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REVIEW

On the relationship between extant innate immune receptors and the evolutionary origins of jawed vertebrate adaptive immunity

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Abstract

For over half a century, deciphering the origins of the genomic loci that form the jawed vertebrate adaptive immune response has been a major topic in comparative immunogenetics. Vertebrate adaptive immunity relies on an extensive and highly diverse repertoire of tandem arrays of variable (V), diversity (D), and joining (J) gene segments that recombine to produce different immunoglobulin (Ig) and T cell receptor (TCR) genes. The current consensus is that a recombination-activating gene (RAG)-like transposon invaded an exon of an ancient innate immune VJ-bearing receptor, giving rise to the extant diversity of Ig and TCR loci across jawed vertebrates. However, a model for the evolutionary relationships between extant non-recombining innate immune receptors and the V(D)J receptors of the jawed vertebrate adaptive immune system has only recently begun to come into focus. In this review, we provide an overview of non-recombining VJ genes, including CD8β, CD79b, natural cytotoxicity receptor 3 (NCR3/NKp30), putative remnants of an antigen receptor precursor (PRARPs), and the multigene family of signal-regulatory proteins (SIRPs), that play a wide range of roles in immune function. We then focus in detail on the VJ-containing novel immune-type receptors (NITRs) from ray-finned fishes, as recent work has indicated that these genes are at least 50 million years older than originally thought. We conclude by providing a conceptual model of the evolutionary origins and phylogenetic distribution of known VJ-containing innate immune receptors, highlighting opportunities for future comparative research that are empowered by this emerging evolutionary perspective.

Keywords Bowfin and gar (Holostei) · Immune system evolution · Joining domains · Origins of V(D)J recombination · Zebrafish

Introduction

It is widely accepted that the adaptive immune system observed in jawed vertebrates evolved from an ancestral innate immune system. However, it took well over 50 years to arrive at this consensus. The search for the origins of the adaptive immune system, that includes immunoglobulin (Ig) genes that encode antibodies and T cell receptor (TCR) genes, began in the 1960s and 1970s with the discovery that antibodies employed Ig domains to bind antigens (Edelman and Gall 1969) and the reporting of antibody responses in sharks and ray-finned fishes (Papermaster et al. 1964; Litman et al. 1971b, a; Sigel et al. 1972). The identification of TCRs a decade later (Allison et al. 1982; Meuer et al. 1983; Haskins et al. 1984) revealed that they, like antibodies, encode extracellular Ig domains to bind antigens (Chien et al. 1984; Hedrick et al. 1984a, b; Yanagi et al. 1984). Ig and TCR sequences have since been identified in clades as disparate as archosaurs, lepidosaurs, amphibians, lungfish, coelacanth, ray-finned fishes, and cartilaginous fishes (Rast and Litman 1998; Pettinello and Dooley 2014; Mashoof and Criscitiello 2016; Flajnik 2018), thereby providing evidence that these are ubiquitous across all major jawed vertebrate lineages.

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Jawless cyclostome vertebrates (lampreys and hagfish) also display an adaptive immune response. However, biochemical and molecular strategies have failed to identify Ig or TCRs in this ancient lineage (reviewed by Khalturin et al. 2004). Instead, they possess an alternative adaptive immune system defined by antigen receptors that utilize leucine-rich repeats (LRRs), rather than Ig domains, for antigen binding. These variable lymphocyte receptors (VLRs) are expressed in cellular lineages comparable to B and T cells in jawed vertebrates (Pancer et al. 2004a, 2005; Li et al. 2013a; Hirano et al. 2013; Holland et al. 2014; Boehm et al. 2018; Sutoh and Kasahara 2021). Collectively, these observations form the basis of the hypothesis that extant Ig and TCR sequences originated from an ancestral sequence present in the common ancestor of jawed vertebrates.

Across all jawed vertebrate lineages, the vast majority of Ig and TCR proteins share the same general structure that includes a single Ig domain of the V (variable) type on the amino terminus for antigen recognition. The carboxy terminus of the V domain undergoes somatic recombination that, depending on the Ig or TCR class, will join a V segment to a D (diversity) segment and a J (joining) segment, or to only a J segment. This process, termed V(D)J recombination, occurs in lymphocytes and is a defining feature of the jawed vertebrate adaptive immune system. An evolutionary origin of V(D)J recombination that coincided with the dawn of jawed vertebrates is well established. However, the sequences representing likely descendants of the innate immune sequences that gave rise to V(D)J recombination have only recently come to light. In this review, we highlight these new insights and their implications. We begin by providing an overview of the role of V(D)J recombination in Ig and TCR sequence diversification. We next review species that encode germline-joined VJ segments in Ig genes. We then discuss innate immune receptors that represent candidate V(D)J evolutionary ancestors due to their encoding of VJ joined exons. We focus particular attention on novel immune-type receptors (NITRs) of ray-finned fishes, as recent work has identified this group of receptors to have deeper evolutionary origins than previously hypothesized. We conclude our review by providing a phylogenetic perspective for a shared origin of Ig-type adaptive and innate immune receptors.

The evolutionary conservation of V(D)J recombination

The defining genetic feature of the adaptive immune system of jawed vertebrates is the process by which Ig and TCR genes undergo RAG-mediated somatic V(D)J recombination in developing lymphocytes. Across all jawed vertebrates, Ig genes undergo recombination exclusively in B lymphocytes whereas TCR genes undergo recombination exclusively in T lymphocytes (Tonegawa 1983; Litman et al. 2010; Flajnik 2018). Although several hundred million years of jawed vertebrate evolution have given rise to clade-specific immunoglobulin isotypes such as IgG in mammals, IgO in platypus or IgT in ray-finned fishes, (Danilova and Amemiya 2009; Sun et al. 2013; Pettinello and Dooley 2014; Mashoof and Criscitiello 2016; Flajnik 2018), the mechanism of V(D)J recombination has remained conserved. The generation of an Ig heavy chain (IgH) from a single B cell begins with a tandem array of gene segments encoding variable (V) type Ig domains adjacent to a tandem array of diversity (D) gene segments followed by a tandem array of joining (J) segments at the heavy chain locus in the germline genome. In the simplest terms, the recombination process selects a single V, a single D, and a single J segment from each array, deletes the genomic region between them, and joins them together into a single VDJ region through interactions with RAG1 and RAG2 recombinases. This single VDJ is then transcribed with downstream constant (C) Ig domains leading to a VDJC$_n$ configuration (e.g., Fig. 1a). A similar process occurs at the Ig light chain (IgL) locus, except recombination is only between a single V and a single J segment (Fig. 1b). Given that each B cell has the ability to select different V, D, and J segments for IgH recombination while also selecting different V and J segments for IgL, V(D)J, recombination has the potential to give rise to a vast pool of antibodies (Fig. 1c).

TCR loci also undergo VJ (e.g., TCRα, TCRγ) or VDJ (e.g., TCRβ, TCRδ) recombination. The partnering of an IgH with an IgL (e.g., Fig. 1), a TCRα with a TCRβ, and a TCRγ with a TCRδ, adds an additional level of diversity to antibodies and TCRs, giving each individual B cell or T cell the potential to make different antibody or TCR heterodimers that work in concert to recognize different pathogens (Litman et al. 2010; Murphy and Weaver 2016; Flajnik 2018). Considering the potential immunogenetic diversity V(D)J recombination can generate, it is not surprising that this aspect of jawed vertebrate immunity has been conserved. However, it also raises the question of how such a system arose in the first place. Given the conservation of V(D)J recombination, it is likely that Ig and TCR genes are derived from a common ancestral Ig gene (Litman et al. 2010; Flajnik 2018). “The” ancestral gene would have encoded a joined VJ sequence that was invaded by a RAG-like transposon leading to the separation of the V segment from the J segment (Agrawal et al. 1998; van den Berg et al. 2004; Eason et al. 2004). As our understanding of the molecular basis of the jawed vertebrate innate immune system continues to mature, new work has shed light on innate immune receptors that may share recent common ancestry with Ig

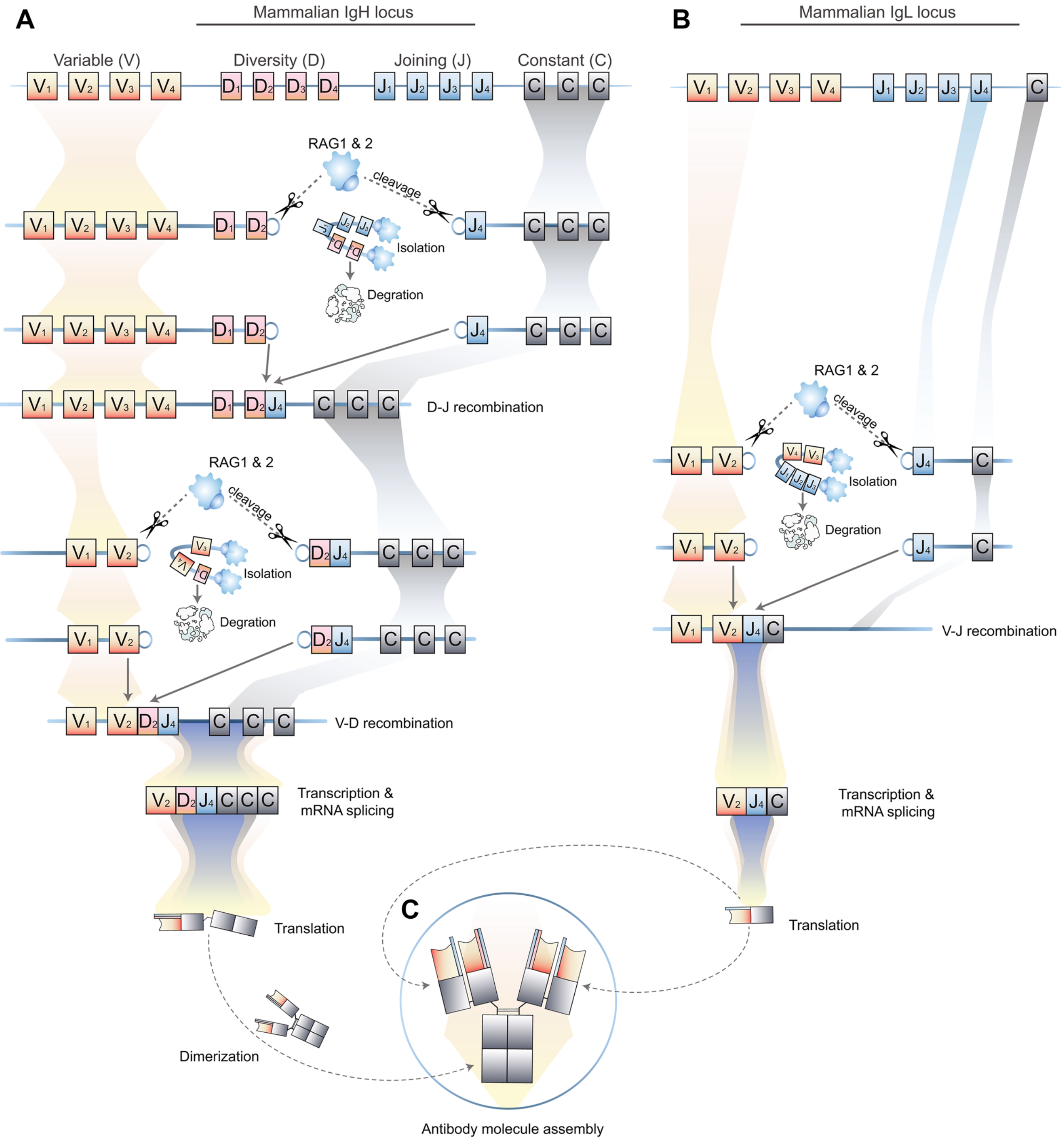


Fig. 1 An overview of V(D)J recombination in jawed vertebrates. **a** VDJ recombination in B lymphocytes involves the selection of a single V, a single D, and a single J gene segment from the IgH locus, and **b** the independent selection of a single V and a single J gene segment from a IgL locus. Within an individual B cell, the RAG1/RAG2 complex recognizes recombination recognition signal (RSS) sequences flanking V, D, and J segments, introduces double stranded breaks at these sites within the genomic DNA, removes the intervening sequence, and subsequently repairs the DNA. The recombined gene and adjacent constant (C) domains are then transcribed with a transmembrane domain to produce a B cell receptor (BCR, not shown) or **c** without a TM domain to generate a secreted antibody. The selection of V, D, and J gene segments is nearly random such that each B cell generates a different antibody. T lymphocytes use the same general mechanism to recombine TCRβ and TCRδ genes (VDJ) and TCRα and TCRγ genes (VJ)

and TCR genes, thereby empowering the generation of new hypotheses and perspectives on the evolutionary origins of V(D)J recombination. We discuss these new insights below.

VJ germline joined Ig and TCR genes

In most jawed vertebrates, Ig and TCR genes are organized in a "translocon" arrangement in which multiple V, (D), and J gene segments are arranged upstream of the constant domain exons such as V_n-(D_n)-J_n-C (e.g., Fig. 1a, b). However, some loci in Chondrichthyans (cartilaginous fish) are arranged as "clusters" of V, (D), and J gene segments with C domain exons such as $(V\text{-}D\text{-}J\text{-}C)_n$ that provide a very different template for gene rearrangements (Dooley and Flajnik 2006; Hsu and Criscitiello 2006; Hsu 2009, 2018; Ott et al. 2021). For example, the heavy chain genes in horned shark (*Heterodontus francisci*) are organized genomically as V-D_1-D_2-J-C-TM with up to 100 independently functioning copies (Kokubu et al. 1987, 1988a; Rast and Litman 1998). Moreover, light chain genes in multiple cartilaginous fish species are organized in clusters that are present as V-J-C and, in many instances, can include the germline V and J sequences as a single gene segment (VJ-C) (Rast et al. 1994; Rast and Litman 1998; Yoder and Litman 2000). In addition, some cartilaginous fish heavy chain loci are also partially or fully germline joined (VD_1-D_2J-C, VD-J-C or VDJ-C) (Kokubu et al. 1988b; Rast and Litman 1998; Yoder and Litman 2000). Over the past several decades, evidence for germline-joined Ig elements have also been reported outside of Chondrichthyans in lineages as evolutionarily divergent as chickens (*Gallus gallus*) (Reynaud et al. 1989) and channel catfishes (*Ictalurus punctatus*) (Ghaffari and Lobb 1999). These germline joined IgH and IgL loci may, therefore, reflect the structure of a shared ancestral Ig gene that has escaped the influence of the RAG1/2 machinery, *or* joined loci derived from unjoined genes reflecting the result of RAG-mediated genome editing within the germline (Yoder and Litman 2000; Lee et al. 2000; van den Berg et al. 2004). Given the rapid proliferation of high quality reference genomes in the last several years, testing these competing hypotheses now represents an achievable and promising avenue of future research.

J gene segments

The V domains of Igs and TCRs possess three complementarity-determining regions (CDRs) that play a primary role in binding antigens. Although the first two CDRs (CDR1 and CDR2) are within the V domain, CDR3 includes the carboxyl-terminus of the V segment, all of the D segment (if present) and the J segment. J segments typically encode 12–15 residues which provide a GxG motif that produces a bulge conformation in the Ig fold (G strand). This bulge conformation is highly conserved in antigen receptors across jawed vertebrates (Eason et al. 2004). An initial description of conserved residues embedded within J domains included GxGTx(L/V) in which "x" refers to any amino acid (Chothia and Lesk 1987). A subsequent analysis revealing FGxGTx-LxV to represent the most conserved residues that form bulges (Eason et al. 2004). For the purpose of this review, we consider the consensus J sequence to be FGxGTx(L/V)xV (Table 1). The variability of the G strand sequence, length, and structure has a major impact on the CDR3, and thus on antigen-binding properties (North et al. 2011; Tsuchiya and Mizuguchi 2016).

VJ joined innate immune receptors

The paired signaling nature of innate immune receptor families

Across vertebrates, a growing number of Ig domain-containing innate immune receptor (IIIR) families have been described that possess inhibitory and activating members. These IIIR families include killer-cell immunoglobulin-like receptors, (KIRs) (Lanier 1998; Vilches and Parham 2002), signal-regulatory proteins (SIRPs) (van Beek et al. 2005; Barclay and Brown 2006), CD300s (Vitallé et al. 2019), triggering receptors expressed on myeloid cells (TREMs) (Allcock et al. 2003), putative remnants of an antigen receptor precursor (PRARPs) (Fu et al. 2019; Ohta et al. 2019), chicken immunoglobulin-like receptors (CHIRs) (Laun et al. 2006; Viertlboeck and Göbel 2011), *Xenopus* MHC-linked Ig superfamily V (XMIV) proteins (Ohta et al. 2006), teleost NITRs, diverse Ig domain-containing proteins (DICPs) and leukocyte immune-type receptors (LITRs) (Montgomery et al. 2011; Wcisel and Yoder 2016), hagfish novel ITAM-containing IgSF receptors (NICIRs), and agnathan paired receptors resembling antigen receptors (APARs) (Suzuki et al. 2005; Haruta et al. 2006). Although present across lineages that have independently evolved over hundreds of millions of years (Alfaro et al. 2009; Near et al. 2012; Prum et al. 2015; Dornburg et al. 2021a), IIIR families have several highly conserved features (Wcisel and Yoder 2016). Inhibitory IIIRs typically encode one or more cytoplasmic immunoreceptor tyrosine-based inhibition motif

Table 1 Representative joining (J) sequences from adaptive and innate immune receptors

Protein	Common name	Genus species	J (and J-like) sequence (`FGxGTx(L/V)xV`)	GenBank ID	Reference
IgH	Human	*Homo sapiens*	**WGQGSLVTV**	P01825.2	[a]
IgLλ	Mouse	*Mus musculus*	**FGGGTKLTV**	P01724.1	[a]
IgLκ	Human	*Homo sapiens*	**FGGGTKVDF**	P01593.2	[a]
TCRα	Mouse	*Mus musculus*	**FGTGTLLSV**	P01739.1	[a]
TCRβ	Human	*Homo sapiens*	**FGSGTRLTV**	P01733.2	[a]
CD8β	Human	*Homo sapiens*	**FGKGTQLSV**	AAB21671.2	[a]
CD8β	Chicken	*Gallus gallus*	**LGTGTQLDV**	Z26484.1	Tregaskes et al. (1995)
CD8β	Axolotl	*Ambystoma mexicanum*	**FGTGTELVV**	AF242416.1	Fellah et al. (2002)
CD8β	Pufferfish	*Takifugu rubripes*	**WKSGILLLP**	AB281056.1	Suetake et al. (2007)
CD79b	Human	*Homo sapiens*	**QGCGTELRV**	BAD96674.1	[a]
CD79b	Chicken	*Gallus gallus*	**NSCGTEIRV**	AB062512	Katsukura et al. (2001)
CD79b	Catfish	*Ictalurus punctatus*	**WGRGTELQV**	CA378285	Sahoo et al. (2008)
SIRPα	Human	*Homo sapiens*	**SGAGTELSV**	NP_001035112.1	[a]
SIRPα	Mouse	*Mus musculus*	**SGGGTEVYV**	NP_031573.2	[a]
SIRPα1	Chicken	*Gallus gallus*	**KGGGTKVSV**	NP_001032920.3	Viertlboeck et al. (2006)
SIRPβ	Human	*Homo sapiens*	**SGAGTELSV**	NP_006056.2	[a]
SIRPβ	Mouse	*Mus musculus*	**SGGGTELLV**	NP_001002898.1	[a]
SIRPβ1	Chicken	*Gallus gallus*	**RGQGTEVSV**	CAJ28362.1	Viertlboeck et al. (2006)
SIRPγ	Human	*Homo sapiens*	**SGPGTEMAL**	NP_061026.2	[a]
NKp30	Human	*Homo sapiens*	**TGNGTRLVV**	NP_667341.1	[a]
NKp30	Rat	*Rattus norvegicus*	**TGNGTRLVV**	NP_861543	[a]
NKp30-like	Frog	*Xenopus tropicalis*	**YGNGTFLNV**	-	BX773925 from: Flajnik et al. (2012)
NKp30-like	Frog	*Xenopus tropicalis*	**HGNGTFLNV**	-	ES685492 from: Flajnik et al. (2012)
NKp30-like	Frog	*Xenopus tropicalis*	**HGKGTFLNV**	-	215–10 from: Flajnik et al. (2012)
NKp30-like	Frog	*Xenopus tropicalis*	**HGNWTFLHV**	-	88–2 from: Flajnik et al. (2012)
NKp30-like	Nurse shark	*Ginglymostoma cirratum*	**TGNGTVVTV**	QTT61900.1	NS344823 from: Kinlein et al. (2021)
NKp30-like	Nurse shark	*Ginglymostoma cirratum*	**FGPGTRLTV**	QTT61904.1	NS173965 from: Kinlein et al. (2021)
NKp30-like	Nurse shark	*Ginglymostoma cirratum*	**KGGGTKLGV**	QTT61902.1	NS334913 from: Kinlein et al. (2021)
PRARP1	Chicken	*Gallus gallus*	**FGDGTRLVV**	QBF67926.1	Fu et al. (2019)
PRARP2	Chicken	*Gallus gallus*	**FGNGTVLLV**	QBF67927.1	Fu et al. (2019)
VJC1$_{406}$ (PRARP)	Frog	*Xenopus tropicalis*	**FANGTTLIV**	XP_031747672.1	Xt-596859 from: Ohta et al. (2019)
VJC1$_{1310}$ (PRAPR-like)	Frog	*Xenopus tropicalis*	**WGGGTFLSV**	-	Ohta et al. (2019)
VJC1$_{258}$ (PRAPR-like)	Frog	*Xenopus tropicalis*	**FGEGTVVRV**	-	Ohta et al. (2019)
Nitr1b	Zebrafish	*Danio rerio*	**MGSGTRLLV**	NM_001309819.1	Yoder et al. (2004)
Nitr9	Zebrafish	*Danio rerio*	**VVKGTRLIV**	AY570237.1	Yoder et al. (2004)
Nitr17	Bowfin	*Amia calva*	**YGNGTFVML**	-	Dornburg et al. (2021b)
Nitr20	Bowfin	*Amia calva*	**FGNGTFVMV**	-	Dornburg et al. (2021b)
TCR-like	Lamprey	*Petromyzon marinus*	**APGGTQLTV**	AY686861	Pancer et al. (2004b)
NICIR1	Hagfish	*Eptatretus burgeri*	**MYPGTKLVV**	AB125060.1	Haruta et al. (2006)
APARA1	Hagfish	*Eptatretus burgeri*	**CGGGTKLEV**	AB177610.1	Suzuki et al. (2005)
APARB1	Hagfish	*Eptatretus burgeri*	**CGEGTKLEV**	AB177615.1	Suzuki et al. (2005)
AmpIgVj-C2	Amphioxus	*Branchiostoma floridae*	**SGDGTRYQLVV**	XP_035664176.1	Chen et al. (2018)

Table 1 (continued)

Protein	Common name	Genus species	J (and J-like) sequence (`FGxGTx(L/V)xV`)	GenBank ID	Reference
Brla-VDB	Amphioxus	*Branchiostoma lanceolatum*	**FGDGQDSQSITVTV**	AY293854.1	Sato et al. (2003)

[a] References are included only for sequences from non-mammalian sequences

(ITIM: S/I/V/LxYxxI/V/L). Engagement of a ligand by the inhibitory receptor can lead to phosphorylation of the ITIM's tyrosine which can then recruit SHP2-containing phosphatases leading to inhibition of immune cell activation (Isakov 1997; Barrow and Trowsdale 2006). Activating IIIRs either encode a cytoplasmic immunoreceptor tyrosine-based activation motif (ITAM: YxxL/Ix$_{(6–8)}$YxxL/I) or possesses an intramembranous charged residue that promotes association with an activating adaptor protein such as DAP10 or DAP12. When an activating receptor binds its ligand, the ITAM's tyrosines can be phosphorylated which stimulates a signaling cascade mediated by Syk family kinases that promote immune cell activation (Isakov 1997; Barrow and Trowsdale 2006). Recent work is now revealing that some of these IIIR families encode VJ sequences that are highly similar to, and may in fact share a common ancestry with, VJ sequences in Ig and TCR genes. These putative evolutionary relatives include natural cytotoxicity receptor 3 (NCR3/NKp30), SIRPs, CD79b, CD8β, PRARPs, and NITRs. We discuss each of these below.

Cephalochordate and agnathan VJ sequences

Recombining Ig and TCR genes only have been identified in jawed (gnathostome) vertebrates. However, genes encoding proteins with strikingly similar architectures have been reported from cephalochordates (amphioxus) and agnathans (lamprey and hagfish). In amphioxus, a single copy gene, AmpIgVjC2, encodes a putative activating receptor with a protein architecture similar to TCRs, including a joined VJ sequence (Fig. 2; Table 1) (Chen et al. 2018). Amphioxus has also been found to possess a structurally distinct gene, Brla-VDB, that encodes five transmembrane domains along with a V and J-like sequence (Sato et al. 2003). The presence of these genes

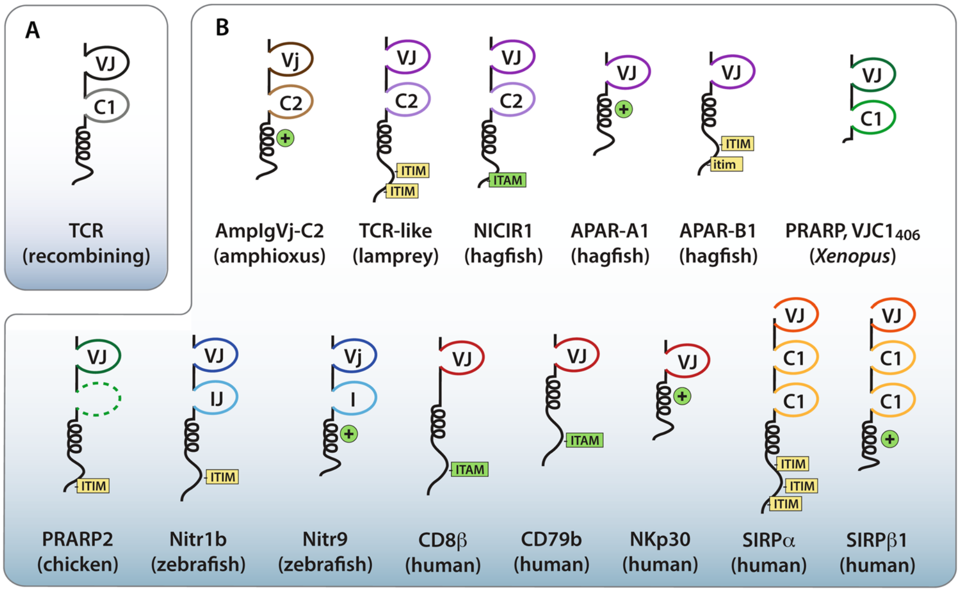


Fig. 2 Protein architecture of select immune-type receptors described in the text. The primary structural features of **a** a prototypic TCR and **b** a number of non-recombining immune receptors that encode joined VJ sequences. Note that AMPIgVj-C2 and TCR-like are predicted to reflect single-copy genes identified only in amphioxus and lamprey, respectively. PRARPs have been identified in birds, reptiles, amphibians, and bony fish, but not in mammals. CD8β and CD79b are encoded by single copy genes identified in most jawed vertebrate lineages. NKp30 is a single copy gene in humans, but multigene families of NKp30-like sequences have been identified in frogs and sharks. All other proteins are representatives of multigene families encoded in multiple species/lineages. The protein structures shown here reflect the species listed in parentheses and may not accurately represent orthologous sequences across all species. Dashed lines in chicken PRARPs reflect degenerated or divergent Ig domains. GenBank numbers for representative sequences are provided in Table 1

in a lineage the diverged prior to the most recent common ancestor of vertebrates, along with a RAG1-like (but not RAG2) sequence (Dong et al. 2005; Zhang et al. 2014), certainly could have set the genomic stage for the origin of V(D)J recombination.

The quest for Ig or TCR sequences from cyclostomes resulted in the discovery of VLRs (see above). However, these efforts also identified an ITIM-containing TCR-like transcript from lamprey (Pancer et al. 2004b) and structurally similar novel ITAM-containing IgSF receptor (NICIR) sequences from hagfish. Both the lamprey TCR-like and hagfish NICIR sequences encode membrane-bound receptors and a membrane distal joined VJ exon as well as a C2 Ig exon. (Fig. 2; Table 1) (Suzuki et al. 2004; Haruta et al. 2006). Subsequently, a family of similar VJ genes encoding paired inhibitory and activating receptors were identified from hagfish and named "agnathan paired receptors resembling antigen receptors" or APARs (Suzuki et al. 2005). The APAR family includes both putative activating forms with charged residues within their transmembrane domain and putative inhibitory forms with cytoplasmic ITIMs (Fig. 2). Collectively, the discovery of these sequences reveals that VJ sequences predate the rise of jawed vertebrates, thereby providing critical context to the origins of adaptive immunity.

Natural cytotoxicity receptor 3 (NCR3/NKp30)

The human genome encodes three distinct natural cytotoxicity receptor genes *NCR1*, *NCR2*, and *NCR3* that respectively encode NKp46, NKp44, and NKp30 (Pessino et al. 1998; Pende et al. 1999; Moretta et al. 2001; Allcock et al. 2003). *NCR1* is encoded near the leukocyte receptor complex (LRC) on chromosome 19. *NCR2 and NCR3* are located approximately 10 Mbp apart on chromosome 6, with the former adjacent to the TREM gene family and the latter within the MHC. All three NCRs encode activating receptors that recognize a broad spectrum of ligands ranging from cellular to viral-, parasite- and bacterial-derived ligands (Kruse et al. 2014). Although NKp46, NKp44, and NKp30 all possess extracellular Ig domains, NKp30 is unique in encoding a VJ domain (Fig. 2; Table 1). This VJ structure is evolutionarily conserved in NKp30-like gene clusters in vertebrate lineages as deeply divergent as Anurans (e.g., *Xenopus tropicalis*) and Chondrichthyans (e.g., nurse shark, elephant shark, whale shark) (Table 1) (Flajnik et al. 2012; Kinlein et al. 2021). These deep evolutionary origins and conservation of VJ suggest that this structure is essential for NKp30 function and may share a recent common ancestry with TCR and Ig V-J sequences.

Signal-regulatory proteins

SIRPα, an inhibitory receptor, was originally identified in humans at the turn of the century with SIRPβ and SIRPγ identified soon after (van Beek et al. 2005). Since then, the human SIRP family has grown to include two predicted SIRP-like receptors (e.g., SIRPδ and SIRPβ2), with all five of the genes clustered on human chromosome 20 (Fig. 2; Table 1) (van den Berg et al. 2004). Although SIRPs contain VJ sequences, they are expressed primarily in myeloid cells. This contrasts with the expression of Ig and TCR genes in lymphocytes and reflects a functional difference between SIRPs and other VJ exon-bearing genes. SIRPα is an inhibitory receptor that binds CD47 (aka integrin-associated protein, IAP) which is a broadly expressed marker of self. Within the context of macrophages, engagement of CD47 by SIRPα initiates a signal that inhibits signaling from phagocytosis receptors (Timms et al. 1998; Vernon-Wilson et al. 2000; Brown and Frazier 2001; Matlung et al. 2017; Oronsky et al. 2020). Corresponding with this putatively more ancient function, SIRPs have been broadly found among mammals and also in avian lineages such as chickens. The chicken SIRP gene cluster has been found to share conserved synteny with the mammalian gene cluster, suggesting analogous functions between these lineages that span the crown of living amniotes (Viertlboeck et al. 2006). Whether SIRPs occur in earlier diverging vertebrates such as amphibians, coelacanths, or ray-finned fishes remains unknown and little investigated (van Beek et al. 2005). As such, we raise the possibility that SIRPs may have even deeper evolutionary origins to motivate future research that could place the evolutionary history of this gene family into the context of the early history of vertebrate VJ exon-bearing gene families.

CD79b

Mammalian CD79a (MB-1/Ig-α) and CD79b (B29/Ig-β) proteins form heterodimers that associate with membrane-bound Ig proteins forming the B cell receptor (BCR). Antigen binding by the Ig leads to B cell activation which relies on the ITAMs within CD79a and CD79b (Tseng et al. 1994; Tanaka and Baba 2020). These loci were first identified in transcripts over 30 years ago (reviewed by (Sakaguchi et al. 1993)), revealing CD79a to possess a single C2-type Ig domain and CD79b to contain a single V-type Ig domain. While functionally associated with the V(D)J recombining IgH and IgL proteins, the human *CD79A* and *CD79B* genes are not linked to the IgH (chromosome 14) or either IgL (chromosomes 2 and 22) loci. These genes are also not linked to each other (chromosomes 19 and 17, respectively). Instead, sequence disparity between these two genes reflects hundreds of millions of years of independent evolution. For example, *CD79B* contains a single VJ exon (Fig. 2; Table 1), and has been reported (or identified by ENSEMBL; http://www.ensembl.org/ release 104) across the majority of jawed vertebrate lineages that span the most recent common ancestor of ray-finned fishes and mammals. Across this broad range of organisms, CD79b V domains possess a J-like sequence suggesting this feature is essential for protein function (Table 1) (Sayegh et al. 2000; Katsukura et al. 2001; Guselnikov et al. 2003; Sahoo et al. 2008). The origin of CD79b remains unclear, with sequences predicted from sharks and skates (*Amblyraja radiata*, GenBank XM_033035416.1; *Rhincodon typus*, GenBank XM_020509696.1; *Callorhinchus milii*, GenBank XM_007907352.2) that may indicate an origin coincident with that of CD79a, given recent reporting of this gene in spiny dogfish (*Squalus acanthias*) (Li et al. 2013b). Future work verifying these predicted evolutionary origins of CD79b is needed.

CD8β

Mammalian CD8 functions as a coreceptor with TCR to recognize antigens displayed by class I MHC molecules. CD8 is made up of two membrane-bound proteins which are expressed on nearly all cytotoxic T cells. CD8 can be present as a CD8α/CD8β heterodimer or a CD8α homodimer. The *CD8A* and *CD8B* genes are adjacent on human chromosome 2 and approximately 2 Mbp from an Ig light chain locus (IgK). Although they do not undergo rearrangement, CD8α and CD8β share significant homology to Ig light chain V domains, and CD8β includes a consensus J sequence (Fig. 2; Table 1). *CD8A* and *CD8B* genes have been identified across all jawed vertebrate lineages with Ig/TCR genes including chicken (Tregaskes et al. 1995), amphibians (Fellah et al. 2002) and teleosts (Suetake et al. 2007; Castro et al. 2011), although the J sequence is not well conserved in teleosts (Table 1). The *CD8A*/*CD8B* locus in chicken and *Xenopus* are linked to two genes (*SPON2*, *FGFRL1*) that are also linked to the novel immune-type receptor (NITR) gene cluster in multiple teleost species (see below). Although teleosts also encode CD8α/CD8β, this linkage has been used to propose that these immune-type molecules may be close evolutionary relatives (Desai et al. 2008).

Putative Remnants of an Antigen Receptor Precursor (PRARPs)

Recently, a family of PRARPs with joined VJ exons were reported from chicken (Fu et al. 2019) and *Xenopus* (Ohta et al. 2019). PRARPs are predicted to play important, but unknown, roles in immunity. For example, PRAPs are expressed in chicken lymphocytes providing an important parallel between these genes and Ig and TCR genes (Fu et al. 2019). In addition, transcripts from chicken reveal that PRARP1 and PRARP2 possess cytoplasmic ITIM or ITIM-like (itim) sequences (Fig. 2) suggesting a functional role in modulating cellular activation (Fu et al. 2019). Although only two genes, *PRARP1* and *PRARP2,* were identified in chicken and a number of other birds, multiple tandem pairs of *PRARP1*/*PRARP2* sequences were identified in the genomes of domestic duck (*Anas platyrhynchos f. domestica*), Chinese alligator (*Alligator sinensis*), and Chinese softshell turtles (*Pelodiscus sinensis*) (Fu et al. 2019). Similarly, *Xenopus tropicalis* and *Xenopus laevis* encode multiple PRARP sequences (named $VJC1_{406}$ genes) as well as two PRARP-like sequences ($VJC1_{258}$ and $VJC1_{1310}$ which was initially reported as a joined IgL sequence) (Ohta et al. 2019). In contrast, a single PRARP sequence is present in the genome of coelacanth (*Latimeria chalumnae*). Whether or not PRARP sequences occur in shark genomes remains unclear (Fu et al. 2019), and PRARPs have not been identified in mammalian or teleost species suggesting that they have been lost in these lineages (Fu et al. 2019). Regardless, all PRARP sequences are predicted to encode a carboxyl-terminal VJ domain (Table 1) and an adjacent second Ig domain. In nearly all lineages, the second Ig domain is a C1-type constant domain, reflecting the organization of IgL sequences (e.g., $VJC1_{406}$ in Fig. 2). An exception to this structure is in avian species where the second Ig domain is degenerated or significantly diverged (e.g., PRARP1 and PRARP2 in Fig. 2). Future research is needed to decipher the evolutionary origins of PRARPs, their structural variants within and between species, as well as the immunological roles they play in various lineages across the vertebrate Tree of Life.

Novel immune-type receptors in bony fish

NITRs have been identified in all major lineages of neopterygian fishes (Teleostei and Holostei; see below) and typically encode two extracellular Ig domains: one of the V-type and one of the I-type, and both Ig domains can include J or J-like sequences (van den Berg et al. 2004;

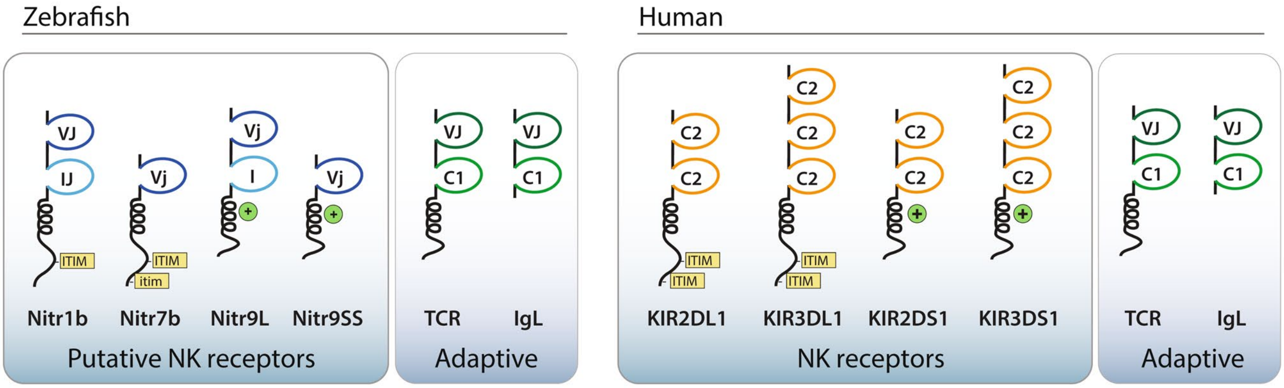


Fig. 3 Neopterygian NITRs and mammalian KIRs share structural and functional similarities. Neopterygian NITRs (e.g., representative zebrafish NITRs on the left) are predicted to function as NK receptors in a mechanism parallel to mammalian KIR NK receptors (e.g., representative human KIRs on the right) (Yoder and Litman, 2011). NITR and KIR families include inhibitory (indicated by cytoplasmic ITIM/itim sequences) and activating (indicated by intramembranous charged residues) forms. It is well established that KIRs are involved in allorecognition (Lanier 1998; Vilches and Parham 2002), and a similar observation has been made for an NITR (Cannon et al. 2008). Both receptor families employ Ig domains for ligand recognition, albeit, NITRs possess V-type and I-type Ig domains, and KIRs possess C2-type Ig domains. Relevant to this review, the majority of NITRs encode J (or J-like, "j") sequences in both V and I domains (e.g., VJ and IJ). In comparison, the general structure of TCR and IgL chain proteins is highly conserved between neopterygian and mammalian species

Eason et al. 2004; Yoder 2009).The NITR extracellular architecture is most similar to TCRs. However, as a family they include inhibitory and activating forms that parallel those of mammalian NK receptors (e.g., KIRs) (Litman et al. 2001; Yoder and Litman 2011). Inhibitory NITRs are identified by the presence of cytoplasmic ITIM or itim sequences and activating forms are identified by the presence of an intramembranous charged residue. Co-immunoprecipitation experiments using recombinant proteins have shown that an activating NITR employs its intramembranous charged residue to partner with the activating adaptor protein, Dap12, and can use Dap12 to mediate an activating signal into NK cells (Yoder et al. 2004; Wei et al. 2007). In contrast, cross-linking an inhibitory NITR can suppress this signaling pathway (Yoder et al. 2001). These structural and functional similarities of NITRs to mammalian NK receptors (Fig. 3) have led to the hypothesis that NITRs play a role as NK receptors in fish. The recent identification of NITRs in holostean species (Braasch et al. 2016; Wcisel et al. 2017; Dornburg et al. 2021b) further challenges early hypotheses addressing the question of when the NITRs arose in vertebrate evolution, which is discussed below.

NITRs: an ancient gene family in ray-finned fishes

NITRs were initially described in the mid-1990s well before current DNA sequencing methods. Although limited by sequencing technology, these pioneering efforts provided the initial clues into NITR protein architecture, diversity, and genomic organization (Strong et al. 1999). Using genomic DNA of southern pufferfish (*Sphoeroides nephelus*), the first NITR sequence was amplified during an effort to amplify V segments of TCR genes in diverse species. The resulting V domain fragment was then used to screen a cDNA library which identified a full-length transcript encoding the prototypical SP-V-I-TM-cyto (SP = signal peptide; V = variable-type Ig domain; I = intermediate-type Ig domain; TM = transmembrane domain; cyto = cytoplasmic domain) NITR configuration and named "Sn193" (Rast et al. 1995). Sn193 and similar sequences were then used to probe a pufferfish genomic library, and a single clone was selected for Sanger sequencing. This 113,426 bp genomic fragment encoded 26 NITR genes/pseudogenes (Strong et al. 1999), a sequencing result that catalyzed over 20 years of subsequent targeted investigations that have refined our understanding of this gene family.

An early review of NITRs gave credence to their ubiquity across teleosts, finding NITRs via BLAST searches in lineages that included Atlantic salmon (*Salmo salar*), Atlantic cod (*Gadus morhua*), Atlantic halibut (*Hippoglossus hippoglossus*), lake whitefish (*Coregonus clupeaformis*), and three-spined stickleback (*Gasterosteus aculeatus*) (Yoder 2009). As all teleosts experienced a genome duplication event early in their history, and NITRs had not been found outside of teleosts, evidence for whether NITRs represented an ancient gene family or an evolutionary novelty that arose after the teleost genome duplication event was equivocal.

However, the recent sequencing of the spotted gar (*Lepisosteus oculatus*) and bowfin (*Amia calva*) genomes soundly reject a teleost genome duplication origin hypothesis (Braasch et al. 2016; Wcisel et al. 2017; Dornburg et al. 2021b; Thompson et al. 2021). Gars and bowfin comprise the sister lineage to teleosts, Holostei, that diverged from the common ancestor of all teleosts prior to the teleost genome duplication event (Wcisel et al. 2020; Dornburg and Near 2021; Thompson et al. 2021). An in depth analysis of bowfin NITRs demonstrated this ancient lineage of fishes encodes a diversity of NITR genes/pseudogenes on par with that of teleosts and likely include activating, inhibitory, and secreted functional forms (Dornburg et al. 2021b). As such, origin and diversity of putative NITR functional forms clearly predate the teleost genome duplication event.

The ubiquity and evolutionary sequence conservation of NITRs

The finding of NITRs in holosteans revealed a similar scope of diversity between holosteans and teleosts (Dornburg et al. 2021b). However, there is also a strong evidence for structural conservation between NITRs of these clades. NITRs have been found to typically possess two extracellular Ig domains across all ray-finned fishes surveyed to date. The first is a membrane-distal V domain that is highly similar to TCR and Ig gene V domains (Litman et al. 2001; Cannon et al. 2008; Yoder 2009). Additionally, NITRs possess a membrane-proximal I-like Ig domain somewhat similar to a constant domain in TCR and IgL. NITR I-domains are characterized by six conserved cysteines, two of which form the disulfide bond required for the Ig-fold as well as four unique cysteines that are not found in any other Ig domain-containing protein family (Yoder 2009; Wcisel and Yoder 2016). Using bowfin NITR I sequences as BLAST queries against publicly available genomes returns canonical NITR sequences from all major lineages of teleosts (Fig. 4), while searches of the elephant shark (*Callorhinchus milii*), lamprey (*Petromyzon marinus*), and hagfish *(Eptatretus burgeri)* genomes on ENSEMBL (http://www.ensembl.org/ release 104) fail to identify any significant hits. Our simple search reveals NITRs to be ubiquitous across teleosts and likely underreported simply due to automatic annotation software naming these loci either derivatives of TCR or IgL.

The majority of NITR V and I domains include a C-terminal J-like sequence which are encoded in single VJ or IJ exons with no evidence for recombination. However, the organization between V and I exons between species is more complex. In pufferfish, the V and I sequences are joined in a single exon (Strong et al. 1999). In contrast, all of the NITR V and I domains in zebrafish are encoded in two exons (Yoder et al. 2001, 2004, 2008). Analyses of the medaka, sea bass, and miiuy croaker genomes further revealed some NITRs with V and I domains as distinct exons and some NITRs with V-I fused exons (Desai et al. 2008; Ferraresso et al. 2009; Meng et al. 2014). Given that NITR V and I domains are encoded in distinct exons in bowfin (Dornburg et al. 2021b), this V-I exon fusion is likely a derived feature reflective of independent intron losses in different teleost lineages. Moreover, comparisons of V and I exons between these species of ray-finned fishes that span hundreds of millions of years of independent evolution have revealed striking sequence similarities.

The sequence conservation of NITRs is underscored by the fact that the majority of early observations made about NITR diversity and their genomic organization in pufferfish have remained consistent when analyzing NITR clusters in other species. While elements of NITR V and I domains are conserved, these early observations revealed a striking asymmetry between these domains. From the analysis of 26 NITR pufferfish sequences, it was clear that the V domains displayed much higher levels of sequence diversity than the I domains in this species. This result has consistently been reflected across other teleosts (e.g., zebrafish, medaka, sea bass) (Hawke et al. 2001; Yoder et al. 2002; Piyaviriyakul et al. 2007; Evenhuis et al. 2007; Desai et al. 2008; Ferraresso et al. 2009; Meng et al. 2014) as well as holostean (bowfin and gar) species (Wcisel et al. 2017; Dornburg et al. 2021b). The asymmetry in sequence diversity can be readily illustrated by a quantification of the variability between representative teleosts and bowfin using Shannon entropy. This simple exercise reveals conservation of sequence across over 300 million years of evolution and strikingly different levels of variability between V and I domains (Fig. 5). Comparisons of NITR V domain diversity to that of immunoglobulins and TCRs further reveal that across species, the regions of NITR V domains with the highest sequence diversity correspond to the CDRs in antibodies and TCRs (Litman et al. 2001; Yoder et al. 2004; Yoder 2009). At the time of this writing, the ligands recognized by NITRs remain unknown. Given the sequence parallels between the V domains of NITRs, Igs, and TCRs, it is highly likely that V domains from different NITRs bind different ligands, offering an exciting avenue of future research.

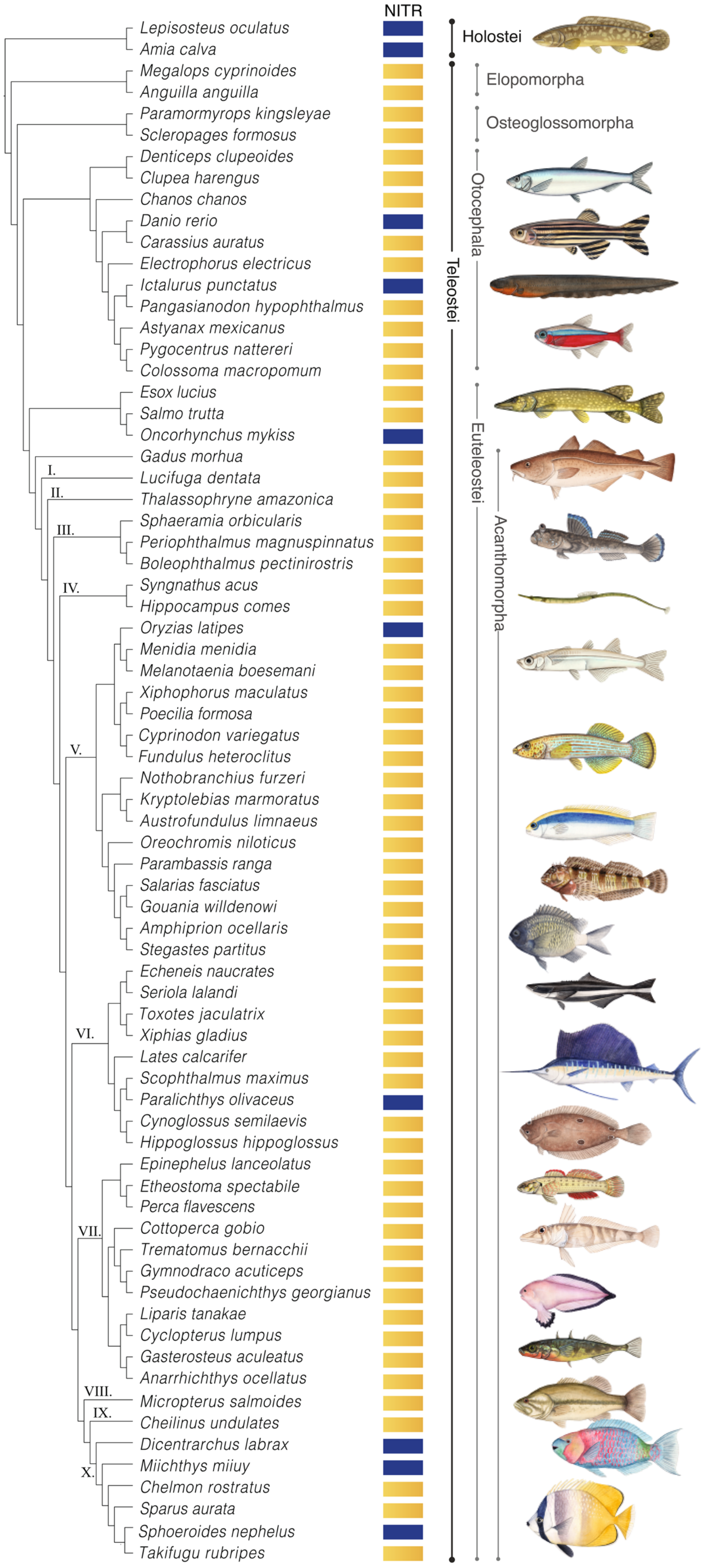


Fig. 4 Distribution of NITRs across the phylogeny of major neopterygian lineages. NITRs can be identified in all major Neopterygii (Holostei and Teleostei) lineages. The phylogenetic tree (left) is adapted from Dornburg and Near (2021). Blue rectangles indicate species from which NITRs were previously reported (Table 2). Yellow rectangles reflect species from which NITRs were identified using BLAST searches of publicly available sequences (terminal taxa). Roman numerals correspond to major lineages of Acanthomorph fishes (Dornburg and Near 2021) for reference: (I) Ophidiiformes, (II) Batrachoidiformes, (III) Gobiiformes, (IV) Syngnathiformes, (V) Blenniiformes, (VI) Carangiformes, (VII) Perciformes, (VIII) Centrachiformes, (IX) Labriformes, and (X) Acanthuriformes. Fish illustrations are provided by T. Near

Table 2 Numbers of NITR sequences reported from neopterygian fishes

Common name	Genus species	# NITR sequences identified	NITRs identified from	References
Bowfin	*Amia calva*	34	RNAseq; reference genome	Dornburg et al. (2021b)
Channel catfish	*Ictalurus punctatus*	18	cDNAs	Hawke et al. (2001); Evenhuis et al. (2007)
European sea bass	*Dicentrarchus labrax*	30	cDNAs; genomic clones	Ferraresso et al. (2009)
Japanese medaka	*Oryzias latipes*	44	cDNAs; reference genome	Desai et al. (2008)
Miiuy croaker	*Miichthys miiuy*	21	cDNAs; whole genome scaffolds	Meng et al. (2014)
Olive flounder	*Paralichthys olivaceus*	1	cDNA	Piyaviriyakul et al. (2007)
Rainbow trout	*Oncorhynchus mykiss*	4	cDNAs	Yoder et al. (2002)
Southern pufferfish	*Sphoeroides nephelus*	26	cDNAs, genomic clones	Strong et al. (1999)
Spotted gar	*Lepisosteus oculatus*	17	RNAseq; reference genome	Braasch et al. (2016); Wcisel et al. (2017)
Zebrafish	*Danio rerio*	39	cDNAs; genomic clones	Yoder et al. (2004); Yoder et al. (2008)

NITR paralog diversification: a mechanism of evolutionary persistence

The NITR gene family has the potential to reveal what may be general evolutionary hallmarks that apply to the diversification and persistence of the V(D)J precursor lineage regardless of whether they are close relatives of the VJ lineage that gave rise to V(D)J. Far from representing a genomic "museum" of ancient sequence diversity that has been maintained, NITRs are characterized by rapid within-lineage gene duplication events. For example, 26 pufferfish NITR V domains can be grouped into 13 different V families with the largest V family including 10 NITRs. Similarly, 39 zebrafish NITR V domains can be

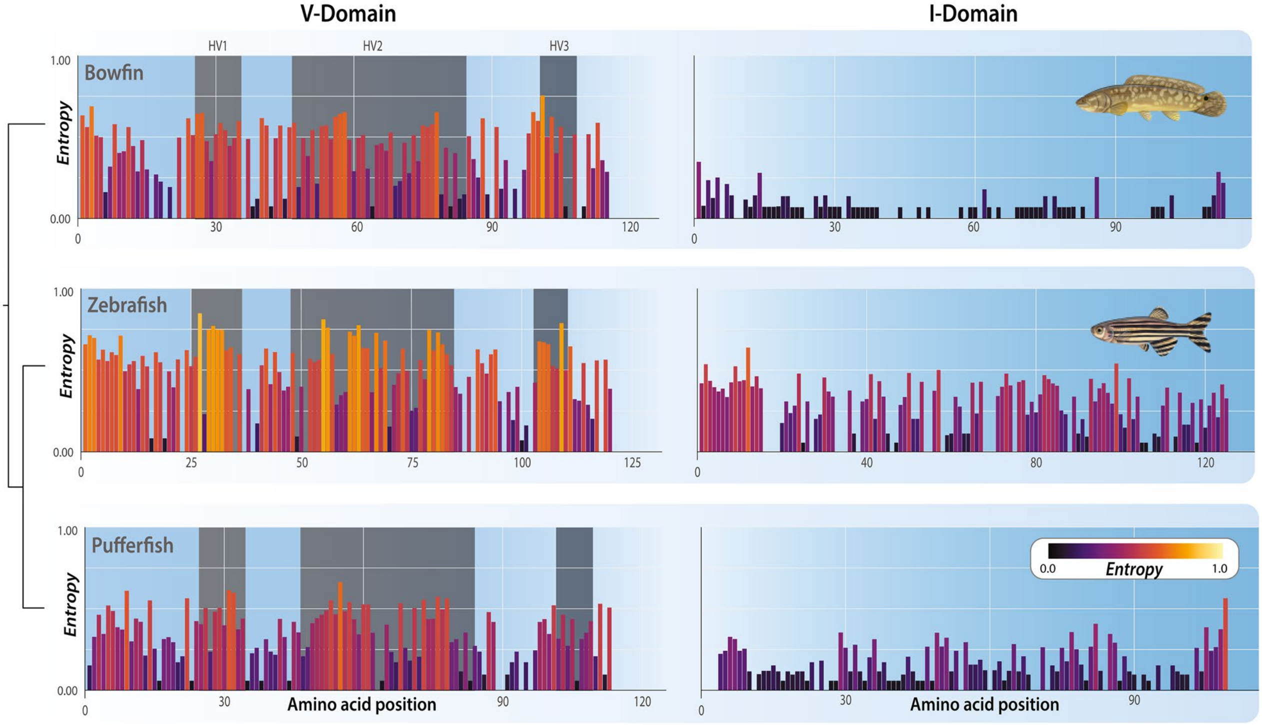


Fig. 5 NITR V domains display much more sequence diversity than NITR I domains. Entropy plots for NITR V (left) and I (right) domains are shown for 17 bowfin (top), 37 zebrafish (middle), and 24 southern pufferfish (bottom) NITRs. The approximate positions of NITR hypervariable regions (HV1, HV2, HV3) that correspond to complementarity-determining regions (CDR1, CRD2, CDR3) in Ig/TCR receptors are indicated by gray shading (Yoder 2009). Entropy plots were generated using the Bios2cor package (Taddese et al. 2021) in R v4.1.0

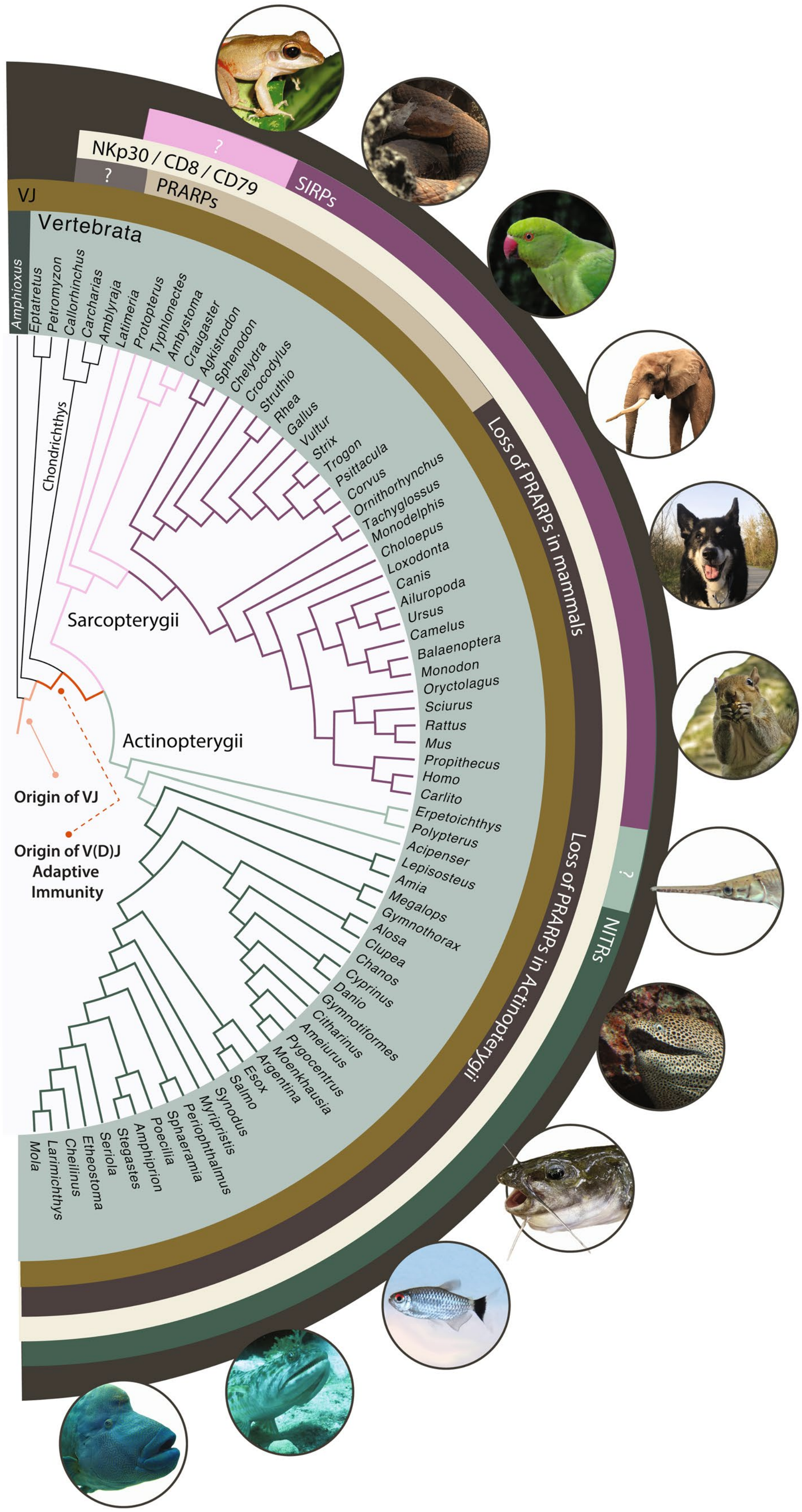
grouped into 14 different V families (Yoder et al. 2008) and 44 medaka NITR V domains can be grouped into 24 different V families (Desai et al. 2008). The high level of sequence evolution has led to the hypothesis that "the clustered organization of these [NITRs] and similar families of innate immune receptors have, and continue to provide the genomic substrate required to persist in the face of evolving pathogenic threats over several hundred million years of teleost evolution" (Dornburg et al. 2021b). Not only does such a result broadly align with expectations of the diversification dynamics of paralogs of any gene (Nei and Rooney 2005; Ferraresso et al. 2009; Inoue et al. 2015; Fernández and Gabaldón 2020), but it also suggests that evolutionary rapid gene birth and death are essential for maintaining immune function and evolutionary longevity of this clustered gene family. The rapid diversification of paralogs also raises a question. What, if any, role do the rapid gene birth and death of NITRs have in shaping the innate immune response within ray-finned fish species?

Over the past several hundred million years, independent ray-finned fish lineages have repeatedly colonized and radiated virtually anywhere water is found (Near et al. 2013). It is clear that habitat transitions that involve fresh to saltwater (Yamanoue et al. 2011; Nakatani et al. 2011; Davis et al. 2012) shifts in thermal regimes (Dornburg et al. 2017), as well as major geographic shifts (Dornburg et al. 2015; Cowman et al. 2017; Siqueira et al. 2019), require a flexible immune response to be successful. What is perhaps less appreciated in the evolution of such transitions, is the degree to which inter-individual variation in gene content provides a wider degree of protection to a population facing novel pathogens (Uhrberg et al. 2002; Vilches and Parham 2002; Tukwasibwe et al. 2020). The full scale of NITR gene-content variation among individuals of a species remains unknown, but preliminary analyses suggest it may be substantial (Dornburg et al. 2021b). Such variability could reflect a biological phenomenon that predates V(D)J, in which gene gain and loss are coupled with meiotic recombination as a substitute for somatic (V(D)J) recombination at the meta-population level. Additionally, the genetic machinery to promote a diversity of VJ immune-phenotypes across a population creates a likely substrate for the evolution of V(D)J recombination. As we move further into the comparative genomics era, further comparative population level studies of NITRs and V(D)J loci across ray-finned fishes have the potential to illuminate fundamental aspects of the evolution of innate and adaptive immunity that has shaped their ability to persist and thrive to the present day.

Considering the relationship between extant innate immune receptors and the evolutionary origins of jawed-vertebrate adaptive immunity

The hypothesis that an ancestral gene encoding a joined VJ sequence was invaded by a RAG-like transposon that gave rise to V(D)J recombination (Agrawal et al. 1998; van den Berg et al. 2004; Eason et al. 2004) raised the question if an ancient lineage of closely related VJ sequence bearing innate immune receptors persist to the present day. As we reviewed above, there are several candidate receptors broadly distributed across vertebrates that may all share a close affinity. While the evolutionary linkage between these receptors is not yet resolved, advances in genome sequencing technology and associated comparative studies have revealed a widespread distribution of these receptors. The broad phylogenetic distribution of these receptors presents the possibility that they either share a close evolutionary affinity, or arose in concert with the rise of the jawed vertebrate adaptive immune system. Assuming that RAG invaded a single VJ in early jawed vertebrates, then subsequent chromosomal duplications likely led to the multiple Ig and TCR loci in extant genomes. Moreover, the earliest vertebrate genome duplication (prior to the split between agnathans and gnathostomes and the invasion of RAG) (Nakatani et al. 2021; Simakov et al. 2020) could have duplicated an ancestral VJ sequence, thereby providing a substrate for additional evolutionary novelty that gave rise to the broad spectrum of VJ-containing receptors and associated functions present today.

In Fig. 6, we provide a conceptual model of the evolutionary origins of different VJ receptors discussed in this review. While CD8β, CD79b, NKp30, and PRARPs all appear to share an origin coincident with the rise of jawed vertebrate adaptive immunity, SIRPs, and NITRs do not. Based on current sequence analyses, the reciprocal absence of NITRs in Sarcopterygians and SIRPs in Actinopterygians suggests the possibility that these receptors may be each other's closest relatives (Fig. 6). This evolutionary scenario remains highly speculative and future work involving detailed investigations of NITRs in early diverging Acintopterygians (sturgeons, paddlefishes, bichirs) and SIRPs in early diverging Sarcopterygians (lungfish, coelacanth, and amphibians) will be critically important for delimiting the early evolutionary history of these ancient gene clusters. Continued functional and sequence-based investigations of all innate immune gene families discussed in this review presents an exciting research frontier with the promise to contextualize not only the origins of jawed vertebrate adaptive immunity, but also the general evolutionary rules that underlie their diversification and persistence.


?
NKp30 / CD8 / CD79
SIRPs
?
PRARPs
VJ
Vertebrata
Loss of PRARPs in mammals
?
NITRs
Loss of PRARPs in Actinopterygii
Amphioxus
Eptatretus
Petromyzon
Callorhinchus
Carcharias
Amblyraja
Latimeria
Protopterus
Typhlonectes
Ambystoma
Craugaster
Agkistrodon
Sphenodon
Chelydra
Crocodylus
Struthio
Rhea
Gallus
Vultur
Strix
Trogon
Psittacula
Corvus
Ornithorhynchus
Tachyglossus
Monodelphis
Choloepus
Loxodonta
Canis
Ailuropoda
Ursus
Camelus
Balaenoptera
Monodon
Oryctolagus
Sciurus
Rattus
Mus
Propithecus
Homo
Carlito
Erpetoichthys
Polypterus
Acipenser
Lepisosteus
Amia
Megalops
Gymnothorax
Alosa
Clupea
Chanos
Cyprinus
Danio
Gymnotiformes
Citharinus
Ameiurus
Pygocentrus
Moenkhausia
Argentina
Esox
Salmo
Synodus
Myripristis
Periophthalmus
Sphaeramia
Poecilia
Amphiprion
Stegastes
Seriola
Etheostoma
Cheilinus
Larimichthys
Mola
Chondrichthys
Sarcopterygii
Actinopterygii
Origin of VJ
Origin of V(D)J Adaptive Immunity

◀**Fig. 6** Proposed model for the evolutionary diversification of VJ sequences and V(D)J recombination. The phylogeny includes all major lineages encoding VJ sequences (brown hemi-circle) with names of representative genera listed. In this model, VJ sequences reflected by Brla-VDB in amphioxus, TCR-like in lamprey (*Petromyzon*), and NICIRs, and APARs in hagfish (*Eptatretus*) were present prior to the appearance of RAG-mediated V(D)J recombination. V(D)J recombination of Ig and TCR genes arose in early jawed vertebrates prior to the evolutionary divergence of Chondrichthyes, Sarcopterygii, and Actinopterygii. CD8β, CD79b, and NKp30 also are identifiable in Chondrichthyes, Sarcopterygii, and Actinopterygii. As CD8 and CD79 mediate the function of BCR and TCRs, they may have coevolved with the recombining Ig and TCR genes. PRARPs have been identified in various sarcopterygians (tan hemi-circle). Given that the possibility of PRARPs in Chondrichthyes remains ("?" in olive gray hemi-circle), it is likely that the absence of PRARPs in actinopterygians and mammals represent independent evolutionary losses. SIRPs have also been identified in a number of Sarcopterygii lineages (dark purple hemi-circle and phylogenetic branches) and may be limited to this group, though investigations in early diverging sarcopterygians are lacking (pink hemi-circle and phylogenetic branches). In contrast, NITRs only have been identified in the teleost and holostean lineages of Actinopterygii (green hemi-circle and phylogenetic branches), with no investigations conducted in bichirs or sturgeon (light green hemi-circle and phylogenetic branches). Although other VJ genes and gene families may still be identified, the parallels in the structure and generalized function of SIRPs and NITRs, and their phylogenetic distribution make it tempting to speculate that they share a common origin. Photo credits: Rachel Etter [catfish] and Alex Dornburg [all others]

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References

Agrawal A, Eastman QM, Schatz DG (1998) Transposition mediated by RAG1 and RAG2 and its implications for the evolution of the immune system. Nature 394:744–751

Alfaro ME, Santini F, Brock C et al (2009) Nine exceptional radiations plus high turnover explain species diversity in jawed vertebrates. Proc Natl Acad Sci U S A 106:13410–13414

Allcock RJN, Barrow AD, Forbes S et al (2003) The human TREM gene cluster at 6p21.1 encodes both activating and inhibitory single IgV domain receptors and includes NKp44. Eur J Immunol 33:567–577

Allison JP, McIntyre BW, Bloch D (1982) Tumor-specific antigen of murine T-lymphoma defined with monoclonal antibody. J Immunol 129:2293–2300

Barclay AN, Brown MH (2006) The SIRP family of receptors and immune regulation. Nat Rev Immunol 6:457–464

Barrow AD, Trowsdale J (2006) You say ITAM and I say ITIM, let's call the whole thing off: the ambiguity of immunoreceptor signalling. Eur J Immunol 36:1646–1653

Boehm T, Hirano M, Holland SJ et al (2018) Evolution of alternative adaptive immune systems in vertebrates. Annu Rev Immunol 36:19–42

Braasch I, Gehrke AR, Smith JJ et al (2016) The spotted gar genome illuminates vertebrate evolution and facilitates human-teleost comparisons. Nat Genet 48:427–437

Brown EJ, Frazier WA (2001) Integrin-associated protein (CD47) and its ligands. Trends Cell Biol 11:130–135

Cannon JP, Haire RN, Magis AT et al (2008) A bony fish immunological receptor of the NITR multigene family mediates allogeneic recognition. Immunity 29:228–237

Castro R, Bernard D, Lefranc MP et al (2011) T cell diversity and TcR repertoires in teleost fish. Fish Shellfish Immunol 31:644–654

Chen R, Zhang L, Qi J et al (2018) Discovery and analysis of invertebrate IgVJ-C2 structure from amphioxus provides insight into the evolution of the Ig superfamily. J Immunol 200:2869–2881

Chien Y, Becker DM, Lindsten T et al (1984) A third type of murine T-cell receptor gene. Nature 312:31–35

Chothia C, Lesk AM (1987) Canonical structures for the hypervariable regions of immunoglobulins. J Mol Biol 196:901–917

Cowman PF, Parravicini V, Kulbicki M, Floeter SR (2017) The biogeography of tropical reef fishes: endemism and provinciality through time. Biol Rev Camb Philos Soc 92:2112–2130

Danilova N, Amemiya CT (2009) Going adaptive: the saga of antibodies. Ann N Y Acad Sci 1168:130–155

Davis AM, Unmack PJ, Pusey BJ et al (2012) Marine-freshwater transitions are associated with the evolution of dietary diversification in terapontid grunters (Teleostei: Terapontidae). J Evol Biol 25:1163–1179

Desai S, Heffelfinger AK, Orcutt TM et al (2008) The medaka novel immune-type receptor (NITR) gene clusters reveal an extraordinary degree of divergence in variable domains. BMC Evol Biol 8:177

Dong M, Fu Y, Yu C et al (2005) Identification and characterisation of a homolog of an activation gene for the recombination activating gene 1 (RAG 1) in amphioxus. Fish Shellfish Immunol 19:165–174

Dooley H, Flajnik MF (2006) Antibody repertoire development in cartilaginous fish. Dev Comp Immunol 30:43–56

Dornburg A, Federman S, Lamb AD et al (2017) Cradles and museums of Antarctic teleost biodiversity. Nat Ecol Evol 1:1379–1384

Dornburg A, Moore J, Beaulieu JM et al (2015) The impact of shifts in marine biodiversity hotspots on patterns of range evolution: evidence from the Holocentridae (squirrelfishes and soldierfishes). Evolution 69:146–161

Dornburg A, Near TJ (2021) The emerging phylogenetic perspective on the evolution of actinopterygian fishes. Annu Rev Ecol Evol Syst 52 https://doi.org/10.1146/annurev-ecolsys-122120-122554

Dornburg A, Wang Z, Wang J, et al (2021a) Comparative genomics within and across bilaterians illuminates the evolutionary history of ALK and LTK proto-oncogene origination and diversification. Genome Biol Evol 13(1):evaa228 https://doi.org/10.1093/gbe/evaa228

Dornburg A, Wcisel DJ, Zapfe K et al (2021) Holosteans contextualize the role of the teleost genome duplication in promoting the rise of evolutionary novelties in the ray-finned fish innate immune system. Immunogenetics 73:479–497. https://doi.org/10.1007/s00251-021-01225-6

Eason DD, Cannon JP, Haire RN et al (2004) Mechanisms of antigen receptor evolution. Semin Immunol 16:215–226

Edelman GM, Gall WE (1969) The antibody problem. Annu Rev Biochem 38:415–466

Evenhuis J, Bengtén E, Snell C et al (2007) Characterization of additional novel immune type receptors in channel catfish, Ictalurus punctatus. Immunogenetics 59:661–671

Fellah JS, Tuffèry P, Etchebest C et al (2002) Cloning and modeling of CD8 beta in the amphibian ambystoma Mexicanum. Evolutionary conserved structures for interactions with major histocompatibility complex (MHC) class I molecules. Gene 288:95–102

Fernández R, Gabaldón T (2020) Gene gain and loss across the metazoan tree of life. Nat Ecol Evol 4:524–533

Ferraresso S, Kuhl H, Milan M et al (2009) Identification and characterisation of a novel immune-type receptor (NITR) gene cluster in the European sea bass, *Dicentrarchus labrax*, reveals recurrent gene expansion and diversification by positive selection. Immunogenetics 61:773–788

Flajnik MF (2018) A cold-blooded view of adaptive immunity. Nat Rev Immunol 18:438–453

Flajnik MF, Tlapakova T, Criscitiello MF et al (2012) Evolution of the B7 family: co-evolution of B7H6 and NKp30, identification of a new B7 family member, B7H7, and of B7's historical relationship with the MHC. Immunogenetics 64:571–590

Fu Y, Yang Z, Huang J, Cheng X et al (2019) Identification of two nonrearranging IgSF genes in chicken reveals a novel family of putative remnants of an antigen receptor precursor. J Immunol 202:1992–2004

Ghaffari SH, Lobb CJ (1999) Structure and genomic organization of a second cluster of immunoglobulin heavy chain gene segments in the channel catfish. J Immunol 162:1519–1529

Guselnikov SV, Najakshin AM, Taranin AV (2003) *Fugu rubripes* possesses genes for the entire set of the ITAM-bearing transmembrane signal subunits. Immunogenetics 55:472–479

Haruta C, Suzuki T, Kasahara M (2006) Variable domains in hagfish: NICIR is a polymorphic multigene family expressed preferentially in leukocytes and is related to lamprey TCR-like. Immunogenetics 58:216–225

Haskins K, Kappler J, Marrack P (1984) The major histocompatibility complex-restricted antigen receptor on T cells. Annu Rev Immunol 2:51–66

Hawke NA, Yoder JA, Haire RN, Mueller MG, Litman RT, Miracle AL, Stuge T, Shen L, Miller N, Litman GW (2001) Extraordinary variation in a diversified family of immune-type receptor genes. Proc Natl Acad Sci U S A 98:13832–13837

Hedrick SM, Cohen DI, Nielsen EA, Davis MM (1984a) Isolation of cDNA clones encoding T cell-specific membrane-associated proteins. Nature 308:149–153

Hedrick SM, Nielsen EA, Kavaler J et al (1984b) Sequence relationships between putative T-cell receptor polypeptides and immunoglobulins. Nature 308:153–158

Hirano M, Guo P, McCurley N et al (2013) Evolutionary implications of a third lymphocyte lineage in lampreys. Nature 501:435–438

Holland SJ, Gao M, Hirano M et al (2014) Selection of the lamprey VLRC antigen receptor repertoire. Proc Natl Acad Sci U S A 111:14834–14839

Hsu E (2018) Immune system receptors in vertebrates: immunoglobulins. Reference Module in Life Sciences. https://doi.org/10.1016/B978-0-12-809633-8.20721-8

Hsu E (2009) V(D)J recombination: of mice and sharks. Adv Exp Med Biol 650:166–179

Hsu E, Criscitiello MF (2006) Diverse immunoglobulin light chain organizations in fish retain potential to revise B cell receptor specificities. J Immunol 177:2452–2462

Inoue J, Sato Y, Sinclair R et al (2015) Rapid genome reshaping by multiple-gene loss after whole-genome duplication in teleost fish suggested by mathematical modeling. Proc Natl Acad Sci U S A 112:14918–14923

Isakov N (1997) ITIMs and ITAMs. The yin and yang of antigen and Fc receptor-linked signaling machinery. Immunol Res 16:85–100

Katsukura H, Murakami R, Chijiiwa Y, Otsuka A, Tanaka M, Nakashima K, Ono M (2001) Structure of the beta-chain (B29) gene of the chicken B-cell receptor and conserved collinearity with genes for potential skeletal muscle sodium channel and growth hormone. Immunogenetics 53:770–775

Khalturin K, Panzer Z, Cooper MD, Bosch TCG (2004) Recognition strategies in the innate immune system of ancestral chordates. Mol Immunol 41:1077–1087

Kinlein A, Janes ME, Kincer J et al (2021) Analysis of shark NCR3 family genes reveals primordial features of vertebrate NKp30. Immunogenetics 73:333–348

Kokubu F, Hinds K, Litman R et al (1988a) Complete structure and organization of immunoglobulin heavy chain constant region genes in a phylogenetically primitive vertebrate. EMBO J 7:1979–1988

Kokubu F, Hinds K, Litman R et al (1987) Extensive families of constant region genes in a phylogenetically primitive vertebrate indicate an additional level of immunoglobulin complexity. Proc Natl Acad Sci U S A 84:5868–5872

Kokubu F, Litman R, Shamblott MJ et al (1988b) Diverse organization of immunoglobulin VH gene loci in a primitive vertebrate. EMBO J 7:3413–3422

Kruse PH, Matta J, Ugolini S, Vivier E (2014) Natural cytotoxicity receptors and their ligands. Immunol Cell Biol 92:221–229

Lanier LL (1998) NK cell receptors. Annu Rev Immunol 16:359–393

Laun K, Coggill P, Palmer S et al (2006) The leukocyte receptor complex in chicken is characterized by massive expansion and diversification of immunoglobulin-like Loci. PLoS Genet 2:e73

Lee SS, Fitch D, Flajnik MF, Hsu E (2000) Rearrangement of immunoglobulin genes in shark germ cells. J Exp Med 191:1637–1648

Li J, Das S, Herrin BR et al (2013a) Definition of a third VLR gene in hagfish. Proc Natl Acad Sci U S A 110:15013–15018

Li R, Wang T, Bird S et al (2013b) B cell receptor accessory molecule CD79α: characterisation and expression analysis in a cartilaginous fish, the spiny dogfish (*Squalus acanthias*). Fish Shellfish Immunol 34:1404–1415

Litman GW, Frommel D, Finstad J, Good RA (1971a) Evolution of the immune response. X. Immunoglobulins of the bowfin: subunit nature. J Immunol 107:881–888

Litman GW, Frommel D, Finstad J, Good RA (1971b) The evolution of the immune response. IX. Immunoglobulins of the bowfin: purification and characterization. J Immunol 106:747–754

Litman GW, Hawke NA, Yoder JA (2001) Novel immune-type receptor genes. Immunol Rev 181:250–259

Litman GW, Rast JP, Fugmann SD (2010) The origins of vertebrate adaptive immunity. Nat Rev Immunol 10:543–553

Mashoof S, Criscitiello MF (2016) Fish Immunoglobulins Biology 5:45. https://doi.org/10.3390/biology5040045

Matlung HL, Szilagyi K, Barclay NA, van den Berg TK (2017) The CD47-SIRPα signaling axis as an innate immune checkpoint in cancer. Immunol Rev 276:145–164

Meng F, Wang R, Xu T (2014) Identification of 21 novel immune-type receptors in miiuy croaker and expression pattern of three typical inhibitory members. Dev Comp Immunol 45:269–277

Meuer SC, Fitzgerald KA, Hussey RE et al (1983) Clonotypic structures involved in antigen-specific human T cell function. Relationship to the T3 molecular complex. J Exp Med 157:705–719

Montgomery BC, Cortes HD, Mewes-Ares J et al (2011) Teleost IgSF immunoregulatory receptors. Dev Comp Immunol 35:1223–1237

Moretta A, Bottino C, Vitale M et al (2001) Activating receptors and coreceptors involved in human natural killer cell-mediated cytolysis. Annu Rev Immunol 19:197–223

Murphy KM, Weaver C (2016) Janeway's immunobiology: ninth international student edition. W.W. Norton & Company

Nakatani M, Miya M, Mabuchi K et al (2011) Evolutionary history of Otophysi (Teleostei), a major clade of the modern freshwater fishes: Pangaean origin and Mesozoic radiation. BMC Evol Biol 11:177

Nakatani Y, Shingate P, Ravi V, Pillai NE, Prasad A, McLysaght A, Venkatesh B (2021) Reconstruction of proto-vertebrate, proto-cyclostome and proto-gnathostome genomes provides new insights into early vertebrate evolution. Nat Commun 12:4489

Near TJ, Dornburg A, Eytan RI et al (2013) Phylogeny and tempo of diversification in the superradiation of spiny-rayed fishes. Proc Natl Acad Sci U S A 110:12738–12743

Near TJ, Eytan RI, Dornburg A et al (2012) Resolution of ray-finned fish phylogeny and timing of diversification. Proc Natl Acad Sci U S A 109:13698–13703

Nei M, Rooney AP (2005) Concerted and birth-and-death evolution of multigene families. Annu Rev Genet 39:121–152

North B, Lehmann A, Dunbrack RL Jr (2011) A new clustering of antibody CDR loop conformations. J Mol Biol 406:228–256

Ohta Y, Goetz W, Hossain MZ et al (2006) Ancestral organization of the MHC revealed in the amphibian Xenopus. J Immunol 176:3674–3685

Ohta Y, Kasahara M, O'Connor TD, Flajnik MF (2019) Inferring the "primordial immune complex": origins of MHC class I and antigen receptors revealed by comparative genomics. J Immunol 203:1882–1896

Oronsky B, Carter C, Reid T et al (2020) Just eat it: a review of CD47 and SIRP-α antagonism. Semin Oncol 47:117–124

Ott JA, Ohta Y, Flajnik MF, Criscitiello MF (2021) Lost structural and functional inter-relationships between Ig and TCR loci in mammals revealed in sharks. Immunogenetics 73:17–33

Pancer Z, Amemiya CT, Ehrhardt GRA et al (2004a) Somatic diversification of variable lymphocyte receptors in the agnathan sea lamprey. Nature 430:174–180

Pancer Z, Mayer WE, Klein J, Cooper MD (2004b) Prototypic T cell receptor and CD4-like coreceptor are expressed by lymphocytes in the agnathan sea lamprey. Proc Natl Acad Sci U S A 101:13273–13278

Pancer Z, Saha NR, Kasamatsu J et al (2005) Variable lymphocyte receptors in hagfish. Proc Natl Acad Sci U S A 102:9224–9229

Papermaster BW, Condie RM, Finstad J, Good RA (1964) Evolution of the immune response. I. The phylogenetic development of adaptive immunologic responsiveness in vertebrates. J Exp Med 119:105–130

Pende D, Parolini S, Pessino A et al (1999) Identification and molecular characterization of NKp30, a novel triggering receptor involved in natural cytotoxicity mediated by human natural killer cells. J Exp Med 190:1505–1516

Pessino A, Sivori S, Bottino C et al (1998) Molecular cloning of NKp46: a novel member of the immunoglobulin superfamily involved in triggering of natural cytotoxicity. J Exp Med 188:953–960

Pettinello R, Dooley H (2014) The Immunoglobulins of Cold-Blooded Vertebrates Biomolecules 4:1045–1069

Piyaviriyakul P, Kondo H, Hirono I, Aoki T (2007) A novel immune-type receptor of Japanese flounder (*Paralichthys olivaceus*) is expressed in both T and B lymphocytes. Fish Shellfish Immunol 22:467–476

Prum RO, Berv JS, Dornburg A et al (2015) A comprehensive phylogeny of birds (Aves) using targeted next-generation DNA sequencing. Nature 526:569–573

Rast JP, Anderson MK, Ota T et al (1994) Immunoglobulin light chain class multiplicity and alternative organizational forms in early vertebrate phylogeny. Immunogenetics 40:83–99

Rast JP, Haire RN, Litman RT et al (1995) Identification and characterization of T-cell antigen receptor-related genes in phylogenetically diverse vertebrate species. Immunogenetics 42:204–212

Rast JP, Litman GW (1998) Towards understanding the evolutionary origins and early diversification of rearranging antigen receptors. Immunol Rev 166:79–86

Reynaud CA, Dahan A, Anquez V, Weill JC (1989) Somatic hyperconversion diversifies the single Vh gene of the chicken with a high incidence in the D region. Cell 59:171–183

Sahoo M, Edholm E-S, Stafford JL et al (2008) B cell receptor accessory molecules in the channel catfish, Ictalurus punctatus. Dev Comp Immunol 32:1385–1397

Sakaguchi N, Matsuo T, Nomura J et al (1993) Immunoglobulin receptor-associated molecules. Adv Immunol 54:337–392

Sato A, Mayer WE, Klein J (2003) A molecule bearing an immunoglobulin-like V region of the CTX subfamily in amphioxus. Immunogenetics 55:423–427

Sayegh CE, Demaries SL, Pike KA et al (2000) The chicken B-cell receptor complex and its role in avian B-cell development. Immunol Rev 175:187–200

Sigel MM, Voss EW Jr, Rudikoff S (1972) Binding properties of shark immunoglobulins. Comp Biochem Physiol A Comp Physiol 42:249–259

Simakov O, Marlétaz F, Yue JX, O'Connell B, Jenkins J, Brandt A, Calef R, Tung CH, Huang TK, Schmutz J, Satoh N, Yu JK, Putnam NH, Green RE, Rokhsar DS (2020) Deeply conserved synteny resolves early events in vertebrate evolution. Nat Ecol Evol 4:820–830

Siqueira AC, Bellwood DR, Cowman PF (2019) Historical biogeography of herbivorous coral reef fishes: the formation of an Atlantic fauna. J Biogeogr 46:1611–1624

Strong SJ, Mueller MG, Litman RT et al (1999) A novel multigene family encodes diversified variable regions. Proc Natl Acad Sci U S A 96:15080–15085

Suetake H, Araki K, Akatsu K et al (2007) Genomic organization and expression of CD8alpha and CD8beta genes in fugu *Takifugu rubripes*. Fish Shellfish Immunol 23:1107–1118

Sun Y, Wei Z, Li N, Zhao Y (2013) A comparative overview of immunoglobulin genes and the generation of their diversity in tetrapods. Dev Comp Immunol 39:103–109

Sutoh Y, Kasahara M (2021) The immune system of jawless vertebrates: insights into the prototype of the adaptive immune system. Immunogenetics 73:5–16

Suzuki T, Shin-I T, Fujiyama A et al (2005) Hagfish leukocytes express a paired receptor family with a variable domain resembling those of antigen receptors. J Immunol 174:2885–2891

Suzuki T, Shin-I T, Kohara Y, Kasahara M (2004) Transcriptome analysis of hagfish leukocytes: a framework for understanding the immune system of jawless fishes. Dev Comp Immunol 28:993–1003

Taddese B, Garnier A, Deniaud M et al (2021) Bios2cor: an R package integrating dynamic and evolutionary correlations to identify functionally important residues in proteins. Bioinformatics 37:2483–2484

Tanaka S, Baba Y (2020) B cell receptor signaling. Adv Exp Med Biol 1254:23–36

Thompson AW, Hawkins MB, Parey E et al (2021) The bowfin genome illuminates the developmental evolution of ray-finned fishes. Nat Genet 53:1373–1384

Timms JF, Carlberg K, Gu H et al (1998) Identification of major binding proteins and substrates for the SH2-containing protein tyrosine phosphatase SHP-1 in macrophages. Mol Cell Biol 18:3838–3850

Tonegawa S (1983) Somatic generation of antibody diversity. Nature 302:575–581

Tregaskes CA, Kong FK, Paramithiotis E et al (1995) Identification and analysis of the expression of CD8 alpha beta and CD8 alpha alpha isoforms in chickens reveals a major TCR-gamma delta CD8 alpha beta subset of intestinal intraepithelial lymphocytes. J Immunol 154:4485–4494

Tseng J, Lee YJ, Eisfelder BJ, Clark MR (1994) The B cell antigen receptor complex: mechanisms and implications of tyrosine kinase activation. Immunol Res 13:299–310

Tsuchiya Y, Mizuguchi K (2016) The diversity of H3 loops determines the antigen-binding tendencies of antibody CDR loops. Protein Sci 25:815–825

Tukwasibwe S, Nakimuli A, Traherne J et al (2020) Variations in killer-cell immunoglobulin-like receptor and human leukocyte antigen genes and immunity to malaria. Cell Mol Immunol 17:799–806

Uhrberg M, Parham P, Wernet P (2002) Definition of gene content for nine common group B haplotypes of the Caucasoid population: KIR haplotypes contain between seven and eleven KIR genes. Immunogenetics 54:221–229

van Beek EM, Cochrane F, Barclay AN, van den Berg TK (2005) Signal regulatory proteins in the immune system. J Immunol 175:7781–7787

van den Berg TK, Yoder JA, Litman GW (2004) On the origins of adaptive immunity: innate immune receptors join the tale. Trends Immunol 25:11–16

Vernon-Wilson EF, Kee WJ, Willis AC et al (2000) CD47 is a ligand for rat macrophage membrane signal regulatory protein SIRP (OX41) and human SIRPalpha 1. Eur J Immunol 30:2130–2137

Viertlboeck BC, Göbel TW (2011) The chicken leukocyte receptor cluster. Vet Immunol Immunopathol 144:1–10

Viertlboeck BC, Schmitt R, Göbel TW (2006) The chicken immunoregulatory receptor families SIRP, TREM, and CMRF35/CD300L. Immunogenetics 58:180–190

Vilches C, Parham P (2002) KIR: diverse, rapidly evolving receptors of innate and adaptive immunity. Annu Rev Immunol 20:217–251

Vitallé J, Terrén I, Orrantia A et al (2019) CD300 receptor family in viral infections. Eur J Immunol 49:364–374

Wcisel DJ, Howard JT 3rd, Yoder JA, Dornburg A (2020) Transcriptome ortholog alignment sequence tools (TOAST) for phylogenomic dataset assembly. BMC Evol Biol 20:41

Wcisel DJ, Ota T, Litman GW, Yoder JA (2017) Spotted gar and the evolution of innate immune receptors. J Exp Zool B Mol Dev Evol 328(7):666–684

Wcisel DJ, Yoder JA (2016) The confounding complexity of innate immune receptors within and between teleost species. Fish Shellfish Immunol 53:24–34

Wei S, Zhou J-M, Chen X et al (2007) The zebrafish activating immune receptor Nitr9 signals via Dap12. Immunogenetics 59:813–821

Yamanoue Y, Miya M, Doi H, et al (2011) Multiple invasions into freshwater by pufferfishes (teleostei: tetraodontidae): a mitogenomic perspective. PLoS One 6:e17410

Yanagi Y, Yoshikai Y, Leggett K et al (1984) A human T cell-specific cDNA clone encodes a protein having extensive homology to immunoglobulin chains. Nature 308:145–149

Yoder JA (2009) Form, function and phylogenetics of NITRs in bony fish. Dev Comp Immunol 33:135–144

Yoder JA, Cannon JP, Litman RT et al (2008) Evidence for a transposition event in a second NITR gene cluster in zebrafish. Immunogenetics 60:257–265

Yoder JA, Litman GW (2000) Immune-type diversity in the absence of somatic rearrangement. Curr Top Microbiol Immunol 248:271–282

Yoder JA, Litman GW (2011) The phylogenetic origins of natural killer receptors and recognition: relationships, possibilities, and realities. Immunogenetics 63:123–141

Yoder JA, Litman RT, Mueller MG et al (2004) Resolution of the novel immune-type receptor gene cluster in zebrafish. Proc Natl Acad Sci U S A 101:15706–15711

Yoder JA, Mueller MG, Wei S et al (2001) Immune-type receptor genes in zebrafish share genetic and functional properties with genes encoded by the mammalian leukocyte receptor cluster. Proc Natl Acad Sci U S A 98:6771–6776

Yoder JA, Mueller MG, Nichols KM, Ristow SS, Thorgaard GH, Ota T, Litman GW (2002) Cloning novel immune-type inhibitory receptors from the rainbow trout, Oncorhynchus mykiss. Immunogenetics 54:662–670

Zhang Y, Xu K, Deng A et al (2014) An amphioxus RAG1-like DNA fragment encodes a functional central domain of vertebrate core RAG1. Proc Natl Acad Sci U S A 111:397–402