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Minireview

Fish 'n' TRIMs

Louis Du Pasquier

Address: University of Basel, Institute of Zoology and Evolutionary Biology, Vesalgasse 1, CH-4051 Basel, Switzerland.
Email: dupasquier@dial.eunet.ch

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Abstract

A novel diversified multigene family of tripartite-motif (TRIM) intracellular receptors with putative antiviral activity has been identified in teleost fish and published in *BMC Biology*. The history of these receptors involves ancient linkage to paralogs of the major histocompatibility complex, and the family has invertebrate precursors.

Multigene families are common in metazoa. They result from genomic duplications and contain a variety of genes, such as those encoding the immunoglobulin superfamily, cadherins, leucine-rich repeats, scavenger receptors, actins, tubulins, keratins, collagens, heat shock proteins and rhodopsins. The largest receptor families, which can contain tens to hundreds of members, comprise mostly either olfactory receptors of the rhodopsin family or immunoreceptors, such as cysteine-rich scavenger receptors (SRCRs) and Toll-like receptors (TLRs) in echinoderms and the nucleotide-binding and oligomerization domain leucine-rich repeat receptors (also known as NOD-like receptors or NLRs) in teleosts, as well as other immunoglobulin superfamily members in teleosts, amphibians, birds and mammals [1].

In such families the immunologist sees a potential source of useful diversity for innate immune recognition and immediately thinks in terms of its adaptive value. The evolutionary biologist reminds us that not all diversity is adaptive and asks for direct evidence of involvement in immunity. At this point the cell biologist confronts the difficult issue of how the expression of large sets of genes is controlled.

Tripartite-motif (TRIM) intracellular receptors have been implicated in control of proliferation, differentiation, development, oncogenesis, apoptosis and antiviral activity in various mammalian species. They comprise a RING finger with E3 ubiquitin ligase activity, a B-box (a type of zinc-finger domain) and a coiled-coil domain mediating oligomerization. Some TRIMs are coupled to the B30.2 module - a fusion of the Pry and Spry domains [2] - which mediates diverse functions in at least six families of human receptor by binding to targets through an interface resembling that of an antibody [3]. The mode of action of the B30.2 domain is mostly unknown, but it may confer antiviral activity on TRIM [4], which is why these molecules are of particular interest. In humans there are 75 TRIMs [5]. All metazoan TRIMs fall into 11 classes [4], all of which contain the above mentioned ring box, one or two B boxes, and a coiled-coil region. The classes are defined according to the absence (class V) or the presence of one to three extra domains.

For the TRIMs discussed in this minireview - that is, those that have been duplicated - these extra domains are: C-terminal subgroup one signature (classes I, II and III), fibronectin type III (classes I and III), B30.2 (classes I and IV), plant homeo- and bromodomains (class VI), and filamin and NHL (NCL1, HT2A, LIN41 repeat) (class VII).

A recent paper by van der Aa *et al*. [6] in *BMC Biology* has now identified a novel large multigene family of TRIM proteins in teleosts, the most abundant infraclass of bony fish, with over 25,000 species. The TRIM proteins are encoded by the *fintrim* (*ftr*) and the *bloodthirsty-related* (*btr*) genes and include a TRIM domain coupled to a B30.2 module. Expression of the genes can be induced by experimental viral infection (by intravenous injection of the viral suspension) in teleost cells and may thus contribute to antiviral defense in these fish. The fish TRIM family is an interesting model because, first, it is amplified uniquely in fish (see [7] for a recent discussion of duplications in the teleost genome) and thus provides a new model for studying the origin, raison d'être and evolution of large multigene families putatively involved in vertebrate immunity; and second, because comparative data may help to determine which segments of the receptors are essential (rather than accessory) to antiviral activity, in particular with respect to the B30.2 domain.

Fish *ftr* diversity and chromosome location

The report by van der Aa *et al*. [6] describes two subsets of the 240 zebrafish TRIMs, which the authors named the *fintrim* (*ftr*) and the *bloodthirsty-related* (*btr*) genes. The 84 *ftr* genes and 33 *btr* genes encode class IV TRIMs (RING finger-B-box-coiled-coil-B30.2 proteins) [4]. The *ftr* genes stem from an unknown ancestor and make up a teleost-specific family encoding proteins induced by viral infection. Ftrs evolved through positive selection of residues in the B30.2 domain homologous to those involved in viral recognition of the mammalian anti-retroviral protein TRIM5α; they are therefore likely to form a family of immune receptors since, in a changing viral environment, diversity of recognition of determinants is a selective advantage for an immune system [6]. The family illustrates a recurrent observation: during evolution, families of genes with non-immune functions in other organisms may become diversified in some species to fulfill functions in immune recognition and defense. Another example of such evolution is shown by members of the immunoglobulin superfamily in invertebrates and vertebrates [8]. In the case of Ftr proteins, the pressure to diversify must have been intense because different species of phylogenetically distant teleosts have generated *ftr* families through different mechanisms: tandem duplications in zebrafish and retrotranspositions in medaka. This convergence suggests again that Ftr diversity has adaptive value. One possibility is that this great diversity compensates for deficiencies in adaptive immunity to viruses.

The other subset of zebrafish TRIMs, the Btrs, stems from an ortholog of mammalian Trim-39. Members of this subset are not documented to have any involvement in immunity, but the fact that the genes are highly amplified whereas *trim-39* is not raises some interesting questions, and studying the origin and diversification of both subsets may prove useful.

Can any possible evolutionary scheme be derived from the position of *ftr* and *btr* genes on the chromosomes? Their presence on 16 of the 25 chromosomes suggests they are very mobile and can diversify independently of each other. However, their multiplicity may partly be due to the fact that, in addition to the two rounds of polyploidization common to gnathostomes, teleosts have had an extra round, which resulted in eight potential paralogous sets of chromosomes instead of the four found in other vertebrates. So do the numerous *ftr* and *btr* clusters match as many paralogous regions? Indeed, it seems that they are all located on chromosomes that also contain major histocompatibility complex (MHC) genes or their paralogs (Figure 1). This pattern fits with the hypothesis that, during the generation of the zebrafish genome, a region containing MHC and TRIM-B30.2 genes duplicated several times, in agreement with the above hypothesis of several genome duplications. If validated by statistical tests, this observation would suggest two things: first, that the *ftr* genes, which are present in a smaller number of clusters than the other TRIM-B30.2 genes, diversified later and specifically in teleosts from one of those early MHC-linked genes; and second, that the MHC-TRIM-B30.2 linkage is ancient and preceded the two rounds of genome duplication common to gnathostomes. Therefore, it is likely that relatives of TRIM-B30.2 existed in invertebrates.

The linkage to MHC and its three paralogous regions [9] is not only ancient, it is also conserved, as several genes encoding proteins with TRIM and B30.2 domains and with antiviral activity are also MHC-linked in various vertebrate lineages, such as birds and mammals [10-12] (Figure 2a), and some are also moderately amplified (such as *TRIM5* genes, with five contiguous members) in a way that is reminiscent of the *ftr* amplification [13]. Was the immunological antiviral function of such receptors a reason to be selected for co-segregation with the region of the MHC containing genes encoding proteins that are associated with inflammatory responses, such as tumor necrosis factor and components of the antigen-presentation machinery? It may have contributed to the creation of a massive assembly of immunity-related genes, which would have had the advantage of being inheritable as a single unit. This linkage may no longer have any selective value in some species, given that it is not apparent in the stickleback, a species of teleosts that has diverged more recently than the zebrafish. In zebrafish, it may simply be a 'fossil' of an ancient, once useful architecture.

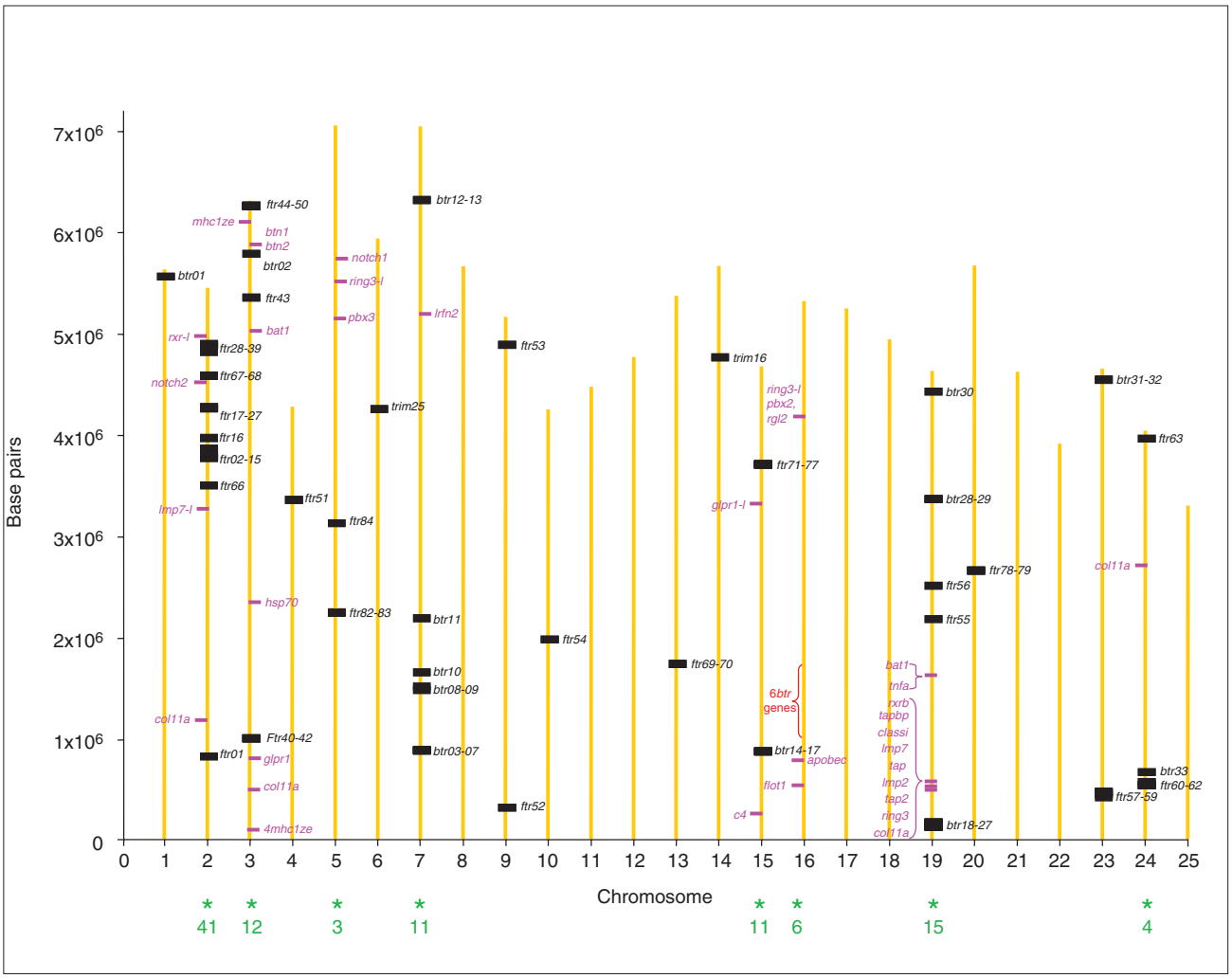

Figure 1
Position of MHC and TRIM-B30.2 genes in the zebrafish genome. Green asterisks denote chromosomes with clusters of *btr* and *ftr* genes, and the number of these genes on the respective chromosome is shown below in green. MHC and MHC-paralogous genes are in magenta. Class II MHC genes have not been indicated; these are on chromosome 8 and not associated with any B30.2 TRIM. In addition, chromosome 16, which is rich in MHC-paralogous genes, has a few Trim-39-like genes not mentioned in [6]. In teleosts many rearrangements have caused the MHC elements that would normally be closely linked to be scattered; in this context, the loose linkage of TRIM genes to MHC genes is not unexpected. Modified from Figure 2 of [6].

The B30.2 domain: origin and mobility

The antiviral properties of TRIM proteins seem to be due mainly to the RING finger-B-box region (in TRIM25) and the B30.2 module (in TRIM5). These two regions work in different ways: through the ubiquitination pathway for the RING finger-B-box region, and by interference with virus decapsidation and replication for the B30.2 module [4].

Very little is known about B30.2 evolution beyond vertebrates. Briefly, the hypothetical evolutionary history of B30.2 is often presented in phases [3-5]: an ancient Spry domain inherited from an ancestral form later became associated with a Pry domain to give the final B30.2 configuration seen only in vertebrates. My analysis of sequences from various invertebrates (Figure 2) shows clearly the presence of a B30.2 domain associated with TRIM-like molecules. In cnidarians, there seems to be two classes of TRIM: class I, with fibronectin type III and B30.2 domains; and class V, without a B30.2 domain. However, in echinoderms, such as the sea urchin, the B30.2 domain is rare. Among the chordates, many classes and many members exist in amphioxus (Cephalochordata), whereas

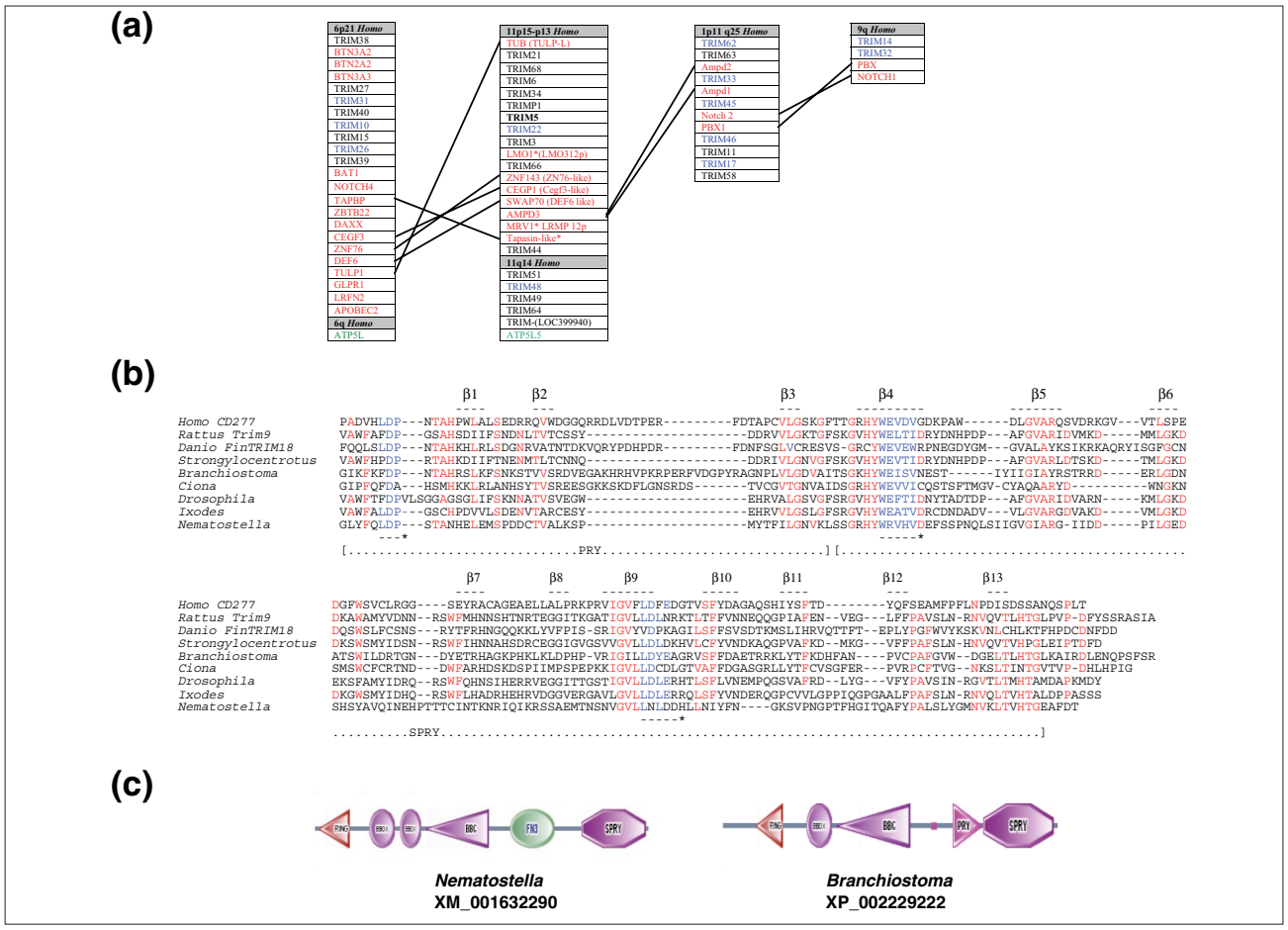

Figure 2

Comparison of sequences and chromosomal positions of TRIM and MHC genes between species. **(a)** Human MHC-paralogous regions. The 1pq, 6p21 and 9q segments are the classical MHC-paralogous regions [8]. For simplicity only selected markers are shown, particularly MHC markers shared with the fish markers shown in Figure 1. For the purposes of this article I identified a set of genes on human chromosome 11p so far not considered as a MHC-paralogous region but that indeed seem to correspond to a group of MHC-paralogous genes that are missing from the group present on chromosome 19p13. Asterisks indicate genes for which paralogs have also been identified on chromosome 12p13, which is also known to contain some MHC-paralogous genes. Two chromosomes also have ATP5L homologs (green) linked to TRIM genes, as also found for three fish species in [6]. TRIMs in blue are those with antiviral activity. Lines indicate paralogous loci. **(b)** Alignment of B30.2 (Pry-Spry) domains across metazoa. Invertebrates are represented by the following sequences: a diploblastic cnidarian (sea anemone, *Nematostella*, XM_001632290), a protostome insect (*Drosophila*, NP_723600.2), a chelicerate (tick, *Ixodes*, EEC02812), a deuterostome echinoderm (sea urchin, *Strongylocentrotus*, XR_026371), a tunicate (sea squirt, *Ciona*, XM_002126032) and a cephalochordate (amphioxus, *Branchiostoma*, XM_002222150). Vertebrates are represented by: a teleost fish TRIM-B30.2 (zebrafish, *Danio*, XM_001332270), human CD277 (*Homo*, NP_001138480, given as an example of B30.2 associated with a molecule other than TRIMs), and rat Trim9 (*Rattus*, NP_569104, of which several invertebrate TRIMs are close homologs). Red, residues conserved in more than 50% of the sequences; blue, regions considered as signatures of the B30.2 domain [2]. Strand prediction (shown above the sequences) is taken from the Phyre server [17]. **(c)** Schematic representations using SMART [18] of TRIM-related proteins from *Nematostella* (close to class I of [5]) and *Branchiostoma* (close to class IV of [6]). The Pry domain is not always suggested by the graphic SMART program, as for *Nematostella* here, but it is visible in the alignment. Data obtained from the ENSEMBL [19], UCSC [20] and Vectorbase [21] servers.

the sea squirt *Ciona* (Urochordata) has only ten TRIMs [5] and only one has so far been found to contain a B30.2 domain. As yet, no association of a B30.2 domain with an extracellular immunoglobulin superfamily domain has been found in invertebrates. The shuffling of the B30.2 exon seems specific to vertebrate butyrophilins (a family of receptors named from a milk protein but that contains several immunologically relevant molecules). This association of a B30.2 domain with a MHC-linked immunoglobulin superfamily member must be old since it is shared between mammals [10-11] and the zebrafish. Indeed, chromosome 3 of *Danio* (zebrafish) contains two

butyrophilin homologs - *btn1*, which encodes a protein containing the B30.2 domain, and *btn2*, which does not - and several MHC markers (Figure 1). The existence of TRIM-B30.2 but the absence of butyrophilin in invertebrates suggests that the B30.2 domain was shuffled from a TRIM into one of the nearby MHC-linked immunoglobulin superfamily members (resembling, for instance, zebrafish *btn2*).

In mammals, the B30.2 domain can be associated with surface, cytoplasmic and nuclear receptors and even with some soluble proteins, such as toxins [2,3]. It remains a mystery why the B30.2 domain is associated with or has colonized so many different genes in some organisms but remains rare among others. However, 'rare' is not the term one would use for the B30.2 domain in teleosts. Following exon shuffling, B30.2 domains similar to those of Ftr proteins expanded tremendously into another large family of zebrafish intracellular receptors, the NOD-like receptors (NLRs) [14,15], which can function as immunoreceptors [16]. These receptors resemble plant disease resistance proteins and are involved in responses to pathogens. Together, the findings that TRIM-B30.2 is present in invertebrates but that B30.2 domains are absent from the NLRs of vertebrates except teleosts suggests that TRIM-B30.2 is older than NLR and that the exon shuffling caused a B30.2 domain to move from a TRIM-B30.2 gene to an NLR gene rather than in the opposite direction.

The existence of hundreds of similar B30.2 domains associated with different intracellular immunoreceptors complicates the analysis of their expression, especially when these receptors are likely to be expressed in the same tissue and sometimes even in the same cells. How are the different receptors compartmentalized and how do they avoid competition? What is the specific role of the B30.2 domains in the various receptors? Fish that diversified massively the B30.2 domains into two categories of receptors, the TRIMs and NLRs, probably under different selection pressures, offer a good model for experiments addressing these questions.

Van der Aa *et al*. [6] have opened Pandora's box, and their work suggests future directions for studies of the phylogenies, linkage and functions of TRIM proteins and their relationships with other immune-system components. Their work calls for more experiments with the teleost model: a more thorough analysis of all TRIM sequences in fish might reveal new trends in the evolution of the family and could enable investigation of the relationships with the related TRIMs without B30.2 or RING-finger-B-box moieties. Tissue localization, regulation of expression and binding specificity must also be determined. This will be difficult, but what is revealed is likely to be fascinating for any biologist. Finally, many unanswered questions about antiviral activity could profit from B30.2 comparative models, among which invertebrates may offer natural variations on the theme of TRIM-B30.2 receptors. Comparative TRIMinologists have many happy days ahead.

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