Anhydrobiosis

Plant desiccation gene found in a nematode

When subjected to drought conditions, some organisms enter a state of suspended animation known as anhydrobiosis[1], surviving for indefinite periods until rehydration allows them to resume normal metabolism. We have identified a gene in the anhydrobiotic nematode *Aphelenchus avenae* that is upregulated in response to desiccation stress and whose encoded protein shares sequence similarity with a late-embryonic gene that is induced in many plants when they are deprived of water. This finding suggests that animals and plants that undergo anhydrobiosis may use common protective strategies against dehydration, and provides a unifying insight into the mechanism of anhydrobiosis.

A characteristic feature of anhydrobiotic organisms is their synthesis of high concentrations of non-reducing sugars during the induction of anhydrobiosis[1]. For example, *A. avenae* accumulates large amounts of trehalose in response to dehydration, and this correlates with its desiccation tolerance[2]. Trehalose protects membranes and proteins from desiccation damage by replacing structural water[1], and contributes to the formation of an intracellular organic glass[3] which is thought to stabilize the cell's contents. In anhydrobiotic plants, sucrose has a similar function[4].

However, several lines of evidence indicate that non-reducing sugars alone are not sufficient to confer a state of anhydrobiosis, and that further adaptations are required[5,6]. A class of proteins known as LEA (for 'late embryogenesis abundant') proteins accumulate in response to water deficit in many plants, and these are particularly abundant in anhydrobiotic plants such as the resurrection plant *Craterostigma plantagineum*, and in maturing seeds and pollen[7]. We therefore investigated whether similar genes are present in *A. avenae*.

We found that several genes are upregulated in *A. avenae* nematodes undergoing anhydrobiosis during exposure to 90% relative humidity for 24 hours. Of particular interest was a strongly induced transcript with a length of about 675 bases (Fig. 1a, b), which encodes a predicted protein of 143 residues. The sequence of this protein indicates that it is a member of the group-3 subclass of LEA proteins[8]; it contains at least four copies of a characteristic 11-mer motif, and further echoes of this motif are present throughout the sequence (Fig. 1c).

The group-3 LEA motif has been proposed to form an amphipathic α-helix that directs the oligomerization of the protein[9]. These proteins are extremely hydrophilic and are resistant to denaturation by heat — prompting suggestions that they help to prevent damage by water stress, for example by acting as hydration buffer, molecular chaperone, ion sink or membrane stabilizer[1,9].

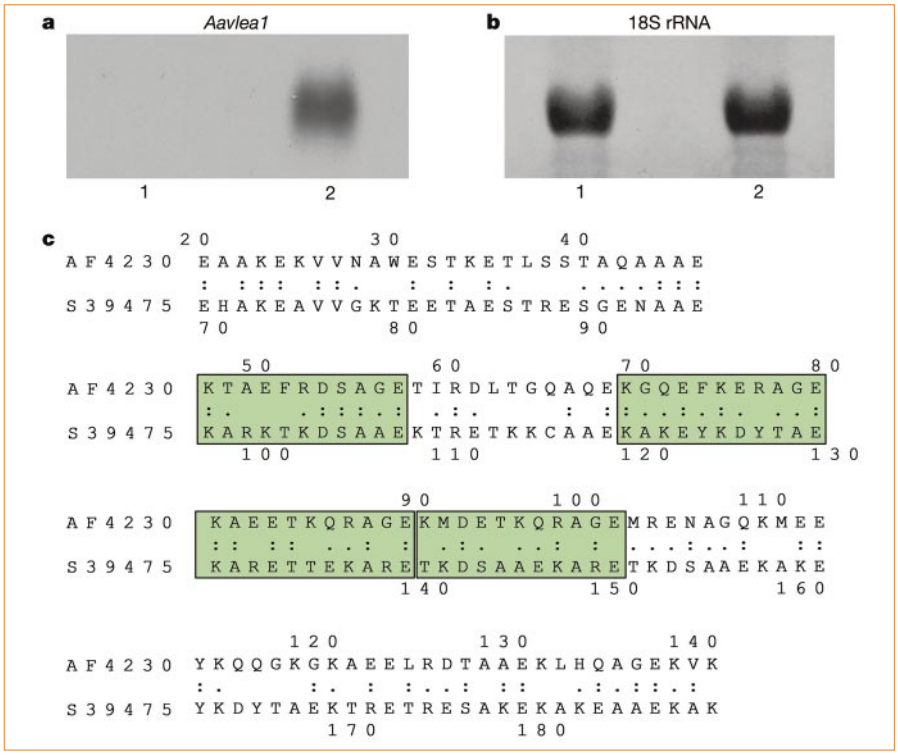

Figure 1 Desiccation induces expression of a protein in the nematode *Aphelenchus avenae* that is homologous with another embryonic protein produced by a plant under similar circumstances. **a,** Expression of the *A. avenae* late-embryonic gene *Aavlea1* in response to desiccation stress, as revealed by northern blotting using an *Aavlea1* probe: lane 1, control RNA from fresh untreated nematodes; lane 2, RNA from nematodes exposed to 90% relative humidity for 24 h. **b,** Same blot, but this time hybridized with an 18S rRNA probe as a loading control. **c,** Local alignment ($P = 2e^{-19}$) of a 121-amino-acid overlap between the predicted sequences of *Aavlea1* (GenBank accession number AF423069) and an embryonic protein from the European white birch (*Betula pendula*; accession number S39475). S39475 was the most closely related LEA-3 sequence to *Aavlea1* that we were able to detect in the GenBank database. There are at least four copies of the characteristic 11-mer LEA-3 motif (highlighted).

Sucrose glasses are stabilized *in vitro* by interaction with a purified group-3 LEA protein from *Typha latifolia* pollen[10]. Non-reducing sugars and LEA proteins may therefore act synergistically[4,10] to promote the formation of a stable 'bioglass' in the cytoplasm of anhydrobiotic plants and in desiccation-tolerant seeds and pollen — the bioglass may trap fragile biological molecules in time and space, and preserve them from desiccation damage. If the newly identified LEA protein in *A. avenae* also stabilizes trehalose glasses in this way during desiccation, the bioglass model for anhydrobiosis can be extended to include anhydrobiotic animals.

Genomic[11] and partial protein[12] sequence information indicates that LEA proteins may be used by other nematodes as a defence against water stress. The genomes of some microorganisms also contain sequences that encode LEA-like proteins (although expression of these has not yet been demonstrated[11]), including the desiccation-tolerant *Deinococcus radiodurans*, which also encodes enzymes for the biosynthesis of trehalose. A bioglass strategy for anhydrobiosis could be widely used in biological systems and may have originated in ancient cell types that were exposed to water stress.

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1. Crowe, J. H., Hoekstra, F. A. & Crowe, L. M. *Annu. Rev. Physiol.* **54,** 579–599 (1992).
2. Madin, K. A. C. & Crowe, J. H. *J. Exp. Zool.* **193,** 335–346 (1975).
3. Crowe, J. H., Carpenter, J. F. & Crowe, L. M. *Annu. Rev. Physiol.* **60,** 73–103 (1998).
4. Sun, W. Q. & Leopold, A. C. *Comp. Biochem. Physiol.* **117A,** 327–333 (1997).
5. Higa, L. M. & Womersley, C. Z. *J. Exp. Zool.* **267,** 120–129 (1993).
6. García de Castro, A. & Tunnacliffe, A. *FEBS Lett.* **487,** 199–202 (2000).
7. Ingram, J. & Bartels, D. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47,** 377–403 (1996).
8. Dure, L. *et al. Plant Mol. Biol.* **12,** 475–486 (1989).
9. Dure, L. *Plant J.* **3,** 363–369 (1993).
10. Wolkers, W. F. *et al. Biochem. Biophys. Acta* **1544,** 196–206 (2001).
11. Dure, L. *Protein Pept. Lett.* **8,** 115–122 (2001).
12. Solomon, A., Salomon, R., Paperna, I. & Glazer, I. *Parasitology* **121,** 409–416 (2000).

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