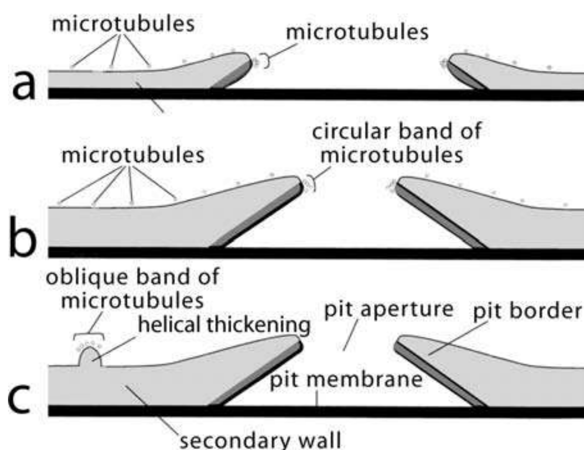


Figure 11.21 Diagram illustrating the association of microtubules with (a) the initial thickening and extension of the border over the pit cavity, (b) continued development of the border, and increase in thickness of the wall between pits, and (c) development of helical thickenings. From Uehara and Hogetsu (1993). Used by permission of Springer-Verlag Wien.

oriented helically and are associated with cellulose microfibril synthesis resulting in an increase in secondary wall thickness between and around the pits (Figs 11.19b, 11.21a, b). During formation of pit borders in the roots of *Pisum sativum* (pea) microfibrils are initially deposited in two arcs on the sides of the pit sites. In face view of pit replicas from freeze-dried sections the microfibrils appeared to Hogetsu (1991) "like a stream flowing around rocks in a river" (Fig. 11.22a). As development of the border continued, the microfibrils curved around the ends and, thus, enclosed the developing aperture (Fig. 11.22b). Following pit development, conspicuous helical bands of microtubules (Figs 11.21c, 11.23a) appeared on the inner surface of the wall between the pits in the pattern of helical, tertiary wall thickenings that developed on the inner surface of the secondary wall (Fig. 11.23b, c) (Uehara and Hogestsu, 1993).

Similar observations have been made also on differentiating tracheary elements in other angiosperms. One additional example will suffice. Early in the differentiation of incipient vessel members of *Aesculus hippocastanum*, microtubules become arranged in a sort of reticulum containing microtubule-free regions (as in *Taxus*) that signal the future position of bordered pits (Chaffey *et al.*, 1999). During pit development, a ring of microtubules appears just inside the developing pit border. As the border grows and the pit opening is reduced, the diameter of the ring of microtubules also decreases (Chaffey *et al.*, 1997).

This intimate association of microtubules with cellulose microfibrils and structural modifications of the cell wall during the differentiation of the secondary xylem in conifers and dicotyledons indicates that microtubules have a significant role in the processes by which these wall modifications occur (Chaffey *et al.*, 1997; Baskin, 2001; Paredez

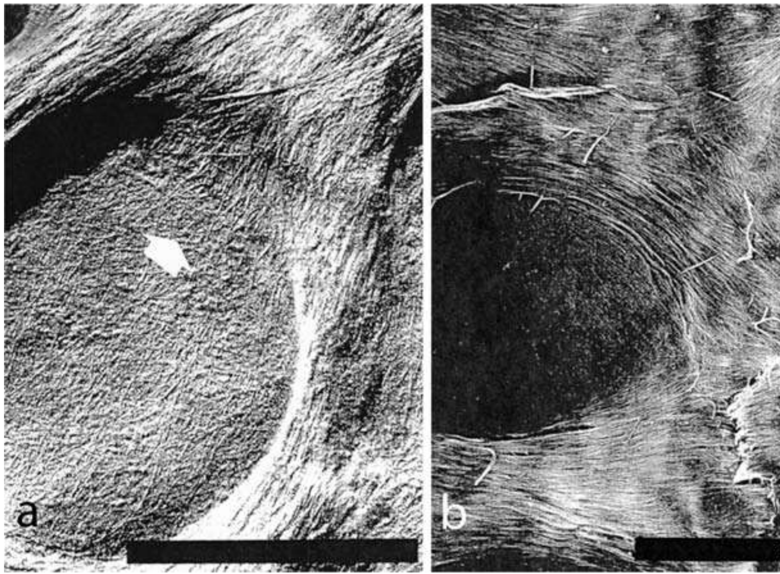


Figure 11.22 Arrangement of cellulose microfibrils in developing bordered pits in tracheary elements from the root of *Pisum sativum* (pea). (a) Initially, microfibrils are deposited in arcs above and below the pit sites. Bar = 2 μm. (b) As development continues, microfibrils are deposited around the ends of the arcs, enclosing the developing pit apertures. Bar = 2 μm. (a, b) From Hogetsu (1991). Used by permission of Springer-Verlag GmbH and Co. KG. © Springer-Verlag Berlin Heidelberg.

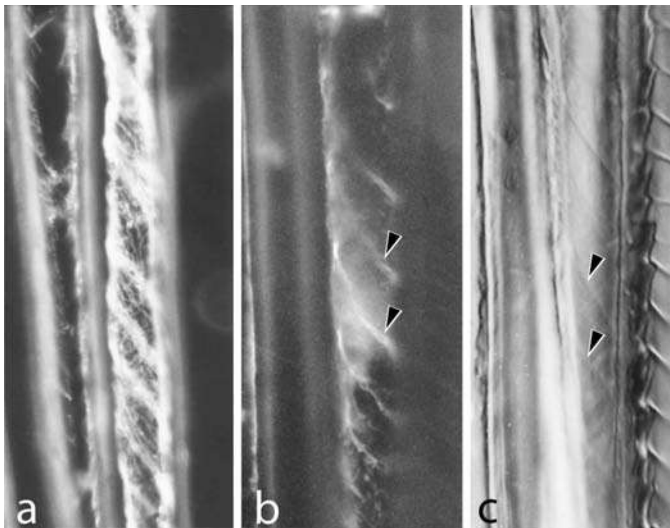


Figure 11.23 Association of microtubules with developing helical wall thickenings in *Taxus* tracheids. (a) Early stage, showing helical bands of microtubules. (b) Bands of microtubules superimposed on developing helical thickenings. (c) Helical thickenings after microtubules have disappeared. Bars (a–c) = 10 μm. (a–c) From Uehara and Hogetsu (1993). Used by permission of Springer-Verlag Wien.

et al., 2006). This viewpoint is supported by experiments utilizing colchicine to destroy the microtubules prior to secondary wall formation which provides strong evidence of their influence in the orientation of wall thickenings. Lacking microtubules, wall thickenings occur in very irregular patterns. Since the early studies of Ledbetter and Porter (1963), Hepler and Newcomb (1964), Wooding and Northcote (1964), and others, the significance of this relationship has been discussed and speculated upon at length, but as yet, there has been no widely accepted explanation of the mechanism by which microtubules influence, or might even control, the synthesis of cellulose. Giddings and Staehelin (1988) hypothesized that parallel microtubules attached to the plasma

membrane by putative protein bridges, form "channels" in which terminal cellulose synthase complexes (rosettes) are restricted. Thus, as the cellulose microfibrils are generated the cellulose synthase complexes are pushed along these channels by the polymerization of cellulose. Initially applied to the alga, *Closterium*, this hypothesis has been extended to vascular plants as well. (See also the more complex hypothesis of "templated incorporation" of Baskin (2001), which accounts for cellulose microfibril alignment in association with microtubules as well as in their absence.) The most convincing evidence for the role of microtubules in the synthesis of cellulose has been provided by Paredez *et al*. (2006). Using techniques that allowed visualization of the movement of Golgi vesicles and cellulose synthase complexes, they demonstrated that the cellulose synthase complexes move within the plasma membrane in parallel trajectories on either side of microtubules. They suggested that the microtubules might actively guide the cellulose synthase complexes and, thereby, control the orientation of newly synthesized cellulose microfibrils. (For more detail, see Chapter 4.)

In differentiating vessel members and other xylem cells in the axial system, actin microfilaments also apparently play a major role in cell wall ornamentation including the formation of simple and bordered pits, helical tertiary thickenings, and the perforations in perforation plates (Chaffey and Barlow, 2002; Chaffey *et al*., 2002). The parallel orientation of microfilaments and microtubules at all of these sites suggests interaction between these components of the cytoskeleton, but the nature of this interaction is unclear. Chaffey *et al*. (2000) have hypothesized, however, that in the development of bordered pits in vessel members F-actin, possibly linked with myosin as actomyosin, might provide the motive force for contraction of the ring of microtubules and microfilaments as the pit aperture decreases in diameter. This viewpoint is further supported and expanded by Chaffey and Barlow (2002) who hypothesize that "an acto-myosin contractile system (a 'plant muscle') is present at the cell plate, the sieve pores, the plasmodesmata within the walls of long-lived parenchyma cells, and at the apertures of bordered pits during their development."

Upon completion of secondary wall formation in tracheary elements, the protoplast may die, as in tracheids and vessel members, or it may persist for several years, as in some wood fibers (Fahn, 1990; Larson, 1994). Just prior to cell death hydrolytic enzymes are believed to enter the cell protoplast from surrounding parenchyma cells, probably through and under the control of plasmodesmata (see Juniper, 1977; Ehlers and Kollmann, 2001), initiating hydrolysis of the regions of primary wall not protected by lignified secondary wall. The effect of hydrolases on the unprotected primary wall may result in the digestion of wall matrix material (e.g., Ohdaira *et al*., 2002), leaving meshworks of cellulose microfibrils which comprise pit membranes. In vessel members entire regions of the primary wall are removed resulting in perforations in the end walls and the formation of perforation plates. It is now well established that the first visual sign of **programmed cell death**, which concludes the differentiation of tracheids and vessel members,