TRANSLOCATION OF A LYOSOMAL ENZYME ACROSS THE MICROSMAL MEMBRANE REQUIRES SIGNAL RECOGNITION PARTICLE

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Using cell-free synthesis, we have previously shown that the early events in the synthesis of one lysosomal enzyme, cathepsin D, are similar to those established for secretory proteins (1,2). Thus, translation of cathepsin D mRNA in vitro yielded preprocathepsin D. When microsomal membranes were present during translation, the NH₂-terminal pre-sequence was cleaved and the resulting procathepsin D was core glycosylated and segregated within the lumen of the microsomal vesicles. The partially established primary structure of the 20 amino acid pre-sequence showed an abundance of Leu residues, which is characteristic of the pre-sequences of many secretory proteins. We therefore proposed that the pre-sequence of preprocathepsin D functions similarly as a signal specifying cotranslational translocation of the nascent lysosomal enzyme across the microsomal membrane.

Recently, two components of the translocation system of microsomal membranes have been isolated and characterized. One is an 11S ribonucleoprotein referred to as signal recognition particle (SRP)(3). The other is an integral membrane protein, referred to as SRP receptor (4) or

ABBREVIATIONS: SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; RM, rough microsomal membranes from dog pancreas; K-RM, 0.5 M potassium acetate-extracted RM; ER, endoplasmic reticulum; SRP, signal recognition particle.
docking protein (5). SRP has been shown to function in the recognition of the signal sequence of secretory proteins (6) and of a transmembrane protein (7) and to cause a site-specific arrest of polypeptide chain elongation shortly after the signal sequence has emerged from the ribosome (8). This translation arrest is released by adding microsomal membranes to the translation system (8). The arrest-releasing activity of the microsomal membranes was localized to a single integral membrane protein, the SRP receptor, which was purified by affinity chromatography on SRP-Sepharose (9).

Our data here indicate that SRP also recognizes the signal sequence of the lysosomal enzyme cathepsin D and that this recognition causes translation arrest which can be released by addition of microsomal membranes. Thus, as observed for secretory proteins and an integral membrane protein, SRP couples the synthesis and translocation of lysosomal enzymes across the microsomal membrane.

Materials and Methods

**Materials.** [35S]Methionine (1000 Ci/mmol) was obtained from New England Nuclear. Protein A-Sepharose CL-4B was purchased from Pharmacia, Trasylol from Mobay Chemical Corp., wheat germ from General Mills, and trypsin, chymotrypsin and *Staphylococcus aureus* nuclease from Boehringer Mannheim.

**Cell-Free Protein Synthesis.** The preparation of porcine spleen poly A+ mRNA, its translation in a *Staphylococcus aureus* nuclease-digested wheat germ cell-free protein synthesizing system, post-translational proteolysis, subsequent immunoprecipitation of cathepsin D, and SDS-PAGE were as previously described (1,2). Dog pancreas rough microsomal membranes (RM), salt-washed RM (K-RM) and purified SRP were prepared as previously reported (10). When added, RM were present in the in vitro translation system at a final concentration of 2 A280 units/ml. When K-RM were included, that amount of salt-extracted membranes derived from 2 A280 units of RM was added per 1 ml of translation mix.

**Results**

As previously demonstrated (1,2), translation of porcine spleen poly A+ mRNA in a cell-free wheat germ translation system and immunoprecipitation with immunoselected antibodies raised against the 30-kD heavy chain (11) of the protease yielded 43-kDa preprocathepsin D on SDS-PAGE and fluorography (Fig. 1, Lane 1). On supplementation of the wheat germ system with microsomal vesicles isolated from dog pancreas, processed and glycosylated 46-kDa procathepsin D was also detected (Fig. 1, Lane 2). The amount of the 46-kDa
Messenger RNA isolated from porcine spleen was translated in a wheat-germ cell-free translation system (final volume, 100 μl) and the in vitro synthesized procathepsin D was purified by immunoprecipitation. Analysis was conducted by SDS-PAGE followed by fluorography of the dried slab gel. Lane 1, immunoreactive translation product synthesized in the absence of RM; Lane 2, immunoreactive translation product synthesized in the presence of RM; Lane 3, immunoreactive translation product synthesized in the presence of K-RM; Lane 4, as Lane 3, except purified SRP was added to a final concentration of 80 units/ml; Lane 5, as Lane 3, except purified SRP was added to a final concentration of 400 units/ml; Lane 6, as Lane 4, except the RM were incubated (2 h, 4°C) posttranslationally with a mixture of trypsin and chymotrypsin (final concentration of each protease, 3 mg/ml); Lane 7, as Lane 6, except SRP was added to a final concentration of 400 units/ml; Lane 8, immunoreactive translation product synthesized in the absence of K-RM, but in the presence of SRP at a final concentration of 400 units/ml.

Protein relative to the 43-kDa protein depended on the concentration of microsomal vesicles. Increasing the concentration of microsomal vesicles increased the synthesis of the 46-kDa product and decreased the 43-kDa product (data not shown). Salt-extraction of the microsomal vesicles prevented translocation of cathepsin D, as it prevented translocation of the secretory protein prolactin (6). Only 43-kDa preprocathepsin D was detected (Fig. 1, Lane 3). When, however, purified SRP was added with the K-RM, 46-kDa procathepsin D appeared, indicating translocation activity was restored (Fig. 1, Lane 4). Increasing the amount of SRP increased the yield of procathepsin D (Fig. 1, compare Lane 4 with Lane 5) and decreased the amount of preprocathepsin D recovered.
To confirm that the 46-kDa protein had crossed the microsomal membrane, the proteases trypsin and chymotrypsin were added post-translationally to the microsomal vesicles (Fig. 1, Lanes 6 and 7). As observed previously (1), the 43-kDa protein was digested but the 46-kDa protein remained intact, indicating that preprocathepsin D was outside the vesicles while procathepsin D was protected inside the lumen of the microsomes.

Translation of porcine spleen mRNA in the presence of K-RM and the higher concentration of SRP yielded two minor additional immunoreactive proteins having apparent Mr of 41,000 and 44,500 (Fig. 1, Lane 5). These proteins were not digested by externally added proteases (Fig. 1, Lane 7, arrows) indicating they had crossed the microsomal membrane and were protected within the lumen of the vesicles. The 41-kDa protein comigrated with the protein detected when procathepsin D synthesized in vitro in the presence of microsomal membranes was treated with endoglycosidase H to remove high mannose carbohydrate (2). Together these results indicate that the 41-kDa protein detected was procathepsin D which had lost its NH₂-terminal signal sequence during membrane translocation but which had not acquired high mannose carbohydrate. The immunoreactive, protected 44.5-kDa protein was probably a partially glycosylated form of procathepsin D. Incomplete glycosylation is frequently detected in vitro for secretory and membrane proteins (13). Detection of increasing amounts of unglycosylated or partially glycosylated procathepsin D in the presence of increasing concentrations of SRP probably indicates that dolichol phosphate is being exhausted (14) by the glycosylation of newly synthesized proteins translocated in an increased amount in the presence of higher concentrations of SRP.

Addition of purified SRP to a wheat germ translation system not supplemented with microsomal membranes selectively inhibited translation of mRNAs coding for secretory proteins but not those coding for cytosolic proteins (6,12). In the absence of K-RM, purified SRP also inhibited translation of cathepsin D mRNA (Fig. 1, compare Lane 8 with Lane 1). This
inhibitory effect was abolished when K-RM were present in the cell-free translation system (Fig. 1, Lane 4).

Discussion

Our results demonstrate that secretory and lysosomal proteins share a common receptor-mediated mechanism for translocation across the ER membrane. SRP, a ribonucleoprotein protein stripped from microsomal membranes by high salt (0.5 M potassium acetate), is required for in vitro translocation of both the secretory protein bovine prolactin (6) and the lysosomal protease cathepsin D. In addition, SRP has been shown to be necessary for the integration of a transmembrane glycoprotein, the delta subunit of the acetylcholine receptor (7). These results suggest that a single mechanism exists for translocation across or integration into the ER membrane and that secretory and lysosomal proteins are co-segregated into and thus mixed within the lumen of the ER. The intracellular biosynthetic pathways of these two classes of proteins must therefore diverge at some point beyond the ER.

The role of SRP in the membrane translocation of lysosomal proteins appears indistinguishable from its role in the translocation of secretory proteins. As observed for secretory proteins, purified SRP inhibits synthesis of the lysosomal protease cathepsin D in a cell-free translation system lacking microsomal membranes. Translation-inhibition is reversed by the addition of microsomal membranes which possess the SRP receptor (4). SRP presumably induces site-specific elongation arrest by recognizing the signal sequence of the nascent polypeptide, as observed for the secretory protein prolactin (8). A discrete NH₂-terminal, SRP-arrested peptide could not be detected for cathepsin D, as it was for prolactin (8), because our cathepsin D antiserum was raised against the COOH-terminal 30-kDa subunit of the lysosomal protease. Translocation was accompanied by signal peptide cleavage and high-mannose glycosylation to yield procathepsin D. The latter two activities appear unperturbed by the salt wash that removed peripheral membrane proteins, for they were detected when only a single component of the salt wash, purified SRP, was added back to the system. However, on the basis of this observation
alone, we cannot rule out the remote possibility that SRP plays some direct role in these processes.

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References