Dehydration tolerance in resurrection plants

How dried flowers

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The ability to survive desiccation is commonly found in seeds or pollen and is widespread in lower organisms, but it is rare in the vegetative tissues of flowering plants. These 'resurrection plants' are so-called because they appear to be dead in the dried state and brought back to life by the addition of water.

Hunting for new resurrection plant species in nature is an art. Armed with a watering can and a notebook, one approach involves watering a likely clump of dried vegetation and returning the next day to see whether the vegetative tissues have regained vitality. Such an amazing sight must have greeted Professor Donald Gaff, who returned to a site in South Africa where healthy plants with striking flowers, each consisting of a purple and white corolla, were now flourishing. Gaff reported these findings in Science[1] and concluded that this plant among 14 other species, *Craterostigma plantagineum* Hochst, was desiccation-tolerant because the mature leaves survived from 15 to approximately 0% relative humidity (Figure 1). Since then, of the quarter of a million species of vascular plants, approximately 300 species have been reported as having the potential to survive desiccation in the vegetative growth phase.

The molecular basis of desiccation tolerance in dicotyledonous angiosperms has been investigated extensively using *C. plantagineum* as a model system[2]. The acquisition of desiccation tolerance in C. *plantagineum* requires the induction of a co-ordinated programme of genetic and biochemical processes during drying. The most prominent metabolic changes that take place during drying are the *de novo* synthesis of proteins and sugars, which are postulated to form the basis of protective mechanisms that limit damage to cellular constituents. Here we will focus on several molecular aspects of desiccation tolerance that may be the basis for desiccation tolerance and have facilitated the laboratory-based discovery of new resurrection plant species.

Key words: *2-octulose, Craterostigma desiccation-tolerant gene (CDT gene), Craterostigma plantagineum, desiccation tolerance, late embryogenesis abundant proteins*

LEA (late embryogenesis abundant) proteins: a link between seed and vegetative desiccation tolerance

In the absence of an obvious function, the LEA class of proteins was named from when and where they were expressed, that is during late embryo development with maximum expression at the point of desiccation. They were first discovered as abundant proteins when embryogenesis in cotton was investigated. The name has stuck ever since, and LEA proteins have consequently been linked to the acquisition of desiccation tolerance in orthodox seeds. In 1990[3], it became clear that LEA proteins also accumulate to extraordinarily high levels in the leaves of *C. plantagineum*, which led to the hypothesis that the same molecules are involved in the tolerance mechanism in seeds and in vegetative tissues of resurrection plants. Since then LEA proteins have also been shown to be expressed in dehydrated tissues other than seeds in desiccation-sensitive plants, such as the genetic model plant *Arabidopsis thaliana*, although perhaps not at the same magnitude. Subsequently, efforts have focused on understanding the function of LEA proteins. Perhaps the two best studied examples from resurrection plants are the *C. plantagineum* proteins: CDeT6-19 and CDeT11-24.

CDeT6-19 and CDeT11-24 are predicted to largely adopt an unstructured rather than a globular conformation. The prediction that both are intrinsically disordered proteins is consistent with NMR studies of recombinant CDeT6-19 produced in *Escherichia coli*, in which no stable structure was identified[4]. Another common feature of both proteins is that they undergo post-translational modification in the form of phosphorylation[5]. CDeT6-19 belongs to the so-called dehydrin class of LEA proteins, which possess highly conserved amino acid motifs. Dehydrins appear to be ubiquitously present in seeds of all higher plants. An unusual but very characteristic feature of this group of proteins is a continuous stretch of serine residues, which appear to undergo phosphorylation. The role of phosphorylation is unclear; however, it may increase the hydrophilicity of the molecules, allow

bloom again

Figure 1. *C. plantagineum* in a desiccated (left) and hydrated (right) state. (Images: E. Fischer.)

shuttling between intracellular compartments, or influence structural stability. In the case of CDeT11-24, which seems to be restricted to *C. plantagineum* and related plant species, several phosphorylation sites are present that coincide with the limited regions of the protein that have the potential to form coiled-coil structures. Given that phosphorylation is a mechanism for influencing the stability and interactions of coiled-coil structures in other proteins, the presence of coiled-coil interaction domains in CDeT11-24 could provide a mechanism for regulated protein oligomerization. CDeT11-24 may therefore combine with itself and/or other desiccation-induced proteins to create a stabilizing network in the desiccated state. *In vitro* experimental evidence supports the theory that LEA proteins can assume a stabilizing role in dehydrating cells. For example, *in vitro* protection assays have shown that CDeT6-19 protein is able to protect enzyme activities from inactivation caused by water depletion[6]. Water loss correlates with changes in enzyme exposure of hydrophobic surfaces, which are ameliorated by the presence of CDeT6-19. This is consistent with the idea that LEAs maintain the hydration shell of proteins and other molecules, effectively replacing water.

Interconversion between 2-octulose and sucrose: osmoprotection derived from an unusual source

A novel feature of *C. plantagineum* is the carbohydrate metabolism during the dehydration and rehydration cycle. *C. plantagineum* contains high amounts of the sugar 2-octulose, which is extremely rare in flowering plants, in fully hydrated leaves. Upon dehydration, the 2-octulose level declines and is converted into sucrose, a process that is reversed during rehydration[7]. As with LEA proteins, a similar correlation

Figure 2. *C. plantagineum* colonizes ephemeral pools in southern Africa. (Image: E. Fischer.)

has been observed in seeds of higher plants between the accumulation of non-reducing sugars, such as sucrose, and the acquisition of desiccation tolerance. The biochemical role of sucrose in this process is not understood; however, a protective role is supported by *in vitro* studies which showed that a wide range of biomolecules are less susceptible to denaturation when dehydrated in the presence of sugars.

The accumulation of sucrose in dehydrated tissues is a common theme in different resurrection plants, although it is derived via different metabolic routes. 2-Octulose is a product of photosynthesis that accumulates in leaves during the light period[8]. It therefore appears that 2-octulose functions solely as a novel storage molecule in readiness for periods of extreme drought, whereupon it is converted into an osmoprotectant.

CDT (*Craterostigma* desiccation -tolerant) genes: RNA regulation

The question arises whether *C. plantagineum* expresses genes that are unique and could be essential for the acquisition of desiccation tolerance. Over ten years ago the unusual *CDT-1* gene was discovered using a genetic approach. No homologues of this gene have so far been identified in any other plant. Constitutive overexpression induces desiccation tolerance in *C. plantagineum* callus tissue coupled with a constitutive expression of a subset of desiccation-induced transcripts. The *CDT-1* gene is transcribed upon desiccation, but it does not code for a polypeptide; it is transcribed both in sense and antisense orientations. Molecular analysis of the mode of action of *CDT-1* suggests that it functions as a regulatory non-protein-coding RNA[10]. *CDT-1* is a member of a large gene family of which all members have features of retrotransposons. Transposition events were traced in the *C. plantagineum* genome. These retro-transposon elements may be responsible for the acquisition of desiccation tolerance, because genes necessary for the desiccation phenotype seem to be activated via small RNAs encoded by *CDT-1* and other members of the family. As a consequence, DNA sequences are capable of directing abundant transcription of genes encoding protective molecules.

Comparative genome analysis in plants analysed so far leads to the speculation that all plants possess the structural genes essential for surviving desiccation, as desiccation is part of the developmental programme during seed development. Most plants produce desiccation-tolerant seeds, but lose this feature during development. Rapid generation of small regulatory RNAs via transposition events seems to be a mechanism that enables plants to adapt the

gene expression programme, thus allowing plants to survive in stressful environments.

Discovering new resurrection plants: exploring diversity within the Linderniaceae

In accordance with the experiences of Gaff[1], desiccation-tolerant species are typically found as pioneers in shallow soils or on rocky outcrops that experience extreme variations in moisture availability (see Figure 2). Using knowledge derived from studying the molecular basis of desiccation tolerance in *C. plantagineum* for nearly 20 years, an alternative search for other resurrection plant species was embarked upon, with the view that general conserved mechanisms of desiccation tolerance exist. An unexpected finding has been *Lindernia brevidens*, a close relative of *C. plantagineum*, which is desiccation-tolerant. Remarkably, *L. brevidens* is endemic to montane rainforests of Tanzania and Kenya, where it never experiences seasonal dry periods[9]. *L. brevidens* has been found exclusively in two fragments of the ancient Eastern Arc Mountains, which were protected from the Pleistocene droughts by the stable Indian Ocean temperature. Mechanisms that confer cellular protection during extreme water loss are well conserved between *C. plantagineum* and *L. brevidens*, including the interconversion of 2-octulose into sucrose and the regulated expression of members of the LEA protein family.

The question remains as to how *L. brevidens* did not lose the ability to adapt to extreme dehydration. One hypothesis that explains the ecological niche occupied by *L. brevidens* is that desiccation tolerance is linked to another, as yet unknown, trait that is important for survival in the rainforest. In this way, the ability to survive desiccation remains; however, the mechanisms that protect the vegetative tissues are never induced in Nature. ■

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