Plant Physiol. (1973) 51, 285–288

Polyribosomes Conserved during Desiccation of the Moss *Tortula ruralis* Are Active[1]

Received for publication June 12, 1972

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ABSTRACT

During desiccation of the moss *Tortula ruralis* (Hedw.) (Gaertn, Meyer and Scherb) polyribosomes are conserved. On rehydration, protein synthesis is rapidly resumed. In the presence of protein synthesis initiation inhibitors ribosome run-off from the conserved polyribosomes takes place, confirming that these retain their activity as intact structures during desiccation.

Tortula ruralis (Hedw.) (Gaertn, Meyer & Scherb) is a poikilohydrous moss with no adaptations to regulate water loss. Many mosses, however, possess physiological adaptations which allow them to suspend their metabolism during periods of drought. Such mosses, of which *T. ruralis* is one, are termed pollacauophytes, and their resistance to desiccation "appears to be exhibited in the cytoplasm" (3).

Many species of bryophytes have been reported to withstand severe desiccation conditions (8), but, to date, little is known of the metabolic mechanisms underlying this drought resistance. Some drought-resistant species of moss undergo a slow decline in the rate of photosynthesis upon desiccation, followed by a rapid recovery on rewetting (10). In other species the rate of respiration increases markedly within hours of rehydration and then falls to near normal levels (6, 15). This enhanced respiration upon rehydration appears to be a general feature of droughted plants (14).

More recently it was observed that the protein synthesizing apparatus (polyribosomes) of the moss *T. ruralis* was conserved during desiccation over activated silica gel (1). On subsequent rehydration the numbers of polyribosomes increased within 2 hr. In this paper, as part of a continuing project to determine the nature of desiccation tolerance in mosses, it is shown that protein synthesis begins rapidly upon rehydration of *T. ruralis* and that this synthesis is carried out on conserved polyribosomes.

MATERIALS AND METHODS

T. ruralis (Hedw.) (Gaertn, Meyer & Scherb) was collected from a wooded habitat close to the city of Calgary. Only the green part of the *Tortula* plant was used for experiments. Preparation of the material and desiccation and rehydration techniques have already been described fully (1).

Extraction of Polyribosomes. Improved extraction of polyribosomes was obtained using a modification of the technique of Jachymczyk and Cherry (9). Moss material (0.5 g) was placed in an ice cold mortar and ground successively in two 3-ml volumes of grinding solution: 0.25 M sucrose, 20 mM KCl, 10 mM magnesium acetate, 50 mM tris-Cl, pH 7.6, 5 mM mercaptoethanol and 1% sodium desoxycholate. The 6 ml of homogenate was transferred to a Duall homogenizer (Kontes Glass Co. Vineland, N.J.), and grinding was completed with five strokes and a half-turn of the ground-glass pestle. The grinding was kept as uniform as possible, and the homogenizers were replaced at regular intervals since, as previously noted in extractions from barley aleurone (5), the quality of the polyribosome preparation varied greatly with the degree of homogenization and age of the homogenizer. After rinsing the homogenizer with a further 2 ml of grinding solution, the homogenate was cleared by centrifugation for 10 min at 15,000*g*. The supernatant was layered over 1.5 M sucrose containing 20 mM KCl, 10 mM magnesium acetate, 50 mM tris-Cl, pH 7.6, 5 mM mercaptoethanol and centrifuged for 1.5 hr at 200,000*g*. The ribosomal pellet thus obtained (consisting of polyribosomes, single ribosomes, and ribosomal subunits) was resuspended in 0.6 ml 20 mM KCl, 10 mM magnesium acetate, 50 mM tris-Cl, pH 7.6, and 5 mM mercaptoethanol with the aid of a motor-driven Teflon pestle. It was then cleared at 15,000*g* for 7 min, and the supernatant, containing a known amount of RNA (estimated by $A_{260\ nm}$) was layered over a 5.5 ml gradient of 10 to 27% sucrose containing 20 mM KCl, 10 mM magnesium acetate, 50 mM tris-Cl, pH 7.6, and 5 mM mercaptoethanol. This was centrifuged at 114,000*g* for 1 hr in a Spinco SW 50.1 rotor. Fractionation of the gradient was carried out using a modified ISCO density gradient fractionator with a 2-mm light path and continually monitoring absorbance at 254 nm. The method used for calculating the percentage of polyribosomes and single ribosomes has been previously reported (1).

Labeling and Extraction of Radioactive Proteins. In most of these experiments, 0.3 g of moss, desiccated over silica gel for 2 hr (20% fresh weight), was placed into the outer rim of a Conway unit and allowed to imbibe in 25 μc of DL-leucine 4,5-T (21.4 c/mmole) (Radiochemical Center Amersham), 100 international units/ml penicillin-streptomycin (Bio-Cult Laboratories, Glasgow) (to eliminate bacterial contamination), and 3 ml of the appropriate imbition solution. Two ml of water were introduced into the central well to maintain the humidity in the unit, a loose fitting lid was placed over the unit, and imbibition was carried out for the desired time. The moss was then washed thoroughly with distilled water to remove excess leucine prior to extraction of proteins.

The extraction technique employed was based on that used previously by Bewley and Black (2). Initial grinding was

[1] Supported by National Research Council of Canada Grant A6352 and an equipment grant from the University of Calgary.

carried out in an ice cold mortar with 3 ml 0.1 M tris-glycine buffer, pH 8.4. Another 3 ml of buffer were added, and after further grinding the homogenate was transferred to a Duall homogenizer and ground vigorously with five or six strokes. The uniformity of homogenization necessary for consistent polyribosome extraction did not appear to be required for the extraction of labeled proteins. After rinsing the homogenizer with 2 ml of the extraction buffer, 8 ml of supernatant were collected by clearing at 17,500*g* for 10 min, and protein was precipitated by adding trichloroacetic acid to a final concentration of 5%. The protein was allowed to precipitate for 10 min in the cold and collected by centrifugation. The pellet was resuspended in a further 5 ml of 5% trichloroacetic acid and then was incubated at 95 C for 15 min. The hot trichloroacetic acid-precipitable material was pelleted after cooling on ice and then taken up in 2 ml 90% formic acid. Aliquots were counted in a standard toluene-ethanol (7:3) scintillator in a Packard Tri-Carb liquid scintillation spectrometer.

Proteins were assayed by the method of Lowry *et al.* (11) following reprecipitation of the proteins from the 90% formic acid with trichloroacetic acid and resuspension in 1 N KOH.

Where the radioactive amino acid was introduced after 15 or 30 min of imbibition, the moss was first rehydrated in 4 ml of the required solution and then processed as described above.

RESULTS AND DISCUSSION

Total RNA and Polyribosome Levels following Desiccation. The modified RNA-extraction technique of Jachymczyk and Cherry (9) gave consistently higher yields of RNA-containing material than the previously reported method (1). This was attributed to the presence of a higher Mg^{2+} (10 mM) levels in the extraction medium; the presence or absence of sodium desoxycholate appeared to make little difference, although it was always included. Table I shows that the total levels of extractable RNA in the ribosomal pellet are the same during the rehydration period as immediately after or before desiccation of the moss for 2 hr over activated silica gel. The distribution of this RNA as single ribosomes or polyribosomes changes. Before desiccation the amount of the RNA as polyribosomes (as measured by absorbance at 254 nm) is about 55% and this falls to around 38% in the dry moss. It remains at this level immediately on rehydration of the moss (about 30 sec after the introduction of water, when the normal morphological form of the moss is resumed once more [1]). Thirty min after rehydration, however, the levels of polyribosomes have risen considerably and within 2 hr are the same as observed in undesiccated moss.

These observations suggest that the protein synthesizing apparatus should be active very rapidly upon hydration of the dry moss. The following experiments were carried out to confirm this and to determine whether it is the conserved polyribosomes which are active or whether (*a*) new messenger or other type of RNA synthesis is a prerequisite for protein synthesis or (*b*) reinitiation of existing mRNAs is essential. If protein synthesis is not totally dependent on either (or both) of these events, then it must be concluded that the polyribosomes conserved during desiccation remain active.

Table I. *Levels of Total RNA and Polyribosomes on Rehydration of T. ruralis*

Condition of Moss	Total RNA[1]	RNA[1] as Polyribosomes
	μg/g initial fresh weight	%
Normal	240	55.5
Total desiccated		38.1
Immediate rehydration	245	38.0
Rehydrated 30 min	235	52.0
Rehydrated 2 hr	240	55.5

[1] Present in the ribosomal pellet (see "Materials and Methods," Extraction of Polyribosomes).

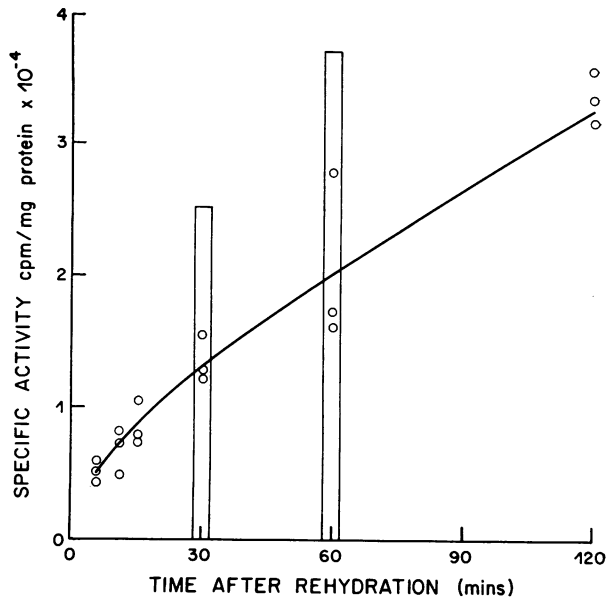

FIG. 1. Protein synthesis on rehydration of 2-hr desiccated *T. ruralis*. Solid line represents the rate of protein synthesis occurring in rehydrated moss. Block diagrams at 30 and 60 min indicate the amount of protein synthesis in undesiccated control moss at these times. Uptake of labeled leucine by the moss is completed within 2 min of rehydration.

Protein Synthesis on Rehydration. As shown in Figure 1, the rate of protein synthesis, as measured by the incorporation of ^{3}H-leucine into protein, rises rapidly within minutes of rehydration of 2-hr desiccated moss. It is presumed that in dry moss the amount of protein synthesis taking place is very low, probably zero, although there appears to be no means of testing this without the introduction of liquid to the moss. The higher rates of protein synthesis in the undesiccated moss are also shown for comparison (Fig. 1). *Tortula* which has been desiccated for periods up to 10 months will conduct protein synthesis on rehydration at a comparable rate to the 2-hr desiccated moss (Bewley, unpublished data). The total length of desiccation, therefore, appears irrelevant insofar as rapid resumption of the translation processes in concerned.

Protein Synthesis in the Presence of Initiation Inhibitors. Aurintricarboxylic acid (Sigma Chemical Co.) and pactamycin are inhibitors of the initiation process of protein synthesis and both are known to be active in plant systems (12, 13). It was reasoned that if the polyribosomes conserved during desiccation of the moss *T. ruralis* remain active, then on rehydration they should be capable of protein synthesis even in the presence of initiation inhibitors, since these inhibitors have no affect on ribosomes actively engaged in protein synthesis on mRNA. Furthermore, with a restriction on reinitiation imposed by the inhibitors, the levels of polyribosomes should fall with time of imbibition as the ribosomes become detached upon completing the reading of the message and are prevented from initiating further protein synthesis.

In Figure 2 a comparison is made of the amounts of poly-

ribosomes over a 30-min rehydration period in the presence and absence of 5×10^{-2} M ATA and 10^{-4} M pactamycin. The procedure outlined in "Materials and Methods" was used for polyribosome extraction at the times indicated. Before extraction, however, it was found to be essential to wash off, with distilled water, all excess inhibitor (particularly ATA[2]) since even in low residual concentrations this reduced the levels of polyribosomes extracted.

Figure 2a shows that the degree of ribosomal material in the form of polyribosomes after 30 sec of rehydration (immediate rehydration) is almost the same in ATA- and pactamycin-treated *Tortula* as in the control moss which was rehydrated on water. As was pointed out previously, the uptake of solution into the moss is achieved within 30 sec. Since the levels of polyribosomes within the inhibitor-treated and control moss are similar, it is obvious that levels of ATA or pactamycin taken up into the moss do not interfere directly with the polyribosome patterns obtained. The recorded changes in these patterns due to the inhibitors at subsequent imbibition times must, therefore, be attributable directly to their action on protein synthesis and not indirectly due to excess present during the extraction procedure.

Fifteen min after rehydration in ATA or pactamycin, the amount of polyribosomes in the moss have been reduced (Fig. 2b), indicating a run-off of three ribosomes from the polyribosomes conserved during desiccation. In contrast, as noted before, the level of polyribosomes have risen in the control moss over this period. After 30 min there is a further slight reduction in polyribosome levels in the inhibitor-treated moss and an increase in the amount of polyribosomes in the control (Fig. 2c).

That protein synthesis is occurring during this period of ribosome run-off was shown by using labeled leucine. Following 2 hr of desiccation the moss was reimbibed on radioactive leucine in the presence or absence of ATA for 15 or 30 min, and the amount of incorporation of this amino acid into protein was determined. In the absence of ATA the level of protein synthesis, as recorded by the incorporation of ^{3}H leucine into protein, within 15 min was 8,480 cpm/mg protein and this doubled to 16,900 cpm/mg protein after a rehydration time of 30 min. In the presence of ATA there was a marked reduction in protein synthesis of 2260 cpm/mg protein and 4640 cpm/mg protein after a reimbibition period of 15 and 30 min respectively. These results show that even in the presence of ATA protein synthesis is taking place, and that the run-off of the ribosomes from the polyribosomes is indeed accompanied by active protein synthesis.

The measurement and comparison of the areas under the single ribosome and polyribosome regions on an ISCO trace following gradient centrifugation can only be regarded as an approximate indication of the levels of active polyribosomes present, particularly since even after ribonuclease degradation of polyribosomes extracted from this moss some 17% of the total absorbance material is still present under the polyribosome areas (Fig. 2 legend and ref. 1). However, even after 30 min of imbibition in the presence of the initiation inhibitors, it appears that complete ribosome run-off has not yet been achieved (polysome levels approximately 30%, Fig. 2c). If the tritiated leucine is supplied to the moss for 15 min, following a 15- or 30-min rehydration period, it is still incorporated into protein. (Table II). Again, the amount of incorporation in the presence of ATA is reduced, but even after a 30-minute preimbibition period on this inhibitor, prior to the addition of leucine, some incorporation into protein is still taking place. The relatively long time required for total ribosome run-off from the conserved polyribosomes can only, at present, be a subject for speculation. It is possible that on some polyribosomes it takes over 30 min for the last ribosomes to complete reading of the mRNA. When one considers the rapid rate of protein synthesis taking place in known bacterial systems this would appear unlikely, although nothing appears to be known about the *in vivo* rate of protein synthesis in plants. Another possible explanation is that the resumption of protein synthesis occurs at different times on different polyribosomes following rehydration. The possibility that some reinitiation of protein synthesis is taking place, even in the presence of ATA, thus maintaining the low level of incorporation cannot be totally

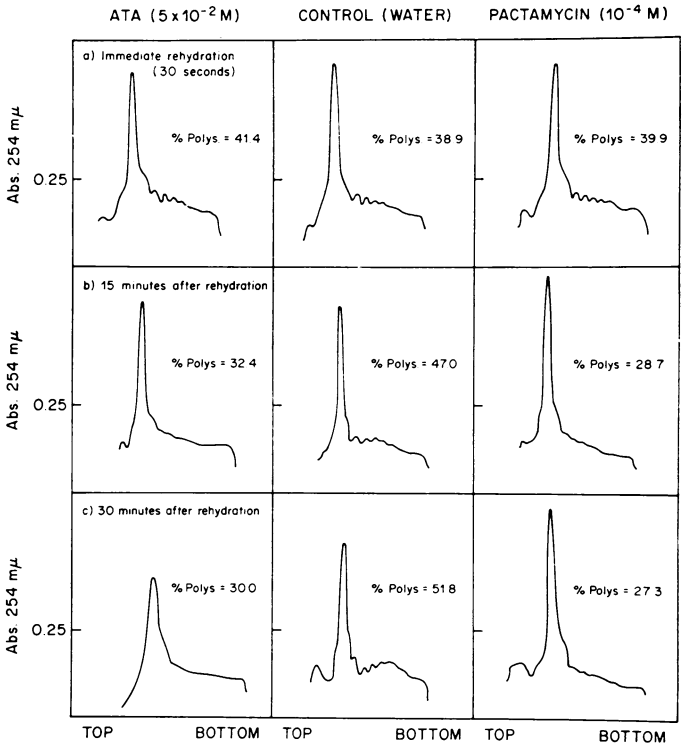

FIG. 2. Ribosome run-off from conserved polyribosomes using ATA and Pactamycin. % Polys: percentage total area in each profile as polyribosomes (see ref. 1 for details). In a parallel series of experiments incubation of the extracted RNA with ribonuclease prior to introduction to the gradient resulted in an OD 254 mμ area reading in the polyribosomal region of about 17% (see ref. 1 for details).

Table II. *Protein Synthesis in Moss Preimbibed before the Introduction of* ^{3}*H-Leucine*

Desiccated moss (0.25 g) was preimbibed on ATA (5×10^{-2} M) or water for 15 or 30 min before transfer to Conway units containing 25 μc DL-leucine 4,5-T, ATA (5×10^{-2} M) or water and 100 international units of penicillin-streptomycin. Protein extraction was carried out 15 min after the time of transfer.

Time of Preimbibition	Protein Synthesis	
	−ATA	+ATA
min	*cpm/mg protein*	
15	10810	2712
30	13430	2650

[2] Abbreviation: ATA: aurintricarboxylic acid.

discounted, although it would appear unlikely with the high concentration (5×10^{-2} M) being used for these experiments.

Parallel incorporation studies using pactamycin were not carried out because of the limited supply available.

CONCLUSIONS

Preliminary studies have indicated that polyribosomes in the moss *T. ruralis* are conserved even after a desiccation period of 24 hr (1). Furthermore, upon subsequent rehydration the levels of polyribosomes increased within 2 hr to those values observed in the undesiccated moss. Although there appears to be no changes in the total amount of extractable RNA, there is an increase in distribution as polyribosomes with increasing time of rehydration. This phenomenon has now been recorded in *Tortula* desiccated for 10 months (Bewley, unpublished).

On rehydration of the moss following a 2-hr desiccation period there is a rapid resumption of protein synthesis. In the presence of ATA and pactamycin, inhibitors of protein synthesis initiation, run-off of ribosomes from the conserved polyribosomes takes place, accompanied by active protein synthesis. The results utilizing these inhibitors demonstrate conclusively that the polyribosomes conserved during total desiccation are active on rehydration.

The question as to whether transcription, as well as translation, is resumed rapidly on rehydration remains to be determined. These studies utilizing initiation inhibitors do suggest, however, that the synthesis of at least two species of RNA, messenger and ribosomal, is not a prerequisite for the protein synthesis observed immediately on rehydration, since in the presence of these inhibitors the translation of both nascent and existing messages would be prevented and, furthermore, newly synthesized ribosomes could not become attached to the initiation sites on either type of message.

This capacity of *Tortula* to maintain a stable protein synthesizing apparatus upon desiccation is of interest when one considers both its poikilohydrous nature and its habitat. It often grows as a primary colonizer on exposed slopes where it is subjected to drought. The ability to conserve the integrity of the polyribosomes can be regarded as an ecological adaptation. It should be noted that the protein-synthesizing capacity of the moss *Bryum pseudotriquetrum*, which grows in an aquatic habitat where it is not normally subjected to drought, is eliminated under water stress conditions and the polyribosomes are irreversibly degraded (Bewley, to be published). This response of *Bryum* is similar to that observed in higher plants subjected to lethal water stress (4, 7).

Acknowledgments—I am grateful to Dr. D. P. Weeks, I.C.R., Philadelphia for kindly donating some of his limited supply of Pactamycin, provided to him by the Upjohn Co., Kalamazoo, Michigan. I am also indebted to Drs. J. D. Duerksen and R. B. Church for their generous loan of equipment and bench space until my own laboratory facilities were established.

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