Plant Physiol. (1984) 74, 21-25
0032-0889/84/74/0021/05/$01.00/0

Plant Desiccation and Protein Synthesis. IV. RNA Synthesis, Stability, and Recruitment of RNA into Protein Synthesis during Desiccation and Rehydration of the Desiccation-Tolerant Moss, *Tortula ruralis*[1]

Received for publication April 13, 1983 and in revised form September 8, 1983

MELVIN J. OLIVER[2] AND J. DEREK BEWLEY*
Plant Physiology Research Group, University of Calgary, Calgary, Alberta T2N 1N4 Canada

ABSTRACT

Upon rehydration of desiccated *Tortula ruralis*, RNA synthesis is immediately resumed; this resumption is quicker in moss recovering from slow drying than from rapid drying. Newly synthesized RNA enters the protein synthetic complex almost immediately upon rehydration, reaching control steady state levels within 2 hours after slow drying and 6 hours after rapid drying. RNA synthesized in the 1st hour following the readdition of water enters into polysomes much earlier after slow drying than after rapid drying. The RNA components of the protein synthetic complex are stable to desiccation at either slow or rapid speeds, although more so following the former drying regime. Immediately upon rehydration, these conserved RNA are readily utilized for protein synthesis, and continue to be so at least 4 hours thereafter. Hence, the speed of desiccation affects the rate at which RNA is synthesized upon subsequent rehydration, as well as the mode of utilization of that RNA.

Upon drying, desiccation-tolerant mosses lose their capability for protein synthesis (2, 3, 14, 17). In *Tortula ruralis*, this is manifested as a decline in polysomes during the drying process; the extent of polysome loss is influenced by the speed at which desiccation occurs (13). Rapid water loss results in the retention of approximately 50% of the polysomes in the dried state; slow water loss causes complete depletion of polysomes. This loss of polysomes during desiccation is not the result of an increase in RNAse activity (9, 10, 16) but rather is the consequence of ribosomal run-off associated with a failure of the reinitiation process (5, 9). In the dried state, *T. ruralis* contains potentially active ribosomes (12), cytoplasmic rRNA (18), and mRNA (11). Although these studies contribute towards our understanding of the effects of severe water stress on the protein-synthesizing complex in tolerant plants, we know less about the recovery of normal metabolic processes upon the release from stress, an equally important aspect in relation to how these plants can withstand desiccation.

Previous studies on the recovery of protein synthesis upon rehydration of *T. ruralis* showed that this process recommences rapidly (3, 13); this is also the case in *Polytrichum commune* (17) and *Neckera crispa* (14). In *T. ruralis*, control levels of polysomes are restored within 2 h of the reintroduction of water to slow-dried moss, even after the moss has been stored in the desiccated state for 10 months (4, 9). However, the speed at which desiccation is administered quite dramatically affects the rate at which protein synthesis recovers. Surprisingly, even though rapid-dried moss contains a significant amount of polysomes (13), slow-dried moss resumes protein synthesis at a faster rate (13). The reason for this discrepancy is not readily apparent, although studies have shown that rapid desiccation is the more damaging to cellular integrity and metabolism, and the slower recovery of protein synthesis may be a manifestation of this disruption (6, 7, 10). RNA synthesis also recovers quickly upon rehydration, at least for slow-dried *T. ruralis* (11). However, it appears from inhibitor studies that, upon rehydration, RNA synthesis (11) is not necessary for protein synthesis to resume, at least initially. Whether or not it is necessary for the survival of the moss during later times of rehydration was not ascertained.

To understand the mechanisms underlying the ability of *T. ruralis* to recover from a desiccation event, we have undertaken a comprehensive study on the effects of drying on the recovery of protein synthesis. This paper deals with the recovery of RNA synthesis upon rehydration and its recruitment into the protein synthesizing complex. We have also attempted to quantify the effect of desiccation upon extant RNA involved in the protein synthetic complex and the role of conserved RNA in rehydration protein synthesis.

MATERIALS AND METHODS

Plant Material. *Tortula ruralis* ([Hedw.] Gaertn, Meyer, and Scherb) was collected from its natural habitat along the Bow River west of the Calgary city limits. The moss clumps were dried at room temperature on the laboratory bench before storage in black polyethylene bags at 5°C. For experimental purposes, the apical 1 cm of the green gametophyte was cut from moss rehydrated for 24 h in diffuse lighting and washed thoroughly with distilled H_2O.

Administration of Desiccation. Rapid desiccation was achieved by placing fresh moss gametophytes of known weights on a disc of Whatman No. 1 filter paper overlying activated silica gel in a closed dish for 24 h. Slow desiccation was administered by placing similar samples in aluminum dishes over a stirred saturated solution of ammonium nitrate (RH 65%) for 24 h. Using these regimes, the air-dried weight (20% of original fresh weight) was obtained within 1.5 and 3.4 h, respectively.

Radioactive Labeling and Rehydration Conditions. To measure RNA synthesis and entry of newly synthesized RNA into the

[1] Supported by Natural Sciences and Engineering Research Council of Canada Grant A6352 to J. D. B.

[2] Present address: Department of Biology, Washington University, St. Louis, MO 63130.

protein synthetic complex upon rehydration, dried moss (300–400 mg fresh weight) was rehydrated in 3 ml distilled H_2O containing 2.5 μCi [^{14}C]uridine (Amersham, 522 mCi/mmol) for various periods of time. In pulse-chase experiments, dried moss (400 mg fresh weight) was rehydrated for 1 h in 3 ml distilled H_2O containing 2.5 μCi [^{14}C]uridine, washed thoroughly with distilled H_2O, and incubated for various times in a 4.8 μg/ml solution of uridine. For RNA stability experiments, 400-mg samples of 24-h hydrated moss were incubated for 4 h in the presence of 3 μCi [^{14}C]uridine, washed with distilled H_2O, and subjected to desiccation at both rapid and slow speeds. These samples were then rehydrated for hourly periods in a 5.4 μg/ml solution of uridine.

RNA Isolation. Radioactive labeled moss was homogenized in 8 ml extraction buffer (0.1 M Tris-acetate [pH 9.0], 0.1 M NaCl, 2 mM EDTA, and 1.0% [w/v] SLS) (19) at 4°C, first in a mortar and pestle and finally in Duall ground grass homogenizer (Kontes Glass Co., NJ). The homogenate was centrifuged at 15,000g for 10 min and the pellet discarded. An aliquot of the supernatant was taken at this point for precursor uptake estimations. RNA in the remaining supernatant was extracted twice with equal volumes of phenol:chloroform:iso-amyl alcohol (50:50:1, v/v/v) (1). The final aqueous extract was made 0.3 M with respect to sodium acetate, and RNA was precipitated overnight at −20°C by the addition of 2.5 volumes 100% ethanol. This precipitate was collected by centrifugation at 15,000g for 10 min, washed twice with 95% ethanol, and dried in a gentle stream of air. The RNA was then dissolved in 1 ml sterile distilled H_2O. The concentration of RNA was determined by measurement of A at 260 nm, assuming that 1 mg/ml RNA has an A of 20 units. Radioactivity was measured by liquid scintillation spectrometry.

Polysome Analysis. The technique for ribosome extraction and analysis was based on that described by Gwóźdź and Bewley (12) and Malek and Bewley (15).

Dry or hydrated radioactively labeled moss (400 mg fresh weight) was ground in 7 ml grinding buffer (50 mM Tris-HCl [pH 8.1], 0.25 M sucrose, 40 mM KCl, 5 mM magnesium acetate, 5 mM mercaptoethanol, and 1% (v/v) Triton X-100) in a mortar and pestle with final cell breakage in a Duall ground glass homogenizer. The homogenate was cleared by centrifugation at 15,000g for 10 min, and the resultant supernatant was layered onto a 1.5-ml 36% (w/v) sucrose pad containing 50 mM Tris-HCl (pH 8.1), 40 mM KCl, 5 mM magnesium acetate, and 5 mM mercaptoethanol. A ribosomal pellet was obtained by centrifugation at 166,500g for 1.5 h in a Beckman Ti50 rotor. This was resuspended in 0.6 ml 50 mM Tris-HCl (pH 8.1) containing 40 mM KCl, 5 mM magnesium acetate, and 5 mM mercaptoethanol, and cleared by centrifugation at 10,000g for 10 min. A 0.4-ml aliquot of the resultant supernatant (containing approximately 0.5 mg of RNA by A_{260} measurement) was layered onto a 5.6-ml linear sucrose gradient (10–50% [w/v]) and centrifuged at 166,500g for 1.25 h in a Beckmann SW 40 rotor. Gradients were analyzed using an ISCO model 640 density gradient fractionator and an ISCO UA-5 gradient monitor with a 2-mm light path, continually recording A at 254 nm. Radioactivity of each fraction (0.2 ml) was determined by liquid scintillation spectrometry.

RESULTS

Recovery of RNA Synthesis during Rehydration. The recovery of RNA synthesis upon rehydration of both slow- and rapid-dried moss, as measured by incorporation of [^{14}C]uridine into total RNA of gametophyte samples from a single moss clump, is depicted in Figure 1. The rate of RNA synthesis upon rehydration after both drying regimes is linear. However, the rate of synthesis for moss which had been dried slowly is higher than in that dried rapidly. This difference in the rate of the recovery of RNA synthesis is not due to differential rates of precursor uptake (results not shown).

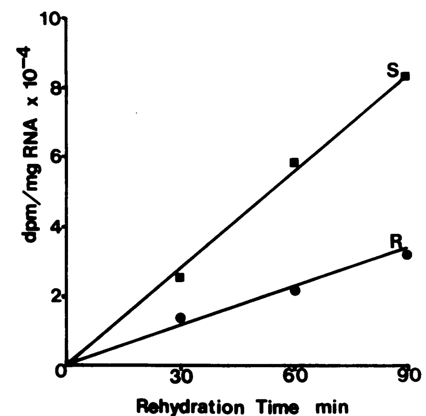

FIG. 1. Incorporation of [^{14}C]uridine into total RNA during the rehydration phase following slow (S) and rapid (R) desiccation of *T. ruralis*. Duplicate dried moss samples (300 mg fresh weight) were rehydrated for the appropriate time intervals in 3 ml water containing 2.5 μCi [^{14}C]uridine. Labeling was stopped by washing with copious amounts of water and immediate extraction of RNA.

RNA Incorporation into Ribosomal Fractions. Slow- and rapid-dried moss was rehydrated in water containing [^{14}C]uridine, with moss samples being removed for extraction at hourly intervals over a 4-h period. Moss hydrated for 24 h prior to exposure to labeled precursor for 1 to 4 h served as the appropriate control. Ribosomal pellets were extracted from these samples and separated by sucrose density centrifugation into polysome (P), ribosome (R), and subunit (S) fractions (Fig. 2).

The time course of entry of newly synthesized RNA into the three components of the ribosomal pellet of 24-h hydrated, control, moss is shown in Figure 2A. The maximum incorporation of radioactive RNA into each component occurs within the 1st h from the addition of label. Then the level of newly synthesized RNA within the ribosomal pellet remains constant for a minimum of 4 h. This is not due to limited availability of precursor (data not shown) and, hence, the constancy is taken to imply that the rate of loss of RNA from the protein synthetic complex equals its rate of replacement. The disturbance of the normal pattern of RNA incorporation by the introduction of a desiccation event, either rapid or slow, is shown in Figure 2, B and C, respectively. In both, there is a decrease in the rate of entry of newly synthesized RNA into all components of the ribosomal pellet, resulting in a delay in the time at which the steady state level of incorporation is attained. The magnitude of the decrease in rate of entry of RNA into the three components, and increase in the time before steady state attainment, is dependent upon the speed at which the moss was desiccated prior to rehydration. Following rapid desiccation (Fig. 2B), the decrease in rate of entry of RNA into the ribosomal pellet results in at least a 4-h delay in the attainment of steady state levels whereas, following slow desiccation (Fig. 2C), this is delayed by only 2 h. Further experiments (not presented) demonstrate that steady state levels of RNA incorporation into the ribosomal pellet are not reached until 6 h after the readdition of water.

In both rehydrated rapid- and slow-dried moss, there is an initially greater incorporation of RNA into the polysome fraction of the complex than into either the subunit or ribosome fractions. At later rehydration times, the distribution of label in these three fractions progresses towards the type of distribution found in the control moss.

Fate of RNA Synthesized during the First Hour of Rehydration. Both rapidly and slowly dried moss were rehydrated in the presence of [^{14}C]uridine and allowed to utilize this RNA precur-

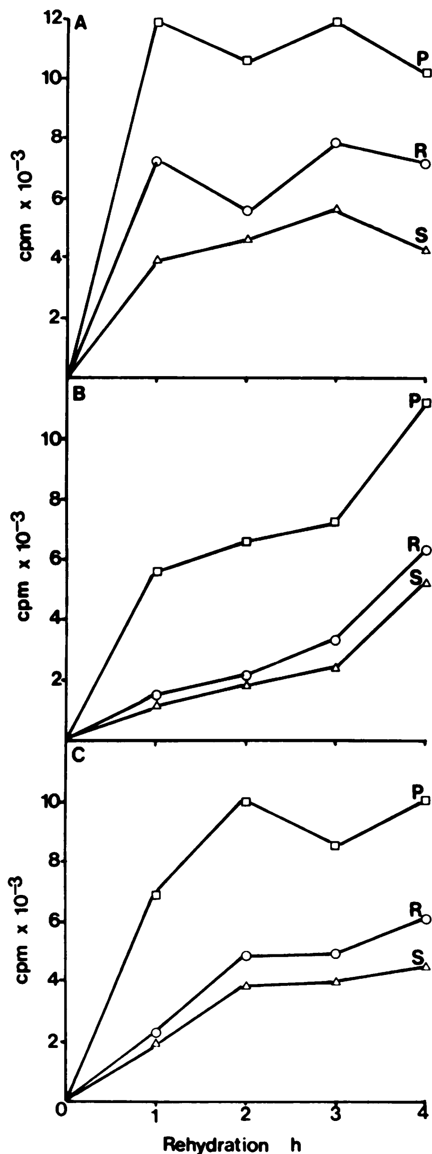

FIG. 2. Time course of entry of newly synthesized RNA components into subunits (S), ribosomes (R), and polysomes (P) of 24 h hydrated (A), rehydrated rapid-dried (B), and rehydrated slow-dried (C) *T. ruralis*. Samples of moss gametophytes (400 mg fresh weight, 80 mg dry weight) were incubated, or rehydrated, in 3 ml water containing 2.5 μCi [^{14}C]uridine for 1, 2, 3, or 4 h before extraction and analysis of the ribosomal pellets, as described in "Materials and Methods."

sor for 1 h. After thorough washing with distilled H_2O, which is known to totally remove all radioactive precursor from the moss (data not shown; 16), the moss was incubated in water containing an excess of unlabeled uridine to dilute out any radioactivity released by RNA catabolism. Samples were taken at 0, 1, 2, and 3 h following the removal of [^{14}C]uridine and subjected to polysome extraction and analysis. The results of this pulse-chase experiment are shown in Figure 3.

RNA synthesized during the 1st h following rapid desiccation enters all three of the ribosomal pellet fractions (S, R, and P) at a steadily increasing rate for the next 2 h (*i.e.* 1-3 h; Fig. 3A) after exposure to an excess of unlabeled uridine. During the 3rd h of the 'chase' period (*i.e.* 3-4 h; Fig. 3A), the levels of this RNA remain constant in the subunit and ribosome fractions, but decline rapidly in the polysomal fraction. Maximum levels of incorporation into the ribosomal pellet of the RNA synthe-

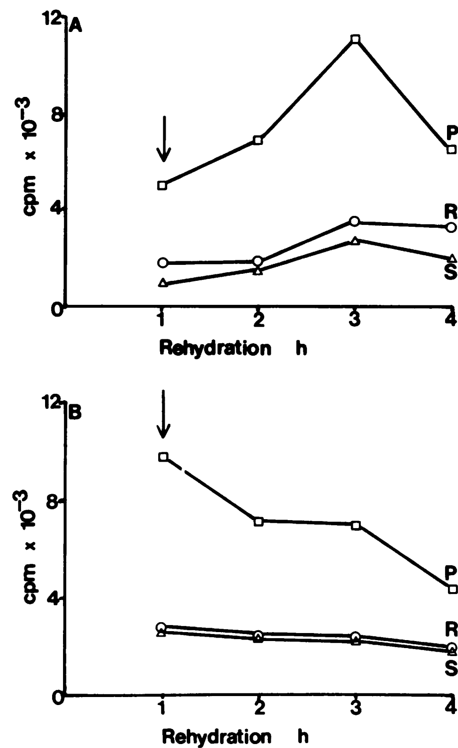

FIG. 3. Entry, location, and persistence within the components of the ribosomal pellet of RNA synthesized during the 1st h of rehydration of rapid- (A) and slow- (B) dried *T. ruralis*. Samples of rapid- and slow-dried moss were rehydrated in 3 ml water containing 2.5 μCi [^{14}C]uridine for 1 h prior to washing and incubation in 3 ml water containing 4.8 μg unlabeled uridine. Ribosomal pellets were extracted and analyzed at 0, 1, 2, and 3 h following the initial 1 h 'pulse' of [^{14}C]uridine. End of pulse, and beginning of chase is marked by an arrow. (S), subunits; (R), ribosomes; (P), polysomes. This experiment was accomplished using two different clumps of moss; hence, the necessity for two controls.

sized in the 1st h of rehydration of rapid-dried moss occurs at 3 h of rehydration (2 h after incubation in excess 'cold' uridine).

Maximum levels of incorporation of newly synthesized RNA into the ribosomal pellet of slow-dried moss occurs at the end of this 1st h of rehydration (*i.e.* prior to incubation in excess cold uridine) (Fig. 3B). Levels of this RNA in the ribosome and subunit fractions remain relatively constant during the 'chase' period, but RNA that has entered the polysome fraction is rapidly lost during this time of exposure to unlabeled uridine.

Stability of the RNA Components to Desiccation and Rehydration. To investigate the stability of RNA to desiccation and rehydration, 24 h hydrated moss was incubated in water containing [^{14}C]uridine for 4 h prior to desiccation. To ensure that no [^{14}C]uridine was present upon readdition of water, the moss was thoroughly washed with water prior to drying. In addition, rehydration was effected in the presence of an excess of unlabeled uridine to prevent interference of any [^{14}C]uridine released from RNA during the resumption of normal metabolism. Samples of dried moss and moss rehydrated for 1 to 4 h were used to obtain ribosomal pellets which were then subjected to component analysis. Moss hydrated for 24 h and labeled with [^{14}C]uridine for 4 h was also subjected to analysis, and thus served to ascertain the control levels of incorporated RNA prior to desiccation. The results of this experiment are depicted in Fig. 4, A and B). Because of logistical considerations of polysome analyses, direct replication of samples is not possible. Nevertheless, the results of identical experiments provide data that are in complete agreement with the trends seen in the figures presented here.

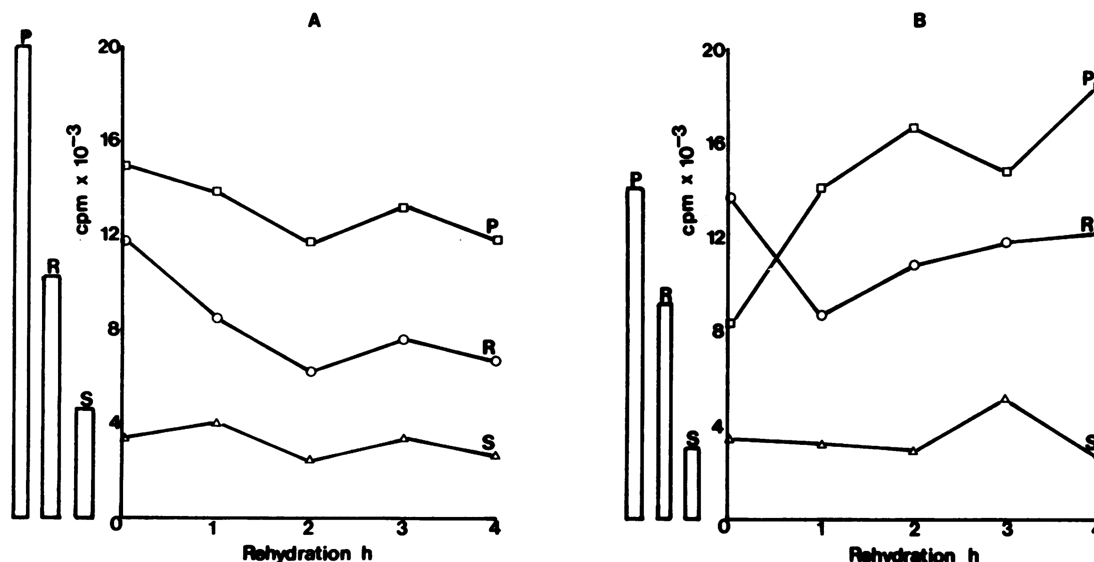

FIG. 4. Stability of the RNA components of subunits (S), ribosomes (R), and polysomes (P) of *T. ruralis* during and after (A) rapid and (B) slow desiccation and rehydration. Hydrated moss was incubated for 4 h in the presence of 3 μCi [^{14}C]uridine prior to desiccation. Rehydration was achieved by placing dried moss in 3 ml water containing 5.4 μg unlabeled uridine. Polysome analysis was implemented as described in "Materials and Methods" after extraction of the ribosomal pellet. Levels of RNA incorporation into the various fractions of the ribosomal pellet prior to desiccation are depicted as histograms to the left of each graph.

During rapid desiccation (Fig. 4A), there was a notable decrease in the level of radioactivity in the polysome fraction, compared to predesiccation control levels (histogram to left of figure), with a small increase in label in the ribosomal fraction. However, this reapportionment of labeled RNA during the desiccation process is not as dramatic as that exhibited during slow drying (Fig. 4B). Here the loss of label from the polysome fraction to the ribosome fraction is such that, in the dried state (0 h of rehydration), the amount of labeled RNA in the ribosome fraction is almost twice that in the polysome fraction. These observations reflect the physical state of the protein synthetic complex found in the dried moss, in that the polysome content of slow-dried moss is considerably less than that of rapid-dried (13). Differences in the amount of label seen in the controls of Figure 4, A and B, are the result of using two different clumps of moss whose uptake of radioactive precursor varied.

Upon rehydration of slow-dried moss, labeled RNA (that which is conserved in the dry state) is redistributed between the three fractions of the protein synthetic complex such that within 1 h its distribution is similar to that found in the predesiccation controls. At later rehydration times, the level of radioactivity in these fractions remains relatively constant or increases. On the other hand, there appears to be an overall loss of labeled RNA during the initial rehydration of rapid-dried moss (Fig. 4A) when compared to the overall levels in the dried or control samples. In the slow-dried moss samples, there is no such loss during the rehydration phase; in fact, the levels of conserved RNA in the complex may increase slightly at later rehydration times.

DISCUSSION

RNA synthesis quickly resumes upon readdition of water to dried *T. ruralis* (Fig. 1), the slow-dried moss exhibiting a faster rate of recovery; this agrees with the observation that protein synthesis recovers more rapidly following slow than rapid drying in this moss (13). That rapid desiccation results in a slower rate of recovery of both RNA and protein synthesis is probably symptomatic of the greater degree of cellular disruption caused by this drying regime (5). Nevertheless, it appears that the RNA synthesized upon rehydration is quickly processed and utilized in the protein synthetic complex. Under normal hydrated conditions, the moss can synthesize, process, and integrate RNA into the protein synthetic apparatus within 60 min following the addition of labeled precursor to the incubation medium (Fig. 2A). Once maximum incorporation of newly synthesized RNA into the complex is attained, a steady state condition is reached where the rate of entry of this RNA is equal to the rate of exit from the complex to the cellular pool. Prior desiccation increases the time needed to reach maximum incorporation levels, and achievement of this steady state. The delay is greater for moss that has been dried rapidly and is probably a consequence of the observed reduced rate of RNA synthesis (Fig. 1).

During the initial stages of RNA incorporation into the ribosomal pellet, there appears to be a bias towards recruitment into the polysomal complex (Fig. 2, B and C). This is confirmed by the results of the pulse-chase experiment (Fig. 3, 0–1 h for both rapid- and slow-dried moss). The kinetics of the subsequent loss of newly synthesized RNA from the polysomal fraction, once maximum incorporation is reached (at 1 h for slow-dried moss and 3 h for rapid-dried moss), can be interpreted to mean that RNA synthesized in the 1st h of rehydration is predominantly messenger RNA. This conclusion is supported by two observations. First, the loss of labeled RNA from the polysomal fraction is rapid once maximum incorporation is reached, characteristic of a molecule that has a fast turnover rate within the complex. Second, the radioactivity lost from the polysomal fraction cannot be accounted for in the ribosomal or subunit fractions (Fig. 3), which would not be the case if the label was associated with ribosomal RNA. That these observations can be explained on the basis of tRNA associated with the polysome complex appears to be unlikely.

These results tend to contradict earlier findings that newly synthesized RNA does not associate with ribosomes until 2 h following the readdition of water, and that once associated with ribosomes it does not participate in protein synthesis *per se* until even later (18). Since the inherent nature of protein synthesis is such that the cellular subunit pool is in a state of dynamic equilibrium with the cellular polysomal pool (8), it is unlikely that incorporation should occur only into a distinct ribosome

pool, as suggested in (18). The difference between the findings presented here and those of Tucker and Bewley (18) are probably a result of the increased sensitivity of the techniques used in this study.

The stability experiments (Fig. 4) further demonstrate the ability of the moss to conserve the major components of the protein synthetic complex during slow desiccation, not only qualitatively but also quantitatively. As a consequence of rapid drying, however, there does appear to be some loss of the RNA components. But, overall, these results demonstrate that not only are the RNA components involved in protein synthesis stable to desiccation, and conserved in the dried state, they are also readily and rapidly utilized for protein synthesis upon rehydration.

LITERATURE CITED

1. Aviv H, P Leder 1972 Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. Proc Natl Acad Sci USA 69: 1408–1412
2. Bewley JD 1972 The conservation of polyribosomes in the moss *Tortula ruralis* during total desiccation. J Exp Bot 23: 692–698
3. Bewley JD 1973 Polyribosomes conserved during desiccation of the moss *Tortula ruralis* are active. Plant Physiol 51: 285–288
4. Bewley JD 1973 Desiccation and protein synthesis in the moss *Tortula ruralis*. Can J Bot 51: 203–206
5. Bewley JD 1979 Physiological aspects of desiccation tolerance. Annu Rev Plant Physiol 30: 195–238
6. Bewley JD, P Halmer, JE Krochko, WE Winner 1978 Metabolism of a drought-tolerant and a drought-sensitive moss. Respiration, ATP synthesis and carbohydrate status. *In* JH Crowe, JS Clegg, eds, Dry Biological Systems. Academic Press, New York, pp 185–203
7. Bewley JD, TA Thorpe 1974 On the metabolism of *Tortula ruralis* following desiccation and freezing: Respiration and carbohydrate oxidation. Physiol Plant 32: 147–153
8. Boulter D, RJ Ellis, A Yarwood 1972 Biochemistry of protein synthesis in plants. Biol Rev Camb Philos Soc 47: 113–175
9. Dhindsa RS, JD Bewley 1976 Plant desiccation: Polysome loss not due to ribonuclease. Science 191: 181–182
10. Dhindsa RS, JD Bewley 1977 Water stress and protein synthesis. V. Protein synthesis, protein stability, and membrane permeability in a drought sensitive and a drought-tolerant moss. Plant Physiol 59: 295–300
11. Dhindsa RS, JD Bewley 1978 Messenger RNA is conserved during drying of the drought-tolerant moss *Tortula ruralis*. Proc Natl Acad Sci USA 75: 842–846
12. Gwóźdź EA, JD Bewley 1975 Plant desiccation and protein synthesis. An *in vitro* system from dry and hydrated mosses using endogenous and synthetic messenger RNA. Plant Physiol 55: 340–345
13. Gwóźdź EA, JD Bewley, EB Tucker 1974 Studies on protein synthesis in *Tortula ruralis*: Polyribosome reformation following desiccation. J Exp Bot 25: 599–608
14. Henckel RA, NA Satarova, SV Shaposhnikova 1977 Protein synthesis in poikiloxerophytes and wheat embryos during the initial period of swelling. Soviet Plant Physiol 24: 754–762
15. Malek L, JD Bewley 1978 Effects of various rates of freezing on the metabolism of a drought-tolerant plant, the moss *Tortula ruralis*. Plant Physiol 61: 334–338
16. Oliver MJ 1982 The role of desiccation in the control of transcription and translation in the moss *Tortula ruralis*. PhD Thesis. University of Calgary, Calgary.
17. Seibert G, K Loris, J Zoellner, B Frenzel, RK Zahn 1976 The conservation of poly-A-containing RNA during the dormant state of the moss *Polytrichum commune*. Nucleic Acids Res 3: 1997–2003
18. Tucker EB, JD Bewley 1976 Plant desiccation and protein synthesis. III. Stability of cytoplasmic RNA during dehydration, and its synthesis on rehydration of the moss *Tortula ruralis*. Can J Bot 53: 94–101
19. Verma DPS, GA MacLachlan, H Byrne, D Ewings 1975 Regulation and *in vitro* translation of messenger ribonucleic acid for cellulase from auxin-treated pea epicotyls. J Biol Chem 250: 1019–1026