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Transfer of Proteins across Membranes

Biosynthesis *in vitro* of Pretrypsinogen and Trypsinogen by Cell Fractions of Canine Pancreas

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Canine pancreas was fractionated into free ribosomes and rough microsomes. Detached ribosomes were prepared by treatment of rough microsomes with detergent. Poly(A)-containing mRNA was extracted from rough microsomes. The biosynthesis of canine pancreatic secretory proteins was studied by comparing proteins synthesized *in vitro* by translation of mRNA or by completion of nascent chains present in free ribosomes, rough microsomes, and detached ribosomes with proteins synthesized in tissue slices using polyacrylamide gel electrophoresis in sodium dodecyl sulfate and subsequent autoradiography.

The banding pattern of authentic secreted proteins synthesized in tissue slices was largely congruent with that obtained from the translation products of rough microsomes indicating that the bulk of the mRNA engaged with rough microsomes codes for secretory proteins.

The banding pattern of translation products from mRNA in the absence of microsomal membranes was not congruent with that of authentic secretory proteins. Primary translation products for trypsinogen and the other serine protease zymogens using mRNA appeared to be larger in molecular weight than authentic proteins by 1000–2000 and are thus designated 'presecretory' proteins.

The banding pattern from the translation products of free ribosomes, which are essentially devoid of membranes, was similar to that of 'presecretory' proteins.

Translation of mRNA in the presence of microsomal membranes yielded a banding pattern for serine protease zymogens congruent with that of the translation products of rough microsomes, and these products were resistant to posttranslational proteolysis, indicating that segregation and processing of these polypeptide chains had taken place during translation *in vitro*.

The exocrine pancreas has served in the past to establish much that is presently known regarding the intracellular pathway of secretory proteins [1]. The exocrine pancreas contains an abundance of rough endoplasmic reticulum with has been characterized as the site of synthesis of exportable proteins. Many of these proteins have been identified by actual or potential enzymatic activity (summarized in [2]), and a number have been characterized by amino acid sequence (summarized in [3]). Accordingly we chose this tissue to probe the early events of biosynthesis of secretory proteins. We have approached this problem by studying the synthesis of proteins *in vitro* by cell fractions and derived subfractions isolated from the exocrine pancreas. Canine pancreas was chosen since ribonuclease levels have been reported to be low [4] and undegraded polyribosomes have previously been isolated from this tissue [5]. In this paper we describe our cell fractionation procedures and present our data on protein biosynthesis *in vitro* using these fractions. The appearance of pretrypsinogen or trypsinogen in the translation products of various cell fractions and derived subfractions is described in detail.

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Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

Definition. A_{260} unit, the quantity of material contained in 1 ml of a solution which has an absorbance of 1 at 260 nm, when measured in a 1-cm pathlength cell.

EXPERIMENTAL PROCEDURE

Procurement and Preparation of the Pancreas for Cell Fractionation

Dogs weighing 20–50 lb (9–23 kg) were fed *ad libitum*. A solution of 20 mg of acepromacine, i.e. 10-[3-

(dimethylamino)propyl]-phenothiazin-2-ylmethyl ketone, was injected intramuscularly 1 h prior to sacrifice as a general sedative. Several minutes prior to sacrifice sodium pentobarbital, 300–500 mg, was administered intravenously. When deep central nervous system depression was achieved (usually within several minutes), the dog was placed on a surgical table, the thorax was opened and the great vessels were severed at the base of the heart to allow rapid exsanguination into the chest cavity. The abdomen was opened by a midline incision, the pancreas (all of the tail and most of the head) was removed with scissors, and the gland was immersed in an ice-cold solution of 0.25 M sucrose in Tris/K/Mg (50 mM Tris-HCl pH 7.5, 25 mM KCl, and 5 mM $MgCl_2$). All subsequent steps were carried out at 3 C. The pancreas was spread on a parafilm strip in the cold room and freed of connective tissue, fat, and large blood vessels. A razor blade was used to cut the pancreas into small pieces which were passed through a tissue press (stainless steel with 1-mm-diameter perforations). Passage through the tissue press removed much of the connective tissue and facilitated subsequent homogenization. The resulting brei was mixed with 2 vol. of 0.25 M sucrose in Tris/K/Mg and was homogenized by 3–4 passes in a Potter-Elvehjem homogenizer with a motor-driven teflon pestle (Arthur H. Thomas Co., Philadelphia, Pa.).

Preparation of Cell Fractions and Subfractions

The homogenate was centrifuged for 10 min at 11000 rev./min ($13000 \times g_{av}$) in an angle rotor (type SS34) of a Sorvall RC-5 centrifuge (Du Pont Co., Newton, Conn.) to yield a postmitochondrial supernatant. 15 ml of the postmitochondrial supernatant were loaded on a discontinuous sucrose gradient containing 5 ml each of 2.2, 1.75 and 1.5 M sucrose in Tris/K/Mg. After centrifugation for 24 h at $140000 \times g_{av}$ in an angle rotor [type A-211 of the IEC centrifuge from Damon/IEC Div., Damon Corp., Needham Heights, Mass.], the layer of 1.75 M sucrose in Tris/K/Mg containing rough microsomes was removed with a syringe and diluted with an equal volume of a 'supernatant' fraction; the latter was arbitrarily defined as the top 10 ml fraction derived from the postmitochondrial supernatant after the 24-h centrifugation step. This fraction is rich in RNase inhibitor (data not shown). Centrifugation of this diluted rough microsome fraction for 30 min at $100000 \times g_{av}$ over a 1-ml cushion of 1.3 M sucrose in Tris/K/Mg in a Spinco no. 40 rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) yielded a pellet of rough microsomes.

Treatment of the diluted rough microsomes fraction with sodium deoxycholate (final concentration of 1%) and subsequent centrifugation for 24 h at $100000 \times g_{av}$ over a 1-ml cushion of 2.25 M sucrose in Tris/K/Mg yielded a pellet of 'detached' ribosomes.

The free ribosome fraction comprised the pellet obtained after the 24-h centrifugation of the postmitochondrial supernatant over the discontinuous sucrose gradient (see above). Pellets of free and detached ribosomes and of rough microsomes were stored at −80 °C.

For the determination of RNA in various subfractions of the homogenate (see Table 1) a fractionation procedure described previously [6] was used.

Cell-Free Protein-Synthesizing Systems and Assays

The two systems for protein synthesis *in vitro* have been described previously [7]. In the 'readout' system polypeptide chains contained in free and detached ribosomes and in rough microsomes are completed *in vitro*. Few chains are synthesized *de novo* because this system does not contain added initiation factors. In the 'initiation system', polypeptide chains are synthesized *de novo*, using isolated mRNA and initiation factors contained in the native small ribosomal subunits.

Initiation System. The reaction mixture (250 µl) contained 25 µmol of KCl, 5 µmol of Hepes·KOH (pH 7.3 at 20 °C), 0.75 µmol of $MgCl_2$, 0.5 µmol of dithiothreitol, 0.25 µmol of ATP, 0.05 µmol of GTP, 1.5 µmol of creatine phosphate, 10 µg of creatine phosphokinase, 10 µCi of a reconstituted protein hydrolysate (algal profile) containing 15 ^{14}C-labeled amino acids, 7.5 nmol each of the five amino acids not present in the algal hydrolysate (asparagine, cysteine, glutamine, methionine, and tryptophan) as well as 0.4 A_{260} unit of native small ribosomal subunits from rabbit reticulocytes as a source of small ribosomal subunits and initiation factors [8], 1.2 A_{260} units of derived large ribosomal subunits obtained from detached ribosomes (see above) of canine pancreas by the puromycin/KCl procedure, 100 µl pH-5 enzymes prepared from a high-speed supernatant of Krebs ascites cells [8], and 0.05 A_{260} unit of poly(A)-containing RNA prepared from canine pancreas rough microsomes[2]. In some cases (indicated in figure legends) the initiation system also contained EDTA-stripped pancreatic microsomes [9].

Readout System. The composition of this system was identical to that of the initiation system except that it contained either free or detached ribosomes or rough microsomes instead of native small ribosomal subunits, derived large ribosomal subunits and mRNA.

Incubation in both systems was at 37 °C. 10-µl aliquots were removed at indicated time intervals and spotted on 3 MM Whatman filter paper disks, which were processed according to Mans and Novelli [10]. Radioactivity was determined in toluene/Liqui-

fluor (New England Nuclear Corp., Boston, Mass.) in a Beckman LS 350 liquid scintillation counter at about 75% efficiency.

Proteolysis of Translation Products

25-μl aliquots removed from the two systems described above after incubation (see figure legends), were cooled to 0–2 °C in an ice bath, and each treated for 3 h at the same temperature with 3 μl of a solution containing trypsin and chymotrypsin (500 μg of each per ml). Proteolysis was terminated by the addition of 1 vol of 10% trichloroacetic acid, and the ensuing precipitate was prepared for sodium dodecyl sulfate/polyacrylamide gel electrophoresis as described below.

Analysis of Translation Products by Dodecyl Sulfate/Polyacrylamide Gel Electrophoresis

25-μl aliquots removed from the two systems after completion of incubation were cooled to 0–2 °C in an ice bath and treated with an equal volume of ice-cold 10% trichloroacetic acid; after 1 h the ensuing precipitate was collected at 0–4 °C by centrifugation in a swinging bucket rotor for 10 min at 2000 × *g*. The supernatant was removed as completely as possible and the precipitate was dissolved by incubation for 20 min at 37 °C in 30 μl of a solution containing 15% sucrose, 5% sodium dodecyl sulfate, bromphenol blue (serving both as a pH indicator for the sample and as a tracking dye for electrophoresis), 100 mM Tris base and 8 mM dithiothreitol (if the solution turned yellow, pH 3, Tris base was added in 1-μl aliquots to restore the blue color, pH 4.5 and higher). Solubilization was completed by incubation in a boiling water bath for 2 min. After cooling to room temperature, 2 μl of a 0.5 M solution of 2-iodoacetamide was added to each sample, and the mixture was incubated for 1 h at 37 °C before a 25-μl aliquot was layered into a slot of a polyacrylamide slab gel.

Rabbit globin, porcine chymotrypsinogen, ovalbumin, and bovine albumin were treated in an identical manner and were used as standards for molecular weight deteminations.

The slab gel (1-mm thick) consisted of a 10–15% acrylamide gradient serving as a resolving gel and a 5% acrylamide stacking gel, both in dodecyl sulfate and buffers as described by Maizel [11]. Electrophoresis was for 20 h and at constant current.

After electrophoresis, the slab gel was stained in a solution containing 0.2% Coomassie brilliant blue, 50% methanol, and 10% glacial acetic acid for 2 h and then destained in 50% methanol and 10% acetic acid. After destaining the gel was soaked in the last solution with 5% glycerol added; this was helpful in preventing the gels from cracking during and after drying on Whatman 3 MM paper.

Autoradiography of Dried Polyacrylamide Gels

Dried gels were exposed to medical X-ray film (Cronex 2D, Du Pont de Nemours and Co., Inc., Wilmington, Del.), generally for a few days. The films were developed by conventional procedures.

Preparation of ^{14}C-labeled secretion products was as described by Scheele [12]. Their analysis by dodecyl sulfate/polyacrylamide gel electrophoresis and autoradiography was as described above for translation products.

RESULTS

Fig. 1 shows the sedimentation profiles of free and detached ribosomes. Both ribosome populations contain polyribosomes. There are considerably more monomers than polyribosomes in free ribosomes while the reverse is true for detached ribosomes. The amount of polyribosomes in the free ribosome fraction was somewhat variable from preparation to preparation; frequently this fraction contained only monomers and no detectable polyribosomes. The detached ribosome fraction on the other hand always contained more polysomes than monomers, occasionally more than shown in Fig. 1. These results are in agreement with Dickman and Bruenger [5] and conclusively indicate that mRNA is isolated in these fractions in relatively undegraded form.

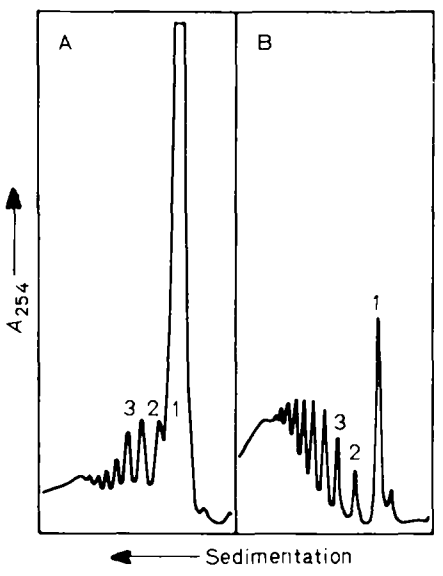

Fig. 1. *Sedimentation profiles of (A) free and (B) detached ribosomes from dog pancreas.* Pellets of free and detached ribosomes were resuspended in ice-cold double-distilled water. 0.1-ml aliquots containing 2.5 A_{260} units were layered on 12.5 ml of 10–40% sucrose gradients in 50 mM KCl, 50 mM triethanolamine, pH 7.4, and 5 mM $MgCl_2$. The gradients were centrifuged at 4 °C in an SB 283 rotor of an IEC centrifuge for 100 min at 190000 × g_{av}. Arrow indicates direction of sedimentation. The monomer, dimer and trimer peaks are designated 1, 2, and 3, respectively

Table 1. *Distribution of RNA in fractions derived from 1 ml canine pancreas homogenate (33% w/v)*

Fraction	RNA	
	µg	(% total)
Nuclei	70	(1.5)
Total ribosomes	4190	(91.7)
Free ribosomes	470	(10.3)
Nonsedimentable RNA	310	(6.8)
Detached ribosomes	3720[a]	(81.4)
Total RNA	4570[b]	(100)

[a] Calculated from amount in total ribosomes minus free ribosomes.

[b] Sum of RNA in nuclei, total ribosomes, and nonsedimentable RNA.

From ultrastructural work it is known that the exocrine pancreatic cell contains abundant rough endoplasmic reticulum and only a few free ribosomes. Using a fractionation scheme developed for rat liver [6] to determine the distribution of RNA in nuclei and in cytoplasmic subfractions, we obtained data compatible with the ultrastructural findings. It can be seen (Table 1) that about 90% of the cellular RNA is present in the ribosome fraction; only slightly more than 10% of the ribosome population is free whereas 90% is membrane-bound.

The isolated cell fractions (free ribosomes, rough microsomes, detached ribosomes) were incubated in a 'readout system' (see Methods) to complete the unlabeled nascent polypeptide chains present in these fractions. Thus the completed chains were labeled *in vitro* by the radioactive amino acids added onto the carboxy-terminal end of the unlabeled nascent chains present in these fractions. The time course of incorporation of labeled amino acids is shown in Fig. 2. It can be seen that chain completion in the rough microsomes required an incubation period between 20–30 min depending on the fraction. Free ribosomes and detached ribosomes are equally active in protein synthesis despite the fact that detached polysomes are relatively more abundant than free polysomes (Fig. 1). This is believed to be due to the cosedimentation of mRNA molecules and 40-S initiation complexes with free ribosomes. Whereas the addition of the initiation inhibitor aurin tricarboxylic acid has no effect on the translation of detached ribosomes, it reduces the translation of free ribosomes by approximately 30%. The additional incorporation of radioactivity due to initiation events in the free ribosomal fraction explains the continued amino acid incorporation seen in this fraction up to 45 min.

The kinetics of incorporation of radioactive amino acids in the 'initiation system' (see Methods) where polypeptide chains were synthesized *de novo* using mRNAs isolated from rough microsomes, continued linearly for 130 min (Fig. 3). The relatively high

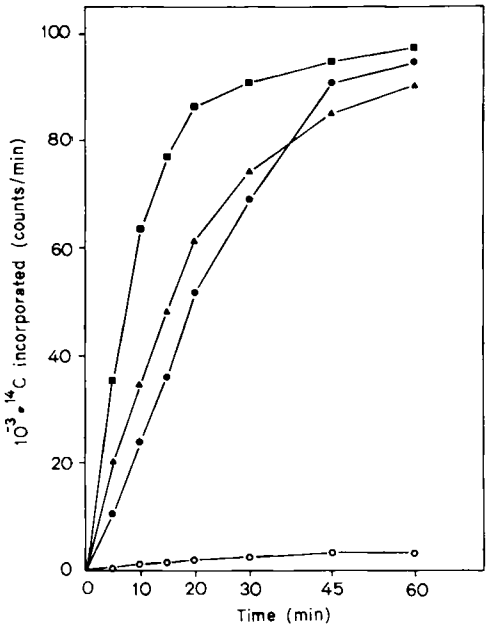

Fig. 2. *Time course of polypeptide synthesis in a readout system.* The system contained either 8.0 A_{260} units of free ribosomes (●), 8.0 A_{260} units of detached ribosomes (▲), 12.0 A_{260} units of rough microsomes (■), or no added cell fraction (○)

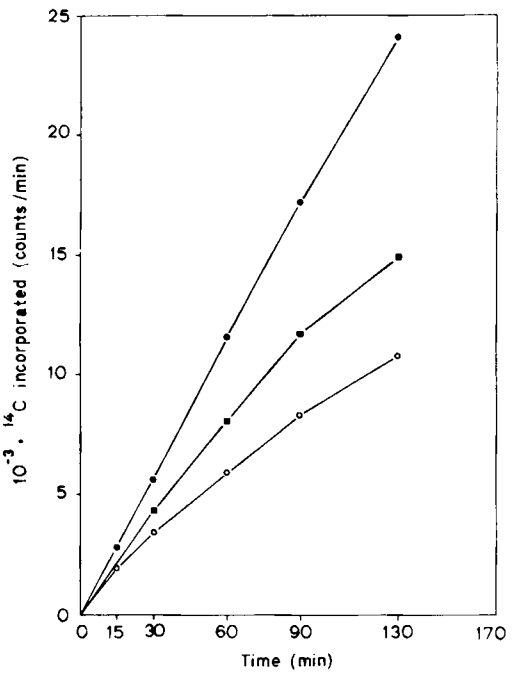

Fig. 3. *Time course of polypeptide synthesis in an initiation system.* Synthesis was measured in the absence of added mRNA (○) or in presence of 0.05 A_{260} unit of mRNA extracted from pancreatic rough microsomes without (●) or with (■) 1.4 A_{260} units of added EDTA-stripped pancreatic microsomes

background of incorporation seen in the absence of added mRNA is due to (a) endogenous mRNA present in the Krebs ascites pH-5.0 preparation and (b) globin mRNA which cosediments with the native small ribo-

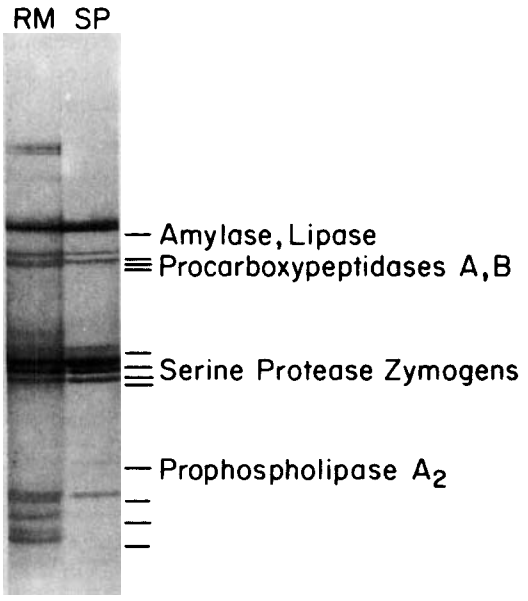

Fig. 4. *Analysis by dodecyl sulfate polyacrylamide gel electrophoresis and autoradiography of polypeptide chains completed by rough microsomes (slot RM) in a readout system and secretion products synthesized by canine pancreas slices (slot SP).* Individual secretory proteins are labeled as identified by Scheele (unpublished results). Among the serine protease zymogens, the polypeptide chain showing the fastest mobility was identified by potential enzyme activity as trypsinogen

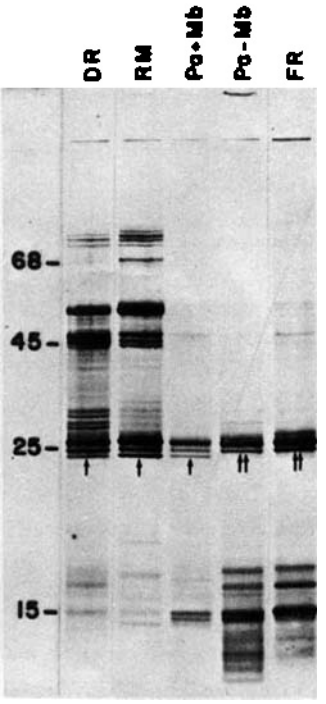

Fig. 5. *Analysis by dodecyl sulfate polyacrylamide gel electrophoresis and autoradiography of polypeptide chains completed at various times and in various systems.* The slots show polypeptide chains completed at the 60-min time point (see Fig. 2) by detached ribosomes (DR), rough microsomes (RM) and free ribosomes (FR) in a readout system and of polypeptides synthesized *de novo* at the 130-min time point in an initiation system (see Fig. 3) in the presence of mRNA extracted from pancreatic rough microsomes without (Pa-Mb) or with EDTA-stripped pancreatic microsomes (Pa + Mb). Single-bar arrow indicates trypsinogen band, double-bar arrow pretrypsinogen band. Numbers refer to $10^{-3} \times M_r$ or marker proteins (see Methods) pointing to their position in a separate slot (not shown) of the slab gel

somal subunits isolated from rabbit reticulocytes. Translation of the former results in polypeptide chains which show a diffuse banding pattern, and translation of the latter results in the α and β chains of globin which are seen at positions corresponding to molecular weights of approximately 15000 in the polyacrylamide gels. Post-translational treatment with trypsin and chymotrypsin results in degradation of these polypeptide chains (see Fig. 6).

Fig. 4 shows an analysis of the translation products of rough microsomes (slot RM) in comparison to radioactively labeled secreted proteins (slot SP) synthesized *in vitro* using canine pancreas slices by dodecyl sulfate/polyacrylamide gel electrophoresis and subsequent autoradiography. Methods used to identify secretory proteins by actual or potential enzymatic activity will be presented elsewhere (Scheele, unpublished). The banding pattern of translation products from incubation of rough microsomes in a readout system shows quantitative and qualitative similarities to that of secreted protein. Quantitative similarities are evident in that major bands constituting the readout products of rough microsomes correspond to similarly intense bands of secreted proteins, indicating that the amount of various secreted proteins is related to the amount of their corresponding mRNAs engaged on membrane-bound ribosomes. Qualitative similarities are evident from the identical mobilities of the secreted proteins.

Fig. 5 shows a comparison of the translation products of the subcellular fractions studied in this paper. Polypeptide chains resulting from the translation of mRNA isolated from canine pancreas rough microsomes by phenol extraction and oligo(dT)-cellulose chromatography (slot Pa-Mb) shows a banding pattern which is not congruent with that of 'authentic' secretory proteins (slot RM). This is clearly seen for the group of protease zymogens (identified in Fig. 4 above which migrate near the 25000-molecular-weight marker. Among this group the band marked by the single-bar arrow has been identified as trypsinogen according to potential enzymatic activity and the double-bar arrow has been identified as pretrypsinogen by radio-sequencing studies [13]. Pretrypsinogen was shown to differ from trypsinogen in that it contains an amino-terminal extension of 15 or 16 amino acid residues. The differences in migration of primary translation products (slot Pa-Mb) and 'authentic' secretory proteins (slot RM) in this area of the gel (serine protease zymogens) is compatible with a

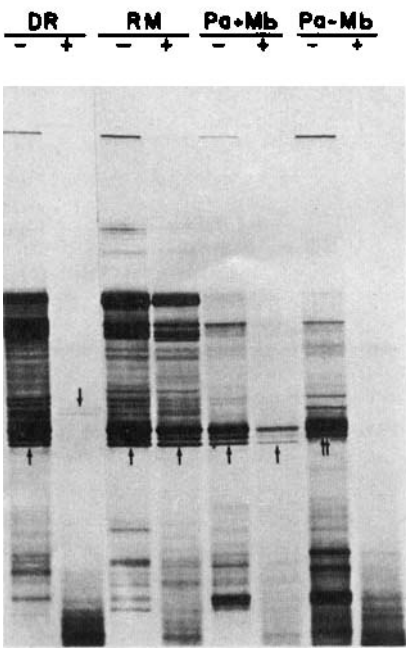

Fig. 6. *Analysis by dodecyl sulfate/polyacrylamide gel electrophoresis and autoradiography of polypeptides synthesized as described in Fig. 2 and 3 followed by incubation in the absence (−) or presence (+) of proteolytic enzymes (see Methods).* Designations of slots as in Fig. 5. Downward pointing arrow designates proteolysis-resistant bands synthesized by detached ribosomes

molecular weight difference for each of these proteins of between 1000 – 2000.

The readout products of free ribosomes (Fig. 5, slot FR) showed a banding pattern very similar to that of proteins synthesized by the translation of mRNA in that only the larger polypeptide chains (presecretory proteins) are present. The larger proteins displayed in slots Pa-Mb and FR were synthesized in the absence of microsomal membranes. In contrast, when pancreatic mRNA was translated in the presence of stripped pancreatic microsomes (slot Pa+Mb) translation products co-migrated with those of 'authentic' secretory protein (slot RM). Proteins displayed in these two slots were synthesized in the presence of microsomal membranes. Finally the readout products of detached ribosomes (Fig. 5, slot DR) are compared to those from rough microsomes (Fig. 5, slot RM). The banding patterns are very similar. Noteworthy is the presence of a band (single bar arrow in slot DR) in the position of 'authentic' trypsinogen.

Fig. 6 shows the effect of combined protease treatment (50 μg/ml, trypsin plus 50 μg/ml chymotrypsin) on the translation products derived from the cell fractions presented in Fig. 5. The translation products derived from detached ribosomes and mRNA in the absence of membranes are degraded to small polypeptide fragments by the protease treatment. Those products derived from rough microsomes and the several products (serine protease zymogens) derived from the translation of mRNA in the presence of stripped microsomal membranes ('reconstituted' rough microsomes) were partially resistant to the protease treatment.

DISCUSSION

Our initial attempts to isolate undegraded polyribosomes from the pancreas of the rat, guinea pig, and pigeon failed most likely due to nucleolysis during cell fractionation. We turned to the canine pancreas since isolation of apparently undegraded polyribosomes was accomplished from canine pancreas by Dickman and Bruenger [5]. These investigators treated a postmitochondrial supernatant with sodium deoxycholate and by subsequent sedimentation through a sucrose cushion obtained a pellet containing both free and detached polyribosomes. In order to separate free from detached polyribosomes we modified the procedure of Dickman and Bruenger: a postmitochondrial supernatant was subfractionated into free ribosomes and rough microsomes and then detached ribosomes were obtained by detergent treatment of rough microsomes. Messenger RNA was isolated from rough microsomes by dodecyl sulfate/phenol extraction and oligo(dT)-cellulose chromatography (B. Dobberstein, P. Lizardi and G. Blobel, unpublished). We then analyzed the products of translation *in vitro* of these fractions and derived subfractions to probe the early biosynthetic events of secretory proteins. We found that the banding pattern of translation products from rough microsomes was largely congruent with that of 'authentic' secreted protein, indicating that for the exocrine pancreas and at this level of resolution there is little if any further proteolytic processing of secretory proteins from their site of segregation within the cisternal space of the rough endoplasmic reticulum to their site of secretion into the extracellular space. (Analysis of the translation products of rough microsomes by the two-dimensional gel technique [12] indicates that the majority of labeled proteins are coincident with enzymatically identified proteins secreted from canine pancreas slices.)

The translation of mRNA derived from rough microsomes resulted in polypeptide chains which were 1000 – 2000 larger in molecular weight than 'authentic' secreted proteins. These chains have been designated as 'presecretory' proteins based on the following observations. First, translation of mRNA in the presence of stripped microsomal membranes results in the processing of polypeptide chains to authentic secreted proteins. Second, radiosequencing studies have identified one of the pancreatic presecretory proteins as pretrypsinogen [13]. In this study pre-

trypsinogen was shown to contain an amino-terminal extension of 15 or 16 residues preceding the amino terminus of authentic trypsinogen. Authentic trypsinogen was identified by its highly characteristic trypsinogen activation peptide containing the sequence Asp-Asp-Asp-Asp-Lys within the first six or seven amino-terminal residues. Furthermore, this peptide extension was shown to be an apparent feature of pancreatic presecretory proteins in other molecular weight ranges, notably those of the other protease zymogens, the two prophospholipases, and the three procarboxypeptidases.

Translation of fractions containing microsomal membranes, rough microsomes and reconstituted rough microsomes, resulted in polypeptide chains which corresponded to secretory proteins. Translation of those fractions devoid of microsomal membranes, mRNA and free ribosomes resulted in polypeptide chains which corresponded with presecretory proteins. These findings indicate that processing (proteolytic cleavage) of presecretory proteins is a feature of the microsomal membrane. That processing of presecretory proteins is closely linked to segregation of these proteins within microsomal cavities is indicated by post-translational treatment of the various cell fractions with trypsin and chymotrypsin (Fig. 6). Fractions containing processed polypeptide chains, rough microsomes and reconstituted rough microsomes, were largely resistant to proteolysis, while fractions containing unprocessed chains, mRNA and free ribosomes, were sensitive to such treatment. These studies suggest that the proteolytic processing enzyme which removes the peptide extension is located within the microsomal vesicle, most probably associated with the inner surface of the microsomal membrane.

Detached ribosomes represent a special case since the protecting vesicle membrane had been solubilized prior to incubation in the translation system. Authentic trypsinogen appears among the readout products of these polyribosomes. Our interpretation of this result is that detached ribosomes contain nascent chains from which the peptide extension had been removed *in vivo* and which are subsequently completed *in vitro*. This finding in association with those reported for detached ribosomes from murine myeloma [7] suggests that the peptide extension of presecretory proteins occurs at the amino terminus and is removed prior to chain completion.

The relative abundance of secretory protein mRNAs associated with free ribosomes was unexpected and may have resulted, in part, from an artefact occurring during tissue procurement. It is conceivable that functional ribosome attachment, postulated in the signal hypothesis to depend on the aggregation of ribosome receptor proteins in a fluid membrane, does not occur at low temperatures; thus during cooling of the tissue from 37 C to 0 C aggregation of these proteins may be more temperature-sensitive than protein synthesis, giving rise to free ribosomes and polysomes containing mRNAs for secretory proteins. Other studies not presented here (Scheele, unpublished) are in support of this hypothesis. Considerations such as this suggest that the 10.3% given for the RNA content of the free ribosomal fraction in Table 1 is a maximal figure.

The findings recorded here on cell fractions and derived subfractions from the canine exocrine pancreas describe many of the early events involved in the biosynthesis of pancreatic secretory proteins. They suggest that trypsinogen and possibly the other serine protease zymogens are synthesized as precursor proteins, differing from their authentic counterparts by a peptide extension approximately 10–20 amino acid residues at their amino terminus. During translation this peptide extension is removed from the nascent polypeptide chain within the microsomal vesicle. Cleavage of this peptide occurs prior to chain completion and by an apparently specific membrane-associated protease.

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