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Introduction to desiccation biology: from old borders to new frontiers

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Abstract

Main conclusion **A special issue reviews the recent progress made in our understanding of desiccation tolerance across various plant and animal kingdoms.**

It has been known for a long time that seeds can survive near absolute protoplasmic dehydration through air drying and complete germination upon rehydration because of their desiccation tolerance. This property is present both in prokaryotes and eukaryotes across all life kingdoms. These dry organisms suspend their metabolism when dry, are extremely tolerant to acute environmental stresses and are relatively stable during long periods of desiccation. Studies aiming at understanding the mechanisms of survival in the dry state have emerged during the past 40 years, moving from in vitro to genomic models and comparative genomics, and from a view that tolerance is an all-or-nothing phenomenon to a quantitative trait. With the prospect of global climate change, understanding the mechanisms of desiccation tolerance appears to be a promising avenue as a prelude to engineering crops for improved drought tolerance. Understanding desiccation is also useful for seed banks that rely on dehydration tolerance to preserve plant genetic resources in the form of these propagules. Articles in this special issue explore the recent progress in our understanding of desiccation tolerance, including the evolutionary mechanisms that have been adopted across various plant (algae, lichens, seeds, resurrection plants) and animal model systems (*Caenorhabditis elegans*, brine shrimp). We propose that the term desiccation biology defines the discipline dedicated to understand the desiccation tolerance in living organisms as well as the limits and time constraints thereof.

Keywords Anhydrobiosis · *Arabidopsis* · *Artemia* · *Caenorhabditis* · *Craterostigma* · Evolution · Heat shock factor · LEA proteins · Longevity · Oxidative stress · *Polypedilum* · *Ulva* · Seed · Transcriptome · *Trebouxia* · *Xerophyta*

Abbreviations

HSP	Heat shock protein
HSF	Heat shock factors
LEA	Late embryogenesis abundant
RH	Relative humidity
TRG	Taxonomically related gene

Introduction

It is common knowledge that water is essential for life. Far from being an inert solvent, water is pivotal to the structure, stability and dynamics of proteins, membranes and nucleic acids. Water guides conformational changes in molecular structure and mediates recognition by discriminating between specific and nonspecific binding (Saenger

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1987; Pocker 2000; Levy and Onuchic 2006). Thus, one of the greatest challenges for all living things is to grow and reproduce in an atmosphere that is very dry in many terrestrial regions. Indeed, upon drying below 90 % relative humidity (RH), desiccation-sensitive cells and organisms suffer damage to all main cell components. Proteins lose their correct conformation and can form toxic aggregates (Prestrelski et al. 1993; Goyal et al. 2005; Tapia and Koshland 2014). Membranes undergo phase transitions causing their leakiness and fusion (Crowe et al. 1992; Hoekstra et al. 2001). RNA and DNA undergo structural rearrangements and fragmentation, and chromatin stability is compromised (Leprince et al. 1995; Faria et al. 2005; Potts et al. 2005; Gusev et al. 2010; Dinakar and Bartels 2012). Also the stress imposed by the loss of water leads to oxidative damage because drying occurs in the presence of oxygen in cells where high rates of electron transport activities are conducive of generating reactive oxygen species (Colville and Kranner 2010; Miller et al. 2010).

Nevertheless, there exist biological mechanisms allowing a small proportion of organisms to survive essentially complete desiccation, which results in dry but viable tissues, containing as little as 0.1 % of their hydrated water content. When water becomes available again, they rapidly imbibe and resume metabolism. In practical terms, desiccation tolerance is defined as the ability to survive drying to equilibrium with air at RH of 50 % (to approximately −100 MPa, Bewley 1979; Gaff and Oliver 2013). The dry state results in the cessation of metabolism and the resulting dry cytoplasm is so viscous that it resembles a solid wherein molecular movements on a nanometer scale are severely limited (Clegg 2001; Buitink and Leprince 2008; Ballesteros and Walters 2011; Walters 2015) and there is little or no ATP present (Leprince and Hoekstra 1998; Erkut and Kurzchalia 2015; Hand and Menze 2015).

Desiccation-tolerant organisms are present in all kingdoms, both in pro- and eukaryotes, including micro-organisms, plants and lower animals. In bacteria, desiccation tolerance has been encountered among many different forms such as akinetes, exospores, myxospores, cysts as well as vegetative cells, the most desiccation-tolerant forms tending to be Gram positive (Potts 1994; Potts et al. 2005). One the most studied species as a model is *Nostoc commune*, a filamentous cyanobacterium that is conspicuous on exposed limestone of karst regions (Billi and Potts 2002; Potts et al. 2005). In fungi, studies on desiccation tolerance have focused mainly on the baker's yeast (Dupont et al. 2014), which has turned out to be a powerful genomic model to test in vivo mechanisms of desiccation tolerance that were previously characterized in vitro (Calahan et al. 2011; Tapia and Koshland 2014). In lower plants, desiccation tolerance occurs in terrestrial macroalgae, quite frequently in bryophytes (Oliver et al. 2005) and apparently in all lichens (Candotto Carniel et al. 2015; Kranner et al. 2008), but only seldomly in pteridophytes (Bewley 1979; Alpert 2005). It is very common in pollens and seeds of Angiosperms. Desiccation tolerance occurs even in leaves and roots of the so-called resurrection plants of which about 300 species have been discovered (reviewed by Gaff and Oliver 2013; Farrant et al. 2015; Giarola and Bartels 2015). In the animal kingdom, desiccation tolerance commonly occurs in three phyla: nematodes (e.g., *Aphelenchus avena* and the dauer stage of the genetic model species *Caenorhabditis elegans*; Erkut et al. 2013), rotifers (Tunnacliffe and Lapinski 2003) and tardigrades (Wełnicz et al. 2011). It is also present in juvenile stages of two additional phyla: the encysted embryo stage of some crustaceans, including the brine shrimp Artemia sp (Crowe 1971; Clegg 2001) and the larvae of a non-biting midge, *Polypedilum vanderplanki* (Gusev et al. 2010, 2014).

A special issue to celebrate recent achievements in our understanding of desiccation tolerance and a highlight of future challenges

This special issue is the result of a workshop entitled "New Frontiers in Anhydrobiosis" that was held in 2014 in Pornichet, France. The workshop enabled scientists from different disciplines (molecular biology, ecology, food science, plant and animal physiology, microbiology, evolutionary biology) to share their progress and exchange ideas on future prospects regarding desiccation tolerance in various kingdoms. It was the latest in a series of meetings that have been organized from the moment when the main hypotheses explaining the mechanisms of desiccation tolerance were forwarded. Such meetings covering both desiccation-tolerant pro- and eukaryotes were held in 1978, 1985 and 2005 and led to the publication of several proceedings (Crowe and Clegg 1978; Leopold 1986; Alpert 2005). A summary of the topics addressed at these meetings is in Fig. 1 to show how research into desiccation tolerance has evolved over the past 40 years. We have invited a number of colleagues to submit papers to this special issue on desiccation tolerance to illustrate some aspects of the topics that were addressed. This special issue is not meant to deliver a comprehensive view of our understanding of desiccation tolerance or to capture the full scientific program of the workshop (see the brief overview in Fig. 1). Instead, it provides selected integrated views illustrating various facets of the mechanisms of desiccation tolerance and emphasizes the recent progress made using functional genomics approaches and transcriptome data mining. Since desiccation tolerance occurs in unrelated phyla, this special issue features both animals and plants

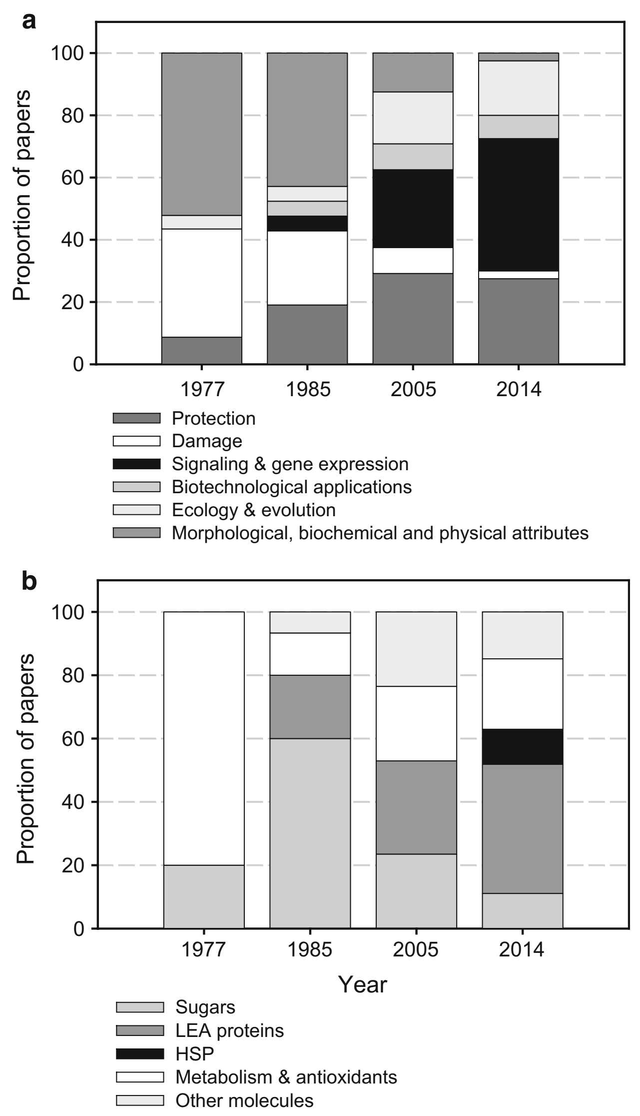

Fig. 1 The evolution of research topics on desiccation tolerance across all organisms that have been studied during the past 40 years. Analyses were performed using key words and abstracts of the proceedings of respective meetings held at the indicated year and published in Crowe and Clegg (1978), Leopold (1986) and Alpert (2005). Data for 2014 were taken from the book of abstracts of the conference entitled "New Frontiers in Anhydrobiosis" that was held in Pornichet, France. **a** Relative contributions of the different features of desiccation tolerance. **b** Relative research efforts devoted to the putative molecular compounds involved in desiccation tolerance

and illustrates that life in the dry state involves remarkably similar mechanisms among living organisms. In this introduction, we will present a brief history of research on desiccation tolerance and argue for a better term than "anhydrobiosis" to describe this field of study. Then, we will highlight the main observations in the papers published in this special issue in the framework of our current knowledge of desiccation tolerance.

A brief history of desiccation tolerance

The most ancient record recognizing the importance of the dry state in biology is probably that of Theophrastus of Lesbos around 370 BC. The Father of Botany taught in the Athenian school and described in details the conditions necessary to keep dry seeds alive and for how long they can be stored. The narrative translated by Sir Arthur Hort (1916) is as follows:

> "[…] It appears that soil and climate make a difference as to whether the seed gets worm-eaten or not, at least they say that at Apollonia on the Ionian Sea beans do not get eaten in this way at all, and therefore they are put away and stored; and about Cyzicus they keep an even longer time. It also makes a great difference to keeping that the seed should be gathered dry, for then, there is less moisture in it. […].
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> […] For propagation and sowing generally seeds one year old seem to be best; those two or three years old are inferior, while those kept a still longer time are infertile, though they are still available as food. For each kind as a definite period of life in regard to reproduction. However, these seeds too differ in their capacity according to the place in which they are stored. For instance, in Cappadocia at a place called Petra they say that seed remains even for forty years fertile and fit for sowing, whole as food it is available for sixty or seventy years; for that it does not get worm-eaten at all like clothes and other stored-up articles, for that the region is, apart from this, elevated and always exposed to fair winds and breezes […]."

Another example illustrating that plant desiccation tolerance was part of common knowledge comes from the use of *Myrothamnus flabellifolia*, a resurrection plant used in traditional medicine in Southern Africa (reviewed in Moore et al. 2007). In Zulu language and folklore, this bush is called 'uvukwabafile', which means "wakes from the dead". This plant, probably the largest known desiccation-tolerant organism, was rediscovered in the early 1900 by FE Weiss. He was amazed by the so-called "miraculous" reviviscence upon rehydration of what appeared to be a dried, dead branch (Weiss 1906). FE Weiss was probably unaware that the first annotated desiccation-tolerant organisms were dried rotifers that resurrected in the presence of water (Keilin 1959; Tunnacliffe and Lapinski 2003), as observed by van Leeuwenhoek in 1702 using his invention of the microscope. Initially, however, van Leeuwenhoek did not think that they were alive in the dry state and was surprised that within an hour of them being wetted they were active (quoted by Alpert and Oliver 2002;

Tunnacliffe and Lapinski 2003). The next 150 years after van Leeuwenhoek's observation saw a vigorous debate between zoologists and philosophers who disputed each other findings—or beliefs—as to whether some organisms are able to survive desiccation and resurrect upon rehydration. This debate was fierce and is well documented in the literature (see references in Keilin 1959; Crowe 1971; Tunnacliffe and Lapinski 2003).

Perhaps, the first scientist to experimentally establish that life can exist in the dry state was Spallanzani who confirmed in 1776 that rotifers are desiccation tolerant following slow drying and can revive after 4 years in dry storage (quoted by Leopold 1986; Tunnacliffe and Lapinski 2003). Duchartre (1852) demonstrated that is was possible to dry immature seeds to a water content equivalent to that of mature seeds without killing them. He saw in this discovery a technological breakthrough to speed up the plant life cycle, but did not address the mechanisms of tolerance. The extraordinary stability of dry seeds started to be documented around 1850 (reviewed by Keilin 1959; Priestley 1986), although there was little understanding of the conditions necessary to keep them alive in the dry state. These first accounts of longevity led a visionary botanist, William Beal, to launch in 1879 a seed storage experiment with the intent of determining the length of time that seeds of some common plants would remain alive during storage in sealed vials. This study, which is still in progress, is probably the longest continuously monitored viability experiment (see the latest viability data in Telewski and Zeevaar 2002). An important milestone in the recognition of desiccation tolerance was the publication by Bernard (1878), the first scientist who noticed that desiccation tolerance was widespread among plants and animals. He demonstrated that removing water is an important factor in bringing the organism into a state of so-called "chemical indifference" and considered that desiccation-tolerant organisms belong to a different category of living things. Throughout the second half of the 19th century, experiments on desiccation tolerance were aimed at addressing one main question: whether or not life is a discontinuous process since no signs of life or metabolism could be observed in the dry state. Dry seeds were used, probably because they were a convenient material to obtain and survival was easy to monitor: if they survived, they germinated. The rationale of these experiments was to subject dry seeds to conditions known to arrest metabolism or to kill living organisms such as incubating them in pure oxygen or noxious gases (Romanes 1893) or at low temperatures (at −53 °C for several weeks; De Candolle 1895) or by plunging them in liquid nitrogen whose method of production had just been invented (Brown and Escombe 1897). None of these treatments were effective in killing those seeds, which led to the suggestion that dry organisms truly represented a discontinuity in life.

The review by Keilin (1959) on anhydrobiosis stimulated the first studies aimed at unraveling the mechanisms of desiccation tolerance by leading researchers such SJ Webb, JS Clegg, JH Crowe and LM Crowe, mainly working on the brine shrimp *Artemia*. Using biochemical and biophysical approaches, these authors posited the role of polyols as a means to replace water molecules in the dry state (Crowe 1971; Crowe et al. 1992). In animals, yeasts and some resurrection plants (Fernandez et al. 2010), this was attributed to the disaccharide trehalose. Recent evidence obtained in anhydrobiotic nematodes shows that trehalose is absolutely required for their survival in the dry state (Erkut et al. 2011). Correlations between sugar content and desiccation tolerance in plants—first in pollens, and then seeds and additional resurrection plants (Hoekstra et al. 2001)—led to suggestion that sucrose and oligosaccharides were acting as surrogates of trehalose. The role of trehalose in particular received much attention in the popular press and even led to fantastic allegations (reviewed in Crowe 2007). Indeed, the sugar alone has proven to be remarkably useful in preserving biomolecules and even intact cells in vitro (Crowe 2007). From these observations and the publicity around the amazing protective effects in vitro, it was extrapolated in the literature that trehalose alone could confer desiccation tolerance. However, trehalose or non-reducing sugars alone are not sufficient to preserve whole, intact organisms in vivo (Ooms et al. 1993; Tunnacliffe and Lapinski 2003; Ma et al. 2005; Dinakar and Bartels 2013), suggesting the need of non-based disaccharide mechanisms.

Among non-based disaccharide mechanisms, the contribution of antioxidants to desiccation tolerance had already been suggested in the 60s by Heckly and collaborators, following studies on lyophilized bacteria that exhibited an oxygen-dependent accumulation of free radicals during dry storage (Heckly and Dimmick 1968). In the 70s, Bewley and collaborators, working on the desiccation-tolerant moss *Tortula ruralis*, suggested that repair mechanisms are important during rehydration from the dry state (Bewley 1979). Late embryogenesis abundant proteins (LEA) were discovered during the molecular characterization of cotton seed development by Dure et al. (1981) and their role in desiccation tolerance was first proposed by McCubbin et al. (1985) based on the physico-chemical characterization of the purified LEA Em protein from wheat germ. With the progress of sequencing technologies, LEA homologues were discovered in other desiccation-tolerant organisms, first in resurrection plants and pollens, then in microbes and more recently in animals. Finally, an important step forward in our understanding of survival in the dry state occurred in the mid-80s when Burke (1986) suggested that glasses could be formed from cell solutes like sugars in dry anhydrous organisms. “Biological glasses” was a concept borrowed from physics and physical chemistry where it was used to explain unusual thermodynamical properties of supercooled liquids. It was, therefore, proposed that glasses might act to fill spaces in a cell during dehydration and that their high viscosity prevents chemical reactions that require molecular diffusion (Buitink and Leprince 2008; Walters 2015).

Moving from “anhydrobiology” to “desiccation biology”

Studies on desiccation tolerance in the late 19th century led to the concept of anhydrobiosis, a term coined by Giard in 1894 (quoted by Tunnacliffe and Lapinski 2003) to refer specifically to the state of suspended animation achieved in the dry state. We propose the term “desiccation biology” instead of anhydrobiology to encompass the breath of studies aimed at unraveling the complexity of adaptive strategies underlying desiccation tolerance across living organisms as well as the limits and time constraints thereof. There are several arguments in favor of “desiccation biology” that are developed in this issue and summarized as follows. There is an increasing number of examples where the water content limit for survival lies between 10 and 20 %, which is far below that considered as water stress and yet above what truly desiccation-tolerant organisms can tolerate (see Dussert et al. 2004 for an interesting example in coffee species). This is illustrated in this issue by Walters for seeds that have lost their desiccation tolerance (i.e., are recalcitrant) during evolution, or can tolerate limited drying depending on their environmental conditions (i.e., have intermediate characteristics between those of recalcitrant and truly desiccation-tolerant seeds) and by Candotto Carniel et al. (2015) for the two symbiotic organisms that form a desiccation-tolerant lichen, one being more tolerant than the other. Understanding the reasons why recalcitrant and intermediate species exhibit various levels of desiccation sensitivity during drying will be helpful to understand desiccation tolerance.

Furthermore, anhydrobiosis does not take into account the “time” component of desiccation tolerance, including the rate of water loss and the longevity in the dry state. Although the dry state brings extraordinary stability to anhydrobiotes, their longevity varies from a few hours to millennia (Crowe 1971; Priestley 1986). The importance of this “time” factor is discussed here by Walters (2015) who argues that discrete categories of seed storage stability in the dry state can be explained through structural and molecular mobility responses to drying within cells. Data on drying human and insect cells presented by Hand and Menze lend support to this hypothesis. While desiccation tolerance in these sensitive cells can be achieved artificially with appropriate preconditioning and loading of protective compounds, these dried cells die within minutes in the dry state. The time component is also reflected in the regulatory mechanisms that are necessary to induce desiccation tolerance before drying and during repair upon rehydration. For example, desiccation tolerance and longevity in seeds in the dry state appear to be acquired in a non-synchronous manner during development and involve different regulatory processes (Chatelain et al. 2012; Verdier et al. 2013). In this issue, Costa et al. (2015) have been able to uncouple desiccation tolerance from storability in germinated radicles of Arabidopsis.

New insights into the mechanisms leading to desiccation tolerance

By presenting a mix of reviews and new experimental evidence across plant and animal kingdoms, this special issue illustrates the myriad of adaptations that are required for survival in the dry state. Despite the diversity, complexity and evolutionary position of these many desiccation-tolerant organisms, these adaptations can be readily classified into five categories that are not mutually exclusive. Using this framework, we summarize below the general topics forming this special issue, but we refer readers also to other excellent reviews for details (Crowe et al. 1992; Hoekstra et al. 2001; Alpert and Oliver 2002; Tunnacliffe and Lapinski 2003; Potts et al. 2005; Moore et al. 2007; Kranner et al. 2008; Wełnicz et al. 2011; Dinakar and Bartels 2013; Gaff and Oliver 2013).

1. Avoidance of mechanical stress both at the macroscopic level (e.g., leaf folding in resurrection plants, coiling of midge and dauer larvae) and the molecular level (composition and bending of phospholipid membranes, plasticity of cell walls). An example of cell wall adaptation to drying is provided by Holzinger et al. (2015) on *Ulva compressa*, a Mediterranean macroalga that survives severe desiccation (−23 MPa) and salinity. These authors present evidence that the flexibility of the cell wall due to pectins contributes to desiccation tolerance. In resurrection plants, the pectin matrix is also considered to maintain cell wall plasticity during desiccation by preventing irreversible polymer adhesion (Moore et al. 2013). To explain the large variation in seed desiccation sensitivity during drying and longevity in the dry state, Walters (2015) calls for a re-evaluation of the mechanical stress imposed by cellular volume changes during drying.

She proposes a new model based on changes in cellular volume as a means to characterize cell responses to water stress both on a temporal and spatial scale, allowing for a quantitative evaluation of desiccation tolerance across species and tissue types.

2. Avoidance of oxidative stress during drying and in the dry state. Several strategies exist to achieve this according to whether or not desiccation-tolerant organisms exhibit photosynthetic activity, the main generator of reactive oxygen species. Here, Farrant et al. (2015) review the tolerance mechanisms developed by the resurrection plant *Xerophyta viscosa*. Furthermore, light might be an additional stressor for dry desiccation-tolerant plants. The danger of photo-oxidation in relation to desiccation tolerance is illustrated by Candotto Carniel et al. (2015) on *Trebouxia* sp., a representative of the green algal genus that occurs in 50 % of lichen species. Both light and the presence of mycosymbiont are critical to the survival of the algae in the dry state because these factors influence the deleterious oxidative burst during rehydration. The authors suggest that each partner does not contribute equally to this desiccation-tolerant symbiosis, the mycosymbiont being the weakest in ability to survive drying.
3. Synthesis of protective compounds to counteract the removal of water molecules and that they are necessary for the structural and functional stability of proteins and membranes in the dry state. The nature and importance of these compounds are reviewed here in the resurrection plant *X. viscosa* by Farrant et al. (2015) and in the nematode *C. elegans* by Erkut and Kurzchalia (2015). Furthermore, Hatanaka et al. (2015) present a detailed characterization of all 27 LEA genes in the desiccation-tolerant midge and Hand and Menze review the LEA genes present in the brine shrimp Artemia. These authors also demonstrate the synergistic effects of LEA proteins and trehalose on the desiccation tolerance of genetically engineered insect and human cells, as previously achieved in yeast (Tapia and Koshland 2014).
4. Metabolism, coordination and regulation during desiccation. The induction of protective mechanisms needs to be coordinated before and/or during drying to allow prolonged survival in the dry state. This coordination is evidenced by the need for various mechanisms of preconditioning that can be necessary to elicit desiccation tolerance (e.g., osmotic stress prior to drying, slow drying and developmental controls when desiccation tolerance is restricted to certain phase of the life cycle). These and the kinetic properties of the responses to drying are suggestive of several coordinated layers of regulation. Examples of preconditioning and responses at the molecular level (i.e., early and late response) are illustrated for plants (germinated seeds of Arabidopsis, Costa et al. 2015) and animals (*C. elegans*, Erkut and Kurzchalia 2015 and *A. franciscana*, Hand and Menze 2015). Metabolomic and transcriptomic studies suggest that metabolism is remodeled by recruiting C, N and S into the synthesis of protective non-reducing sugars, stress proteins and antioxidants while electron transport systems are being deactivated and/or dismantled to avoid or decrease the risk of oxidative stress (Avelange-Macherel et al. 2006; Oliver et al. 2011; Erkut and Kurzchalia 2015). These changes are usually performed at the expense of cellular growth and division. The importance of regulatory control of the balance between catabolism and anabolism in desiccation tolerance has recently been demonstrated on yeast using functional genomics (Calahan et al. 2011; Welch et al. 2013). A survey of yeast mutants defective in genes involved in stress responses such as trehalose synthesis, DNA damage repair, antioxidants, LEA and heat shock protein (HSP) production were capable to survive drying like wild-type cells. Only mutations that block respiration in stationary cells rendered them desiccation sensitive (Calahan et al. 2011). It was later found by the same group that desiccation tolerance can be induced by lowering the growth rate of yeast using nutrient deficiency or non-fermentable C sources (Welch et al. 2013). Here, using a pharmacological approach, Hand and Menze (2015) show that control of energy homeostasis appears to be necessary for *A. franciscana* to tolerate freeze-drying. Also, new evidence is provided by Costa et al. (2015) suggesting a role for energy homeostasis in germinated seeds of Arabidopsis, in which desiccation tolerance is re-established by an osmotic pre-treatment. A gene co-expression network characterizing the transcriptome changes upon re-establishment of desiccation tolerance in seedlings led to the identification of CBSX4, a protein containing a cystathionine β-synthase domain that is characteristic of subunits of the SnRK1 complex. This is an ancient and highly conserved eukaryotic energy sensor that functions as heterotrimeric complexes and its role in seed longevity has been demonstrated (Rosnoblet et al. 2007). Here, mutant analysis suggests that CBSX4 plays a repressive role in desiccation tolerance during osmotic treatment (Costa et al. 2015).
5. Repair mechanisms that can be synthesized before drying and/or during rehydration. These mechanisms received a lot of attention in bryophytes (Bewley 1979; Oliver et al. 2004) where the primary strategy is to repair limited desiccation-induced damage during

rehydration. However, repair mechanisms in desiccation tolerance in other organisms remain poorly understood. Here, Hatanaka et al. (2015) present evidence that in the desiccation-tolerant larvae of midge *P. vanderplanki*, specific LEA genes appear to be up-regulated during rehydration as in desiccation-tolerant mosses. Their observations are consistent with in vitro data showing that PM25, a seed-specific LEA protein from *Medicago truncatula* efficiently dissociates desiccation-induced protein aggregates during their rehydration (Boucher et al. 2010). Altogether, this suggests that certain LEA proteins play a role in repair mechanisms in desiccation-tolerant organisms, particularly during rehydration.

Regulatory and evolutionary aspects of adaptation to desiccation tolerance

The studies reported in this issue show that the mechanisms of desiccation tolerance are remarkably similar despite the big differences in the taxonomic, evolutionary position and complexity of the tolerant organisms. Thus, it is likely that the signatures of the critical adaptive mechanisms will be archived in their genomes. With the development of next-generation sequencing and comparisons between desiccation-tolerant species or sister-group contrasts (comparisons between desiccation-tolerant species and a close desiccation-sensitive relative), it is increasingly possible to address the evolutionary mechanisms that have led to adaptations to the dry state. This special issue reports several examples and hypotheses regarding these mechanisms (Almoguera et al. 2015; Giarola and Bartels 2015; Hatanaka et al. 2015). Gene duplication is considered to be an evolutionary mechanism that adds new biological function to an organism. Based on the expression characteristics of LEA genes and their genomic features in the resurrection plant *Craterostigma plantagineum* and in the desiccation-tolerant midge *P. vanderplanki*, Giarola and Bartels (2015) and Hatanaka et al. (2015) argue that LEA gene duplication and associated *cis*-regulatory gene elements probably evolved to adapt gene expression patterns to desiccation tolerance. Individual copies of duplicated genes can assume new functionalities (Oh et al. 2012). In plants, heat shock transcription factors (HSFs) form a large and complex family which play a role in regulating heat stress. They have also gained new functions by neo-functionalization and divergent expression pattern (Yang et al. 2014), including in desiccation tolerance in seeds. Indeed, seed-specific HSFs can activate a genetic program that contributes to seed longevity and to tolerance against severe stresses in seedlings (reviewed here by Almoguera et al. 2015). These authors show that ectopic expression of two sunflower HSFs in tobacco seedlings leads to the maintenance of elevated amount of HSPs during seedling establishment and delayed leaf senescence when induced by stress. They suggest that homoiochlorophyllous resurrection plant species have evolved towards desiccation tolerance by modifying seed-specific expression of transcription factors to constitutive plant-wide expression leading to the protection of the photosynthetic apparatus against water loss.

In addition to adaptation by gene duplication, stress adaptation can also occur through taxonomically restricted genes (TRG). These genes, also called “lineage-specific genes” or “orphan genes”, are protein-coding genes that do not share sequence similarity outside the lineage and lack assignable function (Oh et al. 2012; Dinakar and Bartels 2013). In the transcriptome of *C. plantagineum*, more than 33 % of the genes are accounted for as TRGs in contrast to 10–20 % in Arabidopsis (Giarola and Bartels 2015), suggesting a role for these genes in desiccation tolerance. This raises the intriguing question as to whether pools of TRG serve as a reservoir of adaptive potential for desiccation tolerance. Finally, as well as these genomic adaptations, epigenetic controls also may play key roles in regulating desiccation tolerance, particularly when the life cycle includes a desiccation-tolerant and a sensitive phase. For example, non-protein regulatory molecules such as non-coding RNAs could contribute to the modulation of expression networks in desiccation tolerance (Giarola and Bartels 2015).

Prospects

Our increasing understanding of desiccation tolerance will continue to challenge our dogmas about water, life and evolution. The rapid development of tools and approaches to delve into genomes, transcriptomes, proteomes and metabolomes is adding to our understanding of life in the dry state. Systems biology approaches will allow to identify and connect the essential genes that make an organism desiccation tolerant and highlight redundancy and synergistic mechanisms, different regulatory pathways leading to survival in the dry state and putative trade-offs between desiccation tolerance and other necessary life-sustaining activities. Functional genomics is now providing a tangible way to test in vivo mechanisms of desiccation tolerance that were previously only confined to in vitro system. Comparative approaches such as sister-group contrasts and ancestor–descendant comparisons will reveal how desiccation tolerance has evolved and adapted to the needs of such a wide range of organisms and ecological niches. The development of technologies that allow us to discriminate between surviving and non-surviving organisms in the dry state at the cellular level, or allow us to characterize and measure non-invasively the very slow movements (or relaxation modes) of macromolecules, residual water and oxygen in the dry cells should step forward to further address the time component of desiccation tolerance.

Because desiccation tolerance occurs over an enormous range of taxa and provides tolerance against extreme environmental stresses, the positive consequences of understanding how this is achieved are large. For example, we need improved technologies to preserve dry biotic pharmaceutical products, to obtain dry and stable living human tissues (Hand and Menze 2015), to preserve our genetic resources and biodiversity (seeds, pollens, meristems), particularly for those species that are difficult to maintain alive for long periods (Walters 2015) and to discover new compounds such as trehalose, whose physical and biochemical properties in the dry state offer a new set of technological opportunities. Understanding how desiccation tolerance evolved will also provide knowledge on genes that can be used to better engineer crops and improve their resistance to, and survival of drought (Crowe 2007; Potts et al. 2005; Farrant et al. 2015).

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