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Late embryogenesis abundant proteins

Versatile players in the plant adaptation to water limiting environments

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Late Embryogenesis Abundant (LEA) proteins accumulate at the onset of seed desiccation and in response to water deficit in vegetative plant tissues. The typical LEA proteins are highly hydrophilic and intrinsically unstructured. They have been classified in different families, each one showing distinctive conserved motifs. In this manuscript we present and discuss some of the recent findings regarding their role in plant adaptation to water deficit, as well as those concerning to their possible function, and how it can be related to their intrinsic structural flexibility.

Key words: water deficit, intrinsically unstructured proteins, hydrophilins, late embryogenesis abundant proteins, drought stress

Abbreviations: ATP, adenosine triphosphate; IUP, intrinsically unstructured proteins; LDH, lactate dehydrogenase; MDH, malate dehydrogenase; LEA, late embryogenesis abundant; MPa, megapascal

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The maturation drying process of embryo development correlates with the acquisition of desiccation tolerance and with the specific induction of a set of proteins first described as Late Embryogenesis Abundant (LEA).[1] These small proteins are ubiquitous in land plants from the earliest taxonomic groups such as mosses, through gymnosperm and angiosperm species. Most of them belong to the "hydrophilins" family, a group of highly hydrophilic, intrinsically unstructured proteins (IUPs) characterized by a biased amino acid composition enriched in Gly and other small residues that favors a flexible conformation in aqueous solutions.[2,3] Although these physicochemical characteristics are widespread to most LEA proteins, differences in their amino acid sequences reveal at least seven groups.[4,5]

Many reports have described LEA proteins induction in vegetative tissues of several plant species under water deficit conditions imposed by the environment or accumulated as part of a developmental program in desiccation tolerant structures or stages (reviewed in refs. 4-6). These accumulation patterns also apply to hydrophilins found in organisms from different kingdoms that have evolved under mild or extreme environmental selective forces.[3,7] We proposed that the physicochemical properties of these proteins have served as a driving force to select proteins capable to preserve and maintain cell functions during the life cycle of organisms from the deleterious effects caused by changes in water availability.[3,8] Although some mechanisms have been proposed,[3,6,9-14] very little is known about how they carry out their functions. Which and how diverse are their targets, is there any cooperation between different LEA proteins and/or other molecules (e.g., sugars), how these proteins provide the hydrogen bonds or reorganize the available water molecules to maintain the functional structure of their ligands, are some of the questions that still remain to be answered.

As is the case for molecular chaperones, the function of LEA proteins has been approached by in vitro assays, where partial dehydration was imposed to enzymes (LDH, MDH, catalase, fumarase, rhodanase) in the presence or absence of LEA proteins from groups 2–4 and groups 6 and 7 belonging to different plant species.[11,15-18] LEA-like proteins from non-plant groups and other hydrophilins, such as bacterial or yeast hydrophilins, and a nematode hydrophilin with a similar 11-mer amino acid repeat found in the group 3 LEA proteins have been tested in vitro as well.[9,11] The results from these experiments have shown that these proteins are able to prevent conformational changes in reporter

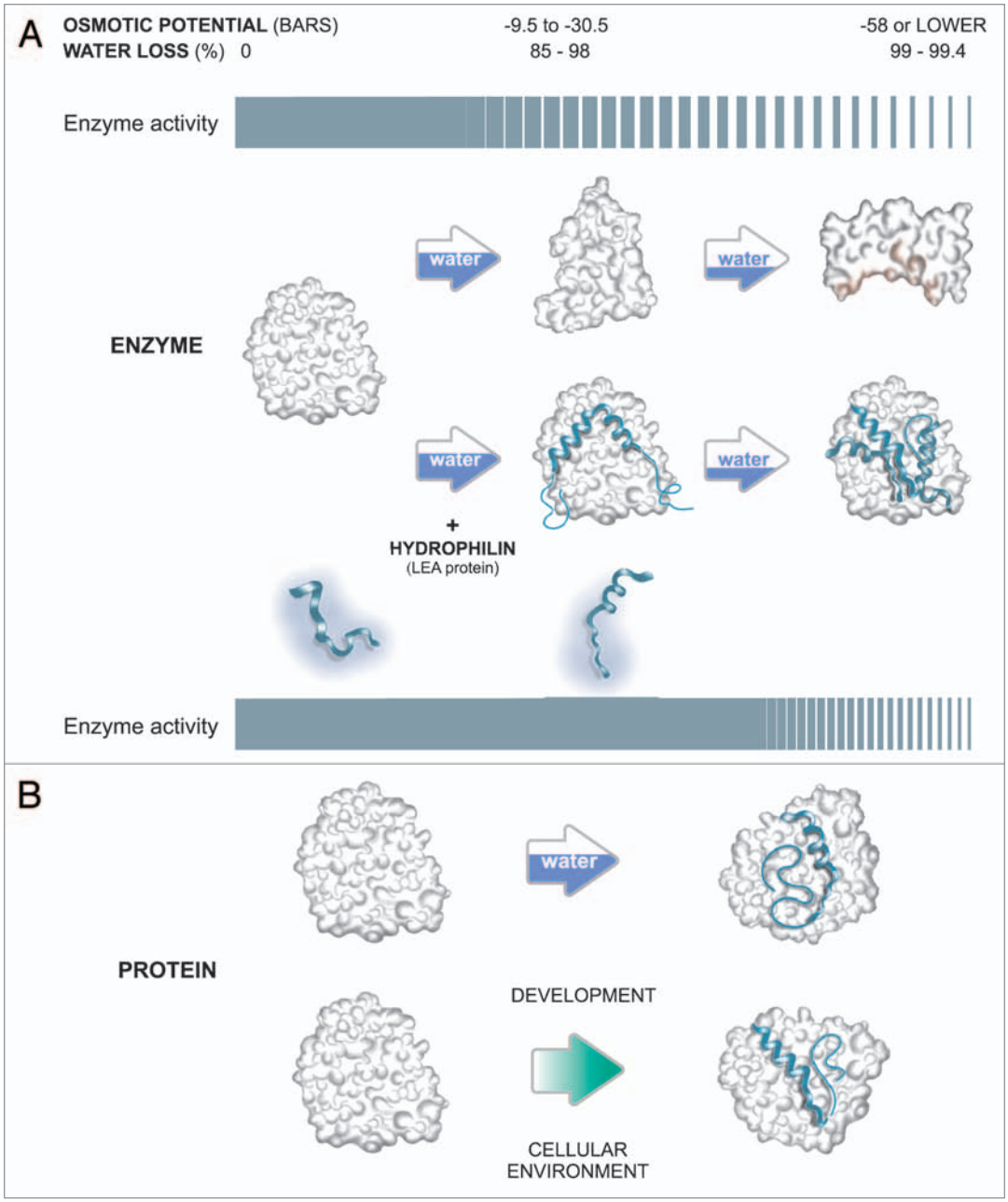

Figure 1. (A) This scheme illustrates a hypothetic model for the function of LEA proteins and other hydrophilins. In this example, under moderate water deficit, an enzyme suffers conformational changes that lead to a decrease in its activity and, under more severe stress conditions, more critical structural modifications lead to the exposure of hydrophobic residues (red shadowing). The presence of LEA proteins (hydrophilins) (green strand) prevents changes in the conformation of the enzyme, as a result of which the enzyme retains its activity, under water limitation conditions. This effect can be achieved at a 1:1 hydrophilin:enzyme ratio under moderate water stress; however, under severe dehydration, the action of more than one hydrophilin per enzyme molecule could avoid further conformational changes that may lead to protein aggregation. Hydrophilins or LEA proteins may be unstructured under optimal water availability and upon water limitation would acquire a conformation depending on the stress levels and/or on the presence of ligands. (B) Under certain environmental conditions, LEA proteins or other hydrophilins could act as molecular "knobs" able to modulate the activity of their ligands by inducing light structural modifications upon binding. These environmental conditions could be a particular cellular environment during development and/or those imposed by nature.

enzymes, induced by water limitation conditions, which led to loss of their activity and, under more severe water restriction to denaturation and consequently to protein aggregation (**Fig. 1A**). This activity does not use ATP and, in contrast to typical molecular chaperones (i.e., heat shock proteins), if added after the dehydration, it is unable to recover the native conformation or the activity of the reporter enzymes. Although many of these experiments have been carried out using an excess of LEA proteins relative to the target enzyme (5:1–100:1); it has also been shown that LEA proteins are able to prevent inactivation of

target enzymes even in a 1:1 ratio, suggesting that protein-protein interactions are necessary for their function to be accomplished (**Fig. 1A**).[11]

The predicted unstructured and/or malleable nature of LEA proteins in aqueous solution envisaged structural modifications upon environmental changes, which have been shown in some cases by in vitro experiments where low water availability environments were able to induce steady secondary structures.[9,19-23] As it has been proposed for the IUPs, this structural plasticity may be necessary for the appropriate recognition of their ligands and/or for the stabilization of these interactions, and it may explain why LEA proteins from different groups, with no sequence similarity, can carry out similar function in vitro. If so, then how can we explain the highly conserved and specific motifs for each group, and moreover why have different groups of LEA proteins been maintained throughout evolution? It is possible that some of these motifs are implicated in the recognition and binding to a specific set of molecular targets and/or in the establishment of a spatial conformation needed to carry out their function with specific sets of ligands. Accordingly, the participation of conserved motifs in the protective function of group 2 and 4 LEA proteins was suggested by freeze-thaw in vitro experiments with mutant versions of these proteins lacking some of the conserved sequences and comparing their activity to their corresponding wild-type forms.[12] Also, these conserved sequences may play a role in the establishment of homo- or hetero-oligomers by facilitating and/or stabilizing the interaction with themselves or with LEA proteins from the same or different groups. The detection of LEA proteins (a group 6 LEA protein from common bean[24] and one Arabidopsis group 4 LEA protein [AtLEA4-2],[8]) in high molecular mass complexes in cell extracts obtained from stressed plants supports this idea. Whether the formation of higher-order structures is part of the mechanism through which LEA proteins achieve their protective function constitutes another challenging question.

Most of the evidence concerning the participation of LEA proteins in plant tolerance to water limiting conditions has been obtained by overexpression experiments (reviewed in ref. 4), yet these data should be taken with caution since ectopic expression may be a misleading evidence for the function of the endogenous protein. However, recently, direct evidence was obtained by carrying out a phenotypical analysis of Arabidopsis plants deficient in group 4 LEA proteins.[8] In this work, it was shown that plants deficient in one, two or the three members of this group are more susceptible than wild-type plants to water deficit. The effect of the absence of these proteins was more evident during germination under hyperosmotic (350 mM mannitol) and high salt concentrations (250 mM NaCl), and during recovery after severe drought treatments (-4.6 ± 0.6 MPa in the substrate) applied to adult plants in the flowering stage. Interestingly, mutant plants not only showed a lower biomass accumulation but also a reduced ability to produce axillary and floral buds when compared to wild-type plants. When Arabidopsis plants at the flowering stage were subjected to a fast and severe water deprivation (-6.45 ± 0.57 MPa in the substrate), rehydrated and allowed to recover, they were unable to produce axillary and floral buds. In contrast, Arabidopsis plants overproducing one of group 4 LEA proteins (*35S*::*AtLEA4-5*::*NOS*) had an increased capacity to recover and generate competent buds upon this treatment when compared to wild-type plants.[8]

Even though the accumulation of this particular LEA protein group was not analyzed in meristematic regions, a high abundance of other LEA proteins has been detected in plants grown under optimal irrigation, in apical and lateral root meristems, as in the case of a group 6 LEA protein from common bean[24] and a group 2 LEA protein in dormant buds from apricot trees during winter.[25] This genetic evidence led to propose that these proteins protect the integrity of meristematic cells and other tissues essential for plant reproduction from the negative effects of water limitation.[8] Accordingly, plants deficient in one of these group 4 LEA proteins exhibit a reduced seed production after recovery from stress in terms of seed numbers albeit not of biomass.[26] A similar result was obtained for plants grown under optimal irrigation, suggesting a shielding role for these proteins on the plant cellular processes required for normal fruit and/or seed development. These new discoveries necessarily lead to new questions: Which cell types could need the presence of LEA proteins under normal plant development? Do these proteins possess additional functions other than preventing the loss of protein native conformations under water limitation, or are there any changes in the water status of plant cells during development that represent a stressful situation for adequate protein function?

Although LEA proteins appear to carry out similar functions, the existence of distinctive families is indicative of functional diversity. Moreover, the fact that the deficiency of different members of group 4 LEA proteins led to detectable phenotypes, and that this is not compensated by other groups of LEA proteins supports this idea.[8] This functional diversity could be related to their particular ligands, or to the characteristics of the cellular microenvironment where they are active, given by the cell type, the tissue or the stress condition, the developmental stage and so forth (see **Fig. 1B**). Functional redundancy does not seem to apply even among members of the same group, since the absence of one, two or the three members of the group 4 LEA proteins led to a susceptible phenotype under water deficit, which also agrees with their differential protein accumulation patterns.[8] This is in tune with the idea that LEA proteins could be performing different functions in different cellular processes in a tissue- or developmental-specific manner, in response to particular stress levels or combination of stressful stimuli, ultimately providing higher plants with mechanisms to adapt to changes in water availability.

Although, as mentioned above, some clues concerning the function of these proteins have been discovered, how the described activities are translated in vivo or whether they are able to perform additional or alternative functions still awaits an answer. To approach these questions one should keep in mind their intrinsically unstructured nature and, therefore, their predictable high structural flexibility. Now, we know that proteins that are intrinsically unstructured are broadly represented in eukaryotes and that they play relevant roles in different cellular processes.[27]

Interestingly, a number of IUPs are integral components of protein complexes suggesting that molecular recognition is involved in their functional mechanism.[28] For some IUPs, it is known that they are disordered under physiological conditions and acquire a defined conformation upon binding to their cellular targets,[29] and for plant IUPs, such as the typical LEA proteins, some results indicate that they acquire certain ordered conformation under low water availability environments.[16,21,23,30,31] When compared with globular or structurally ordered proteins, the structural dynamism that IUPs such as LEA proteins and other hydrophilins exhibit is suggestive of novel functional molecular mechanisms as well as of accomplishment of multiple functions. Moonlighting in LEA proteins is conceivable if they could bind to more than one partner, if they bind to the same partner in different modes (**Fig. 1B**), or if they undergo changes in their intracellular or cell type localization, variations in oligomerization, structural changes due to environmental fluctuations, etc. This characteristic would allow them not only to protect their ligands from the effects of low water availability but also modulate the action of different partner molecules (**Fig. 1B**).

The remarkable high correlation between the accumulation of hydrophilins and water deficit in organisms of all taxonomic kingdoms that suggests that their unstructured nature is essential for their function under such conditions, and the identification of conserved motifs in each family are characteristics that present LEA proteins as a paradigm for the study of IUPs. Clearly, there is still much to know about the structural properties and organization of these proteins and, even more important, about how they are related to their function. Given the complexity of the functional scenario for these proteins, resourceful experimental approaches will be needed to elucidate their in vivo function.

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