

# Distribution of Cytosolic mRNAs Between Polysomal and Ribonucleoprotein Complex Fractions in Alfalfa Embryos<sup>1</sup>

## Stage-Specific Translational Repression of Storage Protein Synthesis during Early Somatic Embryo Development

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### ABSTRACT

Cell-free translational and northern blot analyses were used to examine the distribution of storage protein messages in the cytoplasmic polysomal and mRNA-protein complex (mRNP) fractions during development of somatic and zygotic embryos of alfalfa (*Medicago sativa* cv Rangelander RL-34). No special array of messages was identified in the mRNP fraction; however, some messages were selectively enriched in either the polysome or mRNP fractions, and their distribution pattern varied quantitatively during development of the embryos. During the earliest stages of somatic embryo development, storage protein messages already were present, but there was no detectable accumulation of the proteins. Selective enrichment of messages for the 11S, 7S, and 2S storage proteins occurred in the mRNP fraction during the globular, heart, and torpedo stages of somatic embryogenesis, but the distribution pattern was shifted toward the polysomal fraction at the beginning of cotyledon development. Thus, there was translational repression of storage protein synthesis at the early stage of somatic embryo development that was relieved later. During the cotyledonary development stages in the somatic and zygotic embryos, storage protein synthesis and distribution of the messages were similar in that these specific messages were predominantly in the polysomal fraction.

Control points in the regulation of protein synthesis in plants can be from the initial synthesis of mRNA and its processing to the final modification and targeting of the resultant protein. The synthesis of storage proteins is an integral event in seed development, and the expression of their genes is regulated primarily at the transcriptional level; in vegetative tissues, they are transcriptionally silent (5, 25).

In animal cells there is substantial evidence that mRNPs<sup>2</sup> are an intermediate stage of mRNA translation and represent mRNAs in transit from the nucleus to the polysomes (4). In animal embryo development the presence of untranslated mRNAs is a common observation. For example, during sea urchin development, a rapid synthesis of proteins occurs

immediately after fertilization, which may result from the unmasking of messages stored in repressed mRNPs (9). Stage-specific activation of different mRNA species is also common during early embryonic development in the surf clam (22). The presence and importance of mRNPs in plants has not been subjected to intensive study, although there is ample evidence for the presence of "stored" mRNAs in RNPs in the mature dry seed (10, 18, 20, 24). Their presence during embryogenesis is uncertain, although it has been reported that the mRNA for the  $\beta$  subunit of  $\beta$ -conglycinin increases several days before the accumulation of the protein (14). It is suggested in this case, however, that failure of the protein to accumulate is caused by its initial instability (23).

There are marked quantitative and qualitative differences in storage protein accumulation between zygotic and somatic embryos, including those of alfalfa (*Medicago sativa* cv Rangelander RL-34) (13). To understand the differential regulation of protein synthesis in these two embryo types, we have followed the events that take place during development. Here we relate the extent of storage protein synthesis to the distribution of their specific mRNAs in the polysomal and mRNP, nonpolysomal fractions of the cells of developing embryos. Evidence for a translational control of protein synthesis during the early stages of somatic embryogenesis is presented.

### MATERIALS AND METHODS

#### Somatic Embryo Production

A highly embryogenic clone of alfalfa (*Medicago sativa* cv Rangelander RL-34) isolated by Meijer and Brown (16) was used for the production of somatic embryos. Plants were grown under reduced light intensity (200  $\mu$ E) at 20°C under a 12/12-h light/dark cycle.

Embryogenic callus was induced from petioles grown on B-5h solid medium (11) containing 1 mg/L 2,4-D and 0.2 mg/L kinetin (1) at 25°C and 75  $\mu$ E on a 16/8-h light/dark cycle. After 2 to 4 weeks of growth on solid medium, calli were transferred to suspension culture (B5g medium containing 1 mg/L 2,4-D and 0.1 mg/L naphthaleneacetic acid) and allowed to grow for 7 to 10 d. The culture was sieved through a 500- $\mu$ m nylon mesh followed by a 200- $\mu$ m mesh. The cell suspension collected on 200- $\mu$ m mesh was plated on a solidified hormone-free medium, Boi2Y (6), for embryo develop-

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<sup>2</sup> Abbreviations: RNPs, ribonucleoprotein complexes; LMW protein, low mol wt protein.

ment (25°C, 75  $\mu$ E light intensity, 16/8-h light/dark cycle). Embryos at different developmental stages were collected for the experiments.

### Zygotic Embryos

Alfalfa plants were raised in a growth chamber at 25°C, 16/8-h light/dark cycle, 350  $\mu$ E light intensity, and 75% RH. Flowers were hand-pollinated, and developing embryos were collected as required. Developmental stages were identified on the basis of pod, seed, endosperm, and embryo characteristics (26).

### In Vivo Protein Labeling

Embryos at different developmental stages were labeled with 100  $\mu$ Ci [ $^3$ H]leucine (Dupont Canada Inc., Dorval, PQ) per 50 embryos for 4 h at room temperature. Unincorporated leucine was rinsed away, and the embryos were frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$  until extraction.

### Extraction of Storage Protein

Seed storage proteins were subjected to two sequential extractions (12). The S-1 fraction proteins were extracted with a low salt buffer (200 mM NaCl, 25 mM phosphate buffer [pH 7.0], 10  $\mu$ M leupeptin [Sigma Chemical Co., St. Louis, MO] and 1 mM PMSF [Sigma]). The pellet obtained after centrifugation was reextracted with a high salt buffer of similar composition except that the salt was increased to 1 M NaCl to obtain the S-2 fraction proteins. The 7S and 2S storage proteins were predominantly in the S-1 fraction, whereas the S-2 fraction contained the 11S protein.

Proteins were precipitated using 80% chilled acetone and solubilized in Laemmli buffer (15) before separation by SDS-PAGE. Gels were treated with Enhance (NEN, Lachine, PQ) and dried, and the labeled proteins were detected by fluorography using Kodak X-Omat film. Identification of the storage proteins on gels was according to the method of Krochko *et al.* (13).

### Isolation of Polysomes and Supernatant (mRNP fraction)

Polysomes were isolated according to the method of Bag (3) with slight modifications in the buffers used. Embryos were incubated in cycloheximide (50  $\mu$ g/mL) for 20 min at room temperature and then homogenized in lysis buffer containing 200 mM Tris·HCl (pH 8.5), 400 mM NaCl, 200 mM sucrose, 35 mM MgCl<sub>2</sub>, 25 mM EGTA, 200  $\mu$ g/mL heparin, 0.5% Nonidet P-40, and 50  $\mu$ g/mL cycloheximide. The heparin concentration was increased by a further 300  $\mu$ g/mL before polysomes were isolated by centrifugation of the 20,000g supernatant at 100,000g for 75 min at 4°C in a Beckman ultracentrifuge (Beckman Instruments, Palo Alto, CA) using a 75 Ti rotor. The 100,000g pellet and supernatant were considered as polysome and nonpolysome, or mRNP fractions, respectively.

For sucrose density gradient analysis of polysomes, the 20,000g supernatant was centrifuged in a SW28 rotor (Beckman) at 100,000g for 3.5 h through a 10 to 40% sucrose

density gradient in 200 mM Tris·HCl (pH 8.5), 400 mM NaCl, and 1 mM MgCl<sub>2</sub>. Fractions 1 to 4 (mRNP), 5 to 9 (small polysome), and 10 to 22 (large polysome) were collected.

### Isolation of Poly(A)-Rich mRNA and Cell-Free (in Vitro) Translation

Poly(A)-containing mRNA was isolated by affinity chromatography (2) using oligo(dT)-cellulose, type-III (Collaborative Research, Bedford, MA). Aliquots of mRNA were translated in a micrococcal nuclease-treated rabbit reticulocyte lysate (17) (Promega, Madison, WI) cell-free system using [ $^3$ H]leucine to label the newly synthesized polypeptides. The translation products were separated by electrophoresis on an SDS-polyacrylamide gel (12%) and detected by fluorography.

### Northern Blot Analysis

Poly(A)-rich RNA was fractionated by electrophoresis on 1% agarose gels using a pH 7.0 buffer containing 20 mM Mops, 5 mM sodium acetate, 1 mM EDTA, and 1.1 M formaldehyde. RNA from the gel was transferred to 0.45  $\mu$ m Zeta-bind (Bio-Rad, Richmond, CA) and hybridized against nick-translated  $^{32}\text{P}$ -labeled recombinant plasmid (13).

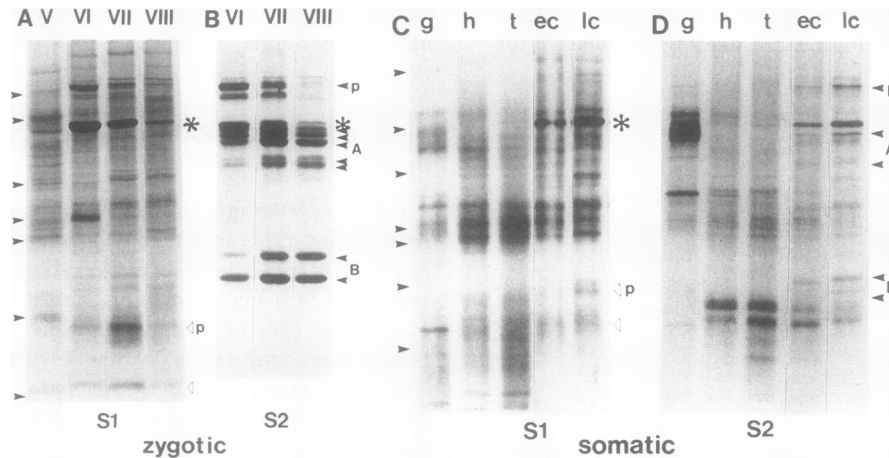
Hybridization was carried out for 24 h at 65°C using the required radioactive probe (10<sup>6</sup> cpm/mL hybridization buffer) in the presence of 1% BSA, fraction V (Boehringer Mannheim Canada Ltd., Laval, PQ), 0.5 M phosphate buffer (pH 7.2), 7% SDS, and 1 mM EDTA. Following hybridization, filters were washed twice at 60°C for 30 min in a buffer containing 0.5% BSA, 40 mM phosphate buffer (pH 7.2), 5% SDS, and 1 mM EDTA. Two final washings of the blots were carried out at 60°C for 20 min in 40 mM phosphate buffer (pH 7.2), 1% SDS, and 1 mM EDTA.

## RESULTS AND DISCUSSION

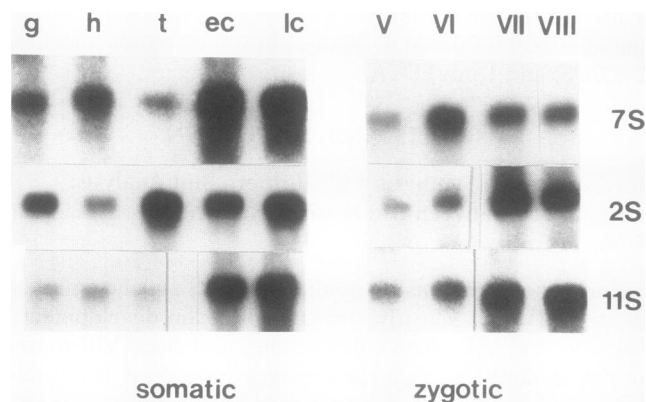
### In Vivo Protein Synthesis and Northern Blot Analysis: Comparison Between Developing Zygotic and Somatic Embryos

For our experiments on alfalfa, we used zygotic embryos at stages IV and V (early and late cotyledon development), stage VI and VII (reserve deposition), and stage VIII (onset of desiccation) (26). Somatic embryos were used at the following stages of development: day 0 (globular), day 3 (heart), day 5 (torpedo), day 10 (early cotyledon), and day 16 (late cotyledon).

The patterns of protein synthesis and, in particular, those of the storage proteins medicagin (11S), alfin (7S), and LMW (2S) were measured by labeling with radioactive leucine and analysis by one-dimensional SDS-PAGE and fluorography (8, 12). Synthesis of 7S protein occurred predominantly at stage VI of zygotic embryogenesis and to a lesser extent at stages VII and VIII (Fig. 1A). The processing of the precursor of 7S to the several component polypeptides (12) takes considerably longer than 4 h (J.E. Krochko, unpublished). Hence, only the 50-kD precursor of this protein is detectable after the relatively short incubation period. A parallel experiment using northern blot analysis with a  $^{32}\text{P}$ -labeled 7S storage



**Figure 1.** *In vivo* protein synthesis during the development of alfalfa embryos. Embryos were labeled with [ $^3$ H]leucine for 4 h. Storage proteins were fractionated by two sequential extractions using low (S-1, A and C) and high salt (S-2, B and D) buffers and were separated by SDS-PAGE. An equal number of counts was loaded in each lane. The developmental stages of the embryos are indicated above the lanes. In somatic embryos (C and D): g, globular; h, heart; t, torpedo; ec and lc, early and late cotyledon, respectively. In zygotic embryos (A and B): V to VIII, developmental stages. Asterisk, Unprocessed alfin; solid arrowheads, medicagin with its acidic (A) and basic (B) polypeptides; open arrow, LMW storage protein; p, precursor form of either the 11S (solid arrowhead) or LMW (open arrow) protein. Solid arrowheads on left side of the lanes indicate markers of 66, 50, 36, 29, 24, 16, and 10 kD.



**Figure 2.** Northern blot analysis of mRNAs in developing somatic and zygotic embryos of alfalfa using recombinant storage protein-specific cDNAs (11S, pCD43; 7S, pCD48; 2S, a polymerase chain reaction product). An equal amount of poly(A<sup>+</sup>) RNA from developing embryos was loaded in each lane. Somatic embryos: g, globular; h, heart; t, torpedo; ec and lc, early and late cotyledons, respectively. Zygotic embryos: Stages V, VI, VII, and VIII. The same probes were developed for the same time for the same embryo type but different times for different embryo types, e.g. the lane for the 7S probe for somatics was developed for the same time but for a different time from the 7S probe for zygotics. Likewise, the 7S probe was developed for a different time from the 11S or 2S probes.

protein cDNA from pea, pCD48 (7), showed that the relative amount of 7S mRNA also was greatest at stage VI (Fig. 2, zygotic). Thus, it appears that the extent of 7S protein synthesis can be correlated with the amount of transcript present in the embryo.

The 2S storage protein also is extracted in the S-1 fraction (12) and is comprised of two polypeptides linked by a disulfide bridge. It runs on gels as a single band of approximately 14 kD under nonreducing conditions (8). Western blot analysis has shown that the 2S protein is present as early as stage V in zygotic embryos, but it is not detectable by Coomassie staining (8) nor is it clearly labeled using a 4-h pulse of [ $^3$ H]leucine at that stage. Its synthesis was first apparent at stage VI, reaching a maximum by stage VII, followed by a decline in synthesis by stage VIII (Fig. 1A). In a similar manner, the relative accumulation of its mRNA, using northern hybridization to a polymerase chain reaction-derived probe from synthetic oligomer (13) was greatest at stage VII (Fig. 2, zygotic), although low amounts of LMW mRNA were detectable at stage V.

The third storage protein is the 11S. It is the major storage protein in zygotic embryos and is made up of disulfide-linked, acidic and basic polypeptides (12) that are soluble in high salt extraction buffer and make up the S-2 fraction. Synthesis of this protein occurs during stages VI to VIII (Fig. 1B), with maximum synthesis being observed at stage VII. Detection of the mRNA for 11S protein, using a  $^{32}$ P-labeled pea legumin (11S) cDNA clone from pea, pCD 43 (7), showed it to be at its maximum during stage VII (Fig. 2, zygotic). Thus, synthesis and accumulation of the seed storage proteins (11S, 7S, 2S) in developing zygotic embryos depend upon

the synthesis and availability of their specific transcripts, *i.e.* their synthesis is regulated primarily at the transcriptional level of gene expression.

The synthesis and accumulation of storage proteins and their messages were measured also in developing somatic embryos. Synthesis of unprocessed 7S was detected faintly in torpedo-stage embryos and increased in amount to the late cotyledon stage (Fig. 1C). The 2S protein was synthesized by the late cotyledon stage, but compared to the zygotic embryos, its synthesis and accumulation was very low and late (Fig. 1, A and C) (13). The mRNAs for 2S and 7S protein increased during the cotyledonary stages of somatic embryo development (Fig. 2), and substantial amounts of these mRNAs for these proteins were detected even at early stages of development, *i.e.*, globular to torpedo stages (Fig. 2, somatic), before accumulation of these proteins was detectable (Fig. 1C). There was no detectable synthesis or accumulation of 11S (Fig. 1D) in early developing somatic embryos (globular to torpedo) even though northern blot analysis showed a small amount of its mRNA to be present (Fig. 2, somatic). Synthesis of this protein occurred primarily during the cotyledon stages (Fig. 1D) and was accompanied by an increase in transcripts for this protein (Fig. 2, somatic).

In zygotic embryos between stages V and VIII, the presence of storage protein messages can be reasonably correlated with the accumulation of their translational products. But at the early stages of somatic embryogenesis there is substantial storage protein message accumulation but not of the proteins. These observations point to the possibility that in somatic embryos some storage protein messages are present but are not being translated during the early stages of development. We cannot rule out the possibility that posttranslational breakdown of proteins takes place during the earlier stages of somatic embryogenesis, although western blots of the extracted proteins using specific storage protein antibodies failed to reveal any breakdown products (13).

### Developmental Changes in Distribution of mRNAs for Storage Proteins between Polysomal and mRNP Fractions of Somatic and Zygotic Embryos

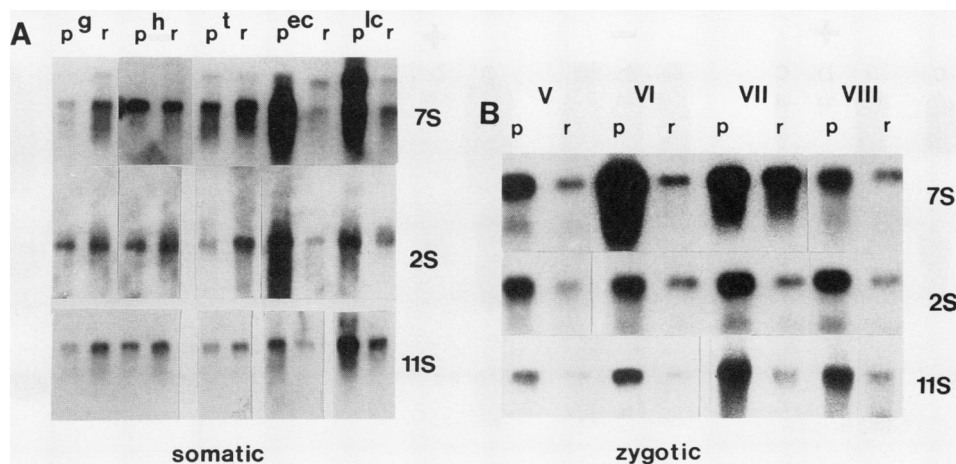
Embryogenesis in plants commences soon after fertilization, but because of their small size and their location within the ovule, it is very difficult to collect sufficient numbers of early zygotic embryos to study the developmental and metabolic changes taking place. In alfalfa, such embryos are almost transparent and <0.5 mm in length. In contrast, somatic embryos, which undergo similar developmental changes including the formation of the globular, heart, torpedo, and cotyledon stages are more easily obtainable and provide a reasonable, but not identical, alternative.

Developing somatic embryos at globular, heart, torpedo, and early and late cotyledon development stages were used to study the distribution of storage protein messages between polysomal and mRNP fractions. For comparison, developing zygotic embryos at stages V to VIII were used.

During the early stages of somatic embryo development a much greater proportion of the population of mRNAs for all the storage proteins was present in the mRNP fraction (Fig. 3A, g-t), but at the beginning of the cotyledon stages this distribution was reversed, shifting from the mRNP to the polysomal fraction (Fig. 3A, ec and lc) along with concomitant accumulation of additional messages (Fig. 2). The storage protein-specific messages were predominant in the polysomal fraction both preceding and during maximal storage protein synthesis in the developing zygotic embryos (Fig. 3B) and even when protein synthesis was declining at the advanced stages of maturation.

### Distribution of Storage Protein-Specific Messages during Sucrose Density Gradient Analysis

From the above observations, it is reasonable to speculate that the messages for storage proteins are inactive in cellular



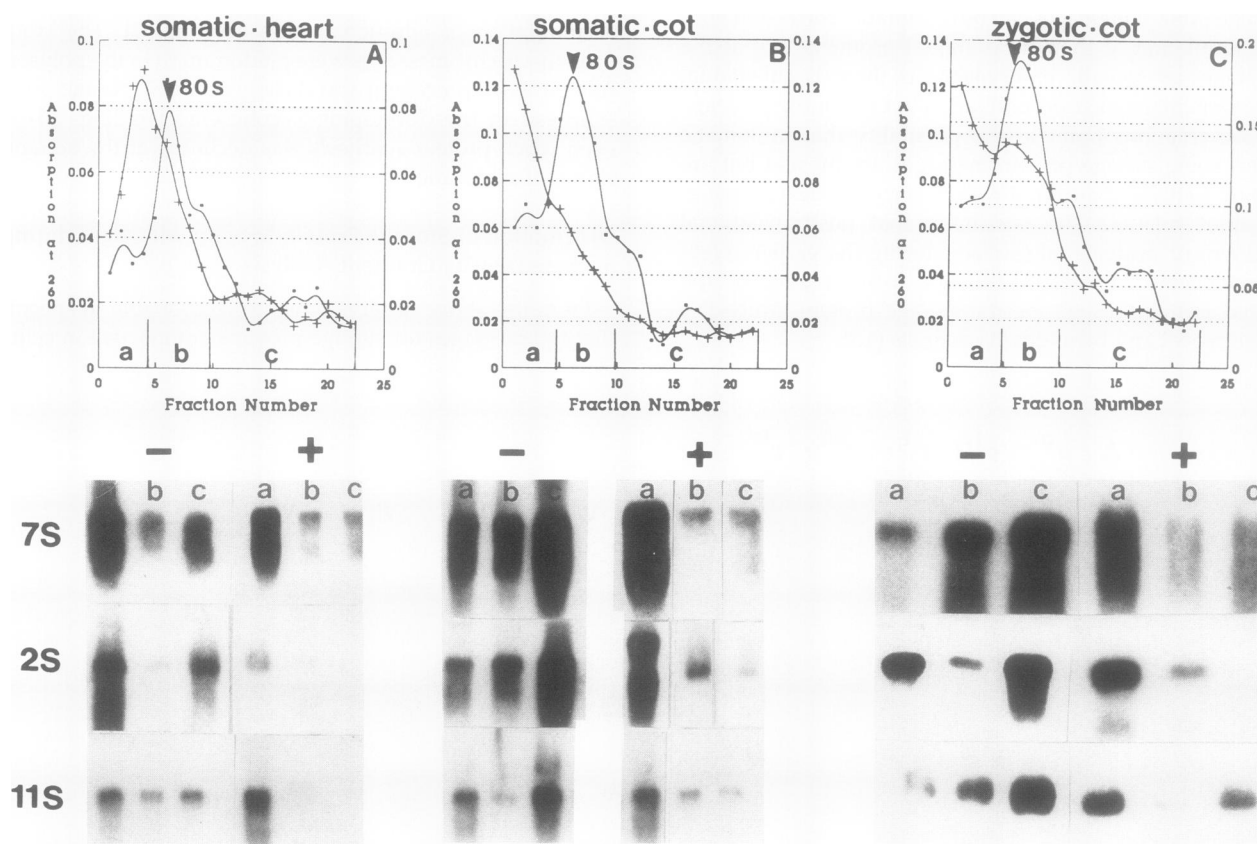
**Figure 3.** Northern blot analysis and subcellular distribution of mRNAs specific for 11S, 7S, and 2S storage proteins during development of somatic and zygotic embryos of alfalfa. mRNAs present in somatic (A) and zygotic (B) embryos in the p (polysomal) and r (mRNP) fractions. Stages of development are as in Figure 2. Paired lanes (p and r) were loaded with an amount of poly(A<sup>+</sup>) mRNA representing equivalent fractions of the original extract, which gives a representation of the normal distribution of storage protein messages between the two fractions. Different times of exposure to the x-ray plates were used for each of the probes.

protein synthesis during the early stages of somatic embryogenesis. They are preferentially distributed as the untranslated mRNP form during the globular, heart, and torpedo stages. The validity of this speculation was reinforced by an analysis of the distribution of the storage protein messages on sucrose density gradients. Fractions obtained from the gradients containing mRNP, ribosomes, or small polysomes and large polysomes were extracted for their total RNA and subjected to northern blot analysis. A parallel experiment was also conducted in which the 20,000g supernatant was treated with 50 mM EDTA for 30 min before running on the sucrose density gradient and analysis of the RNA as above. Treatment with EDTA dissociated the polysomes and caused a shift in the released messages from polysomes to the mRNP fraction (Fig. 4, A-C, top panel). Northern blot analysis of the sucrose density gradient fractions showed the storage protein messages were abundant in the translationally active large polysomal fraction of somatic embryos at the cotyledonary stage (Fig. 4B, c, -EDTA) and of zygotic embryos at the same stage (Fig. 4C, c, -EDTA). EDTA dissociated the polysomal fraction of these embryos (Fig. 4, B and C, +EDTA), and all the storage protein messages then sedimented as mRNPs (Fig. 4, B and C, a, +EDTA). All of the

storage protein messages from an early stage (heart) of somatic embryo development sedimented as mRNPs (Fig. 4A, a, -EDTA) with a small fraction being present in the large polysomes (Fig. 4A, c, -EDTA). In contrast to the situation in cotyledon-stage somatic and zygotic embryos, EDTA treatment had only a small effect on the distribution of storage protein messages in the sucrose gradient (Fig. 4A, c, +EDTA), although the relatively minor fraction of mRNA that sedimented along with the polysomes was dissociated and sedimented as mRNP. These observations lend support to the contention that storage protein messages are preferentially distributed as mRNPs during early somatic embryo development.

#### Analysis of Cell-Free Translational Products of Polysomal and mRNPs from Developing Somatic and Zygotic Embryos

The distribution of mRNA species between the polysomal and mRNP fractions of cells of developing zygotic and somatic embryos was determined by SDS-PAGE analysis of the resultant polypeptides following translation of the messages in a cell-free rabbit reticulocyte system. The paired



**Figure 4.** Subcellular distribution of storage protein-specific mRNAs from somatic and zygotic embryos of alfalfa on sucrose density gradients and the effect of EDTA. A, B, and C: Sedimentation profiles of cytoplasmic RNA extracts of somatic (A) heart and (B) cotyledon stage embryos and (C) zygotic cotyledon stage embryos in the absence (■) or presence (+) of EDTA. RNA (1  $\mu$ g) extracted from (a) mRNP (fractions 1–4), (b) monosomes and small polysomes (fractions 5–9), and (c) large polysomes (fractions 10–22) were used for northern blot analysis against probes for the major 11S, 7S, and 2S storage proteins. Lanes a, b, and c are RNA from the three fractions obtained from sucrose gradients in the absence (–) or presence (+) of EDTA.

lanes in each panel in Figure 5 show the synthesis of proteins from polysomal mRNAs and those in the mRNP fraction extracted from the transition stages of developing somatic and zygotic embryos, *i.e.* when the patterns of storage protein synthesis change (Fig. 1). The first and obvious observation is that mRNAs present in the mRNP fraction can be translated following their separation from the protein component of this fraction using oligo(dT)-cellulose ("Materials and Methods") and transfer to a different protein-synthesizing environment, the rabbit reticulocyte system. The nature of the mRNAs in the polysomal and mRNP fractions changed from the torpedo to the early cotyledon stage in somatic embryos (Fig. 5A, t and ec) and from stage V to VI in zygotic embryos (Fig. 5B). In somatic embryos, the pattern of proteins synthesized by both fractions from the earlier stages (globular and heart) resembled those from the torpedo stage, and the late cotyledon stage messages in both fractions were as in the early cotyledon stage (not shown). Similarly, the *in vitro* protein synthesis patterns directed by messages from stage VII and VIII zygotic embryos changed little from those of stage VI, except for some intensification of bands in the samples derived from the mRNP fraction (not shown), presumably resulting from some specific reduction of synthesis associated with maturation drying at the later stages of development.

The patterns of polypeptides synthesized by polysomal mRNAs and those in the mRNP fraction were qualitatively quite similar in both embryo types (Fig. 5, A and B), with all peptides being common to both fractions. However, there were notable differences in the distribution of certain proteins synthesized by the mRNAs from the two fractions, as determined by the relative intensity of several bands. Of the known storage proteins in somatic embryos, the amount of

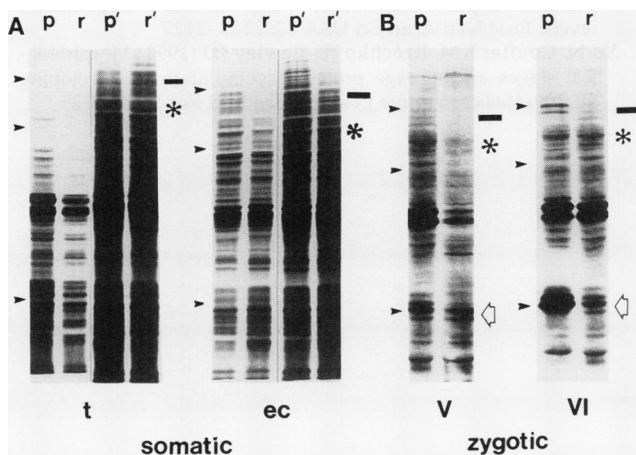
11S message present in the mRNP fraction during the torpedo stage was greater than in the polysomal fraction, whereas in the early cotyledon stage, this distribution was reversed (Fig. 5A, p and r). Unfortunately it was difficult to identify the 2S and 7S products at the torpedo stage, but in the early cotyledon stage, relatively more 7S protein resulted from translation of the polysomal fraction than the mRNPs (Fig. 5A, p and r). In the zygotic embryos at stage VI, synthesis of 11S, 7S, and 2S polypeptides by mRNAs from the polysomal fraction was more evident compared to the mRNP fraction. In addition, the distribution of mRNAs for these storage proteins was greater in the polysome fraction at stage VI than at stage V (Fig. 5B).

The results obtained from these *in vitro* studies show the extent to which storage protein mRNAs are present in the mRNP fraction. Although, in themselves, the patterns are not sufficient to show the redistribution of mRNAs from the mRNP to polysomal fractions at the time of maximal protein synthesis, they are repetitively consistent with the data obtained from our northern hybridization studies.

## CONCLUSION

In this study we observed an underutilization of mRNAs for storage proteins during the development of somatic embryos of alfalfa, particularly in the precotyledonary stages. Several species of mRNA of known and unknown identities were present in the mRNP fraction, including the messages for the major 11S, 7S, and 2S storage proteins. These messages were not defective because they were translatable, after deproteinization, in a cell-free reticulocyte system. The majority of the storage-specific messages sedimented in the mRNP fraction during the globular, heart, and torpedo stages of somatic embryogenesis, but during the cotyledonary stages the distribution was in favor of the polysomal fraction. In zygotic embryos at the cotyledonary stages, the storage protein messages were present to a large extent in the polysomal fraction also.

The reason for the regulation of storage protein synthesis by repression of translation at the early stages of somatic embryogenesis is not known, but, as in zygotic embryos, certain morphological changes presumably must be completed before the onset of reserve deposition. The occurrence of substantial amounts of untranslated mRNAs is known in a variety of eukaryotic cells (21), where they occur as RNPs (19). In animal cells, stage-specific activation of mRNAs is known to occur during embryonic development (9, 22). The underutilization of cytoplasmic mRNAs could be the result of their association with protein ligands, or the rate of initiation of specific messages on ribosomes at some stages of development may limit their translational efficiency, or there could be a limitation to the elongation reaction of protein synthesis. Certainly, in the somatic (and zygotic) embryos, there are few qualitative differences between the proteins synthesized *in vitro* from mRNAs extracted from the active (polysomal) and nonpolysomal mRNP fraction, and yet at different stages of development in somatic embryos the distribution of messages between these two fractions differs. Only at the cotyledonary stage is there a relationship between the accumulation of storage proteins and the recruitment of



**Figure 5.** Differences in polypeptides synthesized *in vitro* by mRNAs from the polysomal and mRNP fractions during the development of somatic and zygotic embryos. Protein synthesis by poly(A<sup>+</sup>) mRNA extracted from (A) somatic (torpedo and early cotyledon stage) and (B) zygotic (stages V and VI) embryos. Lanes p and r are proteins synthesized by the polysomal and mRNP fractions, respectively; p' and r' in A are longer exposures of the same gel. Arrowheads on left side of gels indicate markers of 66, 45, and 20 kD.

messages into the polysome fraction. Even at these later stages in somatic embryos, however, although there is a strong relationship between the amount of 11S and 7S storage protein synthesized *in vivo* and the presence of their mRNAs recruited into polysomes, there is little synthesis of the LMW protein even though its mRNA is abundantly present in polysomes (13). Thus, although protein synthesis at early stages of somatic embryo development may be translationally regulated, at later stages some posttranslational control is in effect also.

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