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Plant Desiccation and Protein Synthesis

III. STABILITY OF CYTOPLASMIC RNA DURING DEHYDRATION, AND ITS SYNTHESIS ON REHYDRATION OF THE MOSS *TORTULA RURALIS*[1]

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ABSTRACT

RNA species from the haploid gametophyte generation of the moss *Tortula ruralis* exhibit typical eukaryotic characteristics. The major ribosomal and soluble RNA species are stable during drying and rehydration. RNA synthesis occurs rapidly on reintroduction of the moss to water and incorporation into high molecular weight RNA fractions was detected after 20 to 30 minutes of rehydration and into low molecular weight fractions after 30-60 minutes. Newly synthesized ribosomal RNA was detected in ribosomes within 2 hours of rehydration, but not in polysomes. It is apparent that the ribosomal and transfer RNA conserved during desiccation is involved in the re-establishment of early protein synthesis during subsequent rehydration and that, initially, there is no requirement for newly synthesized material.

Tortula ruralis (Hedw) Gaertn, Meyer, and Scherb is a common moss of the dry western Canadian prairies, it is poikilohydric and can withstand long periods of drought (2). In our previous studies (13) we have noted that within 24 hr of the addition of water to dried moss cellular organization returns to that observed in the normal unstressed plant. During this time the structural disruptions which the organelles undergo immediately following rehydration appear to be repaired (13, 14). Bewley (1) reported that polysomes could be extracted from rapidly dried moss and that these were conserved during desiccation and reactivated on rehydration (3). These conserved and active polysomes are comprised of cytoplasmic type ribosomes (12). Slow desiccation of *T. ruralis* results in a complete loss of polysomes which reform rapidly on rehydration probably using messenger RNA and ribosomes conserved during desiccation (8). RNA synthesis also occurs in rehydrated moss (4, 5, 8). These observations have led us to determine the stability of the major RNA species upon desiccation and rehydration, to study synthesis of cytoplasmic RNA species during rehydration, and to follow the fate of this newly synthesized RNA into the active protein-synthesizing apparatus. There appears to be no previous reports on RNA synthesis in moss gametophytes, nor upon the effects of desiccation on RNA synthesis in poikilohydric plants.

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MATERIALS AND METHODS

The desiccation, storage, and hydration of the gametophytes of *Tortula ruralis* prior to experimental use has been described (8, 12).

RNA was extracted from 0.5 g fresh weight of moss ground in a chilled mortar in 8 ml of buffer (50 mM sodium acetate [pH 5.1], 1 mM EDTA, 0.5% SDS) using the chloroform-phenol method of Perry *et al.* (10). Samples were applied to 2.6% polyacrylamide gels containing 0.5% agarose, or to 7.5% gels, and separated by electrophoresis at 5 mamps/tube in a buffer containing tris, Na H_2PO_4, EDTA, and 0.2% SDS (pH 7.6) as described by Loening (9). After running for at least 2 hr, the 8-cm disc gels were scanned at 260 nm. For counting, 1-mm slices were cut and digested overnight in 1 ml of 90% Nuclear Chicago solubilizer (Amersham/Searle) before the addition of 10 ml of standard scintillation cocktail in toluene.

Ribosomes were extracted from 1 g of hydrated moss in 8 ml of buffer (50 mM tris-Cl [pH 8.6], 40 mM KCl, 5 mM magnesium acetate, 5 mM 2-mercaptoethanol) containing 0.25 M sucrose and 2% Triton X-100 (4). For sucrose gradient analysis 4 to 5 E_{260} units of ribosomal material were applied in a 0.1- to 0.2-ml sample to the top of a 6-ml, 10 to 32% (w/v) linear sucrose gradient made up in the ribosome extraction buffer. After centrifugation (4) the tubes were punctured and analyzed by absorbance at 254 nm on an ISCO UA-2 gradient analyzer. Fractions of 0.25 ml were collected, and their radioactivity was counted.

RESULTS AND DISCUSSION

Characterization of RNA from *T ruralis*. When RNA extracted from hydrated fresh moss was separated by electrophoresis, at least 11 peaks were regularly observed. We used 2.6% acrylamide gels to identify the slower migrating larger mol wt species (Fig. 1A) and 7.5% gels for those migrating faster (Fig. 1B). Peaks 1 and 3 (Fig. 1A) co-migrated with the 24S and 17S (6) peaks of RNA extracted from wheat germ (5) and with RNA prepared from the moss ribosomal pellet (11, data not presented): these peaks are thus ribosomal RNA. Peaks 2 and 4 (or 5) migrated at the same rate as the ribosomal RNAs from *Escherichia coli*, which were distinct from the RNA of the moss ribosomal pellet (11, data not presented). These two small peaks must be ribosomal RNA of a prokaryotic nature and are probably from chloroplasts or mitochondria. During extraction there was probably a loss, or degradation, of organelle RNA and thus our gel patterns should not be used for relative quantitative estimation of these organelle RNA types. Unidentified minor peaks 5 (or 4), 6, 7, 10, and 11 (Fig. 1, A and B) might be, or include, their degradation products.

Five RNA species were separated on 7.5% gels. Peak 9 was the major peak (Fig. 1B), which co-migrated with the major peak obtained from the moss 100,000*g* supernatant and with the soluble RNA isolated from wheat germ (11; data not presented): this was identified as the soluble RNA fraction. Peak 8 made up quite a large proportion of the low mol wt. RNA extracted from the moss ribosomal pellet (11; data not presented) was tentatively identified as a 5S ribosomal RNA associated with this fraction.

Stability of RNA Fractions following Desiccation. Our ultrastructural studies of the moss cell have shown us that cellular damage occurs on rewetting of the desiccated moss (13). We determined the fate of the cytoplasmic RNA fractions during the early stages of rehydration. Fresh, hydrated moss was washed thoroughly in sterile H_2O water (12), imbibed for 4 hr in 200 μCi of ^{3}H-uridine (uridine 5,6-T, 42 Ci/mmol), washed in unlabeled uridine solution, and then dried over silica gel before storage for 24 hr. RNA was extracted from dry moss and from moss rehydrated for 30, 60, and 120 min, and then separated by electrophoresis. The RNA absorbance profiles did not change

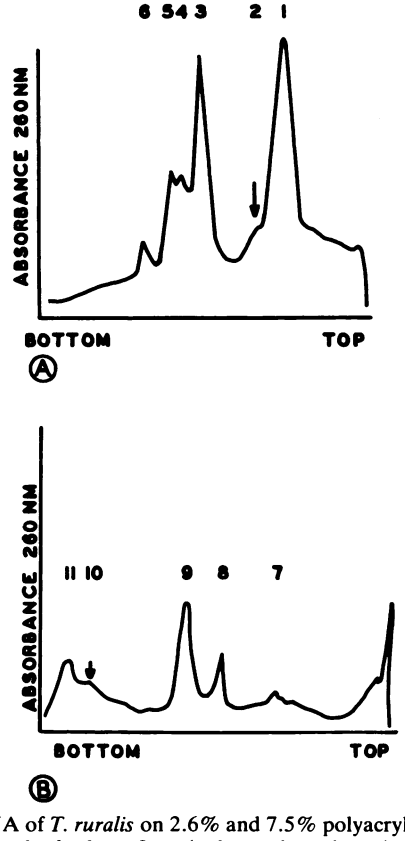

FIG. 1. RNA of *T. ruralis* on 2.6% and 7.5% polyacrylamide gels. A: 2.6%, total length of gel was 8 cm (only top 6 cm shown), electrophoresis time was 2 hr at 5 mamps/tube. B: 7.5%, total length of gel was 8 cm, electrophoresis time as A.

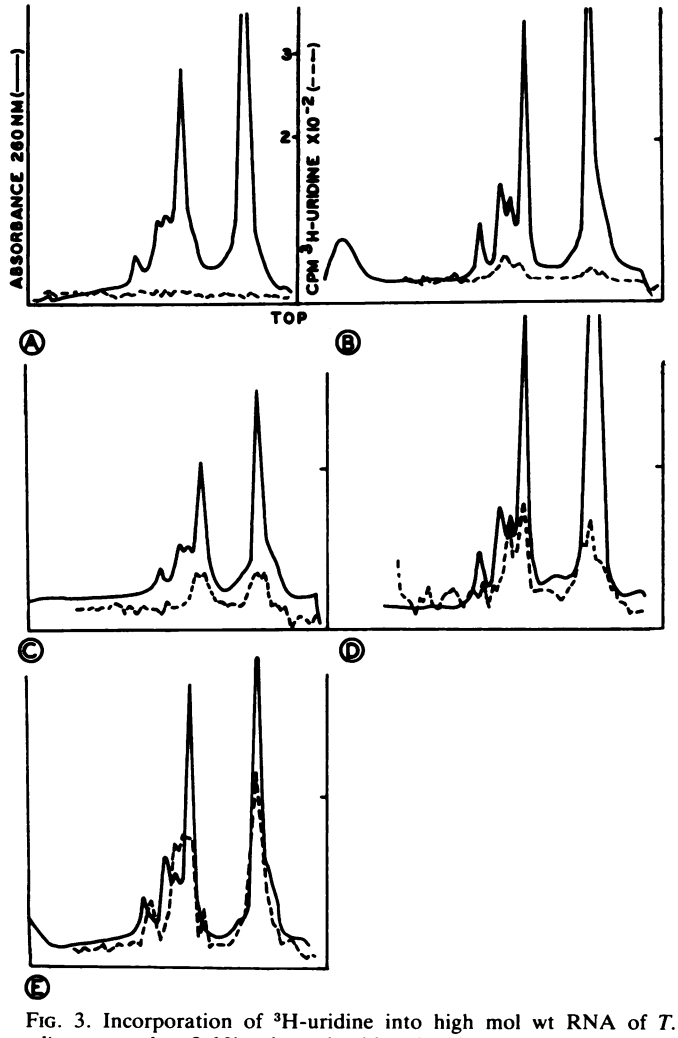

FIG. 3. Incorporation of ^{3}H-uridine into high mol wt RNA of *T. ruralis* separated on 2.6% polyacrylamide gels. Electrophoresis time: 2.5 hr at 5 mamps/tube; gel length 8 cm. Absorbance at 260 nm (——); uridine incorporation (- - -). Rehydration time: A, 10 min; B, 20 min; C, 30 min; D, 40 min; E, 60 min.

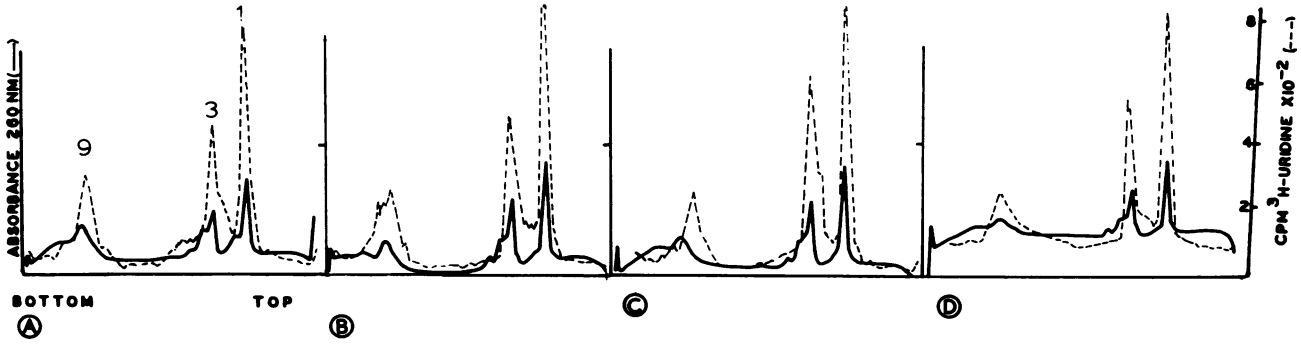

FIG. 2. Stability of *T. ruralis* RNA during rehydration. Dry moss, prelabeled with ^{3}H-uridine, was extracted for RNA (A), or rehydrated for 30 min (B), 60 min (C), and 120 min (D) before extraction and separation on 2.6% gels, length 8 cm. Electrophoresis time: 2 hr at 5 mamps/tube. Absorbance at 260 nm (——); uridine incorporation (- - -). Peaks marked for reference, see Fig. 1 and text.

during the 2 hr of rehydration (Fig. 2, A to D) (the high background absorbance in Fig. 2D is not significant) nor were they substantially different from the profile of the fresh moss RNA (Fig. 1A) (except that the soluble RNA was included in the gels shown in Fig. 2, since all 8 cm is shown). The distribution of the ^{3}H-uridine in the various peaks (equivalent to peaks, 1, 3, and 9 in Fig. 1) was unchanged during rehydration (Fig. 2, A to D) which is indicative of a lack of cytoplasmic RNA degradation, or significantly large turnover induced as a consequence of desiccation or recovery therefrom. Similarly, when prelabeled RNA from dry or 30-, 60-, and 120-min rehydrated moss was separated or 7.5% gels, there was no appreciable change in either absorbance or radioactivity in the RNA fractions (11; data not presented).

RNA Synthesis and Utilization upon Rehydration. Moss dried slowly in atmospheres of high relative humidity contains no polysomes and yet can resume protein synthesis within minutes of being reintroduced to water, even when the majority of RNA synthesis is inhibited (8). We were interested in determining when and what types of RNA were synthesized on rehydration and whether they became involved in early protein synthesis.

The kinetics of RNA synthesis during the early stages of rehydration have been published previously: incorporation of precursor into an RNA fraction occurs within minutes after the reintroduction of moss to water (4, 5, 8, 11). To determine into which RNA fractions labeled precursor was being incorporated, slowly dried moss was rehydrated for various times in 200 μCi of ^{3}H-uridine before RNA extraction and separation on 2.6% (Fig. 3) and 7.5% (Fig. 4) gels. The first indication of incorporation into the major ribosomal peaks was observed after 20 min of rehydration (Fig. 3B), with increasing incorporation into 17S and 25S cytoplasmic RNA species up to 1 hr after the addition of H_2O to the moss (Fig. 3, C to E). Some incorporation into other peaks was observed after longer times of imbibition (Fig. 3, D and E). No heavy RNA precursor molecule was found at earlier times of imbibition. Incorporation of uridine into the soluble RNA fraction on 7.5% gels was not apparent until after 30 min of rehydration (Fig. 4B) and this increased with longer imbibition time (Fig. 4C). The appearance of radioactive label into any distinguishable RNA peak takes at least 20 min to materialize, a time considerably later than the commencement of protein synthesis (8).

Slowly dried moss was rehydrated in 50 μCi of ^{3}H-uridine for various times before extraction of the ribosomal pellet and the fate of the incorporated uridine in the ribosomal and polysomal fractions followed. Moss rehydrated for 10 min (Fig. 5D) contained four or five polysome peaks, a ribosome peak, and subunits. Incorporation of label into the area of the gradient containing the subunits occurred within 1 hr of rehydration (Fig. 5B), but it was only after 2 hr that there was substantial incorporation into the ribosome peak (Fig. 5D). Even at this time, there was no significant incorporation associated with the polysomal regions of the gradient, illustrated by the fact that treatment of the 2-hr ribosomal pellet with RNase prior to loading on the gradient did not cause any redistribution of uridine label (Fig. 5E). These results lend credence to our previous hypothesis (8) that protein synthesis can take place on reassembled polysomes on rehydration of *T. ruralis*, without a requirement for new RNA synthesis.

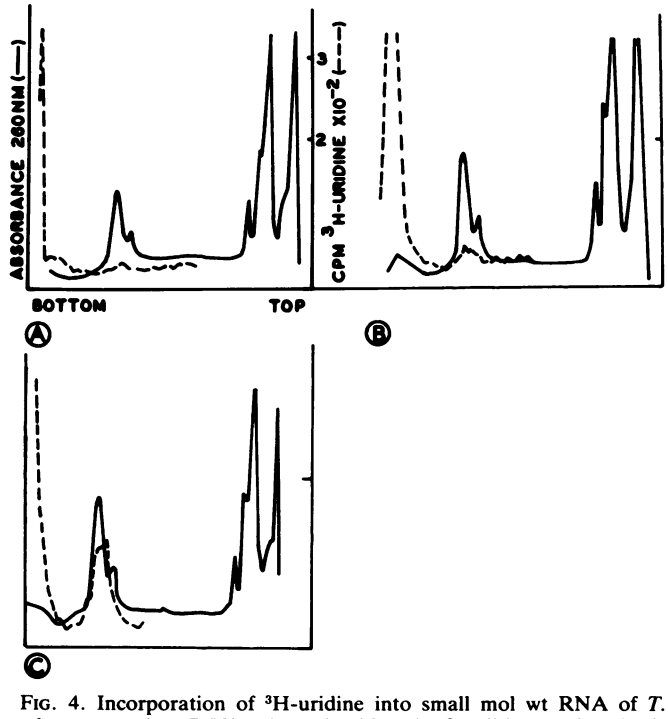

FIG. 4. Incorporation of ^{3}H-uridine into small mol wt RNA of *T. ruralis* separated on 7.5% polyacrylamide gels. Conditions as for Fig. 3; Rehydration time: A, 10 min; B, 30 min; C, 60 min.

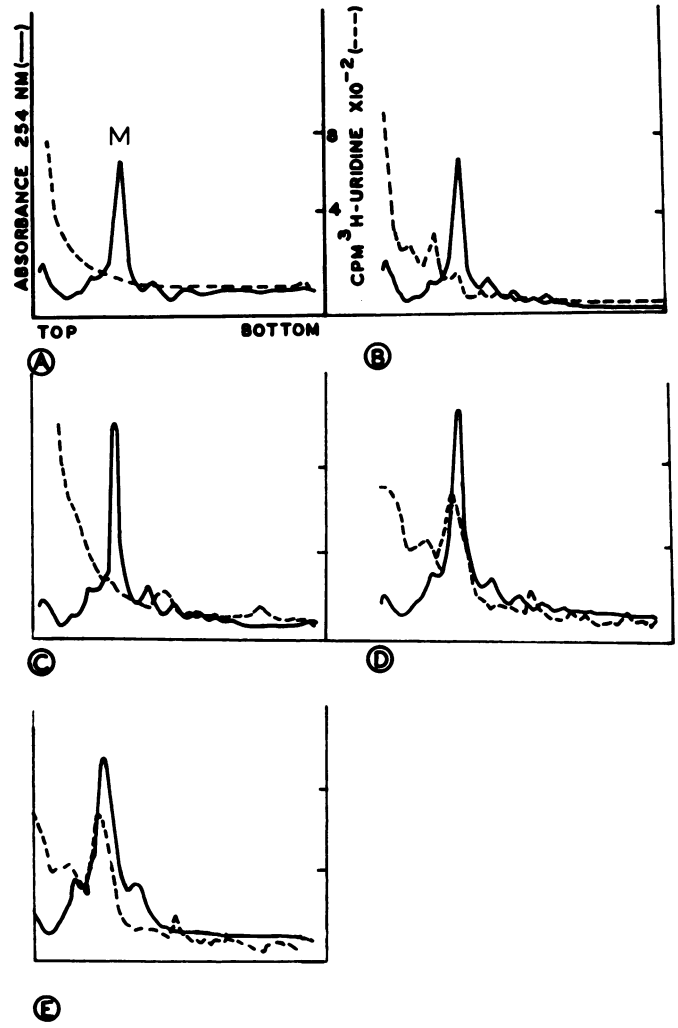

FIG. 5. Time of polysome formation following rehydration of *T. ruralis*. Ribosome profile obtained by centrifugation at 37,000 rpm for 75 min through a 10 to 32% sucrose gradient using a Beckman SW50·1 rotor. Absorbance at 254 nm (——); uridine incorporation (- - -). A: 30 min rehydration; B: 60 min; C: 90 min; D: 120 min; E: extract D treated with 1 μg/ml bovine pancreatic ribonuclease prior to loading on the gradient. M: ribosome peak.

CONCLUSIONS

It is apparent from these studies that cytoplasmic ribosomal and soluble RNA species are stable during desiccation and subsequent rehydration of *T. ruralis*. While renewed RNA synthesis occurs during early stages of reimbibition (4, 5, 8), such synthesis is not essential in the re-establishment of protein synthesis occurring on rehydration (7, 12). Furthermore, RNA incorporated into ribosomes does not appear in polysomes for at least the first 2 hr following imbibition. Tentative evidence against new messenger RNA synthesis can be derived from the fact that the polysome regions of our sucrose gradients were not labeled with uridine. Protein synthesis on rehydration occurs in the presence of actinomycin D (8) and cordycepin (11; R. S. Dhindsa unpublished). We are now attempting to determine if messenger RNA is conserved intact during desiccation and rehydration, for we have shown here that the other RNA components of protein synthesis are preserved following desiccation stress.

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