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Letter to the Editor

Cold-adapted Antarctic fish: The discovery of neuroglobin in the dominant suborder Notothenioidei

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ABSTRACT

Novel globins, such as neuroglobin (Ngb) and cytoglobin, have recently been discovered in many vertebrates. Ngb is mainly expressed in neurons and plays a neuroprotective role during hypoxic stress. Neuronal hypoxia and cerebral ischemia induce Ngb expression; knocking down Ngb expression increases hypoxic neuronal injury *in vitro* and ischemic cerebral injury *in vivo*. Although Ngb was originally identified in mammals, it is also present in fish, including the zebrafish *Danio rerio*. We have discovered the *Ngb* gene to be present in red-blooded notothenioid fish species, and in at least 13 of the 16 species of the white-blooded icefish family Channichthyidae. The deduced amino-acid sequences are well conserved. The retention of the Ngb gene by channichthyids, despite the loss of hemoglobin and myoglobin, appears very intriguing.

The variety of adaptations underlying the ability of modern high-Antarctic notothenioid fish to survive at freezing temperatures represents the extreme of low-temperature adaptations found among vertebrates (di Prisco et al., 2007).

Notothenioidei, the dominant suborder in the Antarctic ocean, have evolved reduction of hemoglobin (Hb) concentration and multiplicity, perhaps as a consequence of temperature stability and other environmental parameters (di Prisco et al., 2007). These adaptations may be advantageous in coping with increased viscosity of body fluids at low temperature. In the Channichthyidae (icefish) family, the blood pigment is absent (Ruud, 1954).

Because oxygen solubility in seawater is inversely proportional to temperature, the cold Antarctic seas are an exceptionally oxygen-rich habitat. Therefore, oxygen uptake and transport are not limiting steps for Antarctic fish. As water temperatures decreased to freezing and ice appeared in the water column, the ancestral Antarctic notothenioid acquired a key evolutionary innovation — antifreeze glycoproteins (AFGPs), a physiological adaptation that allows them to survive and diversify in ice-laden seawater that reaches temperatures of nearly −2 °C (Chen et al., 1997).

Evolutionary gain of new genes, changes in expression pattern changes, and loss of genetic information, especially for Hb and myoglobin (Mb) in Channichthyidae, reflect the specialisation of Antarctic organisms to a narrow range of low temperatures.

The loss of Mb and Hb in icefish, together with enhanced lipid membrane densities, becomes explicable by the exploitation of high oxygen solubility and low metabolic rates in the cold, where an enhanced fraction of oxygen supply occurs through diffusive oxygen flux.

Icefish developed compensatory adaptations that reduce tissue oxygen demand and enhance oxygen transport. Hemoprotein loss is paralleled by dramatic increases in blood volume and capillary diameters. The homeostatic activity of nitrogen monoxide probably facilitated the evolution of these traits (Cheng and Detrich, 2007). Oxygen delivery to tissues occurs by transport of the gas physically dissolved in the plasma. However, the development of these compensatory physiological and circulatory adaptations in icefishes argues that loss of Hb and erythrocytes was probably maladaptive under conditions of physiological stress. Recent studies highlight how the loss of Hb and Mb and, in particular, of their associated nitrogen-monoxide oxygenase activity, may have favoured the evolution of these compensations in icefishes (Sidell and O'Brien, 2006). Icefish genomes retain a small, inactive portion of the adult *α-globin* gene; the other part and the whole adult *β-globin* gene have been deleted prior to diversification of the extant species within the clade (di Prisco et al., 2002). This was supposed to be a feature of all icefishes, but, recently, a survey of the 16 icefish species indicated that the genome of a phylogenetically derived species, *Neopagetopsis ionah*, has a complete, but non-functional, adult αβ-globin complex, more similar to the ancestral condition of a globin-expressing species of the sister group Bathydraconidae than to Channichthyidae (Near et al., 2006).Burmester et al. (2000, 2002) have recently described

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novel globins, such as neuroglobin (Ngb) and cytoglobin, in many vertebrates. Ngb is mainly expressed in neurons and plays a neuroprotective role during hypoxic stress. Evidence includes the observations that neuronal hypoxia and cerebral ischemia induce Ngb expression and that knocking down Ngb expression increases hypoxic neuronal injury *in vitro* and ischemic cerebral injury *in vivo* (Greenberg et al., 2008). However, enhanced expression of Ngb does not seem to be a universal response to all forms of neuronal injury, because in some instances the same response is not observed (Greenberg et al., 2008).

Other suggested roles include reactive oxygen species (ROS) scavenging, signal transduction (Wakasugi et al., 2003) and regulation of apoptotic pathways (Khan et al., 2007). However, the physiological function of Ngb is still unknown. The work of Kahn et al. (2007) demonstrated that overexpression of Ngb produces a beneficial effect in the recovery after experimentally induced strokes, also suggesting a role of this protein in the Alzheimer's disease.

Ngb displays the classical vertebrate folding 3/3 (Pesce et al., 2003; Vallone et al., 2004), and binds oxygen and other ligands.

Although Ngb was originally identified in mammalian species, it is also present in fish including the zebrafish *Danio rerio* (Awenius et al., 2001). Mammalian and fish Ngb proteins share about 50% amino-acid sequence identity. Recently, Watanabe and Wakasugi (2008) suggest that zebrafish Ngb is a cell-membrane penetrating globin.

We have recently discovered that the *Ngb* gene is present in the DNA of red-blooded notothenioid species and in at least 13 of the 16 channichthyid species. The deduced amino acid sequences of *Ngb* gene cloned from three red-blooded species (*Bovichtus variegatus, Dissostichus mawsoni*, and *Gymnodraco acuticeps*) and two icefishes (*Chionodraco myersi*, and *Neopagetopsis ionah*) are well conserved. A nearly full-length α-globin cDNA was also obtained from brain RNA of *D. mawsoni*. This α globin shares high sequence identity with known major Hbs of notothenioids. These findings will soon be published in full details.

The retention of the *Ngb* gene by icefish despite the lack of Hb, and the loss of Mb in most species, is a very intriguing finding. Whether the *Ngb* gene is expressed is the next important question. Also, whether the α-globin mRNA in the brain is from nervous tissue or from circulating blood needs to be definitely verified. If both are expressed in the brain, the functions of these single globins (Ngb, α-globin) need to be ascertained in future studies, because these proteins may have important implications in the physiology and pathology of the brain. To our knowledge, the expression of a single globin gene in non-erythroid cells has been reported in two cases, i.e. in activated macrophages from adult mice and lens cells (Liu et al., 1999), and in alveolar epithelial cells (Newton et al., 2006), thus neural expression remains a possibility.

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