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# Post-Transcriptional Control of Sucrose Synthase Expression in Anaerobic Seedlings of Maize[1]

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## ABSTRACT

**We have examined post-transcriptional control of expression of the anaerobically induced sucrose synthase 1 (SS1) isozyme mRNA encoded by the *shrunken* (*Sh*) gene of maize (*Zea mays* L.). The SS1 transcript level is increased in maize seedling roots during anaerobiosis without a concomitant increase in the SS1 protein level. We show that the anaerobic SS1 RNA was loaded onto polyribosomes and that SS1 proteins produced by *in vitro* translation of polyribosomal RNA from anaerobic roots and immature kernels were indistinguishable based on abundance and apparent molecular weight. [$^{35}$S]Methionine uptake in control and anaerobically stressed seedling roots indicated a detectable, but only slight, increase in radiolabel in the SS1 polypeptide as compared to the sucrose synthase 2 isozyme, SS2. However, this slight increase in [$^{35}$S]methionine uptake did not contribute to a detectable increase in the steady state level of SS1 protein relative to SS2 protein. Chase experiments with unlabeled methionine indicated that SS1 protein was relatively stable in the anaerobic environment. From these results we conclude that SS1 protein was not rapidly turned over in the anaerobic environment and that expression of anaerobically induced SS1 transcripts was blocked at some step beyond polyribosomal loading.**

In maize two sucrose synthase isozymes, SS1 and SS2, are encoded by the *Sh* and *Sus* genes, respectively (3, 6). The *shrunken* (*sh*) mutation on chromosome 9 is associated with a complete loss of the SS1 protein and approximately 40% reduction in endosperm starch content (3, 6). No other phenotype is associated with the *sh* mutation. The *Sh* gene is highly expressed in the developing endosperm resulting in abundant levels of both the SS1 transcript and the SS1 protein (3). The *Sus* encoded SS2 isozyme is detectable in many parts of the plant including the embryo and endosperm (5). The two isozymes are biochemically similar and share immunological cross-reactivity (3, 8).

Biochemical and genetic evidence indicates that maize SS[2] protein is composed of four subunits (3, 8, 25). In maize roots and shoots, where both genes are expressed simultaneously at approximately equal levels, subunits of SS1 and SS2 proteins copolymerize to yield a total of five isozymes comprised of two homotetrameric and three heterotetrameric forms (5). The *sh* mutant lacking the SS1 protein and a *Sus*-null mutant lacking the SS2 protein contain only the SS1 or SS2 homotetramers, respectively (4).

The anaerobic induction of the *Sh* gene at the transcript levels was first shown by Springer *et al.* (23). These data were later confirmed and extended to indicate that the SS1 protein is present during anaerobiosis (19, 20, 24). There is no increase in the level of SS2 transcripts during anaerobiosis. In a previous report we have shown (20) that the anaerobic induction of the *Sh* gene is limited to the SS1 RNA level as no increase in SS1 protein is detected. The SS proteins are detectable at nearly the same levels in anaerobically stressed seedlings as in the control. In the present communication we have further analyzed molecular events relating to the subsequent fate of induced SS1 transcripts and show that post-transcriptional control, specifically beyond polyribosomal loading, is evident in the expression of the *Sh* gene in anaerobically stressed seedlings.

## MATERIALS AND METHODS

### Maize Stocks

All maize (*Zea mays* L.) lines used in this study were Pioneer inbred lines displaying the wild type or nonshrunken phenotype designated Pio 30055 and Pio 3165. Seeds were germinated in flats of vermiculite and grown for 4 to 7 d in a lighted growth chamber. Seedlings to be used for anaerobic treatment were removed from the vermiculite and submerged for 20 h in 5 mM Tris-HCl buffer (pH 7.5). Following anaerobic treatment the roots were frozen in liquid nitrogen and their RNA was isolated. RNA was isolated from kernels 16 d after pollination.

### RNA Analysis

Total RNA was isolated by the method of Chirgwin *et al.* (2). Free and membrane bound polyribosomal RNAs were isolated as described in Jackson and Larkins (15). Total polyribosomal RNA was isolated by a method slightly modified from Jackson and Larkins. Briefly, 15 g of tissue was ground in a coffee mill in the presence of dry ice. Thirty mL of grinding buffer (0.25 M sucrose, 0.2 M Tris-HCl [pH 9], 0.05 M $Na_2EGTA$, 0.06 M magnesium acetate, 0.4 M KCl, and 1.0% Triton-X 100) was added to the tissue. The slurry was

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[2] Abbreviation: SS, sucrose synthase.

strained through cheese cloth and centrifuged at 35,000*g*. The supernatant solution was loaded onto a 5 mL sucrose cushion (1.5 M sucrose, 0.005 M $Na_2EGTA$, 0.04 M Tris-HCl [pH 9], 0.03 M magnesium acetate, 0.1 M KCl) and 2.5 mls of 0.5 M sucrose buffered as above. The polyribosomes were pelleted at 28,000 rpm in an SW28 rotor. The pellets were resuspended in 0.5 M SDS, 1.0 mM Tris-HCl (pH 8), and 0.1 mM $Na_2EDTA$ and were extracted with phenol:chloroform:isoamyl alcohol (24:24:1) to remove the proteins. The RNA solution was adjusted to 1 M ammonium acetate, and the RNA was precipitated with 2.2 volumes absolute ethanol. The RNA was pelleted by centrifugation, resuspended in sterile distilled water, and quantified by spectrophotometry (18).

RNA blots were made by separating the RNA on a formaldehyde-agarose gel (10) and transferring the RNA to nitrocellulose membrane (11, 26). The blots were hybridized in 50% formamide (BRL), 5 × SSC, 5× Denhardt's solution. 0.1% SDS, 0.1 M phosphate buffer (pH 6.5), and 250 μg/mL sheared denatured calf thymus DNA at 42°C for 48 h. The RNA blots were rinsed at 68°C in 0.3 × SSC, 0.1% SDS, 5 mM $Na_2EDTA$. These conditions eliminate cross-hybridization of the SS1 cDNA with the SS2 transcript (data not shown). The DNA source of the radiolabeled probe was gel purified EcoR1 fragments of an SS1 cDNA clone or *Adh*1 cDNA clone (18). A nick translation kit (BRL) was employed using [$^{32}$P]dCTP following the manufacturer's instructions and the activity was typically about $10^8$ cpm/μg of DNA.

### Protein Analysis

*In vitro* translations were done using rabbit reticulocyte lysates obtained from BRL. The reaction was brought up to 500 μL in immunoprecipitation buffer (0.1 M phosphate buffer [pH 7.2], 0.15 M NaCl, 0.5% Triton X-100) and the TCA-precipitable cpm in 10 μL was determined (8). Forty μL of 10% TCA was added to 10 μL of the labeled protein solution to precipitate the proteins. The precipitate was collected on a glass filter and rinsed with vacuum filtration with 3 mL of 10% TCA. The filters were rinsed in absolute ethanol and dried overnight, and the radioactivity was quantified in a scintillation counter.

Total anaerobic proteins were labeled with [$^{35}$S]Met by submerging 3 to 5 seedlings in 45 mL of 5.0 mM Tris-HCl (pH 7.5) to initiate anaerobiosis and adding 1.0 mCi of [$^{35}$S] Met for the required labeling period (23) [$^{35}$S]Met was chased out of the labeled protein fraction with the addition of a 1000-fold molar excess of cold methionine. In all cases total anaerobic treatment was for 20 h. Total soluble proteins were isolated in immunoprecipitation buffer and the radioactivity was quantified as above.

Immunoprecipitation of SS1 and SS2 proteins was done using polyclonal antibodies directed against SS1 by standard methods (7, 8). SS1 and SS2 immunoprecipitation was determined to be quantitative by finding virtually no SS1 or SS2 protein remaining in the extracts upon a second precipitation attempt. SDS-PAGE was done by the method of Laemmli (17). Immunoblot analysis was done as described previously (5); primary antisera in these analyses were raised against purified SS1 protein (19). Fluorography was done by soaking the gel in 1.5 M sodium salicylate for 30 min, and exposing the dried gel to x-ray film. Autoradiography of proteins was performed by transferring the proteins to nitrocellulose paper and exposing the blot to x-ray film.

## RESULTS

### Polyribosomal Loading of SS1-transcripts

RNA blots of total mRNA and free and membrane-bound polyribosomal RNA from anaerobically induced and uninduced maize roots were hybridized with radiolabeled SS1 cDNA or *Adh*1 cDNA to determine if the homologous transcripts were loaded onto polyribosomes. Figure 1 shows the autoradiograph of these blots. The increase in the intensity of hybridization of the probes to mRNA from anaerobic roots showed that there was a significant induction of the SS1 and *Adh*1 mRNAs upon anaerobic stress in the total root mRNA as has been previously reported (Fig. 1, lanes 1 and 4). The probes also hybridized more intensely to the free and membrane bound polyribosomal mRNA reflecting an enrichment of SS1 and *Adh*1 transcripts in both the free and membrane bound polyribosomal RNA fractions from anaerobic roots (Fig. 1, lanes 2, 3, 5, and 6). The elevation in the abundance of the *Adh*1 transcript served as a control to confirm that anaerobic induction occurred in these samples. The greater abundance of *Adh*1 transcripts and SS1 transcripts in the polyribosomal fractions of RNA than in total RNA indicated that the majority of these mRNAs was loaded onto polyribosomes.

### *In Vitro* Translation of Polyribosomal RNA

To determine if the polyribosomal SS1 RNA was capable of directing *in vitro* translation, total polyribosomal RNA was isolated from maize kernels, anaerobically induced roots, and aerobic roots. Immature kernel mRNA was included as a control because this tissue has an abundant level of SS1 protein (3). RNA blot analysis of equal amounts of these RNAs showed that the SS1 mRNA level found in the kernels and the anaerobically induced roots were significantly greater than that found in the aerobic roots (Fig. 2A). These same RNAs were used to direct protein synthesis *in vitro* in the

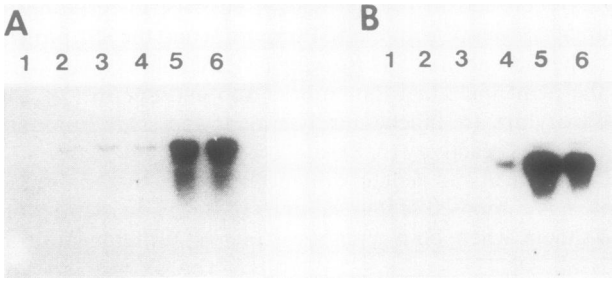


**Figure 1.** RNA blot analysis of RNA from aerobic (lanes 1–3) and anaerobically induced maize root (lanes 4–6). Four μg of total RNA (lane 1 and 4), of free polysomal RNA (lanes 2 and 5), and of membrane-bound polysomal RNA (lanes 3 and 6) were size-fractionated and transferred to nitrocellulose paper. Duplicate blots were hybridized with radiolabeled SS1 cDNA or *Adh* 1-cDNA (A and B, respectively). The blots were exposed to x-ray film (Kodak) for 12 h.

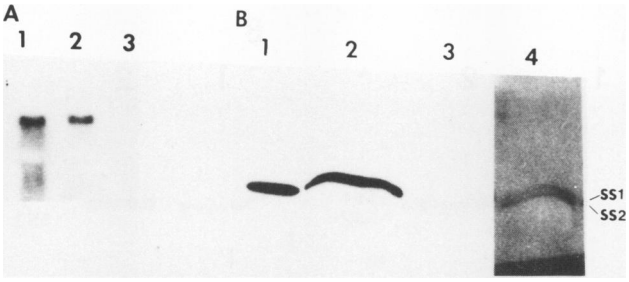


**Figure 2.** RNA blot analysis of maize total polysomal RNAs (A) and analysis of SS1 and SS2 proteins translated *in vitro* from these same RNAs (B). Total polyribosomal RNA isolated from kernels (lane 1), anaerobically induced roots (lane 2), and aerobic roots (lane 3) was analyzed by Northern blot analysis using a radiolabeled SS1 cDNA as described in Figure 1 (A). These RNAs were also translated *in vitro* in the presence of [$^{35}$S]Met. SS1 and SS2 proteins were immunoprecipitated from 8000 cpm of the translation products, separated by SDS-PAGE, transferred to nitrocellulose paper, and fluorographed (B). The gels were exposed to x-ray film for 3 d to make the fluorographs except for lane 4 which is the same as lane 3, but exposed to x-ray film for 14 d.

presence of [$^{35}$S]Met and SS proteins were immunoprecipitated from equivalent cpm of radiolabeled protein. Figure 2B shows the fluorographs of SS1 and SS2 proteins after fractionation by SDS-PAGE. The SS1 protein band was easily visualized in the lanes containing translation products of RNA from anaerobic roots and immature kernels (lanes 1 and 2), but not in aerobic root RNA (lane 3). A longer duration of exposure of lanes 3 (Fig. 2B) did reveal distinct SS1 and SS2 protein bands (Fig. 2B, lane 4). A longer duration of exposure of lanes 1 and 2 (Fig. 2B) also revealed a distinct SS2 protein band approximately equal in intensity to the SS2 protein band shown in Figure 2B, lane 4 (data not shown). The SS2 band was identified by its comigration with the SS2 protein band from *in vitro* translations of RNA from a *Sh* deletion mutant (data not shown). Additional evidence to conclude that the protein band in lane 2 is due solely to SS1 protein is based on the fact that the anaerobic roots show elevated levels of only the SS1 transcript; no detectable increase of SS2 transcripts is seen (19, 20, 24). Comparisons of intensities of the SS1 and SS2 protein bands showed that radioactive SS1 protein translation product was far more abundant in *in vitro* translations of immature kernel and anaerobically stressed root RNA than in the aerobic root RNA. These comparisons also showed that polyribosomal RNA from anaerobically induced root and from kernels directed equivalent amounts of SS1 synthesis as a fraction of radiolabel incorporated into total protein.

The intensities of the SS1 and SS2 protein bands representing the *in vitro* translations of control root RNA indicated these proteins incorporated nearly the same amount of radiolabel (Fig. 2B, lane 4). An equal level of *in vivo* expression of these two genes in maize seedling roots has been described previously (5). The SS proteins translated from anaerobically stressed root and kernel RNAs did not differ significantly in apparent mol wt and reacted with SS1 antibody. We concluded from these data that SS1 transcripts were translated *in vitro* in proportion to their relative abundance and that the majority of the SS1 transcripts from anaerobic roots were indistinguishable from the kernel SS1 transcripts by the criteria used here.

### *In Vivo* Labeling of Maize Root Proteins

The observation that the polyribosomal SS1 transcripts were translatable *in vitro* led us to investigate *in vivo* synthesis and stability of SS1 and SS2 proteins. We radiolabeled anaerobically stressed seedlings with $^{35}$S-Met for 1 and 4 h and aerobic seedlings for 0.5 and 1 h. One thousand cpm of total labeled anaerobic proteins (Fig. 3A, lanes 1 and 2) and 5,000 cpm of total labeled aerobic proteins (Fig. 3A, lanes 3 and 4) were size fractionated and visualized by autoradiography. The distribution of the TCA precipitable radiolabel among a finite number of protein bands on the autoradiograph showed that $^{35}$S-Met was incorporated into total proteins under both labeling conditions. The banding pattern is similar between 1 and 4 h anaerobic pulses and between 0.5 and 1 h aerobic pulses (Fig. 3A). The nearly equal intensities of bands representing size fractionated anaerobic proteins confirmed that the quantification of TCA precipitable counts was correct (Fig. 3A). The SS proteins were immunoprecipitated from 8,000 cpm of total labeled anaerobic protein and from 40,000 cpm of total aerobic protein to examine the accumulation of $^{35}$S-Met in SS1 and SS2 polypeptides with increasing pulse times. These values were determined in previous experiments to be adequate for subsequent visualization of SS1 and SS2 protein. The proteins were size fractionated, transferred to nitrocellulose paper, and visualized first by autoradiography and later by immunostaining. The autoradiograph is shown in Figure 3B, lanes 1 to 4. Because the proteins were immunoprecipitated from equal cpm of either anaerobically or aerobically labeled proteins, changes in the SS band intensities with increasing duration of $^{35}$S-Met pulse reflected changes in

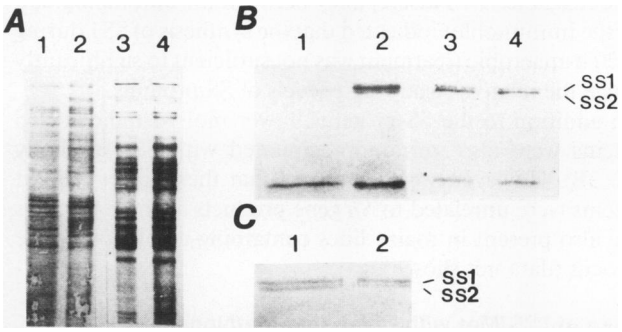


**Figure 3.** Uptake of [$^{35}$S]Met in anaerobically stressed (lanes 1 and 2) and aerobic maize roots (lanes 3 and 4). One thousand cpm of total protein labeled for 1 or 4 h under anaerobic conditions (lanes 1 and 2, respectively) and 5000 cpm of total protein labeled for 0.5 or 1 h under aerobic conditions (lanes 3 and 4, respectively) was separated by SDS-PAGE, transferred to nitrocellulose paper and autoradiographed for 14 d (A, lanes 1 and 2) or 2 d (A, lanes 3 and 4) to visualize the 87 kD proteins. The SS1 and SS2 proteins immunoprecipitated from eight times the cpm of radiolabeled proteins used in A were separated by SDS-PAGE, transferred to nitrocellulose paper, and autoradiographed as above (B). The blot used to make the autoradiograph in B, lanes 1 and 2, was immunostained with SS1 antibody (C).

radiolabeled SS1 and SS2 as a fraction of labeled total protein. The intensity of the SS1 and SS2 protein bands labelled under anaerobic conditions increased during the 1 and 4 h pulse (Fig. 3B, lanes 1 and 2). This increase continued through at least an 8 h pulse (data not shown). Thus SS1 and SS2 proteins contained an increasing fraction of the total incorporated $^{35}$S-Met with increasing pulse time. Conversely, the intensity of the SS bands remained constant in the lanes of the autoradiograph containing the 0.5 and 1 h aerobic pulse samples indicating that SS1 and SS2 protein remained a constant fraction of the total labeled aerobic protein with increasing pulse time (Fig. 3B, lanes 3 and 4). Comparison of the intensities of the SS1 and SS2 bands on the autoradiograph indicated that the relative proportion of radiolabel in anaerobic root extracts was higher in the SS1 polypeptide than in the SS2 polypeptide (Fig. 3B, lanes 1 and 2). By comparing the intensities of the SS1 and SS2 bands from 10,000 cpm, 5,000 cpm, and 2,500 cpm of total radiolabeled anaerobic protein we estimated that SS1 proteins had incorporated at most three times more $^{35}$S-Met than the SS2 protein (data not shown). Furthermore, a comparison of the intensities of the SS1 and SS2 bands on the autoradiograph indicated that the relative proportion of radiolabeled SS1 polypeptide in aerobic root extracts was lower than the SS2 polypeptide (Fig. 3B, lanes 3 and 4).

We immunostained the same blot used to make the autoradiograph in Figure 3B, lane 1 and 2, with SS1 antibody (Fig. 3C, lanes 1 and 2). Bands representing SS1 and SS2 proteins were present in both lanes indicating that SS1 and SS2 protein were successfully immunoprecipitated even in the sample where no radiolabeled SS1 protein was detected (as in Fig. 3B, lane 1). Interestingly the elevated synthesis of SS1 relative to SS2 was not significantly reflected in the steady state level of SS1 and SS2 proteins (Fig. 3C, lanes 1 and 2) as no major difference in staining intensities of the two bands was detectable. This discrepancy between the autoradiograph and the immunoblot indicated that the synthesis of SS1 during the 20 h anaerobic treatment was not sufficient to significantly change the relative steady state levels of SS proteins.

In addition to the SS proteins. lower mol wt radiolabeled proteins were also immunoprecipitated with SS1 antibody (Fig. 3B). However, we determined that these lower mol wt proteins were unrelated to *Sh* gene products as these proteins were also present in maize lines containing a deletion of the *Sh* locus (data not shown).

### Chase of [$^{35}$S]Met with Unlabeled Methionine

The stability of labeled SS protein was further analyzed by labeling anaerobic proteins for 8 h with [$^{35}$S]Met and chasing for 8 h with cold methionine. Total radiolabeled proteins (1000 cpm) from the anaerobic pulse and chase was fractionated by an SDS-PAGE and transferred to nitrocellulose paper. Autoradiography was done (data not shown) to determine that approximately equal cpm were loaded on the gel and used in subsequent immunoprecipitations. The nitrocellulose blot was immunostained to determine the relative steady state of SS1 and SS2 proteins in 1000 cpm of anaerobic root proteins after a 0 and 8 h chase (Fig. 4A, lanes 1 and 2). The amount of total SS1 and SS2 polypeptides became more

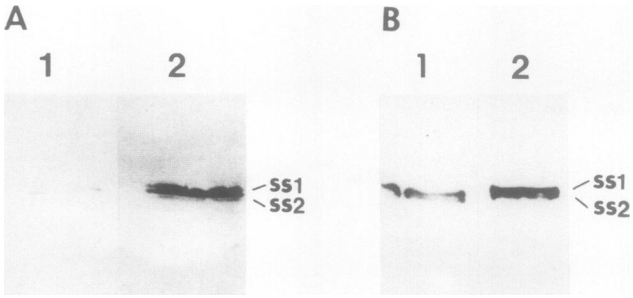


**Figure 4.** Stability of SS1 protein in anaerobically stressed maize roots. Maize roots were labeled for 8 h with [$^{35}$S]Met and chased for 0 or 8 h with unlabeled methionine. One thousand cpm of radiolabeled proteins from the 0 h and 8 h anaerobic chase were separated by SDS-PAGE, transferred to nitrocellulose paper, and immunostained with SS1 antibody (A, lanes 1 and 2, respectively). SS1 and SS2 protein immunoprecipitated from 8000 cpm of total labeled protein from the 0 h chase and the 8 h chase was separated by SDS-PAGE, transferred to nitrocellulose paper, and autoradiographed (B, lanes 1 and 2, respectively). The blots were exposed to x-ray film for 10 d.

abundant in equal cpm of labeled protein with increasing chase time, presumably because more total protein was loaded with increasing chase time to load the same number of cpm. We concluded from these data that the addition of cold methionine to the pulse treatment was an effective chase. Autoradiographs of SS immunoprecipitated from 8000 cpm of radiolabeled protein from the pulse and chase is shown in Figure 4B. A slight increase in the intensity of the SS1 polypeptide after 8 h chase (lane 2) relative to 0 h chase (lane 1) suggested that the radiolabelled SS1 protein was relatively stable in the anaerobic environment. During the course of the chase, the relative intensity of SS1 and SS2 protein bands remained constant, suggesting that these two proteins were equally stable.

## DISCUSSION

In this communication we have analyzed the fate of anaerobically induced SS1 transcripts in maize seedling roots. We conclude the following: (a) the induced SS1 transcripts were loaded onto polyribosomes, (b) the induced transcripts were as translatable *in vitro* as transcripts from maize kernels, (c) the SS1 protein produced in the anaerobic environment was relatively stable.

Our data indicated that during anaerobiosis of maize seedlings a dramatically increased level of SS1 transcripts accumulated on polyribosomes. The SS1 RNA from anaerobically stressed roots was translated *in vitro* as efficiently as SS1 RNA from a source known to accumulate abundant amounts of SS1 protein, the kernel (3). The SS1 protein translated *in vitro* from these two RNAs was of the same apparent mol wt and cross-reacted with SS1 antibodies. *In vivo* labeling experiments indicated that SS1 and SS2 accumulated an increasing fraction of incorporated [$^{35}$S]Met during the 4 h anaerobic pulse. One interpretation of these results is that SS1 protein degradation was slower than its synthesis. After a 1 h anaerobic pulse radiolabeled SS1 protein was not detectable by autoradiography (Fig. 3B, lane 1), though other anaerobic

proteins were easily visualized (Fig. 3A, lane 1). This result is consistent with SS1 protein being synthesized more slowly than other anaerobic proteins. However, it is also possible that rapidly labeled proteins were unusually high in methionine. It is noteworthy that both SS1 and SS2 polypeptides contain only about 2% methionine residues (8). Increasing accumulation of [$^{35}$S]Met in SS proteins with increasing pulse time was specific to anaerobically stressed roots as [$^{35}$S]Met incorporation into SS proteins reached its steady state level during a 0.5 h aerobic pulse. Pulse-chase experiments did not yield any evidence to indicate that SS1 protein was less stable or more rapidly turned over than other anaerobic proteins or the SS2 protein. The fact that SS1 transcripts were loaded onto polyribosomes, were translatable *in vitro*, and that the SS1 protein was relatively stable suggests the failure to accumulate SS1 protein in proportion to the amount of SS1 transcripts is attributable to a markedly reduced translation of SS1 transcripts relative to other anaerobic RNAs. This translational control did not reflect a general decrease in translation since, for example, *Adh*1 transcripts are abundantly translated in anaerobically stressed maize roots (9, 20). The SS1 protein may not be the only inefficiently translated protein in anaerobically stressed roots. The level of aldolase mRNA has been reported to increase 10- to 20-fold in an anaerobically stressed maize roots, though only a 13% increase in the level of protein is detected (12, 16).

In a concurrent study we have compared the cellular localization of SS1 transcripts with SS1 protein by *in situ* hybridization and immunohistological reaction, respectively, in microtome sections of aerobic and anaerobically stressed roots. The data from these studies at the cellular level also supported the present observation that anaerobic induction of the *Sh* locus was manifested primarily at the transcript level. The root apex, however, showed a slight increase in SS1 protein upon anaerobic induction compared to aerobic controls (LJ Rowland, Y-C Chen, P Chourey, personal communication). It is possible that the slight *in vivo* increase of [$^{35}$S]Met incorporated into SS1 polypeptide relative to SS2 polypeptide may be related to the specific increase of SS1 protein observed in the root apex. No change in the steady state level of SS1 was detected, because the amount of protein in the root apex comprises a very small proportion of the protein contained in the entire root.

Several examples of translational control of gene expression in other eukaryotes are available in the literature. For example, β-glucuronidase protein varies as much as 12-fold between different mouse tissues whereas its RNA does not vary more than 3-fold (1). Translational control of ferritin expression in humans has been analyzed. Ferritin transcripts are stored in an untranslated ribonuclear pool and shifted to polyribosomes as the protein is needed. The 5′ untranslated leader sequence of ferritin mRNA confers the translational regulatory property to a heterologous gene in a transformation assay (13, 14). Similarly, it may be possible to identify regions in the well-characterized *Sh* gene (27) that are responsible for the translational control of SS1 transcripts during anaerobic stress. Another level of translational control is based on phosphorylation/dephosphorylation of a protein elongation factor 2, EF2, described in mammalian cells (21). Specifically, phosphorylated EF2 is completely inactive in translation but dephosphorylated EF2 regains activity (21). A similar mechanism is potentially operative in the differential translation of SS1 transcripts in immature kernel and anaerobically stressed seedlings.

It is possible to assign a putative role for the SS1 protein in energy deficient anaerobic roots as the enzyme provides an important link between hexose sugars, UDP-glucose and glycolysis. However, the functional significance of anaerobically induced SS1 transcripts which are, based on the data presented here, inefficiently translated is unclear. We speculate that the SS1 transcripts themselves could be the functional product of the *Sh* gene during anaerobic stress. One interesting possibility is that the primary role of the anaerobically induced SS1 RNA is to sequester ribosomes into nontranslational complexes. Binding the ribosomes in nontranslational complexes could reduce the rate of translation and energy demands in the anaerobic cell, and yet a pool of ribosomes would be available in case of a return to aerobic conditions.

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