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How some plants recover from vegetative desiccation: A repair based strategy

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Abstract

Desiccation-tolerant plants can be grouped into two categories: the 1) desiccation-tolerant plants whose internal water content rapidly equilibrates to the water potential of the environment and 2) the modified desiccation-tolerant plants that all employ mechanisms to retard and control the rate of water loss. Desiccation tolerance can be achieved by mechanisms that incorporate one of two alternatives, viz. cellular protection or cellular recovery (repair). The majority of plants probably utilize aspects of both. Desiccation-tolerant species, in particular the moss *Tortula ruralis*, appear to utilize a tolerance strategy that combines a constitutive protection system and a rehydration-inducible recovery mechanism. The rehydration-induced recovery mechanism of *Tortula ruralis* relies heavily upon a change in gene expression that is mediated by post-transcriptional events rather than the slower reacting transcriptional controls. Findings indicate that it takes a certain amount of prior water loss to fully activate the protein-based portion of the recovery mechanisms upon rehydration.. Utilizing cDNAs representing individual hydrins (proteins whose synthesis is hydration specific) and rehydrins (proteins whose synthesis is rehydration specific), it was determined that if drying rates were slow rehydrin transcripts selectively accumulate in the dried gametophytes. Studies revealed that this storage involves the formation of mRNPs (messenger ribonucleoprotein particles). The identity and possible functions of the rehydrins of *Tortula ruralis* are also under investigation, in particular Tr155, a small rehydrin (24kD) appears to be involved in anti-oxidant production during rehydration.

Introduction

Vegetative desiccation-tolerance has evolved in a relatively small number of plant species. Nevertheless, plants that are capable of vegetative desiccation-tolerance represent most major classes; from the more complex angiosperms (approx. 60 species) and ferns and fern allies (60-70 species) to the less complex taxons that constitute the algae, bryophytes, and lichens where the majority of such spe-

cies reside (Bewley and Krochko 1982, Oliver and Bewley 1997). Desiccation-tolerant plants fall into two main categories; fully desiccation-tolerant plants that can withstand the total loss of free protoplasmic water at any rate and modified desiccation-tolerant plants that can only survive such a stress if water loss is slow. Plant complexity appears to influence which category a plant belongs to. All fully desiccation-tolerant plants studied to date are of the less complex groups of plants; algae, bryophytes or lichens. Modified desiccation-tolerant plants tend to be more complex (ferns, fern allies and angiosperms) although there is at least one bryophyte in this class (Werner *et al.* 1991).

Mechanistically, the means by which desiccation-tolerance is achieved is also dictated by the complexity of the plant and/or the habitat to which it has evolved to exploit. The proposed mechanisms for desiccation-tolerance are founded on three criteria that plants (or plant structures) must meet to survive desiccation (Bewley 1979); (1) limitation of the damage incurred to a repairable level, (2) maintenance of physiological integrity in the dried state, and (3) mobilization of repair mechanisms upon rehydration. These criteria can be simplified into two basic components by which desiccation-tolerance can be achieved; the protection of cellular integrity and the repair of desiccation- (or rehydration-) induced cellular damage, as described by Bewley and Oliver (1992). Plants, in all probability, employ mechanisms that encompass both but as desiccation-tolerance has evolved independently on a minimum of twelve separate occasions (Oliver and Bewley 1997), one would expect that there are examples of plants which span the spectrum of possible combinations of the two strategies; from plants that rely heavily on cellular protection to those that rely more on cellular repair.

Available evidence concerning desiccation-tolerance of modified desiccation-tolerant plants strongly suggests that these tissues and plants utilize mechanisms that rely heavily on inducible cellular protection systems (for reviews see Bartels and Nelson 1994, Bartels *et al.* 1993, Bewley *et al.* 1993, Bewley and Oliver 1992, Burke 1986, Close *et al.* 1993, Crowe *et al.* 1992, Dure 1993, Gaff 1989, Leopold *et al.* 1992, Oliver and Bewley 1996). The protective mechanisms of tolerance appear to involve two major components, sugars and proteins, both of which are postulated to be involved in maintaining cellular integrity during the drying phases (Bewley *et al.* 1993, Crowe *et al.* 1992, Dure 1993, Leopold *et al.* 1992, Oliver and Bewley 1996). The time involved in the induction and establishment of such protective components is thought to be why modified-desiccation tolerant plants do not survive rapid water loss. It is possible that the inducible protective mechanisms evolved in plants capable of limiting water loss, either by morphological or physiological complexities, as a means of invoking desiccation-tolerance on demand. Such a strategy would enable the plant to incur the cost of channelling resources away from growth or reproductive processes only when faced with life threatening water deficits. Less complex plants, *e.g.*, bryophytes and algae, that utilize such a strategy (*i.e.*, modified desiccation-tolerance) may have evolved to take advantage of habitats that only experience desiccation infrequently and/or dry at a slow rate or they have made use of clumping structures that can ameliorate drying rates (clump structures can significantly alter drying rates, (Alpert 1987)).

Fully desiccation-tolerant plants have evolved tolerance mechanisms that allow the plant to survive rapid drying rates (to air dryness within an hour or faster). Since the more complex orders of plants have physiological and morphological adaptations to limit the rate of water loss, it seems that only the less complex plants have acquired (or required) this capability. The evidence available to date (from studies on highly desiccation-tolerant bryophytes) suggests that these mechanisms include a constitu-

tive (rather than inductive) cellular protection component coupled to a rehydration induced recovery process that presumably is designed for the repair of cellular damage. The speed at which desiccation takes place precludes the induction of protective systems which require time for transcription of specific gene sets and the translation of the resulting transcripts into active proteins required for the protective metabolic processes. The plant therefore has to be continuously prepared for a desiccation event and then respond to the cellular disruption it causes when water returns. The constant state of readiness may require a good deal of the energy budget of these plants which may be a contributing factor to their normally slow growth rates (which is true for all of the truly desiccation-tolerant plants studied so far).

In the following discussion we will concentrate on our work with a fully desiccation-tolerant bryophyte, *Tortula ruralis* and we will present our evidence that supports the concept that less complex plants utilize a mechanism of vegetative desiccation-tolerance that is based on constitutive protection and rehydration induced cellular repair. The evidence for a protective mechanism of desiccation-tolerance for modified desiccation tolerant plants can be found in the aforementioned reviews and in an article in this volume (pages 399-403) by Bartels *et al.*(1997).

Desiccation of gametophytic tissues of *T. ruralis* results in a rapid decline in protein synthesis, as in all desiccation-tolerant and intolerant mosses tested so far (Bewley 1972, 1973, Henckel *et al.* 1977, Siebert *et al.* 1976, and M. J. Oliver, unpublished data for *T. caninervis* and *T. norvegica*). This loss of protein synthetic capacity is manifested in a loss of polysomes resulting from the run-off of ribosomes from mRNAs, concomitant with their failure to reinitiate protein synthesis (see Bewley 1979, Bewley and Oliver 1992 for reviews). The rapid loss of polysomes during drying (under "natural" drying rates) and the apparent sensitivity of the initiation step of protein synthesis to protoplasmic drying leads us to the conclusion that the induction of synthesis of "protective" proteins during drying is highly unlikely. This is borne out by the observation that no new mRNAs are recruited into the protein synthetic complex even during slow drying (Oliver 1983, 1991, 1996). The fact that the moss survives rapid desiccation (even when desiccation is achieved in a few minutes in a lyophilizer), also indicates that an inducible protection mechanism is not necessary for survival.

The possible inclusion of a constitutive protection component to the mechanism of tolerance in these plants is strengthened by observations concerning sugar metabolism and the synthesis of proteins purported to have a protective function; *e.g.*, dehydrins.

Sucrose is the only free sugar available for cellular protection in desiccation-tolerant mosses, including *Tortula ruraliformis* and *T. ruralis* (Bewley *et al.* 1978, Smirnoff 1992, Willis 1964). The amount of this sugar in *T. ruralis* gametophytic cells is approximately 10 % dry weight, which is sufficient to offer membrane protection during drying, at least in vitro (Strauss and Hauser 1986). Moreover, neither drying nor rehydration in the dark or light results in a change in sucrose concentration, suggesting it is important for cells to maintain sufficient amounts of this sugar (Bewley *et al.* 1978). The lack of an increase in soluble sugars during drying appears to be a common feature of desiccation-tolerant mosses (Smirnoff 1992).

The existence of dehydrins in desiccation-tolerant vegetative tissues of fully tolerant bryophytes has only recently been reported. Western blots of soluble protein extracts from control, dry and rehydrated gametophytes using purified antibodies raised against the common carboxy-terminus of corn seedling dehydrins (Close *et al.* 1993) show

that *T. ruralis* produces two major dehydrins (80-90 kD and 35 kD). These are present in the hydrated state and do not appear to increase during rapid or slow drying (Bewley *et al.* 1993). In fact, the amount present appears to decrease somewhat during slow drying. A similar result was obtained with the desiccation-tolerant moss *Thuidium delacatulum* (T. L. Reynolds, M. J. Oliver and J. D. Bewley, unpubl. data). Thus, for desiccation-tolerant species (in contrast to those that exhibit modified desiccation-tolerance), proteins that may help accommodate water loss are constitutive.

Although much appears to be precluded during drying of gametophytic tissue of fully desiccation-tolerant bryophytes, there does appear to be some capacity to prepare for a future recovery event. Using cDNA clones corresponding to *T. ruralis* transcripts that are preferentially translated during rehydration (see below and Scott and Oliver 1994), it was determined that several "recovery" transcripts accumulate during slow drying (M.J Oliver unpublished). Analysis of this accumulation during a time of metabolic decline revealed that transcripts are being sequestered in the polysomal fraction of cell extracts. As shown previously (Dhindsa and Bewley 1997) this fraction is actively losing polysomes during slow drying and protein synthesis is inhibited. In fact, when transcript accumulation is at its peak there are no polysomes remaining (Oliver and Bewley 1997). Sucrose density gradient analysis revealed that the transcripts accumulate in a pelletable fraction that sediments near the top of a 10 to 50 % w/v sucrose gradient above, and spreading into, the region of the gradient occupied by the small ribosomal sub-unit (Oliver 1996, Oliver and Bewley 1997, and Wood and Oliver unpublished data). This result is consistent with the hypothesis that, during desiccation, mRNA transcripts are sequestered in mRNA particles (mRNPs). The sequestration of "recovery" mRNAs is not required for desiccation-tolerance or survival since rapidly desiccated moss does not accumulate mRNAs during drying. In fact, available evidence suggests that rapid desiccation results in some loss of mRNAs (Oliver and Bewley 1984b). The implication from this work is that the sequestration of mRNAs required for recovery hastens the repair of desiccation/rehydration-induced damage and thus minimizes the time needed to restart growth upon rehydration. These findings may also explain, in the absence of an inducible dehydrin and sugar response, the ability of *T. ruralis* to "harden" during recurring desiccation events (Schonbeck and Bewley 1981a,b).

As discussed earlier the structural integrity of the dried cells, at least for *T. ruralis* is maintained in the dry state (Platt *et al.* 1994) but, damage, as for all tolerant plants, does occur following rehydration (Oliver and Bewley 1984a). It is thus during the rehydration phase of a wet/dry/wet cycle that one would expect to observe an induction of repair processes.

Early work (see Bewley and Oliver 1992 for review) established the ability of *T. ruralis* and other mosses to rapidly recover synthetic metabolism when rehydrated. The speed of this recovery was dependent upon the prior speed at which desiccation occurred; the faster the rate of desiccation the slower the recovery. In addition, although the pattern of protein synthesis in the first two hours of rehydration of *T. ruralis* is distinctly different from that of hydrated controls, novel transcripts were not made in response to desiccation (Oliver 1991, Oliver and Bewley 1984c). Hence it was suggested that *T. ruralis* responds to desiccation by an alteration in protein synthesis upon rehydration which is in large measure the result of a change in translational control(s). Some changes in transcriptional activity were observed but these did not result in a qualitative change in the transcript population during desiccation or rehydration. Thus it appears that *T. ruralis* relies more upon the activation of pre-existing repair mechanisms for desiccation-

tolerance than it does on either pre-established or activated protection systems.

In a detailed study of the changes in protein synthesis initiated by rehydration in *T. ruralis*, Oliver (1991) demonstrated that the synthesis of 25 proteins is terminated, or substantially decreased, and the synthesis of 74 proteins is initiated, or substantially increased, during the first two hours of hydration. The change in synthesis of these two groups of proteins, the former termed hydrins and the latter rehydrins, is not co-ordinately controlled. The synthesis of hydrins is inhibited upon rehydration of gametophytes that were previously dried to 50 % of their fresh weight, whereas rehydrin synthesis is initiated or stimulated only by a greater water loss, to between 50 and 20 % of their fresh weight. These findings indicate that it takes a certain amount of water loss to fully activate the protein-based portion of the recovery mechanisms upon rehydration This may indicate that there is also a mechanism by which the amount of water loss is "sensed" and "translated" into a protein synthetic response upon rehydration. Perhaps this is a strategy which has evolved to link the amount of energy expended in repair to the amount of damage potentiated by differing extent of drying.

In order to elucidate the function of individual rehydrins, Scott and Oliver (1994) isolated 18 cDNAs that represent putative rehydrins of *T. ruralis*. All 18 rehydrin cDNAs represent mRNAs present in hydrated moss cells, *i.e.*, none represent transcripts exclusive to rehydration, but all are present in greater amounts in polysomes of rehydrated gametophytes compared with those from the fully hydrated moss. This is indicative of a selection of specific mRNAs into polysomes during rehydration, which is consistent with the protein synthesis data and the conclusion that translational controls are important in the rehydration response. Nevertheless, several of the rehydrin cDNAs represent transcripts that also accumulate in the total RNA pool of rehydrated gametophytes, indicating either an increased transcription of rehydrin genes upon rehydration or an increase in rehydrin mRNA stability upon rehydration (Scott and Oliver 1994). This suggests that although desiccation and rehydration do not effect a qualitative alteration in transcription in *T. ruralis*, they may alter transcription of certain genes quantitatively.

Several of the 18 rehydrin cDNAs have been partially or fully sequenced, but only three, Tr288, Tr 155 and Tr213 , have similarity with a previously documented sequences (Oliver, unpublished data). Tr288 has a dehydrin like "K" sequence at the carboxy-terminus of the predicted protein sequence but little similarity with other plant dehydrins other than it is hydrophylic in nature and contains a large number of random coils in its predicted secondary structure. It is also different from other plant dehydrins in that its synthesis is rehydration induced, especially after rapid desiccation. Tr155 is similar to two seed dormancy transcripts, one from barley embryos (Aalen *et al.* 1994) and one expressed during hydration of dormant seeds of *Bromus secalinas* (Goldmark *et al.* 1992). The functions of the proteins encoded by these transcripts is unknown but it is of note that both are related to events associated with seed imbibition (rehydration) and dormancy. Tr213 exhibits a high degree of similarity to polyubiquitins from several plant sources. This may point to an increased need for protein turnover during the recovery from desiccation. Until the function of all rehydrins (or at least the majority) it will remain unclear which cellular processes are important in the repair of desiccation and rehydration induced damage.

In our initial comments we proposed that mechanisms of desiccation-tolerance spanned a range from tissues reliant on cellular protection, to those reliant on repair of desiccation-induced damage. Although the available information is still relatively sparse it does appear to be consistent with

this proposition. Modified desiccation-tolerant plants appear to rely mostly on protective strategies but still retain a significant repair component, and fully desiccation-tolerant plants (at least the bryophytes) are more reliant on repair mechanisms, although even they must limit desiccation-induced damage to a repairable level. Within each of these groups there will be species that are exceptions to this generalization but, regardless of how individual species have developed in their protection-repair strategies, the existence of these alternatives should not be overlooked in future studies.

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