Cell biology in the Antarctic: studying life in the freezer

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Many classical biologists working in Antarctica have 'gone molecular' to study the physiological basis for life on or below the ice. Investigating the remarkable adaptations of specific cells, organelles and molecules to this extreme environment can provide new perspectives on the processes studied in conventional experimental organisms.

Biological research in the Antarctic focuses on survival mechanisms in extreme conditions. Low temperature slows all physiological processes, changes protein–protein interactions, reduces membrane fluidity and increases the viscosity of water. Ice formation can rapidly destroy cell membranes and disrupts osmotic balance. The examination of how cells and organisms respond to such challenges requires a wide range of sophisticated techniques. The development of suitable facilities in the Antarctic has contributed to an explosion of research at molecular and cellular levels. For example, the US Antarctic Program maintains three stations, Palmer on the Antarctic peninsula, the Amundsen-Scott South Pole station and McMurdo on Ross Island. The McMurdo station (Fig. 1; http://www.nsf.gov/od/opp/antarct/usap.htm; also see British Antarctic Survey, http://www.antarctica.ac.uk) is by far the largest of the three, with a summer population of 1,200. The Crary Laboratory at McMurdo (Fig. 1) is well equipped and allows scientists to investigate the same topics as they would in their home labs, but on the uniquely adapted Antarctic organisms. This approach may help identify limiting steps in complex cellular processes, such as protein secretion or neuronal function.

Figure 1 **McMurdo Station on Hut Point, Ross Island. In the summer, the base runs around the clock with a busy heliport (front left) and an ice-pier (rear centre) that becomes the focus of activity when icebreakers and resupply ships make their essential visits. The main science facility is the Crary Lab (three-tier building in the centre). The base has a hospital (red roof), fire station and central galley (large beige building, centre-right), as well as dormitories (ranks of brown buildings in the back) and numerous other maintenance and support buildings.**

Protein secretion and membrane fluidity

Protein secretion is an essential process that is extremely cold-sensitive[1]. The majority of life on earth, however, exists well below the temperatures at which model organisms, such as tissue culture cells and yeast[2,3], are usually grown. But how does protein secretion work in the cold? We found that at low temperatures, the first step of protein secretion — translocation into the endoplasmic reticulum — was more efficient in cold-adapted organisms when compared with temperate species. We identified a limited number of amino acid changes in conserved positions of the translocation channel-forming protein that may constitute cold-adaptation (Römisch *et al.*, unpublished observations). The channel-opening mechanism for protein translocation is not understood, but our sequence data suggest that similar to cold-adapted enzymes, the amino acid substitutions in the channel protein designate hinge regions that allow a conformational change essential for protein translocation across the endoplasmic reticulum membrane[2,4].

Membranes from organisms that exist in cold habitats contain a high proportion of unsaturated fatty acids, which can keep membranes fluid at low temperatures; however, this correlation is limited[5]. For example, in endoplasmic reticulum membranes from an Antarctic fish, we found a high concentration of unsaturated fatty acids, but no concomitant increase in membrane fluidity (Römisch *et al.*, unpublished observations). Our data suggest that membrane rigidity is not a limiting factor for protein translocation across the endoplasmic reticulum membrane in the cold.

Antifreeze

Fish from the polar regions thrive at temperatures close to freezing point (the minimum water temperature at McMurdo is −1.9 °C; freezing occurs at −1.91 °C). To avoid death by freezing, fish in these environments have evolved antifreeze molecules that they secrete into their blood at high concentrations (30–60 mg ml^{-1}; refs 5, 6). Four classes of antifreeze proteins have been characterized: types I and IV are α-helical, whereas types II and III are small globular proteins[7]. In addition, a fifth group, antifreeze glycopeptides, is common in Antarctic fish. These glycopeptides have relative molecular masses (M_r) ranging from 2,400–34,000 (2.4–34K) and a highly repetitive α-helical structure, $(Ala\text{-}Ala\text{-}Thr)_n$, which arose from multiplication of an exon–intron boundary in trypsinogen[8]. The poly-protein precursor has a cleavable

signal peptide and its threonine residues are heavily *O*-glycosylated. The glycoprotein is proteolytically processed into characteristically sized antifreeze glycopeptides before or after secretion. Arctic fish secrete similar glycopeptides into their blood, but these are derived from a different gene — a classic example of convergent evolution[9].

Biological antifreezes adsorb to the surfaces of ice crystals in the blood of these fish and prevent the association of additional water molecules, and thus growth of the ice crystals[6,7,10]. For example, Type III antifreeze has a flat, amphipathic ice-binding surface with polar residues arranged such that they can hydrogen-bond to oxygen on a specific prism plane of the ice crystal[11,12]. Subsequently, Van der Waal's interactions between other residues in the ice-binding surface and the ice itself strengthen to exclude water near the ice surface[11,12]. Recent work suggests the key feature of all protein antifreezes is complementarity between the ice-binding protein surfaces and the ice crystals[13]. Antifreeze molecules can also adsorb to biological membranes and protect them by maintaining lipid bilayer structure during cooling through their phase transition temperatures[14,15].

Heat shock

At high temperatures, cells increase the expression of heat shock proteins (Hsp), specific molecular chaperones that prevent the aggregation of thermally unfolded proteins. What constitutes heat stress depends on the habitat temperature of the organism and the maximal temperature variation. Marine organisms in McMurdo Sound are adapted to survive extremely low temperatures (minimum −1.9 °C) and a narrow temperature range; even in the austral summer, the water temperature rises maximally by 1.5 °C[16]. As a result of adapting to this environment, Antarctic marine invertebrates induce heat shock proteins at 0 °C (National Science Foundation Marine Biology Course, 2000). An Antarctic teleost fish, *Trematomus bernacchii*, has lost its heat shock response altogether. *T. bernacchii* (Fig. 2, left) fails to increase expression of heat shock proteins in response to either thermal or chemical stress[17]. This may be a result of living in a thermal environment that has been extremely stable for the past 14–25 million years[17]. The Hsp70 family, in particular, may be dispensable, because they interact primarily with hydrophobic patches of unfolded proteins[18]. Hydrophobic interactions are relatively weak at low temperatures, so the Hsp70 family will probably not be functional in the cold, nor will hydrophobic patches be important for aggregation of misfolded proteins[2]. *T. bernacchii* die at temperatures of 5–6 °C[19]. The upper lethal temperature is not determined by thermal unfolding of proteins, but most probably by oxygen availability, as discussed below.

Oxygen uptake and delivery

The solubility of oxygen in water is inversely correlated to the water temperature, and the oxygen saturation in McMurdo Sound can reach 100%. Therefore, oxygen uptake and transport are not limiting for Antarctic fish. Erythrocyte counts of red-blooded Antarctic notothenioid fish are an order of magnitude lower than related temperate fish and can be reduced by three orders of magnitude in white-blooded Antarctic icefish[20]. This may be advantageous in coping with increased viscosity of body fluids at low temperature and is partially compensated for by increased blood volume and higher cardiac output[21,22]. The haemoglobin content of erythrocytes is variable in Antarctic fish and is positively correlated to swimming activity[20,23,24]. Haemoglobin can be completely absent in icefish, which can also lack the oxygen binding protein of muscle, myoglobin[25,26].

Reduced levels of oxygen-binding proteins in Antarctic icefish correlate with an increase in the percentage of cell volume containing mitochondria[27,28]. Furthermore, oxygen solubility is higher in membrane lipids than in aqueous solutions, and therefore mitochondrial membranes may act as conduits for oxygen and partially compensate for the lack of haemoglobin and myoglobin[24,28]. Antarctic budding yeast often contain giant mitochondria (Fig. 2, centre), which may support the hypothesis that intracellular membranes are critical for adequate oxygen delivery at low temperature (Römisch *et al.*, unpublished observations).

A further consequence of high oxygen availability in cold water is gigantism of invertebrates[29]. Increased oxygen content of the blood allows a longer circulatory system, and thus a larger body[30]. For example, the volume of the Antarctic giant isopod *Glyptonotus antarcticus* (Fig. 2, right), a relative of woodlice (about 250 µl volume), can excede 50 ml.

Neurobiological adaptations

Neuronal function is dependent on the ability of cell membranes to establish, maintain and rapidly change transmembrane electrical potential. Ion pumps generate electrochemical gradients across membranes, and the ability of neurons to generate trains of action potentials is influenced by the kinetics of channel opening and closing. Channel gating, which is dependent on conformational changes of proteins, is more strongly affected by changes in temperature than those of channel conductance, which depends primarily on diffusion. Communication between neurons at synapses depends on the rates of neurotransmitter release, diffusion and binding, and subsequent neurotransmitter recycling or breakdown.

Striking differences in neuronal function and network architecture between the Antarctic mollusc *Clione antarctica* and a related temperate species have been identified (J. Rosenthal, personal communication). The voltage dependence of sodium channel opening is shifted positively in *C. antarctica*, which raises the neuronal spike threshold and makes it more difficult for the neurons to carry signals (as sodium channels drive regenerative action potentials). However, calcium channels operate over their usual voltage range, and may therefore have an enhanced role in spike generation in the Antarctic species.

Our work includes studies of neuronal function in Antarctic marine crustacea (http://www.zoo.cam.ac.uk/zoostaff/matheson/antarc1.html). At all temperatures examined, the peripheral nerves of the Antarctic isopod *G. antarcticus* (Fig. 2, right) conduct more rapidly than those of a temperate species, but this is not related to differences in axon diameter or myelination. Examination of touch-sensitive mechanoreceptors in *G. antarcticus* demonstrate that sensory transduction is strongly affected by temperature. The number of action potentials elicited by a stimulus increases with temperature from 1.8–7 °C, but then decreases and fails at 21 °C.

In Antarctic fish, nerve conduction fails when freezing sets in (at around −5 °C); conversely, the velocity of propagation increases with temperature (up to 28 °C) in a linear fashion[31]. At low temperature, nerves from Antarctic fish conduct substantially faster and their neuromuscular junctions have higher rates of spontaneous neurotransmitter release (miniature endplate potentials) than those of temperate species, indicating that their presynaptic terminals are relatively closer to neurotransmitter release. This is probably a mechanism for reducing transmission failures at low temperature. However, individual endplate potentials decay more rapidly in the Antarctic fish, thus limiting the post-synaptic response amplitude at low temperatures. In contrast, the responses of vestibular (balance and orientation) neurons in the central nervous system of Antarctic fish are remarkably similar to those of temperate species.

Structural adaptations of enzymes

Life exists even in the most inhospitable regions of Antarctica, such as brine canals in sea ice and snow at the South Pole[32]. The organisms that live in these extreme environments have enzymes with either higher catalytic activity over a temperature range, or specifically at low temperature. The

increased thermolability accompanying these adaptations may be a consequence of increased protein flexibility conferred by amino acid changes that allow cold-adapted enzymes to undergo conformational changes required for activity at low temperature[2–4]. Alternatively, thermolability may be caused simply by a lack of selective pressure, as temperatures in cold habitats are often very stable[2,3,33]. Increased thermolability means that enzymes from psychrophiles (organisms that can only live in the cold) are often difficult to crystallize. Most structural data available are derived from cloning, sequencing, homology modelling strategies, and recently from nuclear magnetic resonance (NMR) studies. The changes that make enzymes work in the cold are subtle and often challenging to identify, as it can be difficult to discern what changes evolved under selective pressure, and which are functionally neutral[33]. For these reasons, directed evolution in the lab is now being used to understand what protein features are important for adaptation to specific parameters[33].

Protein flexibility, and hence enzymatic activity, is determined by intramolecular bonds, interactions between enzyme subunits, substrate binding and interactions with the solvent[2]. These interactions change with decreasing temperature: hydrogen bonds and electrostatic interactions become stronger and hydrophobic interactions are weakened. Enzymes with low optimum temperatures frequently contain specific amino acid replacements when compared with their counterparts from temperate organisms[2,3,34,35]. Furthermore, they tend to have more polar and fewer hydrophobic residues, fewer hydrogen bonds and ion pairs, additional glycine residues, a low arginine to lysine ratio and fewer hydrophobic interactions between subunits[2,3,34,35]. The active sites of cold-adapted enzymes are usually highly conserved; amino acid replacements can occur at the entry to the active site, at hinge regions which permit conformational changes, or at interfaces between domains and subunits[2,4,34].

Cold adaptations in microtubule assembly and dynamics

Structural proteins of the cell are also challenged by the cold. It is well known that microtubules depolymerize when conventional tissue culture cells are incubated on ice. Tubulin from Antarctic fish, however, can polymerize under these conditions[36]. This difference lies in the tubulin dimers themselves, as microtubule-associated proteins do not contribute to polymerization[36]. So how are cold-adapted tubulins different?

Microtubules consist of α and β subunits that form dimers. Tubulin dimers assemble into protofilaments, 13 of which associate laterally to form a hollow cylinder, the microtubule. Tubulins from Antarctic fish contain a small number of amino acid substitutions that map to the lateral inter-protofilament surfaces and to the interior of the α- and β-subunits, but surprisingly, not to the longitudinal inter-dimer surfaces[36]. These alterations seem to stabilize the tubulin monomers in a conformation that favours polymerization and strengthens protofilament interactions. In addition, dynamic instability of microtubules from Antarctic fish is strongly reduced, presumably because the amino acid changes in the tubulin monomers slow the conformational change in the tubulin dimer preceding depolymerization[36]. Microtubules from *Saccharomyces cerevisiae* also display little dynamic instability when compared with mammalian microtubules[37]. Thus, in principle, this characteristic is not detrimental. Furthermore, microtubule-associated proteins can modify microtubule dynamics, and perhaps their activity is pivotal when cells from psychrophilic organisms undergo meiosis or mitosis.

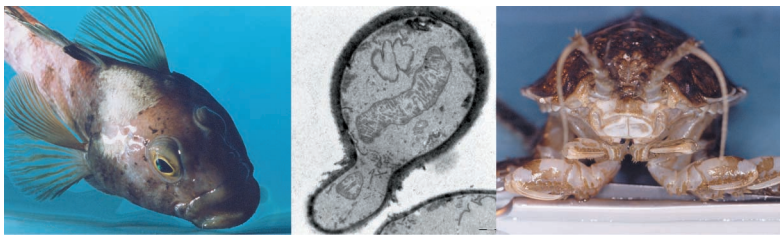

Figure 2 **Experimental organisms. The Antarctic teleost fish T. bernacchii (left; 15 cm total length), budding yeast Cryptococcus antarcticus (centre; 5 μm diameter) and isopod crustacean G. antarcticus (right; 7 cm body width).**

RNA and protein synthesis

The rate of enzymatic reactions are halved when temperature is reduced by 10 °C. Thus, one would expect both synthesis and turnover of macromolecules to be slower at low temperature. Surprisingly, this is not the case, as at −1.5 °C, protein and polyA-RNA synthesis/degradation in Antarctic sea urchin embryos is identical to that in temperate species at 25 °C, and thus 4–6-fold higher than expected[38]. The high level of protein biosynthesis in Antarctic sea urchin embryos is a direct consequence of increased polyA-RNA synthesis[38]. Paradoxically, this high metabolic activity is paired with low respiration rates, suggesting that the energy cost of protein turnover in Antarctic species is only 4% of that of temperate species[38]. The molecular basis for this phenomenally efficient use of metabolic energy is unclear, but it is probably not caused by more efficient ATP synthesis in cold-adapted mitochondria[27].

Molecular cell biology research in the Antarctic is still in its infancy. At a molecular level, the majority of biological phenomena specific to this environment are just beginning to be understood. Much remains to be discovered for anyone interested in life from the cold habitats of our planet. □

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