Plant Physiol. (1988) 86, 725–733
0032-0889/88/86/0725/09/$01.00/0

Polysomes, Messenger RNA, and Growth in Soybean Stems during Development and Water Deficit[1]

Received for publication July 27, 1987 and in revised form November 4, 1987

HUGH S. MASON*[2], JOHN E. MULLET, AND JOHN S. BOYER
Department of Soil and Crop Sciences (H.S.M., J.S.B.) *and Department of Biochemistry and Biophysics* (H.S.M., J.E.M., J.S.B.), *Texas A&M University, College Station, Texas 77843*

ABSTRACT

The polysome status and populations of polysomal mRNA were examined in different regions of dark-grown soybean (*Glycine max* [L.] Merr.) stems that contained either dividing, elongating, or mature (nongrowing) cells. There was a developmental gradient of polysome content in which the dividing tissue had the highest levels and the mature tissue the lowest. A few hours after transplanting the seedlings to vermiculite having low water content (water potential ψ_w = −0.29 megapascals), stem growth rate decreased to 30% of well-watered controls and the polysome content decreased most in the dividing and elongating tissues. After 24 to 36 hours, stem growth and polysome content recovered gradually. *In vitro* translation products of polysomal mRNA from dividing, elongating or mature tissue were examined on two-dimensional gels. In well-watered controls, each of the stem regions was enriched in a small subset of the polysomal mRNA population, probably because of developmentally regulated gene expression. Exposing plants to low ψ_w for 24 hours induced a change in the relative abundance of a small number of polysomal mRNAs in the elongating and mature tissues, but not in the dividing tissue. After 24 to 72 hours at low ψ_w, the changes in polysomal mRNA population were reversed in the elongating tissue. The data indicate that changes in stem growth at low water potential are associated with changes in polysome status and polysomal mRNA in the elongating tissue.

Plant growth is severely inhibited by water deficits, and much work has concentrated on determining the cause of the inhibition (17). Soybean seedlings have provided a great deal of information about changes in plant water status induced by low ψ_w[3] (6, 8, 23). When the seedlings are grown in the dark at 100% RH to prevent transpiration, stem tissue in the elongating region has a ψ_w below that of the mature, non-growing tissue which is in near-equilibrium with the transpiration stream (8). This ψ_w gradient could be due to the extensibility of the cell walls which prevents turgor from reaching the maximum level it would have in cells with the same osmotic potential but rigid cell walls (6). During growth inhibition due to water deficits, turgor is maintained in the elongating tissue of these stems (8, 23) and in several other tissues of agronomic species (21, 22, 36). These data indicate that, although turgor may be important in some tissues (17), decreased turgor cannot explain the inhibition of growth at low ψ_w in all cases. Similarly, turgor was unchanged during auxin-stimulated growth of pea stem or blue light mediated inhibition of stem growth in cucumber and sunflower, which led Cosgrove (11) to suggest that these treatments instead affected cell wall properties.

Metabolic processes, particularly those affecting cell wall extensibility, could be involved in growth regulation. Inhibitors of protein synthesis (*e.g.* cycloheximide) inhibit auxin-induced growth and H^+ excretion at concentrations that do not affect respiration (2, 9, 33). Cleland (10) reported that the capacity for H^+-induced loosening of cell walls was decreased by pretreating oat coleoptiles with cycloheximide. These findings suggest that some short-lived protein(s) may be required for auxin- and H^+-induced effects on growth.

Protein synthesis is rapidly inhibited by water deficits, as indicated by disaggregation of polysomes (polyribosomes) (16, 21, 24, 28). In addition, there are differences between growing and nongrowing tissues with respect to polysome content (12, 34) and the sensitivity of polysomes to disaggregation at low ψ_w (3, 21). These data are consistent with the idea that plant growth requires protein synthesis and that its inhibition could be a factor contributing to reduced growth during water deficits.

Several environmental stresses alter gene expression in plants, including heat, anaerobiosis, and salt (29). Water deficit induces the expression of some genes common only to water deficit and ABA treatments in maize (15). In soybean seedlings, relative proportions of cell wall proteins in growing and non-growing hypocotyl tissues are different and are altered at low ψ_w (7). Thus it seems possible that growth regulation during water deficit is mediated at least partly at the level of gene expression.

Therefore, we proposed as a working hypothesis that growth inhibition due to water deficit involves transcriptional or translational alterations of gene expression that might affect the mechanical properties of the cell wall. Since cessation of cell growth during normal development is probably mediated by such events, we undertook a study of polysome content and polysomal mRNA populations in regions of the soybean stem which contain either dividing, elongating or nongrowing (mature) cells, and we compared the developmental changes with those induced by low ψ_w. We found that water deficit induces changes in the elongating tissue that are similar in some respects to those brought on by maturation. The changes disappear during a period of adjustment when growth rate increases.

MATERIALS AND METHODS

Plant Material. Soybean (*Glycine max* [L.] cv Williams) seeds were surface sterilized in 1% NaOCl for 5 min, rinsed with running tap water for 2 h, sown in vermiculite wetted to runoff with

[1] Contribution of the Texas Agricultural Experiment Station. Partial support for this work was also provided by grants from the Texas Advanced Technology Research Program, the Department of Energy grant DE-FG05-84ER13273 to J. S. B., and the United States Department of Agriculture grant 85-CRC-1-1672 to J. E. M.

[2] Present address: Department of Biochemistry and Biophysics, Texas A & M University, College Station, TX 77843.

[3] Abbreviations: ψ_w, water potential; LP/P, ratio of large polysomes to total polysomes; P/T, ratio of polysomes to total ribosomes.

0.1 mM $CaCl_2$ (5 ml/g vermiculite, $\psi_w = -0.01$ MPa), and grown in the dark at 100% RH and 29 ± 1°C. After 48 h, seedlings were transplanted to vermiculite containing the same amount of water (1×) or 1/8 the amount of water (1/8×, $\psi_w = -0.29 \pm 0.02$ MPa). All seedling manipulations were done in a controlled environment chamber at 100% RH under a green safelight (maximum transmission at 525 nm and negligible transmission <475 nm or >575 nm). Stems (hypocotyls) were harvested at various times after transplanting and dissected into three regions (8): the 5 mm just below the cotyledons (dividing region), the next 15 mm (elongating region), and the remainder (mature region). Tissue was frozen in liquid N_2 within 30 s of harvest and stored at −80°C until extracted.

Growth Measurement. Stem growth was measured photographically using a Nikon FM2 equipped with a 50 mm lens and a No. 1 closeup diopter. Seedlings were arranged in plastic pots sitting inside a large styrofoam box which was maintained at 100% RH by lining the walls with wet blotter paper. A removable window was cut in one wall and covered with transparent plastic wrap on the inside. The top of the box was sealed with a piece of transparent Plexiglas and covered with a black cloth. At appropriate intervals the black cloth was removed, and Kodak Tri-X pan film (ASA 400) was exposed for 1 s at f/5.6 using a green safelight described above. Exposure of the seedlings to the safelight was about 5 s at each exposure. The film was developed according to specifications and the negatives were projected (enlarged about ×20) for measurement of stem lengths.

Isolation of Polysomes and Polysomal RNA. Polysomes were isolated by the method of Davies and Larkins (12) with modifications. Frozen tissue was ground to a fine powder in liquid N_2 with a mortar and pestle. The powder was homogenized with five volumes extraction buffer (0.2 M Tris-HCl, pH 8.5; 0.2 M KCl; 0.25 M RNase-free sucrose; 30 mM $MgCl_2$; 10 mM EGTA, 2.5 mM DTT, 5μg/ml proteinase K). All further operations were conducted at 2 to 4°C. When melted, the homogenate was centrifuged at 3000*g* for 5 min to pellet cell debris and nuclei. The supernatant was made to 2% polyoxyethylene-10-tridecyl ether (nonionic detergent), incubated 10 min, and then centrifuged for 10 min at 12,000*g* in a microcentrifuge or 20,000*g* in a Beckman JS-13.1 rotor. The supernatant (0.6–0.8 ml) was layered on a linear 15 to 60% sucrose gradient in 50 mM Tris-HCl (pH 8.5), 25 mM KCl, 10 mM $MgCl_2$, and centrifuged at 40,000 rpm ($270{,}000g_{max}$) for 2 h in a Beckman SW-41 Ti rotor. Gradients were fractionated with an ISCO model 185 gradient fractionator with an ISCO model UA-5 UV monitor recording the absorbance profile at 254 nm.

Appropriate fractions were collected, made to 2% SDS and 20 mM EDTA, and extracted twice with phenol:chloroform:isoamyl alcohol (25:24:1, v/v). The final aqueous phase was made to 0.3 M potassium acetate (pH 5.5) and precipitated with 2.5 volumes of ethanol at −20°C overnight. The RNA was reprecipitated twice with 0.3 M potassium acetate and ethanol, and the final pellet was dried under reduced pressure. The RNA was dissolved in sterile deionized H_2O at a concentration of 2 mg/ml and stored at −80°C.

Determination of Polysome Size Classes. In order to measure absorbance baselines for polysome profiles, ribosome-free supernatants (obtained by centrifuging the 20,000*g* supernatants for 3 h at $200{,}000g_{max}$) were centrifuged on 15 to 60% sucrose gradients and scanned at 254 nm as described above for polysome fractionation. The baselines were traced onto the absorbance scans of polysome-containing gradients (Fig. 1A), which were divided into regions containing monosomes and ribosome subunits, polysomes containing 2 to 5 ribosomes (SP), and polysomes containing six or more ribosomes (LP). Photocopies of profiles were cut out and weighed to determine the the ratios P/T, which represents the proportion of ribosomes in polysome aggregates, and LP/P, which represents the proportion of polysomes (P = SP + LP) containing ≥6 ribosomes.

***In Vitro* Translation and Fractionation of Products.** Polysomal RNA was heated at 65°C for 5 min and quickly chilled to 2°C just before translation. The RNA was translated in a nuclease-treated, message-dependent wheat germ extract (Amersham) according to instructions in the product literature. The 30 μl assays contained 10 μg polysomal RNA, 10 μCi [^{35}S]methionine (>800 Ci/mmol), K^+ at 140 mM, and 20 units of RNasin. After incubation at 23°C for 60 min, RNase A and DNase I were added to a concentration of 125 μg/ml each. The assays were incubated at 2°C for 15 min and stored at −80°C after determination of TCA-insoluble radioactivity.

Translation products were fractionated by the two-dimensional system of O'Farrell (27). The assays were prepared for isoelectric focusing by drying under reduced pressure and resuspending in 25 μl of 9.5 M urea. An equal volume of lysis buffer (27) was added, and 20 to 50 μl containing 0.8 to 1.6 × 10^6 cpm TCA-insoluble radioactivity were loaded on each 12 cm × 1.5 mm first dimension gel. Proteins were focused at 400 V for 15 h followed by 1000 V for 1 h (7000 V-h total), and the gels were soaked in Laemmli (18) sample buffer for 45 min. The second dimension was SDS-PAGE with a 12% acrylamide resolving gel using the buffer system of Laemmli (18). Second dimension gels were fixed with acetic acid:methanol:water (1:4:5, v/v) and fluorographed (5) using Kodak X-Omat AR or BB-1 x-ray film. Exposure times were adjusted to give approximately equal overall densities between gels, and were about 6 d per 10^6 cpm TCA-insoluble radioactivity on Kodak BB-1 film.

Chemicals. [^{35}S]Methionine (>800 Ci/mmole) was from New England Nuclear. Wheat germ extract for *in vitro* translation was from Amersham. Ampholines were from LKB (Bromma, Sweden). Ribonuclease-free sucrose was from BRL (Gaithersburg, MD). Proteinase K was from Boehringer-Mannheim. RNasin (placental ribonuclease inhibitor) was from ProMega Biotech (Madison, WI). All other chemicals were obtained from Sigma.

RESULTS

Polysome Status of Different Stem Regions. Polysomes were sedimented directly from 20,000*g* supernatants. This prevented losses of the monosomes and ribosome subunits that may occur in the alternate method, which requires polysomes to be pelleted through dense sucrose (12). All profiles (Fig. 1) represent equivalent numbers of stem segments except for the 1/8× mature tissue, which represents twice the number of segments but approximately the same fresh weight as 1× mature tissue. Figure 1 shows that, in 1× stems, ribosomes shifted to the non-polysomal region of the sucrose gradient as the tissue became more mature (Fig. 1, A–C). The large amount of subunits in these profiles may be due to dissociation of 80S ribosomes induced by proteinase K, which was used as an inhibitor of ribonuclease. Proteinase K eliminates artifactual polysomal aggregates which associate via nascent peptide chains that are hydrophobic, but has no other effect on sedimentation characteristics at the concentration used here (1). Differences in polysome size and content were more apparent when gradients were scanned on a more sensitive scale (Fig. 1 insets). These show that, although the polysomes changed with development in both 1× and 1/8× stems, the content of large polysomes was lower in all regions of the 1/8× stems (Fig. 1, D–F) compared to the 1× stems (Fig. 1, A–C). The polysome content decreased most in dividing and elongating tissues (Fig. 1, D–E).

The polysome size class ratios for tissues studied in Figure 1 were calculated using data from several similar experiments (Table 1). The ratio P/T represents the proportion of total ribosomes found in polysomal aggregates, and is usually interpreted as that part of the available ribosomes engaged in protein synthesis. The

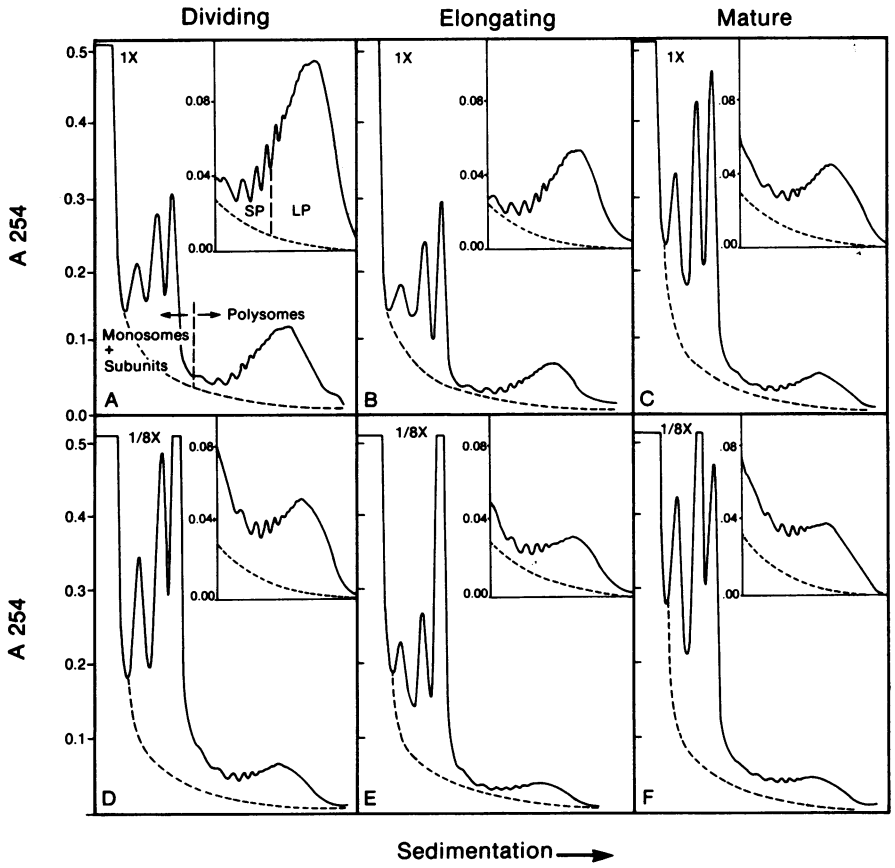

FIG. 1. Polysome profiles from soybean stem segments. Seedlings were harvested 24 h after transplanting to 1× (A–C) or 1/8× (D–F) vermiculite and dissected into dividing, elongating and mature regions. The dashed lines represent absorbance baselines generated by sedimenting post-ribosomal supernatants. The insets show the polysome region of the same samples scanned on a more sensitive scale. The number of segments and fresh weight represented in the profiles are: A, D: 1.7 dividing regions, 0.04 g; B, E: 1.7 elongating regions, 0.09 g; C: 1.7 (1×) mature regions, 0.34 g; F: 3.4 (1/8×) mature regions, 0.32 g. In A (inset): SP = small polysomes, LP = large polysomes.

Table I. *Size Classes of Polysomes from Soybean Stems*

Tissue was harvested 24 h after transfer of the plants to 1× or 1/8× vermiculite. P/T represents the proportion of total ribosomes aggregated into polysomes, and LP/P represents the proportion of polysomes having 6 or more ribosomes. Polysomes were prepared and sedimented as in Figure 1, and the ratios P/T and LP/P were determined by cutting out and weighing appropriate areas of the profiles, using the post-ribosomal supernatant profiles as a baseline. Values are the means ± 1 SD of 3 (dividing, mature) or 4 (elongating) separate determinations.

Treatment	Hypocotyl Region	P/T	LP/P
1×	Dividing	0.48 ± 0.02	0.78 ± 0.05
	Elongating	0.46 ± 0.06	0.81 ± 0.03
	Mature	0.27 ± 0.02	0.67 ± 0.03
1/8×	Dividing	0.25 ± 0.03	0.52 ± 0.07
	Elongating	0.21 ± 0.03	0.61 ± 0.04
	Mature	0.24 ± 0.03	0.58 ± 0.02

proportion of polysomes found in aggregates of 6 or more ribosomes, shown as LP/P, indicates the relative rate of ribosome binding to mRNA (initiation of peptide synthesis), assuming that rates of peptide elongation and termination are constant (35). The data indicate that in 1× seedlings, mature tissue had a much reduced capacity for protein synthesis (P/T) compared to dividing and elongating tissue (Table I), which is consistent with other reports (3, 12, 21, 34). Plants grown at low ψ_w showed low P/T values in all areas of the stems. In addition, low ψ_w decreased the apparent rate of peptide initiation (LP/P) in all the tissues.

Time Course of Changes in Stem Growth and Polysome Ratios. In order to determine whether changes in growth rate were correlated with changes in polysome status, we measured these parameters at various times after transplanting to 1× or 1/8× vermiculite. Figure 2A shows that transplanting to 1× vermiculite had no effect on the rate of stem growth for 10 h, after which there was an increase from 1.6 to 2.3 mm/h. The growth of the 1× stems became difficult to measure photographically after 40 h because of the great stem length and bending, but direct measurements showed that the 1× stems continued to elongate rapidly until 72 h after transplanting (data not shown). Bozarth *et al.* (7) observed similar rapid growth at least until 96 h after transplanting. In 1/8× vermiculite, there was a rapid decrease in growth rate within 1 h that reached a minimum value between 18 and 22 h after transplanting. A subsequent recovery began between 24 and 36 h, and a growth rate of 1.4 mm/h was observed by 72 h. Bozarth *et al.* (7) found that the fresh weights per unit length of elongating and mature stem tissues were the same in 1× and 1/8× stems, which indicates that no water loss occurred in the 1/8× stems.

The polysome status of the elongating stem tissue generally followed the growth rate in both 1× and 1/8× seedlings (Fig.

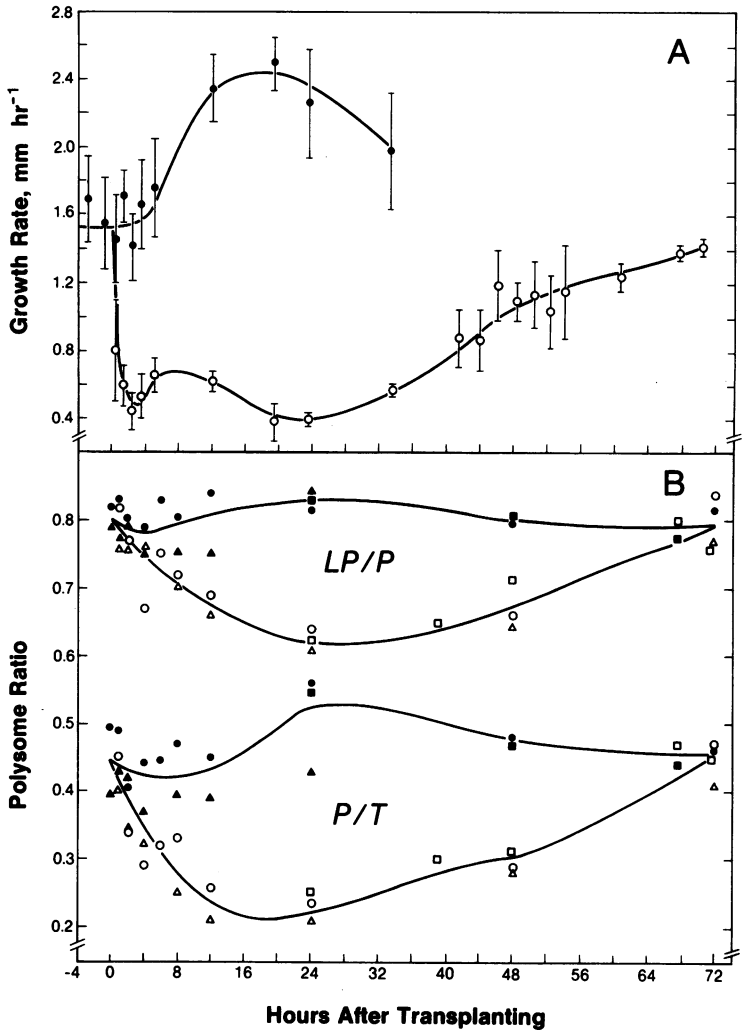

FIG. 2. Seedling growth rate (A) and polysome status of the elongating region (B) of soybean seedlings after transplanting to 1× (closed symbols) or 1/8× (open symbols) vermiculite. Growth rates are the means ± 1 SD of measurements of 5 plants, and the data points are plotted at the midpoint of the time interval between measurements. The experiment was repeated twice with similar results. Polysome ratios were determined from profiles like those in Figure 1, as described in Table I. Data are from 3 separate experiments, indicated by triangles (experiment 1), circles (experiment 2), and squares (experiment 3). The total ribosomal material (in arbitrary units) for a typical experiment (number 1) varied randomly about the means ± SD of 642 ± 55 (1×) and 650 ± 76 (1/8×).

2B). Transplanting seedlings to 1× vermiculite may have caused a slight and transient decrease in P/T and LP/P, but both increased somewhat by 24 h. When seedlings were transplanted to 1/8× vermiculite, polysome ratios decreased after 4 h, and reached minimum values by 24 h. The progressive decrease of LP/P along with P/T suggests that there was a partial block of initiation (16, 35), but this does not preclude the possibility that mRNA availability was reduced (12). Polysome status began to recover by 40 h, and reached levels observed in 1× controls by 72 h after transplanting.

Polysomal mRNA Populations in Stem Tissues. In order to determine what differences existed in polysomal mRNA populations in different regions of the stem and how these were affected by growth at low ψ_w, we translated polysomal mRNA in a wheat germ extract and visualized the products by fluorography of two-dimensional polyacrylamide gels. Figure 3 shows that many mRNAs were found in all stem regions examined (*e.g.*, spots marked 'C' in Fig. 3, A–C). Other spots appeared preferentially in one tissue type (spots marked 'D,' 'E,' or 'M' in Fig. 3, A–C). Although growth at low ψ_w greatly decreased the polysome content in the dividing tissue (Fig. 1D), there were few changes in the polysomal mRNA populations (Fig. 3, A *versus* D). The elongating tissue of the 1/8× plants showed an enhancement of several mRNAs (Fig. 3, B *versus* 3E, spots 1–7, 9, and 10), but some mRNAs decreased that were normally most abundant in elongating tissue (*cf.* Fig. 3, B and E, spots marked 'E'). Interestingly, some of the mRNAs that increased in the elongating region at 1/8× were most abundant in mature tissue of 1× plants (spots numbered 3, 7, 9, and 10 in Fig. 3). Low ψ_w increased some of the same mRNAs in the mature tissue that were enhanced in the 1/8× elongating tissue (*cf.* Fig. 3, C *versus* F, spots 1–4 and 6), but did not change others (spots 5, 7, 9, and 10). Three spots were induced by low ψ_w only in the mature tissue (Fig. 3F, spots numbered 8, 11, and 12).

The increase in polysomal mRNA coding for particular peptides in elongating 1/8× tissue could have arisen by changes in either cellular mRNA levels or distribution of mRNA between polysomes and the nonpolysomal fraction. In order to determine whether these mRNAs were present but untranslated in the cytoplasm of 1× tissue and whether low ψ_w changed the distribution, we translated nonpolysomal mRNA from the elongating region of the stem. This mRNA was prepared from material that sedimented more slowly than 70S on gradients like those in Figure 1. The two-dimensional patterns (Fig. 4) show some differences between the 1× and 1/8× treatments, but only one spot (number 5) could be identified as one of the polysomal mRNAs that showed increased levels at low ψ_w, and it was more prevalent in the cytoplasm of 1/8× stems. Thus it is unlikely that the change in polysomal mRNA population was due to recruitment of mRNA into polysomes from a cytoplasmic nonpolysomal pool of mRNA. Their increased abundance in polysomes probably resulted from enhanced gene transcription and/or increased mRNA stability

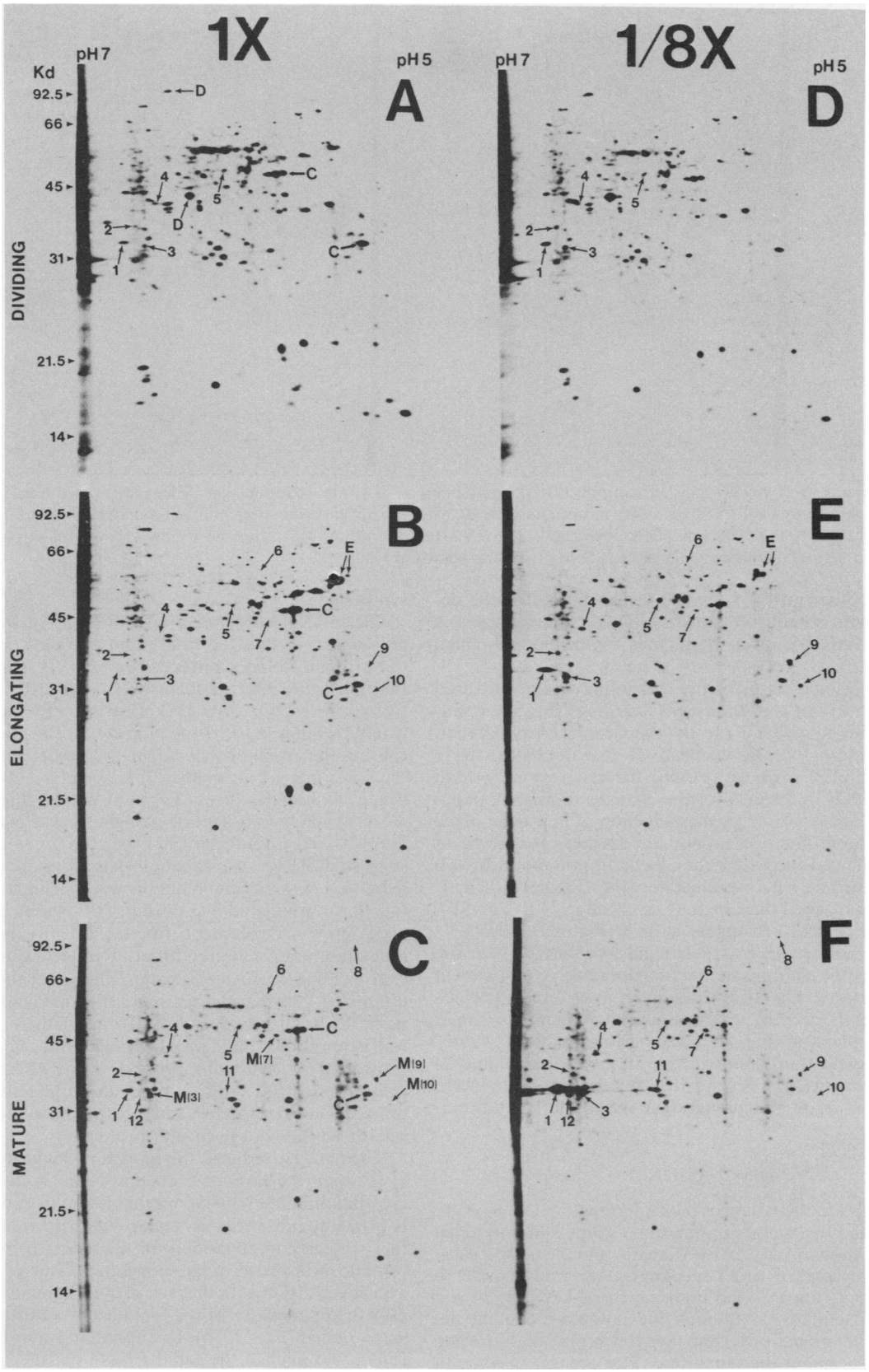

FIG. 3. Fluorographs of *in vitro* translation products of polysomal mRNA from soybean stems. Stems were harvested 24 h after transplanting to 1× (A–C) or 1/8× (D–F) vermiculite and were dissected into dividing (A, D), elongating (B, E), and mature (C, F) regions. The numbers above indicate the pH range of the first dimension, and the numbers at left indicate the mol wt of Bio-Rad standards in the second dimension. Spots marked 'D' in (A), 'E' in (B), and 'M' in (C) are most abundant in dividing, elongating and mature tissues, respectively. Spots marked 'C' are common to all tissues. Spots marked with numbers are affected by low water potential (see text).

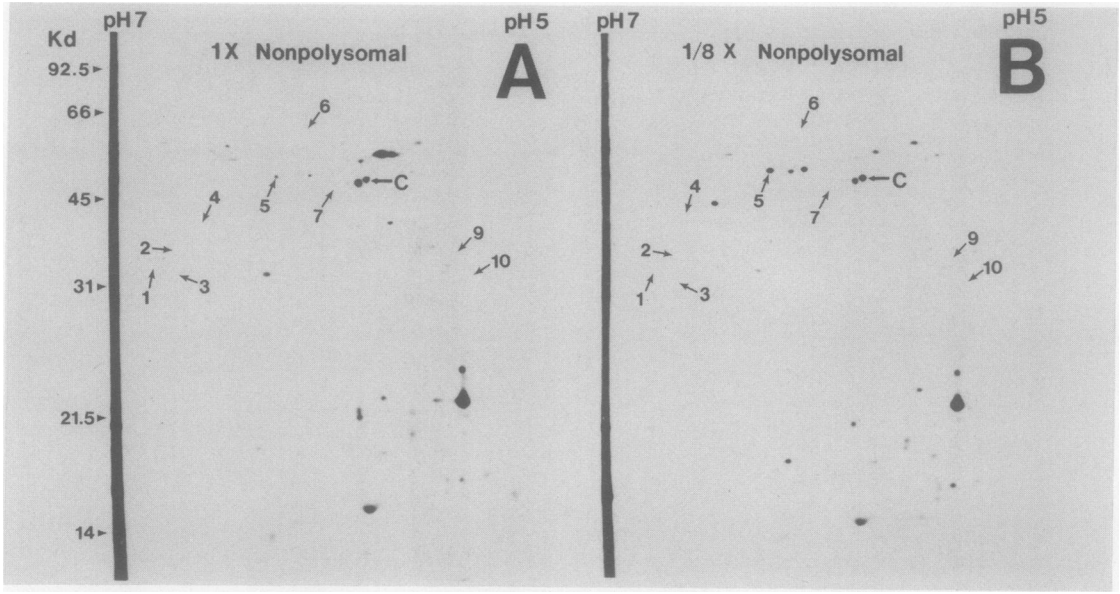

FIG. 4. Fluorographs of *in vitro* translation products of nonpolysomal mRNA from soybean stems. Elongating tissue from stems was harvested 24 h after transplanting to 1× (A) or 1/8× (B) vermiculite. The 20,000*g* supernatants were prepared and sedimented as in Figure 1, and material sedimenting more slowly than 70S was phenol extracted. The RNA in this fraction was translated in the wheat germ extract and the products fractionated as in Figure 3. Numbers indicate positions of spots as shown in Figure 3.

relative to the other mRNAs. Figure 4 also shows that the decreased polysome content in the 1/8× plants did not cause a major shift of mRNAs from polysomes to the nonpolysomal fraction.

Since the polysome status of the elongating tissue recovered during the recovery of growth in 1/8× seedlings (Fig. 2), it was of interest to know whether the polysomal mRNA population of the elongating tissue also changed. If growth changes were mediated by altered gene expression, the recovery of growth might occur either by reversal of the changes in mRNA population seen in Figure 3E, or by the induction of new gene products. The results in Figure 5 support the former case. Some of the mRNAs showed increased abundance in polysomes by 4 h after transplanting to 1/8× vermiculite, (Fig. 5E, spots 1–4, 7, 9, and 10), and reached their highest levels after 24 h (Fig. 5F). These mRNAs began to disappear at 48 h (Fig. 5G), and by 72 h their relative abundance was quite similar to that in 1× tissue (Fig. 5H). This time course is similar to those observed for growth and polysome status (Fig. 2). Interestingly, spots 9 and 10, which appeared to be induced by water deficit in elongating tissue at 24 h after transplanting (Fig. 3E), also occurred in the 1× elongating tissue before transplanting (Fig. 5A), but disappeared 24 h after transplanting (Figs. 3B and 5B). Other mRNAs that were enhanced by low ψ_w in elongating tissue did not follow this pattern.

DISCUSSION

Our data indicate that changes in stem growth at low ψ_w were closely paralleled by changes in polysome status and polysomal mRNA. Both growth rate and polysome content of the elongating region decreased when seedlings were transplanted to vermiculite having low ψ_w, and both increased between 24 and 72 h after transplanting. Although the polysome content decreased somewhat more slowly than the growth rate, both reached minimal levels at about the same time. The changes in some of the polysomal mRNAs behaved similarly. Because the changes occurred in the elongating tissue, they could be associated with the changes in growth. The data do not allow firm conclusions on cause and effect relations, but are consistent with the hypothesis that growth may be affected by alterations in protein synthesis.

The mRNA used for *in vitro* translation was obtained from polysomes, so that the translation products probably represent the proteins being synthesized *in vivo*. It is important to note that the translation of some polysomal mRNAs may be selectively repressed *in vivo* (32). However, the inactive polysomes would be inhibited at elongation steps of peptide synthesis, thus causing the formation of larger polysomes, as was found with the *Cat2* mRNA in maize (32). Since we observed a shift to smaller polysomes (lower LP/P) at low ψ_w (Fig. 2B, Table I), it seems unlikely that peptide elongation was inhibited.

A basis exists for the link between protein synthesis and growth suggested above. An equation describing plant tissue enlargement (6, 11), which combines the water demand and water supply functions formulated by Lockhart (19), shows that, in the steady state, growth depends on five factors: (a) the turgor, (b) the minimum turgor required for growth (yield threshold), (c) a cell wall yielding coefficient (extensibility), (c) the water potential difference between the cells and the surroundings, and (c) the tissue hydraulic conductance. Three of these (yield threshold, wall extensibility, and tissue hydraulic conductance) are probably controlled by metabolic processes that affect the mechanical properties of cell walls or hydraulic conductivity of cell membranes. These properties could be modulated by changes in the rate of synthesis of particular proteins.

Since low ψ_w reduced the growth of soybean stems somewhat faster than the polysome content (Fig. 2), the initial cause of growth inhibition may be unrelated to polysome loss. The cause is probably not a loss of turgor, since previous studies showed that similarly treated soybean seedlings maintained turgor by osmotic adjustment in the elongating tissue of the stem (8, 23). This is consistent with the lack of correlation between turgor and growth observed in other osmotically adjusting tissues during water deficit (21, 22, 36). Disruption of water flow to elongating cells could explain the rapid growth decline. A drop in the ψ_w of the xylem, which occurs when plants are transplanted to vermiculite having low ψ_w, could cause a disruption of the radial gradient of water potential between the transpiration stream and the growing cells (6, 8). Reestablishment of a favorable gradient would be required for growth to continue, and this could be

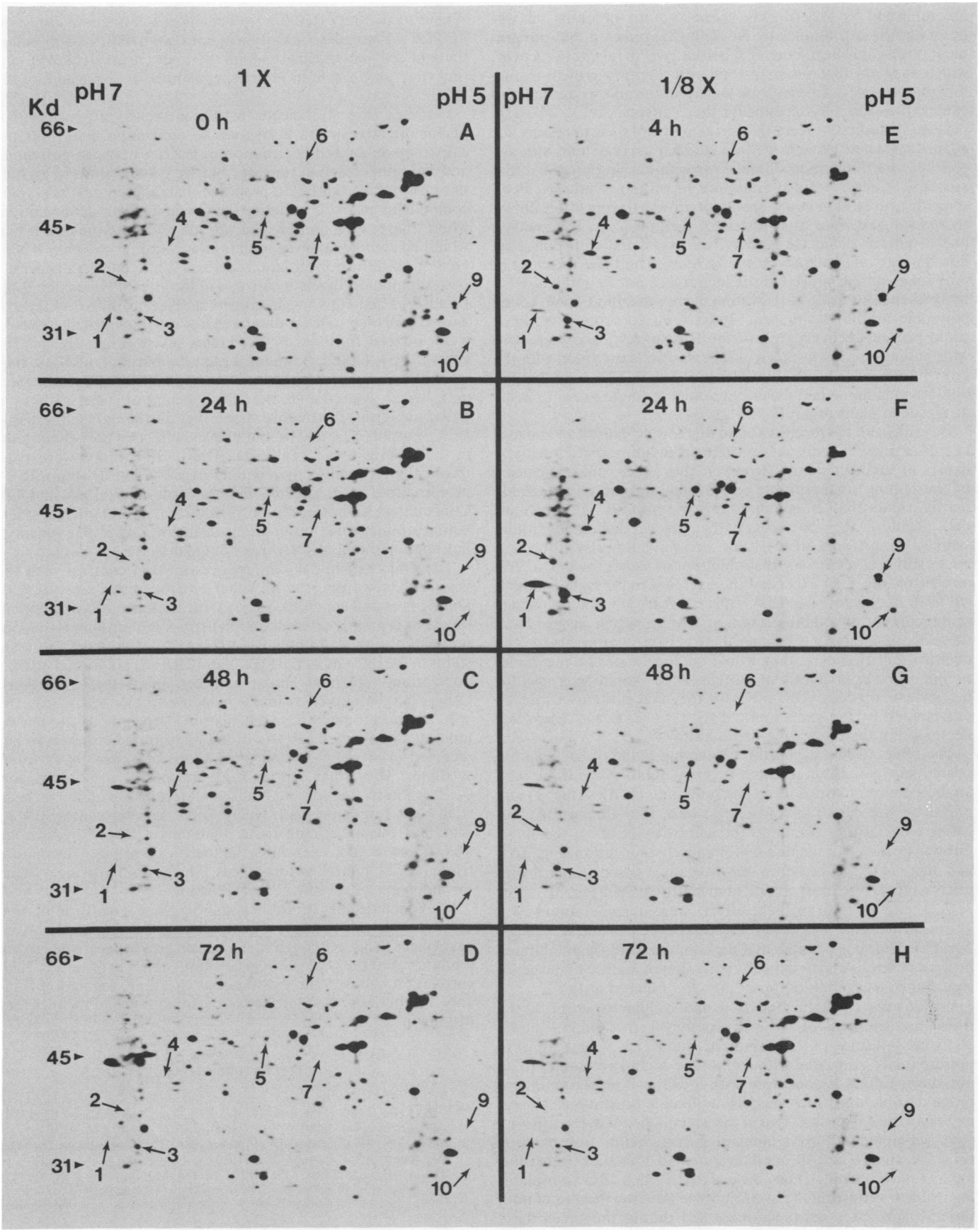

FIG. 5. Time course of changes in *in vitro* translation products of polysomal mRNA from the elongating region of soybean stems. Polysomal RNA was translated and products fractionated as in Figure 3. Treatments and time after transplant are: A: 1×, 0 h (untransplanted); B: 1×, 24h; C: 1×, 48h; D: 1×, 72 h; E: 1/8×, 4 h; F: 1/8×, 24 h; G: 1/8×, 48 h; H: 1/8×, 72 h. Numbered arrows indicate positions of spots as shown in Fig. 3. Numbers at left indicate positions of mol wt markers as in Figure 3.

accomplished by lowering the tissue osmotic potential, as was observed (8, 23). Studies by Nonami (25) indicate that the gradient is reestablished about 10 h before growth begins to recover, which suggests that other factors were limiting growth during this interval. A decreased rate of protein synthesis and altered gene expression may account for these effects.

Several studies indicate that protein synthesis is necessary for auxin-stimulated growth (2, 9, 33). This is consistent with the concept of a population of labile 'growth-limiting proteins' that are rapidly depleted in the absence of protein synthesis. Polysome disaggregation could thus limit growth during water deficit by reducing the rate of synthesis of such proteins. By analogy, loss of nitrate reductase activity was associated with polysome loss during water deficits in maize leaves, and the recovery of polysome content preceded the increase in nitrate reductase activity upon rewatering (24). Although growth inhibition preceded polysome loss in the present study, reduced protein synthesis could prevent recovery of growth. This suggestion is consistent with findings that polysome content was well correlated with the growth rates of four different plants at low ψ_w (21), and that the non-growing tissue had fewer polysomes than the growing tissue of the same plant organ (3, 12, 21, 34).

Bozarth *et al.* (7) recently found that low ψ_w caused an increase in salt-extractable cell wall protein in dividing and elongating tissues of soybean stems. However, this effect could be caused by the failure to incorporate wall protein into an unextractable matrix, rather than an increased rate of synthesis. Although our data (Table I, Fig. 2B) suggest that low ψ_w decreased protein synthesis in growing stem tissues, accumulation of wall protein might still occur, because the inhibition of stem growth (to 30% of control, Fig. 2A) exceeded the decrease in polysome content (to 50% of control, Fig. 2B). Bozarth *et al.* (7) saw no change in the content of soluble protein at low ψ_w, which suggests that the cells maintained a balance between protein synthesis and degradation. However, they would not have detected the depletion of a small population of proteins which might be crucial for the growth process. The mRNAs for such proteins could be represented by the spots marked 'E' (Fig. 3, B and E), which decreased in the elongating tissue at low ψ_w.

The effect of low ψ_w on the elongating tissue of soybean hypocotyl may be interpreted as a reversible shift towards the metabolic state of mature tissue. The polysome profiles (Fig. 1) and polysome size class ratios (Table I) were very similar for elongating tissue of 1/8× seedlings and mature tissue of 1× seedlings. Growth in 1/8× vermiculite inhibited stem elongation (Fig. 2A), and increased the presence in elongating tissue of particular mRNAs that were most abundant in mature tissue (Fig. 3E, spots 3, 7, 9, and 10). All of these effects were maximal about 24 h after transplanting and were reversed between 24 and 72 h (Figs. 2 and 5). Thus it is possible that the combined effects of inhibited protein synthesis and enhanced expression of mature tissue genes contributed to the inhibition of growth observed at low ψ_w.

We do not know the function and cellular location of the translation products of the mRNAs that were enhanced in 1/8× tissue, but some of them (Fig. 3E, spots 1, 3, 9, and 10) were preferentially associated with membrane-bound polysomes (data not shown). This is consistent with a cell wall or plasma membrane destination, which suggests a possible function in the regulation of cell expansion. Two of the water deficit-induced mRNAs appeared in the pattern of elongating tissue before transplanting (Fig. 5A, spots 9 and 10), and disappeared 24 h after transplanting to 1× vermiculite (Figs. 3B and 5B) or 48 h after transplanting to 1/8× vermiculite (Fig. 5G). Since the growth rates of both the 1× and 1/8× seedlings increased during the times these mRNAs disappeared (Fig. 2A), they may be associated with growth inhibition. Interestingly, several of the products (Fig. 3E, spots 1–3, 9, and 10) had mobilities in the second dimension similar to that reported for a soybean seed peroxidase (M_r = 37 kD) (31). Peroxidase activity was correlated with growth potential and cell wall plasticity along the mung bean stem (14), and thus may play a role in growth regulation, as suggested by Fry (13).

Our data (Fig. 4) indicate that the increased presence of particular mRNAs in the polysomes of elongating tissue was not caused by an altered distribution of mRNA between polysomes and the nonpolysomal fraction, but we cannot entirely exclude this possibility. Lodish (20) proposed that different mRNAs could compete for available initiation factors, and that under conditions where these were limiting the rate of protein synthesis, a bias would be created favoring initiation on "strong" mRNAs having greater affinities for initiation factors. While our data suggest a partial initiation block at low ψ_w (reduced LP/P values in Table I and Fig. 2B), this mechanism is unlikely to have caused the altered mRNA profile for three reasons. First, some of the mRNAs were induced from barely detectable levels (Figs. 3E and 5E, spots 1, 2, 4, 6, and 7), and if these were 'strong' mRNAs, they would be more prevalent in polysomes of unstressed plants. Second, low ψ_w caused a greater enhancement of some mRNAs in polysomes from the mature tissue than in the elongating tissue (Fig. 3F, spots 1, 3, and 6), but had less effect on polysome status in the mature tissue (Table 1). Third, LP/P ratios were most drastically reduced in the dividing tissue (Table I), but mRNA profiles were least affected in this tissue (Fig. 3, A and D). Concomitant alterations in polysome content and mRNA translation products also occur in aged and wounded pea epicotyl, but nonpolysomal mRNA was not examined (30).

The effects of low ψ_w on gene expression contrast with the drastic changes reported for other environmental stresses (29). During heat and anaerobic stresses, the normal pattern of protein synthesis is greatly altered, and the stress proteins are the major products observed. Although low ψ_w greatly altered the polysome status in the present work (Figs. 1 and 2B), the polysomal mRNA populations remained similar with certain notable exceptions. Others have observed a similar response to low ψ_w (4, 15).

In addition to the mRNA changes in elongating tissue, low ψ_w induced some novel mRNAs in the mature tissue that were not detected in dividing or elongating tissue of either 1× or 1/8× seedlings. These may represent a very specific response to low ψ_w. It is interesting to note that the mature tissue of the soybean stems lost turgor pressure when plants were grown at low ψ_w (26), but the elongating tissue maintained turgor by osmotic adjustment (8, 23). Therefore, the specific response in the mature tissue may be related to turgor loss, which could provide a signal for induction of specific gene expression.

We conclude that ψ_w low enough to cause growth inhibition also causes changes in gene expression in the elongating tissue of soybean stems. Although some time elapses between the growth inhibition and the changes in gene expression, the otherwise high correlation between the two suggests that gene regulation is involved in both the maintenance of the inhibition and the recovery of growth.

LITERATURE CITED

1. ABE S, E DAVIES 1985 Quantitative isolation of undegraded polysomes from aged pea tissue in the absence of contaminants and artifacts. Plant Cell Physiol 26: 1499–1509
2. BATES GW, RE CLELAND 1979 Protein synthesis and auxin-induced growth: inhibitor studies. Planta 145: 437–442
3. BEWLEY JD, KM LARSEN 1982 Differences in the response to water stress of growing and non-growing regions of maize mesocotyls: protein synthesis on total, free and membrane-bound polyribosome fractions. J Exp Bot 33: 406–415
4. BEWLEY JD, KM LARSEN, JET PAPP 1983 Water stress-induced changes in the pattern of protein synthesis in maize seedling mesocotyls: a comparison with the effects of heat shock. J Exp Bot 34: 1126–1133
5. BONNER WM, RA LASKEY 1974 A film detection method for tritium-labelled

proteins and nucleic acids in polyacrylamide gels. Eur J Biochem 46: 83–88
6. BOYER JS, AJ CAVALIERI, E-D SCHULZE 1985 Control of the rate of cell enlargement: excision, wall relaxation, and growth-induced water potentials. Planta 163: 527–543
7. BOZARTH C, JE MULLET, JS BOYER 1987 Cell wall proteins at low water potential. Plant Physiol 85: 261–267
8. CAVALIERI AJ, JS BOYER 1982 Water potentials induced by growth in soybean hypocotyls. Plant Physiol 69: 492–496
9. CLELAND RE 1971 Instability of growth limiting proteins of the *Avena* coleoptile and their pool size in relation to auxin. Planta 99: 1–11
10. CLELAND RE 1983 The capacity for acid-induced wall loosening as a factor in the control of *Avena* coleoptile cell elongation. J Exp Bot 34: 676–680
11. COSGROVE DJ 1983 Photocontrol of extension growth: a biophysical approach. Phil Trans R Soc Lond B 303: 453–465
12. DAVIES E, BA LARKINS 1972 Polyribosomes from peas. II. Polyribosome metabolism during normal and hormone-induced growth. Plant Physiol 52: 339–345
13. FRY SC 1980 Gibberellin-controlled pectinic acid and protein secretion in growing cells. Phytochem 19: 735–740
14. GOLDBERG R, A IMBERTY, M LIBERMAN, R PRAT 1986 Relationships between peroxidatic activities and cell wall plasticity. *In* H Greppin, C Penel, Th Gaspar, eds, Molecular and Physiological Aspects of Plant Peroxidases. University of Geneva, Switzerland, pp 209–220
15. HEIKKILA JJ, JET PAPP, GA SCHULTZ, JD BEWLEY 1984 Induction of heat shock protein messenger RNA in maize mesocotyls by water stress, abscisic acid, and wounding. Plant Physiol 76: 270–274
16. HSIAO TC Rapid changes in levels of polyribosomes in *Zea mays* in response to water stress. Plant Physiol 46: 281–285
17. HSIAO TC, E ACEVEDO, E FERERES, DW HENDERSON 1976 Water stress, growth, and osmotic adjustment. Phil Trans R Soc Lond B 273: 479–500
18. LAEMMLI UK 1970 Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685
19. LOCKHART JA 1965 An analysis of irreversible plant cell elongation. J Theor Biol 8: 264–275
20. LODISH HF 1974 Model for the regulation of mRNA translation applied to haemoglobin synthesis. Nature 251: 385–388
21. MASON HS, K MATSUDA 1985 Polyribosome metabolism, growth and water status in the growing tissues of osmotically stressed plant seedlings. Physiol Plant 64: 95–104
22. MATSUDA K, A RIAZI 1981 Stress-induced osmotic adjustment in growing regions of barley leaves. Plant Physiol 68: 571–576
23. MEYER RF, JS BOYER 1981 Osmoregulation in soybean seedlings having low water potentials. Planta 151: 482–489
24. MORILLA CA, JS BOYER, RH HAGEMAN 1973 Nitrate reductase activity and polyribosome content of corn (*Zea mays* L.) having low leaf water potentials. Plant Physiol 51: 817–824
25. NONAMI H 1986 Mechanism of cell enlargement at low water potential. PhD dissertation. University of Illinois at Urbana-Champaign, IL
26. NONAMI H, JS BOYER, E STEUDLE 1987 Pressure probe and isopiestic psychrometer measure similar turgor. Plant Physiol 83: 592–595
27. O'FARRELL PH 1975 High resolution two-dimensional electrophoresis of proteins. J Biol Chem 250: 4007–4021
28. RHODES PR, K MATSUDA 1976 Water stress, rapid polyribosome reduction and growth. Plant Physiol 58: 631–635
29. SACHS MM, T-HD HO 1986 Alteration of gene expression during environmental stress in plants. Annu Rev Plant Physiol 37: 363–376
30. SCHUSTER AM, E DAVIES 1983 Ribonucleic acid and protein metabolism in pea epicotyls. II. Response to wounding in aged tissue. Plant Physiol 73: 817–821
31. SESSA DJ, RL ANDERSON 1981 Soybean peroxidases: purification and some properties. J Agric Food Chem 29: 960–965
32. SKADSEN RW, JG SCANDALIOS 1987 Translation control of photo-induced expression of the *Cat2* catalase gene during leaf development in maize. Proc Natl Acad Sci USA 84: 2785–2789
33. THEOLOGIS A, TV HUYNH, RW DAVIS 1985 Rapid induction of specific mRNAs by auxin in pea epicotyl tissue. J Mol Biol 183: 53–68
34. TRAVIS, RL, JM ANDERSON, JL KEY 1973 Influence of auxin and incubation on the relative level of polyribosomes in excised soybean hypocotyl. Plant Physiol 52: 608–612
35. VASSART G, JE DUMONT, FRC CANTRAINE 1971 Translational control of protein synthesis: a simulation study. Biochim Biophys Acta 247: 471–485
36. WESTGATE ME, JS BOYER 1985 Osmotic adjustment and the inhibition ofleaf, root, stem and silk growth at low water potentials in maize. Planta 164: 540–549