Antarctic Fish *IgT*, a Weird Option of Immunoglobulin Genes

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Abstract

Antarctic fish are an extraordinary example of adaptation to the extreme cold environment where significant gene modification events have played a crucial role. Due to the well-documented plasticity of its gene locus, the Immunoglobulin (Ig) molecule represents a valuable model for understanding evolutionary modifications occurred in the Antarctic teleost species. We have reported, in a recent paper, the first identification of *IgT* heavy chain gene in the Antarctic Notothenioid teleost *Trematomus bernacchii*; it encodes three differently sized *IgT* transcript variants, named Long, Short and Shortest. Genomic analysis revealed that, within the intron between the exons coding for the entire first and second constant domains, a reminiscence of the ancestral second exon was present. We have disclosed an unsuspected complexity of the *Ig* genes of Antarctic fish, reinforcing the general concept of a high variability of these genes across teleost species.

Commentary

Antarctic fish, most of which belonging to the Perciform Suborder Notothenioidei, have acquired, during their evolution, specific features that allow them to thrive, at present, at cold and stable temperature (-1.86°C) [1]. The morphological evolution and diversification of Antarctic Notothenioid teleosts into over 120 species is one of the best examples of adaptive radiation in the marine environment. New ecological chances, for instance the extinction of antagonists, the colonization of vacant niches, or the emergence of key innovative features such as the gain of antifreeze glycoproteins have allowed them to live in cold habitats where other species would die. The evolution of the antifreeze glycoprotein genes from a trypsinogen-like gene [2] is a striking innovation, in terms of genetic fitness, that guarantees the survival. However, in the course of their evolution, Antarctic fish have undergone also some "disaptations", as mentioned by Montgomery and Clements [3], namely regressive changes or disappearance of traits in some species, such as the loss of erythrocytes [4] and the hemoglobin [5] in the Channichthyidae family, also known as icefish. They have completely lost the β-globin genes but maintain non-functional genomic remnants of α-globin genes.

The analysis of genome data from the Antarctic endemic fish provides a helpful information regarding processes that led to evolutionary adaptation, e.g. genome modifications and epigenetic control of gene expression [6], and that are crucial in influencing the capacity of Notothenioids to respond to striking environmental changes. However, despite a growing interest in the genome-wide study, it is not exhaustive enough to provide a clear picture because of the limited number of sequenced genomes. Over the last decade, there has been an explosion of molecular-based analyses, due to the development of high-throughput technologies, shedding light on the genomic basis of cold adaptation of Notothenioids. So, we have learned from integrative genomic and transcriptomic analyses that genome

evolution, under constant cold conditions, has resulted in extensive expansions of specific gene families of utmost importance, since involved in reducing stress effects derived from cold temperatures [7]. This was followed by an increase of gene expression and gene functions that contributed to physiological fitness of Antarctic Notothenioids under cold conditions, such as anti-oxidation, anti-apoptosis, lipid and protein metabolism, and immune response.

In the teleost species studied to date three *IgH* chain isotypes, *IgM*, *IgD*, and *IgT* have been identified. The discovery of *IgT* in teleosts has changed the old paradigm that *Ig* isotypes evolved distinct roles into systemic and mucosal compartments during tetrapod emergence, confirming that an *Ig* isotype dedicated to mucosal immunity arose much early in evolution, sharing common features with mammalian mucosal immune response [8]. In the last two decades or so, we have disclosed several peculiar features of Antarctic teleost *Ig* genes, thus contributing to the understanding of the evolutionary changes that have occurred in the *Ig* gene loci [9-12]. We have recently reported the first identification of *IgT* heavy chain constant region sequence in a cold adapted teleost species, *Trematomus bernacchii* [13]. Interestingly, this is a strange case of three differently sized *IgT* transcript variants, named Long, Short and Shortest, not found for the *IgM* isotype in any of the Antarctic species we have previously investigated. The Long and Short variants are encoded by indel alleles, whereas the Shortest variant originates by alternative splicing that leads to Cτ2 skipping. The loss of almost the entire Cτ2 domain together with the conservation of some amino acid residues, typical of hinge regions, in the remaining domain, would more likely represent another sophisticated and distinctive hallmark of the evolution of the Antarctic fish *IgH* locus. The most intriguing side of the story came out when we looked at genomic level in an attempt to find a comprehensive explanation of the presence of the three variants.

The *IgT* heavy chain constant region sequence was further analyzed with respect to that of *Bovichtus diacanthus*, a sub-Antarctic species, belonging to a phylogenetically basal lineage of Notothenioids. This analysis allowed us to show that the architecture of the intron between the second and third expressed Cτ exons in *T. bernacchii* was quite

well conserved in terms of size and degree of sequence conservation compared to that of *B. diacanthus*. In contrast, the intron between the first and second expressed Cτ exons revealed signs of significant modifications either in sequence or length, containing besides a reminiscence of the ancestral Cτ2 exon. This is not new to Antarctic fish *IgH* locus since, in an earlier work, we had highlighted the presence of a large insertion, containing up to four 39-nucleotide extra exons, within the Cμ4-TM1 intron [14]. The insertion of this region, which is antiparallel to an upstream region in the same gene locus, causes a significant modification of the mRNA splicing mechanism. The resulting membrane-bound form of the *IgM* heavy chain consists of only two constant *Ig* domains, and includes up to four short extra exons encoding a significantly long Extracellular Membrane-Proximal Domain. These results can be ascribed to important gene modification events that occurred during the radiation of Nototheniidae, initiated about 24 MYA [15]. In the case of *IgT* gene, the weird side of the evolutionary history of Antarctic fish *Ig* genes came out again: instead of gaining some extra exons, it has partially lost the Cτ2 exon, retaining a trace of it within the intron between the first and second expressed Cτ exons. The resulting exonic remnant, by virtue of the conservation of the acceptor and donor sites, can then undergo an in-frame splicing and thus be included in the mature transcript. This scenario underlines the important role of introns not only in fulfilling their functions in the mRNA processing, and genome architecture, but also, from an evolutionary perspective, as an optimal substrate upon which evolution has acted, leading to the gain of modified genes.

As we speculated in our report, the evolutionary novelties observed either for *IgM* or *IgT* of Antarctic fish might represent the effects of random events occurred during Nototheniidae radiation, accompanied by successive losses of several parts of the genome and the insertion into the genome of some transposable elements. The former could represent one of a series of hypothetical stages through which occurred the remodelling process of the pre-existing ancestral gene that gave rise to the functional gene copy. The latter may have contributed by causing structural alterations of the *IgH* locus, such as duplications and/or deletions in the insertion sites, leading eventually to the loss of most Cτ2 exon. This is line with the well-known concept that fish genome diversity has been further shaped by lineage-specific events over time such as genome expansion and/or contraction. The presence of multiple gene clusters in *Danio rerio* is an example of genome diversification derived from local duplications, or the emergence of intronless genes in *Oryzias latipes* as an impact of retrotransposition mechanisms. Here, we should also bear in mind the classical view, recently re-evaluated, about the key role of the Recombination-Activating Gene (RAG) transposon, and the two rounds of whole-genome duplication in the birth of the adaptive immune system in Gnathostomata [15,16]. Duplications of genes are frequent also in Notothenioids and our previous investigations on *IgH* gene locus multiplicity by Fluorescence In Situ Hybridization (FISH) revealed an *IgH* gene locus duplication in several Antarctic fish species, with the duplicated genes located and expressed on different chromosomes [17]. Recent information indicates that the genome expansion occurred in parallel with phyletic diversification of Notothenioidei and repetitive elements, e.g. Gypsy and Copia LTR retro transposons, SINE elements, Mariner/Tc1 transposon, may have contributed to the differential expansion of their genomes and of specific gene families [18,19].

Of note, the architecture of the *IgT* heavy chain region is particularly heterogeneous since comprises different numbers of constant domains: four domains are found in most species [20],

whereas three are present in *Gasterosteus aculeatus* and two in *Takifugu rubripes*. Moreover, *IgT* in *Cyprinus carpio* are even more heterogeneous since they are chimeric molecules consisting of a Cμ1 domain and a Cτ domain [21]. Considering these differences observed in the Cτ domain number, it is not surprising that Antarctic fish *IgT* lack almost an entire domain, but rather that they retain a very limited portion of it, implying a specific structural significance. In fact, the presence of a very short Cτ domain containing cysteine, glycine, and proline residues suggests that this region may function as a sort of hinge between the Cτ1 and Cτ2 domains, recalling the functional role of the region connecting the Cμ2-Cμ3 domains, we have previously described in the Antarctic *IgM* heavy chain [9]. However, the *IgM* hinge region, which is coded as a 3' extension of the Cμ2 exon, is remarkably longer than those of non-Antarctic teleosts, providing higher flexibility; and also highly polymorphic either at the splicing sites, giving rise to hinges of different lengths, or at several sites where most substitutions are non-synonymous, favouring the purifying selection [11]. Somehow, the "short Cτ2 domain" of *IgT* could play a key structural role as a flexible portion of the molecule that is required under the extreme environmental constraints. Also, the possible involvement of its conserved cysteine in different disulfide bonds forming differently sized multimers cannot be excluded.

The discovery of such a peculiar *IgT* in Antarctic fish raises the question: which is the driving force that made the modifications, introduced by random events in *Ig* genes, a fortunate happenstance? Clearly, understanding the evolutionary forces that, by remodelling genes, generated a good product will undoubtedly have a remarkable impact on our knowledge of the dynamics of organisms' adaptation to the environment where they live. The recent global climate changes are causing an increase in temperatures for most areas of the Southern Ocean; it would therefore be fascinating to predict how the plasticity of immune genes of Antarctic teleost species can cope with increased environmental stress conditions.

In view of these considerations, I think that Antarctic fish will never cease to amaze us by reinventing pre-existing genes with some novel as well as unique characteristics that are critically important for exerting their function in the constantly cold environment. However, there are still many dark side points awaiting clarification. For this reason, Antarctic fish certainly deserve considerable attention and further efforts to be investigated.

Author Contributions

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