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Hypoxic Stress-Induced Changes in Ribosomes of Maize Seedling Roots[1]

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ABSTRACT

The hypoxic stress response of *Zea mays* L. seedling roots involves regulation of gene expression at transcriptional and posttranscriptional levels. We investigated the effect of hypoxia on the translational machinery of seedling roots. The levels of monoribosomes and ribosomal subunits increased dramatically within 1 hour of stress. Prolonged hypoxia resulted in continued accumulation of nontranslating ribosomes, as well as increased levels of small polyribosomes. The return of seedlings to normal aerobic conditions resulted in recovery of normal polyribosome levels. Comparison of ribosomal proteins from control and hypoxic roots revealed differences in quantity and electrophoretic mobility. *In vivo* labeling of roots with [^{35}S]methionine revealed variations in newly synthesized ribosomal proteins. *In vivo* labeling of roots with [^{32}P]orthophosphate revealed a major reduction in the phosphorylation of a 31 kilodalton ribosomal protein in hypoxic stressed roots. *In vitro* phosphorylation of ribosomal proteins by endogenous kinases was used to probe for differences in ribosome structure and composition. The patterns of *in vitro* kinased phosphoproteins of ribosomes from control and hypoxic roots were not identical. Variation in phosphoproteins of polyribosomes from control and hypoxic roots, as well as among polyribosomes from hypoxic roots were observed. These results indicate that modification of the translational machinery occurs in response to hypoxic stress.

Plants respond to environmental stress conditions (heat shock, drought, and flooding) through changes in gene expression. For example, the transfer of *Zea mays* L. seedlings from air to low oxygen conditions results in dramatic changes in protein synthesis in root cortex and other nonphotosynthetic tissues (2, 15). Hypoxic or anaerobic stress of seedling roots causes a decrease in overall protein synthesis and a near-complete repression of normal protein synthesis within 1 h. After 5 h of hypoxic stress of seedlings, a group of about 20 polypeptides accounts for 70% of the proteins synthesized by roots (18). These so-called anaerobic proteins include glycolytic enzymes (2) and other proteins required for metabolism under low oxygen conditions (8). If hypoxic stress is removed before cell death occurs, then the normal pattern of protein synthesis is resumed.

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The changes in gene expression which result from hypoxic stress are regulated at both transcriptional and posttranscriptional levels (6, 7, 18, 21). The transcriptional regulation of the anaerobic response involves a rapid increase in steady-state levels of mRNAs which encode anaerobic proteins (6). The posttranscriptional regulation of the anaerobic response is known to involve selective translation of mRNAs which encode anaerobic proteins and/or repression of translation of mRNAs which encode normal aerobic proteins (16–18). As a first step toward elucidation of the posttranscriptional regulation of the anaerobic response, we have examined the translational machinery of both control (aerobic) and hypoxic (anaerobic) seedling roots. We report here that a low-oxygen environment induces a decrease in levels of polyribosomes and an increase in levels of monoribosomes and free ribosomal subunits. In addition, hypoxic stress results in physical and chemical modifications of the protein component of ribosomes.

MATERIALS AND METHODS

Plant Material

Zea mays L. (Pioneer Hibred, inbred line B73) kernels were surface sterilized with 0.025% (v/v) sodium hypochlorite and germinated on trays in the dark for 5 to 7 d.

Experimental Treatments

Hypoxic stress was induced by complete immersion of intact seedlings in induction buffer (0.5 mM Tris-HCl [pH 8.0], 7.5 μg/mL chloramphenicol) in a closed 1 gallon Mason jar into which 100% (v/v) argon was bubbled into the buffer. Trays of control seedlings were left uncovered in the dark for 2 h prior to harvest. Harvested seedling roots were immediately frozen in liquid nitrogen.

In Vivo Labeling of Roots

Primary seedling roots were labeled *in vivo* by placing the apical 2 cm into induction buffer containing 150 μCi [^{35}S] met/mL or 90 μCi [^{32}P]Pi/mL for 3 h. Two roots were labeled per milliliter of induction buffer. Hypoxic labeling was carried out in a closed Mason jar into which 100% (v/v) argon continuously flowed. Labeling of control roots was carried out in an open Mason jar containing moist paper towels.

Polysome Isolation

Ribosomal subunits (40S, 60S), monoribosomes (80S) and polyribosomes, collectively termed polysomes, were isolated

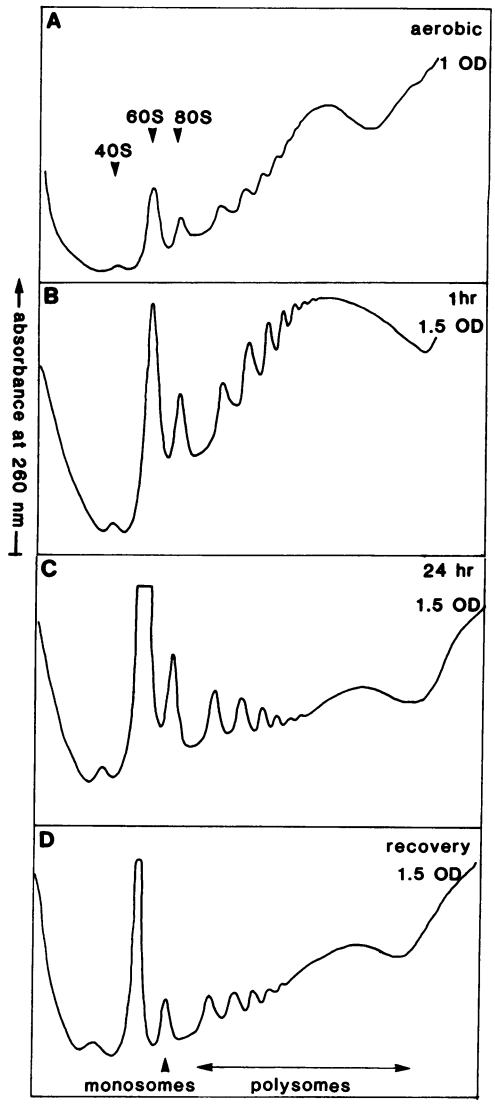

Figure 1. Absorbance/sedimentation profiles (260 nm) of polysomes on sucrose gradients. Polysomes were purified from roots of seedlings grown in air (A), submerged in water under argon for 1, 24 h (B, C), or submerged for 24 h and transferred to air for 2 h (D). Polysomes were purified from a detergent treated root extract by centrifugation for 3.5 h through a sucrose cushion. The polysome pellets, which contained polyribosomes, monoribosomes (80S), and a portion of the total ribosomal subunits (40S and 60S), were loaded onto continuous 15 to 65% (w/v) sucrose gradients, centrifuged and fractionated with an ISCO gradient fractionater linked to an A_{260} nm UV monitor.

from seedling roots. Roots were ground under liquid nitrogen and suspended in extraction buffer (0.2 M Tris-HCl [pH 9.0], 0.4 M KCl, 25 mM EGTA, 35 mM $MgCl_2$, 0.2 M sucrose, 50 μg/mL cycloheximide, 50 μg/mL chloramphenicol). Further manipulations were carried out on ice or at 4°C. The extract was filtered through four layers of sterile cheesecloth and two layers of milk filters. Nonionic detergents were added (1% (v/v) Triton X-100, 1% (w/v) Brij 35, 1% (v/v) Tween-40, 1% (v/v) NP-40) and the extract was incubated on ice for 30 min to solubilize membrane polysomes. The extract was centrifuged at 30,000*g* for 20 min. The S-30 supernatant was layered over a sucrose cushion (1.75 M sucrose, 40 mM Tris [pH 9.0], 0.2 M KCl, 5 mM EGTA, 30 mM $MgCl_2$, 50 μg/mL cycloheximide, 50 μg/mL chloramphenicol), and centrifuged at 105,000*g* for 3.5 h, unless indicated otherwise. The polysome pellet was resuspended in 200 to 400 μL resuspension buffer (40 mM Tris [pH 8.4], 0.2 M KCl, 5 mM EGTA, 30 mM $MgCl_2$, 50 μg/mL cycloheximide, 50 μg/mL chloramphenicol). Eighty millimolar β-glycerophosphate was included in all buffers used in the purification of *in vivo* phosphorylated polysomes.

Sucrose Gradient Analysis of Polysomes

Polysomes (1–1.5 A_{260}) were loaded onto 5 mL, 15 to 65 (w/v) sucrose gradients (40 mM Tris [pH 8.4], 0.2 M KCl, 0.1 M $MgCl_2$, 50 μg/mL cycloheximide, 50 μg/mL chloramphenicol) and centrifuged at 115,000*g* for 1 h in a swinging bucket rotor. The gradients were analyzed at 0.2 sensitivity with an ISCO gradient fractionator linked to an A_{260} nm UV monitor and chart recorder. Polysome size classes were determined as described previously (13) except that a blank sucrose gradient was used to set the baseline for the gradient profiles.

Gel Electrophoresis

Proteins in the polysome pellet were analyzed by two-dimensional NEpHGE[3] according to O'Farrell *et al.* (14). RNA was removed from polysomes by addition of 1 mM magnesium acetate and 67% (v/v) acetic acid, followed by centrifugation. The RNA pellet was reextracted and proteins were precipitated from the combined supernatants with four volumes of cold acetone and then pelleted by centrifugation. Polysomal proteins were separated in the first dimension in NEpHGE (pH 3.5–10) tube gels for 1600 V h. Gels were soaked in equilibration buffer (50 mM Tris-HCl [pH 6.8], 5% (v/v) 2-mercaptolethanol, 10% (v/v) glycerol, 2% (w/v) SDS) for 2 min and boiled for 2 min. Proteins were separated in the second dimension by electrophoresis on 15% (w/v) polyacrylamide, 0.1% (w/v) SDS Laemmli gels (10). Proteins were visualized by silver-staining and fluorography.

In vitro Labeling of Sucrose Gradient Fractions

Polysomes were separated on sucrose gradients as described above and the gradients were collected in 0.3 mL fractions. The fractions were diluted with 1 volume of 2× kinase buffer (50 mM Tris [pH 7.5], 20 mM $MgCl_2$, 20 mM DDT) containing 10 μCi/mL γ-[^{32}P]ATP. The kinase reaction, using endogenous kinase activity, was carried out for 2 h at room temperature and protein was precipitated by addition of 2 volumes of cold ethanol and centrifugation. The polysome pellet was resuspended in SDS loading buffer (10 mM Tris-HCl [pH 6.8], 1% (v/v) 2-mercaptoethanol, 2.5% (v/v) glycerol, 4% (w/v) SDS, 0.01% (w/v) bromophenol blue), boiled for 5 min and separated by electrophoresis on a 15% (w/v) polyacrylamide, 0.1% (w/v) SDS Laemmli gel (10). Total proteins were visualized by silver-staining and labeled proteins by fluorography.

[3] Abbreviation: NEpHGE, nonequilibrium pH gel electrophoresis.

Table I. *Size Class Distribution of Ribosomes in Polysome Pellets from Maize Seedling Roots*

The ratios of polyribosomes to total ribosomes (P/T) and large polyribosomes to total polyribosomes (LP/P) were determined for the polysome profiles shown in Figure 1. A scan of a blank sucrose gradient was used as the baseline for the profiles. The large polyribosomes have greater than five ribosomes.

Treatment	P/T	L/P
Control	0.79	0.90
1 h hypoxic	0.78	0.85
24 h hypoxic	0.29	0.34
Recovery	0.29	0.53

RESULTS

Polyribosome, Monoribosome, and Ribosome Subunit Levels

We examined the levels of ribosome subunits (40S, 60S), monoribosomes (80S ribosomes), and polyribosomes (>80S ribosomes) in polysome pellets isolated from control and hypoxic stressed seedling roots (Fig. 1). A rapid increase in monoribosomes occurred within 1 h of anaerobiosis (Fig. 1, A and B). After 24 h of stress, high levels of ribosome subunits and monoribosomes were observed, as well as increased levels of small polyribosomes (Fig. 1C). When 24 h stressed seedlings were allowed to recover briefly in air for 2 h, we observed a reduction in levels of ribosome subunits and monoribosomes and reaccumulation of large polyribosomes (Fig. 1D). The ratios of polyribosomes to total ribosomes (P/T) and polyribosomes containing more than five ribosomes to total polyribosomes (LP/P) were determined for the profiles shown in Figure 1. These calculations indicate a decrease in both total polyribosomes and large polyribosomes in response to low oxygen conditions. In addition, these measurements suggest that roots allowed to recover for 2 h showed an increase in the level of large polyribosomes and no change in the level of total polyribosomes. When polyribosome size classes from control and 24 h hypoxic roots were compared in four separate experiments we observed that hypoxia stimulated a 39 ± 4% reduction in the P/T ratio and a 38 ± 6% reduction in the LP/P ratio (Table I).

Ribosomal Proteins

We investigated the hypoxic stress-induced changes in the protein component of ribosomes. Proteins in the polysome pellet were separated by two-dimensional gel electrophoresis (NEpHGE/SDS-PAGE) and visualized by silver-staining (Fig. 2). The majority of proteins visualized were basic proteins which ranged in molecular mass from 5 to 35 kD. The molecular mass distribution and basic nature of these proteins was similar to that described previously for plant ribosomal proteins (5). A minor, variable class of acidic ribosomal proteins was also observed.

Comparison of the ribosomal proteins from control and hypoxic stressed roots revealed a number of differences in protein quantity and electrophoretic mobility (Fig. 2, A and B). Both control (open arrows) and hypoxic (closed arrows) condition specific proteins were resolved. In addition, we observed that the small ribosomal proteins (<20 kD) from stressed roots migrated at a reduced rate in the NEpHGE dimension. This change in electrophoretic mobility indicates that these proteins are less basic in stressed root cells. Ribosomes isolated from hypoxic roots consisted mostly of monoribosomes and 60S ribosomal subunits, whereas ribosomes from control roots were mostly polyribosomes. Thus, the difference in electrophoretic mobility and protein composition of polysomes from control and hypoxic roots could

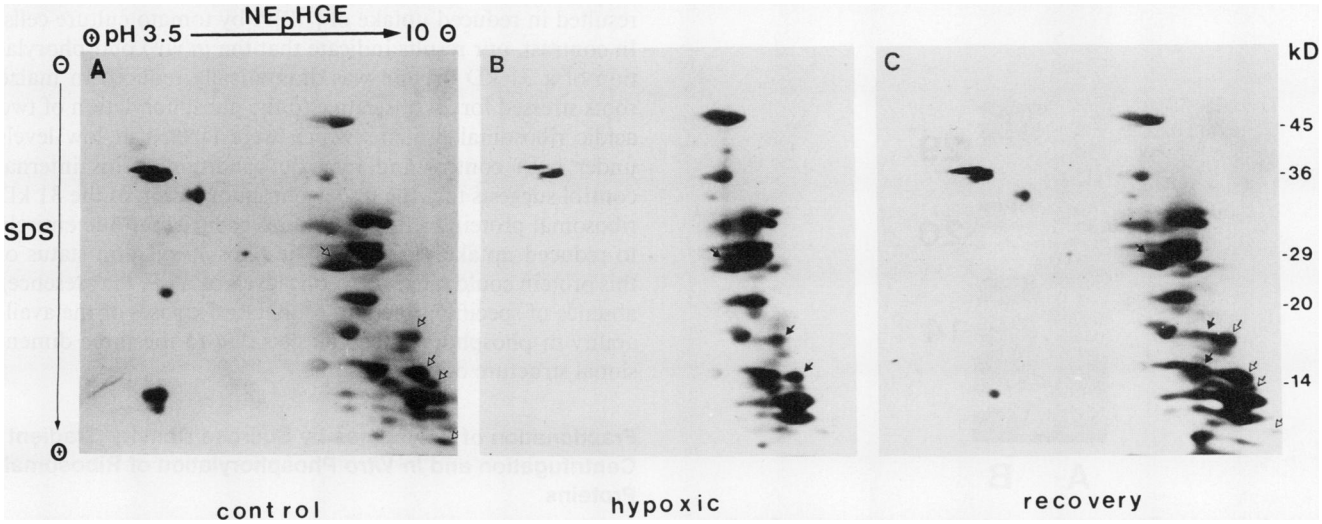

Figure 2. Silver-stained ribosomal proteins from control and hypoxic roots separated by two-dimensional, NEpHGE/SDS-PAGE. Polysomes were isolated from roots of seedlings grown in air (A), submerged in argon for 24 h (B), or submerged in argon for 4 h and allowed to recover in air for 2 h (C). RNA was removed from the polysome pellets and ribosomal proteins were fractionated in pH 3.5 to 10 NEpHGE gels in the first dimension, and in 15% (w/v) polyacrylamide-SDS gels in the second dimension.

reflect the observed difference in polyribosome/monoribosome ratios, or demonstrate condition specific characteristics of ribosomal proteins. Not surprisingly, NEpHGE analysis of polysomes isolated from roots which were allowed to recover briefly from anaerobic stress revealed a mixture of the basic ribosomal proteins observed in control and stressed roots (Fig. 2C).

In Vivo Synthesis of Ribosomal Proteins

Sachs *et al.* (18) characterized the proteins synthesized by seedling roots during hypoxia using two-dimensional, native/SDS-PAGE. Such electrophoretic conditions are inappropriate for separation of ribosomal proteins since highly basic polypeptides do not migrate into native gels. Highly basic proteins can be resolved by one-dimensional SDS-PAGE. Therefore, SDS-PAGE was used to determine whether ribosomal proteins are synthesized by seedling roots during hypoxic stress. Five-d-old seedling roots were labeled *in vivo* with [^{35}S]Met under normal and hypoxic conditions. Polysomes were purified and proteins were separated by SDS-PAGE and fluorographed (Fig. 3). We observed that the synthesis of polysomal proteins during hypoxia was reduced 10-fold, as determined by the incorporation of [^{35}S]Met into TCA insoluble polypeptides. A similar reduction in the synthesis of soluble proteins during hypoxia was also measured. The labeled proteins that were detected were similar in size to ribosomal proteins (5), in contrast to the soluble anaerobic proteins described previously which ranged in size from 87 to 31.5 kD (18). In comparing the *in vivo* synthesized polysomal proteins of hypoxic (Fig. 3A) and aerobic (Fig. 3B) roots, we observed that more proteins were labeled under control than under stress conditions, and that the proteins labeled under the two conditions were of different apparent molecular weights.

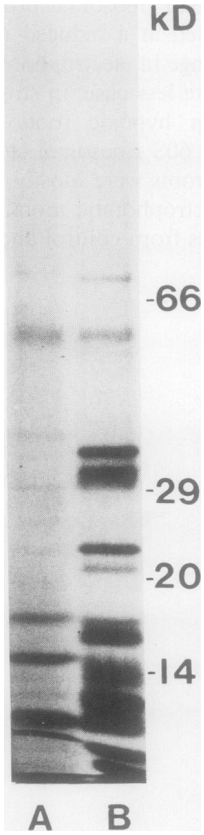

Figure 3. *In vivo* [^{35}S]Met labeling of polysomal proteins. Seedling roots were labeled with [^{35}S]Met (150 μCi/mL) for 3 h under the following conditions: hypoxic after 24 h of preinduction (A), control (B). Polysomes were isolated, separated by 15% SDS-PAGE, and visualized by fluorography.

In Vivo Phosphorylated Ribosomal Proteins

The phosphorylation of certain ribosomal proteins occurs under normal growth conditions in all eukaryotic organisms that have been examined (20). The *in vivo* phosphorylation of maize ribosomal proteins was examined to further characterize differences between the translational machinery of control and hypoxic roots. Intact seedling roots were labeled *in vivo* with [^{32}P]Pi for 3 h, and polysomes were isolated. A fourfold reduction in the incorporation of [^{32}P]Pi into RNA-free polysomal protein, and an eightfold reduction of [^{32}P]Pi into polysomal RNA were observed. Polysomal proteins were separated by two-dimensional, NEpHGE/SDS-PAGE and visualized by fluorography (Fig. 4). Under normal, aerobic conditions the major phosphorylated protein observed was a basic, 31 kD polypeptide (Fig. 4A). The phosphorylation of this protein was dramatically reduced in seedling roots which had been stressed for 24 h (Fig. 4B). Two acidic proteins of 36 and 10 kD were phosphorylated at very low levels in both control and hypoxic roots during the 3 h labeling period.

Scharf and Nover (19) observed previously that, in tomato culture cells, a 30 kD 40S ribosomal subunit protein is rapidly dephosphorylated in response to heat shock. Two hours of anaerobic, osmotic or salt stress, reportedly, had no effect on the phosphorylation of the 30 kD protein. These stresses resulted in reduced uptake of [^{32}P]Pi by tomato culture cells. In contrast, our results indicate that the *in vivo* phosphorylation of a 31 kD protein was dramatically reduced in maize roots stressed for 24 h, relative to the phosphorylation of two acidic ribosomal proteins which were labeled at low levels under both control and hypoxic conditions. This internal control suggests that the under-phosphorylation of the 31 kD ribosomal protein in hypoxic maize roots is not due entirely to reduced uptake of [^{32}P]Pi. The phosphorylation status of this protein could reflect cytosolic levels of ATP, the presence/absence of specific protein phosphatases/kinases or the availability of phosphoryl acceptor sites due to the three dimensional structure of the ribosome.

Fractionation of Polysomes by Sucrose Density Gradient Centrifugation and *In Vitro* Phosphorylation of Ribosomal Proteins

In vitro phosphorylation of ribosomal proteins by kinases which copurify with polysomes has been observed previously (3). *In vitro* phosphorylation of polysomal proteins was carried out to examine differences between ribosomal subunits, mon-

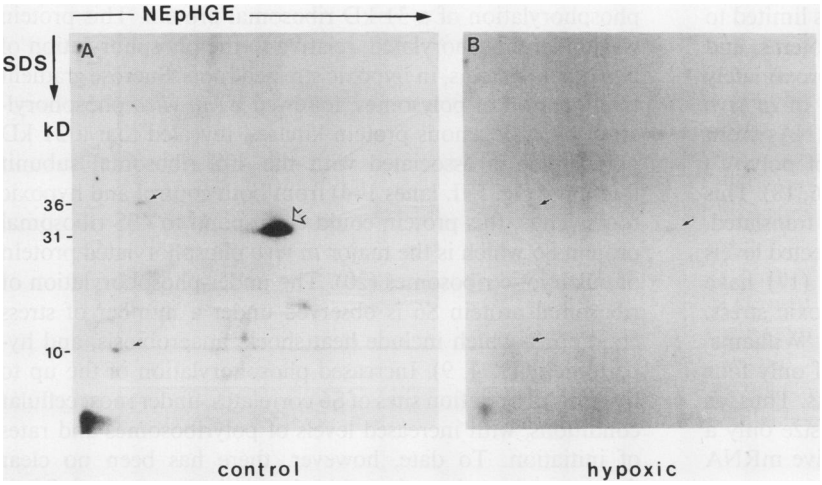

Figure 4. *In vivo* phosphorylation of ribosomal proteins with [^{32}P]Pi. Seedling roots were *in vivo* labeled with [^{32}P]Pi (90 μCi/mL) for 3 h under the following conditions: control (A), hypoxic after 24 h of preinduction (B). Polysomes were isolated and proteins were separated by two-dimensional, NEpHGE/SDS-PAGE as in Figure 2 and visualized by fluorography.

oribosomes and polyribosomes from control and hypoxic roots. In this *in vitro* assay, the pattern of protein phosphorylation relates to ribosome structure, presence of phosphoryl group acceptor sites, endogenous kinase activities, or a combination of these factors. Polysomal pellets were isolated from control and hypoxic treated roots, separated by sucrose density gradient centrifugation and collected in 14 fractions. Endogenous protein kinases in each fraction were activated by addition of kinase buffer and γ-[^{32}P]ATP. The proteins were separated by SDS-PAGE and visualized by silver-staining and fluorography.

Figure 5 shows the A_{260} nm profiles of the polysome pellets fractionated on sucrose gradients (I) and fluorographs of the *in vitro* phosphorylated proteins separated by SDS-PAGE (II). We observed condition specific differences in the *in vitro* kinased proteins of ribosomal subunits, monoribosomes, and polyribosomes of both control and hypoxic roots. For example, 21.5 and 15 kD phosphoproteins were visible in polyribosome fractions (7–14) from control roots and not detected in polyribosome fractions from hypoxic roots. In contrast, 25, 24, 23, and 20 kD phosphoproteins appeared to be specific to hypoxic polysomes. An 18.5 kD phosphoprotein was detected in control monoribosome fractions, as well as hypoxic monoribosome and polyribosome fractions. The polyribosome fractions of hypoxic roots contained certain proteins which increased in abundance with polyribosome size (31.5, 25, 24, and 18.5 kD) and others which decreased in abundance with polyribosome size (35 and 31 kD). With the exception of a 16 kD protein, the phosphoproteins corresponded to ribosomal proteins which were visible on the silver-stained gel (not shown).

DISCUSSION

Hypoxic stress of maize roots is known to cause a rapid shutdown of normal protein synthesis. Sachs *et al.* (18) dem-

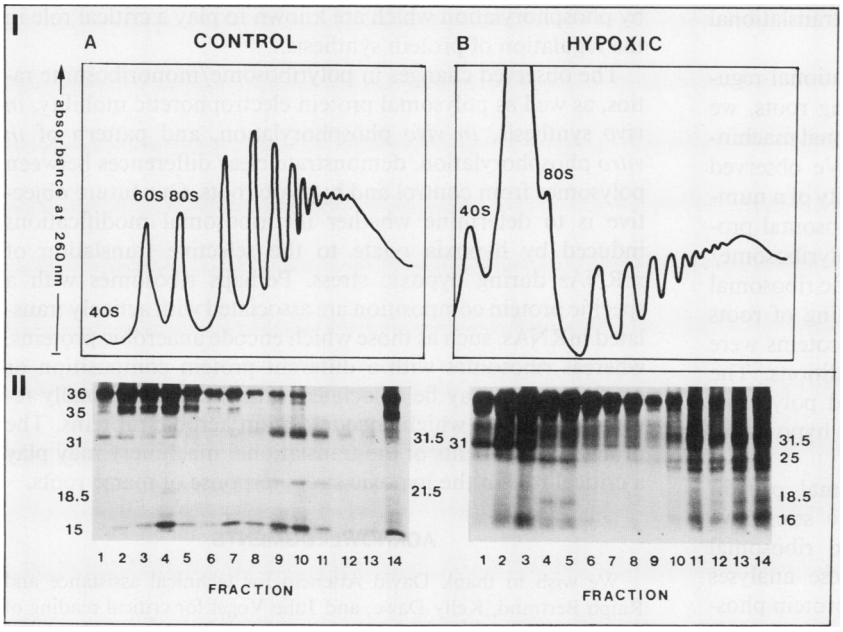

Figure 5. Separation of polysomes on sucrose density gradients followed by *in vitro* phosphorylation of ribosomal proteins. Polysomes from control (A) and 24 h hypoxic (B) roots were prepelleted through sucrose cushions for 18 h, and separated by centrifugation on continuous 15 to 65% (w/v) sucrose gradients. The gradients were analyzed as in Figure 1 and fractionated. Absorbance 260 nm profiles (I). Fluorographs of *in vitro* phosphorylated proteins separated by SDS-PAGE (II). The apparent mol wt of the phosphorylated proteins are indicated.

onstrated that after 1 h of stress protein synthesis is limited to a group of 33 kD polypeptides, the transition proteins, and after 5 h of stress protein synthesis is limited to approximately 20 proteins, the anaerobic proteins. Comparison of *in vivo* protein synthesis and *in vitro* translation of mRNAs from hypoxic stressed roots revealed that a number of poly(A^+) mRNAs are not translated during hypoxic stress (16, 18). This indicates that certain mRNAs are selectively translated, whereas others are stable but not translated at expected levels under anaerobic conditions. Russell and Sachs (17) have examined the response of soybean roots to hypoxic stress. Their results indicate that anoxia on roots of the 'Williams' variety of soybean results in selective synthesis of only four polypeptides from a large pool of poly(A^+) mRNAs. Thus, as compared to maize, anoxic soybean roots synthesize only a subset of the anaerobic proteins, but show selective mRNA accumulation and translation.

Our analysis of polysome profiles revealed that maize seedling roots exposed to hypoxic stress rapidly accumulate monoribosomes and ribosome subunits, concomitant with a decrease in levels of polyribosomes. Lin and Key (11) reported that hypoxic stress and dinitrophenol treatment cause rapid disaggregation of polyribosomes and accumulation of monoribosomes in soybean cell cultures. They determined that the loss of polyribosomes did not occur when cell cultures were treated with cycloheximide which inhibits elongation of protein synthesis. In addition, they observed that polyribosome levels increase once the stress is removed, even when RNA synthesis is inhibited. We observed a rapid increase (within 1 h) in monoribosome and ribosomal subunit levels in hypoxic stressed maize roots. This accumulation of monosomes could result from impairment of translation at the level of initiation or elongation. The accumulation of small polyribosomes (disomes, trisomes), which occurs after prolonged periods of hypoxic stress (24 h), could reflect inhibition or reduced rates of elongation of translation of certain mRNAs. An increase in monoribosomes and decrease in polyribosome levels observed in response to heat shock in *Drosophila* (12) and drought stress in soybean (13) is indicative of the translational regulation of these stresses.

As a first step in the elucidation of the translational regulation of the hypoxic response of maize seedling roots, we compared the protein component of the translational machinery from control and hypoxic stressed roots. We observed differences in quantity and electrophoretic mobility of a number of ribosomal proteins. The variations in ribosomal proteins could reflect the observed differences in polyribosome/monoribosomes ratios, as well as condition specific ribosomal proteins or protein modifications. *In vivo* labeling of roots with [^{35}S]Met revealed that newly synthesized proteins were incorporated into polysomes under stress conditions. The observed differences between the *in vivo* labeled polysomal proteins of control and hypoxic roots indicate hypoxia-induced changes in ribosome composition.

To address whether modification of ribosomal protein phosphorylation occurs in response to hypoxic stress, we compared *in vivo* and *in vitro* phosphorylated ribosomal proteins from control and hypoxic roots. These analyses revealed hypoxia-induced changes in ribosomal protein phosphorylation. A dramatic change was detected in the *in vivo* phosphorylation of a 31 kD ribosomal protein. This protein was underphosphorylated, relative to the phosphorylation of two other proteins, in hypoxic stressed roots. Sucrose gradient fractionation of polysomes, followed by *in vitro* phosphorylation by endogenous protein kinases, revealed that a 31 kD polypeptide is associated with the 40S ribosomal subunit fractions (Fig. 5 II, lanes 1–4) from both control and hypoxic roots. Thus, this protein could correspond to 40S ribosomal protein S6 which is the major *in vivo* phosphorylated protein of eukaryotic ribosomes (20). The under-phosphorylation of ribosomal protein S6 is observed under a number of stress conditions, which include heat shock, anaerobiosis, and hypertonicity (1, 4, 9). Increased phosphorylation of the up to five phosphorylation sites of S6 correlates, under most cellular conditions, with increased levels of polyribosomes and rates of initiation. To date, however, there has been no clear demonstration that the phosphorylation status of S6 is causally related to the rate of translation initiation or protein synthesis (20). The under-phosphorylation of a 31 kD, 40S ribosomal protein may reflect some level of translational control in hypoxic maize seedling roots.

An *in vitro* assay of endogenous ribosomal kinases and phosphoryl acceptor sites was used to probe for differences in polysomes from control and hypoxic roots. This analysis revealed condition specific differences in the pattern of *in vitro* phosphorylated proteins of ribosomal subunits, monoribosomes, and polyribosomes. Polyribosomes from control and hypoxic roots showed both quantitative and qualitative differences in their pattern of protein phosphorylation. In addition, the phosphoproteins of the polyribosome fractions from hypoxic roots were heterogeneous. Unlike the polyribosomes of control roots, the levels of certain phosphoproteins of hypoxic roots increased with polyribosome size, whereas the levels of others decreased. The variations in ribosomal protein phosphorylation could be due to condition specific differences in ribosome conformation, protein composition, and/or endogenous kinase activity. These differences might also reflect the dynamic, covalent modifications of proteins by phosphorylation which are known to play a critical role in the regulation of protein synthesis.

The observed changes in polyribosome/monoribosome ratios, as well as polysomal protein electrophoretic mobility, *in vivo* synthesis, *in vivo* phosphorylation, and pattern of *in vitro* phosphorylation, demonstrate clear differences between polysomes from control and hypoxic roots. Our future objective is to determine whether the ribosomal modifications induced by hypoxia relate to the selective translation of mRNAs during hypoxic stress. Perhaps ribosomes with a specific protein composition are associated with actively translated mRNAs, such as those which encode anaerobic proteins, whereas ribosomes with a different protein composition or conformation may be associated with the translationally repressed mRNAs which encode certain aerobic proteins. The protein components of the translational machinery may play a critical role in the hypoxic stress response of maize roots.

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