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DeVries: the Art of not freezing fish

Lloyd Peck discusses the impact of two classic *Science* papers, the first by Art DeVries and Donald Wohlshlag, published in 1969 and the second by DeVries alone published in 1971 on freeze tolerance in Antarctic fish.

Art DeVries transformed our understanding of low-temperature biology with two publications in 1969 and 1971. The first paper was with Donald Wohlshlag, an icon of low-temperature biology of that era. They showed that the freezing point of Antarctic fishes and their blood was depressed well below what it was expected to be based on their salt contents (DeVries and Wohlshlag, 1969). In the second paper (DeVries, 1971), Art demonstrated that the mechanism for achieving this was through the production of glycoprotein antifreezes.

In the early 1960s, marine biology was changing because of the spread of scuba diving, which was also catching on in Antarctica, and it was there, as a Research Assistant in 1962, that DeVries carried out the first winter study of fish biology. He then began a long series of summer visits to McMurdo Station where he turned out to be the right man in the right place at the right time. Although depth ranges of fish had been identified and related to characteristics such as metabolic rate, he made some of the first underwater observations of Antarctic fish living in intimate contact with ice, and demonstrated that three species in particular, *Trematomus borchgrevinki* (now *Pagothenia borchgrevinki*), *T. bernacchii* and *T. hansoni*, even use crevices and holes in ice formations as refuges from seal predators. In contrast, some of the deeper water fish such as liparids and zoarcids froze when brought to the surface and came into contact with ice crystals. Earlier studies had shown that some Arctic fish, like the liparids and zoarcids were supercooled and lived only in deep waters where there could be no contact with ice crystals to initiate freezing (Scholander et al., 1957). Research into freezing resistance at this time was, however, dominated by a search for dissolved small solutes, then the only recognised physical mechanism that would lower the freezing point to avoid freezing.

In his first iconic paper, DeVries used a Fisk osmometer to measure blood serum freezing points and showed they were around –2.0°C, which is slightly below the freezing point of seawater (–1.9°C) and well over 1°C below the freezing point of blood serum from non-polar fish (DeVries and Wohlshlag, 1969). This paper also reported the concentrations of sodium chloride and other small solutes in the blood, and showed that they accounted for only half of the observed freezing point depression, whereas a glycoprotein isolated from the serum by chromatography following heat precipitation and centrifugation accounted for another 30% of the freezing point depression. Later work showed that Antarctic notothenioids exhibit a freezing point depression of their blood serum over twice that of fish from warmer waters (Fig. 1). Slightly less than half of the freezing point depression in Antarctic notothenioids is due to elevated NaCl levels and other low molecular weight solutes (osmotic concentration of 550–625 mOsm kg^{-1} compared with 320–380 mOsm kg^{-1} for temperate and tropical fish), lowering the freezing point to about –1.1°C. The remainder of the freezing point depression to –2°C or below is due to the action of antifreeze glycoproteins (Fig. 1).

The second major paper (DeVries, 1971) used the technique of watching very small ice crystals melt and grow in capillary tubes with very fine temperature variations, with and without the presence of the antifreeze glycoprotein. These trials showed that the action of the antifreeze glycoprotein produced a large separation between the melting point of the ice

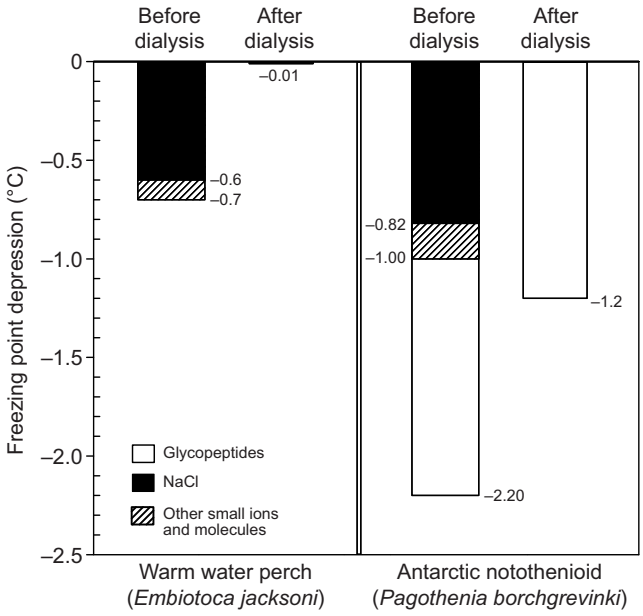

Fig. 1. The effect of NaCl, other small ions and molecules, and glycopeptides on the freezing point of blood plasma from a warm-water fish and an Antarctic notothenioid. The higher salt content of the plasma of *P. borchgrevinki* gives a larger freezing point depression than in the warm water *E. jacksoni*, but over half the freezing point depression in the Antarctic species is due to glycopeptides. Based on Eastman and DeVries, 1986.

Classics is an occasional column, featuring historic publications from the literature. These articles, written by modern experts in the field, discuss each classic paper's impact on the field of biology and their own work.

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crystal and the freezing point, or the temperature at which ice grew rapidly, referred to as a thermal hysteresis. By inactivating the antifreeze glycoprotein using borate, DeVries showed that the likely active site on the molecule was associated with the *cis*-hydroxyl groups of carbons 3 and 4 of the galactose residues. In this paper, he also suggested that the mechanism for halting ice crystal growth was by adsorption of the glycoprotein onto the crystal face growth sites, to prevent the attachment of water molecules and inhibit crystal growth. This is now the accepted explanation for antifreeze action, as opposed to the previous leading hypothesis that antifreeze glycoproteins acted by structuring water.

These two papers and a series of accompanying works (e.g. DeVries et al., 1970) effectively started a new field of research into biological antifreezes that now includes labs in the USA and other countries, including Canada, Japan, China, Israel, Germany and Norway. Hundreds, possibly thousands, of papers have been published on this topic. We now know that the many size isoforms of antifreeze glycoproteins in Antarctic notothenioid fishes are encoded in large families of polyprotein genes (Chen et al., 1997) and different antifreeze proteins are found in teleost fishes in the Antarctic and Arctic (DeVries and Cheng, 2005). These large gene families arose by gene duplication, enabling the synthesis of large quantities of protective antifreeze glycoproteins and antifreeze proteins. All of them work the same way as DeVries had proposed initially in 1971 and later confirmed with James Raymond in 1977 (Raymond and DeVries, 1977), in what is now called the adsorption–inhibition mechanism. Since then, antifreeze glycoproteins have become widely used in industry in a broad range of applications, including controlling the texture and fluidity of low-temperature compounds in products such as ice-cream.

The dramatic advance in molecular and genomic technologies over the past two decades has had a marked effect on this field. Antifreeze-like ice-binding proteins have now been isolated from a wide range of taxa, including bacteria, diatoms and copepods, and in the latter it is thought that the genes encoding antifreeze-like ice-binding proteins may have been acquired by horizontal gene transfer (Kiko, 2010). Genomic advances have also allowed the identification of multiple gene copies for antifreeze glycoproteins and antifreeze proteins; in the Antarctic toothfish, 14 antifreeze glycoprotein polyprotein genes have been mapped, each encoding multiple antifreeze glycoprotein molecules (as many as 30–40), underscoring the evolutionary importance of this adaptation (Nicodemus-Johnson et al., 2011). The antifreeze glycoproteins of Antarctic fish evolved from a pancreatic trypsinogen gene (Chen et al., 1997) and this is recognised as a key evolutionary adaptation conferring a dramatic survival advantage in notothenioid fish that other taxa were unable to match in the Southern Ocean.

Recently, it has been demonstrated that not only do antifreeze proteins and antifreeze glycoproteins inhibit the growth of ice crystals, but they also inhibit the melting of the ice crystals that can persist in fish tissues in the laboratory for at least 24 h at 1°C above the equilibrium freezing/melting point, and for several days at slightly lower temperatures (Cziko et al., 2014). During summer warming episodes at high latitude in McMurdo Sound, water temperatures and thus Antarctic fish body temperatures occasionally rise above the equilibrium melting point, but antifreeze-bound and stabilised internal ice crystals fail to melt, and thus superheated ice occurs naturally inside fish in field conditions. There has been debate over the past decade or more as to how ice crystals are removed from tissues. One hypothesis was that at warm times the ice melts. It now seems that this may not easily happen, at least in the perennially frigid high-latitude waters. Several questions now arise, including: do notothenioid fishes experience adverse physiological consequences, e.g. inflammatory responses, or blocking of blood vessels, resulting from the retention of ice that does not melt within their bodies? There is evidence that the spleen is involved in ice sequestering because of the preponderance of ice crystals found there (Praebel et al., 2009; Evans et al., 2010). It has also been shown that the larvae of some Antarctic notothenioids lack sufficient antifreeze glycoproteins, but are still resistant to freezing, and the mechanism is thought to reside with an unblemished integument that effectively resists the inward propagation of ice crystals (Cziko et al., 2005). So, although Art Devries has had over five decades of leading research into how cold polar fish survive in temperatures where they really should freeze, there are still many sizeable questions for him to grapple with.

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