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Rapid Changes in Levels of Polyribosomes in *Zea mays* in Response to Water Stress[1]

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ABSTRACT

Sucrose gradient profiles of polyribosomes from the coleoptilar node region of seedlings of *Zea mays* L. were obtained without pelleting and redispersion of the particles. Water stress caused a shift of ribosomes from the polymeric to the monomeric form, starting about 30 minutes after stress initiation and when the water potential of the tissue began to decrease measurably. After about 4 hours of stress (a decrease in tissue water potential of about 5 bars), most of the higher polymers of ribosomes had shifted to monoribosomes. Release of stress caused the ribosomes to revert from monomeric to polymeric form after a lag period apparently determined by the extent of prior stress. Use of bentonite and isolation of polyribosomes from combined stressed and control tissue gave results indicating that the reduced polyribosomal level was not an artifact caused by ribonuclease during isolation.

Incubating roots in cycloheximide (2 micrograms per milliliter) had no effect on the proportion of polyribosomes in control roots, but it prevented the loss of polyribosomes caused by stress. Since cycloheximide inhibits the release of nascent polypeptide from polyribosomes, it appears possible that stress-effected loss in polyribosomes occurs only if polypeptides can be terminated and released.

Water deficit in plants is thought to affect protein synthesis (5). Leaves of sugar beet subjected to prolonged water stress contained less protein than control leaves (16). Prior water stress reduced the subsequent incorporation of amino acids into proteins in tobacco leaf discs (2). There are also cursory indications (4, 7, 19) that the levels of polyribosomes (polysomes) may be reduced by stress. This has not been studied, however, either in detail or with up-to-date methods of isolating polysomes.

The change in protein is only one of the myriad metabolic alterations observed in plants under water stress (10). To study the causal relationship between plant processes and water stress, it is essential to identify the early changes in the plant at the onset of water deficiency. The effect of water deficit of short duration on polysomes from etiolated maize seedlings was studied. A rapid response in polysomal level to relatively small changes in water status of the tissue was observed.

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MATERIALS AND METHODS

Maize (*Zea mays* L., var WF9 × M14) seedlings were first germinated and grown for 2¾ days in the dark at 27 to 29 C on paper towels moistened with 0.1 mM $CaCl_2$ in glass trays covered with Saran Wrap (Dow Chemical Co.) slit at several places for aeration. Water stress was routinely initiated by transferring seedlings to dry paper towels in open trays in the same germination chamber. Stress varied somewhat from seedling to seedling, depending on position and exposure of roots to air. Alternatively stress was effected by briefly dipping the roots of the seedlings in a mannitol solution (containing 0.1 mM $CaCl_2$) and then replacing them on paper towels moistened with the same solution in covered trays. The dipping was repeated three times, 15 to 20 min apart, to maintain a thin film of mannitol around the roots.

The seedlings were rewatered by dipping them in 0.1 mM $CaCl_2$ and then transferring them to paper towels moistened with 0.1 mM $CaCl_2$, in covered trays.

Polysomes were isolated from the rapidly growing coleoptilar node region. The homogenizing buffer (10 mM tris-Cl, 2 mM $MgCl_2$) was at pH 8.5. Isolation at pH 8.5 yielded a slightly higher proportion of polysomes and more total ribosomes per unit of tissue than at pH 6.5. High pH prevents the association of endogenous ribonuclease with ribosomes (11) and reduces the activity of maize ribonuclease (unpublished).

Segments encompassing the node and the tissue about 4 mm on both sides of the node were cut and immediately buried in pulverized dry ice. Thirty segments, weighing about 0.8 g, were partially thawed and placed in 1.7 ml of ice-cold homogenizing buffer containing 0.81 mg of bentonite (11) in a micro-cup (internally ribbed, 1.4-cm diameter, 5-ml brim capacity) of a Virtis homogenizer. The tissue was homogenized at ice temperature according to a procedure suggested by Dr. J. L. Key (personal communication). The homogenizer was quickly turned to full speed (45,000 rpm) and immediately off again, three times. Surprisingly, homogenizing the tissue in the Virtis tended to yield a slightly higher percentage of polysomes than did pulverizing in powdered Dry Ice. The homogenate was clarified at 17,500 rpm (Sorvall SS-34 rotor) for 10 min. The supernate (0.4 or 0.5 ml) was directly placed on the sucrose gradient without pelleting the ribosomes. A discontinuous gradient, consisting of the "isokinetic" gradient of Noll (15), 15 to 28% of sucrose and totaling 10.3 ml, with 1.0 ml of 7.5% sucrose layered on top, was prepared within 3 hr of use. The extra layer allowed good separation of the monoribosomes (monosomes) from the soluble material without prolonged centrifugation, thus minimizing pelleting of the largest polysomes. All sucrose solutions were in the homogenizing buffer. The tube was centrifuged in the SB-283 rotor of an IEC B-60 ultracentrifuge at 41,000 rpm for 1 hr at 1 to 4 C.

The tube content was pumped with an ISCO (Instrumentation Specialties Co., Lincoln, Neb.) fractionator, model 180, through a flow cell with a light path of 2 mm. Absorbance at 254 nm was recorded with an ISCO UA-2 ultraviolet analyzer.

To determine the proportion of polysomes, the areas under the monomeric and polymeric peaks, arbitrarily divided (dotted lines, Fig. 1a), were measured with a planimeter. The absorbance base line was assumed to be horizontal. The proportion of monosome was probably overestimated because of contamination by the soluble material.

The amount of total ribosomes obtained varied from extraction to extraction, probably because the degrees of homogenization varied. Estimated polysomal percentage in duplicate samples often differed by several points. Hence, small differences in polysomes, when noted herein, were always verified by repeating the experiment several times.

Water potential was determined by the procedure of Shardakov (1). About 0.18 ml each of a series of sucrose solutions, differing from each other in water potential by 1 bar, was added to separate covered vials, each containing six node segments. After the contents stood for 30 to 50 min with intermittent swirling, a minute amount of methylene blue was added. A drop of the colored bathing solution was introduced into the original solution to see whether it sank or floated. The water potential of the tissue was assumed to be that of the solution (interpolated to ¼ bar) with density which remained unchanged during contact with the tissue. Duplicates measured generally agreed within 1 bar. This method, being rapid, is particularly suited for use with rapidly growing tissue. It minimizes probable changes in tissue water potential caused by accumulation of solutes and softening of the cell wall during growth. It has been suggested (13) that a short time of tissue immersion, as in this study, may lead to errors caused by solutes escaping from the cut cells. This should not be a significant shortcoming in the present work since cutting the standard node segment into four pieces or blotting the cut ends of the segments did not materially alter the water potential subsequently measured. Further, Dr. D. Henderson (personal communication) has found that the water potentials of maize leaves determined by this method agreed well with those determined with a pressure bomb.

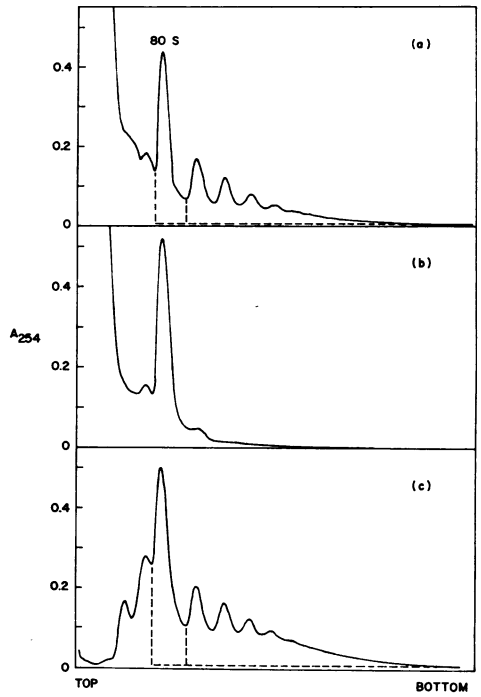

FIG. 1. Sedimentation profiles of ribosomes from coleoptilar node segments of unstressed maize seedlings. a: Clarified homogenate. The proportion of polysomes, estimated from the areas under the monosomal (80 S) and polysomal peaks, was 57%. b: Clarified homogenate treated with ribonuclease. The homogenate, containing ¼ as much material as that in part a, was incubated at 20 C for 2 min with pancreatic ribonuclease (1 μg/ml). c: Ribosomes after pelleting and redispersion. Polysomes were estimated to be 61%. An aliquot of the clarified homogenate used in part a was layered on 1 ml of 20% sucrose in homogenizing buffer and centrifuged in the A-321 angle rotor at 60,000 rpm for 1 hr. Pelleted ribosomes were gently dispersed in the homogenizing buffer, clarified at 17,500 rpm for 8 min, and layered on the gradient.

RESULTS

Isolation and Separation. The sedimentation profile of the extract from control seedlings (no water stress) shows a dominant amount of soluble material at the meniscus and a series of ultraviolet-absorbing peaks (Fig. 1a). The main peak was identified as the monosome by simultaneously sedimenting isolated 80 S ribosomes (9) in another gradient. The material in the polysomal region shifted to the monosomal peak when treated with ribonuclease (Fig. 1b). The two small peaks to the left of the monomer are assumed to be the 60 and 40 S subunits (9) and were often inadequately separated from the soluble material.

Pelleting of polysomes from the extract and redispersion had a variable effect on the sedimentation profile. The proportion of polysomes was not materially altered in some cases (Fig. 1, a *versus* c) but was either increased or decreased in others (data not shown). Presumably the sedimentation profile of the extract reflects more closely the situation *in vivo* since artifactual aggregation of ribosomes due to pelleting (9) and possible shearing upon redispersion are avoided. The procedure without pelleting is rapid (3.5 hr from tissue harvest to completion), thus minimizing degradation and centrifugation time.

Effect of Water Stress. Stressing seedlings on dry paper towels caused a progressive shift of ribosomes from polymeric to monomeric form (Fig. 2). The shift started about 30 min after transferring to dry towels, as suggested by data in Figure 2 and confirmed by several other experiments covering the period of 20 to 40 min. At about this time, water potential of the node segment also began to decrease measurably (Fig. 2 and other data not shown). After about 4 hr of stress, the polysomal peaks became inconspicuous and the water potential was reduced to −10 to −12 bars. More severe evaporative conditions in the germinating chamber brought about still more rapid declines in polysomal content and tissue water potential.

Rewatering seedlings that had been stressed for 4 hr to −10.5 to −11 bars effected no noticeable change in polysomal profile in 20 to 30 min but gave increases in both polysomes and water potential in 50 to 60 min (Fig. 3). Recovery in polysomes and water potential was apparently complete in about 2.5 hr (Fig. 3). In other experiments where stress was more severe (4.5 hr stress, to −11 to −12 bars), there was no measurable recovery in water potential in the first 50 min. Recovery in polysomes did not begin until about 2 hr after rewatering. Apparently, the duration of the lag period is determined by the extent of prior stress. In all experiments, the percentage of polysomes during recovery did not significantly exceed that of the control. This is in contrast to the transitory over-recovery in growth rate of maize leaves (12) when stress is released.

The largest stress achieved in the node (a decrease of 5 or 6 bars in water potential) is still relatively mild. Although node segments stressed 4 hr were moderately flaccid, their water content (*e.g.*, 85.7%) was only 1 or 2% less than that of the control (*e.g.*, 87.1%). No difference in relative water content (relative turgidity) was detected between stressed and control segments. The small

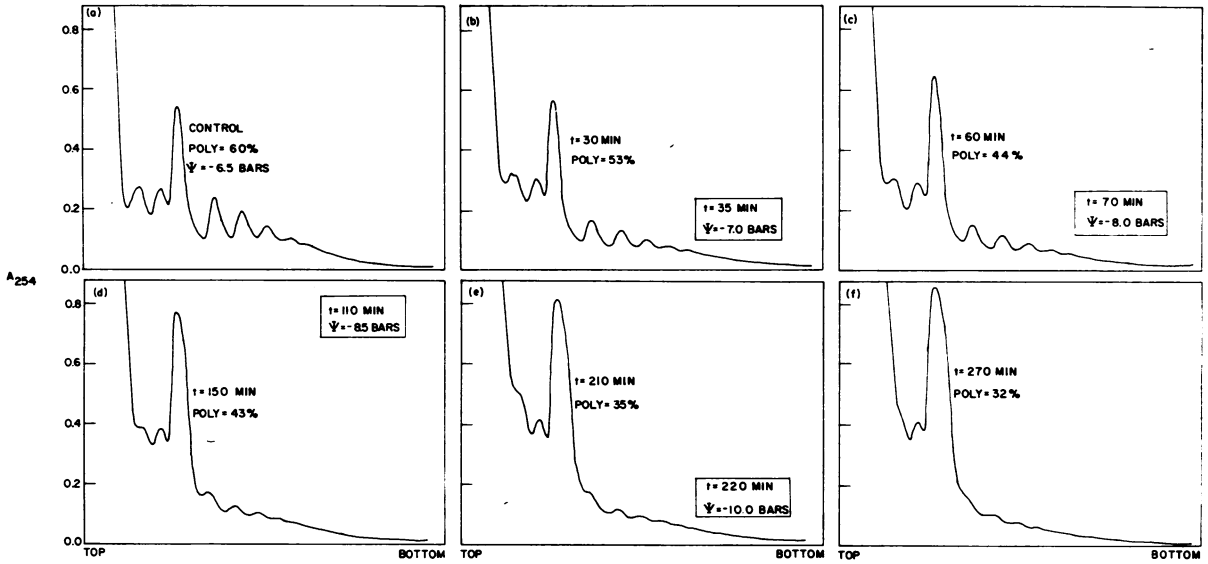

FIG. 2. Effects of water stress on polysomes and water potential in coleoptilar node segments. Stress was initiated by transferring seedlings to dry paper towels. Values adjacent to the monosomal peak denote the time (t) from the start of stress to sampling for polysome analysis and the estimated percentage of polysomes (poly). Values in the box denote the time (t) from the start of stress to sampling for the determination of water potential and the water potential (Ψ). Control (a) consisted of seedlings kept growing on moistened paper towels in covered trays. Percentages of polysomes and water potential are single determinations and generally consistent with values obtained in other experiments.

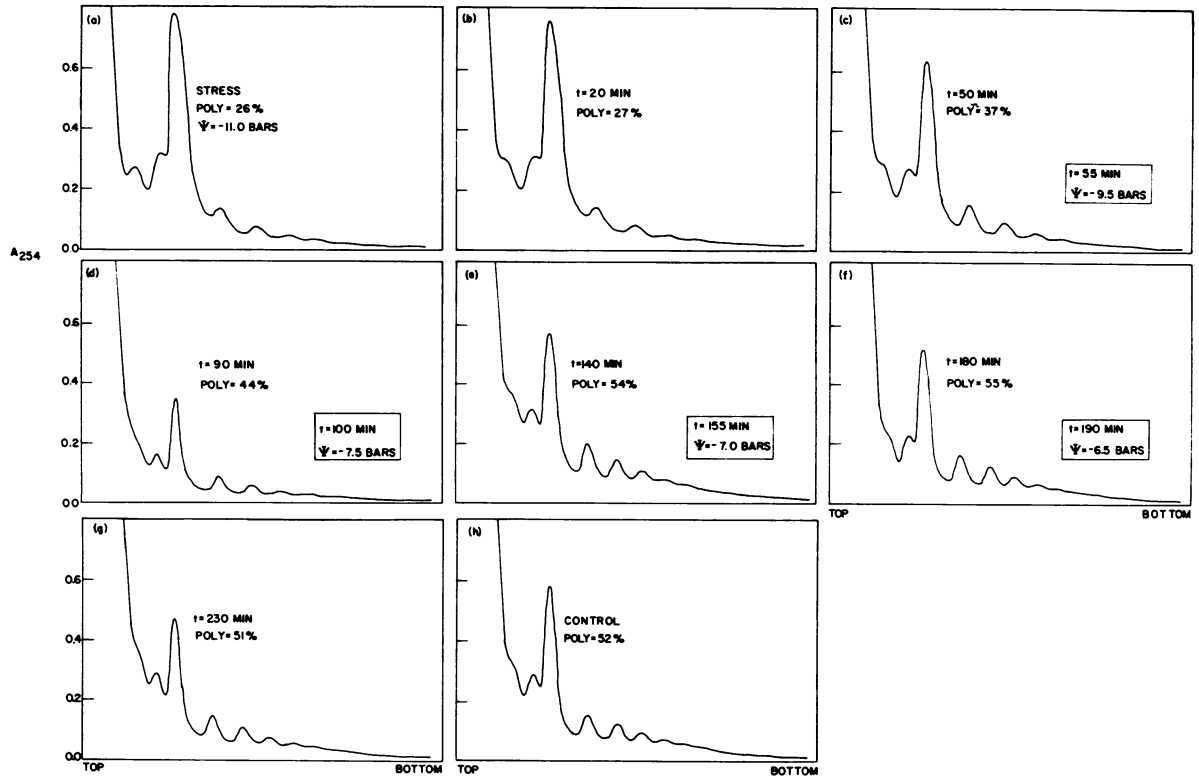

FIG. 3. Recovery in polysomes and water potential in the coleoptilar node segments upon rewatering of water stressed seedlings. Seedlings were stressed for 4 hr on dry paper towels (a) and then rewatered. t denotes the time from rewatering to sampling. Other notations are the same as for Figure 2. Control sample (h) was taken at the end of the recovery period. Polysomal percentages for two other controls, sampled at the beginning and the middle of the recovery period, were respectively, 50 and 57%.

water deficits and the tendency of the tissue to continue growing when floated on water, either at room temperature or 5 C, made the measurements inaccurate.

The control tissue, although growing vigorously, was fairly low in water potential, probably because of the way the seedlings were grown. Only a small portion of the roots were in direct contact with the wet paper towels; most of the roots were actually growing in nearly saturated air.

Roots of seedlings stressed for 3 or 4 hr on dry paper towels were desiccated irreversibly, although shoots retained most of

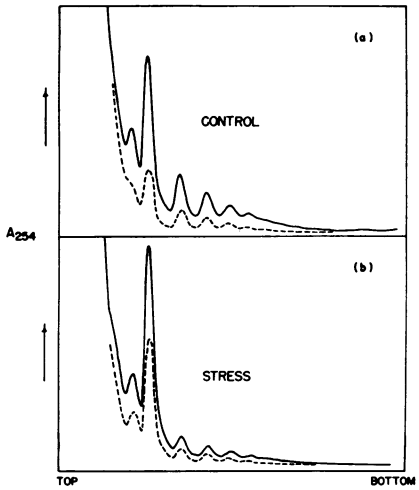

FIG. 4. Polysomes in coleoptilar node segments of control (a) and stressed seedlings (b) as affected by the amount of bentonite used for isolation. —: standard amount of bentonite; – –: 3× standard amount of bentonite. The polysomal percentages in the control tissue were 50 and 47% and, in the stressed tissue, 31 and 30%, respectively, for 1 × and 3 × bentonite. Seedlings were stressed for 2.5 hr on dry paper towels. Twenty node segments were ground in pulverized Dry Ice with a mortar and pestle. After the Dry Ice had sublimated and when the mixture began to thaw, 1 ml of cold 10 mM sodium phosphate, pH 6.5, 1 mM in $MgCl_2$, was added together with standard amount of (0.54 mg) or 3 × bentonite (1.6 mg). The resultant homogenate, partially frozen, was thawed by shaking, then clarified, and layered on sucrose gradient. Similar effects of bentonite were observed when control and stressed tissue was homogenized with the Virtis homogenizer at pH 8.5.

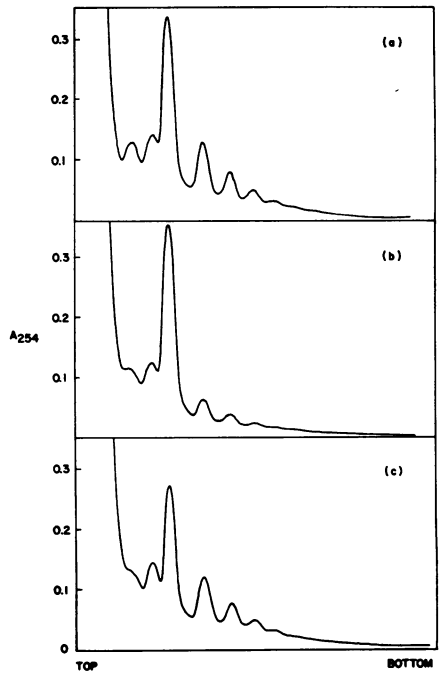

FIG. 5. Effects of cycloheximide on polysomes in root apices during stress by mannitol. a: Control, 51% polysomes: b: 1-hr stress, 34% polysomes; c: 1-hr cycloheximide pretreatment followed by 1-hr stress in the presence of cycloheximide, 56% polysomes. Stress was effected by dipping. Roots were dipped in 0.1 mM $CaCl_2$ in part a, in 0.6 M mannitol in part b, and in cycloheximide (2 μg/ml) and then 0.6 M mannitol containing 2 μg of cycloheximide per ml in part C. All solutions contained 0.1 mM $CaCl_2$. Sixty root apices (5 mm long) were homogenized in 1.6 ml of homogenizing buffer containing 0.27 mg of bentonite.

their moisture. Upon rewatering, shoot growth resumed, but only small portions of roots proximal to the seed remained alive; lateral roots were initiated from these portions many hours later.

The effect of water deficits on polysomes was confirmed by other methods of stressing. Polysomal percentage was lowered by immersing seedlings for 2 to 3 hr in solutions of 0.7 M mannitol in 0.1 mM $CaCl_2$, as compared to immersing in 0.1 mM $CaCl_2$ alone. Although air was vigorously bubbled through the solutions, seedlings incubated in $CaCl_2$ alone contained less polysomes than those left growing on moist paper. Oxygen supply was apparently still restricted in spite of the aeration. To eliminate this difficulty, the procedure of dipping the roots of seedlings periodically in mannitol ("Materials and Methods") was adopted. Periodically dipping roots in 0.1 mM $CaCl_2$ did not reduce polysomal content.

Dipping roots in mannitol was very effective in stressing roots (see below), but not shoots of the same seedling. The node segments showed only a slight reduction in polysomes and a decrease of 1 to 2 bars in water potential 3 hr after their roots were dipped in 0.6 M mannitol.

Ribonuclease Activity during Isolation. Prolonged and severe water stress increases the ribonuclease level in plants (6). The possibility existed that the reduction in polysomes in stressed tissue was an artifact caused by increased ribonuclease activity during isolation. Doubling or tripling the ribonuclease inhibitor (bentonite) used in the standard procedure reduced the amount of each ribosomal species (monomer, dimer, etc.) through adsorption of the particles but did not noticeably change the distribution among polymers and monomer, either for stressed tissue or for control (Fig. 4). Without any bentonite the proportion of polysomes was reduced (*e.g.*, to 39% for control). A mixture of one-half stressed and one-half control tissue yielded polysomal percentages intermediate to those from stressed and control alone, indicating little or no degradation of control polysomes by the stressed tissue and its contents. The ribonuclease level in the stressed segments, determined (11) in several experiments, showed no increase after 2 hr of stress and only a hint of an increase after 4 hr as compared with the control. In contrast, the proportion of polysomes began to decrease after 30 min. These results suggest that the difference in polysomes between stressed and control tissue was not caused by ribonuclease activity after cells were ruptured.

Effect of Cycloheximide. Cycloheximide inhibits the elongation of peptide chain and the release of nascent polypeptide from polysomes. Treating roots with cycloheximide (2 μg/ml) did not affect the polysomal profile of the control roots but prevented the shift of polysomes to monosomes brought about by stress (Fig. 5).

DISCUSSION

This study demonstrates a clear and rapid response in polysomal population to relatively small changes in plant water status. Previously the effects of water stress on polysomes have been reported (7, 19), but definitive data were lacking.

The decrease in polysomes, along with the reduction in CO_2 assimilation, is the earliest metabolic alteration resulting from water stress elucidated so far. In the study of water stress, attempts were rarely made to differentiate between early and later events among the numerous changes in stressed plants. In elucidating the causal relations between stress and plant processes, it is essential to identify and study the early events, since they are less complicated by the effect of stress on development of the plant and physiological age of plant parts. Early changes in processes are not readily detectable by measuring cumulative quantities such as composition or enzyme levels. Changes in these would become evident only long after the pertinent proc-

esses are affected. The rates of processes should be studied directly whenever possible. Polysomal levels presumably reflect the rate of protein synthesis.

I used the node segment as the source of ribosomes to minimize complications due to differences in cellular development. Shoots were shorter from seedlings stressed for several hours than from control, but since cell division occurs at the node and zones of expansion extend above and below, node segments of the same length from stressed and control seedlings should contain cells at about the same stages of ontogeny.

In this study, with the short duration of stress, recovery in polysomes after rewatering was completed in as short a period as 2.5 hr. Tobacco leaves, when subjected to stress of several days, did not recover fully the ability to incorporate amino acids until 1.5 to 3 days after stress release (2).

Lin and Key (14) found that anaerobiosis shifted polysomes to monosomes in soybean roots and that cycloheximide blocked the shift as well as protein synthesis and release of nascent polypeptides. They concluded that the change of polysomes to monosomes during the anaerobic treatment occurs only if polypeptide chains are completed and released. The effect of cycloheximide on polysomes during water stress is reminiscent of the results of Lin and Key. It would be reasonable to assume that the completion of polypeptide chains is also necessary for the stress-effected reduction in polysomes. This in turn implies that a lack of chain initiation may be the cause of the reduction. It has been suggested that protein synthesis is controlled at the initiation step (14).

How water deficits at the moderate level encountered in this study (in contrast to the severe dehydration used in some work, *e.g.*, Ref. 3) reduce polysomes is an intriguing question. The decrease in water potential was 6 bars at most, which corresponds to a decrease of less than 0.5% in the activity of water[2] and should be too small to affect substantially reactions in which water participates directly. Speculations (5, 18) as to changes in configuration of plant macromolecules due to changes in water potential within the physiological range are not supported by evidence. In the few available studies of macromolecules in solutions (*e.g.*, Ref. 17), alteration in configuration occurred only when water potential was reduced to levels much lower than that in living mesophytic plants. In this study, the water content of the tissue was reduced only 1 or 2% by stress. Hence there should be no marked change in solute concentration or ionic strength in the cell. Changes in pressure potential probably accounted for most of the change in total water potential in the node. Reactions and macromolecular configuration in the liquid phase, however, should be insensitive to a few bars of change in pressure.

On the other hand, growth of cells, because of the internal push required to extend, is extremely sensitive to changes in turgor pressure of such magnitude. Elongation of *Nitella* cells is reduced by decreases in turgor pressure of fractions of a bar and stopped by a decrease of 3 bars (8). Elongation of maize leaves is similarly affected by decreases in tissue water potential of similar magnitude (12). Preliminary data, obtained by continuously monitoring elongation of shoots of etiolated seedlings with a sensitive position transducer (12), indicate that reduction in expansive growth preceded reduction in polysomes at the onset of water stress and recovery in growth preceded recovery in polysomes after rewatering. This suggests the possibility that the change in polysomes results from and is not the cause of changes in expansive growth.

[2] Calculated with the equation $\Psi = (RT/\bar{v}_w) \ln a_w$, relating water potential (Ψ) to activity of water (a_w), where R, T, and $\bar{v}_w$ are, respectively the gas constant, absolute temperature, and partial molal volume of water.

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