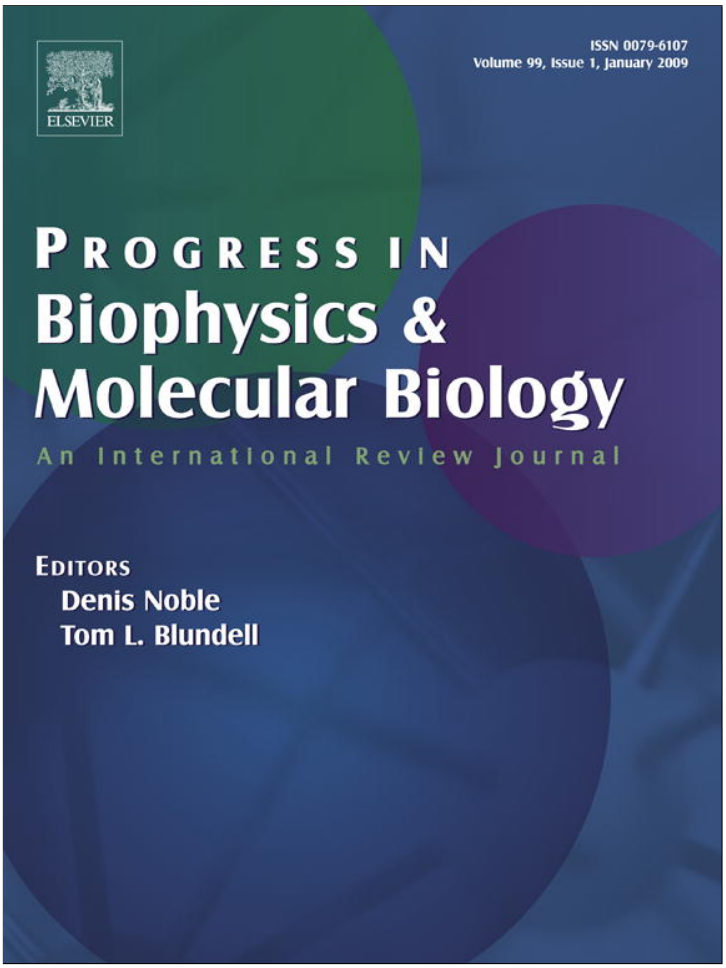

Progress in Biophysics and Molecular Biology 99 (2009) 1–6

Contents lists available at ScienceDirect

Progress in Biophysics and Molecular Biology

journal homepage: www.elsevier.com/locate/pbiomolbio

Review

When cells lose water: Lessons from biophysics and molecular biology

Julio J. Caramelo [a,b], Norberto D. Iusem [c,*]

[a] Fundación Instituto Leloir and IIBBA-CONICET, Av. Patricias Argentinas 435, Buenos Aires, Argentina
[b] Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina
[c] Departamento de Fisiología, Biología Molecular y Celular, e Instituto IFIByNE-CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina

ARTICLE INFO

Article history:
Available online 17 October 2008

Keywords:
Drought
Desiccation
Abiotic stress
LEA proteins
Natively unfolded

ABSTRACT

Organisms living in deserts and anhydrobiotic species are useful models for unraveling mechanisms used to overcome water loss. In this context, late embryogenesis abundant (LEA) proteins and sugars have been extensively studied for protection against desiccation stress and desiccation tolerance. This article aims to reappraise the current understanding of these molecules by focusing on converging contributions from biochemistry, molecular biology, and the use of biophysical tools. Such tools have greatly advanced the field by uncovering intriguing aspects of protein 3-D structure, such as folding upon stress. We summarize the current research on cellular responses against water deficit at the molecular level, considering both plausible water loss–sensing mechanisms and genes governing signal transduction pathways. Finally, we propose models that could guide future experimentation, for example, by concentrating on the behavior of selected proteins in living cells.

Contents

1. Introduction

Biologists are naturally curious about plant species dwelling in arid zones that are permanently exposed to drought stress. Tolerance to water deficit probably first occurred in organisms as primitive as bryophyte mosses and was evolutionarily important for the conquest of land by plants (Rensing et al., 2008). Even more intriguing is the existence of plants and animals that can survive almost complete water loss by entering a reversible state of metabolic arrest (Tunnacliffe and Lapinski, 2003). This phenomenon, called anhydrobiosis, was first described by Van Leeuwenhoek in 1702 for small asexual invertebrates (Tunnacliffe and Lapinski, 2003). Bacteria, yeast, plants and trees living in deserts as well as resurrection plants have thus been excellent models with which to explore molecular responses to water loss (Billi and Potts, 2002; França et al., 2005; Bernacchiaa and Furinib, 2004; Frankel et al., 2003; Brosché et al., 2005). At the physiological level in plants, drought seems to select for higher water-use efficiency, defined as the ratio of crop yield to water uptake; this trait is attributable to decreased stomatal conductance—that results in less transpiration—and increased root biomass (Blum, 2005;

Abbreviations: ABA, abscisic acid; CD, circular dichroism; DSR, drought stress response; ER, endoplasmic reticulum; FTIR, Fourier transform infrared spectroscopy; LEA, late embryogenesis abundant; ROS, reactive oxygen species; UPR, unfolded protein response.

* Corresponding author.
E-mail address: norbius@fbmc.fcen.uba.ar (N.D. Iusem).

0079-6107/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.pbiomolbio.2008.10.001

Heschel and Riginos, 2005; Knight et al., 2006; Song et al., 2008). Since drought is currently a major agronomic problem (Marris, 2008), it has been studied at various levels of biological organization using many different experimental methodologies.

2. How much do we know about molecular responses to stress in general?

Heat shock is probably the best understood molecular stressor, and the unfolded protein response (UPR) pathway has been intensively studied (Ron and Walter, 2007). Central to the UPR are Ire1p, ATF6 and PERK—three conspicuous endoplasmic reticulum (ER) membrane-bound proteins that transduce protein misfolding signals from the lumen of the ER towards the nucleus (Lee et al., 2002). Other types of laboratory-induced stress that cause unfolding (i.e., inhibition of protein glycosylation, reducing conditions, and mobilizing Ca^{2+} from the ER) also lead to UPR; this process is also recognized in plants (Martinez and Chrispeels, 2003).

Responses to drying, freezing or salt-induced stress (which are mechanistically related) share some features with those against thermal stress. The rationale is that the molecular crowding generated by water loss triggers the aggregation of macromolecules (Goyal et al., 2005), which may also occur upon temperature increase due to protein unfolding (Sasahara et al., 2007). To the best of our knowledge, UPR has not been examined under water-deficit stress. Nevertheless, its occurrence should not be surprising since protein aggregation is known to be alleviated by chaperones during both typical heat shock-induced UPR and drought stress in plants (van Gemeren et al., 1997; Liu et al., 2006). However, water loss probably brings about unique responses (Shinozaki et al., 2003).

At this point, it is important to distinguish between "drought stress tolerance" and "desiccation tolerance". Both conditions share some basic features, but desiccation tolerance usually refers to an extreme situation defined by survival with water content lower than 5% (0.05 g H_2O/g dry weight) in adapted organisms (several lower plants, algae, lichens, and invertebrates). Desiccation tolerance probably requires additional protective factors beyond those needed under milder drought stress conditions. In plants, the physiological response to regain turgor and health is thought to be a consequence of (i) hormonally mediated induction of genetic programs, mainly by abscisic acid (ABA) (Bernacchiaa and Furinib, 2004; Cuming et al., 2007), (ii) long-term accumulation of protective molecules (Ramanjulu and Bartels, 2002) and (iii) closure of leaf stomata (Brodribb and Holbrook, 2003).

3. Cellular responses to water-deficit stress

Living cells do not take water for granted. Hydration is a major driving force in the protein folding process, compensating for the reduction of the conformational entropy of the polypeptide chain (Imai et al., 2007). Therefore, water loss brings about serious consequences: in addition to protein aggregation, stressed cells must contend with oxidative stress (Chaves and Oliveira, 2004), changes in membrane fluidity and cytoplasmic viscosity (Hoekstra et al., 2001) and damaged DNA (França et al., 2007). Consequently, cells react defensively by initiating several signal transduction pathways that result in the accumulation of different transcripts, proteins, sugar molecules and lipophilic anti-oxidants, almost always concomitant with increased ABA levels (Cuming et al., 2007; Ramanjulu and Bartels, 2002).

Organic osmolytes are small solutes used by numerous water-stressed organisms to maintain cell volume. Some of them consist of amino acids and their derivatives, polyols and neutral sugars (or charged sugars in thermophilic organisms). Such osmolytes are often called "compatible solutes", a term indicating a lack of detrimental effects on cellular macromolecules (Yancey, 2005). Compatible solutes appear in high amounts (up to 20% total dry mass) upon osmotic adjustment but, in contrast to ions, do not inhibit cytosolic enzymes. Interestingly, they exert a protective effect by stabilizing biological membranes under stress conditions (Hincha and Hagemann, 2004). For instance, sucrose, trehalose and sorbitol protect liposomes from leakage of soluble markers and from membrane fusion during drying and rehydration. In addition, these molecules lower the lipid phase transition temperature *in vitro*, probably by interacting with the lipid polar headgroups.

In situations of extreme desiccation, sugars can replace the lost shell of water around macromolecules, H-bonding with proteins and thus alleviating damaging effects caused by drying (the water replacement hypothesis, Potts, 1994). This might well be a protective mechanism in desiccated resurrection plants, which accumulate sucrose (Peters et al., 2007). In contrast, trehalose, known to act as a protective solute in bacteria and protozoa, is hardly detected in most plants, although the genes needed for its biosynthesis are present in *Arabidopsis* (Wingler, 2002). The formation of an intracellular glass (glassy matrix) at ambient temperatures with mechanical properties of a plastic solid (Koster, 1991) is another mechanism by which sugars might protect cells during severe desiccation. Glass formation could be beneficial since the resulting increase in viscosity limits chemical reactions requiring diffusion, thus ensuring stability.

Among the proteins that accumulate in both mild and severe dehydration, the most extensively characterized are those belonging to LEA superfamily, which are classified into three major groups based on particular amino acid sequence motifs (Tunnacliffe and Wise, 2007). There are more than fifty LEA-encoding genes in the *Arabidopsis thaliana* genome, most of which have abscisic acid response elements (ABRE) in their transcriptional enhancers (Hundertmark and Hincha, 2008). Despite the various roles proposed for LEA proteins, their precise function has not been fully uncovered.

Aquaporins—widespread transmembrane channel proteins that regulate water flux—are also associated with water status. Aquaporins have been identified by their two highly conserved asparagine–proline–alanine (NPA) boxes, which are important for the formation of their water-permeating pore (Ishibashi, 2006). Aquaporin gene expression in plants suffering from drought stress varies depending on the particular aquaporin gene, water stress level and plant organ (Galmés et al., 2007).

More recently, genome-wide transcriptomic approaches have produced similar results; many genes have been implicated in the drought stress response (DSR). Of these, about 10% encode transcription factor genes (Seki et al., 2002). On the other hand, proteomics-based strategies have yielded expected proteins such as LEA, ASR (Maskin et al., 2001) and others known to be involved in basic metabolic pathways such as ATP production, photosynthesis, protein synthesis and folding, oxidative stress tolerance and cytoskeleton reorganization (Boudet et al., 2006; Gazanchian et al., 2007).

4. Biophysical approaches

Several laboratories have investigated conformational changes suffered by proteins upon water stress by using spectroscopic methods. Among them, Wolkers et al. (1999) and Oldenhof et al. (2006) measured the Fourier Transform Infrared Spectroscopy (FTIR) spectra of whole cells between 1800 and 1500 cm^{-1}, corresponding to the amide-I and -II absorption bands of the protein backbones and the sugar OH band. These experiments show that, upon slow drying, a large assortment of proteins acquires a more ordered state and that sugars form a tighter H-bonding network.

Conventional screening and cloning of differentially expressed transcripts or, more recently, proteomic strategies have allowed purification of diverse drought-induced LEA proteins. Subsequent elegant *in vitro* assays followed by FTIR and far-UV circular dichroism (CD) revealed that LEA (or LEA-like) proteins in solution are natively unfolded and then fold properly upon desiccation (Goyal et al., 2003; Gilles et al., 2007; Tolleter et al., 2007; Goldgur et al., 2007). Such natively unfolded polypeptides contain unstructured regions spanning not less than 70% of the sequence. They share certain features including low complexity, high flexibility, low mean hydrophobicity and relatively high net charge (Uversky et al., 2000). Drying-induced protein folding was confirmed by the following observations: (i) the CD spectrum of those proteins in aqueous solution appeared typical of a random coil structure, with 200-nm minimum ellipticity; (ii) under drying or in the presence of agents such as trifluoroethanol, the spectrum became typical of that of α-helices, with high ellipticity at 192 nm and minima at 208 and 222 nm. A limitation of these CD studies is the difficult interpretation of small bands at 210–230 nm. Wise and Tunnacliffe (2004) have produced an appealing model portraying protein filaments generated from a more ordered state by desiccation. It remains unknown whether the folded state confers a new and possibly protective function. However, such speculation is tempting given the accumulation and folding upon drying of these proteins.

5. Dehydration-triggered functionality of LEA proteins

LEA proteins are abundant (occurring not only in seeds) and timely synthesized, and they fold as a consequence of drought stress. Many potential roles have been proposed for this widespread, heterogeneous and expanding protein family. In fact, the ASR family can be classified as a new group of LEA proteins as they are small, hydrophilic, and glycine-rich and present in seed, pollen and desiccated vegetative tissues, with transcription factor activity (Maskin et al., 2007). Purified LEA proteins, either cytoplasmic or mitochondrial, have been used in *in vitro* protection assays to evaluate chaperone (anti-aggregation) activity as determined by light scattering, or protection of liposome integrity or enzyme activity upon drying (Dong et al., 1995). The main conclusion of these studies is that LEAs are usually able to protect other proteins or membranes, in a fashion similar to sugars (Hoekstra et al., 2001), perhaps by acting as water replacement molecules. Since such protection occurs not only after drying cycles but also in buffers at slightly elevated temperatures without any previous drying (Goyal et al., 2005), the relevance of the folded state of LEAs remains uncertain. This point could be investigated by performing similar experiments with mutated LEA bearing helix-breaking amino acids. Alternatively, since LEAs (but not proteins such as BSA, the typical control in these protection experiments) behave similarly irrespective of their sequence, their properties may be ascribed to their polyelectrolyte nature.

Another possibility is that these proteins have different functions at different degrees of water content. For example, in high water content situations, they may act as molecular sponges (exploiting their high hydration capacity) and ion chelators to minimize injury to membranes and proteins; such activity was demonstrated by proton NMR and differential scanning calorimetry for dehydrin, a group 2 LEA protein (Tompa et al., 2006). In addition, these proteins may have anti-oxidant properties that minimize the damaging effects of reactive oxygen species (ROS), as strongly suggested for a group 5 LEA from *A. thaliana* (Mowla et al., 2006). However, at low water content and high cytoplasmic viscosity, these proteins may confer stability to the cytoplasmic glassy matrix, as suggested by studies of dried carrot somatic embryos (Wolkers et al., 1999). Such activity may occur via increased glass transition temperature (T_g) of sugars (Wolkers et al., 2001). In this scenario, LEAs and sugars have been proposed to form a tight hydrogen bonding network together in the dehydrated cytoplasm.

At this point, it should be noted that *in vivo* studies on the conformational changes of LEAs have yet to be undertaken, although such studies for other proteins have been performed in animal systems by utilizing fluorescent probes (Roberti et al., 2007). For instance, a LEA protein with cyan and yellow fluorescent proteins fused to its ends could reveal stress-induced compaction *in vivo* (Fig. 1), following a principle similar to that employed for cameleon calcium indicators (Miyawaki et al., 1997).

6. The challenge of identifying the primary sensor of water stress

As in other types of environmental cues, primary sensors of water deficit are elusive. Theoretical models (Fig. 2) may therefore be helpful for generating a whole picture of the stress response in order to guide future research. Early and essential plasma membrane-bound components of a pathway devoted to sensing external changes in osmolarity or inner turgor pressure have been clearly identified, first in yeast and later in plants. Their role was inferred from their strategic subcellular localization, their rapid phosphorylation, and the triggering of their kinase activity by osmotic variation or loss of the cell wall (Reiser et al., 2003; Urao et al., 1999). Also worth mentioning is the recent evidence that histidine kinase ATHK1-defective *Arabidopsis* mutant plants are unable to withstand water stress (Wohlbach et al., 2008). However, a primary osmosensing role has been unambiguously proved only in prokaryotes, specifically for the ABC transporter (operon OpuA) from *Lactococcus lactis*, which functions in a pure reconstituted liposome system that responds to a hyperosmotic medium (van der Heide and Poolman, 2000).

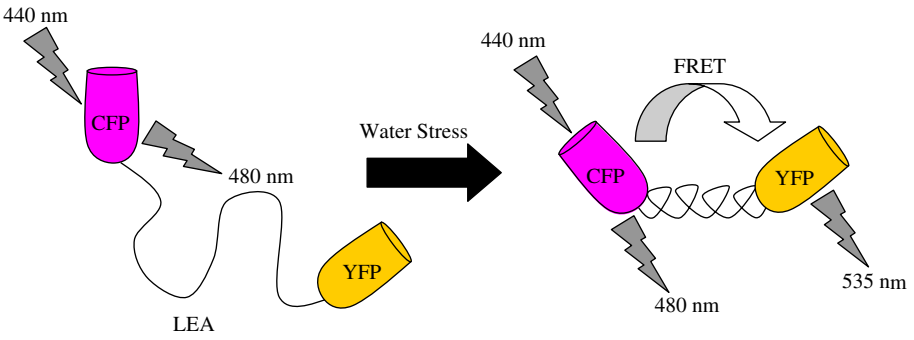

Fig. 1. A possible strategy for following structural transitions of LEA proteins *in vivo*.

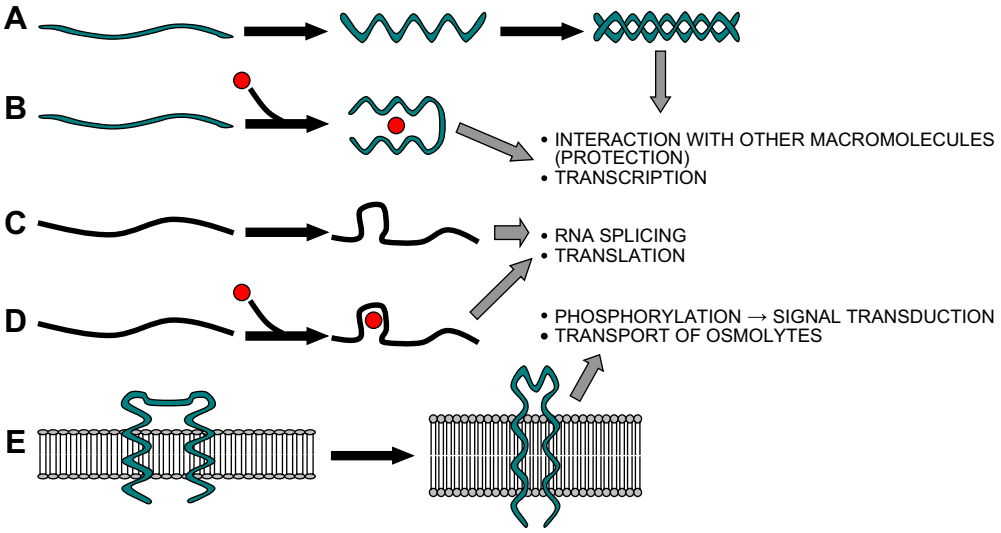

Fig. 2. Possible mechanisms for water stress sensing. Lower water potential may induce a conformational change either in pre-existing proteins such as transcription factors and/or LEA-like proteins (A) or in mRNAs encoding proteins relevant to DSR (C). In the case of proteins, the secondary structure could become more ordered and the quaternary structure could become more complex, resulting in improved biological activity. Alternatively, increased concentration of a small solute could promote the folding of either ligand-sensitive proteins (B) or aptamer mRNA domains (D) (Cheah et al., 2007), bringing about the same final responses as in A and B. Not mutually exclusive from the previous models, a loss of cell turgor (overcoming the cell wall structural support) could increase the cell membrane lateral pressure, thus triggering a conformational change of integral membrane proteins (E); this results in either transporter activity leading to influx of osmolytes or cytoplasmic kinase-mediated initiation of intracellular signal transduction.

On the other hand, transcription factors involved in drought signal transduction cannot be ruled out as primary sensors. Some of these, for instance, the DREB1 family (Liu et al., 1998), recognize known specific target enhancer sequences, such as the short DRE (desiccation-responsive element) present in rd29A (Kasuga et al., 2004). These might be early sensors, but this role has not been confirmed, as no detailed structural studies have been performed other than deletion studies. Judging from the amino acid sequence (many hydrophobic residues), such proteins do not seem to be natively unfolded; thus, a desiccation-induced LEA-like conformational change is unlikely.

A drying-sensitive master transcription factor can be conceptualized that, when folded into the proper conformation, binds to and up-regulates target genes encoding synthetic enzymes of disaccharides and ABA. The experimental confirmation of such a model would be difficult but is probably worthwhile. One such experiment, inspired by the work of Shinozaki (Seki et al., 2002; Liu et al., 1998), could consist of analyzing the kinetics of transcriptome microarray profiles, paying particular attention to patterns obtained immediately after the beginning of stress, followed by identification (i.e., by simple-hybrid cDNA libraries in yeast) of proteins that bind to the enhancer of the early-expressed genes. Another strategy could be genetics-based, similar to that followed by Zhu's group for cold stress (Ishitani et al., 1998), searching for mutants defective in reporter gene regulation by the promoter/enhancer of an upstream gene. Regulatory sequences of the DREB1A gene would constitute ideal targets for the discovery of drought-responsive master transcription factors.

7. A biotechnological dream: engineering drought tolerance

It is hard to imagine that a single gene could confer full drought tolerance, as this trait is known to be determined by multiple genes. However, despite our limited knowledge of stress-associated cell metabolism (Vinocur and Altman, 2005), single transgenes encoding transcription factors have been reported to confer improved drought resistance in the laboratory (Kasuga et al., 1999; Jeanneau et al., 2002; Yang et al., 2005; Karaba et al., 2007). Because they act very soon after the ABA level increases, they might be considered authentic master genes. Nevertheless, it is important to note that undesired phenotypes usually appear in the transgenic plants and that real and efficient tolerance in the field has not yet been convincingly reported.

8. Perspectives

More work is required to determine whether the *in vivo* expression of the molecules described herein is part of a drought stress response program or a mere display of general stress injury. Although the downstream components of the DSR have been extensively characterized, scarce information is available regarding molecules that initially trigger this response (Fig. 2). The search for individual drought-induced genes and proteins may therefore be over, as it has failed to produce a holistic picture of DSR. We thus envision that the next decade of research will provide insight into the molecular basis of both the damage caused by drought and the response mounted against it, with a particular emphasis on master factors. Biophysical methods are likely to uncover novel protein functions in plants by revealing changes in structure as a consequence of stress. An alternative approach could consist of exploring epigenetic modifications of genes involved in DSR. However, similarly to behavioral adaptations in animals (Weaver et al., 2007), epigenetic events probably represent the consequences rather than the causes of specific gene activation. Finally, new strategies designed for studies of water-deficit stress such as those proposed herein may be adopted by specialists in other areas of biological research to elucidate shared mechanisms involved in various types of stress.

Acknowledgements

We wish to thank the anonymous reviewers for their constructive comments and criticisms on a previous version of this manuscript. This work was supported by grants from Universidad de Buenos Aires (UBA), Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) and Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Argentina. J.J.C. and N.D.I. are

members of the Carrera del Investigador Científico, CONICET, Argentina.

References

Bernacchiaa, G., Furinib, A., 2004. Biochemical and molecular responses to water stress in resurrection plants. Physiol. Plant. 121, 175–181.

Billi, D., Potts, M., 2002. Life and death of dried prokaryotes. Res. Microbiol. 153, 7–12.

Blum, A., 2005. Drought resistance, water-use efficiency, and yield potential—are they compatible, dissonant, or mutually exclusive? Aust. J. Agric. Res. 56, 1159–1168.

Brodribb, T.J., Holbrook, N.M., 2003. Stomatal closure during leaf dehydration, correlation with other leaf physiological traits. Plant Physiol. 132, 2166–2173.

Brosché, M., Vinocur, B., Alatalo, E.R., Lamminmäki, A., Teichmann, T., Ottow, E.A., Djilianov, D., Afif, D., Bogeat-Triboulot, M.B., Altman, A., Polle, A., Dreyer, E., Rudd, S., Paulin, L., Auvinen, P., Kangasjärvi, J., 2005. Gene expression and metabolite profiling of *Populus euphratica* growing in the Negev desert. Genome Biol. 6, R101.

Boudet, J., Buitink, J., Hoekstra, F.A., Rogniaux, H., Larré, C., Satour, P., Leprince, O., 2006. Comparative analysis of the heat stable proteome of radicles of Medicago truncatula seeds during germination identifies late embryogenesis abundant proteins associated with desiccation tolerance. Plant Physiol. 140, 1418–1436.

Chaves, M.M., Oliveira, M.M., 2004. Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. J. Exp. Bot. 55, 2365–2384.

Cheah, M.T., Wachter, A., Sudarsan, N., Breaker, R.R., 2007. Control of alternative RNA splicing and gene expression by eukaryotic riboswitches. Nature 447, 497–500.

Cuming, A.C., Cho, S.H., Kamisugi, Y., Graham, H., Quatrano, R.S., 2007. Microarray analysis of transcriptional responses to abscisic acid and osmotic, salt, and drought stress in the moss *Physcomitrella patens*. New Phytol. 176, 275–287.

Dong, A., Prestrelski, S.J., Allison, S.D., Carpenter, J.F., 1995. Infrared spectroscopic studies of lyophilization-induced and temperature-induced protein aggregation. J. Pharm. Sci. 84, 415–424.

França, M.B., Panek, A.D., Eleutherio, E.C., 2005. The role of cytoplasmic catalase in dehydration tolerance of *Saccharomyces cerevisiae*. Cell Stress Chaperones 10, 167–170.

França, M.B., Panek, A.D., Eleutherio, E.C., 2007. Oxidative stress and its effects during dehydration. Comp. Biochem. Physiol. A Mol. Integ.r Physiol. 146, 621–631.

Frankel, N., Hasson, E., Iusem, N.D., Rossi, M.S., 2003. Adaptive evolution of the water stress-induced gene Asr2 in *Lycopersicon* species dwelling in arid habitats. Mol. Biol. Evol. 20, 1955–1962.

Galmés, J., Pou, A., Alsina, M.M., Tomàs, M., Medrano, H., Flexas, J., 2007. Aquaporin expression in response to different water stress intensities and recovery in Richter-110 (*Vitis* sp.): relationship with ecophysiological status. Planta 226 (3), 671–681.

Gazanchian, A., Hajheidari, M., Sima, N.K., Salekdeh, G.H., 2007. Proteome response of *Elymus elongatum* to severe water stress and recovery. J. Exp. Bot. 58, 291–300.

Gilles, G.J., Hines, K.M., Manfre, A.J., Marcotte Jr., W.R., 2007. A predicted N-terminal helical domain of a Group 1 LEA protein is required for protection of enzyme activity from drying. Plant Physiol. Biochem. 45, 389–399.

Goldgur, Y., Rom, S., Ghirlando, R., Shkolnik, D., Shadrin, N., Konrad, Z., Bar-Zvi, D., 2007. Desiccation and zinc binding induce transition of tomato abscisic acid stress ripening 1, a water stress- and salt stress-regulated plant-specific protein, from unfolded to folded state. Plant Physiol. 143, 617–628.

Goyal, K., Tisi, L., Basran, A., Browne, J., Burnell, A., Zurdo, J., Tunnacliffe, A., 2003. Transition from natively unfolded to folded state induced by desiccation in an anhydrobiotic nematode protein. J. Biol. Chem. 278, 12977–12984.

Goyal, K., Walton, L.J., Tunnacliffe, A., 2005. LEA proteins prevent protein aggregation due to water stress. Biochem. J. 388, 151–157.

Heschel, M.S., Riginos, C., 2005. Mechanisms of selection for drought stress tolerance and avoidance in *Impatiens capensis* (Balsaminaceae). Am. J. Bot. 92, 37–44.

Hincha, D.K., Hagemann, M., 2004. Stabilization of model membranes during drying by compatible solutes involved in the stress tolerance of plants and microorganisms. Biochem. J. 383, 277–283.

Hoekstra, F.A., Golovina, E.A., Buitink, J., 2001. Mechanisms of plant desiccation tolerance. Trends Plant Sci. 6, 431–438.

Hundertmark, M., Hincha, D.K., 2008. LEA (late embryogenesis abundant) proteins and their encoding genes in *Arabidopsis thaliana*. BMC Genom. 9, 118.

Imai, T., Harano, Y., Kinoshita, M., Kovalenko, A., Hirata, F., 2007. Theoretical analysis on changes in thermodynamic quantities upon protein folding: essential role of hydration. J. Chem. Phys. 126, 225102.

Ishibashi, K., 2006. Aquaporin subfamily with unusual NPA boxes. Biochim. Biophys. Acta 1758 (8), 989–993.

Ishitani, M., Xiong, L., Lee, H., Stevenson, B., Zhu, J.K., 1998. HOS1, a genetic locus involved in cold-responsive gene expression in *Arabidopsis*. Plant Cell 10, 1151–1161.

Jeanneau, M., Gerentes, D., Foueillassar, X., Zivy, M., Vidal, J., Toppan, A., Perez, P., 2002. Improvement of drought tolerance in maize: towards the functional validation of the Zm-Asr1 gene and increase of water use efficiency by overexpressing C4–PEPC. Biochimie 84, 1127–1135.

Karaba, A., Dixit, S., Greco, R., Aharoni, A., Trijatmiko, K.R., Marsch-Martinez, N., Krishnan, A., Nataraja, K.N., Udayakumar, M., Pereira, A., 2007. Improvement of water use efficiency in rice by expression of HARDY, an *Arabidopsis* drought and salt tolerance gene. Proc. Natl. Acad. Sci., USA 104, 15270–15275.

Kasuga, M., Liu, Q., Miura, S., Yamaguchi-Shinozaki, K., Shinozaki, K., 1999. Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. Nat. Biotechnol. 17, 287–291.

Kasuga, M., Miura, S., Shinozaki, K., Yamaguchi-Shinozaki, K., 2004. A combination of the *Arabidopsis* DREB1A gene and stress-inducible rd29A promoter improved drought- and low-temperature stress tolerance in tobacco by gene transfer. Plant Cell Physiol. 45, 346–350.

Knight, C.A., Vogel, H., Kroymann, J., Shumate, A., Witsenboer, H., Mitchell-Olds, T., 2006. Expression profiling and local adaptation of *Boechera holboellii* populations for water use efficiency across a naturally occurring water stress gradient. Mol. Ecol. 15 (5), 1229–1237.

Koster, K., 1991. Glass formation and desiccation tolerance in seeds. Plant Physiol. 96, 302–304.

Lee, K., Tirasophon, W., Shen, X., Michalak, M., Prywes, R., Okada, T., Yoshida, H., Mori, K., Kaufman, R.J., 2002. IRE1-mediated unconventional mRNA splicing and S2P-mediated ATF6 cleavage merge to regulate XBP1 in signaling the unfolded protein response. Genes Dev. 16, 452–466.

Liu, Q., Kasuga, M., Sakuma, Y., Abe, H., Miura, S., Yamaguchi-Shinozaki, K., Shinozaki, K., 1998. Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in *Arabidopsis*. Plant Cell 10, 1391–1406.

Liu, D., Zhang, X., Cheng, Y., Takano, T., Liu, S., 2006. rHsp90 gene expression in response to several environmental stresses in rice (*Oryza sativa* L.). Plant Physiol. Biochem. 44, 380–386.

Marris, E., 2008. Water: more crop per drop. Nature 452, 273–277.

Martinez, I.M., Chrispeels, M.J., 2003. Genomic analysis of the unfolded protein response in *Arabidopsis* shows its connection to important cellular processes. Plant Cell 15, 561–576.

Maskin, L., Gudesblat, G.E., Moreno, J.E., Carrari, F.O., Frankel, N., Sambade, A., Rossi, M., Iusem, N.D., 2001. Differential expression of the members of the Asr gene family in tomato (Lycopersicon esculentum). Plant Sci. 161, 739–746.

Maskin, L., Frankel, N., Gudesblat, G., Demergasso, M.J., Pietrasanta, L.I., Iusem, N.D., 2007. Dimerization and DNA-binding of ASR1, a small hydrophilic protein abundant in plant tissues suffering from water loss. Biochem. Biophys. Res. Commun. 352, 831–835.

Miyawaki, A., Llopis, J., Heim, R., McCaffery, J.M., Adams, J.A., Ikura, M., Tsien, R.Y., 1997. Fluorescent indicators for Ca2+ based on green fluorescent proteins and calmodulin. Nature 388, 882–887.

Mowla, S.B., Cuypers, A., Driscoll, S.P., Kiddle, G., Thomson, J., Foyer, C.H., Theodoulou, F.L., 2006. Yeast complementation reveals a role for an *Arabidopsis thaliana* late embryogenesis abundant (LEA)-like protein in oxidative stress tolerance. Plant J. 48, 743–756.

Oldenhof, H., Wolkers, W.F., Bowman, J.L., Tablin, F., Crowe, J.H., 2006. Freezing and desiccation tolerance in the moss *Physcomitrella patens*: an in situ Fourier transform infrared spectroscopic study. Biochim. Biophys. Acta 1760, 1226–1234.

Peters, S., Mundree, S.G., Thomson, J.A., Farrant, J.M., Keller, F., 2007. Protection mechanisms in the resurrection plant *Xerophyta viscosa* (Baker): both sucrose and raffinose family oligosaccharides (RFOs) accumulate in leaves in response to water deficit. J. Exp. Bot. 58, 1947–1956.

Potts, M., 1994. Desiccation tolerance of prokaryotes. Microbiol. Rev. 58, 755–805.

Ramanjulu, S., Bartels, D., 2002. Drought- and desiccation-induced modulation of gene expression in plants. Plant Cell Environ. 25, 141–151.

Reiser, V., Raitt, D.C., Saito, H., 2003. Yeast osmosensor Sln1 and plant cytokinin receptor Cre1 respond to changes in turgor pressure. J. Cell Biol. 161, 1035–1040.

Rensing, S.A., et al., 2008. The *Physcomitrella* genome reveals evolutionary insights into the conquest of land by plants. Science 319, 64–69.

Roberti, M.J., Bertoncini, C.W., Klement, R., Jares-Erijman, E.A., Jovin, T.M., 2007. Fluorescence imaging of amyloid formation in living cells by a functional, tetracysteine-tagged alpha-synuclein. Nat. Methods 4, 345–351.

Ron, D., Walter, P., 2007. Signal integration in the endoplasmic reticulum unfolded protein response. Nat. Rev. Mol. Cell Biol. 8, 519–529.

Sasahara, K., Yagi, H., Naiki, H., Goto, Y., 2007. Heat-induced conversion of beta(2)-microglobulin and hen egg-white lysozyme into amyloid fibrils. J. Mol. Biol. 372, 981–991.

Seki, M., et al., 2002. Monitoring the expression profiles of 7000 *Arabidopsis* genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. Plant J. 31, 279–292.

Shinozaki, K., Yamaguchi-Shinozaki, K., Seki, M., 2003. Regulatory network of gene expression in the drought and cold stress responses. Curr. Opin. Plant Biol. 6, 410–417.

Song, W.Y., Zhang, Z.B., Shao, H.B., Guo, X.L., Cao, H.X., Zhao, H.B., Fu, Z.Y., Hu, X.J., 2008. Relationship between calcium decoding elements and plant abiotic-stress resistance. Int. J. Biol. Sci. 4, 116–125.

Tolleter, D., Jaquinod, M., Mangavel, C., Passirani, C., Saulnier, P., Manon, S., Teyssier, E., Payet, N., Avelange-Macherel, M.H., Macherel, D., 2007. Structure and function of a mitochondrial late embryogenesis abundant protein are revealed by desiccation. Plant Cell 19, 1580–1589.

Tompa, P., Bánki, P., Bokor, M., Kamasa, P., Kovács, D., Lasanda, G., Tompa, K., 2006. Protein–water and protein–buffer interactions in the aqueous solution of an intrinsically unstructured plant dehydrin: NMR intensity and DSC aspects. Biophys. J. 91, 2243–2249.

Tunnacliffe, A., Lapinski, J., 2003. Resurrecting Van Leeuwenhoek's rotifers: a reappraisal of the role of disaccharides in anhydrobiosis. Philos. Trans. R Soc. Lond. B Biol. Sci. 358, 1755–1771.

Tunnacliffe, A., Wise, M.J., 2007. The continuing conundrum of the LEA proteins. Naturwissenschaften 94, 791–812.

Urao, T., Yakubov, B., Satoh, R., Yamaguchi-Shinozaki, K., Seki, M., Hirayama, T., Shinozaki, K., 1999. A transmembrane hybrid-type histidine kinase in *Arabidopsis* functions as an osmosensor. Plant Cell 11, 1743–1754.

Uversky, V.N., Gillespie, J.R., Fink, A.L., 2000. Why are "natively unfolded" proteins unstructured under physiologic conditions? Proteins 41, 415–427.

van der Heide, T., Poolman, B., 2000. Osmoregulated ABC-transport system of *Lactococcus lactis* senses water stress via changes in the physical state of the membrane. Proc. Natl. Acad. Sci. 97, 7102–7106.

van Gemeren, I.A., Punt, P.J., Drint-Kuyvenhoven, A., Broekhuijsen, M.P., van't Hoog, A., Beijersbergen, A., Verrips, C.T., van den Hondel, C.A., 1997. The ER chaperone-encoding bipA gene of black *Aspergilli* is induced by heat shock and unfolded proteins. Gene 198, 43–52.

Vinocur, B., Altman, A., 2005. Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. Curr. Opin. Biotechnol. 16, 123–132.

Weaver, I.C., D'Alessio, A.C., Brown, S.E., Hellstrom, I.C., Dymov, S., Sharma, S., Szyf, M., Meaney, M.J., 2007. The transcription factor nerve growth factor-inducible protein "a" mediates epigenetic programming: altering epigenetic marks by immediate-early genes. J. Neurosci. 27, 1756–1768.

Wingler, A., 2002. The function of trehalose biosynthesis in plants. Phytochemistry 60, 437–440.

Wise, M.J., Tunnacliffe, A., 2004. POPP the question: what do LEA proteins do? Trends Plant Sci. 9, 13–17.

Wohlbach, D.J., Quirino, B.F., Sussman, M.R., 2008. Analysis of the *Arabidopsis histidine* kinase ATHK1 reveals a connection between vegetative osmotic stress sensing and seed maturation. Plant Cell 20, 1101–1117.

Wolkers, W.F., Tetteroo, F.A., Alberda, M., Hoekstra, F.A., 1999. Changed properties of the cytoplasmic matrix associated with desiccation tolerance of dried carrot somatic embryos. An In situ Fourier transform infrared spectroscopic study. Plant Physiol. 120, 153–164.

Wolkers, W.F., McCready, S., Brandt, W.F., Lindsey, G.G., Hoekstra, F.A., 2001. Isolation and characterization of a D-7 LEA protein from pollen that stabilizes glasses in vitro. Biochim. Biophys. Acta 1544, 196–206.

Yancey, P.H., 2005. Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. J. Exp. Biol. 208, 2819–2830.

Yang, C.Y., Chen, Y.C., Jauh, G.Y., Wang, C.S., 2005. A Lily ASR protein involves abscisic acid signaling and confers drought and salt resistance in *Arabidopsis*. Plant Physiol. 139, 836–846.