Plant Desiccation and Protein Synthesis

II. ON THE RELATIONSHIP BETWEEN ENDOGENOUS ADENOSINE TRIPHOSPHATE LEVELS AND PROTEIN-SYNTHESIZING CAPACITY[1]

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ABSTRACT

Rehydration of *Tortula ruralis* in 2,4-dinitrophenol inhibits protein synthesis, polysome formation, and ATP production. Polysomes are conserved intact and are active *in vitro* in hydrated *Tortula* placed in this chemical, although *in vivo* protein synthesis is inhibited. Hydrated moss placed under nitrogen in the dark shows a reduced capacity for ATP and protein synthesis, but polysomes are conserved. During anaerobiosis in light, ATP and protein synthesis are unaffected. Rehydration of slow-dried *Tortula* in nitrogen in the dark results in reduced *in vivo* protein synthesis, but not polysome formation; this reduction is much less in the light. Slow-dried moss, but not fast-dried, has a greatly reduced ATP content in the dry state, but this rapidly returns to normal levels on rehydration. The prolonged burst in respiration observed previously on rehydration of *Tortula* is not paralleled by ATP accumulation. Changes in energy charge in all treatments tested follow the changes in ATP. The aquatic moss, *Hygrohypnum luridum*, which is intolerant to drought, loses ATP during fast drying and this is not replenished on subsequent rehydration.

We consider that the relationship between levels of ATP and protein synthesis is more meaningful during rehydration of mosses (the time when repair to desiccation-induced cellular damage can occur) than during desiccation, and that drought-induced cessation of protein synthesis may not be mediated directly through ATP availability.

Previous studies on the mosses *Tortula ruralis* and *Hygrohypnum luridum* have demonstrated their respective capacity or incapacity to withstand desiccation stress (2, 4). Rapid desiccation of *Tortula* leads to the conservation of intact polyribosomes in the dried state, and slow desiccation results in the conservation of separated messenger and ribosomal components (9, 10). Desiccation of *Hygrohypnum* results in a loss of polyribosomes and, unlike *Tortula,* this moss does not resume protein synthesis on subsequent rehydration (3, 4). Recently, we have found that under conditions of increasing stress, *Tortula* exhibits a gradual decrease in respiration and

immediately upon rehydration there is a pronounced burst of respiration (5). On hydration of seeds, a correlation has been found between germination capacity and levels of adenosine phosphates (8, 13, 15, 17), and this may be related, in turn, to protein synthesizing capacity (14).

In this study we have determined in *Tortula* the effects on protein synthesis of conditions known to deplete ATP levels. The effects of drying and rehydration on ATP and protein synthesis in *Tortula* and *Hygrohypnum* were then followed. Consideration has also been given to the energy charge as a possible mechanism for controlling protein synthesis.

MATERIALS AND METHODS

Tortula ruralis ([Hedw.] Gaertn, Meyer, and Scherb) and *Hygrohypnum luridum* (Hedw.) Jenn. were collected and processed for experimentation as described previously (4, 19).

Three hundred milligrams of cut and washed moss were used for polyribosome extraction (9) and for *in vivo* protein synthesis studies (3). The technique for *in vitro* protein synthesis has been fully described (9).

Determination of Adenosine Phosphates. Adenosine phosphates were extracted from 300 mg of moss by grinding in a mortar in 3 ml of 35% perchloric acid (6) which was used to kill the plant at the end of the treatment period. Two more 3-ml volumes of perchloric acid were added and the material was transferred to a Duall ground glass homogenizer (Kontes Glass Co., Vineland, N. J.) for further grinding. The homogenate was kept in ice for at least 15 min, and then was neutralized with 1 N NaOH containing 0.2 N $NaHCO_3$. After further time on ice, the supernatant was collected by centrifugation and frozen at −22 C overnight. After thawing, a further clearing spin was carried out and the supernatant was made up to a standard volume (35 ml) with distilled H_2O. After appropriate dilution with H_2O, ATP was assayed after the method of Addanki *et al.* (1). Triplicate moss samples were used for each treatment.

Firefly lantern extract (Sigma FLE-250) was made up according to the instructions of the manufacturer, and after standing overnight at 4 C, it was filtered immediately before use. To a small vial (capacity 1.6 ml) was added 0.1 ml of firefly extract in 50 mM potassium arsenate and 20 mM magnesium sulfate (pH 7.4), and 0.9 ml distilled H_2O. This was placed in a glass scintillation vial and counted for 6 sec in a Packard Model 3375 Tri-Carb Liquid Scintillation Spectrometer (gain 52%, discriminator 50–1000, with the coincidence switch in the 'off' position). The material to be assayed (0.1 ml) was then injected into the vial and recounted for 6 sec immediately after the scintillation vial was 'loaded' into the machine. Counts per second of the sample minus blank were compared

with those of an ATP standard curve, linear between 1 and 40 pmoles.

The above extracts were stored frozen at −22 C for 10 days before total adenosine phosphate levels were determined. Selected treatments were appropriately diluted in 0.5 M HEPES, pH 7.5, and 0.5 M magnesium acetate. ATP, ADP, and AMP were determined according to Ching and Ching (8), except that the incubation was for 25 min at 34 C. The ATP assay was then conducted as described above. Energy charge was calculated in the normal manner (6, 8).

Boiling water extraction of adenosine phosphates from moss was compared with our perchloric acid technique and with boiling ethyl alcohol extraction (18). The former gave results almost identical to those obtained when perchloric acid was used, and the latter was less efficient and less convenient. Results presented on ATP levels were obtained from extracts diluted with water, and those on E.C. were from separate estimations of ATP, ADP, and AMP of selected extracts diluted in HEPES and magnesium acetate. The ATP estimations of the latter were close (within ± 15%) to those of the former.

RESULTS

Effects of 2,4-Dinitrophenol and Anaerobic Conditions.

Three hundred-milligram lots of hydrated or slowly dried moss were placed in Petri dishes containing 4 ml of DNP[3] (1 mM) or water. Capacity for protein synthesis was measured by adding 10 μCi of L-leucine 4,5-T (NEN, 42.7 Ci/mmole) for 30 min intervals at zero time, or after 0.5, 1, 2, or 4 hr. At these times polysomes were extracted from duplicate, unlabeled samples and analyzed directly on a sucrose gradient (3), or their synthetic activity was tested in vitro (9). Slow-dried moss, that was rehydrated in water, resynthesized polysomes, but in DNP this capacity was greatly reduced (Table I). Protein synthesis in the presence or absence of DNP reflected the levels of polysomes. Dried moss (from storage) (19) which has been rehydrated for at least 24 hr can be regarded as hydrated or control moss, because by this time Tortula has resumed its normal morphological, biochemical and structural (20) state. Placing this hydrated moss in DNP for up to 4 hr caused a large decrease in protein synthesis (over 90%) but only a 30% decrease in polysomes. When extracted polysomes were assayed in vitro, in the presence of ATP and added generating system (9), they were still active (Table I), indicating no irreversible effects on them of DNP-mediated ATP depletion. DNP itself does not directly retard protein synthesis, for when 1 mM DNP was added to the in vitro system, there was no reduction in polysome-directed incorporation of leucine into polypeptide. It is also worth noting here that 1 mM DNP did not affect the luciferin-luciferinase ATP assay system either (data not presented). Treatment of hydrated moss with 1 mM sodium cyanide resulted in a similar decrease in uptake of the isotope and its in vivo incorporation into protein over a 4 hr period (data not presented).

One further action of DNP on the hydrated moss was to decrease the uptake of radioactive leucine, which suggests that uptake is an energy-requiring process. The question arises as to whether the DNP- (and nitrogen-) induced decrease in protein synthesis in vivo was attributable to a lack of ATP required directly for some event of protein synthesis or was a consequence of a diminished supply of labeled leucine available in the cell for incorporation into protein. We do not have an unequivocal answer. The correlation between

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[3] Abbreviations: DNP: 2,4-dinitrophenol; E.C.: energy charge.

Table I. *Effects of 2,4-Dinitrophenol on Protein Synthesis, Polysome Levels and Activity, in Rehydrated and Hydrated Tortula ruralis*

Treatment	Time	Protein Synthesis[1]	Inhibition of Synthesis	Inhibition of Leucine Uptake	Polyribosomes[2]	In vitro[3] Protein Synthesis
	hr	cpm/mg protein × 10⁻³	%			cpm/mg rRNA × 10⁻³
Rehydrated moss						
+H₂O	0.5	16.0			30.1	
+DNP		0.8	95		10.4	
+H₂O	1	32.0			38.0	
+DNP		5.7	83		13.9	
+H₂O	2	46.0			39.7	
+DNP		3.8	92		4.5	
Hydrated[4] moss						
+H₂O	1	36.0				
+DNP		2.0	95	89	28.3	9.4
+H₂O	2	42.0				
+DNP		2.8	93	73	28.4	8.2
+H₂O	4	56.0			38.2	11.5
+DNP		2.8	95	88	26.7	6.4

[1] Over a 30-min period immediately before the indicated time.
[2] Value for percentage of polyribosomes was obtained after the ribonuclease treatment of ribosomal pellet had been subtracted (2).
[3] Incorporation by ribosomal pellet extracted at times indicated (9).
[4] Previously rehydrated for at least 24 hr, when the normal physiological state of the moss is regained (20).

Table II. *Effects of Anaerobiosis on Protein Synthesis, Polysome Levels and Activity, in Hydrated Tortula ruralis*

Treatment	Time[1]	Protein synthesis[2]	Inhibition of Synthesis	Inhibition of Leucine Uptake	Polyribosomes[3]	In vitro Protein Synthesis[4]
	hr	cpm/mg protein × 10⁻³	%			cpm/mg rRNA × 10⁻³
In light						
Air	2	42.6				
N₂		32.6	24		28.6	13.0
Air	4	37.6				16.3
N₂		30.0	20		27.5	14.3
Air	6	43.9			36.0	
N₂		24.6	44		21.8	
In darkness						
Air	2	31.5			31.0	
N₂		3.0	90	58	22.9	14.4
Air	4	35.0			29.0	15.0
N₂		4.6	87	48	24.0	14.4
Air	6	34.0			24.6	
N₂		2.2	94	48	19.0	

[1] Total time in air or N₂ before protein synthesis was determined or polyribosomes were extracted.
[2] Over a 30-min period immediately following the indicated time.
[3] Value for percentage of polyribosomes was obtained after the ribonuclease treatment of ribosomal pellet had been subtracted (2).
[4] Incorporation by ribosomal pellet extracted at times indicated (9).

decreased uptake and decreased protein synthesis was reasonably good in DNP-treated moss, but this correlation was much poorer in moss kept under anaerobic conditions (Tables II and III). Furthermore, we have noted that the amount of label incorporated into protein in the presence of DNP or nitrogen was never more than a few percent of total uptake. These observations suggest to us that the decrease in labeled

Table III. *Effect of Anaerobiosis on Protein Synthesis and Polysome Levels in Rehydrated Tortula ruralis*

Treatment	Time	Protein Synthesis[1]	Inhibition of Synthesis	Inhibition of Leucine Uptake	Polyribo-somes[2]
	hr	*cpm/mg protein* $\times$ *10^{-3}*		*%*	
In light					
Air	0.5	22.0			36.0
N₂		10.0	54	55	35.4
Air	1	22.0			35.9
N₂		15.0	33	68	31.2
Air	2	28.0			36.0
N₂		14.0	50	74	30.0
In darkness					
Air	0.5	21.1	85	52	41.7
N₂		3.2			36.7
Air	1	24.6	90	72	43.5
N₂		2.6			34.6
Air	2	21.3	90	78	41.5
N₂		2.3			35

[1] Over a 30-min period immediately before the indicated time.

[2] Value for percentage of polyribosomes was obtained after the ribonuclease treatment of ribosomal pellet had been subtracted (2). Polyribosomes were extracted after imbibition of moss for the indicated times.

precursor in protein was not attributable to the precursor's more limited uptake, but rather to its limited utilization for synthesis.

Hydrated moss was transferred to anaerobic conditions in the normal light in which our experiments are conducted (diffused daylight and fluorescent laboratory light), or in darkness (under a dim green safelight) by placing it into a vessel through which N₂ was continuously passed. After 6 hr in N₂, moss maintained in the light showed a decrease in protein synthesis 50% less than moss kept in darkness (Table II). In both light and darkness, anaerobiosis induced a relatively small decline in polysomes and in their *in vitro* activity. When slow-dried moss was rehydrated in light in N₂, a reduction in *in vivo* protein synthesis occurred, but this was more greatly reduced in darkness (Table III). Uptake of leucine was inhibited to a similar extent in both instances, and considerable polysome reformation occurred irrespective of whether the moss was rehydrated in light, dark, N₂, or air.

The influence of DNP and N₂ on ATP synthesis is shown in Figures 1 and 2. Hydrated moss placed in nitrogen in the dark became depleted in ATP, reaching a minimum within 2 hr, showing that our conditions for this treatment were indeed anaerobic. On reintroduction to air, normal ATP levels were regained within 30 min. Moss kept under the same anaerobic conditions in the light showed little change in ATP; similar results were obtained with moss kept in air in darkness. The E.C. for hydrated *Tortula* and for that maintained in light in N₂ for 2 hr were similar, but *Tortula* in N₂ in darkness for 2 hr had a markedly decreased E.C. (Fig. 1). Moss kept in light and air for the duration of this experiment exhibited no changes in ATP or E.C. (data not presented). When ATP was depleted in N₂ in darkness for 2 hr there was no net loss of total adenosine phosphates (*e.g., Tortula* kept under N₂ in darkness or light contained 3.52 and 3.55 μmoles/g fresh weight of adenosine phosphates, respectively, compared with 3.59 μmoles/g in moss maintained 4 hr in light in air). Rehydration of slow-dried moss in 1 mM DNP prevented any increase in ATP over 1 hr compared with the dramatic increase within 5 min in water-rehydrated moss (Fig. 2).

ATP and Energy Charge on Drying and Rehydration. Moss was desiccated rapidly or slowly (10) and ATP content of the dried moss was measured (Fig. 2). Fast-dried moss retained the same ATP levels as the hydrated controls. Slow-dried moss showed the same trend down to 34% fresh weight, even though there was a marked loss of polysomes at this stage (ref. 10, and Fig. 2). A further 14% loss in fresh weight resulted in a 62% decrease in ATP. The E.C. decreased from 0.76 to 0.52 in the slow-dried moss (Table IV). Both ATP and E.C. returned to normal values within 5 min of reintroduction of water to the slow-dried moss (Table IV and Fig. 2); protein synthesis resumed within this time period too (10). Fast-dried moss retained a fairly high polysome content (2, 3), and no decrease in ATP or E.C. occurred either (Table IV and Fig. 2). A small
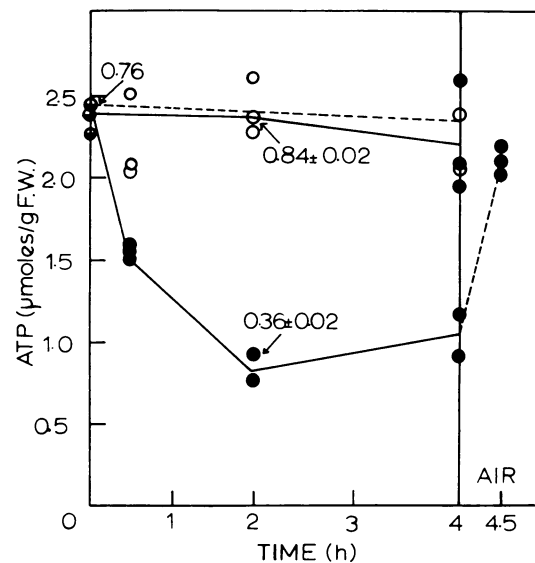

FIG. 1. Effects of anaerobiosis on ATP content and E.C. of hydrated moss. ●———●: N₂, dark; ○———○: N₂, light; ●--●: air, dark. Moss was in the hydrated state at the beginning of the experiment (zero time). Points indicate degree of variability between three replicates. Figures arrowed to certain points in the graph represent the E.C. (± SD) at that time.
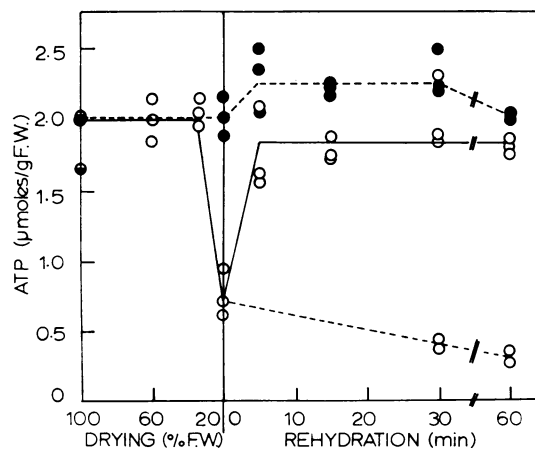

FIG. 2. Changes in ATP content and E.C. during drying and dehydration of fast- and slow-dried moss. ●--●: fast-dried moss; rehydrated in H₂O; ○———○: slow-dried moss; rehydrated in H₂O; ○--○: slow-dried moss, rehydrated in 1 mM DNP. All treatments were performed under lighted conditions. For details on drying treatments and times see reference 10. One hundred per cent fresh wt. represents ATP in control moss rehydrated for 24 hr.

Table IV. *Energy Charge of Quickly and Slowly Dried Tortula ruralis and during Rehydration*

Hydrated moss has an E.C. of 0.76 (see Fig. 1).

| Treatment | Energy Charge[1] | | |
| | Time of rehydration | | |
	0 min	5 min	30 min
Fast-dried	0.72 ± 0.07	0.76 ± 0.03	0.68
Slow-dried	0.52 ± 0.04	0.76	0.725 ± 0.025

[1] ± SD of duplicate samples rehydrated on water in light.

increase in ATP, but not in E.C., occurred on rehydration of fast-dried moss, but this (if at all significant) was small in comparison to the burst in ATP synthesis occurring in the rehydrated slow-dried moss. Total adenosine phosphate levels were maintained more or less equally in slow- and fast-dried moss on drying and rehydration (data not presented).

ATP and Energy Charge in Dried and Rehydrated Hygrohypnum luridum. Hygrohypnum is an aquatic moss and intolerant to drought (4). ATP content of hydrated, fast-dried and rehydrated moss are shown in Table V, along with the related E.C. Dried moss maintained less than 50% of the level of ATP found in moss which had been grown in the greenhouse in humid conditions, and had never been subjected to water stress. In hydrated Hygrohypnum, ATP levels and E.C. were lower than those of Tortula, but they are obviously sufficient because this moss continues to flourish in the greenhouse. Rehydrated Hygrohypnum, unlike Tortula, showed a decline in ATP and E.C., although total adenosine phosphate levels were more or less equal throughout (1.72–1.90 μmoles/g fresh weight).

DISCUSSION

Slow-dried moss contains no polysomes because during the drying process ribosomes run off the message, and there is a restriction to reassociation which is probably attributable to failure of the ribosome and message to carry out the initiation reaction (10). ATP is believed to be involved in this factor-dependent process (16) in wheat embryos and it is widely recognized for its involvement in the aminoacylation of tRNA in both pro- and eukaryotes. For in vitro protein synthesis to be catalyzed by moss ribosomes, there is an absolute dependence upon ATP (9), although we do not know yet which steps show this dependence. The possibility arises that restrictions on protein synthesis imposed by desiccation stress are mediated via a limited supply of ATP. In dry seeds, which have separated ribosomes and message, ATP content is very low (7, 13–15) and when protein synthesis resumes on rehydration, ATP levels quickly rise (14). One might expect a similar correlation of events in Tortula.

Rehydration of slow-dried moss in DNP resulted in very limited protein synthesis, polysome reformation, and ATP synthesis. However, when hydrated moss was placed in DNP there was a marked decrease in protein synthesis but not in extractable polysomes. This means that polysomes already present in the hydrated moss could not complete the translation process in the presence of DNP. This could be attributable to failure of aminoacyl tRNA formation, because a restriction on the reinitiation process alone would allow for polysome run off, as occurs in the presence of known initiation inhibitors (3). Limiting ATP levels would also result in limited

GTP synthesis. GTP is necessary for peptide chain elongation. One further possibility is that the termination process of protein synthesis might be ATP-dependent, but nothing is known about this event in plants.

The maintenance of relatively high amounts of polysomes in the presence of DNP (and N_2) over 4 to 6 hr also serves to indicate a lack of endogenous ribonuclease activity in Tortula under conditions where polysomes are inactive. We have previously pointed to a lack of involvement of this enzyme during drought-induced polysome depletion (10).

Hydrated Tortula subjected to anaerobic conditions reacted differently in darkness than in light. Tortula maintained in light showed a maximum decrease in in vivo protein synthesis of 44% after 6 hr in N_2, compared with 94% for plants in darkness. Reduction in polysome levels in darkness was small (23%) in relation to the fall in protein synthesis. When soybean roots were kept under anaerobic conditions for as little as 1 hr, polysomes became depleted and the in vitro activity of the extracted ribosomal pellet was reduced to around 20% (12). In contrast, reduction of in vitro activity of the extracted ribosomal pellet from anaerobic, dark-imbibed Tortula was less than 5%; the same conditions depleted in vivo synthesis by nearly 90% (Table II).

Slow-dried moss was rehydrated in aerobic or anaerobic conditions in light and darkness (Table III). The decrease in in vivo protein synthesis under N_2 in light was considerably less than that in darkness, although it is interesting to note that there was almost equal polysome reformation as in the aerobic controls. It is possible that in the moss rehydrated in N_2 in darkness there is sufficient ATP and aminoacyl tRNA present (unlikely in view of the DNP results) or produced (more likely) to allow for polysomes to be formed, but insufficient amounts for maintenance of their in vivo activity. Polysomes formed in the light under N_2 are 3 to 5 times more active than those formed in darkness. This increased in vivo synthetic capacity in light in rehydrated and hydrated Tortula could be attributable to ATP being formed by reactions occurring within the irradiated chloroplasts (assuming such ATP is even available to the cytoplasm) or to utilization within the cell of O_2 produced by photosynthesis.

There is a good correlation between in vivo protein synthesis and endogenous ATP levels (Tables II and III, and Fig. 1). Recently, the importance of the energy charge has been emphasized (6), and it has been suggested that an E.C. of about 0.8 must be maintained for growth and an E.C. above 0.5 is necessary for viability. The E.C. is around 0.8 in hydrated moss (Fig. 1) and also in moss in light under N_2. In darkness in

Table V. *Effects of Desiccation on ATP Levels and Energy Charge of Hydrated and Desiccated Hygrohypnum luridum*

Treatment	ATP[1]	E.C.[2]
	$\mu moles/g$ fresh wt	
Hydrated[3]	0.57 ± 0.07	0.63
Fast-dried		
Dry	0.27 ± 0.02	0.48 ± 0.01
Rehydrated		
5 min	0.11 ± 0.04	
30 min	0.07 ± 0.02	
60 min	0.04 ± 0.02	0.30 ± 0.07
210 min	0.05 ± 0.02	

[1] ± SD of triplicate extractions.
[2] ± SD of duplicate extractions.
[3] Moss which was maintained in the greenhouse in the hydrated state (4).

N_2, however, the E.C. fell to 0.36, when ATP and protein synthesis levels were depleted. While a correlation between E.C. and germination capacity has been shown in seeds (7, 8, 13, 14), it has been pointed out (8) that the phenomenon of E.C. is derived from studies on prokaryotic bacteria (6). Eukaryotic plants could have compartmental synthesis and utilization of various adenosine phosphates in nuclei, chloroplasts, and mitochondria, and thus E.C. calculations derived from extracted ATP, ADP, and AMP might not be indicative of the availability of these at any one particular site within the cytoplasm.

A small increase in ATP, but not in E.C., occurred on rehydration of the fast-dried moss but this was small in comparison with the burst in ATP synthesis in rehydrated slow-dried moss. These results are somewhat surprising, for on rehydration of slow-dried moss we have observed a very large burst in respiration, which was even greater in rehydrated fast-dried moss (5). This burst can be maintained for several hours, yet ATP levels became constant in 5 min in rehydrated slow-dried moss and increased little in rehydrated, fast-dried moss (ref. 5, and Fig. 2). It is possible that much of the observed respiratory burst is uncoupled from ATP production and only sufficient ATP synthesis occurs to maintain a constant pool. Alternatively, a large amount of ATP could be synthesized, but utilized at the same rate. Because respiratory bursts have often been observed when droughted plants are rehydrated (5, 20 and references therein), in the light of our results it would seem that the relationship between enhanced resaturation respiration and ATP synthesis could be studied profitably. As far as mitochondria in *Tortula* are concerned, these become very swollen on rehydration and return to their normal size within 24 hr (20).

We conclude, on the basis of our experiments with *Tortula*, that there is a correlation between ATP levels, E.C., and protein synthesis, at least during rehydration of slow-dried moss and in moss subjected to DNP or anaerobic conditions. Because ATP and E.C. remain high in fast-dried and rehydrated moss, in which polysomes are conserved, there might be a correlation here also. We are more skeptical of the potential involvement of ATP in controlling the switching-off process of protein synthesis during desiccation. Protein synthesis ceases in fast-dried moss while ATP levels and E.C. are still high. On slow drying, polysomes are considerably depleted at 43% fresh weight and yet ATP levels have not declined at all. Thus, while we have shown that reduced ATP levels decrease protein synthesis, decreased protein synthesis resulting from drought need not be mediated through a decline in ATP. We assume in our argument, however, that there is no compartmentation of ATP away from the sites of protein synthesis.

In a previous study of "acid-soluble organic phosphorus" levels in the poikiloxerophytic moss *Neckera crispa*, Henckel and Pronina (11) found no decreases during drying (rate of drying unstated), nor during gradual drying of the drought-resistant, blue-green *Stratonostoc commune*. They did not, however, observe any respiratory burst on remoistening after dehydration. They concluded that the maintenance of the "acid-soluble phosphorus" at the same level after dehydration as before is important in drought resistance because energy is transferred to the "appropriate submicroscopic structures throughout drying" and this is used for the formation and maintenance of these labile structures.

On the other hand, we consider the capacity for repair of membranes and organelles on rehydration to be one important adaptation to drought resistance, as well as some resistance to irreparable damage during drying (see also ref. 20). Thus, depletion of ATP during drying is of no consequence if high levels can be resynthesized during rehydration, when repairs are effected. Henckel and Pronina (11) found a big decrease in "acid-soluble phosphorus" levels in dried hygrophytic mosses *Plagiochasma asplenioides* and *Atrichum undulatum* during drying, as we found for *Hygrohypnum*. We presume hygrophytic mosses lack an effective repair mechanism, or there is insufficient ATP produced to prime this mechanism, or organelle damage is so extensive that ATP production and organelle repair is irreversibly disrupted.

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