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Signal Recognition-like Particles Are Present in Maize*

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We show that maize storage protein translocation across microsomal membranes is mediated by signal recognition particles (SRPs) similar to those described in animal systems (Dobberstein, B. (1978) *Hoppe-Seyler's Z. Physiol. Chem.* 252, 955–962; Walter, P., and Blobel, G. (1980) *Proc. Natl. Acad. Sci. U. S. A.* 77, 7112–7116). We have prepared a high salt extract from endosperm cell homogenates, from which a ribosome-free fraction was obtained. This fraction is enriched in an SRP-like factor which apparently corresponds to a ribonucleoprotein particle that sediments at about 12 S. The RNA moiety of this 12 S particle is complex, showing a three-band electrophoretic pattern and sedimenting at about 8 S. The fraction restores translocation competence of salt-washed maize microsomes as tested by using a pre-zein message. In contrast to canine SRPs, the maize SRP-like component does not cause a translation arrest of maize storage proteins (zein) in a wheat germ cell-free system.

During development of maize seeds, endosperm cells synthesize and accumulate storage proteins: zeins and glutelins-2 (1, 2). The synthesis is developmentally regulated and occurs on polysomes bound to the endoplasmic reticulum (ER).[1] Subsequently, the polypeptides cross the membrane and remain sequestered within the ER cisternae, forming vesicles called protein bodies (3). It is well established that the transport pathway of maize storage proteins contains only one sorting step localized at the level of the ER (4). It was shown that a cleavable signal peptide is present in all these proteins, and the corresponding sequences were inferred from the analysis of cDNA and genomic clones (5–7).

Two components of the translocation machinery across the ER have been identified in animal systems: the signal recognition particle (SRP) (8) and the docking protein (SRP receptor) (9). Animal SRP is an 11 S ribonucleoprotein that contains six polypeptide chains and a 7 S RNA. The functions of the SRP and the docking protein are well established (see review in Ref. 10) and can be readily assayed *in vitro*. Walter and Blobel (11) have reconstituted heterologous SRP using the corresponding proteins and 7 S RNA from different animal sources. Studies on the secondary structure of SRP 7S RNA from mammals, amphibia, and insects demonstrate that these structures have been extensively conserved throughout animal evolution (12, 13).

Plants constitute another large branch in the evolution of eucaryotes. It has been shown that dog pancreas microsomes can process and translocate maize storage proteins (3) and pea vicilins (14). In addition, Bassüner *et al.* (15) have demonstrated that canine SRP actively mediates storage protein translocation in *Vicia faba*. These findings suggest for cereals and legumes a translocation mechanism similar to that described for animal secretory proteins. The aim of this work is to answer the question of whether an SRP-like particle actually plays a role in mediating maize storage proteins transport and accumulation in endosperm cells. In this study, we have purified a 12 S particle from maize endosperm that shows an *in vitro* activity for zein translocation across ER membranes. The particle has a complex RNA moiety, making it different from SRP 7 S RNA in animals.

MATERIALS AND METHODS

Plant Material—Seeds from the inbred maize W64A (*Zea mays* L.) were harvested 20 days after pollination, frozen immediately in liquid nitrogen, and stored at −70 °C. Endosperms were isolated from kernels after removing pericarps and embryos by hand. Wheat germ lysate was prepared essentially as Roberts and Patterson (16).

Isolation of Microsomal Membranes—Dog pancreas microsomes, salt-washed and nuclease-treated (K-RM), were prepared as described (17, 18). Maize rough microsomes were purified by a modification of the procedure described by Burr and Burr (19). Maize endosperms (16 g) were homogenized in a chilled mortar in the presence of quartz sand and 16 ml of buffer A (50 mM Tricine (pH 8.00), 100 mM KCl, 6 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, 1 mM PMSF) containing 20% sucrose. The homogenate was filtered through one layer of nylon cloth and centrifuged for 5 min at 500 × g and for 8 min at 2,500 × g. The supernatant was layered onto linear gradients of 35–70% sucrose in buffer A previously overlaid with 1.5 ml of 22.2% sucrose in the same buffer. The gradients were centrifuged at 140,000 × g_{av} for 2 h at 4 °C in a Beckman SW 40 rotor. Rough microsomes were obtained by collecting and pooling the membrane bands of the gradient (the second band from the top in each tube). Nuclease-treated maize microsomes (MN) were obtained from rough microsomes after micrococcal nuclease treatment. The microsomes were suspended in buffer B (50 mM Hepes (pH 7.6), 1 mM DTT, 20% glycerol); adjusted to 1 mM EDTA, 2 mM $CaCl_2$, and 100 units/ml micrococcal nuclease (Boehringer Mannheim); and incubated for 15 min at 25 °C. The nuclease treatment was stopped by bringing the membranes to 4 mM EGTA. Salt-washed maize microsomes (MNK) were obtained from MN (in this case, treated with 5,000 units/ml micrococcal nuclease) by increasing the salt concentration to 500 mM KOAc. After incubation on ice for 10 min, the sample was centrifuged for 1 h at 100,000 × g_{av} in a Beckman SW 60 rotor through a 1-ml cushion of 17%

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[1] The abbreviations used are: ER, endoplasmic reticulum; SRP, signal recognition particle; K-RM, salt-washed and nuclease-treated dog pancreas microsomes; MN, nuclease-treated maize microsomes; MNK, salt-washed and nuclease-treated maize microsomes; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; DTT, dithiothreitol; PMSF, phenylmethylsulfonyl fluoride; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid.

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sucrose in buffer C (50 mM Hepes (pH 7.6), 500 mM KOAc, 10 mM EDTA). Salt extraction was performed twice, and the final pellet was resuspended in buffer B to give a final concentration of about 6 A_{280} units/ml. Control experiments were carried out with MN and MNK to ensure the absence of endogenous translational activities.

Isolation of SRPs—Dog pancreas SRP was purified according to Walter and Blobel (18). Maize SRP-like factor was isolated by a procedure which is an adaptation of that described by Walter and Blobel (18, 20). Dissected endosperms (90 g) were ground in a chilled mortar in the presence of quartz sand and 70 ml of buffer D (50 mM triethanolamine (pH 7.5), 100 mM KOAc, 6 mM $Mg(OAc)_2$, 1 mM EDTA, 1 mM DTT, 1 mM PMSF, 8.5% sucrose), and the suspension obtained was filtered through one layer of nylon cloth. The KOAc concentration was brought to 500 mM by the addition of 4 M KOAc (pH 7.5). The mixture was incubated on ice for 15 min and centrifuged for 10 min at 1,000 × g_{av}. The supernatant was distributed in Beckman SW 40 tubes which contained a 1-ml cushion of 17% sucrose in buffer E (50 mM triethanolamine (pH 7.5), 500 mM KOAc, 6 mM $Mg(OAc)_2$, 1 mM EDTA, 1 mM DTT, 1 mM PMSF). After centrifugation for 3 h at 140,000 × g_{av}, the postribosomal supernatants, including the upper half of the cushions, were pooled and diluted with 1 volume of buffer F (50 mM triethanolamine (pH 7.5), 6 mM $Mg(OAc)_2$, 1 mM EDTA, 1 mM DTT, 1 mM PMSF, and 0.01% Nikkol (octaethylene glycol mono-*N*-docecyl ether, Nikko Chemicals, Tokyo)). The sample was passed through a 6-ml DEAE-Sepharose CL-6B column (16 ml/h) previously equilibrated with buffer G (50 mM triethanolamine (pH 7.5), 250 mM KOAc, 5 mM $Mg(OAc)_2$, 1 mM DTT, and 0.01% Nikkol). After loading, the column was washed with 10 volumes of buffer G. The material retained in the column was eluted with 1.5 volumes of buffer H (50 mM triethanolamine, 650 mM KOAc, 5 mM $Mg(OAc)_2$, 1 mM DTT, and 0.1% Nikkol). This sample was loaded onto 11.5-ml linear gradients of 5–20% sucrose in buffer H. After centrifugation at 4 °C for 21 h at 192,000 × g_{av}, the gradients were collected and monitored at 254 nm using an Isco gradient fractionator. Aliquots of all fractions were stored for further analyses. The fractions corresponding to the 12 S peak were pooled and diluted with buffer F to a final concentration of 250 mM KOAc. The sample was concentrated by passing through a 600-μl DEAE-Sepharose column as described above. The final concentration of this extract was 1–2 A_{260} units/ml.

RNA was purified using a phenol/chloroform extraction procedure, and the resultant samples were analyzed by electrophoresis in 7 M urea and 7% acrylamide gels as described by Maniatis and Efstratiadis (21). After electrophoresis, the gels were stained with ethidium bromide at 0.4 g/ml, and the RNA bands were visualized with UV light.

In Vitro Transcription and Translation—A wheat germ cell-free system was used in all translation and translocation experiments. *In vitro* translation of polysomal RNA from maize endosperm was carried out as described (3) using [^{35}S]methionine as a labeled precursor.

In vitro transcriptions were done according to Stueber *et al.* (22) using pDS6-derived plasmids and *Escherichia coli* RNA polymerase (Pharmacia LKB Biotechnology Inc.). Zein messenger was obtained by transcription of the pDS-M_1 *Hpa-Dra* plasmid (23) (kindly provided by B. Dobberstein, European Molecular Biology Organization, Heidelberg, West Germany). The messenger coding for the human platelet-derived growth factor peptide was synthesized by transcription of the PDGF5 plasmid (kindly provided by R. Gentz, Hoffmann-La Roche). This protein has no signal peptide and is not translocated across microsomal membranes. It was used as a control in the *in vitro* translations. The concentrations of microsomes and SRPs in the translation reactions were as follows: K-RM, 0.4–0.8 A_{280} unit/ml; MNK, 0.1–0.5 A_{280} unit/ml; canine SRP, 0.2–0.4 A_{260} unit/ml; and maize SRP, 0.1–0.2 A_{260} unit/ml. All translation experiments were carried out in the presence of Nikkol at 0.002% to stabilize SRP activity as described by Walter and Blobel (8).

Affinity experiments were carried out as described by Meyer and Dobberstein (24). Salt-washed microsomes from dog pancreas or maize endosperm (0.01 A_{280} unit) were preincubated at 0 °C for 30 min with canine or maize SRP (0.004 A_{260} unit) in 50 mM KOAc. After preincubation, the membranes were pelleted at 15,000 × g_{av} for 15 min and resuspended with 20 μl of translation mixture containing zein messenger. Translation was carried out as described above.

Aliquots of translation mixtures were treated with 100 μg/ml proteinase K at 4 °C for 30 min. The reactions were stopped by adding PMSF (the final concentration being 10 mM). After precipitation in 12.5% trichloroacetic acid, translation products were analyzed by SDS-PAGE (25) (15% acrylamide; 150:1 ratio of acrylamide to bisacrylamide) and fluorography.

When necessary, radioactive bands localized in the dried gel were cut out, and radioactivity was determined by liquid scintillation counting.

RESULTS

Effect of Canine SRP on Maize Microsome Translocation Competence—Using *in vitro* translocation assays, Torrent *et al.* (3) demonstrated that translation of polysomal RNA mainly produces storage preproteins and that rough microsomes process these proteins. In this paper (Fig. 1, *lanes c* and *d*), we show that the addition of nuclease-treated microsomes to an RNA *in vitro* translation assay promotes a partial processing and translocation of zein polypeptides. However, in the presence of salt-washed maize microsomes (Fig. 1, *lanes e* and *f*), the translocation of synthesized storage proteins is not observable. These findings raised the question of whether MNK would require a salt-extractable component for storage protein translocation, as was previously shown for canine pancreas microsomes (8). To explore this possibility, we studied the effect of canine SRP on our translocation system.

A coupled transcription/translocation system was used as isolated endosperm RNA populations were too complex. Zein messenger was synthesized by transcription of the pDS-M_1 *Hpa-Dra* plasmid (23). A single zein is obtained from this messenger by *in vitro* translation (Fig. 2, *lane 1*). The synthesis of the pre-zein is strongly inhibited by the presence of canine SRP in the wheat germ cell-free system (Fig. 2, *lane 3*).

As shown in Fig. 2 (*lanes b, e,* and *h*), dog pancreas SRP restores the translocation competence of maize salt-washed membranes. A control experiment was carried out with dog pancreas microsomes for comparison with a homologous system. Only when the SRP is present in the assay together with the highest MNK concentration tested (Fig. 2, *lanes g–i*) is the degree of translocation equivalent to that found for the control homologous system (Fig. 2, *lanes j–l*). These results indicate that the maize translocation system appears to be

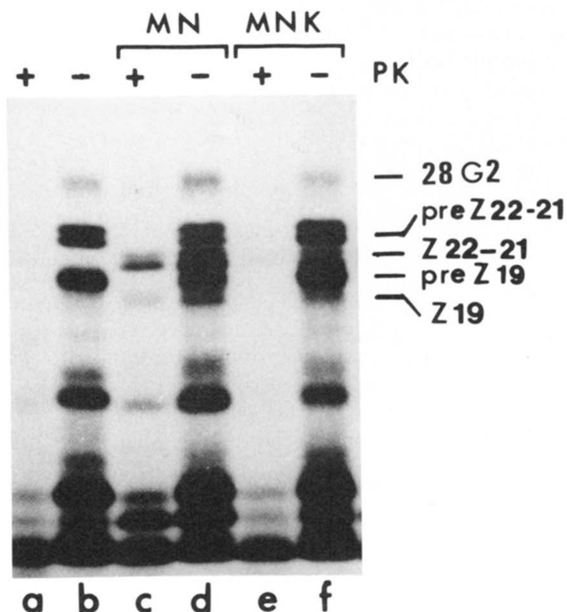

FIG. 1. **Translocation ability of MN and MNK.** Endosperm RNA was translated in the absence of membranes (*lanes a* and *b*) or in the presence of MN (*lanes c* and *d*) or MNK (*lanes e* and *f*). Protease protection experiments were as described under "Materials and Methods." *In vitro* synthesis was carried out in a wheat germ cell-free system, and the translation products were analyzed by SDS-PAGE and fluorography. Positions (in kilodaltons) of mature zein (*Z*), pre-zein (*pre Z*), and glutelin-2 (*G2*) polypeptides are indicated to the right. *PK*, proteinase K.

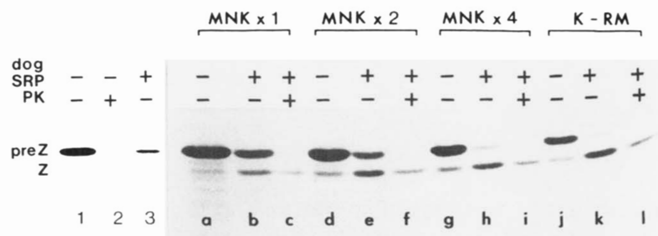

FIG. 2. **Effect of dog pancreas SRP on translocation competence of nuclease-treated and salt-washed maize microsomes.** Zein mRNA derived from plasmid pDS-M_1 was translated in the presence of increasing MNK concentrations (*lanes a–i*; MNK × 1 concentration being 0.1 A_{280} unit/ml). Canine SRP was added where indicated. Translocation across canine K-RM is included as reference (*lanes j–l*). Controls without membranes are shown (*lanes 1–3*). Proteolysis with proteinase K (*PK*) was carried out as described under "Materials and Methods." Shown is a fluorogram depicting the translation products of *in vitro* synthesis. *pre Z* and *Z* refer to pre-zein and mature zein translation products, respectively.

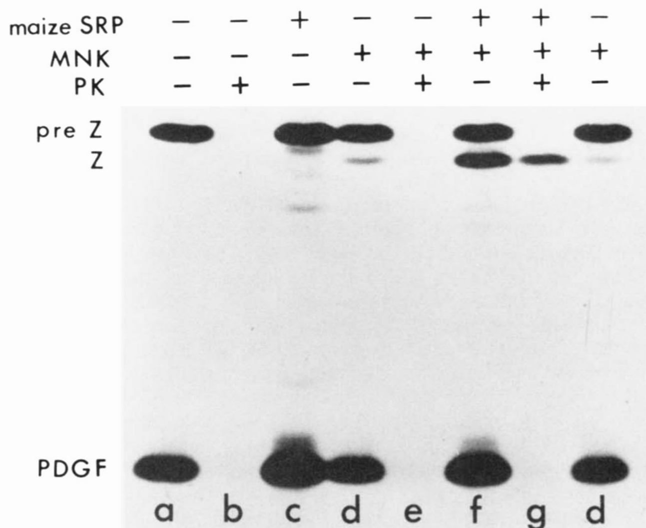

FIG. 3. **Activity assay of maize SRP determined in wheat germ cell-free system.** An aliquot of maize SRP extract (0.2 A_{260} unit/ml) was added to a translation/translocation assay programed with the zein mRNA derived from plasmid pDS-M_1 in the presence (*lanes f* and *g*) or absence (*lane c*) of salt-washed maize microsomes (0.5 A_{280} unit/ml). Control experiments in the absence of maize SRP are included (*lanes a, b, d,* and *e*). Proteolytic protection of the translocated polypeptides is also shown. A human platelet-derived growth factor (*PDGF*) transcript was translated simultaneously in all incubation assays. Shown is a fluorograph of the translation products separated by SDS-PAGE.

analogous to the mammalian system.

Membrane concentration seems to be an important factor in this *in vitro* system. In the presence of dog SRP, the yield of translocation for zein polypeptides correlates with the increasing amounts of MNK (Fig. 2, *lanes b, e,* and *h*). Although the membranes were submitted to several salt-wash treatments, some translocation background was observed for MNK in the absence of the SRP (Fig. 2, *lanes a, d,* and *g*). In fact, the presence of a wheat SRP in the cell-free system cannot be excluded.

Maize SRP Activity on Zein Translocation—Since dog SRPs were active in translocating zeins across maize membranes, this may suggest that a maize SRP could be present in the endosperm cells. Further experiments were carried out in an attempt to isolate these particles from endosperm extracts. To isolate SRP-like particles from maize, we used a method based on the intracellular distribution studies on canine SRP (2). Maize SRP activity was tested in several fractions of the purification process.

As expected and in agreement with other authors (8), maize SRP activity was observed only in fractions corresponding to the 12 S peak in the last step of purification (see Fig. 5*A*).

Two approaches were used to determine the possible presence of SRP activity in this fraction. The first approach was based on the recovery of the MNK translocation competence by direct addition of maize SRP (0.2 A_{260} unit/ml) to a wheat germ translation system (Fig. 3). Maize MNK membranes were added at the optimal concentration tested (0.5 A_{280} unit/ml; see Fig. 2). It was observed that membrane concentrations higher than 0.5 A_{280} unit/ml significantly inhibit zein translation. We included a platelet-derived growth factor peptide transcript as a control in all incubations. This protein acts as a translation marker and allows the quantification of the translocation relative to the translational efficiency in each case.

It is known that canine SRPs inhibit translation of secretory proteins in a wheat germ system (27). Addition of maize SRP does not result in an arrest of translation of zein in the absence of membranes (Fig. 3, *lane c*), and it induces an increase of about 25% of both zein and platelet-derived growth factor peptide translation, as was deduced from the band counting. Since this effect is shown to be similar in proteins with or without signal peptide, we cannot exclude the possibility of some degree of contamination by initiation or elongation factors that would induce the observed stimulation.

As expected, MNK membranes by themselves cannot translocate pre-zeins (Fig. 3, *lanes d* and *e*). Interestingly, maize SRP is able to restore MNK translocation (Fig. 3, *lanes f* and *g*). A pre-zein band was always observed in the maize SRP translation/translocation assay. Although maize SRP was always concentrated before assaying, the recovery of translocation capacity of MNK was never higher than 50%, as was deduced from the band counting of the total synthesized products. The control experiment (Fig. 3, *lane d*) shows a 5% translocation background.

The second approach (in order to establish the presence of active maize SRP in the purified fraction was based on the potential of this particle to specifically bind to microsomal membranes (24). According to SRP subcellular distribution studies for dog pancreas (20), the SRP is mainly found in the membrane-bound state at 50 mM KOAc. Therefore, prior to translation, we incubated maize and dog SRPs either with MNK or with K-RM at 50 mM KOAc (Fig. 4, *lanes b, d,* and *e*). Control experiments were conducted in the absence of both maize and canine SRPs (see Fig. 4, *lanes a* and *c*). Translation began after the addition of zein mRNA and a wheat germ system to the membrane-bound SRP pellet. The results shown in Fig. 4 indicate that processing and translocation levels of MNK and K-RM do not differ significantly when dog SRP is bound to these membranes (Fig. 4, *lanes b* and *d*). In contrast, the total translocation across MNK preincubated with maize SRP is higher than the translocation obtained across MNK preincubated with canine SRP (Fig. 4, *lanes d* and *e*). It should be noted that, in the translocation assay with canine SRP MNK, the pre-zein band is not observable. This is due to the elongation arrest produced by canine SRP in the wheat germ translation system (27) (see Fig. 2, *lane h*). In the experiment described here, the presence of maize SRP does not promote an enhancement of pre-zein translation. This finding would indicate that several components which normally copurify with maize SRP extract are removed after maize SRP-membrane complex recovery by centrifugation. From the results expressed in Fig. 4, we can

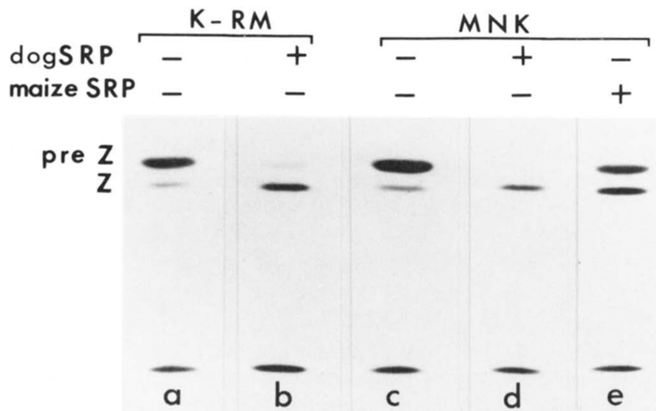

FIG. 4. **Activity assay of canine and maize SRPs bound to membranes.** K-RM and MNK membranes were preincubated with canine or maize SRP extract. After pelleting, the incubated membranes were added to a wheat germ translation system containing zein mRNA derived from plasmid pDS-M_1. Translation products were analyzed by SDS-PAGE and fluorography. *Lanes b* and *d*, canine SRP preincubated with K-RM and MNK, respectively; *lane e*, maize SRP preincubated with MNK membranes; *lanes a* and *c*, translations in the absence of SRPs (control assays). *Bottom bands* correspond to electrophoretic front.

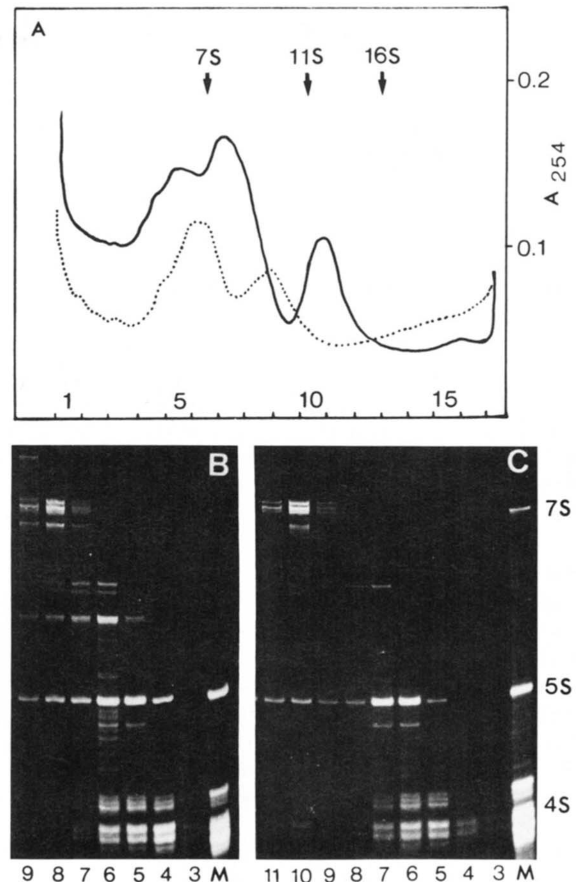

FIG. 5. **Characterization of maize SRPs by sucrose gradient and RNA analysis.** The postribosomal supernatant eluted at 650 mM KOAc from a DEAE-Sepharose column was layered on top of a sucrose gradient and processed as described under "Materials and Methods." The profile of the gradient recorded at 254 nm is shown in *A* (——). Simultaneously, a protein-depleted aliquot of the DEAE eluate (after phenol/chloroform extraction) was also analyzed by a sucrose gradient (*A*, ····). *Arrows* indicate the positions of sedimentation coefficient markers. Gradients were fractionated, and fractions of 750 μl were collected. Aliquots of both gradient fractions were analyzed by their RNA content by urea/polyacrylamide gel electrophoresis. *B*, RNAs from gradient fractions of the protein-depleted DEAE eluate (see *A*, ····). *C*, RNAs from gradient fractions of the complete eluate (see *A*, ——). Numbers of profile fractions correlate identically with those of RNA gels. *Lanes M* in *B* and *C* show a mixture of RNA markers: 7 S RNA from dog SRP, 5 S rRNA from *E. coli*, and tRNA from calf liver.

conclude that translocation levels are improved when a homologous system of SRP/membrane is used in an *in vitro* transcription/translation system.

Characterization of Maize SRP-like Particles—The results described above show that the fraction corresponding to the 12 S peak in the sucrose gradient enhances the zein translocation competence of maize membranes. The question then arises if this activity is related to a ribonucleoprotein particle (maize SRP) as was described in animal systems. All fractions collected from the sucrose gradient analysis (Fig. 5*A*) were analyzed for their RNA content (Fig. 5*C*). As shown in Fig. 5, fractions of the lowest sedimentation coefficient contain mostly 4 S tRNA and ribosomal RNA, whereas fraction 10 (corresponding to particles of about 12 S) is associated with an RNA of approximately 8 S. It should be noted that, whereas canine SRP has a single RNA band of 7 S (see Fig. 5*C*, *lane M*), maize SRP shows three RNA species of different apparent molecular weights. These three RNA electrophoretic bands are observable even for the first steps of preparation (*i.e.* the crude high salt extract; data not shown), and no sign of degradation was observed during the different steps of purification. To check the reproducibility of this RNA moiety, we purified the particle both from maize microsomes as described in "Materials and Methods" and from protein bodies isolated according to Ludevid *et al.* (2). In all cases, three RNA major bands were obtained (data not shown). In addition, different denaturing electrophoresis gels (21, 26) were used in order to rule out the possibility that secondary structures could account for differences in mobility of a single 8 S RNA.

We also examined the distribution of the 8 S RNAs by sucrose gradient analysis from a protein-depleted DEAE-Sepharose eluate. An aliquot of this eluate was treated with phenol/chloroform prior to loading onto the sucrose gradient, and a RNA profile was obtained (Fig. 5*A*, ····). Interestingly, it is observed that the three RNA bands undergo a shift from fraction 10 (12 S) (Fig. 5*C*) to fraction 8 (8 S) (Fig. 5*B*), indicating the presence of proteins associated with these RNA species.

DISCUSSION

The results presented in this paper strongly support the view that SRP-like particles are present in maize endosperm and demonstrate that maize storage proteins use a translocation machinery which is essentially similar to that described for secretory and integral membrane proteins of mammalian cells (10).

We have previously shown that maize endosperm rough microsomes are able to translate and translocate maize storage proteins (3) in an *in vitro* system. In this work, we show that, after salt washing of nuclease-treated microsomes, a loss of translocation capacity of these membranes takes place. A similar observation has also been described for dog pancreas microsomes (28), where it has been demonstrated that a high salt treatment efficiently removes the SRPs (8). It was therefore assumed that putative maize SRPs should behave similar to canine SRP, and so we attempted to prepare maize particles according to canine SRP purification procedures.

We have obtained an endosperm fraction which is enriched in 12 S particles. The particles contain an RNA which is heterogenous, as seen by electrophoresis, showing a three-band pattern; this RNA sediments as a whole at about 8 S. A

recent report (29) suggests the presence of an SRP-like component in wheat germ extracts. These authors did not describe any heterogeneity for the 7 S RNA of wheat germ 11 S SRP. However, in our preparations, it should be noted that, through the different steps of the purification, these three RNA electrophoretic bands remain and that no degradation was observed. In fact, preliminary results[2] on the molecular analysis of these 8 S RNA species indicate the existence of substantial heterogeneity at the sequence level. Moreover, analysis of 7 S cDNA clones from *Drosophila* (13) and man (30) indicates the presence of sequence microheterogeneity among the 7 S RNA populations. Since genes coded in the maize genome frequently appear as multigenic families, the heterogeneity in the 8 S RNA may reflect such a situation.

As would be expected, maize 12 S SRP appears to be a ribonucleoprotein. This can be concluded from the differences in the sedimentation coefficient that have been observed between the complete particle (12 S) and the protein-depleted particle (8 S). We assayed to analyze the protein moiety of the particle (data not shown), but, so far, identification of specific proteins appears to be difficult due in part to the small amounts available of maize 12 S SRP samples and in part to minor contaminations of ribosomal proteins.

Our data show that maize SRP extracts can restore translocation competence of maize salt-washed membranes. This demonstration of the *in vitro* activity of maize SRP confirms the presence of this particle in maize endosperm. Under optimal conditions, maize SRPs are able to promote at least 50% of translocation of zein polypeptides. On the other hand, we show that dog SRP can stimulate translocation across salt-washed maize microsomes. Previously, it was demonstrated that the docking protein is the natural receptor for canine SRP in dog pancreas ER and that this interaction is necessary for SRP-mediated translocation (19). On this basis, we can postulate the existence of an analogous receptor in maize endosperm ER, and we take advantage of this fact in the functional affinity experiments. It is apparent that the homologous system formed by canine SRP and K-RM works as expected, and no pre-zein is observed in the presence of SRP. Both dog and maize SRPs bound to maize salt-washed microsomes are efficient in processing and translation of the pre-zein message, with the efficiency higher for the homologous SRP-membrane complex. We also observe that maize SRP does not arrest translation of pre-zein in the *in vitro* wheat germ cell-free system. This is in agreement with the results obtained by Prehn *et al.* (29) for wheat germ SRP. The arresting effect is one of the mechanisms of action described for canine SRP (31). However, it appears that such dog SRP behavior takes place only in a wheat germ system (heterologous system) (32), and it cannot be extended to all secretory or integral proteins (33).

From all the data described up to now, it follows that the difference in activity between SRPs from animal and plant systems is not dependent on the structure of the signal peptide; thus, special attention must be paid to SRP-ribosome and SRP-ER membrane interactions. In order to improve the present knowledge on translocation mechanism in maize, we believe that studies on 7 S RNA structure and SRP reconstitution, using plant homologous systems, should be carried out.

[2] N. Campos, C. Zwieb, and J. Palau, unpublished data.

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