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How do Animal Mitochondria Tolerate Water Stress?

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Occurrence of mitochondria-targeted LEA gene in animals increases organelle resistance to water stress.

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Abstract

The vast majority of animal species do not tolerate severe water stress, but the encysted embryo of the brine shrimp *Artemia franciscana* is an exceptionally useful organism to investigate physiological mechanisms for enduring extreme environmental insults. Any substantial reduction in cellular water poses a threat to survival. Nevertheless anhydrobiotic animals survive virtually complete loss of cellular water. The mechanisms that govern "life without water" (anhydrobiosis) are still not well understood. With certain exceptions, it seems that a recurring strategy for tolerating severe water loss involves the accumulation of both low molecular weight solutes (e.g. trehalose or other polyol) and highly hydrophilic macromolecules such as Late Embryogenesis Abundant (LEA) proteins, which were first described about 20 years ago in plant seeds. New studies show that LEA proteins found in animals not only protect proteins in the cytosol during desiccation, but also confer resistance to water stress, including freeze tolerance, to the mitochondrion.

TEXT

Invertebrates from several different phyla including nematodes, rotifers, tardigrades, and arthropods are able to survive reversible dehydration down to 2% tissue water.[1,2,3] An important animal for the study of animal desiccation tolerance is the encysted embryo of the brine shrimp *Artemia franciscana*. It is among the most stress resistant life-history forms in the animal kingdom and might be considered an animal extremophile *par excellence*.[4] Brine shrimp experience desiccation and temperatures below freezing during their normal life cycle. Wholesale removal of cellular water serves to aggregate macromolecules, disintegrate subcellular structures in non-adapted cells, and suspend metabolism due to removal of the water layer around proteins and

membranes.[5] Freezing at rapid cooling rates may cause cellular injury by intracellular ice formation, whereas slow rates can cause severe dehydration and unfolding of proteins due to increased salt concentrations in the unfrozen fraction of the remaining water.[6]

Lessons from Brine Shrimp

Tolerance to water stress in *A. franciscana* is most likely governed by several different mechanisms that are in place to protect cells and tissues during water loss, as well as to repair injuries after rehydration. Biochemical strategies in brine shrimp include the accumulation of high concentrations of the protective solute trehalose,[7] increased expression levels of several types of small heat shock proteins (e.g. p26, Hsp 21, Hsp 22),[8,9,10] and Late Embryogenesis Abundant (LEA) proteins[11]. LEA proteins were first described in orthodox (non-recalcitrant) seeds where their accumulation correlates with desiccation tolerance of the developing plant embryo. These proteins have been proposed to act as a hydration buffer by sequestering ions and by stabilizing other proteins and membranes via direct interaction.[12,13] Additional functions may include formation of structural networks and stabilization of sugar glasses.[14,15] In order to survive water stress it is mandatory to protect the integrity of the outer plasma membrane and to preserve the form and function of intracellular organelles such as the mitochondrion. Protection of the mitochondrion during water stress in brine shrimp embryos is governed by at least one organelle-specific LEA protein (AfrLEA3m), which in all likelihood works synergistically with to the non-reducing sugar trehalose.[16]

Anhydrobiotic Plants and Animals: The Same on the Inside?

LEA proteins found in plants can be grouped into 6 superfamilies of which Group 3 protein homologues have also been found in nematodes and Group 1 and 3 in arthropods. *In silicio*-predicted subcellular localizations for plant group 3 LEA proteins include cytosol, ER, chloroplast, and the mitochondrion[17, 18, 19]. Direct experimental evidence for a mitochondria-targeted plant Group 3 LEA protein (PsLEAm) was presented by Grelet et al.[20]. PsLEAm significantly reduced desiccation injury in mitochondrial enzymes and was later shown to protect from membrane fusion liposomes that were air-dried and subsequently rehydrated [13]. The recently discovered gene *AfrLEA3m* from *A. franciscana* [16] encodes a 307 amino acid polypeptide that is highly enriched in hydrophilic and charged residues. The mRNA expression levels of *AfrLEA3m* are several folds higher in desiccation tolerant embryos than in intolerant larvae. The polypeptide belongs to the family of Group 3 LEA proteins and represents the first example of an organelle-targeted LEA protein in animals. Biochemical studies to elucidate its function are underway and it remains to be seen if AfrLEA3m, similar to the plant homolog, stabilizes both proteins and membranes during desiccation. Analogous to plants, the finding that LEA proteins in anhydrobiotic animals are targeted to different subcellular compartments suggests that similar strategies are employed to enable anhydrobiosis in both kingdoms. Therefore, it is quite tempting to speculate that a LEA protein targeted to the endoplasmic reticulum still awaits its discovery in an anhydrobiotic animal. However, intracellular compartments other than the mitochondrion could be stabilized by non-LEA proteins such as p26, which can be found in the nucleus of encysted brine shrimp embryos[21]. Table 1 gives an overview of proteins in *A. franciscana* that serve possible roles in resistance to water stress and their subcellular locations.

Future Directions for Biostabilization

We found that mammalian hepatoma cells that express a chimeric protein composed of the first 70 N-terminal amino acids of AfrLEA3m and green fluorescence protein readily incorporate the construct into their mitochondria[16]. Recently we confirmed this finding for a construct composed of the complete LEA protein fused to a blue fluorescence protein (unpublished observations). These results demonstrate the highly conserved nature of the protein import machinery for mitochondria from mammalian and invertebrate cells, and indirectly, of the targeting sequence as well.

If isolated mammalian mitochondria are frozen in the presence of trehalose (trehalose present outside but not inside the matrix) some functions of the outer membrane are maintained, but pivotal bioenergetic functions of the inner membrane are compromised[22]. Rat liver mitochondria that are loaded with trehalose in the matrix (i.e., trehalose present on both sides of the inner membrane) show significantly higher inner membrane integrity after desiccation than those without trehalose loading. Still, irreversible damage occurs at water contents below 0.2 g water/g solids[23]. These result demonstrated that to confer complete desiccation tolerance to a complex structure such as the mitochondrion takes more than optimized loading of trehalose. AfrLEA3m may not be the magic bullet in engineering desiccation tolerance. Nevertheless, the opportunity to investigate the impact of combining AfrLEA3m and trehalose for biostabilization of mitochondria might bring us one step closer to the exciting possibility of engineering desiccation tolerant mammalian cells and tissues.

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Table 1

Proteins with possible roles in resistance to water stress and/or recovery afterwards in *A. franciscana*.

Protein	**Acc. No***	**Class**	**Localization$**	**Reference**
AfrLEA1	ACA47267	Group 3 LEA	Cytosol (*Is*)	11
AfrLEA2	ACA47268	Group 3 LEA	Cytosol (*Is*)	11
AfrLEA3m	ACM16586	Group 3 LEA	Mitochondrion (*Ex*)	16
Group 1 LEA	ABR67402	Group 1 LEA	Cytosol (*Is*)	17
p26	ABX89317	Small HSP[1]	Nucleus, Cytosol (*Ex*)	21
Artemin	AAL55397	Ferritin family	Cytosol (*Ex*)	24
Hsp 21	ABD19712	Small HSP[1]	Cytosol (*Ex*)	9
Hsp 22	ABD19713	Small HSP[1]	Nucleus (*Ex*)	8

$*Is* = *in silicio* predicted, *Ex* = experimental

[1]HSP = heat shock protein

* = NCBI accession number