Translocation of Globin Fusion Proteins across the Endoplasmic Reticulum Membrane in *Xenopus laevis* Oocytes

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**Abstract.** We have studied the translocation of a normally cytoplasmic protein domain across the membrane of the endoplasmic reticulum in cell-free systems and in *Xenopus laevis* oocytes. Coding regions for the normally cytoplasmic protein globin were engineered in frame either 3' or 5' to the coding region for the signal sequence of either *Escherichia coli* b-lactamase or bovine preprolactin, respectively, in SP6 expression plasmids. RNA transcribed from these plasmids was microinjected into oocytes as well as translated in cell-free systems. We demonstrate that both in vivo and in vitro, a previously amino-terminal signal sequence can direct translocation of domains engineered to either side. Moreover, the domain preceding the signal sequence can be as large as that which follows it. While, in general, cell-free systems were found to faithfully reflect translocation events in vivo, our results suggest that a mechanism for clearance of signal peptides after cleavage is present in intact cells that is not reconstituted in cell-free systems.

A growing body of evidence suggests that translocation of newly synthesized secretory and membrane proteins across the endoplasmic reticulum membrane is directed by a series of interactions between discrete sequences within the nascent chains and receptors (1). The mechanism by which this occurs has been studied by two general approaches. One approach used fractionation and reconstitution of translocation activity to identify critical components. This approach led to the discovery of signal recognition particle (SRP)¹ and the SRP receptor and to elucidation of the phenomenon of elongation arrest (4, 5, 12, 13, 18, 19, 22, 23, 25). A second approach involves the use of molecular genetic techniques to prepare plasmids encoding altered proteins which contain domains that direct the translocation process (i.e., signal and stop-transfer sequences) placed in an unusual location. This second approach has demonstrated that normally cytosolic proteins can be redirected to the endoplasmic reticulum lumen by a signal sequence, that translocation can be uncoupled from translation, and that amino-terminal signal sequences have the intrinsic ability to direct translocation of both amino and carboxyl flanking domains (9, 15, 16).

One limitation of both approaches has been that they were carried out in cell-free systems. To study translocation events in vivo, we decided to microinject *Xenopus* oocytes. They present several advantages for our purposes: large numbers of constructions can be rapidly introduced by microinjection of the same (unpurified) transcript that is used for cell-free translation studies (3); expression is sufficiently massive that, in many cases, the resultant translation products immunoprecipitated from one oocyte labeled with [³⁵S]methionine for 1 h can be detected after a 1-d exposure following SDS PAGE and autoradiography; a variety of probes including RNA (6), antibodies (14, 21), cell fractions (17), and drugs (2) could potentially be rapidly introduced.

As a first step towards corroborating and extending findings from cell-free systems, we have studied the translocation of globin domains in engineered fusion proteins. In one fusion protein, the globin domain is preceded by the signal sequence for b-lactamase, and in a second set of fusion proteins the globin domain itself precedes the signal sequence of preprolactin. Our results show that a signal sequence is sufficient to direct translocation of a cytoplasmic protein, and that preprolactin is capable of directing the translocation of both amino and carboxyl flanking domains in oocytes, thus extending our previous findings in cell-free systems. Our results also show that, contrary to the results obtained in cell-free systems, the signal sequence and associated amino-terminal domain generated by signal peptidase cleavage seems to be rapidly and preferentially degraded in oocytes. In contrast, the “passenger” domain from which the signal sequence has been cleaved, as well as residual full-length precursor in the cytosol, are longer-lived.

These observations demonstrate that, in general, events observed in cell-free systems are a faithful reflection of events occurring in living cells. However, some additional translocation-related events not reconstituted in vitro may be assayed in *Xenopus* oocytes.

**Materials and Methods**

**Materials**

Rabbit anti-human globin antisera was obtained from Cappel Laboratories,
Constructions

All constructions were engineered into the pSP64 vector which was obtained from Promega Biotech. A chimpanzee α-globin cDNA cloned into the Pst site of pBR322 was kindly provided by S. Liebhaber, University of Pennsylvania School of Medicine, Philadelphia, PA. This plasmid, pMC18, was cut with XmnI in the presence of ethidium bromide, followed by timed digestion with Bal31, re-cut with NcoI, treated with Klenow, and T4 DNA ligase. From the resulting transformants a perfect fusion of the lactamase signal sequence with the initial methionine of globin was recovered and subcloned into pSP64 as a Hind III–Pst I fragment. This plasmid was termed pSP125E. pSPG1E was derived from pSP125E by deletion of the signal sequence coding region and a portion of 3′ untranslated region as a result of cleavage with Bgl II, Nco I, treatment with Klenow, and T4 DNA ligase. Details for the pSPGPI construction can be found in reference 14. pSPG2P was derived from pSPG1P by subcloning a BssH II–BstE II fragment containing two globin coding regions into the BssH II–BstE II sites of pSPGPI.

In Vitro Synthesis of Capped Transcripts

Cesium-purified plasmids were linearized at sites in the 3′ untranslated region, extracted in phenol/chloroform, ethanol precipitated, and dissolved in water. Translation was carried out in 10-μl volumes containing 2.5 μg linearized DNA, 20 μg SP6 polymerase, 0.25 mg/ml calf liver tRNA, 0.5 mM ATP, 0.5 mM CTP, 0.5 mM UTP, 0.1 mM GTP, 0.5 mM GpppG (caps), 10 mM dithiothreitol, 2 mM spermidine, 6 mM MgCl2, 40 mM Tris-Cl, pH 7.5, and 0.9 U/ml human placental RNase inhibitor. Reactions were carried out at 40°C for 1 h and aliquots of total reaction mix were used directly for injection or in vitro translation.

Cell-free Translation

4-μl aliquots of total transcription products were added to translation reactions of 20-μl vol containing 8.6 μl of rabbit reticulocyte lysate prepared as described (1). 1 μCi/ml [35S]methionine, 0.2 mM each of the other 19 amino acids, methionine, 16 mM Tris–HCl (pH 7.5), 100 mM KCl, 2 mM MgCl2, 0.44 mM spermidine, 2 mM dithiothreitol, 0.9 mM GTP, 1 mM ATP, 10 mM creatine phosphate, 0.4 mg/ml creatine phosphokinase, 0.1 mg/ml calf liver tRNA. Reaction mixtures were incubated at 24°C for 60 min in the absence or presence of 2 U/ml dog pancreas rough microsomes (prepared as described, reference 24).

Posttranslational Protease Protection

After a 1-h incubation at 24°C in the presence of membranes, translation reaction mixtures were chilled on ice, adjusted to 10 mM CaCl2, and divided into equal aliquots of 5 or 10 μl. Some were treated with proteinase K (dissolved in 10 mM CaCl2, 50 mM Tris [pH 7.5], and preincubated at 37°C for 15 min) at a final concentration of 0.3 mg/ml either in the presence or absence of 1% Nikkol (a nonionic detergent used to disrupt the lipid bilayer). Proteinase digestion was stopped by the addition of 2 mM phenylmethylsulfonyl fluoride (PMSF) and samples were immediately heat shocked to 100°C, then incubated at 100°C for 10-15 min. Samples were diluted 20-fold in a solution of 1% Triton, 0.1 M Tris–HCl (pH 8.0), 100 mM EDTA, 100 mM NaCl, subjected to immunoprecipitation with either 0.5 μl anti-prolactin antiserum or 4 μl anti-globin antiserum and protein A-Sepharose-CL4B, followed by SDS PAGE.

Microinjection of Xenopus Oocytes

Xenopus oocytes were manually dissected and were subsequently injected and labeled in modified Barth's saline solution (MBSH) containing [35S]methionine as described (6, 20). MBSH contains 88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO3, 0.82 mM MgSO4, 0.33 mM Ca (NO3)2, 0.41 mM CaCl2, 10 mM Hepes (pH 7.6).

Immunoprecipitation of Oocyte Translation Products

Medium and oocytes were separated, and the medium was then diluted in 4× vol Buffer A (1% Triton X-100, 0.1 M Tris–HCl (pH 8.0), 0.1 M NaCl, 0.01 M EDTA, 1 mM PMSE); oocytes were homogenized in 20 μl/oocyte of Buffer A. 2-10 μl antiserum was added to each sample, and samples were incubated overnight at 4°C. After incubation, samples were centrifuged in an Eppendorf centrifuge at 10,000 g for 1 min to remove aggregates, and the supernatants were then transferred to a fresh tube containing 15 μl of a 50% slurry of protein A-Sepharose in Buffer A. Samples were incubated for 1 h at 4°C, washed three times with Buffer A, and twice subsequently with buffer containing 0.1 M Tris–HCl (pH 7.5) and 0.1 M NaCl to remove residual Triton.

Figure 1. Line diagrams of relevant regions of SP6 expression plasmids.
Figure 2. Cell-free translation of pSPI25E. Transcription-linked wheat germ translations programmed with pSPI25E were carried out as described in Materials and Methods. Reactions were carried out either in the absence (lanes A and B), or presence (lanes C-E) of dog pancreas rough microsomes. Some samples were subsequently treated with trypsin in the absence (lane D) or presence (lane E) of 1% Triton. Samples were immunoprecipitated with anti-globin antiserum (lanes B-E), or nonimmune serum (lane A), subjected to SDS PAGE, and viewed by autoradiography. Downward-pointing arrowheads indicate the signal sequence-containing precursor, pre125E; upward-pointing arrowheads indicate the processed molecule, 125E, from which the signal sequence has been cleaved.

Pulse-Chase

Oocytes were injected with in vitro-synthesized transcripts, preincubated for 5 h, and pulse-labeled for 1 h in MBSH containing 5 mCi/ml 3S-met. Labeled medium was then removed, oocytes were briefly washed in cold MBSH, and then incubated for varying lengths of time in MBSH containing 10% fetal calf serum and 20 mM unlabeled methionine.

Subcellular Fractionation of Injected Oocytes

Freshly labeled oocytes were homogenized with 40 μl/oocyte at 4°C in an isoosmotic buffer containing 10% (wt/vol) sucrose, 150 mM NaCl, 10 mM MgAc, 20 mM Tris–HCl (pH 7.6) (Buffer B). 500 μl of total homogenate was layered over a 1-ml cushion of 20% sucrose (wt/vol), 50 mM NaCl, 10 mM MgAc, 20 mM Tris–Cl (pH 7.5) (Buffer C), and spun at 15,000 g for 30 min in the ultracentrifuge. The top 50 μl (cytosolic components) was removed, the rest of the supernatant aspirated, and the pellet (vesicles) resuspended in Buffer A.

Proteolysis of Oocyte Vesicles

Freshly labeled oocytes were homogenized with a ground glass homogenizer in 40 μl/oocyte of Buffer B. Each sample was divided into three aliquots: the first aliquot was a control with no added protease, and the other two aliquots were treated with 0.3 mg/ml Proteinase K in the presence or absence of 1% Triton X-100. All samples were incubated for 3 h at 4°C. Protease digestion was stopped by the addition of 2 mM PMSF followed by boiling in 2× vol 2% SDS in 0.1 M Tris-Cl (pH 8.9) for 15 min. Samples were then diluted 20-fold in a solution of 1% Triton, 0.02 M Tris (pH 8.0), 2 mM EDTA, 20 mM NaCl, and subjected to immunoprecipitation followed by gel electrophoresis.

Results

We have previously shown that the lactamase signal sequence together with the first five amino acids of authentic lactamase is sufficient to direct globin to the lumen of microsomal vesicles in cell-free systems (9). We initiated the present study by extending these initial observations using a construct encoding a perfect fusion between the signal and globin coding regions placed behind the SP6 promoter (pSPI25E). Fig. 1 displays line diagrams of the relevant coding regions for plasmid pSPI25E and other plasmids studied here. RNA was transcribed from this plasmid using SP6 polymerase, and was translated in a wheat germ cell-free system in the

Figure 3. (a) Subcellular fractionation and proteolysis of GIE (globin) and BP3 (prolactin) in Xenopus oocytes. Oocytes were injected with SP6 transcripts of pSpgIE (lanes A, B, E-G) or pSpbp3 (lanes C, D, H-J), labeled with [35S]methionine, and vesicles prepared as described. Cytosolic (lanes A and C) and vesicle (lanes B and D) fractions were separated as described. Samples were assayed by protease protection (lanes E-J). Samples were incubated in the absence (lanes E and H) or presence (lanes F, G, I, and J) of proteinase K, some with 1% Triton (lanes G and J) added. Samples were immunoprecipitated either with anti-globin (lanes A, B, E-G) or antiprolactin (lanes C, D, H-J) antisera and subjected to SDS PAGE. Gels were fluorographed, and bands were visualized by autoradiography. Large arrowheads indicate mature prolactin and small arrowheads indicate globin. (b) Expression and subcellular localization of 125E in Xenopus oocytes. Oocytes were either injected with pSPI25E transcripts (lanes A, B, E-J) or were not injected (lanes C and D) and subsequently labeled for 1 h as described. Some of the injected oocytes were separated into cytosolic (lane E) and vesicle fractions (lane F). Other injected oocytes were homogenized in isoosmotic buffer and were subsequently treated with proteinase K in the absence (lane H) or presence (lane I) of detergent, or were untreated (lane G), as described in Materials and Methods. Samples were immunoprecipitated with anti-globin serum (lanes A, C, E-F) or nonimmune serum (lanes B and D), analyzed by SDS PAGE, and viewed by autoradiography.
absence and presence of microsomal membranes prepared from dog pancreas. In the absence of membranes, a single globin-immunoreactive product (termed pre125E), which was of higher molecular weight than authentic globin, was observed (Fig. 2, lane B). In the presence of membranes, an additional globin-immunoreactive band (termed 125E), which co-migrated with authentic globin, was observed (Fig. 2, lane C). To determine the localization of these two protein species, proteases were added after completion of translation. Pre125E was localized outside of the microsomal vesicle (i.e., in the topologic equivalent of the cytosol), since it was degraded by added proteases. However, 125E was quantitatively protected from protease (Fig. 2, lane D) unless nonionic detergent was added to solubilize the protecting lipid bilayer (Fig. 2, lane E), thus indicating the microsomal vesicle lumen as its location.

To determine the subcellular localization of 125E in vivo, transcripts were microinjected into Xenopus oocytes. Control oocytes were co-injected with transcripts encoding a cytosolic protein (GIE, authentic full length globin without a signal sequence) and a normally secreted protein (BP3, full-length preprolactin). Fig. 3 a shows that after subcellular fractionation, GIE was localized to the cytosol (lanes A and B), whereas BP3 was in the vesicle fraction (lanes C and D). When injected oocytes were homogenized in an isosmotic buffer and subsequently incubated with proteinase K, GIE was completely degraded (Fig. 3 a, lanes E–G), whereas BP3 was completely protected from digestion (Fig. 3 a, lanes H–J). When pSP125E transcripts were injected into Xenopus oocytes, a single globin-immunoreactive product was observed after a 1-h pulse with [35S]methionine (Fig. 3 b, lane A). Neither nonimmune control precipitations (Fig. 3 b, lanes B and D), nor anti-globin immunoprecipitation of mock-injected oocytes (Fig. 3 b, lane D) demonstrated globin-immunoreactive products under these conditions. This product, which co-migrated with 125E, was demonstrated by cell fractionation to be located quantitatively in the vesicle fraction of the oocyte (Fig. 3 b, lanes E and F). Moreover, proteolysis demonstrated that, like its cell-free counterpart (see Fig. 2), the mature 125E product was localized to the vesicle lumen (Fig. 3 b, lanes G–I). We conclude that both in intact cells and in cell-free systems, an amino-terminal signal sequence is sufficient to direct a normally cytoplasmic protein across the endoplasmic reticulum membrane. Elsewhere we will demonstrate that this mature globin localized to the ER lumen can be transported through the secretory pathway and secreted into the medium (Simon, K., and V. R. Lingappa, manuscript in preparation).

Having established that a simple substrate displays similar translocation behavior in vitro and in vivo, we studied more complex constructions. Recently, we engineered a globin coding region 5' to the normally amino-terminal signal sequence of bovine prolactin. Plasmid pSPG1 encodes a fusion protein comprised of the initial 109 amino acids of globin followed in frame by the signal sequence (30 amino acids) and the entire coding region of bovine prolactin (199 amino acids). When pSPG1 was expressed in a cell-free transcription–translation system, the former amino-terminal prolactin signal sequence still directed translocation of the prolactin domain with concomitant cleavage of the signal sequence (15). Moreover, the globin domain flanking the signal on the amino terminus was also translocated, albeit with apparent reduced efficiency.

To extend these studies, we constructed a similar chimeric protein in which part of a second globin-coding region was engineered into that of pSPG1. This new plasmid, pSPG2P, encoded 250 amino acids of globin (parts of two globin-coding regions arranged in tandem) followed by the preprolactin signal sequence (30 amino acids) and mature prolactin (199 amino acids) (see Fig. 1). When expressed in the transcription-linked translation system from reticulocyte lysate, a single polypeptide of 45,000 D, termed preG2SP, was observed (Fig. 4, lane A); this product was both globin and prolactin immunoreactive (Fig. 4, lanes A, C, and F). When membranes were present during translation, two new products were observed. One of these membrane-dependent products, termed G2SI, was globin but not prolactin immunoreactive, and this peptide was the predicted size for the globin domains joined to the signal sequence (Fig. 4, lane C). The other product, termed PI, was prolactin but not globin immunoreactive, and it co-migrated with mature bovine prolactin (Fig. 4, lane F). Digestion with protease revealed that, as observed previously with GSI (15), some, but not all, G2SI chains were protected from protease in the absence of detergent (Fig. 4, lane D). In the same aliquot, essentially all PI chains were protected from protease in the
absence of detergent (Fig. 4, lane G). Thus, enlarging the domain preceding the signal sequence appeared to have little effect on either promoting or preventing translocation of that domain. These results have been reconfirmed by using glycosylated domains (15).

These results suggest that translocation of a single globin domain engineered 5′ to a signal sequence was not a fortuitous result limited to domains of a narrow range of sizes. To determine whether the unusual translocation events which were consistently observed in cell-free systems also occurred in intact cells, pSPGP1 and pSPG2P transcripts were expressed in Xenopus oocytes.

Oocytes were injected with transcripts of pSPGP1 and pSPG2P, and after an overnight preincubation to allow for equilibration of RNA, they were continuously labeled with [35S]methionine for 3 h. Newly synthesized products were immunoprecipitated with either anti-prolactin (Fig. 5, lanes A and C) or anti-globin (lanes B and D) serum and analyzed by SDS PAGE. Immune-reactive bands corresponding to the translation products in cell-free systems were observed. In contrast to previous cases (e.g., I2SE and prolactin, see Fig. 3), the products expressed in oocytes from both pSPGP1 and pSPG2P included full-length precursor (pre-GSP and preG2SP). These results demonstrate that although oocytes are highly efficient at translocation of correctly targeted chains, full-length precursors can be detected when translocation efficiency is sufficiently perturbed.

The localization of products was first assessed by cell fractionation, which revealed that P1 and G2S1 were predominantly associated with vesicles, whereas the preG2SP precursor was localized predominantly to the cytosol (Fig. 6). Localization was also studied by protease protection assay. Consistent with the expectations from cell fractionation, preG2SP was almost completely degraded, whereas P1 was protected from proteases unless nonionic detergents were added to disrupt the protecting lipid bilayer (Fig. 7, lanes D–F); quantitative densitometry revealed that 20–30% of total G2S1 chains were protected. Similar cell fractionation and proteolysis results were obtained when transcripts of pSPGP were expressed in oocytes (data not shown). Thus, when a previously amino-terminal signal sequence is engineered to an internal location, both flanking domains can be translocated across the endoplasmic reticulum membrane in cell-free systems and in vivo.

In contrast to our results in cell-free systems, where the GSI (15) and G2S1 chains (Fig. 4) were generated in a 1:1 ratio with P1, much less GSI and G2S1 than P1 was detected in oocytes (see Fig. 5). Densitometric quantitation corrected for methionine content showed that the globin-containing chains we detected represented only 10% of the amount expected if each prolactin molecule had been generated from a peptidase-cleaved full-length precursor. One possible explanation for the non-stoichiometric amounts of globin-containing prolactin chains seen in oocytes is that translation had actually initiated from an internal methionine within the globin-prolactin domains. If this were the case, P1 over-expression would simply reflect a preference for initiating translation at the internal rather than the initial AUG. To test this possibility, injected oocytes were incubated in the presence of hydroxyeul-

**Figure 5.** Expression of pSPGP and pSPG2P in Xenopus oocytes. Groups of eight oocytes were injected with pSPGP (lanes A and B) or pSPG2P (lanes C and D) transcripts, preincubated overnight, and labeled for 3 h with [35S]methionine as described. Oocytes were then homogenized in Buffer A, and each set was divided into two aliquots which were immunoprecipitated with anti-prolactin (lanes A and C) or anti-globin (lanes B and D) serum, respectively. Downward-pointing arrowheads indicate preGSP and preG2SP; smaller upward-pointing arrowheads indicate peptides migrating to the position of authentic prolactin in lanes A and C, and indicate immunoreactive globin fragments generated from cleavage of the internal signal sequence in lanes B and D. Much larger amounts of precursor are observed in lanes B and D as compared with lanes A and C because 10x vol was used in lanes B and D in order to visualize the faint globin-immunoreactive bands.

**Figure 6.** Subcellular fractionation of G2P expressed in Xenopus oocytes. Oocytes were injected with pSPG2P transcripts, preincubated overnight, and then labeled for 6 h with [35S]methionine as described. Groups of 10 injected oocytes were homogenized in isosmotic buffer and separated into a cytosolic (lanes A and C) and vesicle (lanes B and D) fraction. Each fraction was immunoprecipitated with anti-globin (lanes A and B) or anti-prolactin (lanes C and D) antiserum, respectively. Samples were analyzed by SDS PAGE, and viewed by autoradiography. Downward-pointing arrowheads refer to preG2SP; small upward-pointing arrowheads refer to chains migrating to the position of authentic prolactin in lanes C and D and to the immunoreactive globin fragments generated from cleavage of the signal sequence in lanes A and B.
Proteolysis of G2P in Xenopus oocytes. Groups of 15 oocytes were injected with pSPG2P transcripts and then labeled for 6 h with [35S]methionine as described. Labeled oocytes were homogenized in isoosmotic buffer and digested with proteinase K in the absence (lanes B and E) or presence (lanes C and F) of 1% Triton; lanes A and D show total products in the absence of proteolysis. Each sample was then divided into two aliquots, and one set was immunoprecipitated with anti-prolactin (lanes A–C), and the other with anti-globin (lanes D–F) serum. Samples were analyzed by SDS PAGE and viewed by autoradiography. Downward-pointing arrowheads refer to G2SP; upward-pointing arrowheads refer to chains migrating to the position of authentic prolactin (lanes A–C) or to immunoreactive globin fragments (lanes D–F). Equivalent amounts of oocyte products were used for lanes A–C and D–F; however, the former lanes were from a shorter exposure, to avoid overexposure of the prolactin-reactive bands.

Discussion

We initiated these experiments for several reasons. First, we wished to extend our earlier results which suggested that, when placed in an internal location, a previously amino-terminal signal sequence could direct the translocation of both amino- and carboxy-terminal domains. In the present results of a pulse–chase experiment in which all products generated from both fusion proteins were followed through time. Whereas the amounts of precursor and mature prolactin (localized to the cytosol and secretory pathway, respectively) remained relatively constant, both the GSI and G2SI chains were rapidly degraded with a half-time of ~30 min. Consistent with these observations, P1 (but not GSI or G2SI fragments) can be detected in the incubation medium after a long chase (data not shown). At least one event (rapid degradation), apparently specific for cleaved signal sequences and their associated domains is not reconstituted in our cell-free systems. We suggest that this process, detected by the use of engineered probes with lengthened signal-associated domains, may reflect the normal metabolism of signal sequences after cleavage.
study, we show that this phenomenon occurs even when the domain which precedes the signal is the same size as that which follows it. Second, we wished to corroborate these surprising findings in vivo in order to rule out the possibility that the observed translocation reflected a permissiveness unique to cell-free systems. Results presented here demonstrate that such internalized signal sequences can function to translocate both flanking domains in intact cells. It should be noted that the experiments presented here show yields and not rates of assembly, since translocation into the endoplasmic reticulum can occur posttranslationally (16, 17, 26).

In addition to addressing these questions, this study yielded some unexpected findings. Cell-free systems typically yield incomplete conversion of nascent precursors to processed authentic chains. This is in contrast to the lack of observed precursor in living cells and tissues and has been presumed to reflect inefficiency of signal–receptor recognition in a fractionated, heterologous system. Consistent with this interpretation, increase in the concentration of available receptors (by adding increasing membrane concentration) resulted in increased efficiency of translocation in vitro (10). Investigation of these phenomena in Xenopus oocytes has been revealing. We have detected precursors in oocytes, and this observation establishes the Xenopus oocyte as an in vivo system in which probes of translocation might be studied with greater sensitivity by measuring changes in the relative amounts of precursor and products which accumulate. Thus, it might be possible to approach directly such previously inaccessible problems as the role of SRP-mediated elongation arrest in vivo by microinjection of SRP.

The finding that signal-bearing cleavage fragments were grossly under-represented in oocytes was at variance with the results from cell-free systems. Since full-length precursor and full-length globin appeared reasonably stable in the cytosol, and processed i25E (globin), as well as mature prolactin, appear stable in the endoplasmic reticulum lumen and secretory pathway, we suspect that the extremely rapid degradation of the processed signal-containing globin domains reflects a fate peculiar to signal-containing cleavage fragments. However, we cannot exclude the possibility that the instability of GSI and G2SI may be caused by their inability to be completely translocated across the endoplasmic reticulum membrane.

Signal sequences are generated stoichiometrically with secretory and membrane proteins, and in some tissues specialized for secretion (pituitary or pancreas, for example) the quantities of stored secretory product constitutes a large percentage of total cell protein, thus it seems likely that an efficient mechanism for clearance of cleaved signal sequences might exist. Attempts to study the fate of cleaved signal peptides have been hampered by the difficulty in detecting the small, hydrophobic, cleaved peptide. For this reason, the true fate of cleaved signal sequences has remained largely an unresolved problem. By joining a passenger domain to the amino terminus of a signal sequence, the size of the resultant cleaved, signal-bearing domain has been greatly increased. Moreover, the globin domain now serves to identify the fragment such that it can be immunoprecipitated and easily detected by standard SDS PAGE. While we cannot rule out the possibility that such engineering has altered the fate of the cleaved signal itself, the distinctive kinetics of degradation of the two different signal-bearing cleavage fragments compared to even the same domains when part of a full-length precursor suggests that the rapid degradation of signal-bearing globin fragments occurred by a mechanism normally used for signal degradation. Our findings provide the basis for an assay in which we can attempt to localize the signal clearance machinery by reconstituting these events in vitro using oocyte cell fractions.

To date, most progress in defining the mechanisms of targeting to and translocation across the endoplasmic reticulum membrane derives from studies in cell-free systems. While the results of this study reconfirm that the translocation behavior of altered substrates in cell-free systems is largely faithful to the corresponding events in vivo, our results identify at least one translocation event which cannot be studied in currently available cell-free systems; namely, the fate of cleaved signal sequences. Xenopus oocytes programmed with the engineered substrates described here and in conjunction with appropriate antibodies should permit elucidation of this issue. Studies are in progress to exploit these and other implications of engineered substrate function in Xenopus oocytes.

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