In Vitro Uptake and Processing of Maize Auxin-Binding Proteins by ER-Derived Microsomes

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We have analyzed auxin-binding proteins from maize encoded by the *Zm-ERabp* gene family. Open reading frames of cDNA clones predict proteins containing N-terminal hydrophobic signal sequences. In vitro studies show that the Zm-ERabp1 protein can be translocated into ER-derived microsomes where it is processed and glycosylated. A cDNA clone encoding the Zm-ERabp4 protein predicts an open reading frame with a signal sequence that shows striking differences in charge distribution, in comparison to the signal sequence of Zm-ERabp1. Two translation products are synthesized from the Zm-ERabp4 transcript in the in vitro system, but only one of them is translocated into maize endosperm microsomes, indicating that specific cotranslational modifications in the primary sequence remaining after processing may play a role in the cellular trafficking of the Zm-ERabp4 protein.

Key words: Auxin-binding protein — In vitro transport — Processing — Trafficking — *Zea mays*.

The phytohormone auxin regulates plant growth and development, presumably by influencing one or more aspects of metabolism in various target tissues (Brummell and Hall 1987). At the cellular level, the primary response to auxin is observed several minutes after application of this phytohormone and affects the plasma membrane (Ephritikhine et al.), the Golgi apparatus (Ray 1985), the ER (Buckhout et al. 1981), the nucleus (Theologis 1986) and the cell wall (Kutschera and Briggs 1987). It is generally thought that receptor proteins could play an important role in the primary perception of phytohormones and therefore it is expected that the elucidation of the structural and functional characteristics of phytohormone receptor proteins might contribute to our understanding of the molecular mechanisms of phytohormone action. The search for auxin receptors has led to the identification of soluble and membrane-associated auxin-binding sites which are operationally termed auxin receptors (Hertel 1981, Palme et al. 1991). Of the many auxin-binding sites, "site I" has been the most thoroughly studied (for recent reviews see Jones 1990, Napier and Venis 1991, Palme and Schell 1991). "Site I" of maize corresponds to a protein named Zm-ERabp1 (*Zea mays* endoplasmic reticulum (ER)-associated auxin-binding protein). This protein was purified to homogeneity from microsomal fractions of maize coleoptiles and found to bind 1-naphthylacetic acid with a K_D of 2.4×10^{-6} M (Shimomura et al. 1986, Venis 1987, Hesse et al. 1989, Palme et al. 1990). Its primary structure was deduced from isolated cDNAs and was shown to contain amino- and carboxy-terminal signals which presumably are responsible for its targeting to—and retention within—the ER (Hesse et al. 1989, Inohara et al. 1989, Tillmann et al. 1989). We have found additional, related proteins of similar size which differ in their amino-terminal primary sequence as well as in their recognition by Zm-ERabp1-specific antibodies (unpublished results). Interestingly, polyclonal antibodies raised against Zm-ERabp1 also recognize antigenically related proteins in plasma membranes of tobacco protoplasts (Barbier-Brygoo et al. 1989, 1991).

The search for cDNAs that encode Zm-ERabp1 related proteins has led to the identification of Zm-ERabp4. Expression studies have shown that the gene encoding *Zm-ERabp4* is preferentially expressed in the female reproductive organs of maize (Hesse et al. 1989, 1993), possibly reflecting the well-documented role played by auxins in fruit development. Although the Zm-ERabp1 and Zm-ERabp4 proteins show a high degree of similarity in their primary amino acid sequences, the amino-terminal signal peptide sequences are very different (Hesse et al. 1993).

Abbreviations: Zm-ERabp, *Zea mays* auxin-binding protein; ER, endoplasmic reticulum; SRP, signal recognition particle.

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Proteins that are intracellularly transported to their final destination via the secretory pathway enter this pathway at the membrane of the ER: these proteins are recognized, targeted to and translocated across the membrane of the rough ER. A cytosolic ribonucleoprotein-complex is formed when the signal sequence is bound by the signal recognition particle (SRP) shortly after emergence from the ribosome. Targeting occurs via the SRP-translation complex by docking with a rough ER-specific SRP receptor known as "docking protein" (for review see Rapoport 1990). Translocation of the imported protein proceeds from the amino- to the carboxy-terminal end. In most cases, the amino terminal signal peptide is removed cotranslationally. Since the active site of the signal peptidase is located in the luminal side of the ER membrane (Walter et al. 1979), processing can take place only after a significant part of the imported protein, including the signal peptide, has been translocated across or buried into the ER membrane.

The knowledge of the intracellular location of the different isoforms of the Zm-ERabp family is fundamental if we are to understand the in vivo function or functions carried out by these proteins. We have focussed our research in this direction, and, as a starting point, we have set up an in vitro system that allows the processing and translocation of Zm-ERabp proteins in the presence of ER-derived microsomes. In the present work, we study the translocation of Zm-ERabp1 and Zm-ERabp4 proteins and demonstrate that a cotranslational modification of the nascent peptide can potentially affect its competence for translocation across the ER membrane.

Material and Methods

Enzymes and chemicals—Restriction enzymes, staphylococcal nuclease and glycopeptidase F were obtained from Boehringer Mannheim, Federal Republic of Germany. T4 DNA ligase was purchased from Bethesda Research Laboratories. RNA polymerase from *Escherichia coli* was obtained from Pharmacia-LKB Biotechnology Inc. (Sweden) and proteinase K was from Sigma. Unless stated otherwise all enzymes were used as instructed by the manufacturer. The cloning methods that were used are described in Sambrook et al. (1989) or Ausubel et al. (1989). L[^{35}S]methionine was purchased from Amersham International. Prestained molecular mass protein markers were from Biorad (F.R.G.).

In vitro transcription translation system—To carry out the in vitro transcription-translation experiments, the *Zm-ERabp1* and *Zm-ERabp4* cDNAs were cloned into the plasmid pDS6 (Bujard et al. 1987). The pDS6 derived plasmids were transcribed in vitro with *Escherichia coli* RNA polymerase, and the resulting mRNAs were translated in a wheat germ cell-free system as described by Stueber et al. (1984).

The wheat germ extract used in the translation experiments was prepared essentially as described by Roberts and Patterson (1973). To test for membrane translocation, canine pancreas microsomes or maize endosperm microsomes were included in the translation mix. Canine pancreas microsomes were prepared and treated with staphylococcal nuclease as described by Walter and Blobel (1983). Maize endosperm microsomes were isolated and treated with nuclease as described by Campos et al. (1988a).

Protease digestion—Digestions with proteinase K were done in 9 μl aliquots of the appropriate translation mixture. Incubation was performed for 30 min at 4°C with either 0.09 mg ml^{-1} of proteinase K or 0.09 mg ml^{-1} of proteinase K containing 1% sodium deoxycholate. Proteolysis was stopped by the addition of phenylmethylsulfonyl fluoride (2 mg ml^{-1}, final concentration).

Glycopeptidase digestion—Aliquots of the translation mixture were supplemented with 0.8 U of glycopeptidase F from *Flavobacterium meningosepticum* (PNGase F, EC 3.22.18) and an equal volume of the appropriate buffer to bring the solution to a final concentration of 150 mM $NaPO_4$ (pH 7.5), 10 mM EDTA, 1% Triton X-100 and 0.2% 2-mercaptoethanol. Samples were incubated for 5 hours at 37°C. The reaction was stopped by the addition of 12.5% TCA (final concentration), the pellets were washed once with acetone and dissolved in buffer for SDS-PAGE (Laemmli 1970). The gels were prepared for fluorography as described by Skinner and Griswold (1983). Films were preflashed as described by Laskey and Mills (1975).

Results

Structures of the Zm-ERabp signal peptides—Signal peptides involved in translocation into the ER typically have three distinct domains: a positively charged amino-terminal area, followed by a hydrophobic core and a polar carboxy-terminal region (von Heijne 1989). Amino acid sequences of the signal peptides of the Zm-ERabp1 and Zm-ERabp4 proteins were deduced from the corresponding cDNA clones (Hesse et al. 1989, 1993). The amino-terminal signal peptides are shown in Figure 1 (Zm-ERabp1, amino acid residues 1 to 38; Zm-ERabp4, amino acid residues 1 to 41). Structural analysis of these sequences revealed three structural domains characteristic for signal peptides. For Zm-ERabp1 the N-terminal domain contains a hydrophilic amino-terminal area (n-region) that carries negatively charged amino acids (aspartic and glutamic acid residues in positions 4 and 7). In addition an arginine residue is located at position 15. The hydrophobic core region has 12 amino acid residues (positions 19 to 30) and is bounded by polar amino acids. A polar carboxy-terminal region (c-region) is located at the end of the signal peptide. This region is preceded by a proline residue which is thought to

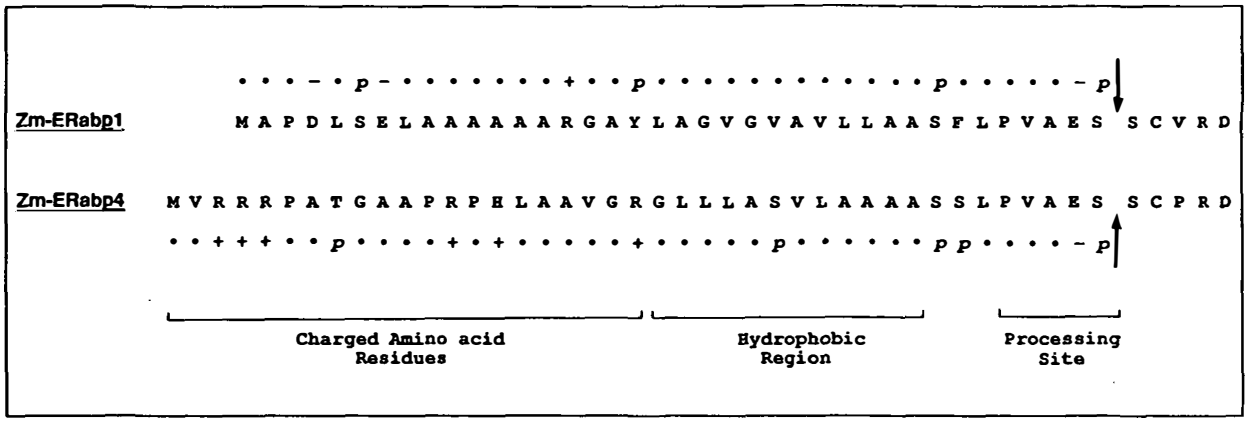

Fig. 1 Comparison between the signal peptides of Zm-ERabp1 and Zm-ERabp4 proteins. +, positively charged amino acid residue; p, polar amino acid residue; ●, hydrophobic amino residue; −, negatively charged amino acid residue.

interrupt the ordered secondary structure of the signal sequence thereby establishing the fidelity of signal peptidase cleavage. According to empirical rules describing signal peptide characteristics (von Heijne 1983, 1986, 1989, Perlman and Halvorson 1983), small and uncharged amino acids are usually found at positions −1 and −3. This was found to be the case for the Zm-ERabp1 sequence which has a serine (position 38) and an alanine (position 36) residue preceding the signal peptidase cleavage site (between serine residues 38 and 39). Whereas the signal peptidase cleavage site is identical in Zm-ERabp1 and Zm-ERabp4 signal peptides the rest of their amino acid sequences share little or no homology. For example, the Zm-ERabp4 signal peptide lacks the negatively charged amino acid residues found in the Zm-ERabp1 signal at positions 4 and 7. Instead Zm-ERabp4 has three positively charged residues (arginine residues in position 3 to 5). In total, the Zm-ERabp4 signal peptide contains six positively charged residues as compared to only one arginine residue in the Zm-ERabp1 signal peptide. Furthermore, the hydrophobic core region is interrupted by polar amino acid residues.

In vitro translocation of the Zm-ERabp1 protein—To investigate whether structural differences found in the Zm-ERabp signal peptides play a role in cotranslational processing and ER transport we inserted the *Zm-ERabp* coding sequences into a vector which allows in vitro synthesis of capped mRNA using *Escherichia coli* RNA polymerase (Figure 2). Translation of *Zm-ERabp* specific RNAs in a wheat germ cell-free system in the presence of [^{35}S]methionine yields highly specifically labeled proteins. These proteins can be directly analyzed by SDS-PAGE and visualized by autoradiography. Figure 3 (lane 1, pre) shows that *Zm-ERabp1* specific mRNA was translated into a protein with a molecular mass of approximately 22 kDa corresponding to the unprocessed Zm-ERabp1 precursor protein. This protein was sensitive to proteolytic digestion with proteinase K (Figure 3, lane 2). Addition of canine pancreas microsomes resulted in a reduction in molecular mass to approximately 18.5 kDa, indicating that the signal peptide had been processed (Figure 3, lane 3, indicated with p). Two additional forms of the Zm-ERabp1 protein were also synthesized in the presence of dog microsomes: the Gp protein and the mp protein (Figure 3 lane 3; see also Figure 4 lane 1). The mp protein appears as a very minor product that is barely visible. Most probably, the mp protein represents a processed form of the Zm-ERabp1 protein carrying an as yet unidentified modification that was generated during or after translocation of the peptide into dog pancreas

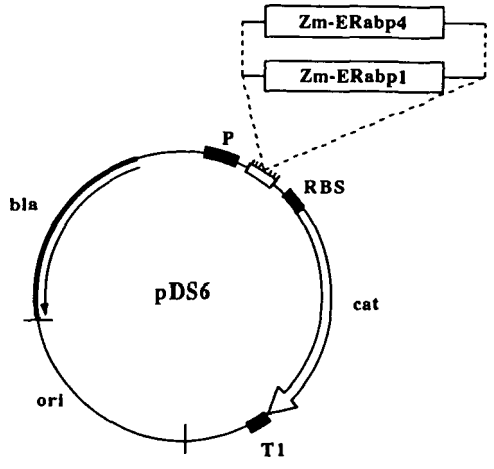

Fig. 2 Coupled transcription-translation vector pDS6-Zm-ERabp1 and pDS6-Zm-ERabp4. *Zm-ERabp* coding sequences were inserted in the restriction site between coli-phageT5 promoter (P) and an efficient transcriptional terminator (T_1). RBS, ribosomal binding site; cat, chloramphenicol transferase gene; ori, origin of replication, bla, β-lactamase gene.

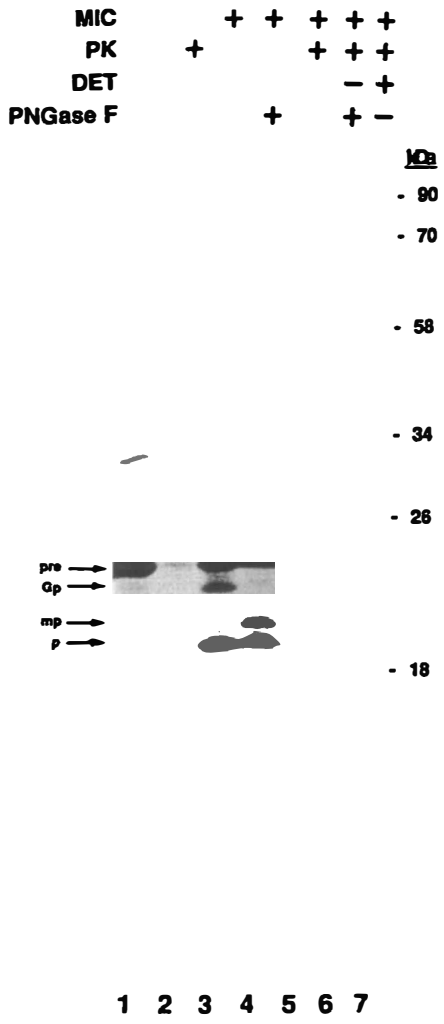

Fig. 3 Translocation of the Zm-ERabp1 protein into canine pancreas microsomes. The Zm-ERabp1 message was translated either in the presence or absence of canine pancreas microsomes (MIC), as indicated above the figure. After translation, proteinase K treatment (PK) was performed either in the presence or absence of sodium deoxycholate (DET). After proteinase K treatment the samples were digested with glycopeptidase F (PNGase F). Finally, the proteins were separated in a 15% polyacrylamide gel. The film was exposed for 4 hours. The position of molecular mass markers is indicated on the right.

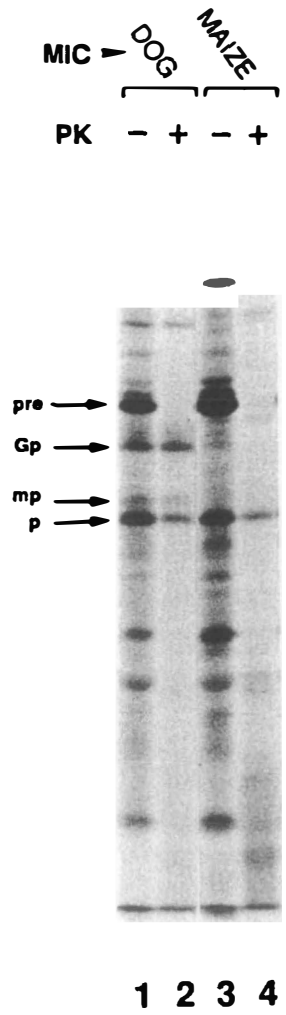

Fig. 4 Translocation of Zm-ERabp1 protein across canine pancreas membranes or maize endosperm membranes. The Zm-ERabp1 message was translated in the presence of canine pancreas microsomes (lanes 1 and 2) or maize endosperm microsomes (lanes 3 and 4). After translation, proteinase K (PK) treatment was performed (lanes 2 and 4). Proteins were separated in a 15% polyacrylamide gel. The film was exposed for 5 hours.

microsomes. The Gp protein corresponds to a processed and glycosylated form of the Zm-ERabp1 protein as shown by glycopeptidase F treatment (compare lanes 3, 4 and lanes 5, 6 in Figure 3). Upon hydrolysis of the carbohydrate moiety, the Gp protein is converted to the mp protein which becomes then very apparent. We conclude that the Gp protein carries two modifications in addition to processing: glycosylation and the unknown modification just mentioned.

Translocation was examined by posttranslational addition of proteinase K with or without detergent. Protein species that have been translocated into, and sequestered within, the canine pancreas microsomes should be protected from proteolytic digestion, but sensitive to protease digestion when the membrane is disrupted by detergent. The extent of translocation was determined by comparing the amount of protein protected from proteinase K digestion in the absence of detergent to the amount present before digestion. The results shown in Figures 3 and 4 show that p, Gp and mp forms of Zm-ERabp1 had been imported into the canine pancreas microsomes (compare

lanes 3, 5 in Figure 3 and lanes 1, 2 in Figure 4). When proteinase K digestion was performed in the presence of sodium deoxycholate all proteins were degraded because the ER membranes had been disrupted (Figure 3, lane 7).

Comparison of processing and translocation of Zm-ERabp1 protein in the presence of canine pancreas microsomes and maize endosperm microsomes did not reveal any differences in the electrophoretic mobility of processed Zm-ERabp1 protein (p; Figure 4). This indicates that both translocation systems process the Zm-ERabp1 protein and it is highly likely that in vitro processing of the Zm-ERabp1 protein by the signal peptidase occurs at the same cleavage site as it does in vivo (Hesse et al. 1989). The fact that the maize microsomes produce only the p form as processed and translocated protein further indicates that the p form, and not the mp form, is the primary processed product.

It is clear from Figure 4 that glycosylation of the Zm-ERabp1 protein occurs only in the presence of canine pancreas microsomes and not in the presence of maize endosperm microsomes (compare lanes 2 and 4). However, this is not surprising. At the stage at which microsomes were prepared from maize endosperm, the translation and translocation machineries were engaged mainly in the synthesis and accumulation of storage proteins (Larkins et al. 1984, Campos et al. 1988a). In addition, a sub-population of maize microsomal membranes was selected during the corresponding purification method (Burr and Burr 1981), on the basis of a high efficiency for the synthesis and translocation of maize storage proteins. Since these proteins are not glycosylated, we do not expect significant levels of glycosylation activity in the final microsomal sample from maize endosperm.

In vitro translocation of the Zm-ERabp4 protein— When Zm-ERabp4 cDNA was transcribed by *Escherichia coli* RNA polymerase and the resulting mRNA was translated in the wheat germ cell-free system in the absence of microsomes, we obtained two major proteins which were sensitive to proteinase K digestion as shown in Figure 5 (lanes 1 and 2, preA and preB). This surprising result prompted us to check whether any additional start or stop codon had been introduced as an artifact in the cDNA or in the expression vector during cloning. DNA sequencing showed that no nucleotide changes were introduced that could induce the synthesis of a novel protein during in vitro translation in our wheat germ cell-free system. In addition, to exclude the possibility that amino-terminal processing by proteases had occurred we used protease inhibitors and verified the amino acid sequence by N-terminal sequence analysis (data not shown).

It was of interest to see whether these proteins were processed and transported in vitro into canine pancreas microsomes. The results in Figure 5 (lanes 3 to 7) show that six proteins were protected against proteinase K digestion, indicating that they had been translocated into the microsomes (lane 5, proteins pA, pB, GpA, GpB, mpA and mpB). The pA and pB proteins correspond to processed non-glycosylated forms of the Zm-ERabp4 protein, whereas proteins GpA and GpB (lane 5) correspond to processed and glycosylated forms of the Zm-ERabp4 protein; this was demonstrated by glycopeptidase F treatment (Figure 5, compare lane 5 with 6). The proteins obtained after glycopeptidase F digestion, i.e. mpA and mpB, did not comigrate with the processed forms, pA and pB, in-

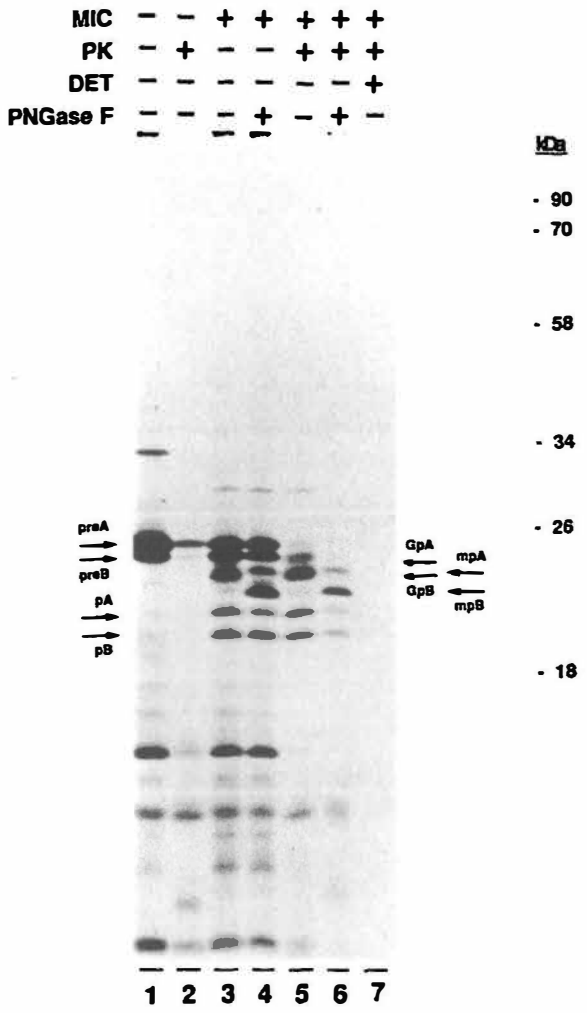

Fig. 5 Translocation of Zm-ERabp4 protein into canine pancreas microsomes. The Zm-ERabp4 message was translated either in the presence or absence of canine pancreas microsomes (MIC), as indicated at the top. After translation, proteinase K treatment (PK) was performed either in the presence or in the absence of sodium deoxycholate (DET). After proteinase K treatment the samples were digested with glycopeptidase F (PNGase F). Finally, the proteins were separated in a 15% polyacrylamide gel. The film was exposed for 4 hours. The position of the molecular weight markers is indicated on the right.

dicating that these proteins carry an additional posttranslational modification (Figure 5, see lane 6). A close inspection of lanes 3 and 5 in Figure 5 shows that low amounts of the mpB form were already present in the corresponding samples before glycopeptidase F treatment. It is not possible to distinguish the mpA form in lanes 3 and 5. Most probably, this protein is present but is not apparent in the complex electrophoretic pattern because it comigrates with the GpB protein. The parallelism existing between the mpA and mpB forms of the ZmERabp4 protein and the mp form of the Zm-ERabp1 protein suggest that the same unknown covalent modification has been introduced in all of them by dog pancreas microsomes.

From our experiments, we conclude that the Zm-ERabp4 related proteins, preA and preB, behave like the Zm-ERabp1 preprotein in the in vitro system. All these proteins are translocated, processed and glycosylated in the presence of dog pancreas microsomes. The size differences between preA and preB are unlikely to be located at the N-terminal presequence of Zm-ERabp4 since, otherwise, processing would have resulted in only one processed protein. We therefore conclude that the cotranslational modification occurs at an as yet unknown position within the mature portion of the Zm-ERabp4 protein.

When Zm-ERabp4 mRNA was translated in the presence of maize endosperm microsomes we observed, however, that only a protein corresponding to preB was translocated (Figure 6). This protein was correctly processed into pB (compare lanes 1, 2 with lanes 4, 5 in Figure 6). The preA protein, however, is not translocated by the maize microsomes. This failure does not seem to be due to a lack of translocation activity in the membranes because equivalent samples of microsomes were able to correctly translocate other control proteins as Zm-ERabp1, At-ERabp1 (Palme et al. 1992) and maize storage proteins (results not shown). The results indicate that the preA preprotein is specifically discriminated by the maize endosperm translocation machinery.

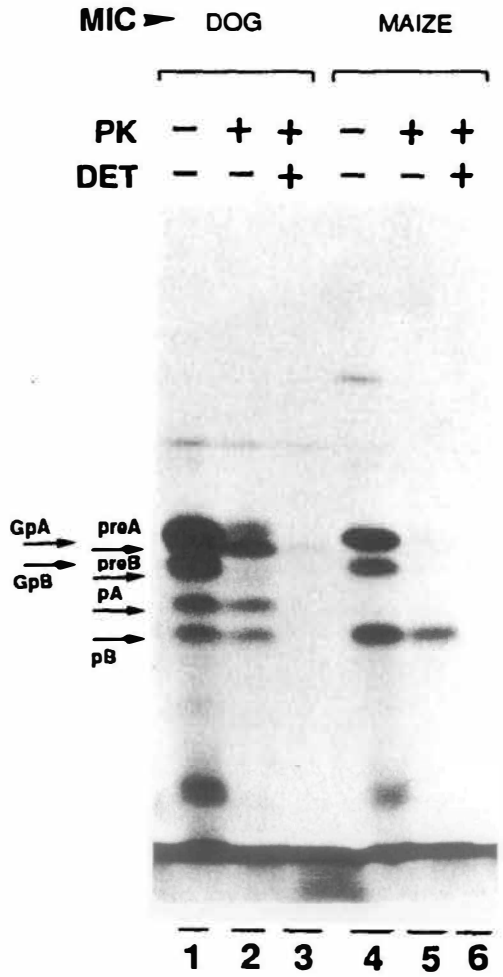

Fig. 6 Translocation of the Zm-ERabp4 protein across canine pancreas membranes or maize endosperm membranes. The Zm-ERabp4 message was translated in the presence of canine pancreas microsomes or maize endosperm microsomes as indicated. After translation, proteinase K treatment (PK) was performed either in the presence or absence of sodium deoxycholate (DET). Proteins were separated in a 12.5% polyacrylamide gel. The film was exposed for 4 hours. Note that a slight reduction in acrylamide concentration has a significant effect in the relative mobility of the preA and preB preproteins versus the GpA and GpB glycosylated proteins (compare with Figure 5). The apparent simplification of the electrophoretic pattern achieved by this reduction makes more obvious the relationship between the protein bands of lanes 1 and 4 in Figure 6.

Discussion

Signal peptides have been described for many plant precursor proteins destined for translocation across the ER membranes (Bassüner et al. 1984). Analysis of wheat germ and maize endosperm cell-free extracts has demonstrated that translocation mechanisms are similar to those operating in mammalian cells (Prehn et al. 1987, Campos et al. 1988a, b, Rapoport 1990). Translocation across the ER of plant cells requires a component with properties that are similar to the mammalian SRP. The maize endosperm ribonucleoprotein complex (SRP) has a sedimentation coefficient of 12S and contains a RNA of 8S (Campos et al. 1988a). In contrast to animal systems, there is a high variability in the 8S RNA population of maize endosperm. The maize 8S RNAs are slightly larger than the canine 7S RNA and sequence analysis has revealed that, although primary sequences show little similarities, the secondary structure of maize endosperm 8S RNAs and canine 7S RNA are strongly conserved (Campos et al. 1988b). Although several differences between mammalian and wheat germ translocation systems have been observed, it was found that, in

many cases, these systems could substitute for each other (Prehn et al. 1987, Campos et al. 1988a).

As several auxin-binding proteins from maize and *Arabidopsis* were found to contain a hydrophobic signal sequence at their amino-terminus, we analyzed translocation and processing of maize auxin-binding proteins in vitro. Although in vitro systems in most cases do not reach an efficiency allowing translocation of all competent translation products, we have demonstrated in this study that the Zm-ERabp proteins are translocated into microsomes. Our results suggest that this group of auxin-binding proteins are also translocated into the ER lumen in vivo. This confirms previously reported experiments where we were able to release the Zm-ERabp1 protein from ER enriched maize coleoptile fractions by treatment with Na_2CO_3 demonstrating that this protein is a luminal constituent of the ER in maize cells (Hesse et al. 1989).

Our demonstration of Zm-ERabp translocation into the ER is consistent with the presence of the KDEL motif at the carboxy-terminus of these proteins. This signal is known to facilitate retention of proteins in the lumen of the ER and thereby prevent its export through the Golgi apparatus (Munro and Pelham 1987, Pelham 1990, Hesse et al. 1989, Inohara et al. 1989, Palme et al. 1992).

In view of the marked diversity in primary structure of signal peptides it has been suggested that secondary or tertiary structures are critical for the successful interactions of this domain with the translocation and processing apparatus of the ER (Finkelstein et al. 1983). This correlates with our finding that, despite differences found in primary amino acid sequences, ERabp signal peptides from maize and *Arabidopsis* are sufficient for translocation into the ER in vitro (Palme et al. 1992). The structural differences between Zm-ERabp1 and Zm-ERabp4 signal peptides may still reflect functional differences in vivo which could result in varying uptake rates into the ER or release of a portion of Zm-ERabp4 into the cytosol. The full answer to this question will require detailed in vivo expression analysis of these proteins.

In vitro expression of the cDNA coding for Zm-ERabp4 resulted in two translation products (preA and preB) instead of one. This indicates that the wheat germ cell-free system is able to covalently modify the Zm-ERabp4 protein but not the Zm-ERabp1 protein. The presence of protein modification activities in wheat germ translation extracts is not surprising and has been previously described (Garcia et al. 1988). Since the presence of two different forms of the Zm-ERabp4 protein (pA and pB) is still apparent after processing of the corresponding preproteins by dog microsomes, we conclude that the observed modification is not located in the signal peptide but in the mature portion of the protein. It is possible that the covalent modification could result from partial carboxy-terminal proteolysis. This is an interesting possibility as removal of the KDEL motif in vivo could result in further export of the protein to other cellular locations such as the plasma membrane. This process could be consistent with the presence of proteins in the plasma membrane of the outer epidermal cells of maize coleoptiles that are antigenically related to Zm-ERabp1 (Hesse et al. 1989) and in the plasma membrane of tobacco protoplasts (Barbier-Brygoo et al. 1989, 1991); this area deserves further investigation. In particular, it would be very interesting to see whether the apparent decrease in molecular mass from 21 to 20.5 kDa (Shimomura et al. 1988) observed during purification of Zm-ERabp1 from maize coleoptile corresponds to the removal of carboxy-terminal amino acids.

However, the presence of two bands corresponding to the Zm-ERabp4 protein could also be due to a cotranslational modification consisting in the covalent linkage of an unknown molecule to the peptide in the wheat germ system. We favor this possibility against partial proteolysis of the carboxy terminus, since only one of the two forms of the Zm-ERabp4 protein (pre B) is competent for translocation across maize endosperm microsomes. It seems unlikely that partial proteolysis of the carboxy terminus could prevent translocation of the protein. On the other hand, it is possible that the addition of an extra moiety in the nascent peptide could hinder translocation or might even be a specific signal for disassembly of the translocation machinery. If this kind of phenomena occurred in vivo it could provide a way to abort translocation and release the protein into the cytoplasm. Although it is not possible to evaluate, at present, whether this mechanism actually has biological significance in vivo, it is worth noting that, in vitro, we observe abortion of translocation only in the homologous system: maize protein synthesized in a wheat germ system in the presence of maize microsomes. The fact that dog microsomes are able to translocate and process the two forms of the Zm-ERabp4 protein might indicate that the heterologous system is less specific. However, we can not rule out the opposite interpretation. Dog pancreas microsomes might be more competent and better suited than maize endosperm microsomes to reproduce in vitro the translocation process. Our observation that the translocation process can be aborted or prevented in in vitro systems is not an exception. Previous work demonstrated that the core protein of hepatitis B virus is targeted to the ER, but the translocation of this protein can be aborted after processing of the precore sequence (Garcia et al. 1988, Bruss and Gerlich 1988). More interestingly, experiments performed with the At-ERabp1 protein from *Arabidopsis thaliana*, a counterpart of Zm-ERabp proteins, also demonstrated that the translocation of this protein can be aborted after processing of the signal peptide (Palme et al. 1992).

Our work opens very interesting possibilities in what concerns the intracellular trafficking of the Zm-ERabp proteins and the mechanism of protein translocation. On the

other hand, it is clear that several important questions remain to be solved. It is essential to know, for instance, if our observations in vitro are biologically significant. Our present goal is to answer these questions by analyzing the expression of the Zm-ERabp cDNAs in vivo.

N.C. gratefully acknowledges a EMBO longterm fellowship. This work was supported by grants from HFSPO and EC-Bridge.

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