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Molecular adaptations in Antarctic fish and bacteria

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Abstract

Marine organisms, living in the cold waters of the Southern Ocean, are exposed to high oxygen concentrations. Cold-adapted organisms have developed networks of defence mechanisms to protect themselves against oxidative stress.

The dominant suborder Notothenioidei of the Southern Ocean is one of the most interesting models, within vertebrates, to study the evolutionary biological responses to extreme environment.

Within bacteria, the psychrophilic Antarctic bacterium *Pseudoalteromonas haloplanktis* TAC125 gives the opportunity to explore the cellular strategies adopted *in vivo* by cold-adapted microorganisms to cope with cold and high oxygen concentration.

Understanding the molecular mechanisms underlying how a range of Antarctic organisms have responded to climate change in the past will enable predictions as to how they and other species will adapt to global climate change, in terms of physiological function, distribution patterns and ecosystem balance.

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1. Introduction

The Antarctic biota 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. Isolation and environmental history have led to a unique biota, both on land and in the sea. Many groups of organisms became extinct in the Antarctica as a result of the increasingly extreme climatic conditions.

Nowadays, the growing interest in polar marine organisms is closely related to the impacts of current climate change. The interaction of the environment with the genome of species (within a group) governs the

impact of climate change on each community. The impacts of climate change will depend on the rate of 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. Species living in thermostable environments are highly specialised within a narrow temperature range. While thermal acclimation capacity still exists among Antarctic notothenioid fish in the short/medium range (Seebacher et al., 2005; Lannig et al., 2005), marine invertebrates may be more vulnerable to warmer temperatures. 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 managing of polar ecosystems.

When alteration of the environment occurs, the ensuing selective pressure affects traits important for

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species fitness. During these changes, populations can (i) escape by migrating to unchanged habitats, leading to local extinction but persistence of the species elsewhere; (ii) adjust to the new conditions through phenotypic plasticity without altering the genetic constitution; (iii) adapt to the changed conditions through genetic changes within the process of evolution (Holt, 1990; Davis et al., 2005). Phenotypic plasticity and adaptation may prevent local extinction. However, genetic (evolutionary) and phenotypic responses differ significantly, because the latter includes a non-genetic, plastic component important to understand the respective roles in responding to environmental changes (Gienapp et al., 2008).

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). These environmental conditions may cause the production of high levels of reactive oxygen species (ROS), able to oxidise proteins, DNA and lipids and leading to extensive injury of cellular components and cell death. Therefore, cold-adapted organisms must develop an effective and intricate network of defence mechanisms against oxidative stress.

Comparative analyses of same-tissue 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 into physiological fitness of Antarctic notothenioids under the extreme polar conditions (Chen et al., 2008). Many of their up-regulated genes are involved in the antioxidant function, suggesting that augmented defenses against oxidative stress are fundamental in a cold and oxygen-rich environment.

The dominance of notothenioid fishes is the result of some of the most interesting evolutionary biological responses to the advent of the extreme conditions of the Southern Ocean. Living in a stable, extremely cold, and well-oxygenated marine environment, this group has evolved adaptations in biochemical and physiological functions, several of which are unique.

Within vertebrates, fishes allow addressing physiological mechanisms at multiple levels of biological organisation from molecules to whole organism, including responses to environmental changes. However, the most significant constraint to research on Antarctic fish is the current lack of genomic sequence data. In contrast, this is not a major problem in Antarctic microorganisms with their small genomes. The possibility to sequence whole bacterial genomes may provide the

necessary amount of data, allowing to reject or accept some of the classical hypotheses currently invoked in protein thermal adaptation. Thanks to their short generation times, bacteria can be used in experiments designed to discriminate plastic and microevolutionary responses. Most bacteria can be cultivated in the laboratory, thus it is possible to change growth conditions and investigate how the transcriptome changes in response.

The development of genomics is increasing the analysis of genome sequences of cold-adapted bacteria, allowing comparison with mesophilic and thermophilic counterparts. Cold-adapted bacteria are generally acknowledged to adopt a variety of adaptive strategies to maintain activity and metabolic function despite challenging conditions. Best performances are made possible through peculiar features which altogether lead to higher flexibility of key parts of the molecular structure of many proteins (D'Amico et al., 2006). The publication of the genome sequence and annotation of the psychrophilic Antarctic bacterium *Pseudoalteromonas haloplanktis* TAC125 (Médigue et al., 2005), isolated in coastal sea water in the vicinity of the French station Dumont d'Urville (Terre Adélie), provides a unique opportunity to study the cellular strategies adopted by cold-adapted bacteria to cope with cold and high-oxygen concentration (Médigue et al., 2005).

2. Molecular adaptations in Notothenioidei

The expansion of the ice sheet due to abrupt cooling in the late Eocene scoured the continental margin, leading to shelf habitat loss and alterations in the marine fauna (Clarke and Crame, 1992; Clarke, 1993; Eastman and McCune, 2000; Aronson and Blake, 2001). Much of the Eocene fish fauna became extinct (Eastman, 1993; Eastman and McCune, 2000), providing vast ecological opportunities for species that succeeded to adapt to habitat change and could thus survive. The ancestral notothenioid, presumably a shallow benthic fish, was able to exploit the changing habitat, and, in the absence of significant niche competition, diversified into the dominant suborder that makes up almost half (46%) of today's Antarctic fish species (Eastman, 2005).

Bovichtidae, Pseudaphritidae, Eleginopidae, Nototheniidae, Harpagiferidae, Artedidraconidae, Bathydraconidae and Channichthyidae are the families of the suborder (Eastman, 2005). Bovichtidae, Pseudaphritidae and Eleginopidae, known as non-Antarctic notothenioids, 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 (Near, 2004).

Notothenioid species living in the Antarctic coastal regions, at the freezing point of sea water (near -1.9°C), are strictly stenothermal (Eastman, 1993, 2005) and highly specialised in their low and narrow temperature window (Somero and DeVries, 1967; Somero et al., 1996; Pörtner et al., 1999, 2000; Peck and Conway, 2000; Peck et al., 2002). The evolution of *stenothermy* in the Southern Ocean was certainly driven by the great constancy of water temperature, whose variations rarely exceeds 2°C (Somero and DeVries, 1967; Podrabsky and Somero, 2006; Pörtner, 2006; Cheng and Detrich, 2007).

However, although Antarctic notothenioids are among the most stenothermal known, they still possess mechanisms to resist to acute heat stress. Their inability to acclimate to elevated temperatures within the long range may arise from their ineffectiveness to change gene expression as their body temperature changes and therefore from the failure of heat-shock response (HSR). The Antarctic notothenioid *Trematomus bernacchii*, for instance, is unable to increase the synthesis of any class of heat-shock proteins following thermal stress (Hofmann et al., 2000; Place et al., 2004).

Notothenioids thriving in more temperate waters, being eurythermal species, have physiologies more typical of cold-temperate environments.

The absence of a heat-shock response in Antarctic notothenioids, but its presence in temperate New Zealand notothenioids (Hofmann et al., 2005), suggests that evolution in a cold, stable environment has led to depletion of some genetic responses. The differences in gene regulation are accompanied by differences in the biochemical and functional features of constitutively expressed heat-shock proteins between Antarctic and cold-temperate notothenioids (Place and Hofmann, 2005). Better knowledge of the acclimation capacities of Antarctic and non-Antarctic notothenioids will help in predicting the potential impacts of climate change on these fishes. Modifications of the genetics of Antarctic notothenioids in response to cold were recently analysed by Chen et al. (2008). Investigations of the genotypic changes in Antarctic notothenioids provided evidence that evolution in the freezing Southern Ocean has produced remarkable transcriptomic shifts and gene expansion in Antarctic notothenioids with respect to related and unrelated temperate species. Increased gene copies in the

Antarctic species compared with non-Antarctic notothenioids (*Bovichtus variegatus* and *Eleginops maclovinus*) indicates duplication of preexisting genes, probably contributing to improved fitness of Antarctic notothenioids (Chen et al., 2008).

Notothenioids are remarkable examples of adaptation to extreme cold. Recent studies showed fundamental aspects of phenotypic plasticity, showing that, while adjustments during juvenile or adult life are usually reversible, exposure to environmental fluctuations during developmental stages may cause life-long effects (Johnston et al., 2003; Pörtner et al., 2007).

Examples of cold adaptation include efficient microtubule assembly at temperatures as low as -1.9°C (Detrich et al., 1989, 2000; Redeker et al., 2004), enzyme-structural constraints (Fields and Somero, 1998; Russell, 2000; Hochachka and Somero, 2002; Fields and Houseman, 2004; Johns and Somero, 2004), decreased membrane fluidity (Römisch et al., 2003), constraints in aerobic energy supply, mitochondrial functioning and the capacity of anaerobic energy production (Johnston et al., 1998; Pörtner, 2006), higher levels of ubiquitin-conjugated proteins in tissues as evidence for cold denaturation of proteins *in vivo* (Todgham et al., 2007).

In high-Antarctic notothenioids, the complete loss of the NADH-6-dehydrogenase (ND6) mitochondrial gene, fundamental in the respiratory function (Papetti et al., 2007), was particularly surprising, because the absence of this gene had never been reported in any other mitochondrial genome. The presence of ND6 gene in sub-Antarctic notothenioids highlights a different evolutionary history. Antarctic notothenioids are able to survive by developing functional compensatory changes in the mitochondrial respiratory system. Bai and Attardi (1998) demonstrated that revertant clones of a mouse cell line (4AR, with a mitochondrial mutation in ND6), were able to suppress the respiratory dysfunction by re-establishing the Complex I (NADH-quinone oxidoreductase) assembly, of which ND6 is part (Bai et al., 2005; Deng et al., 2006). Antarctic notothenioids might cope with complete lack of ND6 because partial impairment of respiratory function would result in incompletely coupled mitochondria and heat production through proton leakage (Brand, 2000), potentially advantageous for ectothermal organisms exposed to constant subzero temperatures (Papetti et al., 2007). Almost simultaneously with the submission of this review, a paper described translocation of the ND6 gene and tRNA^{Glu} from their canonical position (between the ND5 and cytochrome b genes in vertebrates) to the

Control Region in high-Antarctic notothenioids (Zhuang and Cheng, in press). Basal notothenioids had the usual mitochondrial-gene order.

One major example of adaptations evolved by Antarctic notothenioids is the acquisition of genes for antifreeze glycoproteins (AFGPs). AFGPs allow to avoid freezing by binding water molecules, thus preventing growth of ice crystals in the blood and other body fluids (DeVries, 1988; Cheng and DeVries, 1991). Produced by pancreatic tissue and the anterior portion of the stomach (Cheng et al., 2006), AFGPs of notothenioids are a family of polymers composed of a glycotriptide monomeric repeat, -Thr-Ala-Ala-, with each Thr linked to the disaccharide galactose-N-acetylgalactosamine (DeVries, 1988; Cheng and DeVries, 1991). The absence of any detectable AFGP coding sequence in some non-Antarctic families of the suborder Notothenioidei (Cheng et al., 2003), is consistent with this cold adaptive acquisition. In *Notothenia angustata* and *Notothenia microlepidota*, living in New Zealand, genes encoding AFGPs are present and low amounts of the proteins can be detected in their blood (Cheng et al., 2003). The most parsimonious explanation for these observations is that these species or their progenitors were originally in Antarctic waters and subsequently during the late Miocene escaped to north from waters south of the Antarctic Polar Front.

Specialised hematological features are striking adaptations developed by the Antarctic ichthyofauna during evolution at low temperature. Red-blooded Antarctic notothenioids differ from temperate and tropical species in having fewer erythrocytes and reduced hemoglobin (Hb) concentration and multiplicity. In cold Antarctic waters, which have a much higher concentration of dissolved oxygen than elsewhere, and with the selective evolutionary pressure relaxing oxygen transport, red-blooded notothenioids have evolved a decrease in Hb oxygen affinity (Verde et al., 2006).

The blood of the 16 “icefish” species of Channichthyidae (Eastman, 1993), the most phylogenetically derived family, lacks Hb (Ruud, 1954). The increased solubility of oxygen in water allows icefish to transport sufficient oxygen in physical solution rather than by a specific oxygen carrier. Icefish retain genomic DNA sequences closely related to the adult α -globin gene(s) of its red-blooded notothenioid relatives, whereas its ancestral β -globin sequences have been deleted (Cocca et al., 1995; Zhao et al., 1998; di Prisco et al., 2002). The discovery within the icefish family of two distinct genomic rearrangement, both leading to the functional

inactivation of the locus, seems to point towards a multi-step mutational process (Near et al., 2006). These fish cope with the lack of an oxygen carrier with increased blood volume and higher cardiac output (Egginton et al., 2002); they have large gills, highly vascularised, scaleless skin, which favours cutaneous respiration, and enlarged heart with specialised volume pump performance. This heart is unable to cope with either increasing temperature fluctuations or cardiac wall stresses associated with higher systemic pressure demands. This vulnerability highlights the costs of cardiac cold adaptation in the icefish (Egginton et al., 2002; Johnston et al., 2003).

The loss of Hb in icefish is paralleled by the loss of myoglobin (Mb) in several icefish species through at least 4 mutational events (Sidell et al., 1997; Grove et al., 2004; Sidell and O'Brien, 2006). Mb has also been lost in many notothenioids, at least in certain tissues. No notothenioid has Mb in its skeletal locomotory muscle (Sidell et al., 1997). Recently, Hendgen-Cotta et al. (2008) have showed in Mb-knockout mice that Mb produces and scavenges nitrogen monoxide (NO) under deoxygenated and oxygenated conditions, respectively. When it acts as a reductase (Hendgen-Cotta et al., 2008), deoxygenated Mb generates NO from circulating nitrite in cardiac muscle cells under hypoxic stress, where it suppresses the production of ROS in mitochondria, protecting the muscle cells from damage. Excess NO is reconverted to nitrate by oxy Mb acting as a dioxygenase. The hearts of Mb-knockout mice do not recover from experimentally imposed ischemia; these mice show no evidence of nitrite-induced reduction in the damage to heart tissue caused by blood-vessel blockage (Cossins and Berenbrink, 2008). These observations may help to better understand icefish physiology and the compensatory adaptations evolved in the cardiovascular system of these natural knockouts.

While there is no doubt about the adaptive value of AFGPs, Hb/Mb loss in icefish was suggested not to be selectively neutral, but rather maladaptive, as indicated by the development of compensatory adaptations that enhance oxygen delivery, such as cutaneous uptake of oxygen and decreases in metabolic oxygen demand (Sidell and O'Brien, 2006; Cheng and Detrich, 2007).

Selective processes have provided the fish heart with a powerful repertoire of either phenotypic (acclimatory) adjustments (Driedzic et al., 1996; Gamperl and Farrell, 2004) and/or evolutionary (genetic) adaptation (Pelster, 2003), so that cardio-specific response patterns can contribute to the stress response at the organism level. On the basis of these considerations, Garofalo et al. (2009) hypothesised that the icefish cardiac enlargement, rather than being an innovation, can be viewed as

a typical example of phenotypic plasticity of the fish heart, that is a morpho-functional refinement of the basic notothenioid heart design. The icefish cardiomyocytes proliferate in response to the increased hemodynamic overload and mitochondrial remodelling; the increase of the myocardial mass does not modify the basic histological design of the heart (Garofalo et al., 2009).

Recent studies highlight how the loss of Hb and Mb, their associated NO-oxygenase activity and subsequent elevation of NO levels (two fold those observed in red-blooded notothenioids) could explain the unique cardiovascular and physiological traits that have evolved in icefish (Sidell and O'Brien, 2006). Since Hb and Mb are key proteins in NO homeostasis (Barouch et al., 2002), the icefish, as natural knockouts for Hb/Mb, represent a unique example to investigate whether these disruptive losses may have evolved cardiac modifications and susceptibility to NO taking into account the recent evidence provided by experimentally produced Mb-knockout mice (Hendgen-Cotta et al., 2008).

The recent discovery of the neuroglobin (Ngb) gene in the brain of red-blooded notothenioids and in at least 13 of the 16 icefish species (Fig. 1) suggests a crucial biological function of Ngb (Cheng et al., 2009a,b; Verde et al., 2009). The finding that icefish retain the Ngb gene despite having lost Hb, and Mb in most species, may potentially have important implications in the physiology and pathology of the brain. Ngb, a monomeric heme-containing globin, expressed in retinal neurons and fibroblast-like cells, is able to bind oxygen and NO (Burmester et al., 2000; Dewilde et al., 2001; Van Doorslaer et al., 2003; Pesce et al., 2003; Vallone et al., 2004;). Although a number of hypotheses [signal transduction (Wakasugi et al., 2003),

involvement in Alzheimer's disease (Khan et al., 2007), protection of neurons against the deleterious effects of hypoxia and ischemia (Sun et al., 2001, 2003)] have been put forward, its functional role is still uncertain (Hankeln et al., 2005). There is no doubt that Ngb has an essential, conserved function and is beneficial to neurons (Burmester and Hankeln, 2009) and supplies oxygen to the retina, similar to Mb in the myocardium and skeletal muscle (Schmidt et al., 2003). One of the most important physiological function attributed to Ngb is the ability to neutralise the neurotoxic effects of ROS (Brunori et al., 2005) and reactive nitrogen species (Herold et al., 2004; Wang et al., 2008). In an extreme oxygen-rich environment, this neuroprotective role has potential implications in our understanding of the function of this protein and suggests future avenues of investigation.

Within tetrameric Hbs of red-blooded notothenioids, there is another aspect in which Antarctic fish Hbs are exceptional. Although structurally and functionally analogous to mammalian Hbs, Antarctic fish Hbs follow a peculiar oxidation pathway when exposed to air or treated with chemical agents. The α and β chains of these proteins undergo distinct oxidation processes. Particularly unusual is the strong tendency of the β chains to form hexacoordinated bis-histidyl adducts in the ferric state (hemichrome) (Ricchio et al., 2002; Vitagliano et al., 2004, 2008; Vergara et al., 2007, 2008). In the absence of exogenous ligands, Ngb (Pesce et al., 2004), similar to Antarctic fish Hbs, displays hexacoordination with distal His E7 coordinating directly with the heme iron, either in ferrous or ferric forms. The occurrence of endogenous coordination with His as internal ligand is probably due to the need to protect the active site from peroxide stress (Herold et al., 2004). Considering that Antarctic fish Hbs have high peroxidase activity, the exchange between hemichrome and pentacoordinated forms may play a distinctive physiological role, still to be elucidated, in Antarctic teleosts (Vergara et al., 2009).

The peculiar stenothermal phenotype of Notothenioidei reflects evolutionary adaptations to the environment for optimal function under stable conditions. The discovery that Antarctic notothenioids have lost genetic information, possibly essential for life in warmer waters, suggests that Antarctic stenothermal organisms may be vulnerable to global warming. As pointed out by Pörtner et al. (2007), the shift from Hb-mediated oxygen transport and delivery to mechanisms based on diffusion may cause higher vulnerability of icefish to warmer temperatures. This feature would be deleterious, if not lethal, in warmer waters.

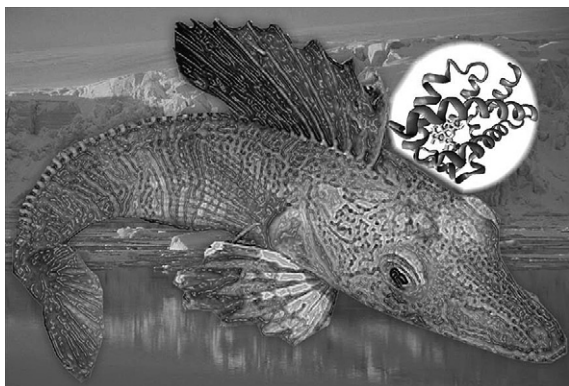


Fig. 1. Cartoon showing the icefish (*Pagetopsis macropterus*) and the presence of Ngb in its brain. The cartoon reports the structure of mouse Ngb (PDB ID: 1Q1F), the only 3D structure available in Protein Data Bank (PDB), together with human Ngb.

In the light of the ongoing climate change, will Antarctic fish tolerate environmental warming? Their thermal specialisation poses Antarctic stenothermal organisms at considerable future risk that may result in the loss of critical biological functions (Peck et al., 2004).

3. Molecular adaptation in Antarctic bacteria: *P. haloplanktis* TAC125

Our knowledge of the diversity of Antarctic marine organisms is still in its infancy. Only an adequate understanding of microbial diversity and genome linked capabilities in polar environments will enable us to reach an ample overview of polar-ecosystem structure and function as well as to establish the effects of climate change.

Genomics is a high-profile science that impacts on all areas of biology, and it is not surprising that it is now playing an increasing role in Antarctic research. Genomic research provides useful tools for studying natural selection in action and investigating the link between organisms and environment by environmental genomics.

Numerous microorganisms have successfully colonised low-temperature habitats. The knowledge of polar marine microorganisms from ecological and genomic perspectives is in the early phase of an exponential growth. Some bacterial polar microbial genomes are already present in GenBank, accompanied by publications: the Euryarchaeota *Methanogenium frigidum* and *Methanococcoides burtonii* (Saunders et al., 2003), the γ -proteobacterium *Colwellia psychrerythraea* 34H (Méthé et al., 2005) and *P. haloplanktis* TAC125 (Médigue et al., 2005) and the δ -proteobacterium *Desulfotalea psychrophila* (Rabus et al., 2004).

Recently, the genome of the *Exiguobacterium sibiricum* strain isolated from 3 million year old permafrost was sequenced and annotated (Rodrigues et al., 2008). The authors showed that *E. sibiricum* is constitutively adapted to cold with differential gene expression between 4 °C and 28 °C (Rodrigues et al., 2008).

Evolution has allowed these cold-adapted organisms not to simply survive, but to grow successfully in the extreme conditions of cold habitats, through a variety of structural and physiological adjustments in their genomes. These strategies include synthesis of unique factors, such as cold-shock proteins (Cavicchioli et al., 2000), molecular chaperones (Motohashi et al., 1999; Watanabe and Yoshida, 2004), compatible solutes (Carpenter and Crowe, 1988; Pegg, 2007) and structural modifications leading to the maintenance of membrane fluidity (Russell, 1998; Chintalapati et al., 2004). In addition to adaptations at the cellular level, a key

adaptive strategy is the modification of enzyme kinetics, allowing the maintenance of sufficient reaction rates at thermal extremes. Enzyme catalysis is based on increased flexibility in certain regions of cold-active enzyme architecture and high activity with a concomitant increase in thermolability (Georlette et al., 2004). However, the adaptations to protein architecture essential to cold-active enzymes are still not well understood, and this study is an active area of investigation (Marx et al., 2004). Nevertheless, the biochemical properties of cold-active enzymes make them attractive for exploitation in biochemical, bioremediation, and industrial processes (Feller and Gerday, 2003).

Among cold-adapted bacteria, the genus *Colwellia* (Deming and Eicken, 2005), within γ -proteobacteria, provides an unusual case, i.e. all characterised members are strictly psychrophilic (requiring temperatures of -20 °C to grow on solid media) living in stably cold marine environments, including deep sea and Arctic and Antarctic sea ice (Deming and Junge, 2005). Many members of this genus produce extracellular polymeric substances relevant to biofilm formation and cryoprotection (Krembs et al., 2002) and enzymes capable of degrading high-molecular-weight organic compounds. The genome sequence of *C. psychrerythraea*, an obligately psychrophilic Arctic bacterium, has provided an important opportunity to better understand its potential functions in the marine environment and to gain insight into adaptation (Méthé et al., 2005). Genome analyses reveal a variety of metabolic capabilities and roles in carbon and nutrient cycling, including some that may be useful to bioremediation in cold environments.

Cold-adapted bacteria have developed programmed responses to strong oxidative stress. *C. psychrerythraea* (Méthé et al., 2005) seems to have faced high oxygen concentration by developing an enhanced antioxidant capacity owing to the presence of several genes that encode catalases and superoxide dismutases. In contrast, the genome sequence of *P. haloplanktis* TAC125 reveals that the bacterium copes with increased oxygen solubility by enhancing production of oxygen-scavenging enzymes and deleting entire metabolic pathways, such as those which generate ROS as side products. The remarkable deletion of the ubiquitous molybdopterin-dependent metabolism in the *P. haloplanktis* TAC125 genome (Médigue et al., 2005) and the number of proteins involved in scavenging chemical groups can be seen in this perspective. Dioxygen-consuming lipid desaturases achieve both protection against oxygen and synthesis of lipids, making the membrane fluid. These characteristics

make this bacterium not only a model for the study of adaptation to cold marine conditions but also an attractive tool for biotechnology production of proteins (Médigue et al., 2005).

A further sign, which may be related to the peculiar features of the Antarctic habitat, may be the synthesis of bacterial Hbs and flavoHbs, surprisingly versatile proteins serving several biological functions. These molecules are bound to fulfil an important physiological role, including protection of the cell from nitrosative and oxidative stress and delivering oxygen to respiring cells. Multiple genes encoding 2-on-2 (2/2) Hbs and one for flavoHb have been discovered in the genome of *P. haloplanktis* TAC125 (Médigue et al., 2005; Giordano et al., 2007), suggesting that specific and different functions may be associated to these two classes of proteins (Giordano et al., 2007). 2/2Hbs are small oxygen-binding hemoproteins, characterised by a structural 2/2 fold (Fig. 2) different from the typical 3-on-3 motif found in classical Hbs (Pesce et al., 2000). 2/2Hbs are able to bind oxygen, CO and NO, albeit with different affinity. The high affinity for oxygen suggests that 2/2Hbs function as oxygen scavengers rather than oxygen transporters (Wittenberg et al., 2002; Ouellet et al., 2003). Similar to Antarctic fish Hbs (Ricchio et al., 2002; Vitagliano et al., 2004, 2008; Vergara et al., 2007, 2008) and Ngb (Pesce et al., 2004), a *P. haloplanktis* TAC125 recombinant 2/2Hb (Giordano et al., 2007) shows a predominance of

a hexacoordinated species in the ferric and ferrous forms (Verde et al., 2009). The physiological role of hexacoordinated Hbs is not well understood, even in these monomeric Hbs and several roles have been suggested. The finding of hexacoordination in monomeric and tetrameric cold-adapted Hbs suggests a common physiological mechanism for protecting cells against oxidative chemistry in response to high oxygen concentration. Other roles have been hypothesised: oxygen scavenger under hypoxic conditions (Burmester et al., 2000, 2002), terminal oxidases (Sowa et al., 1999), oxygen-sensor proteins (Hargrove et al., 2000; Kriegl et al., 2002), proteins involved in NO metabolism (Smaghe et al., 2008). About the latter function, bacterial cells have developed mechanisms for NO detoxification, because of cytotoxic effects of NO (Poole, 2005; Poole and Hughes, 2000). The homeostasis of NO is achieved through a balance between its production and consumption. At high concentrations, NO is not a messenger but a toxic molecule. Its behaviour in biological systems is complicated by its ability to react with oxygen and ROS leading to the production of RNS (Poole and Hughes, 2000). In a rich-oxygen environment and cold stress more than one defence mechanism for NO detoxification may be important. In the genome of *P. haloplanktis* TAC125, besides the hexacoordinated 2/2Hb gene, there is also a gene encoding a flavoHb, a protein with two domains, the heme-containing oxygen-binding domain, and a FAD-containing reductase domain, involved in detoxification of NO (Poole et al., 1996).

The presence of 2/2Hb and flavoHb genes in the cold-adapted *P. haloplanktis* TAC125 raises questions on the structural and functional features evolved by proteins (in comparison with their mesophilic counterparts) in response to thermal adaptation and to the ability to cope with extreme environments.

4. Conclusions

The evolutionary and geographical history of the Antarctic has produced a unique environment, rich in species adapted to extreme conditions. Given the evolutionary history and current environmental setting, the Antarctic biota provides many opportunities to address fundamental biological problems, linking genome to survival of organisms and the functioning of ecosystems.

The application of structural and functional genomic approaches to the examination of Antarctic organisms is growing and the future is promising. The coming years will see an increase in the production of Antarctic genomic sequence data, which provide

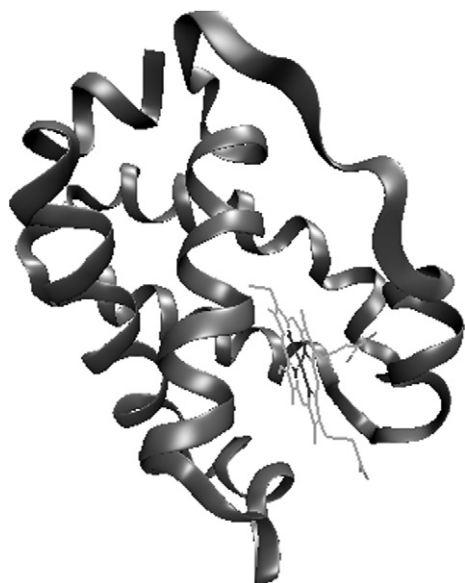


Fig. 2. X-ray structure of *Paramoecium caudatum* 2/2Hb, the first 3D structure of a 2/2Hb (PDB ID: 1UVY), that shows the typical 2/2 folding.

information across the whole range of biological levels from DNA to ecosystem, linking genomics to physiology, microbiology and ecology.

Icefishes are a suitable model to enhance the knowledge on the function of globins in the brain, and especially about their role in species devoid of Hb and Mb. In particular, modern Notothenioides appear to be the result of an extraordinary natural experiment, as they possess the exceptional physiological features (both adaptive and non-adaptive) engineered by organisms that live at permanently cold temperatures.

The Antarctic bacterium *P. haloplanktis* TAC125, although other genomes from psychrophilic bacteria have been sequenced, is considered as one of the best model organisms for the structural/functional genome analyses, since over the last years a wide array of genetic tools and controlled cultivation strategies have been developed.

Climate change will differentially favour species with wide thermal windows, short generation times, and a range of genotypes among populations (Pörtner and Farrel, 2008). Stenothermal marine species appear particularly vulnerable to even small increases in temperature (Clarke et al., 2007; Peck et al., 2009). Therefore, it is essential to understand the complexities of their response to changing environmental temperatures, not only at the individual species level, but also as implications for the whole ecosystem. It will be important to ascertain which species have phenotypic-plasticity capacity, and then focus the attention on those organisms that are most likely to be negatively affected by climate change. The development of biomarkers for environmental stress, e.g. heat-shock proteins, frequently proposed for their highly conserved genes, may provide tools for understanding why some species are more resistant or more temperature tolerant than others and for enabling us to assess organism stress in the ecosystem context (Tomanek and Sanford, 2003; Hamer et al., 2004; Clarke and Peck, 2009).

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