SYNTHESIS AND INTERACTIONS OF CYTOPLASMIC MEMBRANES IN THE PANCREATIC EXOCRINE CELLS

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SUMMARY

The synthesis and interactions of cytoplasmic membranes involved in the intracellular transport of secretory proteins have been investigated in a system of guinea pig pancreas slices pulse labeled in vitro with ^{14}C-L-leucine. It is suggested that the membranes of the rough endoplasmic reticulum and Golgi complex were synthetized independently of each other while the membranes of zymogen granules derive from preexisting structures, probably membranes of Golgi origin.

Biochemical and morphological evidence clearly indicates that in pancreatic acinar cells (1-4), as well as in other protein secreting cells (5-7), secretory proteins are always segregated by membranes. Thus, following their synthesis in the attached ribosomes, such proteins are transported through a series of functionally interconnected membrane-bound compartments which involve, in sequence, the rough endoplasmic reticulum (RER), the Golgi complex and finally the secretory granules, the content of the latter being ultimately discharged in the extracellular space by exocytosis.

While it is clear that the different membranes specifically involved play a central role in these processes, unequivocal information concerning their synthesis and interactions is still lacking. Based on indirect evidence it is generally assumed that these membranes are synthetized in the RER. Some of them would then separate from RER and transform into Golgi membranes, with a balance maintained by a simultaneous transformation of other Golgi membranes into secretory granule membranes (8-11).

In previous studies on the pancreas of the guinea pig we have demonstrated that membrane subfractions can be obtained from rough microsomes (derived from the RER smooth microsomes (primarily contributed by elements of the Golgi complex) and from secretory (zymogen) granules (ZG) by removing the non-membrane components

present in these fractions while leaving the structure and composition of membranes apparently unaffected (12, 13). The synthesis and interactions of these membranes have now been investigated in a system of pancreatic slices where proteins were pulse labeled in vitro with ^{14}C-L-leucine.

METHODS

Male albino guinea pigs weighing 450-500 g were fasted 18-20 hr. Preparation and in vitro incubation of pancreatic slices were carried out as described in references 3 and 13. The slices were pulse labeled with ^{14}C-L-leucine (uniformly labeled; 20 µM; SA 260 mC/mmole; NEN, Langen, Germany) for 5 min, then washed with warm chase incubation medium (containing 2 mM ^{12}C-L-leucine) and reincubated in the latter for various times (0 to 150 min).

Cell fractionation. Highly purified membrane subfractions were isolated from rough and smooth microsomes by the procedure described in detail in 13. This involves, in sequence, incubation of rough and smooth microsomes (isolated as in 12 with minor changes) in 1 M KCl, 5 mM $MgCl_2$, 0.5 mM puromycin (14) for 4 hr at 4°, recovery of partially purified membranes by sucrose gradient centrifugation and washing with 0.2 M $NaHCO_3$ buffer, pH 7.8. Such treatments release the bulk of non-membrane components known to be present in microsomes. Thus by analytical and enzymological criteria it was established that membrane subfractions retain $<$ 5% of the ribosomes, $<$2% of the secretory proteins and $<$ 2% of the absorbed soluble proteins originally present (13). ZG membranes were isolated as described in 12. Such fraction appears composed exclusively by membrane ghosts, due to the total release during the purification procedure of digestive enzymes originally contained within ZG. Chemical and radiochemical assays were carried out as in 13.

RESULTS

Results are summarized in Fig.1. It is evident that at the end of the 5 min pulse incorporation a high protein specific radioactivity was found associated with both the rough and smooth microsome membranes, the ratio of rough to smooth being $\sim$1.3. In rough membranes a consistent decrease of protein specific radioactivity was found after 20 min of chase incubation; between 20 and 80 min there was an additional small decrease, with no further change at the last time-point (150 min). In smooth microsomal membranes the early decrease of protein labeling was less marked. No change could be detected at 80 min, whereas between 80 and 150 min there was a further moderate decrease.

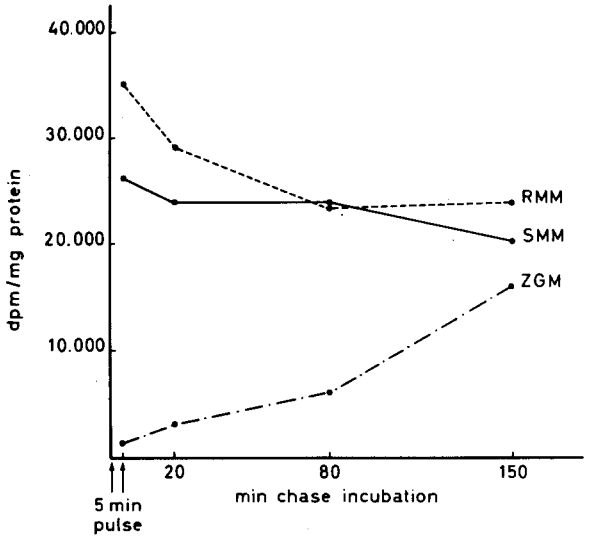

Fig.1.- Specific radioactivity of proteins in membrane fractions isolated from pancreatic slices pulse labeled for 5 min and incubated up to 150 min in chase. Values given are averages of either 5 (0 min in chase), 3 (20 and 80 min in chase), or 2 (150 min in chase) experiments. Variations from one experiment to another were less than 15%.
RMM = rough microsome membranes; SMM = smooth microsome membranes; ZGM = zymogen granule membranes.

A completely different curve was found with ZG membrane proteins. These were practically unlabeled at the end of the pulse; during chase incubation a virtually linear increase of labeling was observed, so that after 150 min the protein specific radioactivity of ZG membranes approached that of smooth microsome membranes.

DISCUSSION

As clearly demonstrated by Jamieson and Palade, in a system of pancreatic slices incubated in vitro proteins can be pulse labeled using radioactive leucine as a tracer (3). Therefore, changes in protein specific radioactivity of cell fractions during post pulse incubation are due to intracellular transport rather than to continued synthesis.

By using such a system we have observed that at the end of a short pulse incorporation both rough and smooth microsome membrane proteins are highly labeled. It is known that in the guinea pig pancreas rough microsomes are a pure fraction and contain fragmentated and resealed cisternae of RER, while smooth microsomes are contributed primarily by Golgi vesicles and cisternae but also contain a sizable amount of contaminants which most probably are unlabeled at the end of the pulse (such as

pieces of plasma membrane and pinocytotic vesicles (3, 12, 15). The presence of these could account, at least in part, for the lower specific radioactivity of the smooth membrane proteins compared to rough. During chase incubation the radioactivity of the proteins of both membrane subfractions remained high. A moderate decrease was observed in the first 20 min particularly in rough membranes; this could be due, at least in part, to the presence of trace amounts of highly radioactive secretory proteins which in our system are known to be transported early from microsomes to ZG during chase incubation (3, 4). In contrast, the small decrease of labeling of smooth membrane proteins between 80 and 150 min cannot be attributed to secretory proteins since most of these are known to leave the microsomes before 80 min of chase. Also the progressive increase of radioactivity in ZG membrane proteins must be attributed to the membranes themselves since our preparations appear free of non-membrane contaminants both by enzymological (12) and analytical (unpublished) criteria.

Taken as a whole these data seem to suggest that the Golgi membranes are synthetized independently from the bulk of RER membranes. Since the Golgi complex itself is not endowed with protein synthetizing capacity the membrane proteins could be synthetized elsewhere and immediately assembled in the Golgi together with phospholipids to yield complete membranes. Possible sites of membrane protein synthesis could be either the free ribosomes (as already suggested in a different system (16) or specialized areas of the RER, from which the membranes would be immediately transferred to the Golgi complex.

However our data do not rule out the possibility that some RER membranes might be transformed into Golgi membranes. The way this transformation could occur is not clear, but it seems possible that, besides other processes, it could imply the insertion of "new" proteins into "old" membranes. Therefore, the high protein specific radioactivity of Golgi membranes at the end of the pulse could be due not to the independent synthesis of these membranes but rather to the insertion of a few highly labeled "transforming" proteins on pre-existing, non-labeled ER membranes. Experiments to check these possibilities are in progress.

As far as the ZG membranes, our data clearly suggest that they are not synthetized as such but rather derive from pre-existing structures. Strong morphological evidence indicates the Golgi complex as the source of ZG membranes (2, 17, 18). Our observation that the increase of protein specific radioactivity in ZG membranes is much larger than the simultaneous decrease in smooth microsomal membranes probably depends

on the fact that in pancreatic acinar cells of fasted guinea pigs there are many more Golgi than ZG membranes. Therefore the transfer from the Golgi complex to ZG of enough membranes to increase markedly the specific radioactivity of the proteins of the latter could result in only a small change in the membranes of the first type.

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