Signal recognition particle triggers the translocation of storage globulin polypeptides from field beans (*Vicia faba* L.) across mammalian endoplasmic reticulum membrane

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Hybridization-selected mRNAs coding for individual storage globulin polypeptides of field beans (*Vicia faba* L.) were translated in a cell-free system. Added mammalian signal recognition particle (SRP) recognizes cleavable signal peptides of the major vicilin and both legumin polypeptide precursors and induces translational arrest. The latter can be released by potassium-washed membranes (K-RM) leading to shortened polypeptides protected against proteases. Thus, SRP and K-RM function in a similar way with plant polypeptides as described for mammalian secretory proteins [(1981) J. Cell Biol. 91, 557–561]. Obviously, the initial steps in the biosynthesis and processing of plant storage globulin polypeptides are principally identical to those of animal secretory proteins.

Plant storage globulin polypeptide *Cell-free translation of individual mRNA species*
Signal peptide *Signal recognition particle* *Microsomal membrane* *Cotranslational processing*

1. INTRODUCTION

Legumin- and vicilin-like proteins, the major storage globulins of developing legume cotyledons consist of a number of polypeptide components which are: (i) synthesized on membrane-bound polysomes [1–3]; (ii) sequestered by and transiently associated with the RER [4–6]; (iii) likely to pass through the Golgi apparatus in an appropriate stage of cell development [4,7]; (iv) finally intracellularly located in membrane vesicles, called protein bodies [8,9].

A preliminary account of these results has been presented at the *3rd Symposium on Seed Proteins* held at Gatersleben, GDR, August 31 – September 2, 1983 [30]

Abbreviations: ER, endoplasmic reticulum; RER, rough endoplasmic reticulum; hPL, human placental lactogen; PMSF, phenylmethylsulfonyl fluoride; RM, rough microsomes; K-RM, potassium-washed rough microsomes; SRP, signal recognition particle

When mRNA coding for storage globulin polypeptides was injected into *Xenopus* oocytes the polypeptides were found to be secreted out of the cells indicating that the intracellular storage process in plant cells must have steps in common with extracellular secretion in animals [10]. Indeed, evidence for NH_2-terminal signal sequences guiding the storage globulin polypeptides across the ER membrane has been obtained for pea and french bean vicilin-like polypeptides by nucleotide sequence data of the cDNA or the genes [11,12]. Evidence was also obtained by cell-free translation of mRNA: For legumin-like polypeptides of soybean the presence of NH_2-terminal extensions was deduced for in vitro synthesized precursor molecules [13], whereas cleavable signal peptides for pea vicilin polypeptides were suggested by in vitro reconstitution experiments using microsomal membranes from dog pancreas [3]. However, with total cellular mRNA from developing plant cotyledons a clear precursor-product relationship is not easy to establish since mixtures of different polypeptides are synthesized which are, within

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each legumin or vicilin globulin group, immunologically related.

Here we solved this problem by the use of individual mRNA species purified by cDNA hybridization-selection coding for field bean storage globulin polypeptides [14]. Furthermore, the translation machinery contained in dog pancreas rough microsomes (RM) was fractionated into signal recognition particles (SRP) and potassium-washed rough microsomes (K-RM), devoid of SRP. The role of these constituents in co-translational translocation of mammalian secretory protein was discovered in [15–21].

We provide for the first time evidence for the translocation of field bean globulin polypeptides across the ER membrane in a way similar to that demonstrated for animal secretory proteins. Dog pancreas SRP, interacting with the signal peptide, exerts a site-specific translational arrest which is released by K-RM, leading to translocation of the nascent polypeptide chains into the lumen of the microsomes. Simultaneously, the NH_2-terminal signal peptide is cleaved off by the signal peptidase.

2. EXPERIMENTAL

Poly(A)-containing RNA from developing field bean cotyledons (*Vicia faba* L. var. minor cv. Fribo), pre-hPL mRNA and BMV RNA were isolated as in [22–24]. Plasmids of a cDNA bank were used to select individual mRNAs from field bean poly(A)-containing RNA by nitrocellulose filter hybridization [25]. The identity of the cDNA clones used has been established [14] and will be published in detail (in preparation). SRP and K-RM were isolated as in [17] except that EDTA-washed RM were employed. Cell-free translation in the wheat germ system using [^{3}H]leucine (6.92 TBq/mmol, 3.8 μM final conc.) or [^{35}S]methionine (45 TBq/mmol, 0.6 μM final conc.) was performed as in [22], except that Nikkol [17] and EGTA (M. Wiedmann, personal communication) were present in all translation assays at final concentrations of 0.002% and 4 mM, respectively. Final concentrations of 120 mM K^+ and 3.2 mM Mg^{2+} were used. Ten units of translocation activity for SRP and 1 unit for K-RM were used per 25 μl translation mixture [18]. K-RM were added 6 min after the start of translation. Post-translational proteolysis was carried out as follows: the incubation mixture was made 2 mM in tetracaine and 0.1 mM in puromycin and maintained for 8 min at 22°C [26]. The mixture was divided into two halves and subjected to proteolysis with Proteinase K (40 μg/ml) in the absence or presence of 0.5% Triton X-100 for 0.75 h at 4°C [27]. The reaction was terminated by addition of Contrycal (750 units/ml) and PMSF (2 mM) and by further incubation for 10 min at 4°C. Determination of radioactivity, immunoselection, labeling of authentic globulins, sample denaturation, SDS-gel electrophoresis and fluorography were performed as in [10]. The samples were denatured in the presence of 2-mercaptoethanol if not stated otherwise. X-ray film exposures were performed for 2 days with [^{35}S]methionine and for 15 days with [^{3}H]leucine, if not indicated otherwise. From translocation assays equal volumes of the initial translation mixture (10 μl) were applied to the gel slots; the corresponding tracks on the fluorographs can therefore be directly compared.

3. RESULTS

To study the processing of discrete storage globulin precursor polypeptides it was essential to purify individual mRNA species. For this purpose we made use of cDNA clones [14,28,29] bound to nitrocellulose filters and selecting mRNAs specific for Met-containing legumin, Met-free legumin and Met-free vicilin precursor polypeptides; i.e., preprolegumin A, preprolegumin B and previcilin (cf. [22,30]. Such plasmid-selected mRNA species stimulated the in vitro amino acid incorporation 1.5 to 13-fold over the endogenous background synthesis (fig.1). Only single storage globulin-related polypeptide bands were observed on SDS-gels if the stimulation was sufficiently high. This result demonstrates the high specificity of the selection procedure.

In a first set of experiments the processing of pre-hPL was studied primarily to prove the functioning of the translocation system. The synthesis of pre-hPL was efficiently suppressed in the presence of dog pancreas SRP (fig.2, track 2 *vs* 1). If K-RM were added, the translational arrest was released and the mature product was observed (track 4). K-RM alone did not affect the synthesis of pre-hPL and were inactive in processing (track 3). On the

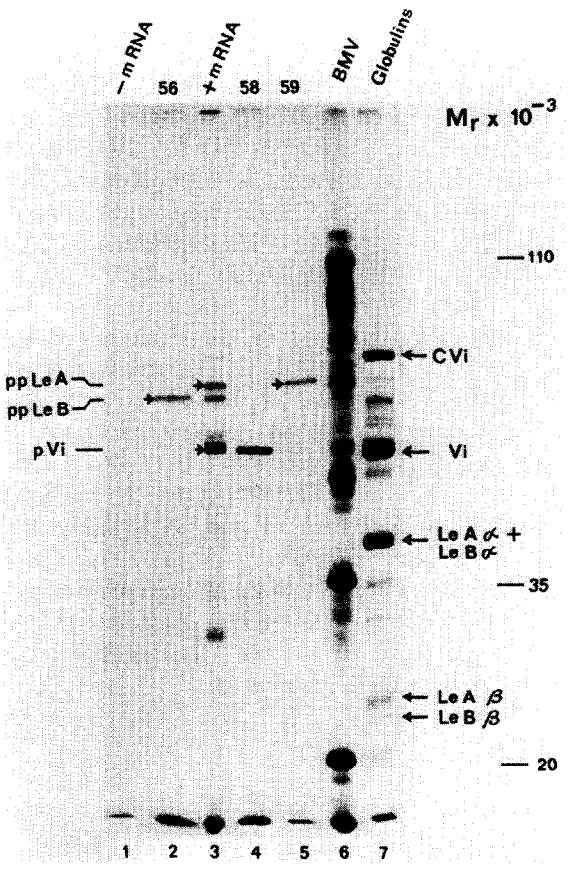

Fig. 1. Cell-free translation of hybridization-selected mRNAs coding for *Vicia faba* storage globulin polypeptides. Individual mRNAs were purified by filter hybridization using plasmids of field bean cDNA clones (indicated by a number at the top of the figure) and translated in a wheat germ cell-free system. Shown is a fluorograph obtained after SDS-gel electrophoresis (9.5% acrylamide) of the [^{3}H]leucine labeled translation products. +mRNA indicates the addition of total poly(A)-containing RNA from developing field bean cotyledons (track 3) whereas −mRNA shows endogenous translation products of the wheat germ system (track 1). The storage globulin-related in vitro translation products of total as well as individual mRNAs are designated as ppLe A, preprolegumin A (Met-containing); ppLe B, preprolegumin B (Met-free); pVi, previcilin (Met-free), (tracks 2–5). Authentic storage globulin polypeptides (^{14}C-labeled) are shown in track 7: CVi, convicilin; Vi, vicilin (Met-free); Le A_α and Le B_α, alpha chains of legumin subunit type A or B, respectively; Le A_β and Le B_β, beta chains of legumin subunits type A or B, respectively. The sizes of the translation products are estimated from those of the BMV RNA coded products (track 6).

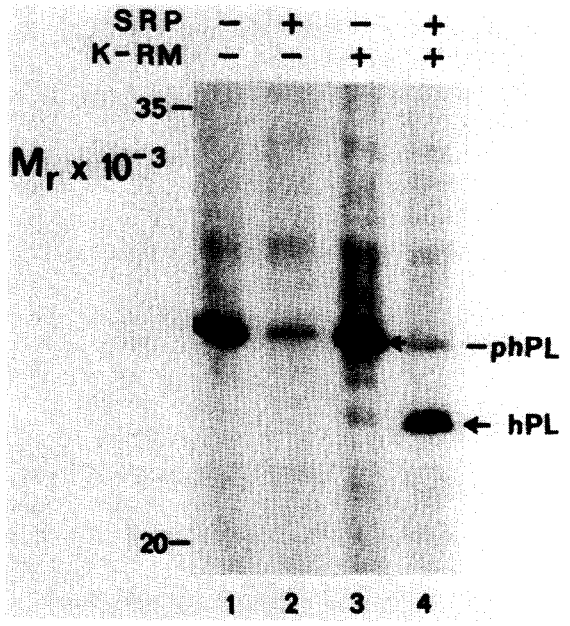

Fig. 2. The effect of SRP and K-RM on the in vitro synthesis and processing of pre-hPL. Pre-hPL mRNA was translated in the absence (tracks 1 and 3) or in the presence of SRP (tracks 2 and 4). K-RM were added to the translation mixtures run in tracks 3 and 4. Shown is a fluorograph of [^{3}H]leucine-labeled samples separated on a 10.6% acrylamide SDS-gel.

other hand, the synthesis of the non-secretory cytoplasmic protein globin was not inhibited by SRP (not shown). These results confirm earlier experiments with preprolocatin and pre-growth hormone [18–20], and support the general importance of SRP function in mammals.

Very similar results were obtained when mRNA coding for legumin A precursors was translated (fig.3). In the presence of dog pancreas SRP the synthesis of legumin A precursor (M_r 67 000) was strongly inhibited (track 4 *vs* 3). However, translation was restored when K-RM, which by themselves did not affect translation (track 5), were added too (track 6). The processed product was smaller than the original precursor by about M_r 1500. It was resistant to Proteinase K attack in the absence of the membrane solubilizer Triton X-100 (track 8) but was efficiently degraded in its presence (track 7). These results indicate that the legumin polypeptides were translocated across the ER membranes and shortened by removal of the signal peptide. Similar observations were made for

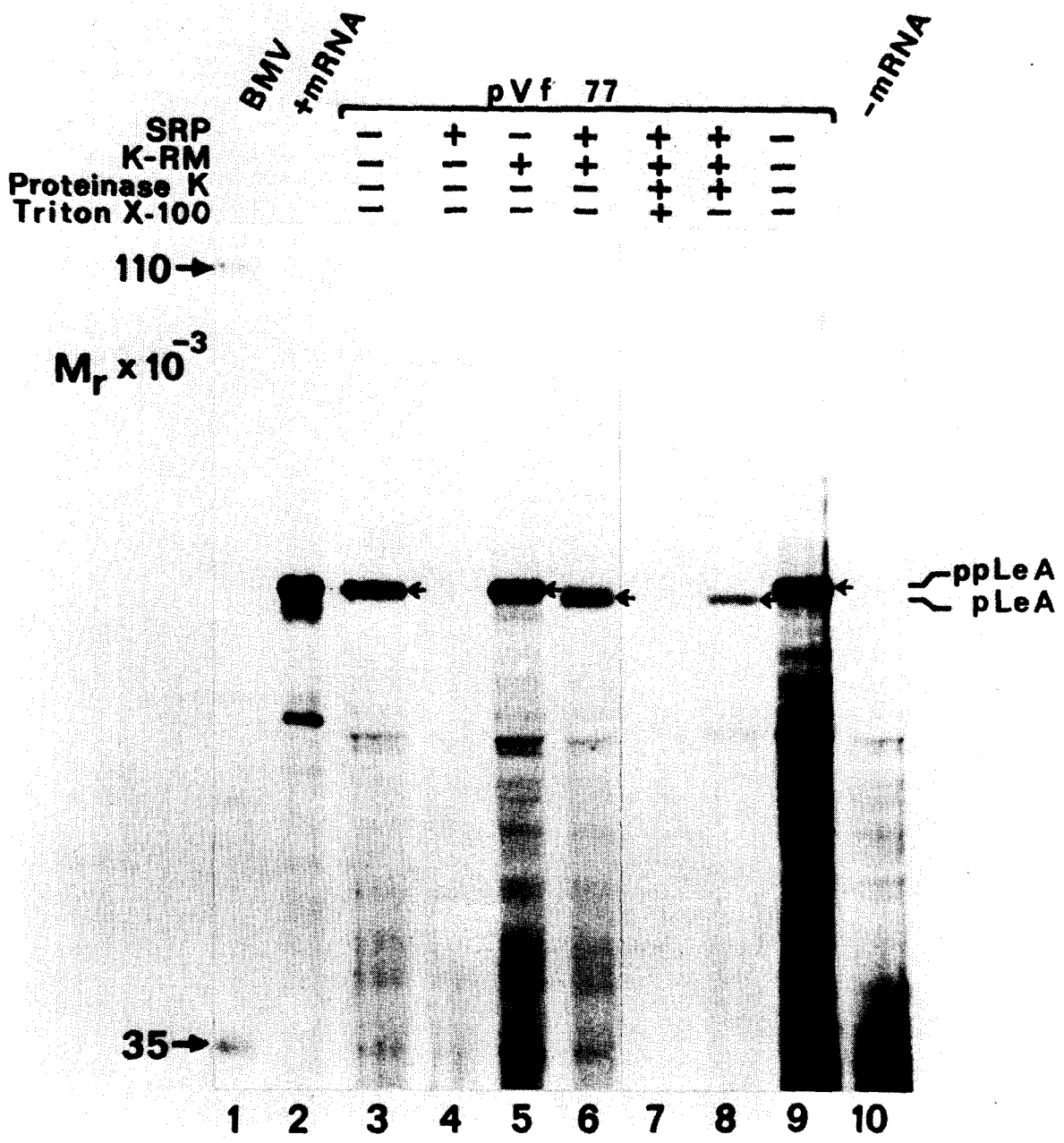

Fig. 3. The effect of mammalian SRP on the in vitro synthesis and processing of prolegumin A (pLe A) precursor polypeptides. Specific mRNA coding for preprolegumin A was isolated using the cDNA plasmid pVf 77 and translated in the cell-free system with [^{35}S]methionine either in the absence (tracks 3, 5, 9) or in the presence of SRP (tracks 4, 6–8). K-RM were added to samples run in tracks 5–8. Post-translational proteolysis was performed in the absence (track 8) or in the presence of Triton X-100 (track 7). Tracks 1 and 2 show BMV and field bean poly(A)-containing RNA coded products, respectively, whereas endogenous translation products are separated in track 10. The right part of the 8% acrylamide SDS-gel (tracks 7–10) was exposed twice as long as the left one. Abbreviations in fig. 1.

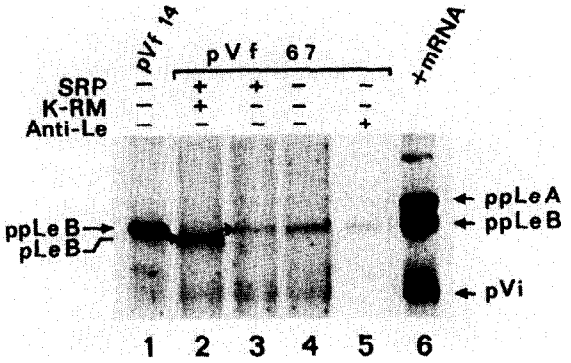

Fig. 4. The effect of mammalian SRP and K-RM on the in vitro synthesis and processing of prolegumin B (pLe B) precursor polypeptides. Specific mRNA was isolated using the cDNA clones no.14 (track 1) or 67 (tracks 2–5). Translation with [^{3}H]leucine was carried out in the presence or absence of SRP and K-RM as indicated. Immunoprecipitation with anti-legumin IgG of the sample shown in track 4 was performed (track 5). A fluorograph of a 9.5% acrylamide SDS-gel is shown. Abbreviations in fig. 1.

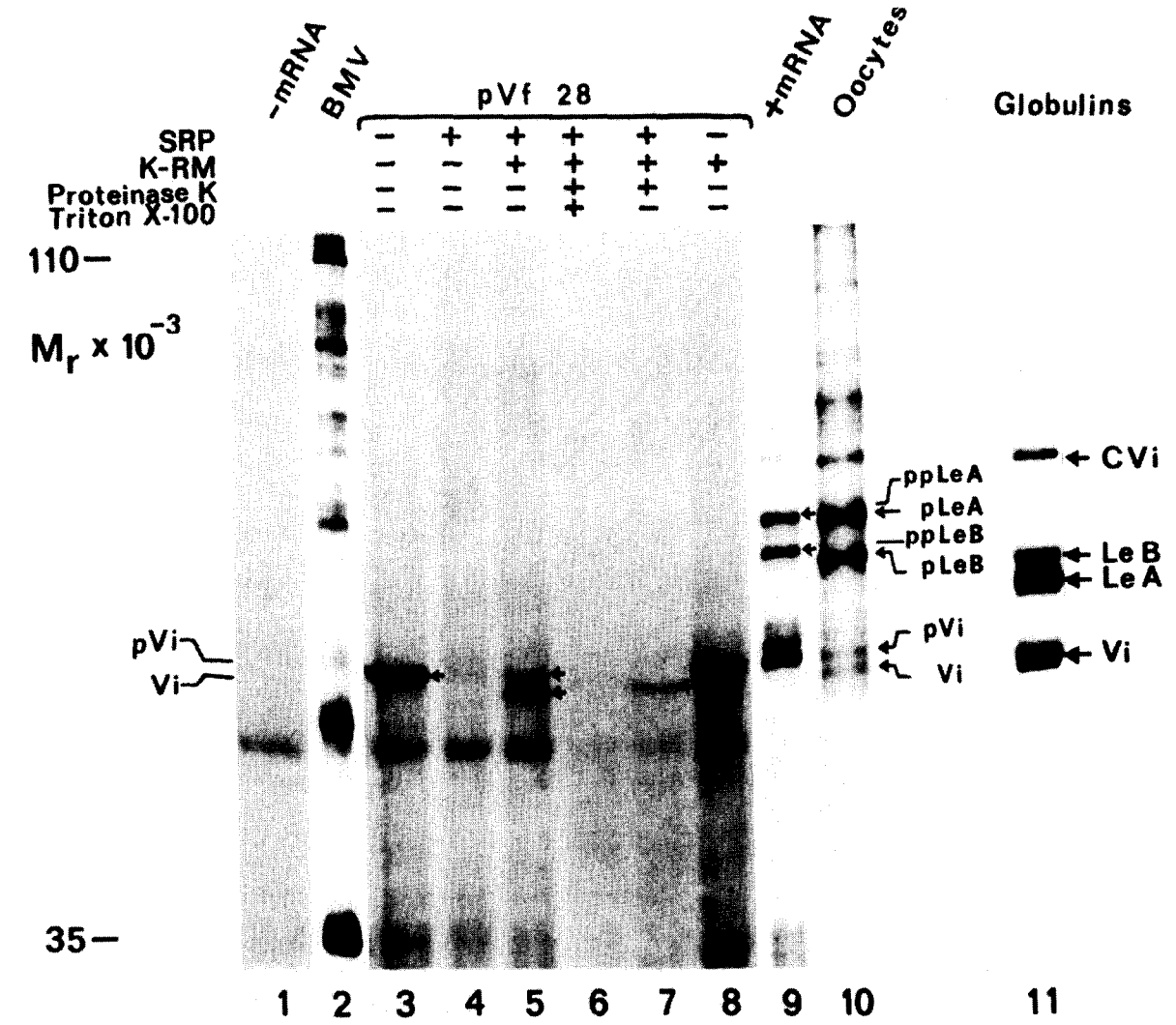

Fig. 5. The effect of mammalian SRP and K-RM on the in vitro synthesis of vicilin precursor polypeptides. Purified mRNA was translated using [^{3}H]leucine and the products were analysed on a 8% acrylamide SDS-gel. SRP and K-RM were present as indicated. Post-translational proteolysis was performed in the absence (track 7) or in the presence of Triton X-100 (track 6). Track 10 shows the products obtained after injection of poly(A)-containing RNA from developing field bean cotyledons into *Xenopus* oocytes. Authentic storage globulins were denatured before electrophoresis in the absence of SH-reagents (track 11, cf. fig.1, track 7). Tracks 1 and 3–8 were exposed 3 times longer than the rest of the gel. Abbreviations as in fig. 1, except Le A and Le B, indicating authentic legumin subunits type A (Met-containing) and type B (Met-free), respectively.

legumin B precursor polypeptides (M_r 64000) (fig.4). The resulting legumin polypeptides are still precursors to the mature legumin A and B subunits, since they can not be cleaved by SH-reagents into the acidic α- and basic β-chains (cf. fig.1, track 7). The mature subunits are most likely formed by further proteolytic processing yielding one α- and one β-polypeptide chain held together by disulfide bonds [22, 29].

The function of SRP and K-RM both in translation and translocation of plant polypeptides was also demonstrated for the predominant vicilin precursor (fig.5). Part of the vicilin pre-polypeptide (M_r 54000) remained unprocessed (track 5) and is completely degraded by post-translational proteolysis, whereas the product of lower M_r (52000) was protected (track 7). Removal of the signal peptides in vitro is also suggested by the fact that the polypeptides synthesized in the presence of SRP and K-RM correspond in size to those obtained in *Xenopus* oocytes after injection of total mRNA (fig.5, track 10), as well as to products obtained after in vitro read out of field bean polysomes [10,22,30,31]. Size comparison in SDS-gels appears to be valid since both legumin subunits and possibly the main vicilin subunit are non-glycosylated [32].

4. DISCUSSION

Recently, appreciable details of the mechanism of protein translocation across ER membranes have been elucidated. SRP was discovered [15–17, 21] which recognizes signal peptides and caused an elongation arrest [18–20], whereas an SRP receptor present in K-RM was found to release this arrest [33–35]. Further components involved in co-translational translocation of polypeptides are likely to exist in K-RM. Since, however, our knowledge is mainly based on studies with bovine preprolactin and pre-growth hormone mRNAs, confirmatory data from other species are desirable. Using pre-hPL mRNA to test the functioning of our translocation system we provide an additional example for the role of SRP in translocation of mammalian secretory proteins.

More importantly, we describe for the first time the use of individual plant storage globulin mRNA, dog pancreas SRP and K-RM (devoid of SRP) for co-translational translocation of plant polypeptides, providing evidence that mammalian SRP and signal peptidase recognize plant signal peptides. This result, together with the observation that dog pancreas SRP recognizes even a procaryotic signal peptide if synthesized in a eucaryotic cell-free translation system [36], indicates a ubiquitous importance of signal sequences. Our findings also support our previous conclusion that storage globulin polypeptides of legumes use a translocation mechanism across the ER membrane homologous to that of animal secretory proteins [10] which most likely involve an SRP-like factor present in the plant cell.

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