

EVOLUTIONARY ADAPTATIONS IN ANTARCTIC FISH: THE OXYGEN-TRANSPORT SYSTEM

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ABSTRACT

Understanding molecular adaptations evolved in response to environmental temperature changes is essential, because temperature affects the kinetic energy of molecules and modifies molecular interactions, macromolecular stability/functioning and membrane features. Environmental oxygen availability may also play an important role in the evolution of polar marine organisms, as suggested by the physiological and biochemical strategies adopted by these organisms to acquire, deliver and scavenge oxygen. This review summarises the current knowledge on the structure and function of hemoglobins of fish living in Antarctic habitats. The variety of adaptations underlying the ability of Antarctic fish to survive at temperatures permanently close to freezing is unique among teleosts. The dominant perciform suborder Notothenioidei affords an excellent study group for elaborating the evolution of biochemical adaptation to temperature. The availability of notothenioid taxa living in a wide range of latitudes (Antarctic, sub-Antarctic, and temperate regions) offers a remarkable opportunity to study the physiological and biochemical characters gained and, conversely, lost in response to cold, besides the possibility to reconstruct the likely evolutionary events modulating the ability of these fishes to carry oxygen in freezing habitats.

Although oxygen can be transported in freely dissolved form most animals rely on one or more protein carriers to deliver it to the respiring tissues. Compared to temperate and tropical species, high-Antarctic notothenioids have evolved reduced hemoglobin concentration/multiplicity. The Antarctic family Channichthyidae (the notothenioid crown group) is devoid of hemoglobin. All extant icefish species lack hemoglobin and many have lost myoglobin expression. In these species, oxygen delivery to tissues occurs by transport of the gas physically dissolved in the plasma.

Keywords: Antarctic; cold adaptation; evolution; fish; hemoglobin.

RESUMO

ADAPTAÇÕES EVOLUTIVAS EM PEIXES ANTÁRTICOS: O SISTEMA DE TRANSPORTE DE OXIGÊNIO. Compreender as adaptações moleculares envolvidas na resposta às mudanças na temperatura ambiental é essencial, pois a temperatura afeta a energia cinética das moléculas e modifica as interações moleculares, a estabilidade/funcionamento das macromoléculas e as características da membrana. A disponibilidade de oxigênio no ambiente pode também ter um importante papel na evolução dos organismos marinhos polares, como indicado pelas estratégias fisiológicas e bioquímicas adotadas por estes organismos para adquirir, transportar e trocar oxigênio. Esta revisão resume o conhecimento atual da estrutura e funcionamento das hemoglobinas de peixes que ocorrem em ambientes Antárticos. A diversidade de adaptações que sustentam a habilidade de peixes antárticos sobreviverem em temperaturas permanentemente próximas do congelamento é única entre os teleósteos. A dominante sub-ordem Perciforme Notothenioidei apresenta-se como um excelente grupo de estudo para melhorar o conhecimento sobre a evolução das adaptações bioquímicas à temperatura. A ocorrência de nototeniídeos em uma ampla variedade de latitudes (Antártica, sub-Antártica e regiões temperadas) oferece uma oportunidade notável para estudar as características fisiológicas e bioquímicas obtidas e, por outro lado, perdidas em resposta ao frio, além de tornar possível a reconstrução dos eventos evolutivos que provavelmente modularam a habilidade desses peixes de transportar oxigênio em ambientes extremamente frios.

Embora o oxigênio possa ser transportado livremente na sua forma dissolvida, a maioria dos animais depende de um ou mais tipos de proteínas carreadoras para entregar o oxigênio aos tecidos. Quando comparadas às espécies temperadas e tropicais, os nototeniídeos da região Antártica, propriamente dita, desenvolveram reduzida concentração/multiplicidade de hemoglobinas. A família de peixes Antárticos Channichthyidae (*crown group* nototeniíde) não apresenta hemoglobina. Todas as espécies de *icefish* (peixes-do-gelo) não possuem hemoglobinas e muitas também não produzem mioglobinas. Nessas espécies, o transporte de oxigênio aos tecidos ocorre através do gás fisicamente dissolvido no plasma.

Palavras-chave: Antártica; adaptações ao frio; evolução; hemoglobina.

RESUMEN

ADAPTACIONES EVOLUTIVAS EN PECES ANTÁRTICOS: EL SISTEMA DE TRANSPORTE DE OXÍGENO. Comprender las adaptaciones moleculares que han evolucionado en respuesta a los cambios de temperatura del medio ambiente es esencial, porque la temperatura afecta la energía cinética de las moléculas y modifica las interacciones moleculares, la estabilidad de las macromoleculares, sus características y el funcionamiento de la membrana. La disponibilidad de oxígeno ambiental desempeña un papel importante en la evolución de los organismos marinos polares, como se evidencia en las estrategias fisiológicas y bioquímicas adoptadas por estos organismos para adquirir, gastar y usar oxígeno. Esta revisión resume el conocimiento actual sobre la estructura y función de la hemoglobina de los peces que viven en hábitats antárticos. La amplia variedad de adaptaciones que permiten que los peces antárticos tengan la capacidad para sobrevivir de forma permanente en temperaturas cerca de la congelación es única entre los teleosteos. El suborden Notothenioidei, perteneciente a los Perciformes, es un excelente grupo para el estudio de la evolución y adaptación bioquímica a la temperatura. La gran variedad de taxones de nototénidos que viven en una amplia variedad de latitudes (Antártida, sub-antárticas, y las regiones templadas) ofrece una oportunidad extraordinaria para estudiar las características fisiológicas y bioquímicas adquiridas y perdidas por este grupo en respuesta al frío, además de la posibilidad de reconstruir los eventos más probables que direccionaron la evolución de la capacidad de transportar oxígeno en hábitats polares.

Aunque el oxígeno puede ser transportado en su forma libre disuelta, la mayoría de los animales dependen de una o más proteínas para entregarlo a los tejidos para la respiración. En comparación con especies de zonas templadas y tropicales, los nototenoideos de la alta Antártida han evolucionado reduciendo la concentración y multiplicidad de hemoglobina. La familia antártica Channichthyidae (el grupo con corona de los Nototénidos) carece de la hemoglobina. Todas las especies de peces existentes que viven en el hielo carecen de hemoglobina y muchas han perdido la expresión de la mioglobina. En estas especies, el aporte de oxígeno a los tejidos se produce por el transporte del gas fisicamente disuelto en el plasma.

Palabras clave: Antártida; adaptaciones al frío; evolución; hemoglobina.

THE ANTARCTIC NOTOTHENIOIDEI

During many million years, the Antarctic biota, both on land and in the sea, has evolved under the influence of a suite of geological and climatic factors, including geographic isolation of the landmass and continental shelves, extreme low temperature and intense seasonality (Clarke & Crame 1992). Over geological time, environmental conditions and habitats changed dramatically; consequently, many groups of organisms became extinct (Eastman 1993).

Nowadays the growing interest in polar marine organisms is closely related to the impacts of current climate change. The impacts of climate change will

depend on the rate of ongoing temperature changes and on the genotype and ecology of species. More negative impacts are expected in species physiologically specialised with respect to temperature and with limited acclimation capacity (Pörtner 2010). Species living in thermostable environments are highly specialised within a narrow temperature range. Antarctic marine invertebrates may be more vulnerable to warmer temperatures (Peck *et al.* 2009a, 2009b), while thermal acclimation capacity still exists among Antarctic fish in the short/medium time range. The rate of impact of current changes in relation to the capacity of extant species to acclimate or adapt is a crucial study area for future management of polar ecosystems.

Over the past million years, the Antarctic shelf has been subjected to tectonic and oceanic events that began to alter the composition of the fish fauna and to initiate the process of faunal replacement (Clarke & Crame 1992). Fragmentation of Gondwana into the modern southern continents and the displacement of the Antarctic continent to its current geographic location have been the most significant events responsible for these changes. The crucial opening of the Drake Passage between southern South America and the Antarctic Peninsula occurred 23.5-32.5 million years ago (mya) (Thomson 2004), and possibly even as early as 41 mya (Scher & Martin 2006). The Drake Passage led to the development of the Antarctic Circumpolar Current (ACC) and this in turn was at least partially responsible for the cooling of Antarctic waters from near 20°C to the present extreme values near -1.9°C, the equilibrium temperature of ice and sea water (Clarke 1983).

The Antarctic Polar Front (APF), the northern boundary of the ACC, is a well-defined, roughly circular oceanic system, running between 50°S and 60°S. Along the Front, the surface layers of the north-moving Antarctic waters sink beneath the less cold and less dense sub-Antarctic waters, generating virtually permanent turbulence. Just north of the Front, the water temperature has an abrupt rise of approx. 3°C, a critical factor for the isolation and adaptation of the ecosystem. The APF greatly limited the opportunities for migration, forcing the shallow-water fish fauna to either adapt to the changing climate or become extinct (Clarke 1983, Eastman 1993).

One group of teleost fish, the perciform suborder Notothenioidei, became largely dominant as a consequence of success in adapting to the challenging environmental conditions, for example low temperature, sea ice, habitat reduction and seasonality of primary production (Clarke & Johnston 1996). The ancestral notothenioid stock probably arose as a sluggish, bottom-dwelling teleost species that evolved some 40-60 mya in the shelf waters (temperate at that time) of the Antarctic continent and experienced extensive radiation, dating from the late Eocene, approx. 24 mya (Near 2004).

The high-Antarctic notothenioids, distributed south of the APF, live at the freezing point of sea water (near -1.9°C), and die at temperatures of 4-6°C (Somero & DeVries 1967). Non-Antarctic

notothenioids, which comprise sub-Antarctic as well as temperate species, are found north of the APF and compose 22% (28 of 129 species) of notothenioid biodiversity (Eastman 2005).

Bovichtidae, Pseudaphritidae, Eleginopidae, Nototheniidae, Harpagiferidae, Artedidraconidae, Bathydraconidae and Channichthyidae are the families of the suborder (Eastman 2005). All Bovichtidae (except one species), monotypic Pseudaphritidae and Eleginopidae and some species of Nototheniidae inhabit waters north of the APF. These species encounter water temperatures of approximately 5°C-15°C (Eastman 2005, di Prisco *et al.* 2007).

Molecular phylogeny has recently begun to provide indications about the time of radiation in the Antarctic. Initial divergence took place about 40 mya during the Eocene (Near 2004).

Bovichtidae, Pseudaphritidae and Eleginopidae essentially never experienced near-freezing water temperatures because they presumably diverged and became established in waters around areas corresponding to New Zealand, Australia and South America before Antarctica became isolated. The phylogenetically basal bovichtids, pseudaphritids and eleginopids do not possess antifreeze glycoprotein (AFGP)-gene sequences in their genomes, indicating that they diverged well before the tectonic isolation and cooling of Antarctica (Cheng *et al.* 2003). The Antarctic clades thus probably evolved after the complete separation of Antarctica and the following isolation of the Southern Ocean from the surrounding water masses due to the formation of the APF. The subsequent cooling of Antarctic waters and expansion of ice sheets led to the extinction of the original temperate fauna (Eastman 2005). Notothenioids, in the absence of competition, filled all ecological niches made available. As a consequence of their extensive radiation, Antarctic notothenioids are considered one of the few examples of marine species flocks (Eastman & McCune 2000).

MOLECULAR ADAPTATIONS IN NOTOTHENIOIDEI

The variety of adaptations underlying the ability of modern Antarctic fish to survive at the freezing temperatures of the environment represents the extreme of low-temperature adaptations among vertebrates.

In an extreme environment such as Antarctica, one of the most important driving forces in the evolutionary adaptations of marine organisms is the enhanced oxygen solubility in the cold waters of the Southern Ocean (Chen *et al.* 2008). Comparative analyses of transcriptome profiles of the Antarctic notothenioid fish *Dissostichus mawsoni* and temperate/tropical fishes showed that evolution in the cold produced genomic expansions of specific protein gene families involved in physiological fitness of Antarctic notothenioids under the extreme polar conditions (Chen *et al.* 2008).

Differences that characterise notothenioids (Table 1) include efficient microtubule assembly at temperatures as low as -1.9°C (Detrich *et al.* 1989, 2000), apparent loss of inducible heat-shock

response (Hofmann *et al.* 2000, Place *et al.* 2004, Place & Hofmann 2005) still possessed by some non-Antarctic notothenioids (Hofmann *et al.* 2005), enhanced enzyme-structural flexibility (Field & Somero 1998), changes in membrane fluidity (Romisch *et al.* 2003), constraints in aerobic energy supply, mitochondrial functioning and capacity of anaerobic energy production (Pörtner 2006), higher levels of ubiquitin-conjugated proteins in tissues as evidence for cold denaturation of proteins *in vivo* (Todgham *et al.* 2007). Some of these differences are not adaptive in a strict evolutionary sense, for example, loss of heat-shock response, gene expression loss of hemoglobin and myoglobin, higher level of ubiquitinated proteins (Somero 2010).

Table 1. Some peculiarities typical of Notothenioidei.

Feature	Consequence	Reference
Membrane change	Increasing fluidity of membranes	Romisch <i>et al.</i> 2003
Heat-shock response	Apparent loss of inducible heat-shock response	Clark & Peck 2009
Tubulin structure	Microtubule assembly	Detrich <i>et al.</i> 1989, 2000
Protein/enzyme structure	Maintaining high catalytic efficiency	Fields & Somero 1998
AFGP biosynthesis	Avoiding body-fluid freezing	Cheng & DeVries 1991
Genome plasticity	Up-regulation of specific genes involved in the anti-oxidant functions	Chen <i>et al.</i> 2008
Globin-gene loss	Reduction of blood viscosity	di Prisco <i>et al.</i> 2002
Agglomerular kidney	Urinary conservation of small antifreeze molecules	Eastman & DeVries 1986
Mitochondrial changes	Increase content in muscle	Guderley 2004

Interesting results showed that *Bovichtus variegatus* (family Bovichtidae) expresses heat-shock proteins in response to heat stress, whereas *Notothenia angustata*, a non-Antarctic notothenioid living in New Zealand waters, does not display the stress-inducible heat-shock protein synthesis (Hofmann *et al.* 2005). These results suggest that heat-shock response, the up-regulation of heat-stress sensitive genes, was lost after evolution in the subzero, stenothermic environment of Antarctic waters during the divergence of Bovichtidae from the other Antarctic notothenioid families. In addition, Buckley & Somero (2009) show that in cold-adapted *Trematomus bernacchii* no transcriptional up-regulation of heat-shock genes

occurs during heat shock and that the level of up-regulation of gene expression under heat stress is highly changed, compared to expression changes measured in temperate, eurythermal fish.

ANTIFREEZE GLYCOPROTEINS (AFGPs)

The biosynthesis of AFGPs is one of the most important evolutionary adaptations discovered in high-Antarctic fish. AFGPs allow to avoid freezing by binding frozen water, thus preventing growth of ice crystals in the blood and other body fluids (Cheng & DeVries 1991). Produced by pancreatic tissue and the anterior portion of the stomach (Cheng *et al.*

2006), AFGPs are a family of polymers composed of a glycotriptide monomeric repeat, -Thr-Ala-Ala-, with each Thr linked to the disaccharide galactose-N-acetylgalactosamine (Cheng & DeVries 1991).

High-Antarctic notothenioids have ample gene families for the production of large amounts of AFGPs. In non-freezing environments, where the antifreeze function becomes unessential, the AFGP function is reduced as observed in non-Antarctic notothenioid fishes (in Arctic fish, AFGP biosynthesis occurs only in winter). In *N. angustata* and *N. microlepidota*, living in cool-temperate waters, the AFGP system is reduced with very low blood AFGP concentration and only two to three genes showing some replacements in the -(Thr-Ala-Ala)- repeat (Cheng *et al.* 2003). One of the Ala residues is occasionally substituted by Pro. *Dissostichus eleginoides*, a non-Antarctic notothenioid of the family Nototheniidae, appears to have no functional AFGP sequences, consistent with its non-Antarctic distribution. However, the apparent absence of AFGP genes in *D. eleginoides* is intriguing because the AFGP gene was thought to have evolved once, before the Antarctic notothenioid radiation, at the base of the family Nototheniidae. The hypothesis is that the species had the primordial AFGP genotype, lost or mutated following its migration to non-Antarctic habitats (Cheng *et al.* 2003).

The study of freezing avoidance in Notothenioidei is now developing along new perspectives, linked to the recent discovery of AFGP-deficient, but freeze resistant notothenioids in early life stages (Cziko *et al.* 2006). The absence of AFGP production in larvae suggests that suitable freezing resistance may temporarily be afforded by alternative mechanisms.

THE HEMOGLOBINS IN NOTOTHENIOIDEI

Since hemoglobins of all animal species have the same heme group, differences in their properties, including oxygen affinity, electrophoretic mobility and pH sensitivity, must result from the interaction of the prosthetic group with specific amino-acid residues in the primary structure. For this reason, fish hemoglobins have been the object of extensive studies in the last few years, not only for their structural characteristics, but also because they offer the possibility to investigate the evolutionary history of these ancient genes in species living in a large

variety of environmental conditions (Weber & Jensen 1988). Fish hemoglobins, similar to other vertebrate hemoglobins, are tetrameric proteins consisting of two identical α and β subunits, each of which contains one oxygen-binding heme group. These subunits are paired in two dimers, $\alpha_1\beta_1$ and $\alpha_2\beta_2$.

Within different species, the transport of oxygen can be modulated by changes in the hemoglobin structure and allosteric-ligand concentration (ATP for most teleost fish), and by changes in the expression of multiple hemoglobins likely to display different functional features. During evolution, complex and sophisticated molecular mechanisms, e.g. modulation by pH, carbon dioxide, organophosphates and temperature, have been developed to regulate oxygen transport by hemoglobin.

Unlike most mammals, including humans, fish often exhibit hemoglobin multiplicity, usually taken as a sign of phylogenetic diversification and molecular adaptation, which results from gene-related heterogeneity and gene-duplication events (Dettaï *et al.* 2008). Oxygen-affinity differences in erythrocytes can also be the result of sequential expression of different hemoglobins, variable concentrations of allosteric effectors, differential response of hemoglobins to effectors (Verde *et al.* 2006, di Prisco *et al.* 2007). The capacity of fish to colonise a large variety of habitats appears to have evolved in parallel with suitable modulation of their hemoglobin system at the molecular/functional level.

Specialised hematological features are striking adaptations developed by the Antarctic ichthyofauna during evolution at low temperature. In the seven red-blooded notothenioid families the hemoglobin level is reduced, the erythrocyte number is an order of magnitude lower than in temperate fish, and is reduced by over three orders of magnitude in the 16 "icefish" species of Channichthyidae (Eastman 1993) in which hemoglobin is absent (Ruud 1954). Icefish retain genomic DNA sequences closely related to the adult α -globin gene(s) of its red-blooded notothenioid ancestors and contemporaries, whereas its ancestral β -globin-gene sequences have been deleted (di Prisco *et al.* 2002). The discovery within the icefish family of two distinct genomic rearrangements, both leading to functional inactivation of the *locus*, seems to point towards a multi-step mutational process (Near *et al.* 2006).

In channichthyids, no carrier has replaced hemoglobin and the oxygen-carrying capacity of the blood is only 10% compared to that of red-blooded fish. They compensate the lack of an oxygen carrier with large gills, increased blood volume and higher cardiac output (Egginton *et al.* 2002); moreover, they have highly vascularised, scaleless skin, which favours cutaneous respiration.

The loss of hemoglobin in icefishes is paralleled by the loss of myoglobin in 6 icefish species through at least 4 mutational events (Sidell & O'Brien 2006). Despite the costs associated with loss of these hemoproteins, the constantly cold and oxygen-saturated waters of the Southern Ocean provided an environment in which fish are able to survive even without oxygen-binding proteins.

In comparison with temperate species, Antarctic notothenioids have lost globin multiplicity, leading to the hypothesis that in the Antarctic thermostable environment the need for multiple hemoglobins may be reduced. Most Nototheniidae have a single major hemoglobin generally accompanied by minor or embryonic components (Verde *et al.* 2006, di Prisco *et al.* 2007). In most Nototheniidae, embryonic α and β globins are expressed in trace or limited amounts in the adult stage, but in at least three species, namely *Trematomus newnesi* (D'Avino *et al.* 1994), *Pagothenia borchgrevinki* (Ricci *et al.* 2000) and *Pleuragramma antarcticum* (Tamburrini *et al.* 1996), embryonic globins are expressed at significant levels (approx. 25% of the total). Adults of species of the modern families Artedidraconidae and Bathydraconidae lack the minor or embryonic globins.

Further evidence that the role of hemoglobin in red-blooded Antarctic fish may have become reduced, because of development of additional different and specialised functions, comes from molecular studies (Bargelloni *et al.* 1998) providing compelling evidence for positive selection of hemoglobin in *Gymnodraco acuticeps* of the family Bathydraconidae, the sister group to Channichthyidae lacking hemoglobins and their genes. *Gymnodraco acuticeps* (a sit-and-wait predator) showed a significantly higher rate of non-synonymous (amino-acid replacing) than synonymous (silent) substitutions in the β -globin DNA sequence with respect to the majority of notothenioids (Bargelloni *et al.* 1998).

The evolutionary development of an alternative physiology based on hemoglobin-free blood may adequately work in the cold for notothenioids in general, and the benefits may include reduced costs for protein synthesis (Pörtner *et al.* 2007).

Unlike most high-Antarctic notothenioids, some sub-Antarctic and temperate notothenioids display higher multiplicity of hemoglobins. These multiple hemoglobins may differentially work in response to temperature differences and fluctuations (much larger than in the Antarctic), presumably to cope with the small or large temperature changes in the respective habitats north of the Polar Front (di Prisco *et al.* 2007). The hemoglobin multiplicity may reflect dynamic life style and different environmental conditions encountered.

The oxygen affinity of hemoglobins of many high-Antarctic notothenioids is quite low (di Prisco *et al.* 2007). This feature is probably linked to the high oxygen concentration in the cold sea. In contrast, the affinity is higher in hemoglobins of non-Antarctic notothenioids. The relationship between high affinity and habitat features remains an open question as far as its structural basis is concerned. Spectroscopic and modelling studies on the hemoglobins of temperate and sub-Antarctic notothenioids have shown that all the non-conservative replacements in the primary structure of the α and β chains leave the conformation and electrostatic field surrounding the heme pocket essentially unmodified with respect to the hemoglobins of high-Antarctic notothenioids (Verde *et al.* 2004).

The decreased oxygen affinity of hemoglobins at lower pH values in the physiological range is known as alkaline Bohr effect (reviewed by Riggs 1988). In many teleost hemoglobins, the complete loss of the subunit cooperativity in binding oxygen and the inability to saturate the ligand sites at low pH, even at high oxygen pressure, is a distinctive property with respect to the Bohr effect. This feature is known as the Root effect (Brittain 2005).

A general reduction in the Root effect is noticed during the evolution of the Antarctic notothenioids (di Prisco *et al.* 2007), corresponding to a variable scenario pertaining to the choroid *rete mirabile*. The physiological role of the Root effect is to secrete oxygen against high oxygen pressures into the swimbladder (when present) and the choroid *rete*

mirabile (Wittenberg & Wittenberg 1961, Wittenberg *et al.* 1964).

Antarctic fish lack the swimbladder. Among high-Antarctic notothenioids, many species have lost the choroid *rete*, although several retain portions of the *rete* and/or small vestigia of the choriocapillaris (Eastman 1993, 2006). Because high-Antarctic notothenioids still have hemoglobins endowed with Root effect also when the choroid *rete* is absent, this function may undergo neutral selection. It has been argued that the possession of the Root effect may undergo neutral selection pressure in the simultaneous absence of *retia mirabilia* and presence of high hemoglobin buffer capacity, as in some basal ray-finned fishes and in the ancestors of teleosts (Berenbrink *et al.* 2005). This may be the case in notothenioids with increased number of His residues (which confer buffer capacity) in the hemoglobin primary structure (Verde *et al.* 2008). In fact, a role for some His residues as modulators of the Root effect has recently been postulated (Mazzarella *et al.* 2006).

The multiple losses of the ocular oxygen-secretion mechanism in notothenioids would not necessarily be associated with degenerated eyes or less visually oriented life styles. In fact, an alternative oxygen-supply route to the retina by a system of hyaloid capillaries is especially well developed in several notothenioid species that have lost the choroid *rete* (for example, Eastman & Lannoo 2004).

CONCLUDING REMARKS

Gene expression patterns and, even more so, loss of genetic information, especially for myoglobin and hemoglobin in notothenioids, reflect the specialisation of Antarctic organisms to a narrow range of low temperatures. These modifications become explicable by exploitation of high-oxygen solubility at the low metabolic rates in the cold, where an enhanced fraction of oxygen supply occurs through diffusive oxygen flux. Conversely, limited oxygen supply to tissues upon warming is an early cause of functional limitation (Pörtner *et al.* 2007).

The evolutionary development of an alternative physiology based on hemoglobin-free blood may adequately work in the cold for notothenioids in general. The benefits due to hemoglobin loss include reduced costs for protein synthesis and oxygen

transport. However, as pointed out by Pörtner *et al.* (2007), the shift from hemoglobin-mediated oxygen transport to mechanisms based on diffusion may account for higher vulnerability of icefishes, and of notothenioids in general, to warmer temperatures.

The southern polar environment is experiencing significant climatic change, as shown by sea-ice reductions at the western side of the Antarctic Peninsula (Clarke *et al.* 2007). Antarctic notothenioids that have had a long evolutionary history at constant temperatures may be uniquely vulnerable to Global Warming (Somero 2005).

Novel globins, such as neuroglobin and cytoglobin, have recently been described in many vertebrates (Burmester *et al.* 2000). Neuroglobin is able to bind oxygen and other ligands and it is transcriptionally induced by hypoxia and ischemia (Brunori & Vallone 2007). It is mainly expressed in retinal neurons and fibroblast-like cells and plays a neuroprotective role during hypoxic stress. The recent discovery of neuroglobin in the brain of red-blooded notothenioids and in some channichthyid species opens the question: what is the role of neuroglobin in fishes lacking hemoglobin and myoglobin (Cheng *et al.* 2009a, 2009b). The finding that icefish retain the neuroglobin gene, despite having lost those encoding hemoglobin and myoglobin in most species, is very intriguing.

Recently, Chen *et al.* (2008) have reported genome-wide studies of transcriptional and genomic changes associated with cold adaptation in Antarctic notothenioids. Their results strongly suggest that evolution in the cold has produced dramatic genomic expansions and/or upregulations of specific gene families. Many of these up-regulated genes are involved in the antioxidant function, suggesting that augmented capacities in antioxidative defence are important components in evolutionary adaptations in cold and oxygen-rich environment.

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