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Fish antiviral tripartite motif (TRIM) proteins

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ABSTRACT

Tripartite motif (TRIM) family or RBCC proteins comprises characteristic zinc-binding domains (a RING (R), a B-box type 1 (B1) and a B-box type 2 (B2)) and coiled-coil (CC) domain followed by a C-terminus variable domain. There are about 80 different TRIM proteins in human, but more than 200 in zebrafish with several large gene expansions (*ftr* > 70 genes; *btr* > 30 genes; *trim35* > 30 genes). Repertoires of *trim* genes in fish are variable across fishes, but they have been remarkably diversified independently in a number of species. In mammals, TRIM proteins are involved in antiviral immunity through an astonishing diversity of mechanisms, from direct viral restriction to modulation of immune signaling and more recently autophagy. In fish, the antiviral role of TRIM proteins remains poorly understood. In zebrafish, fish specific TRIMs so called *fintrims* show a signature of positive selection in the C terminus SPRY domain, reminding features of mammalian antiviral trims such as TRIM5. Expression studies show that a number of trim genes, including many *fintrims*, can be induced during viral infections, and may play a role in antiviral defence. Some of them trigger antiviral activity *in vitro* against DNA and RNA viruses, such as FTR83 that also up-regulates the expression of type I IFN in zebrafish larvae. The tissue distribution of TRIM expression suggests that they may be involved in the regionalization of antiviral immunity, providing a particular protection to sensitive areas exposed to invading pathogens.

1. Introduction

The tripartite motif (TRIM) protein family was initially identified as a set of proteins containing a RING finger motif followed by other cysteine-based motifs called B Boxes [1–3]. TRIM typically contain three zinc-binding domains: a RING (R), a B-box type 1 (B1) and a B-box type 2 (B2), and a coiled-coil (CC) domain. Most TRIM proteins also have an additional domain at the C-terminus that classifies TRIM proteins into nine main classes ([4]) further extended to eleven classes to Ozato's nomenclature [5] (Fig. 1). Alternative classifications were later proposed based on phylogenetic studies [5–7]. The RING domain of the tripartite motif has a ubiquitin (Ub) E3 ligase activity, and catalyzes the formation of chains of Ub or Ub-like peptides (ie SUMO, ISG15, …) via binding to a E2 Ub-conjugating enzyme [5,8,9]. Thus, TRIMs control post-translational modification of many proteins via the activity of the RING domain, determining their location, promoting the assembly of complexes, or sending targets to degradation. The role of B boxes is less clear, but it seems that they are important for self-association and interactions with other proteins. The CC domain promotes the formation of anti-parallel homodimers of TRIM proteins [10]. Variable C-terminus domains can mediate a number of biochemical functions (Fig. 1) and can be predictive of subcellular localization [4,11], cellular function or protein-protein interaction. Among TRIM C-terminal domains, B30.2 domains (also named PRY-SPRY) are particularly frequent in antiviral TRIMs [5,12,13]; they have a β-barrel structure and mediate protein-protein interactions and RNA-binding properties [13] through the variable loops at the top of the domain [14]. In absence of enzymatic activity, B30.2 domain might constitute a scaffold promoting assembly of protein complexes [15,16].

TRIM proteins are implicated in many physiological functions including regulation of transcription, cell proliferation, signal transduction, development, autophagy and immunity [13]. They constitute key regulators of many diseases including cancer and infectious diseases, but also inflammatory diseases and neurologic disorders [17]. However, during the last 10 years, the TRIM family has emerged as a major player in antiviral defence in vertebrates. Since Chelbi-Alix et al. discovered that PML/TRIM19 overexpression conferred resistance against VSV and Influenza virus [18], an impressive number of TRIMs appeared

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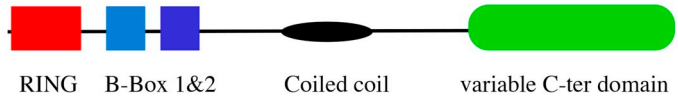
A



B

TRIM protein classes		Domain structure
Class I	(Metazoans)	R-B1-B2-CC-COS-FN3-B30.2
Class II	(Metazoans)	R — B2-CC-COS
Class III	(Birds and Mammals)	R-B1-B2-CC-COS-FN3
Class IV	(Chordates)	R-B1-B2-CC-B30.2
Class V	(Metazoans)	R-B1-B2-CC
Class VI	(Bilaterians)	R-B1-B2-CC-PHD-BROMO
Class VII	(Metazoans)	R-B1-B2-CC-FIL-NHL
Class VIII	(Plants, Fungi, Metazoans)	R-B1-B2-CC-MATH
[(1)]Class IX	(Metazoans)	R-B1-B2-CC-ARF

[(1)] Two additional classes have been proposed by Ozato *et al*. [5]: class X: R-B1-B2-CC-FIL and class XI: R-B1-B2-CC-TM

Fig. 1. Structure and diversity of TRIM proteins.
A. Domain structure of a typical TRIM protein. B. Definition of TRIM classes based on the C-terminal domains: FN3 (fibronectin type III domain), COS (C-terminal subgroup one signature domain), PHD finger/BROMO domain, Ig/NHL (Immunoglobulin/NCL-1, HT2A and Lin-41 repeats) MATH (meprin and TRAF homology domain), ARF (ADP-ribosylation factor domain) and B30.2 domain.

to be implicated in antiviral immunity, through many different mechanisms interfering at different stages of viral cell cycles. These mechanisms have been recently reviewed in detailed for mammalian TRIMs in Refs. [19–21].

TRIMs can inhibit viral replication. The best-known example is probably the α isoform of TRIM5 for primate retroviruses [22,23]. The interaction of the B30.2 domain of rhesus TRIM5α with HIV-1 capsid leads to efficient restriction of the virus through a complex combination of molecular mechanisms involving premature viral un-coating, proteasome-dependent degradation and IFN induction. TRIM22 also restricts HIV-1 via another mechanism, controlling the binding of the Sp1 transcription factor to the retrovirus LTR decreasing transcription efficiency [24]. PML/TRIM19 [25] and TRIM37 have also been shown to restrict HIV-1 replication [26].

Another mechanism involves intra-cellular viral neutralization is exemplified by TRIM21. Binding the Fc region of IgM/G/A via its B30.2 domain, TRIM21 sends virus opsonized with Abs to degradation by the proteasome [27], and induces a pro-inflammatory response [28].

Many TRIMs play a key role in the induction of virus-induced signaling at multiple levels. Thus, TRIM5α acts as a PRR for the retroviral capsid lattice [29]. TRIM25 mediates Retinoic Acid-Inducible Gene (RIGI) activation, ligating poly-Ub chains onto the N-terminal CARD of RIG-I, which induces downstream signaling [30]. TRIMs 4 and 38 also regulate the RIG-I pathway positively, ligating poly-Ub or SUMO (small ubiquitin-like modifier) respectively, to RIG-I and MDA-5 [31,32]. TRIM65 also promotes RLR signaling, but ubiquitinates MDA5 at the RNA helicase domain, leading to activation of IRF3 and induction of IFN [33].

Alternatively, other TRIMs inhibit RLR signaling, as shown for example for TRIM13 and TRIM59, thus promoting viral replication [34,35]. The STING pathway that is activated by recognition of cytosolic DNA, is also regulated by several TRIMs including TRIM21, TRIM30, TRIM32, TRIM38 and TRIM56 [19].

TRIMs can affect TLR signaling as well, either via interaction with IRFs (TRIM21 [36,37]), TRAF6 (TRIM38 [38]) or TRIF (TRIM38 and 56 [39,40]). Finally, TRIMs are also implicated in positive or negative regulation of NLR signaling pathways. For example, TRIM33 ubiquitinates DHX33 and promotes NLRP3 activation [41], while TRIM27 triggers NOD2 degradation [42].

Importantly, large-scale functional studies revealed that TRIMs are frequently modulators rather than direct activators of antiviral immune response [43]. When human TRIMs were screened for their ability to activate innate immune pathways, among 50% of human TRIMs appeared to be able to promote the production of IFN and/or pro-inflammatory cytokines in ectopic expression conditions [43,44], while only a few were described as IFN inhibitors. .Thus, the TRIM family has emerged as one of the most important set of antiviral effectors and regulators of antiviral defence at least in the mammalian species that have been investigated.

Likely driven by a genetic arms race with viruses, this protein family exhibits a remarkable diversification across Vertebrate species, which is most spectacular among teleost fish [45]. In this review, we summarize the current knowledge about the diversity and antiviral function in fish TRIM proteins, in the context of their evolution across Metazoans.

2. Diversity of TRIM proteins in fish in their evolutionary context

Most TRIM classes are found across metazoans, or are restricted to a part of this group: classes I, II, V, VII, X, and IX are present across Metazoa, class VI (RBCC-PHD-BR) across bilaterians, while class IV (RBCC-B30.2) is restricted to Chordates, and class III (RBCC—COS—FN3) has been found only in birds and mammals. However, class VIII, that comprises *trim37* in human, has a much wider taxonomic distribution: RBCC-MATH proteins have been found in fungi, in plants, and in several groups of Protozoans, indicating that TRIM proteins appeared early in eukaryotic evolution [7]. An activity against HIV-1 has also been reported for TRIM37 [26], suggesting that ancient TRIMs might have been involved in antiviral mechanisms from the start. Interestingly, TRAFs (TNF Receptor-Associated Factors) [46], another family of ubiquitin ligases, contains a MATH domain, which may suggest a common origin [7] for these two families implicated in innate immune signaling.

The number of TRIM proteins in invertebrate genomes is generally low [45,47], although some gene expansions have been observed, eg for *trim37* in mosquitoes [7]. The TRIM family is highly diversified in vertebrates, which coincides with the presence of a complex antiviral innate immunity based on the IFN system. Thus, there are about 200 *trim* genes in zebrafish, and 80 in human [48]. An estimation of the number of *trim* genes present in a genome is provided by the number of predicted proteins containing a B-box-type zinc finger domain, since this domain is (quasi)-exclusively present in *trims* [49]. The variation of this number across fish species is illustrated in Table 1. While class I TRIM have very similar counterparts in species as distant as human and *Trichoplax* [47], new classes of TRIM appeared in vertebrates and

Table 1
Number of proteins containing B-box-type zinc finger domain (interpro IPR000315) across fish genomes[a].

Species (alphabetic order)	Number of proteins containing IPR000315
Amazon molly (*Poecilia formosa*)	190
Asian bonytongue (*Scleropages formosus*)	109
Ballan wrasse (*Labrus bergylta*)	369
Bicolor damselfish (Stegastes_partitus)	121
Big-finned mudskipper (*Periophthalmus magnuspinnatus*)	99
Burton's mouthbrooder (*Haplochromis burtoni*)	118
Cave fish (*Astyanax mexicanus*)	186
Channel catfish (*Ictalurus punctatus*)	189
Climbing perch (*Anabas testudineus*)	197
Clown anemonefish (*Amphiprion ocellaris*)	147
Cod (*Gadus morhua*)	137
Fugu (*Takifugu rubripes*)	66
Greater amberjack (*Seriola dumerili*)	155
Guppy (*Poecilia reticulata*)	163
Japanese medaka (*Oryzias latipes*)	136
Mangrove rivulus (*Kryptolebias marmoratus*)	136
Midas cichlid (*Amphilophus citrinellus*)	116
Mummichog (*Fundulus heteroclitus*)	132
Northern pike (*Esox lucius*)	121
Ocean sunfish (*Mola mola*)	90
Orange clownfish (*Amphiprion percula*)	139
Paramormyrops kingsleyae	124
Platyfish (*Xiphophorus maculatus*)	194
Red-bellied piranha (*Pygocentrus nattereri*)	229
Sheepshead minnow (*Cyprinodon variegatus*)	144
Spiny chromis (*Acanthochromis polyacanthus*)	114
Stickleback (*Gasterosteus aculeatus*)	151
Spotted gar (*Lepisosteus oculatus*)	96
Swamp eel (*Monopterus albus*)	97
Tetraodon (*Tetraodon nigroviridis*)	77
Tiger tail seahorse (*Hippocampus comes*)	62
Tilapia (*Oreochromis niloticus*)	190
Tongue sole (*Cynoglossus semilaevis*)	84
Turbot (*Scophthalmus maximus*)	97
Zebrafish (*Danio rerio*)	216
Zig-zag eel (*Mastacembelus armatus*)	156
Human (*Homo sapiens*)	139
Mouse (*Mus musculus*)	71

[a] Fish genomes assemblies present in Ensembl (release 94, Oct 2018) were analysed, as well as human and mouse genomes for comparison.

evolved very fast, with frequent bursts of expansions via various mechanisms. Comparative analysis of the repertoire of TRIM proteins across this phylum unveiled different types of evolutionary patterns. The *trim* genes belonging to class IV (RBCC-B30.2), encoding many factors involved in antiviral defence in human, appeared to be particularly prone to duplication and branch-specific expansion [48,50]. In contrast, most genes from other classes are conserved and possess orthologs in human and zebrafish or pufferfish.

In fact, several class IV TRIMs are also evolutionary stable, such as TRIM25 that is represented in most species by a single well-conserved gene. In contrast, many class IV genes known in human to have antiviral functions, are not found in fish: TRIM5, TRIM21, TRIM22 are among those genes. A particular feature of TRIM in fish is the presence of specific multigenic subsets. Thus, the finTRIMs ("fish novel TRIM" or ftr) were first identified in rainbow trout as virus-induced gene [51], suggesting that they were involved in antiviral immunity. FinTRIMs have a typical RBCC-B30.2 structure, and their most similar counterparts in human are TRIM25 and 39. However, these TRIM have orthologous representatives only in fish and the finTRIM constitute a distinct gene subset. The zebrafish genome harbors a striking diversity of finTRIMs, with more than 80 genes distributed in clusters on different chromosomes [52]. A phylogenetic analysis revealed different subsets suggesting lineage-specific diversification events, and the number of fintrim genes varies greatly across fish species. Conserved syntenies were observed only for the oldest fintrims, and the evolution of these genes has been very fast. While the finTRIM expansion in the zebrafish genome apparently occurred mainly by local duplication, leading to a number of gene clusters of highly similar genes, many medaka fintrim genes are intronless, suggesting they were generated by retrotransposition [48]. Remarkably, finTRIM B30.2 domains are most closely related to those present in a large set NOD-like receptors (NLR) found only in fish, indicating that the evolution of TRIMs and NLRs in fish was connected by exon shuffling [52]. Table 2 shows an updated repertoire of fintrim genes in the current (GRCz11) genome assembly of the zebrafish. Two other TRIM expansions are found in fish genomes, each having a unique counterpart among human class IV genes: trim39 and trim35. The trim39-like genes, are also named *btr* for "bloodthirsty related trim", based on the first zebrafish trim gene *bloodthirsty*, described to be involved in erythrocyte differentiation [53] and belongs to this subset. They are about 30 btr genes in the zebrafish genome, and they are also duplicated in other fish, for example in the pufferfish [48]. TRIM35 also constitute a multigenic subset in fish (with about 35 genes in zebrafish, but only 6 in pufferfish). In contrast to ftr, trim35 duplications are ancient in the fish tree, since many trim35 genes found in stickleback (*Gasterosteus aculeatus*) are part of synteny clusters present in medaka (*Oryzias latipes*) and pufferfish [48].

In the coelacanth, the closest living representative to the divergence point between fish and tetrapods, class IV genes show the same trend and comprise several multigenic gene subsets [54]. Interestingly the ancestral *trim* gene from which these expansions originated remains elusive. In fact, two expansions are most similar to finTRIM and BTR, but phylogenetic analyses do not support an orthology relationship. These observations do not support the hypothesis of an ancestral presence of finTRIM, btr and trim35 in the common ancestors of fish and tetrapods, consistent with the propensity of repeated expansion and diversification suggested by studies in other groups of vertebrates.

Overall, while many TRIM belonging to different classes are found across Metazoans, class IV TRIM has a fast evolutionary dynamic and its diversification in vertebrates coincides with the emergence of the antiviral IFN system [48,50].

3. Fish *trim* are inducible by viruses and some have antiviral activities

3.1. Many TRIM are induced by type I IFN and viral infection

Several trim genes are significantly induced by viral infections, suggesting that they may be effectors or regulators of the antiviral innate response. As a typical and highly expressed interferon stimulated gene (ISG), trim25 is one of the TRIM that has been the most often detected in transcriptome studies of virus infected cells or tissues in multiple fish species [55–58]. Individual fish TRIM characterization also revealed IFN and virus inducibility of several fish TRIM *in vitro* and *in vivo* following virus infection and/or poly I:C treatments in grouper (*Epinephelus coioides*) (TRIM8, [59]; TRIM13, [60]; TRIM16L, [61]; TRIM32, [62]; TRIM35, [63]; TRIM39, [64]; TRIM62 [65]) and common carp (*Cyprinus carpio*) (TRIM32, [66]; TRIM47 [67]). Members of Class IV multigenic TRIM subsets have been also identified as differentially expressed genes, generally with moderate fold changes. In fact, finTRIMs have been initially identified as a gene induced by VHSV in rainbow trout head kidney leukocytes *ex vivo* [51,52]. In the zebrafish, several finTRIM have been found induced by SVCV [68]; with chikungunya virus, a potent IFN inducer [69], *trim8, 14, 25* as well as 27 *fintrim* genes (*ftr*2, 4, 7–9, 11–14, 16, 19, 22, 26, 30, 33, 34–36, 41, 42, 53, 56, 57, 59, 64, 72, 73), one *trim35* and 4 bloodthirsty-like genes (btr04, 24, 27, 29) were found to be up-regulated in larvae by RNAseq analysis (Levraud et al., unpublished). In rainbow trout, twenty trout finTRIM genes were up-regulated in a fibroblast cell line after incubation with inactivated VHSV. Fold changes were consistently moderate (< 3) [57,70]. A homolog of finTRIM was also up-regulated by ISAV in

Table 2
Repertoire of zebrafish fintrim genes in the Zv11 genome assembly.

Gene name	Genomic location	C-ter domain	Ensembl ID
ftr01	Chromosome 2: 11,670,270–11,677,705	yes	ENSDARG00000003909
ftr02	Chromosome 2: 42,236,118–42,242,734	no	ENSDARG00000079412
ftr04	Chromosome 2: 42,260,021–42,261,298	no	ENSDARG00000093932
ftr05	Chromosome 2: 47,869,807–47,870,649	no	ENSDARG00000078065
ftr06*(ftr03)	Chromosome 2: 42,247,560–42,250,254	no	ENSDARG00000018506
ftr06*	Chromosome 2: 42,294,944–42,308,424	yes	ENSDARG00000053450
ftr07	Chromosome 2: 42,312,889–42,314,218	no	ENSDARG00000078446
ftr08	Chromosome 2: 42,318,012–42,321,154	no	ENSDARG00000077412
ftr09	Chromosome 2: 42,330,828–42,333,229	no	ENSDARG00000095345
ftr10	Chromosome 2: 42,344,304–42,345,571	no	ENSDARG00000092023
ftr11	Chromosome 2: 42,708,674–42,713,902	yes	ENSDARG00000038646
ftr12	Chromosome 2: 42,715,995–42,722,040	yes	ENSDARG00000053366
ftr13	Chromosome 2: 43,128,362–43,130,930	yes	ENSDARG00000079537
ftr14	Chromosome 2: 43,131,147–43,135,700	yes	ENSDARG00000053293
ftr14l	Chromosome 13: 18,533,005–18,539,920	yes	ENSDARG00000078254
ftr15	Chromosome 2: 43,144,938–43,151,358	yes	ENSDARG00000074118
ftr19 (ftr27)	Chromosome 2: 47,945,359–47,951,567	yes	ENSDARG00000094131
ftr22	Chromosome 2: 47,900,506–47,904,090	no	ENSDARG00000076969
ftr23	Chromosome 2: 47,905,735–47,911,747	yes	ENSDARG00000067985
ftr24	Chromosome 2: 47,916,204–47,923,803	yes	ENSDARG00000008396
ftr25	Chromosome 2: 47,927,026–47,929,283	no	ENSDARG00000093651
ftr26	Chromosome 2: 47,935,476–47,938,411	no	ENSDARG00000092961
ftr29	Chromosome 2: 59,221,948–59,231,111	yes	ENSDARG00000075355
ftr30	Chromosome 2: 59,190,666–59,205,441	yes	ENSDARG00000095448
ftr32	Chromosome 2: 59,245,240–59,247,811	no	ENSDARG00000093203
ftr33	Chromosome 2: 59,255,193–59,265,537	yes	ENSDARG00000095032
ftr34	Chromosome 2: 59,278,841–59,285,407	yes	ENSDARG00000079614
ftr35	Chromosome 2: 59,295,260–59,303,406	yes	ENSDARG00000089388
ftr36-1# (ftr34)	Chromosome 2: 59,312,776–59,327,299	yes	ENSDARG00000087057
ftr36-2# (ftr65)	Chromosome 2: 59,135,878–59,145,027	yes	ENSDARG00000111722
ftr37	Chromosome 2: 59,221,948–59,231,111	yes	ENSDARG00000092745
ftr38	Chromosome 2: 59,367,429–59,376,399	yes	ENSDARG00000095678
ftr39ps	Chromosome 2: 59,382,428–59,390,464	–	ENSDARG00000079030
ftr41	Chromosome CHR_ALT_CTG3_2_4: 48,997,558–49,005,409	yes	ENSDARG00000100250
ftr42	Chromosome 3: 48,965,923–48,980,319	yes	ENSDARG00000114992
ftr43	Chromosome 3: 4,534,566–4,552,624	yes	ENSDARG00000076106
ftr50-1@ (ftr46)	Chromosome 3: 4,576,089–4,591,643	no	ENSDARG00000054153
ftr50-2@	Chromosome 3: 4,555,758–4,557,653	no	ENSDARG00000115041
ftr50-3@	Chromosome 3: 4,571,886–4,574,372	no	ENSDARG00000116623
ftr50-4@	Chromosome 3: 4,606,718–4,612,324	no	ENSDARG00000112545
ftr51	Chromosome 4: 76,468,839–76,494,299	yes	ENSDARG00000029105
ftr52ps	Chromosome 9: 3,153,466–3,166,159	no	ENSDARG00000011652
ftr53	Chromosome 9: 17,415,241–17,417,666	no	ENSDARG00000041220
ftr54	Chromosome 10: 25,819,996–25,823,258	yes	ENSDARG00000043850
ftr55	Chromosome 19: 15,423,057–15,434,813	yes	ENSDARG00000009161
ftr55alt	Chromosome CHR_ALT_CTG2_1_7: 42,714,293–42,718,619	yes	ENSDARG00000115336
ftr56	Chromosome 19: 12,398,544–12,404,590	yes	ENSDARG00000030954
ftr57	Chromosome 23: 5,736,226–5,743,144	yes	ENSDARG00000074360
ftr58	Chromosome 23: 6,856,469–6,865,962	yes	ENSDARG00000071238
ftr59	Chromosome 23: 6,845,635–6,852,296	yes	ENSDARG00000095864
ftr59alt	Chromosome CHR_ALT_CTG3_2_4: 49,020,286–49,027,056	yes	ENSDARG00000116989
ftr60	Chromosome 24: 6,025,538–6,029,314	no	ENSDARG00000095609
ftr61	Chromosome 24: 6,034,990–6,038,073	yes	ENSDARG00000055520
trim16l (ftr62)	Chromosome 24: 6,353,394–6,359,270	yes	ENSDARG00000055476
ftr64	Chromosome 4: 71,973,211–71,983,381	yes	ENSDARG00000101514
ftr66	Chromosome 2: 37,762,924–37,770,331	yes	ENSDARG00000005327
ftr67	Chromosome 2: 51,065,777–51,087,077	no	ENSDARG00000098820
ftr69	Chromosome 13: 46,196,354–46,200,240	yes	ENSDARG00000052646
ftr70	Chromosome 13: 46,183,639–46,193,697	no	ENSDARG00000076019
ftr72	Chromosome 15: 41,752,335–41,756,507	yes	ENSDARG00000026704
ftr73	Chromosome 15: 41,746,720–41,749,364	yes	ENSDARG00000095298
ftr76	Chromosome 15: 41,716,721–41,720,215	yes	ENSDARG00000076093
ftr79	Chromosome 20: 26,920,681–26,929,685	no	ENSDARG00000093972
ftr80	Chromosome 13: 33,452,096–33,458,790	yes	ENSDARG00000067661
ftr82	Chromosome 5: 30,707,133–30,715,225	yes	ENSDARG00000055647
ftr83	Chromosome 5: 30,741,730–30,753,167	yes	ENSDARG00000025403
ftr85	Chromosome 20: 7,084,154–7,104,637	yes	ENSDARG00000034707
ftr86	Chromosome 15: 509,126–516,750	yes	ENSDARG00000076839
ftr87	Chromosome 2: 51,037,175–51,059,691	yes	ENSDARG00000102557
ftr88	Chromosome 15: 41,735,557–41,744,529	yes	ENSDARG00000097679
ftr90§	Chromosome 15: 41,711,743–41,734,723	yes	ENSDARG00000040536
ftr90§	Chromosome 15: 41,721,050–41,734,639	yes	ENSDARG00000097373
ftr91	Chromosome 15: 41,757,442–41,762,565	yes	ENSDARG00000098004
ftr92	Chromosome 23: 45,887,202–45,897,900	yes	ENSDARG00000095825

(*continued on next page*)

Table 2 (*continued*)

Gene name	Genomic location	C-ter domain	Ensembl ID
ftr93	Chromosome 2: 696,244–707,152	yes	ENSDARG00000039852
ftr94	Chromosome CHR_ALT_CTG3_2_4: 48,977,752–48,992,148	yes	ENSDARG00000115401
ftr95	Chromosome 2: 59,174,872–59,178,801	yes	ENSDARG00000073735
ftr96 (ftr16)	Chromosome 2: 43,742,574–43,745,175	yes	ENSDARG00000032043
ftr97-1& (ftr78)	Chromosome 20: 26,931,496–26,937,453	no	ENSDARG00000055436
ftr97-2&	Chromosome 20: 26,892,761–26,895,591	yes	ENSDARG00000095125
ftr98	Chromosome 8: 39,827,198–39,838,660	no	ENSDARG00000052332
ftr99 (ftr84)	Chromosome 5: 37,040,853–37,047,166	yes	ENSDARG00000035339

Genes names between () correspond to annotated genes as reported in t [48] for Zv9, that point to current members of the family in GRCz11. Changes in assembly and annotation explain why the current gene numbering is discontinuous. Several genes share the same name in Zv11; * ENSDARG00000018506 and ENSDARG00000053450 are named ftr06; # ENSDARG00000087057 and ENSDARG00000111722 are named ftr36; @ The genes ENSDARG00000054153, ENSDARG00000115041, ENSDARG00000116623 and ENSDARG00000112545 are all named ftr50; § ENSDARG00000040536 and ENSDARG00000097373 are overlapping and are both named ftr90; & ENSDARG00000055436 and ENSDARG00000095125 are named ftr97. The B30.2 domain is sometimes not present in the protein model in Ensembl, which is indicated by "no" in the column "C-ter domain".

macrophage-like Atlantic salmon kidney cells (ASK) [71]. These genes were most similar to zebrafish *ftr 8, 13, 14, 16, 22, 23, 61* and *67* [57]; however, their identification would require an exhaustive annotation of finTRIM in the rainbow trout genome, which is not available yet. *trim* genes with B30.2 domains, likely comprising finTRIM orthologs, have also been found induced by NNV in cod using a micro-array transcriptome approach [72]. Additionally, bloodthirsty-like genes (12 trout genes similar to *btr 2, 4, 6, 16, 20, 21, 26*) and trim35-like genes (10 trout genes) were also up-regulated [57]. Altogether, these observations indicate that these trim genes participate to the antiviral innate response. Their moderate level of expression and induction suggest that their role could be related to the regulation of the response, rather than to direct effector functions.

In a recent work, Chernyavskaya et al. described two zebrafish mutants in *uhrf1* (Ub-like, with PHD and RING finger domains 1) or *dnmt1* (DNA methyltransferase 1) genes, showing global DNA hypomethylation and an unexpected expression of type I IFN response. In fact, a deregulation of retroposons in these mutants led to STING activation, and to induction of genes of the IFN pathway, including several trim [73]. Comparison between RNAseq analyses of 120dpf larvae from *uhrf1* and *dnmt1* mutants and transcriptomes from Chikungunuya infected larvae [55] indicated that the pattern of expression of the top10 significantly up- and down-regulated genes was very similar including type I IFN gene and IFN signaling pathway (*mxA, Isg15, stat1a and b,* …). *Trims* and *fintrims* expression were correlated with type I IFN induction: no *fintrim* expression (except of *ftr69*) was reported in *uhrf1* mutant at 55hpf, a time-point with weak type I IFN response. In contrast, at 120hpf, *uhrf1* mutant RNAseq data showed significant induction of *trim25, trim35, ftr08, 07, 09, 11* (with fold changes from 4 to 3.4) and lower induction (about twofold) of *ftr42, 06, 29, 56, 10, 35, 22 and 43*. In comparison, the *dnmt1* mutant also showed *trim25* and *trim35* induction but a wider *ftr* stimulation implicating *ftr10, 11, 09, 06, 04, 91, 05, 69, 42, 72, 56, 57, 34, 22 and 35* (with fold changes from 3.7 to 2). In this analysis, *ftr01* was down-regulated (fold change 0.44), illustrating the complexity of the regulation of the expression of the members of the *ftr* family and, probably, of their implication into the antiviral immune response.

Overall, Table 3 summarized IFN modulation activities (inducer, enhancer or repressor) and antiviral functions reported for conserved TRIM (between human and zebrafish). Human TRIMs constituted potent antiviral proteins acting through IFN modulation while fish TRIMs appeared as positive and negative regulators of IFN signaling pathways.

3.2. Evidences for antiviral activity of fish TRIMs

Over the last decade, publications about fish TRIMs mainly focused on the description of expression patterns at steady state and infectious conditions in different tissues. In contrast to mammalian TRIMs, antiviral activities of these proteins remain poorly described.

Grouper TRIM8, TRIM32 and TRIM39 (a homolog of zebrafish BTR) have been identified as antiviral effectors against DNA and RNA viruses. TRIM8, (with a RBBCC domain structure) and TRIM32 (RBBNHL) showed an antiviral activity against the Singapore grouper iridovirus (SGIV) and a nodavirus, the red-spotted grouper nervous necrosis virus (RGNNV), under ectopic expression in grouper spleen (GS) cells. TRIM8 overexpression led to a reduction of viral induced cytopathic effect, a decrease of viral gene transcription and of viral protein expression. TRIM8 also triggered proinflammatory cytokines expression (including TNFα, IL-1*β*, and IL-8) and type I-IFN signaling pathway, respectively involved in SGIV and RGNNV inhibition through distinct antiviral mechanisms [59]. Ectopic expression of TRIM32 similarly induced interferon signaling and proinflammatory cytokines expression, while repressing STING signaling pathway [62]. TRIM39 (RBCC-B30.2) overexpression had a similar impact on the same viruses. Interestingly, the depletion of the RING E3 ligase domain reverted this phenotype and led to an increase of the viral gene transcription for both viruses [64]. In absence of well-defined antiviral mechanisms, it was also observed that the RING is critical for the TRIM39 inhibitory effect on cell cycle progression [64]. RING was also required for the antiviral effect of TRIM25 [74]. In contrast, TRIM13 (RBCC), TRIM16L (RCC-B30.2), TRIM62 (RBCC-B30.2) and TRIM35 (RBCC-B30.2) from the same species have been identified as negative regulators of IFN signaling pathway [60,63,65]. Interestingly, mouse TRIM13 interacts with MDA5 and negatively regulates MDA5-mediated type I IFN production [35]. Grouper TRIM62 promoted RGNNV infection [65]. In common carp, TRIM32 and TRIM47 were the only TRIMs described as antiviral effectors and inducers of type I-IFN response, interfering with SCVC replication *in vitro* [66,67].

The large fish specific subset of trim genes, called finTRIM, has also been implicated in antiviral defence. *fintrim* inducibility by viral infection and by type I IFN is a first hint of their implication in antiviral immunity (see above). Additionally, their B30.2 domain appeared highly diverse, the variable sites being predominantly located in the variable loops of the domain [52]. Comparing the B30.2 sequences of zebrafish ftr, the hypervariable positions within the B30.2 suggested that it could interacts with ligands as primate TRIM5α does for retroviral proteins: the sites subjected to diversifying (ie 'positive') selection were concentrated in the variable loop 1, between the β-strands 2 and 3 of the domain. In TRIM5α, this loop determines the lentivirus specificity of restriction [75,76]. This distribution of positively selected sites in the loop β2–3 of the ftr B30.2 domain strongly supports the idea of a diverse repertoire of ligands, possibly from viruses. Interestingly, signatures of positive selection of the same region were also found in B30.2 domain of the other zebrafish expanded trim subsets, btr and trim35 [48], which extended the argument. finTRIM characterization also revealed conservation of RING E3 ligase activity [77,78], that had

Table 3
Antiviral activity and IFN modulation of conserved trim genes.

Domain structure		Human genes[x,c]				Zebrafish genes				
		ensembl gene ID	IFN enh.	IFN ind.	antiviral		Ensembl gene ID	IFN enh	antiviral	References
Genes with 1 to 1 orthology relationship between human and zebrafish										
Class I										
trim1	RBCC−COS−FN−B302	ENSG00000080561	+		+	trim1	ENSDARG00000034871	–		
trim9	RBCC−COS−FN−B302	ENSG00000100505	+	+	ND	trim9	ENSDARG00000039123	–		
trim18/mid1	RBCC−COS−FN−B302	ENSG00000101871	+		ND	trim18/mid1	ENSDARG00000060482			
trim36	RBCC−COS−FN−B302	ENSG00000152503	+		ND	trim36	ENSDARG00000062794			
trim67	RBCC−COS−FN−B302	ENSG00000119283	+	+	ND	trim67	ENSDARG00000108787			
Class II										
trim54	RBCC-COS	ENSG00000138100	+		ND	trim54	ENSDARG00000029907			
Class IV										
trim16[a]	.. BCC-B302	ENSG00000108448	ND		ND	trim16 (isg)	ENSDARG00000010673	–	–	[60]
trim25	RBCC-B302	ENSG00000121060	+		+	trim25 (isg)	ENSDARG00000104396	+	+	[48,73]
trim62	RBCC-B302	ENSG00000116525	+		+	trim62	ENSDARG00000060901	–	–	[64]
	RBCC-B302	ENSG00000141569	+		ND	trim65	ENSDARG00000077098			
trim69/trimless/rnf36	RBCC-B302	ENSG00000185880	ND		ND	trim69/trimless/rnf36, isg	ENSDARG00000060688			
trim104/bspry	[a]CC-B302	ENSG00000119411	ND		ND	trim104/bspry	ENSDARG00000104875			
Class V										
trim13[a]	RB	ENSG00000204977	±		+	trim13 (isg)	ENSDARG00000010010	–	–	[59]
trim59	RBCC	ENSG00000248710	ND		+	trim59	ENSDARG00000079238			
RNF207	RBCC	ENSG00000158286	ND		ND	RNF207	ENSDARG00000012409			
Class VI										
trim33	RBCC-PhD-Bromo	ENSG00000197323	ND		ND	trim33	ENSDARG00000016181			
trim66/102[a]	.. BCC-PhD-Bromo	ENSG00000166436	+	+	ND	trim66/102	ENSDARG00000054332			
Class VII										
trim32	RBCC-..−NHL	ENSG0000011940	+		+	trim32	ENSDARG00000102505	+	+	[61,65]
trim45	RBCC-Fil-..	ENSG00000134253	+		+	trim45	ENSDARG00000076781			
trim71	RBCC-Fil-NHL	ENSG00000206557	+		ND	trim71	ENSDARG00000075593			
Class VIII										
trim37	RBCC-MATH	ENSG00000108395	+		+	trim37	ENSDARG00000076473			
Class IX										
trim23	RBCC-ARF/SAR	ENSG00000113595	+		±	trim23	ENSDARG00000069420			
Other human trim like proteins						Other human trim like proteins				
trim44like[a]	..BCC	ENSG00000166326			+	trim44like	ENSDARG00000051761			
domain structure		Human genes				Zebrafish genes				
		ensembl gene ID	IFN enh.	IFN ind.	antiviral		Ensembl gene ID	IFN enh	antiviral	References
Genes with with two zebrafish co-orthologs (presumably ohnologs)										
Class I										
trim46	RBCC−COS−FN−B302	ENSG00000163462	ND		ND	trim46a	ENSDARG00000012367			
						trim46b				
Class II										
trim55	RBCC-COS	ENSG00000147573	+		ND	trim55a	ENSDARG00000029596			
						trim55b	ENSDARG00000058158			
trim 63	RBCC-COS	ENSG00000158022		+	ND	trim63a	ENSDARG00000028027			
						trim63b	ENSDARG00000086685			
Class V										
trim8	RBCC	ENSG00000171206	+		+	trim8a	ENSDARG00000060729	+	+	[58]
						trim8b	ENSDARG00000090512			
Class VII										
trim2	RBCC-fil-NHL	ENSG00000109654	ND		ND	trim2a	ENSDARG00000031817			
						trim2b	ENSDARG00000076174			
trim3	RBCC-fil-NHL	ENSG00000110171	+		ND	trim3a	ENSDARG00000063711			

(*continued on next page*)

Table 3 (*continued*)

Domain structure	Human genes[x,c]				Zebrafish genes				
	ensembl gene ID	IFN enh.	IFN ind.	antiviral		Ensembl gene ID	IFN enh	antiviral	References
					trim3b	ENSDARG00000005397			
Trim subsets expanded in zebrafish with a unique human counterpart									
Class IV									
trim35	RBCC-B302	ND		+		> 30 genes in zebrafish	–	–	[48,62]
trim39	RBCC-B302	ND		+		> 30 genes in zebrafish	+	+	[51,63]

[a] While they lack the R or the CC motifs that have been lost, a few genes are traditionally comprised in the trim classification. They have been kept in this table.

[x] Regarding enhancement of IFN response (IFN enh) + indicates positive regulators, - negative regulators and ± TRIMs which have been described both as positive and negative regulators.

[c] Regarding antiviral activities (antiviral) + denote antiviral TRIMs while – indicate TRIMs with proviral activity.

been shown to be critical to establish antiviral activity of mammalian TRIMs [19,43].

A first direct evidence for a role of finTRIM in the antiviral response came from the characterization of two basal finTRIM, *ftr82* and *ftr83* with conserved structure and genomic context across fishes [77]. This phylogenetic position suggested that these genes may have generic functions, possibly different from those of the main set of *ftr* diversified - and potentially specialized - during fish evolution. Ectopic expression of FTR82 and FTR83 in epithelioma papulosum cyprini (EPC) cells revealed strong antiviral activity of FTR83 against three fish rhabdoviruses SVCV, IHNV and VHSV. This effect was confirmed *in vivo* as *FTR83* depletion allows higher SVCV replication in zebrafish larvae, supporting a protective role of FTR83. This antiviral activity relied on both RING E3 ligase and B30.2 domains as established by the absence of antiviral activity from deletion mutants of FTR83, and from chimeras constructed with FTR82 and 83 domains. FTR83 expression triggered induction of the type I-IFN signaling pathway in EPC cells, indicating that it can play a role in antiviral defence through this mechanism. As mentioned above, *ftr83* mRNA was mainly expressed in tissues exposed to the environment and pathogens, such as gills, and a lower levels in hematopoietic tissues. Interestingly, expression levels of *ftr83* transcripts, that were variable across individuals, were well correlated to the expression of type I IFN. While ftr83 is not an ISG, this correlation suggested that the basal expression of ftr83 could promote a sustained level of IFN in surfaces exposed to pathogens [77].

4. Fintrims: important factors for the regionalization of fish antiviral responses?

The expression data reported so far, mainly from RT-QPCR, suggest that most fish *trim* genes are often expressed in the central nervous system, hematopoietic and mucosal tissues as shown on a tentative heatmap based on refs [48] [59–63,65,67] (Fig. 2). However, a systematic profiling would be necessary to get insight into the potential tissue specific functions of the different members of the family.

Focusing on zebrafish *ftr*, the distribution of several members of the family was also studied in large scale *in situ* hybridization screens using ISH method to describe gene expression pattern on whole-mount zebrafish embryos. Such studies provided a description of ubiquitous finTRIM expression (*ftr29, 65, 72, 81*) in zebrafish embryos (0-72dpf) and also showed tissue restricted expression profiles, for example for *ftr82* and *ftr83* at the same stages. While *ftr82* was first detected in the whole organism, it was later restricted to the lateral mesoderm and pronephritic mesoderm, then to the pronephros and hematopoietic organ of larvae [79]. This pattern was confirmed in by the group of Thisse, who similarly showed early periderm and ventral mesoderm signals evolving in epidermis, heart, mesenchyme, otic vesicle (24-28hpf) before reaching gut, heart, liver, pharynx and vein at 72hpf [80]. Ftr83 showed a more restricted pattern with ISH signal detected in periderm, before expression in pronephritic duct, peripheral olfactory organ and pharynx [80]. Phylogenetic studies revealed that *ftr82* and *83* constitute basal members of the finTRIM family [77]. Evaluation of *ftr83* expression pattern by ISH in 3dpf larva and RT-QPCR on various adult isolated tissues, showed a restricted pattern of expression to gills, skin, pharynx, and to a much lesser extent to other hematopoietic tissues. Interestingly, the expression level of *ftr83* and of the type I IFNφ1 was highly correlated in the gills of adult fish, suggesting that *ftr83* might act locally in areas constantly exposed to pathogens. Importantly, *ftr83* was expressed at a higher level than other finTRIMs and was *not* induced by type I IFN, hence its pattern of expression in healthy fish likely reflects the regions in which it exerts its antiviral activity [77]. In the same study, a wider expression range was found for *ftr82*, the closest relative of *ftr83,* with highest expression levels in the gut without evidence of immuno-modulatory activity. In another report, *ftr82* was suggested to play a role in vascular development, a knockdown after morpholino injections apparently leading to impairment of

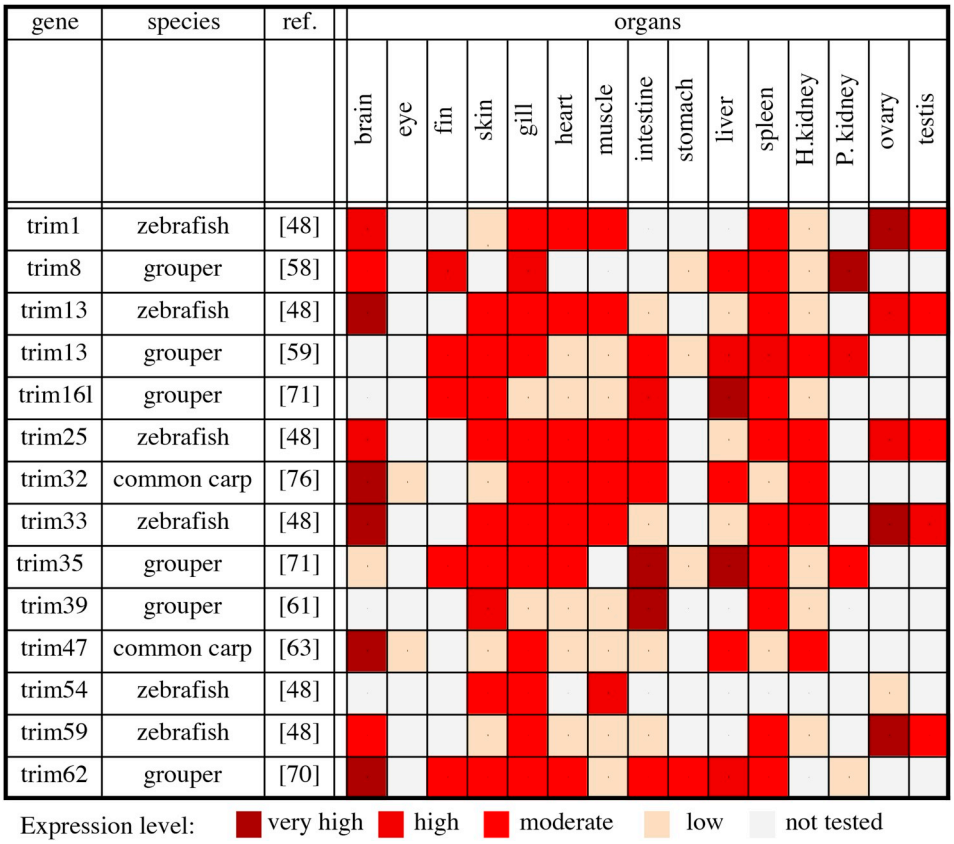


Fig. 2. Expression patterns of fish TRIMs.

intersegmental vessel growth mediated by Notch/VEGF pathway interconnexion with *ftr82* [81]. These approaches were successfully conducted only for finTRIMs with relatively high basal level expression, which allowed ISH signal detection. Other finTRIM members typically exhibit very low basal expression in zebrafish larvae (RNAseq analysis, Levraud et al., unpublished and [82]).

finTRIMs expression patterns were also evaluated by RT-QPCR. Luo et al., reported that ftr12, ftr67, ftr84 and ftr82 are highly expressed at the early stage (from 0 to 8dpf) and described a regionalized finTRIM expression in adult zebrafish with high expression of ftr12, ftr51, and ftr67 in the gill, intestines, and liver, respectively [83]. *ftr82*, *ftr83*, and *ftr84* were mostly expressed in the kidney. However, these data based on RT-QPCR raise the issue of primer specificity; with clusters of highly similar sequences, the finTRIM family is particularly difficult to study with this approach, systematic sequencing of PCR products being necessary to determine what members have been amplified in the assays. In absence of these data, the expression profiles are difficult to compare across tissues.

The general implication of TRIMs in the modulation of innate immune responses, suggests that their tissue-dependent pattern of expression, or induction, may constitute an important level of regulation of both intrinsic and inducible responses. For example, TRIM9, a brain specific TRIM, has recently been described as a negative regulator of the NFκB pro-inflammatory signaling pathway activation in various cell lines and primary cultures of rat neuronal cells [84]. While the regionalization of immunity appears more and more important to avoid adverse effects of defence mechanisms, the exploration of cell type- and tissue-specificity of TRIM expression proteins will likely provide new insights about their importance in the fine-tuning of the host-pathogen interactions. Indeed, the immune response is strongly dependent of the tissue environment in term of specialized immune cells, local cytokine production and even microbiota for skin and mucosae. The integrity of tissues during the reaction is also critical, which influences the choice of effector mechanisms, as side effects of a wrong defence response may be deleterious [85]. Highly sensitive sites such as eye, brain and testis are protected from dangerous side-effects of inflammation and immune responses; the properties of such “immune privileged” regions, for example their capacity to tolerate heterologous grafts, were discovered a long time ago [86,87] and showed that immune responses are compartmentalized and differ between tissues. As mucosae and skin are particularly exposed to invading pathogens, their local immunity should induce effective responses while maintaining tissue integrity and homeostasis [88].

As modulators of type I IFN expression and responses, TRIMs likely participate to the mechanisms of regional variation of IFN responses, which remains poorly understood. Although most cell types are able to up-regulate type I interferon (IFN) upon viral infection, the amplitude and kinetics of the innate antiviral response and the repertoire of IFN stimulated genes (ISGs) are inconstant across different sites (e.g. Ref. [89]). This has been also observed in fish. For example, in zebrafish embryos infected by Chikungunya virus, using *in situ* hybridization, it was found that the general ISG expression pattern (well illustrated by *isg15*) was in liver, gut and vessels, but some genes had specific subpatterns; e.g. *rsad2* was poorly expressed in the gut and *isg12.2* in the liver [55]. Similarly, partially overlapping but distinct patterns were observed for *irf1b* and *isg15* in *uhrf1* mutant zebrafish with exuberant IFN expression [73]. The antiviral immunity within the nervous system illustrates the variability of the IFN response between tissues, and its importance. In mice, an elevated homeostatic type I IFN was found in neurons, higher than the basal expression in fibroblasts. This was critical and sufficient for early control of viral infection, but many ISGs had a low basal expression in neurons suggesting unique IFN signaling [90]. The situation can be further complexified by immuno-regulatory functions of type I IFN itself, that can play anti-inflammatory role as for example in the gut, in addition to its antiviral effect [91]. In addition, different cell types of the gut respond very differently to type I IFNs

[88]. With its exquisite imaging tractability, the zebrafish model constitutes a favourable context and an important tool to address this question at the whole body level, in a vertebrate model.

5. Conclusions

Among TRIMs present in Metazoans, antiviral activities have been characterized for members of classes that are either ancient (class VIII, class I) or recent (i.e., for example class IV, that is restricted to vertebrates). Overall, the current knowledge about the TRIM family across species suggests that these proteins generally play diverse and important roles in antiviral defence. The most striking trait of antiviral TRIMs is probably the diversity of mechanisms in which they are involved. As suggested in Ref. [45], fish TRIM will certainly offer natural variations on the theme of antiviral cytoplasmic receptors, and may reveal a wealth of novel antiviral mechanisms of restriction and immune modulation. The diversity of TRIM repertoires in finfish, that is the largest class of vertebrates with > 25000 species, also provide a excellent illustration of the evolutionary plasticity of the family, and of its capacity to generate large and fast gene expansions that can be co-opted for antiviral functions.

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