relatively straightforward mechanisms, for example by the modification of particular receptors[14,15]. *Hymenoepimecis*'s manipulation of its spider host is probably the most finely directed alteration of behaviour ever attributed to an insect parasitoid.

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Phytochemistry

Heat-stable antifreeze protein from grass

We have discovered an antifreeze protein[1] in an overwintering perennial ryegrass, *Lolium perenne*. The protein is stable at 100 °C and although it is a less effective antifreeze than proteins found in antarctic fish and insects, it is better at preventing ice recrystallization. This property enables grasses to tolerate ice formation in their tissues without being damaged, suggesting that the control of ice-crystal growth rather than the prevention of freezing may have evolved to be the critical factor in their survival at very low temperatures.

Frost-tolerant plants undergo a process of cold acclimation[2,3], during which perennial grasses accumulate a boiling-tolerant protein that inhibits ice recrystallization. We extracted the protein responsible for this activity from cold acclimated leaves of *L. perenne* and cloned its complementary DNA by using the polymerase chain reaction[4]. We found that it had an open reading frame encoding a protein of 118 amino acids (GenBank accession number, AJ277399) and relative molecular mass 11.765K, with six potential *N*-glycosylation sites containing the conserved N-X-S/T glycosylation motif.

Although this boiling-tolerant antifreeze protein (AFP) belongs to a new class of plant proteins and shares no lengthy sequence homology with any other AFP or protein sequence, some of its properties fit with the general pattern for AFPs. It is very hydrophilic, being rich in asparagine (25%), valine (16%), serine (15%) and threonine (10%), and having very few amino acids with aromatic or hydrophobic side chains. The primary structure has a series of highly conserved, 7-amino-acid repeat sequences with regularly spaced serine and threonine residues that may be able to hydrogen-bond with an ice surface. Growth of a single ice-crystal hemisphere from a dilute solution of the protein, and subsequent surface-etching of the ice hemisphere[5], produced a distinctive pattern with six-fold symmetry, demonstrating that the protein was specifically binding to ice on the primary prism plane (Fig. 1a,b).

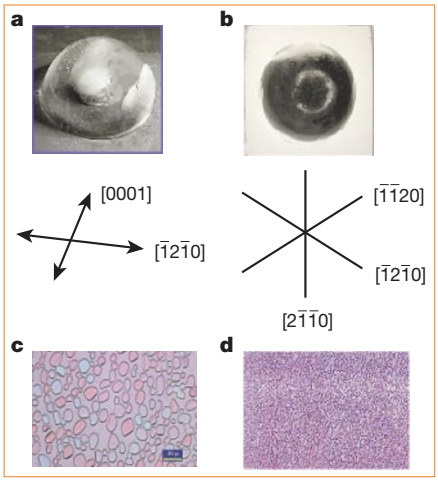

Figure 1 *Lolium* antifreeze protein (AFP) binding to ice and its effect on ice recrystallization. *Lolium* AFP binds specifically to an ice-crystal surface with six-fold symmetry. **a,b,** Ice-etching determination of the binding planes using the hemisphere technique[5]: **a,** three elongated patches positioned on the primary prism plane; and, **b,** the planes symmetrically arranged around the crystal's *c*-axis. **c,d,** Influence of *Lolium* AFP on ice recrystallization. The recrystallization inhibition assay shows crystal growth after 60 min at −6 °C; *Lolium perenne* (**d**) inhibits recrystallization of ice in dilute concentrations relative to growth with the 30% sucrose control (**c**). Scale bar, 50 μm.

The Fourier-transform infrared spectrum of this grass AFP in solution at room temperature revealed a high solvent-exposed β-sheet content which may be exposed at the ice-binding surface[6], as proposed for several other antifreeze proteins, including that from carrot and types II and III from fish. The spectrum was the same in the presence of ice, suggesting that the conformation of the *Lolium* AFP does not change on binding to ice, unlike that of the insect *Dendroides canadensis* thermal-hysteresis protein, which does[7].

Our *Lolium* antifreeze protein had a significantly higher specific activity in an ice-recrystallization inhibition assay[8] than other antifreeze proteins. Growth of ice crystals in 30% sucrose solution was completely inhibited at AFP concentrations below 10 μg ml^{-1} (Fig. 1c,d), which is at least 200 times less in molar terms than the type III AFP from ocean pout (*Macrozoarces americanus*).

In contrast, *Lolium* AFP shows a low thermal hysteresis (the lowering of the temperature at which ice forms on cooling while the melting temperature remains unaltered[9]), with the highest measurable value being 0.1 °C in water and 0.45 °C in 30% sucrose; these values are much lower than the 1.0–1.5 °C reported for fish AFP[10] and the 5–6 °C reported for insect proteins[11], although these too are increased by sucrose[12].

Mechanisms previously proposed to explain how AFPs work all imply some correlation between thermal hysteresis effects and recrystallization inhibition[13]. Our discovery that there is no correlation between these relative activities of the antifreeze protein from *L. perenne* raises questions about the nature of the AFP mechanism and indicates that different classes of AFP may interact with ice in different ways. We propose that the thermal hysteresis activity of the grass protein is unlikely to serve an important protective function at the very low temperatures survived by overwintering grasses, whereas its capacity to control the growth of ice crystals may protect it against damage to the plant cellular structure.

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