

# Between a Rock and a Dry Place: The Water-Stressed Moss

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**ABSTRACT** The earliest land plants faced a suite of abiotic stresses largely unknown to their aquatic algal ancestors. The descendants of these plants evolved two general mechanisms for survival in the relatively arid aerial environment. While the vascular plants or ‘tracheophytes’ developed tissue specializations to transport and retain water, the other main lineages of land plants, the bryophytes, retained a simple, nonvascular morphology. The bryophytes—mosses, hornworts, and liverworts—continually undergo a co-equilibration of their water content with the surrounding environment and rely to a great extent on intrinsic cellular mechanisms to mitigate damage due to water stress. This short review will focus on the cellular and molecular responses to dehydration and rehydration in mosses, and offer insights into general plant responses to water stress.

**Key words:** Abiotic/environmental stress; ABA; poikilohydry; *Tortula ruralis*; bryophytes; *Physcomitrella patens*.

## DROUGHT AVOIDANCE, DROUGHT TOLERANCE, AND DESICCATION TOLERANCE

To survive on land, primordial plants had to either avoid water stress or adapt to manage it. Many drought-sensitive green algae, bryophytes, and tracheophytes avoid water stress by colonizing hydric or heavily shaded ecological niches or growing in short, dense clusters that limit moisture evaporation. The evolution of mechanisms to withstand water stress presumably arose from individuals uniquely suited to occupy the outer limits of mesic niches. The tracheophytes (seed plants, ferns, and lycophytes), the most structurally complex land plants, evolved extensive vascular systems and protective barriers against water loss. Although all tracheophytes possess specialized reproductive cells that naturally experience a period of dehydration—seeds, pollen, and spores—drought tolerance in vegetative tissues is rare among vascular plants. In contrast, many species of bryophytes have adapted to life in mesic and xeric niches without the benefit of water transport and retention systems. Bryophytes are *poikilohydric*: possessing a water content equivalent to that of their environment. Because poikilohydric plants undergo a continual co-equilibration of tissue water content with the surrounding air, bryophytes occupying mesic or xeric habitats are considered drought- or desiccation-tolerant. Although the lack of conductive tissues has limited their physical stature, it has not severely limited the diversity or ecological breadth of bryophytes; their 25 000 representatives are found in

deserts, mountains, woodlands, steppes, and bogs across every continent.

Mosses have a relatively simple lifecycle and minimalist architecture that belies their adaptability to water stress. The characteristic leafy, aerial gametophores readily observed in nature arise from linear, branching protonemal filaments that grow in contact with the humid substratum. Mature, haploid gametophores produce gametangia. Like ferns but unlike seed plants, water is required for fertilization, as the flagellated sperm must swim to the egg. Fertilization yields a diploid sporophyte atop the gametophore. Spore mother cells within the sporophyte undergo meiosis to produce tetrads of haploid spores, which germinate into haploid protonemal filaments. At all stages, moss lacks proper roots and stems, water-conducting vascular tissue, and porous epidermal stomata, common properties of tracheophytes that minimize water loss. Yet, the predominating aerial shoots of the gametophore grow above the hydrated substratum, exposed to air. Their characteristic growth in clusters undoubtedly retards water evaporation from individual gametophores. Given their

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exposed surface area, however, the leaf-like blades of the gametophore, the phyllidia, are especially vulnerable to water loss. The phyllidia of many mosses are only a single cell layer in thickness, making water loss from any given phyllidial cell equally inevitable and potentially problematic. A desiccated phyllidium, however, can regenerate new protonemal filaments following a return to moist conditions, which eventually generate new gametophores (Stark et al., 2005). The continual cycle of water loss-and-gain that the moss cell endures dictates that cell-intrinsic mechanisms must largely limit damage incurred not only from the initial water loss, but also from the sudden influx of water upon rehydration.

Water stress-tolerant mosses may be desiccation-tolerant, or drought-tolerant yet desiccation-sensitive. *Physcomitrella patens* is the most commonly used experimental moss model of water stress tolerance. *P. patens* is commonly found at the margins of temperate lakes and rivers. This moss is drought-tolerant, able to withstand brief periods of moderate water stress (Cumming et al., 2007; Frank et al., 2005). A sustained vapor pressure of  $-10$  MPa is lethal to most land plants. *P. patens* can survive no longer than 10 min of exposure to an atmosphere of  $-100$  MPa (Charron and Quatrano, unpublished results); it is thus considered desiccation-sensitive. In contrast to *P. patens*, the moss genera *Tortula* and *Polytrichum* consist of extremophiles, commonly inhabiting arid niches and able to tolerate remarkably low vapor pressures. *T. caninervis* can survive years stored at vapor pressures as low as  $-540$  MPa, equivalent to atmospheric conditions of 2–4% relative humidity (Oliver et al., 2005). *P. formosum* is capable of recovering after storage for 1 week at  $-400$  MPa (Proctor et al., 2007). *Tortula* and *Polytrichum* spp. are thus considered desiccation-tolerant. These desiccation-tolerant mosses undergo similar morphological and physiological crises to drought-tolerant and drought-sensitive mosses. Rehydration of desiccation-tolerant mosses can be very rapid, however. Whereas rehydration of *P. patens* or *P. formosum* occurs over a period of 1–2 h (Charron and Quatrano, unpublished results; Proctor et al., 2007), it takes only 2 min in the case of *T. ruralis* (Oliver et al., 2005), with return to normal function taking only a few hours.

The toolkit with which to explore water relations in drought- and desiccation-tolerant moss has recently been fortified by several key resources. *P. patens* is an attractive experimental model for water stress studies, not only because it is drought-tolerant, but also because it is well suited to reverse genetic approaches. It is readily cultured axenically, easily transformed, and it undergoes homologous recombination with a frequency comparable to that of *Saccharomyces cerevisiae* (Cove, 2005; Kamisugi et al., 2005, 2006; Schaefer and Zryd, 1997). Other mosses such as *Ceratodon purpureus* are also amenable to genetic manipulation (Trouiller et al., 2007). The genome of *P. patens* was published last year ([www.mossgenome.org/](http://www.mossgenome.org/); Rensing et al., 2008), and the second generation of an expression microarray is available ([www.mogene.com/](http://www.mogene.com/)). Several other molecular biology and bioinformatics tools now exist for *P. patens* (Bezanilla

et al., 2005; Perroud and Quatrano, 2006; Quatrano et al., 2007), and diverse efforts are currently focused upon the discovery of genes involved in the stress responses of this moss. Another drought-tolerant moss, *C. purpureus*, has been used extensively in experiments exploring tropism in moss (Cove and Quatrano, 2006), and is the subject of a genetic mapping project (McDaniel et al., 2007). Regarding the desiccation-tolerant mosses, *Tortula* has so far proven refractory to protoplasting (and thus transformation) and *Polytrichum* spp. have not been successfully grown axenically. Despite these setbacks, their desiccation tolerance has prompted studies of gene expression (*Tortula*) and morphology (*Polytrichum*) following water stress. The collection of  $>10$  000 ESTs generated from a cDNA library of rehydrated *T. ruralis* is publicly available, and is an important resource to study mechanisms conferring tolerance to near-complete desiccation (Oliver et al., 2004).

## THE CELLULAR IMPACT OF WATER STRESS

Water stress embodies two stresses—both dehydration and rehydration—that together impact the architecture of drought and desiccation-tolerant moss cells in characteristic ways. The careful preparation of dehydrated tissue through the use of either anhydrous preparation or direct freeze-fracture methods has shown that although the plasma membrane of *P. formosum* and *T. ruralis* becomes highly convoluted and labyrinthine in the dehydrated state, it remains intact (Platt et al., 1993; Pressel et al., 2006). In fact, ultrastructurally, the plasma membrane as well as organellar membranes of desiccated *T. ruralis* appear as typical lipid bilayers with dispersed intramembranous particles (Platt et al., 1993). The nuclei, chloroplasts, and mitochondria of dried cells lose their elongated shape and become round or ovoid, possibly due to the loss of scaffolding from the depolymerized microtubule cytoskeleton (Pressel et al., 2006). The dehydration results in a generalized condensation of cellular contents, with fragmentation of the central vacuole system, increased viscosity of the cytoplasm, condensation of chromatin, and dense packing of ribosomes (Pressel et al., 2006; Proctor et al., 2007). As the cell rapidly approaches homeostasis during rehydration, the protoplasts swell against the cell wall, and the cells become convex (Proctor et al., 2007). The sub-cellular components gradually return to a normal shape and disposition, potentially dependent on the reforming microtubule cytoskeleton for re-scaffolding (Pressel et al., 2006; Proctor et al., 2007). The speed of recovery is, in general, inversely proportional to the rate and intensity of the stress, but most species recover some normal morphology within minutes, and rebuild their finer architecture within 1–2 d (Pressel et al., 2006; Proctor, 2001; Proctor et al., 2007).

Water stress results in widespread cellular damage stemming from two critical, simultaneous events: the generation

of reactive oxygen species (ROS) and the loss and subsequent influx of water that surrounds biomolecules. Aerobic metabolism and photosynthesis continuously release ROS, and ROS increase during dehydration. ROS accelerate protein denaturation, chlorophyll degradation, DNA damage, and lipid peroxidation and de-esterification. These are deleterious, and the production of ROS must be buffered by enzymatic or molecular antioxidants if the cell is to survive. Genes encoding enzymatic antioxidants (superoxide dismutase, ascorbate peroxidase, dehydroreductases, and glutathione reductase) are up-regulated in water-stressed plants, suggesting induction of a protective mechanism against ROS (Franca et al., 2007). In addition, molecular (non-enzymatic) antioxidants (i.e. ascorbate, glutathione, tocopherols, polyols, carotenoids) protect resurrection plants during drying, and may protect moss at the point at which insufficient water remains for enzymatic reactions to occur (Franca et al., 2007).

Water not only drives lipid bilayer formation, but also directs and maintains proper protein conformation. During dehydration, the shell of hydration surrounding biomolecules gradually diminishes and is eventually lost. Unmitigated, loss of the water that is normally hydrogen-bonded to lipids and proteins has a devastating impact on the biophysical properties and conformation of membranes, catalytic proteins, and structural proteins. In drought conditions, damage is thought to be minimized through preferential hydration: the crowding of 'compatible solutes' around the little water that remains hydrogen bonded with lipids and proteins, creating a protective shell of solute:water:biomolecule (as reviewed in Hoekstra et al., 2001). Compatible solutes may include sugars, oligosaccharides, proline, polyols, glutamate, and other osmolytes, as well as certain hydrophilic proteins such as heat shock proteins and LEA proteins. During total desiccation, compatible solutes, particularly sugars, ensure proper protein conformation and prevent unwanted protein aggregation, membrane fusion, and membrane phase transitions through intimate crowding around proteins and lipids, substituting for water in the critical hydrogen bonds. Compatible solute-lipid polar head group interactions are instrumental in maintaining the liquid crystalline phase of the lipid bilayer, in which polar head groups (and thus acyl chains) remain loosely spaced. Without them, the membrane lipids can pack tightly following water loss, producing a liquid:gel transition and lateral phase separations that irreversibly damage the membrane. When compatible solutes have stabilized the dried cell from the inside out, a 'glassy matrix' forms. Immobilization within a glassy matrix ensures that the cellular constituents are preserved in a near-native biophysical state until the return of water. Genetic studies in yeast and transgenic plants have recently uncovered a causal relationship between the accumulation of compatible solutes (proline and fructans, respectively) and tolerance to drought (Takagi, 2008; Valluru and Van den Ende, 2008). Moreover, a yeast LEA-like heat shock protein HSP12 can prevent leakage of a molecular tracer from drying

liposomes, presumably through interactions with bilayer lipids (Sales et al., 2000).

## DROUGHT TOLERANCE: PRE-CONDITIONING AND ABA

Upon emergence from a state of drought, re-wetted survivors face several predicaments: (1) the cellular architecture may need extensive reconstruction; (2) photosynthetic, respiratory, and biosynthetic systems need to be repaired and restarted; (3) ROS need to be scavenged and neutralized; (4) ion leakage across the plasma membrane needs to be remedied. In the event of gradual or moderate dehydration, constitutive mechanisms of protection particular to the moss species (e.g. the rapid conversion of starches to compatible solutes) can act in concert with activated stress response protocols to ensure survival of drought-tolerant mosses.

Comparative molecular profiling analyses have recently illuminated hundreds of molecular components of abiotic stress responses in *P. patens* (Cho et al., 2006; Cuming et al., 2007; Sun et al., 2007). Although a clearer picture of cellular pathways in the response of moss to water stress will undoubtedly emerge as individual components are characterized, the best understood response is that mediated by the hormone abscisic acid (ABA). ABA has long been recognized for its central role in directing the processes involved in the protection of tracheophyte seeds during seed development and vegetative tissue during water stress. Seed maturation involves a gradual desiccation process. During this process, ABA activates the expression of a subset of genes that harden the embryo against the desiccation and imbibition processes (Finkelstein and Gibson, 2002). In water-stressed tracheophyte vegetative tissue, the principal and immediate response role of ABA is the closing of leaf stomata, thereby limiting the transpiration of water from the largest surface areas of the plant (Rock et al., 2009). Mosses neither produce seeds nor possess stomata, yet ABA has emerged as a critical regulatory component in the protection-based defense against abiotic stress. *P. patens* has the requisite enzymes for ABA synthesis, degradation, and modification (Nishiyama et al., 2003). ABA gradually accumulates in drying moss to six-fold over basal levels, and remains elevated long after the initial dehydration (Werner et al., 1991). A single, low-dose application of ABA is sufficient to brace drought-tolerant mosses against drought, salt, temperature, and osmotic stresses (Beckett, 2001; Cuming et al., 2007; Frank et al., 2005; Hoang et al., submitted; Nagao et al., 2006; Werner et al., 1991). ABA pretreatment may simulate the natural accumulation of ABA in response to seasonal humidity fluctuations progressing towards dry, hot conditions, invoking the ABA-dependent stress response.

ABA response mechanisms in moss are best understood at the transcriptional level. *P. patens* has a full complement of ABA-dependent transcriptional activation machinery (Marella et al., 2006). ABA promotes gene expression from promoters containing ABA-response elements (ABREs) following the

activation of ABRE-binding transcription factors (Rock et al., 2009; Zhang et al., 2005). Canonical ABRE hexamers are over-represented in the promoters of *P. patens* genes induced by dehydration and ABA application (Cuming et al., 2007; Kamisugi and Cuming, 2005). Moreover, *P. patens* has several ABRE-binding transcription factors, the ABI3-like regulators (Marella et al., 2006). Transgenic moss in which a wheat ABRE promoter drives expression of a reporter gene readily responds to ABA, activating gene expression through the wheat ABRE (Knight et al., 1995). Moreover, *P. patens* ABI3A expressed in an *Arabidopsis thaliana* *abi3* mutant partially complements the mutant phenotype (Marella et al., 2006), suggesting that the ABA pathway allowing tracheophytes to tolerate seed desiccation and vegetative water stress also allows bryophytes to tolerate water stress.

The LEA (Late Embryogenesis Abundant) genes are those that are most highly induced in response to water stress or ABA treatment. The genes encoding LEAs, so named for their abundant expression in desiccating seeds, have a broad phylogenetic distribution across all taxonomic kingdoms (Battaglia et al., 2008). LEAs comprise a large protein family grouped according to specific polypeptide repeats and domain structure (Battaglia et al., 2008). The promoter regions of many *P. patens* LEA genes contain hexamers conforming to putative ABREs (A. Cuming, personal communication). The ABRE of *P. patens* LEA-1 is required for transcriptional activation following ABA treatment, and ABA-triggered expression of other LEA genes, dehydrinA/PpLEA2, and several group 3 LEAs may likewise be mediated by ABREs (Cuming et al., 2007; Kamisugi and Cuming, 2005; Saavedra et al., 2006). DehydrinA (DHNA) knockout moss is impaired in its recovery from rehydration, but not in its tolerance to water loss (Saavedra et al., 2006). Likewise, Tr288, a *T. ruralis* protein with 78% similarity to *P. patens* DHNA, is also thought to be involved in cellular protection, perhaps repair, during rehydration (Velten and Oliver, 2001). Their abundance in dehydrating/rehydrating tissue, considered together with an energy-independent mechanism of protection, points to a physical role in protection for the LEAs. The hydrophilic, disordered regions spanning the length of the LEAs are thought to fold upon water loss into amphipathic alpha-helices that stabilize cellular membranes (Koag et al., 2003; Velten and Oliver, 2001; Wolkers et al., 2001). Dehydrins can indeed bind to phospholipid vesicles (Koag et al., 2003). Experimentally induced molecular crowding, however, has a negligible effect on the secondary structure of dehydrins, which retain their disordered characteristics (Mouillon et al., 2008). Thus, it is premature to speculate on the general importance of folding of the LEAs for drought tolerance. Mounting biophysical evidence does, however, indicate that LEAs are involved in the formation of a glassy matrix. When added to a model experimental system, a purified LEA protein promoted the formation of a sucrose-LEA glass (Wolkers et al., 2001). In a later study using in-situ Fourier transform infrared spectroscopy, Oldenhof et al. (2006) demonstrated that ABA pretreatment promoted the formation

of a glassy matrix in dehydrated *P. patens* tissue, likely through LEA accumulation.

Water stress and elevated ABA levels induce many additional, varied responses through transcriptional activation. Two recently identified responses counter the production of ROS. Plants regulate their pools of photoreactive tetrapyrrole intermediates by shuttling them between sub-cellular compartments. The tetrapyrrole protoporphyrin IX (PPP9) is a key substrate in the formation of siroheme, chlorophyll (in the plastid), and heme (in the mitochondria). Transport of PPP9 into the mitochondrion is facilitated by the mitochondrial membrane protein TspO. TspO expression and activity increase in response to water stress or ABA treatment in *P. patens*, presumably moving PPP9 away from the site most apt to support photo-oxidation, into the compartment of heme biosynthesis (Frank et al., 2007). Heme is a cofactor for the ROS scavengers catalase and peroxidase, and therefore an equilibrium shift towards heme synthesis (brought about by increased availability of PPP9) generates active ROS scavengers. Indeed, when TspO knockout lines are stressed, PPP9 accumulates in the plastids and cytoplasm, and the plants suffer from excessive hydrogen peroxide buildup, increased lipid peroxidation, and cell death (Frank et al., 2007). Oxidative stress is also managed by the induction of the monodehydroascorbate reductase (MDHAR) genes. Three separately encoded MDHAR proteins reduce monodehydroascorbate, a ROS produced in stressed *P. patens* (Lunde et al., 2006). The MDHAR genes are differentially transcribed: forms 1 and 3 contain consensus ABREs and coupling elements in their promoter regions, and are transcribed following water stress or treatment with ABA. In contrast, form 2, which contains only a weak ABRE sequence and no coupling element, is not responsive to either treatment (Lunde et al., 2006). These examples demonstrate that by regulating the expression and/or activity of one gene product, plants can in part protect against oxidative stress brought on by water limitations.

The aquaporins have emerged as important components of the transcriptional response to water stress in moss. Aquaporin gene expression in *P. patens* is stimulated by water stress and ABA (Cuming et al., 2007). Aquaporins increase cellular water permeability, but their role in mosses has been unclear, as poikilohydric plants do not regulate their water potential. A recent report has shed light on the possible function of aquaporins in drought tolerance. The expression of three aquaporin genes in *P. patens* is restricted to gametophore tissue (Liénard et al., 2008), notable because this tissue grows surrounded by air, and contains more endogenous ABA than protonemal tissue (Perroud and Quatrano, unpublished results). The deletion of two aquaporin genes results in greater water loss from gametophore tissue, with knockouts wilting faster than wild-type gametophores during moderate water stress (Liénard et al., 2008). The authors hypothesize that the targeted deletion abrogates the limitation of water loss by diminishing normal transpiration. Phyllidia lacking aquaporins would dry from the

arid-facing upper surface while not compensating for water loss through aquaporin-based influx from the moist-facing lower surface. Thus, drought-tolerant moss lacking aquaporins would suffer most noticeably in moderate water stress conditions, while they would appear normal in moist conditions (Liénard et al., 2008). Interestingly, a rehydration cDNA library from *T. ruralis* includes transcripts with high homology (~90%) to the *P. patens* aquaporins, suggesting that they may also be involved in the recovery of desiccation-tolerant moss (Oliver et al., 2004).

Several cellular responses to drought-induced ABA accumulation or ABA treatment are not yet mechanistically linked to gene activation. The protection that ABA provides *P. patens*, and two additional drought-tolerant mosses, *Funaria hygrometrica* and *Atrichum androgynum*, is, however, most likely partially attributable to protein synthesis (Beckett, 1999; Werner et al., 1991). The proteome of water-stressed or ABA-treated *P. patens* changes notably, presumably eliciting diverse downstream effects (Cho and Quatrano, unpublished results). De-novo protein synthesis following ABA treatment in *P. patens* results in the production of the compatible solute theandrose (Nagao et al., 2006). Stressed *A. androgynum* and *P. patens* both accumulate compatible solutes, an adaptive response facilitating the selective hydration of membrane lipids and proteins and formation of a glassy matrix (Mayaba et al., 2001; Nagao et al., 2005; Oldenhof et al., 2006). The sugars are likely released from the catabolism of starches, as chloroplasts of desiccated *P. formosum* and ABA-treated *P. patens* are visibly depleted of starch deposits (Nagao et al., 2005; Pressel et al., 2006). Although not yet formally linked to protein synthesis, ABA pretreatment of *A. androgynum* also reduces K<sup>+</sup> leakage during rehydration (Beckett, 2001). In nature, the extent of K<sup>+</sup> leakage from *A. androgynum* varies seasonally, such that K<sup>+</sup> loss occurring in the dry season is limited, while, in the wet season, it is much greater, suggesting that ABA accumulation triggers the production of hardening protein(s) that limit damage (Beckett and Hoddinott, 1997). Additional adaptations suspected to link ABA with protein synthesis include increased non-photochemical quenching (dissipating excess energy, thereby preventing the generation of ROS), suppressed H<sub>2</sub>O<sub>2</sub> production, and increased stabilization of membrane lipids (Guschina et al., 2002; Mayaba et al., 2001, 2002).

While ABA clearly has a central role in the protection of bryophytes against water stress, it is clear that some mosses also employ an ABA-independent pathway. ABA-independent transcription occurs through the activation of dehydration-responsive elements (DREs) in dehydration-induced gene promoters (Liu et al., 2007). In vascular plants, the binding of the DREB family of transcription factors to DREs is well recognized as a potent activator of transcription in response to water stress (Agarwal et al., 2006). *P. patens* contains at least one DREB, PpDBF1, and *T. ruralis* has two distinct cDNAs with high homology to PpDBF1. Both the *P. patens* DREB and the putative *T. ruralis* DREB are expressed following water stress

(Cuming et al., 2007; Oliver et al., 2004), and DRE-like elements have been found in 5' sequences upstream of dehydration-induced genes (Cuming et al., 2007). PpDBF1 activates transcription from *A. thaliana* DRE-containing reporter constructs, and when over-expressed in tobacco plants, protects them from drought stress (Liu et al., 2007). These findings indicate that moss may have a cohort of drought-responsive genes expressed independently of the ABRE, thus not dependent on the hardening periods classically characterized by ABA accumulation.

## DESICCATION TOLERANCE: CONSTITUTIVE PROTECTION AND REPAIR

Like drought-tolerant mosses, desiccation-tolerant mosses also have constitutive and inducible systems to counter water stress. *T. ruralis*, the desiccation-tolerant moss best studied at the molecular level, contains a relatively high level of sucrose (~10% of dry mass), which remains constant during dehydration and rehydration (Bewley et al., 1978). Furthermore, it constitutively expresses the dehydrins, indicating that it is prepared to weather potential water loss (Bewley et al., 1993). ABA does not appear to be a key factor in drought and desiccation tolerance in *T. ruralis*: this moss neither contains nor responds markedly to ABA (Bewley et al., 1993). Thus far, only one account of inducible transcription in *T. ruralis* has been described: slow drying induces the expression of 'early light inducible proteins' that likely prevent photodamage to the chloroplast from free, excited chlorophyll (Zeng et al., 2002). The constitutive protection responses thus appear largely sufficient to protect desiccation-tolerant mosses during moderate or gradual water stress.

*Tortula* species often grow in exposed habitats and endure repeated cycles of drying and re-wetting. During the rapid dehydration that can occur over the course of daytime in arid climates, little time may exist for inducible responses to reach fruition. Furthermore, during severe or long-term dehydration, *T. ruralis* must stabilize its cellular components, *in the absence of water*, in a state of suspended animation. While its high sugar content presumably promotes the formation of a protective glassy matrix, a robust repair system must restore order and function when plants experience these severe impositions.

We currently understand less about the breadth of repair responses than that of protective responses. However, one parsimonious mechanism of resource conservation underscores how well suited *T. ruralis* is to thrive in water-limiting climates. This strategy was uncovered upon the comparative profiling of the gametophore proteome before and during rehydration. Earlier work had demonstrated that protein synthesis rapidly declines during dehydration (Dhindsa and Bewley, 1977). Oliver and colleagues found that the proteome of metabolically labeled proteins changes significantly following rehydration: in as little as 2 h after *T. ruralis*

rehydration, altered expression of nearly 80% of the newly synthesized proteins was noted (Oliver, 1991). The declining biosynthesis of 'hydrins', proteins present in hydrated gametophores, was concomitant with a substantial increase in 'rehydrins', proteins unique to rehydrated plants (Oliver, 1991). The appearance of rehydrins is not due to new transcription. Rather, existing transcripts encoding the rehydrins accumulate during drying, becoming sequestered and stabilized within protein/mRNA complexes (Wood and Oliver, 1999). These mRNPs serve as storage complexes for the rapid translation of rehydrins after the return of water: upon recovery, nearly 400 unique transcripts are recoverable (Wood and Oliver, 1999). The selective sequestration of rehydrin transcripts demands no metabolic expenditure, presumably allowing plants to quickly replace proteins that are critical for its survival. It is unclear whether rehydrin transcripts share common 'sequestration signals' selecting them for retention in mRNPs when drying first commences.

A less economical means to replenish molecular constituents and repair damaged cellular components is through de-novo transcription. In addition to producing rehydrins from pre-existing mRNAs, *T. ruralis* also mounts an impressive transcriptional response to desiccation following rehydration. Expression profiling was used to catalog the >10 000 individual cDNAs present in rapidly dehydrated *T. ruralis*, which was subsequently allowed to rehydrate for 2 h. (Within a cDNA pool sampled at 2 h following the re-introduction of water, both the protected rehydrin mRNA pool and newly transcribed mRNA pool would be represented.) While over 40% of the total cDNAs that passed quality control encoded unknown proteins, seven of the 30 most abundant cDNAs encoded LEA-like proteins (Oliver et al., 2004). This analysis also revealed that of 80% of the transcripts assignable to a gene ontology based on homology, the majority map to metabolism pathways (Oliver et al., 2004). These findings have three compelling implications. The first is that the first desiccation-tolerant moss examined at the cDNA level has a significant cohort of transcripts that thus far have not been found in tracheophytes or the drought-tolerant *P. patens* and may be unique. The second is that a key component of the response of a desiccation-tolerant moss is through a group of genes (the LEAs) also highly up-regulated in water-stressed or ABA-treated drought-tolerant mosses. One *T. ruralis* rehydrin, Tr288, is a LEA that is rapidly synthesized at the level of translation shortly after rehydration (Scott and Oliver, 1994). Although Tr288 has been further characterized, the mechanism of its action remains as obscure as that of the LEAs in *P. patens* and tracheophytes (Velten and Oliver, 2001). The third implication is that a primary response of recovering *T. ruralis*, whether by de-sequestration of protected transcripts or new transcription of repair response genes, is to quickly replace unsalvageable biomolecules. The two repair mechanisms put into play *after* drying has occurred—the translation of de-sequestered rehydrin mRNAs and the transcription of a subset of unique, enigmatic, or common stress

response genes—likely ensure that *T. ruralis* survives extreme water stress.

## PERSPECTIVES

The natural variation within the mosses, their morphological simplicity, their relatively simple genomic composition, and their evolutionary tenacity make mosses attractive as both experimental plants and subjects for ecology and evolution. *Physcomitrella* is fast approaching 'model plant' status: the sequencing of the *P. patens* genome and refinement of molecular biology and bioinformatics tools for this moss have paved the way for diverse discovery initiatives. Comparative genomic analyses between *P. patens* and vascular plants have already revealed much about land plant evolution and genome structure (Rensing et al., 2008). Expression profiling of *P. patens* using oligonucleotide microarrays is now routinely used to define conserved and unique stress response pathways in this descendant of the earliest land plants (Charron and Quatrano, unpublished results; Cho and Quatrano, unpublished results; Cuming et al., 2007). Targeted gene replacement and RNAi-mediated gene silencing have begun to pinpoint critical components of *P. patens* developmental, biosynthetic, and signaling pathways (Harries et al., 2005; Khandelwal et al., 2007; Perroud and Quatrano, 2006, 2008; Vidali et al., 2007). However, the expansion of shared moss biology resources would open up invaluable opportunities to use moss as a versatile model plant. Forward genetics approaches such as T-DNA-mediated insertion and fast neutron bombardment-mediated deletion would permit the unbiased discovery of genes responsible for phenotypes of interest. Optimized methods for routine axenic growth of yet-to-be-cultured species would facilitate the establishment of uniform cultures to feed into discovery pipelines. Synteny maps would contribute to our understanding of karyotypic evolution in land plants. Finally, sequencing of the genome of a desiccation-tolerant moss (i.e. *Tortula*) or a second drought-tolerant moss (i.e. *Ceratodon*), when used in conjunction with existing *P. patens* tools, would undoubtedly serve to clarify mechanisms of stress tolerance in poikilohydric plants. Our group (in collaboration with several others) will continue to advocate the development of these and other desired resources, with the goal of promoting mosses to true model plants.

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