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Bioinformatics Analysis of the *FREM1* Gene—Evolutionary Development of the IL-1R1 Co-Receptor, TILRR

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Abstract: The TLRs and IL-1 receptors have evolved to coordinate the innate immune response following pathogen invasion. Receptors and signalling intermediates of these systems are generally characterised by a high level of evolutionary conservation. The recently described IL-1R1 co-receptor TILRR is a transcriptional variant of the *FREM1* gene. Here we investigate whether innate co-receptor differences between teleosts and mammals extend to the expression of the TILRR isoform of *FREM1*. Bioinformatic and phylogenetic approaches were used to analyse the genome sequences of *FREM1* from eukaryotic organisms including 37 tetrapods and five teleost fish. The TILRR consensus peptide sequence was present in the *FREM1* gene of the tetrapods, but not in fish orthologs of *FREM1*, and neither *FREM1* nor *TILRR* were present in invertebrates. The *TILRR* gene appears to have arisen via incorporation of adjacent non-coding DNA with a contiguous exonic sequence after the teleost divergence. Comparing co-receptors in other systems, points to their origin during the same stages of evolution. Our results show that modern teleost fish do not possess the IL-1RI co-receptor TILRR, but that this is maintained in tetrapods as early as amphibians. Further, they are consistent with data showing that co-receptors are recent additions to these regulatory systems and suggest this may underlie differences in innate immune responses between mammals and fish.

Abbreviations

IL-1: interleukin 1; IL-1RI: interleukin 1 receptor type I; TILRR: Toll-like and Il-1 receptor regulator; NF-κB: nuclear factor kappa B; NCBI: National Center for Biotechnology Information; FREM-1: FRAS Related Extracellular Matrix gene 1.

1. Introduction

The innate immune system is generally well conserved throughout the animal kingdom with the same characteristic features of regulatory components present in species ranging from insects to mammals [1]. Activation is induced through members of the Toll-like and IL-1 receptor (TIR) family, characterized by the cytoplasmic TIR domain [2]. The high level of conservation of the intracellular domains of both Toll and IL-1R1 and of cytoplasmic regulatory components is consistent with a signaling system that is broadly conserved throughout evolution prior even to the divergence of plants [3] and animals [4]. However, it is increasingly recognized that mechanisms of ligand recognition and co-receptor association, a potent regulator of signal amplification at the level of the receptor complex, are less well conserved [5,6]. In evolutionary terms, such co-receptors appeared relatively late in the development of their respective signaling networks which they control.

Recent studies have revealed that fish, which have been shown to possess certain inflammatory receptors, may lack co-receptors found in more modern organisms, suggesting that signaling mechanisms in earlier species are functionally distinct and less refined. Thus, for example the zebrafish (*Danio rerio*) possesses two paralogs of TLR4, neither of which is stimulated by LPS, and lacks the co-receptors MD2 and CD14 [7,8]. Similarly, phylogenetic studies of the synteny of the syndecan genes in fish and tetrapods has revealed that while the four mammalian syndecan genes arose due to gene duplication, Syndecan 1 (an FGFR co-receptor) is absent from fish genomes probably as a result of deletion following this duplication event [9].

We recently identified the IL-1R co-receptor, TILRR (Toll-like/IL-1 receptor regulator), a 715 amino acid heparan sulfate glycoprotein encoded within the gene for the extracellular matrix protein FREM1 [10]. FREM1 has a distinct function in embryogenesis and development, and is ubiquitously expressed [11].

TILRR binds the cell membrane through a *C*-terminal lectin domain, associates with IL-1R1 and increases receptor expression and ligand binding. TILRR association potentiates recruitment of the MyD88 adapter and receptor signal amplification, and enhances activation of NF-κB and inflammatory genes [10]. We earlier confirmed expression and function of TILRR in mouse and human cells [10].

The current studies examine the presence of TILRR throughout evolution and demonstrate that TILRR is a transcriptional variant of *FREM1* whose transcriptional start site lies within the intronic sequence of *FREM1*. TILRR is lacking in early species such as teleosts and invertebrates, being first identifiable in amphibians. These findings highlight that although innate immunity is evolutionarily

ancient, refinements to the system have continued to arise until more recently and that important differences exist between model organisms used to study inflammation.

2. Results and Discussion

In order to determine the evolutionary development of the IL-1RI co-receptor TILRR, we identified the TILRR isoform of *FREM1* within the genomes of multiple organisms and defined the source of the TILRR peptide sequence within the nucleotide sequence of the *FREM1* loci. Alignment of the peptide sequences of human TILRR and FREM1 show that they are identical from R17 of TILRR (R1481 of FREM1). This TILRR/FREM1 consensus region is encoded by exons 25 onwards in the human *FREM1* gene.

To predict the location within the Human *FREM1* locus where *TILRR* transcripts initiate, we analysed the Ensembl annotation of the 2,179 amino acid encoding Human *FREM1* gene (ENST00000452036). Studying the cDNA sequence of this transcript revealed R1481 to be encoded within a residue overlap splice site and due to ligation of the final two nucleotides of exon 24, and the first of exon 25 (Figure 1). A lack of homology between the 16 N-terminal TILRR residues and any other part of FREM1 suggested that no early *FREM1* coding exon is ligated to exon 25 to encode TILRR. Therefore, we reasoned that the *N*-terminus (translational start site) of TILRR must reside within a sequence of *FREM1* not incorporated into the processed *FREM1* mRNA. We hypothesized that the *N*-terminus of TILRR could be located by examining the annotation of the *FREM1* transcript, prior to the first exon common to both *TILRR* and *FREM1* (exon 25). Translation in all three reading frames of the intronic nucleotide sequence immediately preceding exon 25 produced the unique 16 TILRR *N*-terminal residues that we previously sequenced using MALDI-TOF [10]: MVTQESMLKAALPLFT followed by R17 and the remaining residues up to Q155. As the *N*-terminus of TILRR is produced by a series of consecutive nucleotides immediately prior to and in frame with the third nucleotide of the R1481 codon of FREM1, during RNA processing, *FREM1* mRNA transcripts arise when exon 24 is ligated to a 3' splice acceptor site prior to the third nucleotide of the R1481 codon, whereas exon 1 of *TILRR* commences within intron 23–24 and runs into exon 24 without the requirement for such an acceptor splice site (Figure 1B). This initiation of a novel "orphan" gene from a non-coding sequence is a recently described mechanism, which in many cases allows the organism to adapt to novel conditions [12–14].

Analysis of the Mouse *Frem1* locus (ENSMUSG00000059049) in the same manner as Human *FREM1,* revealed a similar splicing mechanism: the *N*-terminal 16 residues of Mouse TILRR (MGTQEPMLKAALPLFA, as we previously showed by peptide sequencing) [10] are encoded by an intronic nucleotide sequence upstream of exon 25 of the *Frem1* transcript. This is consistent with the suggestion that *TILRR* and *FREM1* mRNAs arise due to alternative transcriptional initiation of the *TILRR* mRNA within intron 24–25 of the *FREM1* gene.

Since analysis of both the Human *FREM1* and Mouse *Frem1* loci clearly identified the 5' *TILRR* coding sequence within the intron preceding exon 25, we reasoned that examining this region in other species would allow determination of whether each organism possesses an ortholog of *TILRR*.

Figure 1. (**A**) Schematic diagram of Human and Mouse *FREM1* pre-mRNA. (**B**) DNA sequence and peptide translation of human and mouse *FREM1* in region of boundary between exons 24 and 25 (upper panel) and TILRR showing genomic sequence and translated peptide.
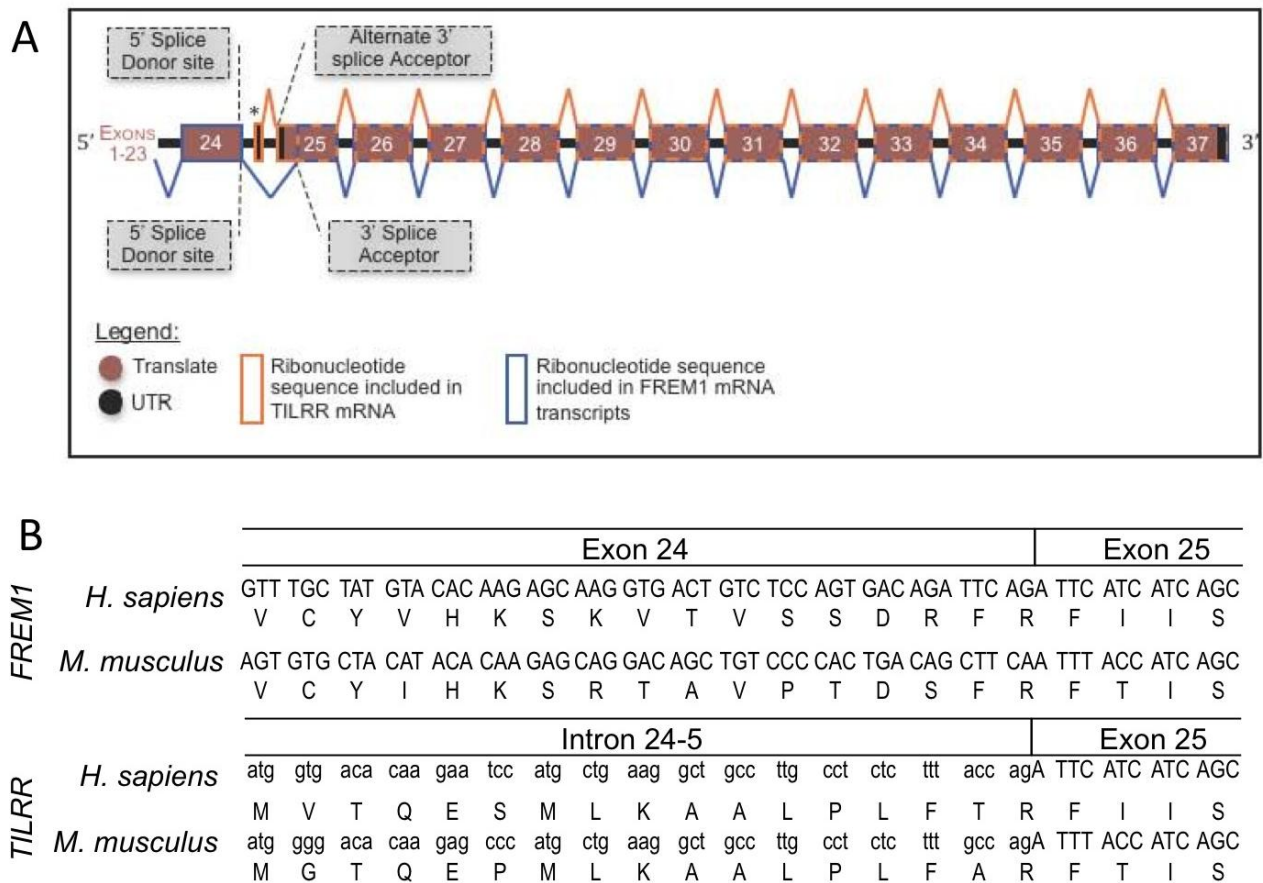
(A) Exons numbered as displayed in the ENSEMBL transcript (ENST00000452036). The diagram outlines the splicing events producing mature *FREM1* mRNA (shown in grey) and the alternate splice events within the intronic region of exons 24–25, which give rise to *TILRR* mRNA (shown in black). As indicated by ENST00000380894, *TILRR* transcripts include a 5' UTR ending within the intronic region of the annotated *FREM1* exons 24–25. Following *FREM1* exon 25, splicing events for both *FREM1* and *TILRR* are identical. * As of the time of writing, it is unknown how far upstream the 5' UTR of *TILRR* extends upstream of exon 25. (B) Intron 24–25 and Exon 25 of *FREM1* are also Exon 1 and 2 of *TILRR*.

We therefore extended our investigation to examining the genomes of 37 tetrapod organisms to identify the 5' end of the *TILRR* coding sequence within each Frem1 ortholog. We used the predicted *FREM1* cDNA transcript sequences to identify the region of each locus corresponding to the exon containing alternative 3' splice acceptor sites as in exon 25 of Human *FREM1*. The preceding nucleotides were translated in frame with the nucleotide sequence of the identified exon and the resulting peptide sequence aligned with the TILRR N-termini. For 33 tetrapod species, 16 consecutive amino acids could be produced in frame with R17, suggesting that a strongly conserved *TILRR* homolog exists in these organisms (Figure 2).

Although four tetrapod *FREM1* loci (*C. familiaris*, *D. ordii*, *T. syrichta* and *E. telfairi*) could not immediately be translated into the 5' TILRR residues, we found that all four possess a single nucleotide alteration compared to the consensus TILRR peptide sequence, but that with this exception the consensus *N*-terminal TILRR sequence was preserved, indicating these species are likely to possess the TILRR isoform of FREM1 (Figure 3). Alternatively, in these species *TILRR* may constitute a pseudogene, which produces a non-functional protein, although it is highly likely that all tetrapod homologues arose from a common ancestor. Future studies are needed to assess the significance of these mutations in signal amplification of the TIR domain.

Since we had identified TILRR orthologues in all tetrapod species studied, we next analysed teleost *FREM1* homologs using annotations of all identified teleost *FREM1* orthologs in the Ensembl database. All teleost species possessed at least one *FREM1* orthologue. However, in these organisms no such *FREM1* ortholog contained a conserved 5' coding sequence indicative of a *tilrr* transcript (Figure 4).

Figure 2. Comparison of TILRR *N*-terminal sequence.

Organism	TILRR N-terminus
V.pacos-ENSVPAG00000005812	MVTREPMLKAALPLFAR 17
T.belangeri-ENSTBEG00000013718	MVTPEPMLKAALPLFAR 17
F.catus-ENSFCAG00000004349	MVTQDPMLKAALPLFAR 17
H.sapiens-ENSG00000164946	MVTQESMLKAALPLFTR 17
P.troglodytes-ENSPTRG00000020782	MVTQESMLKAALPLFTR 17
G.gorilla-ENSGGOG00000014708	MVTQESMLKAALPLFTR 17
P.pygmaeus-ENSPPYG00000019211	MVTQESMLKAALPLFTR 17
O.cuniculus-ENSOCUG00000010379	MVTQESMLKAALPLFAR 17
S.tridecemlineatus-ENSSTOG00000003243	MVTQESMLKAALPLFAR 17
O.princeps-ENSOPRG00000017066	MVTQESMLKAALPLFAR 17
M.murinus-ENSMICG00000015934	MVTQESMLKAALPLFAR 17
M.lucifugus-ENSMLUG00000016591	MVTQESMLKAALPLFAR 17
P.vampyrus-ENSPVAG00000014241	MVTQESMLKAALPLFAR 17
C.jacchus-ENSCJAG00000012240	MVTQESMLKAALPLFAR 17
M.mulatta-ENSMMUG00000008128	MVTQESMLKAALPLFAR 17
E.caballus-ENSECAG00000015567	MVTQESMLKAALPLFAR 17
E.europaeus-ENSEEUG00000001483	MVTQESMLKAALPLFAR 17
C.porcellus-ENSCPOG00000011267	MVTQESMLKAALPLFAR 17
B.taurus-ENSBTAG00000020141	MVTQESMLKAALPLFAR 17
O.garnettii-ENSOGAT00000011883	MVTQESMLKAALPLFAR 17
S.araneus-ENSSARG00000010446	MVTHESMLKAALPLFAR 17
M.domestica-ENSMODG00000014986	MVKQASMLKATLPLFAR 17
M.eugenii-ENSMEUG00000012357	MVKQASMLKATLPLFAR 17
T.truncatus-ENSTTRG00000008037	MVTQESMLKATLPLFAR 17
P.capensis-ENSPCAG00000015286	MVKQESMLKAALPLFAR 17
C.hoffmanni-ENSCHOG00000008350	MVKQESMLKAALPLFAR 17
L.africana-ENSLAFG00000008954	MVKQESMLKAALPLFVR 17
M.musculus-ENSMUSG00000059049	MGTQEPMLKAALPLFAR 17
R.norvegicus-ENSRNOG00000022309	MGTQEPMLKTALPLFAR 17
X.tropicalis-ENSXETG00000003300	MGKQEPMLKAPLPLFAR 17
G.gallus-ENSGALG00000005426	MVNQEHMLKTTLPLFAR 17
T.guttata-ENSTGUG00000004583	MVNREQMLKTALPLFAR 17
A.carolinensis-ENSACAG00000017780	MVKQEHMLKTALPLFAR 17
Consensus sequence	* . ***:.****.*

The 17-residue TILRR N-terminal sequences as identified within FREM1 gene records including Human and Mouse TILRR, with consensus sequence.

Figure 3. Identification of single base mutations responsible for non-homologous sequence in *C. familiaris*, *D. ordii*, *T. syrichta* and *E. telfairi*.

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Tetrapod FREM1 orthologs
H.sapiens              atggtgacacaagaatccatgctgaaggctgcc-ttgcctctctttaccagATTC
ENSG00000164946        -M--V--T--Q--E--S--M--L--K--A--A- -L--P--L--F--T--R--F-
T.syrichta             atg cggcaagagaatccatgctgaaggctccc-ttgcctctctttgccagATTC
ENSTSYG00000004164     -M     -A--R--E--S--M--L--K--A--P- -L--P--L--F--A--R--F-
D.ordii                ctggtgacacaagaatccatgctgaaggctgcc-ttgcctctctttgccagATTC
ENSDORG00000014455     -L -V--T--Q--E--S--M--L--K--A--A- -L--P--L--F--A--R--F-
E.telfairi             atggtgaaagcaga tccatgctgaaggctgcc-ttgcctctctttgtcagATTC
ENSETEG00000015868     -M--V--K--A-   -S--M--L--K--A--A- -L--P--L--F--V--R--F-
C.familiaris           atggtgacacaagattccatgctgaaggctgcc ttgcctctctttgccagATTC
ENSCAFG00000001537     -M--V--T--Q--D--S--M--L--K--A--A- -L--P--L--F--A--R--F-
                        **  *   *   *** ***************** ** ***********  ***★★★★
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For *C. familiaris*, *D. ordii*, *T. syrichta* and *E. telfairi*, analysis of the nucleotide sequences and corresponding translations revealed that single base mutations (highlighted in **black**) are responsible for non-homologous *N*-terminal sequences. *H. sapiens* TILRR is also shown for comparison.

Figure 4. *FREM1* orthologs in fish lack the TILRR *N*-terminus.

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Teleost frem1a orthologs
D.rerio Zv7            tgtaggacatactccttgctcaatatccatcaatctgcatcatccctatagGTTC
ENSDARG00000069473     --V--G--H--T--P--C--S--I--S--I--N--L--H--H--P--Y--R--F-
G.aculeatus            taagggatcagttcagaatcctaacatataatctgtttttatgtttttgccagGTTT
ENSGACG00000000932     --K--G--S--V--Q--N--P--N--I-----S--V--L--C--F--A--R--F-
O.latipes              cgttgggattttgtagcaccctaacaccggttcctctgcatgcttgaacagGTTT
ENSORLG00000020663     --V--G--I--L-----H--P--N--T--G--S--S--A--C--L--N--R--F-
T.nigroviridis         ccgggcggacctgctccagtcgccgcaccacaacccgtctgtctcctcagGTTT
ENSTNIG00000005289     --R--A--D--L--L--Q--S--P--H--H--N--P--V--C--L--L--R--F-
T.rubripes             cccttgtcgagagaggtaaatgcacctgtcagcttttggatatctctttagGTTT
ENSTRUG00000006301     --P--C--R--E--R-----M--H--L--S--A--F--G--Y--L--F--R--F-

frem1b orthologs
D.rerio                AAGCTGAATTCTTCAAATCTTTTCTGACACAAATCATGTTCTCTCCTGCAGCTTT
ENSDARG00000062402     --S-----I--L--Q--I--F--S--D--T--N--H--V--L--S--C--S--F-
A.carolinensis         AAATTCACAATAGTTTATTATCTCCCTGTTCTTTCTTTTTTTCTGAAATAGTTTT
ENSACAG00000007607     --N--S--Q-----F--I--I--S--L--F--F--L--F--F-----N--S--F-
X.tropicalis           GAAATCTGCATATCTTTAGGATTCTATAACCTTATTCTTATTTCTATTTAGTTTC
ENSXETG00000008875     --K--S--A--Y--L-----D--S--I--T--L--F--L--F--L--F--S--F-
G.aculeatus            GTTGGTGTGATCTCCAATCTGCTTCCAACCAAACTGTCTTTGTCTTCACAGTTTT
ENSGACG00000003138     --L--V-----S--P--I--C--F--Q--P--N--C--L--C--L--H--S--F-
O.latipes              CCTTGATGTTCATGCTTGAAAGAATGTGATTGTGTGTTTGTTCCTGTACAGTTTT
ENSORLG00000003500     --L--D--V--H--A-----K--N--V--I--V--C--L--F--L--Y--S--F-

H.sapiens              atggtgacacaagaatccatgctgaaggctgcc-ttgcctctctttaccagATTC
ENSG00000164946        -M--V--T--Q--E--S--M--L--K--A--A- -L--P--L--F--T--R--F-
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Diagram of *FREM1*s orthologues in teleost fish, demonstrating lack of potential TILRR *N*-termini like those identified in other organisms, and suggests that TILRR *N*-termini do not exist within fish *frem1*.

We next examined invertebrate species for orthologues of *FREM1/TILRR*. Using BLASTP, we were unable to locate orthologues of either the consensus regions shared between *Frem1* and *Tilrr* or the *Tilrr* 5' sequence in non-vertebrates (*Drosophila melanogaster*, *Ciona intestinalis*, *Caenorhabditis*

elegans). We therefore concluded that invertebrates do not possess *FREM1*, nor a *TILRR* orthologue. Thus, *FREM1* arose after the evolution of vertebrates, but TILRR only becomes detectable after the divergence of the teleosts.

To investigate possible mechanisms for the absence of *TILRR* in teleosts, we examined the exon/exon boundary sequences of *FREM1* in human, mouse, xenopus and four teleosts (*Danio rerio* [zebrafish], *Oryzias latipes* [medaka], *Takifugu rubripes* [fugu] and *Gasterosteus aculeatus* [stickleback] and the corresponding intron/exon sequences that encode for TILRR in tetrapods but not in teleosts (Figure 5). We found that whereas the exonic and particularly translated sequence of *FREM1* were reasonably well conserved even between mammals and telosts, comparison of the intronic region encoding for TILRR in tetrapods reveals marked divergence. Although all introns end with the major spliceosomal AG consensus splice site, the actual intronic sequences diverged markedly between teleosts and mammals. Although N termini often vary greatly in length and sequence between homologues, in zebrafish, medaka and stickleback translation of the ORF of the contiguous intronic sequence preceding the shared *frem1* exon leads to a stop codon between the shared exon and the earliest possible methionine start codon (Figure 5). In Fugu there is a methionine in the N-terminal sequence that could represent a TILRR start codon, although there is no equivalent of this in the human sequence (Figure 5). Given the otherwise high conservation of the *frem1* gene between teleosts, it seems unlikely that Fugu possess a TILRR homologue when the other three teleosts do not. It seems likely that *TILRR* arose through a major alteration of intronic sequence, rather than a more subtle perturbation that gave rise to generation of novel intronic transcription binding sites.

We therefore conclude that the *TILRR* isoform of *FREM1* is present only in tetrapod organisms, presenting two possibilities for the origin of *TILRR*. This may reflect that it originates from an ancestor common to both teleost and tetrapod organisms that arose after the invertebrates, and was lost in *FREM1* paralogs prior to the evolution of modern teleosts. Alternatively, (an explanation we favour) *TILRR* may have originated following the divergence of a common ancestor into the tetrapod lineage, hence its first detection within amphibian *FREM1* (Figure 6).

Either possibility suggests that, in contrast to a majority of IL-1RI complex components, which are present in primitive species such as *D. rerio* [7,9], *TILRR* is not involved in IL-1R1 controlled responses to pathogenic invasion in ancestral or modern day teleosts.

The work in this study shows the maturation of the IL-1 receptor complex within the timeframe between the divergent evolution of teleost fish and tetrapod amphibians some 360–450 million years ago [15]. The conservation of *TILRR* within the genomes of tetrapod organisms likely represents refinement of IL-1 signaling over the course of vertebrate evolution, to allow increased sensitivity of system control through ligand concentration and receptor levels.

The finding that *TILRR* does not exist in any teleost studied suggests that distinct components of the vertebrate IL-1R1 complex may have evolved at different stages of the evolutionary tree, perhaps reflecting functions related to *TILRR* controlled environmental sensing and attachment. The lack of *TILRR* expression in primitive species, such as *D. rerio*, in addition to the absence of Syndecan 1 and the TLR4 co-receptors [8,9], also supports the notion that inflammatory signaling regulatory mechanisms in mammals are not all synonymous with that of lower vertebrates. Common features of the co-receptors of these systems are related to ligand/receptor interactions and receptor sensitivity to ligand, allowing for increasing variability and specificity over a range of ligand and receptor levels.

Similarly, recently identified *TILRR* mutants demonstrate selective regulation of distinct cellular responses related to inflammation and cell survival, thus contributing refined control and increased specificity [16].

Figure 5. Comparative alignment of coding sequence of FREM1 and TILRR in mammals, amphibians and teleosts.
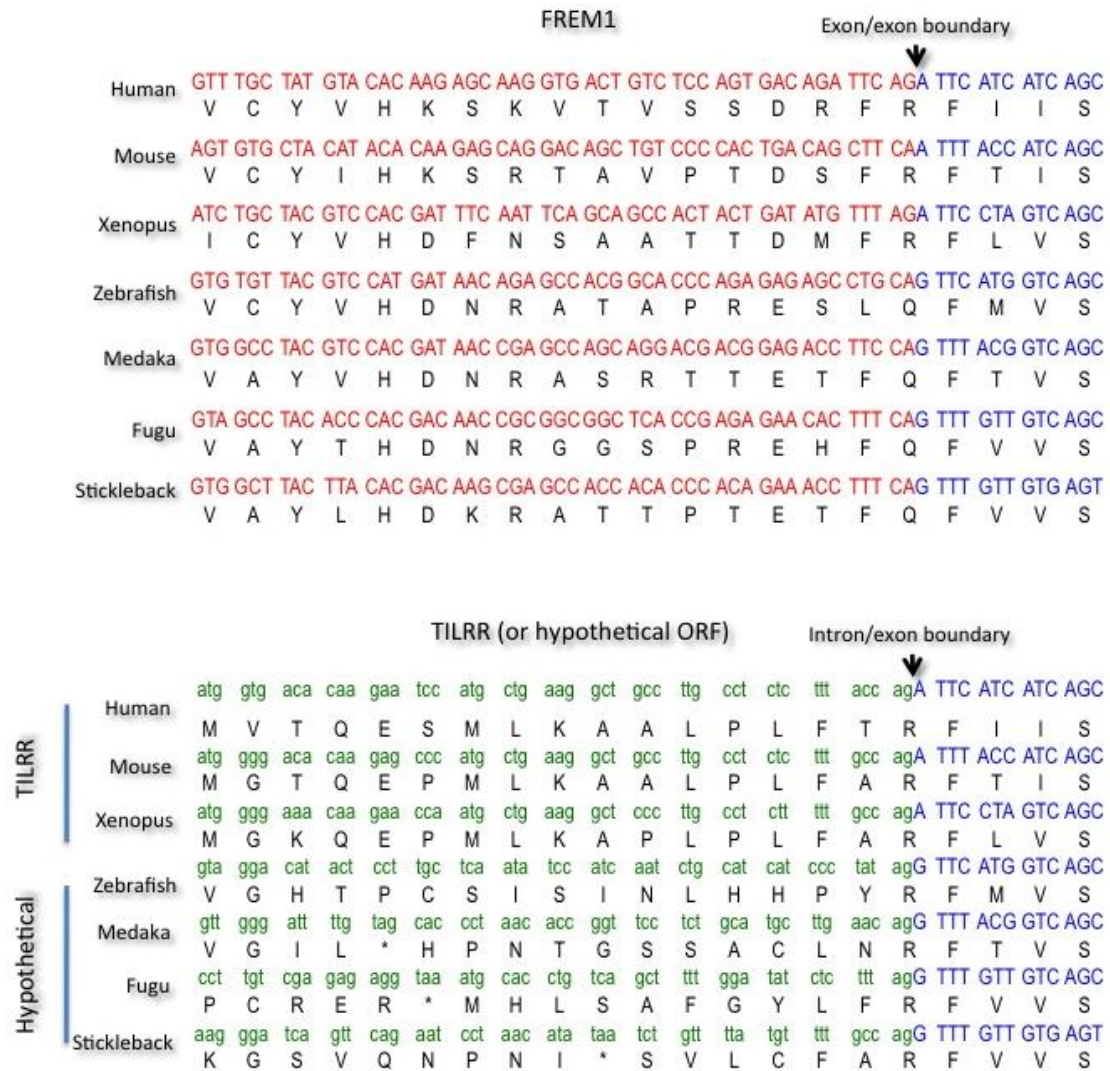
Upper panel: Alignments of the exon/exon sequence coding for FREM1 in human, mouse, xenopus, medaka, zebrafish, stickleback and fugu (red shows sequence for exon 24, blue exon 25) with translated protein sequence. Lower panel: Alignments of the intron/exon sequence coding for TILRR in human, mouse, xenopus, medaka, zebrafish, stickleback and fugu (green shows intron sequence for intron immediately preceding exon 25, blue exon 25) with translated protein sequence (for human mouse and xenopus) and hypothetical translated protein sequence for teleosts to show the protein sequence if the intronic sequence were included in a teleost TILRR orthologue in the same manner as mammals or amphibians. Note the presence of premature stop codons (*) in three of the four teleost sequences (a stop codon arises in the zebrafish intronic sequence upstream of that shown) with only Fugu possessing a methionine downstream to the stop codon that could represent a putative start ATG.

Figure 6. Evolutionary development of FREM1 and presence of putative TILRR sequence.
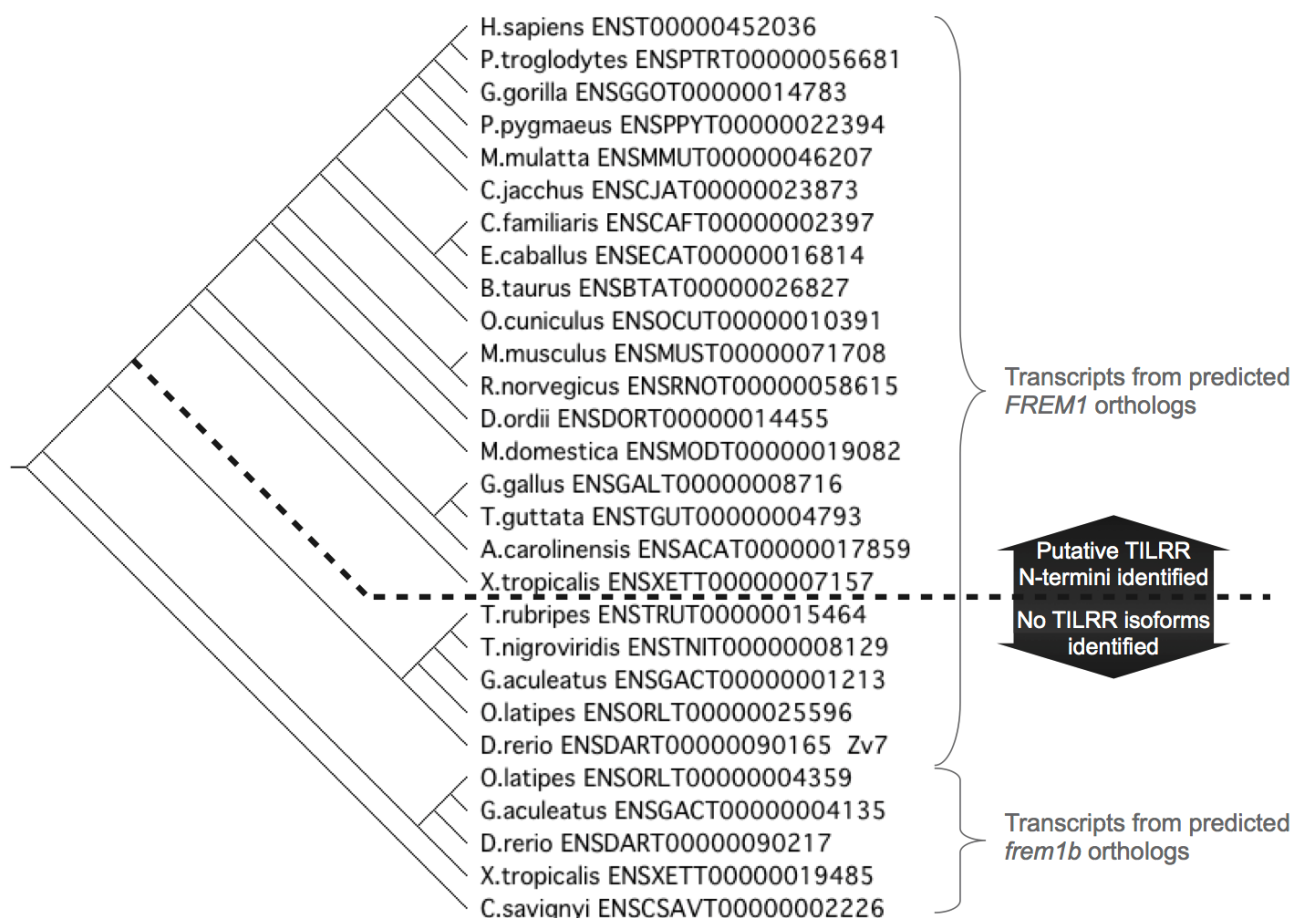
Slanted cladogram displaying the evolutionary divergence of FREM1 in organisms for which complete Frem1 peptide sequences could be identified within Ensembl. FREM1 protein sequences were obtained from the Ensembl transcripts as listed for each organism as orthologs of human FREM1. Representation of multiple Frem1 transcripts indicates the presence of a FREM1 paralog.

3. Experimental Section

3.1. Obtaining Predicted Frem1 Peptide Sequences

Genbank [17] was used as a source for TILRR and FREM1 peptide sequences. GeneIDs and NCBI [18] Reference sequences for the peptides used to compare with predicted transcripts are listed below (Table 1).

Table 1. Accession numbers of FREM1 sequences.

Organism	GeneID	Protein Name	NCBI Reference sequence
H. sapiens	158326	FREM1 isoform 2 (TILRR)	NP_001171175
		FREM1isoform 1 precursor	NP_659403
M. musculus	329872	FREM1 precursor	NP_808531
D. rerio	100216326	Frem1a	NP_001177237
	557221	Frem1b	NP_001131130

Ensembl was used to locate predicted *FREM1* gene loci, identified as orthologs of the Ensembl annotation of Human *FREM1* (ENSG00000164946) or of Zebrafish *frem1b* (ENSDARG00000062402) [19].

Predicted Ensembl *FREM1* ortholog transcripts are shown in Supplementary Figure 1.

Previous studies of *D. rerio* Frem1 used probes deduced by sequence analysis to analyse expression of the two orthologs, Frem1a and Frem1b, which we aligned to the predicted protein sequences as listed in Ensembl. By this method, we found that *D. rerio* Frem1a had a greater similarity to the ENSDART00000090165 transcript annotation in the Zv7 genome as compared to the more recent Zv8 genome annotation. Conversely, the *frem1b* transcript had perfect similarity to ENSDART00000090217 on Zv8. For these reasons we used the Zv7 annotation of ENSDART00000090165 as a basis for the exon structure of *frem1a*, and the Zv8 annotation of ENSDART00000090217 as a basis for the exon structure of *frem1b*.

3.2. Sequence Alignments

All peptide and nucleotide sequence alignments were performed using CLUSTALW and CLUSTALX. A slanted cladogram of FREM1 peptide sequences was constructed using the UPGMA algorithm in CLUSTALX displayed using TreeViewX.

4. Conclusions

Our data show that TILRR is a recent addition to the IL-1RI signaling system. These findings, and those of others looking at evolutionary development of regulatory pathways, are consistent with a role for co-receptors in modulating response control in higher organisms. This opens interesting possibilities for investigating development of regulatory intermediates and delineating mechanisms underlying the increased sensitivity and complexity characteristic of maturation of biological systems during evolution.

Acknowledgments

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