Intracellular Transport and Storage of Secretory Proteins in Relation to Cytodifferentiation in Neoplastic Pancreatic Acinar Cells

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ABSTRACT The pancreatic acinar carcinoma established in rat by Reddy and Rao (1977, Science 198:78-80) demonstrates heterogeneity of cytodifferentiation ranging from cells containing abundant well-developed secretory granules to those with virtually none. We examined the synthesis intracellular transport and storage of secretory proteins in secretory granule-enriched (GEF) and secretory granule-deficient (GDF) subpopulations of neoplastic acinar cells separable by Percoll gradient centrifugation, to determine the secretory process in cells with distinctly different cytodifferentiation. The cells pulse-labeled with [3H]leucine for 3 min and chase incubated for up to 4 h were analyzed by quantitative electron microscope autoradiography. In GEF neoplastic cells, the results of grain counts and relative grain density estimates establish that the label moves successively from rough endoplasmic reticulum (RER) → the Golgi apparatus → post-Golgi vesicles (vacuoles or immature granules) → mature secretory granules, in a manner reminiscent of the secretory process in normal pancreatic acinar cells. The presence of ~40% of the label in association with secretory granules at 4 h postpulse indicates that GEF neoplastic cells retain (acquire) the essential regulatory controls of the secretory process. In GDF neoplastic acinar cells the drainage of label from RER is slower, but the peak label of ~20% in the Golgi apparatus is reached relatively rapidly (10 min postpulse). The movement of label from the Golgi to the post-Golgi vesicles is evident; further delineation of the secretory process in GDF neoplastic cells, however, was not possible due to lack of secretory granule differentiation. The movement of label from RER → the Golgi apparatus → the post-Golgi vesicles suggests that GDF neoplastic cells also synthesize secretory proteins, but to a lesser extent than the GEF cells. The reason(s) for the inability of GDF cells to concentrate and store exportable proteins remain to be elucidated.

Intracellular aspects of the process of protein secretion have been unraveled in the adult pancreatic exocrine acinar cell by the classical autoradiographic studies of Caro and Palade (4) and Jamieson and Palade (20-22). These studies have identified six successive steps in the secretory process namely, synthesis, segregation, intracellular transport via the Golgi complex, concentration, intracellular storage of discharge (for detailed discussion of this process see references 18 and 27). The existence of such a secretory process in a variety of highly differentiated eucaryotic cell types is now well documented (5, 6, 8, 15, 18, 27, 47). Information regarding the acquisition of various steps of the secretory process during cytodifferentiation is, however, lacking. The studies with developing embryonic rat pancreas have provided a greater understanding of the sequential events involved in the histogenesis, the differentiation of the secretory apparatus, and the regulation of expression of pancreas specific genes in this organ (24, 28, 42). Further delineation of intracellular and molecular events such as the secretory process is complicated by the co-emergence of ductal and islets cells in this very small fetal organ where numerous transitional events occur during 14-21-d gestation. This makes it difficult to isolate pancreatic acinar cells and their protodifferentiated precursors for detailed studies. The transplantable pancreatic acinar cell carcinoma of rat established by Reddy and Rao (30) provides...
a suitable model system for analyzing certain aspects of cellular differentiation and exocrine pancreas specific gene expression in neoplasia.

The pancreatic acinar cell carcinoma demonstrates a spectrum of cellular differentiation ranging from cells that contain abundant secretory granules to those devoid of secretory granules (31). Recently, we have separated two subpopulations of neoplastic cells from the dissociated pancreatic carcinoma cell population (3). One neoplastic cell subpopulation termed secretory granule-enriched fraction (GEF) contains highly differentiated acinar cells which essentially resemble mature normal pancreatic acinar cells; the second subpopulation designated secretory granule-deficient fraction (GDF) contains cells with immature cytodifferentiation (3). Despite this phenotypic variation, immunofluorescence studies suggested that neoplastic acinar cells with or without secretory granules contain variable amounts of at least six secretory proteins (14). It is pertinent, therefore, to investigate how genetic information is variable amounts of at least six secretory proteins (14). It is pertinent, therefore, to investigate how genetic information is processed and expressed by these cells.

**MATERIALS AND METHODS**

**Chemicals**

L-[3,5,4-3H]leucine (specific activity 56.8 Ci/mM) was obtained from ICN Pharmaceuticals (Irvine, CA), class IV collagenase (172 U/mg) from Sigma Chemical Co. (St. Louis, MO), soybean trypsin inhibitor (STI) from Worthington Biochemical Corp. (Freehold, N J), bovine serum albumin (BSA) from Sigma Chemical Co. (St. Louis, MO), and Pharmacia Fine Chemicals (Uppsala, Sweden). Hanks' balanced salt solution (HBSS) and minimal essential medium (MEM) without l-leucine and l-glutamine were purchased from Grand Island Biological Company (Grand Island, NY). Ilford L-4, a nuclear research emulsion, was purchased from Polysciences, Inc. (Paul Valley Industrial Park, Warrington, PA). Formvar in ethylene dichloride was obtained from Ladd Research Industries (Burlington, VT). All other chemicals were reagent grade.

**Transplantation of Pancreatic Acinar Carcinoma**

Small 1- to 2-mm fragments of pancreatic acinar carcinoma (30) were transplanted i.p. into the abdominal mesentery of weanling male F344 rats as previously described (3). These implants grew for the most part as single, large encapsulated tumors between 3 to 5 wk. The tumor-bearing animals were starved to exclude trypan blue.

**Preparation of Single Cell Suspensions**

Acinar carcinoma fragments were prepared at room temperature in Krebs' Ringer bicarbonate (KRB) buffer containing 14 mM glucose, 2.5 mM Ca<sup>++</sup>, 1.2 mM Mg<sup>++</sup>, 0.1 mg STI, a complete l-amino acid supplement, 100 U penicillin/ml, 100 #g streptomycin/ml, and 0.2% (wt/vol) BSA (3, 44), and dissociated into single cells using EDTA chelation and collagenase digestion (15-min first digestion and 45-min second digestion) procedure (1) as previously described (3). Viability of the dissociated acinar carcinoma cells was monitored by their ability to exclude trypan blue.

**Separation of GEF and GDF Neoplastic Cells**

Dissociated pancreatic acinar carcinoma cells were fractionated on Percoll:HBSS gradients as previously described (3). GEF and GDF neoplastic cell populations were diluted to a final concentration of 5 x 10<sup>6</sup> cells/ml for use in autoradiographic studies. For general morphologic studies an aliquot of each cell fraction was processed for electron microscopy.

**Pulse-labeling and Chase**

GDF and GEF subpopulations were diluted to 5 x 10<sup>6</sup> cells/ml and preincubated in MEM with 0.4 nM leucine for 15 min at 37°C. The cells were then centrifuged and washed in MEM without l-leucine and gently resuspended into 10-ml volume (5 x 10<sup>6</sup> cells/ml) of prewarmed MEM minus leucine. This suspension was then added to 2.5 MCI of [3H]leucine lyophilized in a siliconized flask resulting in a final concentration of 250 #Ci of radio-labeled precursor/ml cell suspension (3). Immediately after 3-min pulse, the cell suspension was pelleted through the prewarmed chase medium containing a BSA cushion and 4.4 mM [3H]leucine. The cell pellet was resuspended in KRB buffer with 4.4 mM [3H]leucine and 0.2% BSA and chased for 5, 10, 20, 30, 60, 120, 180, and 240 min. At the end of the pulse and at each chase interval, 1.0-ml aliquots of cell suspension were added to ice-cold (4°C) chase medium, centrifuged, and fixed in 2.5% glutaraldehyde after three washes. Cells were postfixed in 1% OsO<sub>4</sub>, dehydrated, stained en bloc for 60 min in 0.5% uranyl acetate, and embedded in Epon as previously described (3).

**Preparation of Autoradiograms**

For light microscope autoradiography, 0.5-μm-thick sections were cut from the Epon-embedded blocks and processed as previously described (31). For electron microscope autoradiography, ultrathin sections displaying pale gold interference color were cut and mounted on 100- and 200-mesh Formvar coated copper grids and coated with a monolayer of I<sub>2</sub>-L4 emulsion. The grids were exposed for up to 4 wk and developed for 2 min in D-19 at 18-20°C. Washed in double distilled water, and fixed for 1 min in 20% sodium thiosulfate/2% potassium metabisulfite.

**Analysis of Autoradiograms**

**Sampling of Cells**

Autoradiograms were photographed on a JEOL 100-CX microscope at a primary magnification of ×7,000, and printed at a final magnification of ×17,500. Each micrograph included one cell, and where necessary a montage of prints was made to include all grains overlying a particular cell profile. Only cell profiles possessing a nucleus and/or the Golgi region were sampled. A total of 400-1,300 grains were examined for each time interval for each subpopulation of cells examined by high resolution electron microscopic autoradiography. Under the conditions used in this study, the resolution of the autoradiographic method is 1.45 Å as determined by Salpeter and Bachmann (35). The location of each developed grain was determined according to the simple grain density method (35-37). Briefly, for each grain localized on a micrograph for each time interval a series of concentric circles on a plastic overlay was fitted over each silver grain. The center of the best fit circle was then taken as the autoradiographic grain center. The grain compartment over which the grain center fell was then reported and scored on the electron micrograph.

**Selection of Grain Compartments**

Cytoplasmic grain compartments were modeled essentially as outlined by Salpeter and Farquhar (36). The modified grain compartments were selected as follows: first compartment 1, rough endoplasmic reticulum (RER), which was divided into (a) RER/IN representing grains overlying the RER lumen and limiting membrane and (b) RER/OUT representing a 1-HD (half-distance) rim around all RER profiles. Next is compartment 2, the Golgi region, which includes the Golgi cisternae and the large Golgi vacuoles (this compartment was divided into three regions: [a] GOL/C, the Golgi cisternae, [b] GOL + ER, a 2-HD rim of RER on the forming [er] face of the Golgi [see Ehrenreich et al. (7) for further description], and [c] GOL + CV, a 2-HD rim of condensing vacuoles located at the maturing [em] face [or secretory face] of the Golgi apparatus). Compartment 3 is the post-Golgi vesicles, which is divided into (a) POG/VIN, condensing vacuoles proper, and (b) POG/OUT + CYT, a 2-HD rim of surrounding cytoplasm. Compartment 4 is secretory granules, which is divided into (a) ZG/IN, grains with centers above secretory granules, and (b) ZG + CYT, a 2-HD rim of cytoplasm surrounding secretory granules. Compartment 5 is the plasma membrane and a 2-HD rim (GHDPM), compartment 6 the nucleus, compartment 7 the cytoplasmic matrix excluded by other compartments, compartment 8 the mitochondria, compartment 9 lysosomes and vacuoles associated with phagocytosis, and compartment 10 miscellaneous elements of the cytoplasm not included in compartments 1 through 9. During the allocation of grains to specific compartments partial credit was given whenever a grain center fell within more than one of the above defined compartments. The "grain percent" was determined as outlined by Salpeter and Melton (37). The allocation of grains to specific cellular compartments was devised such that the shielded zones were added to the primary compartment to yield one grain total for these pooled compartments. Specifically, the shielded pooled zones for the RER (RER/IN and RER/OUT = RER total), the Golgi complex (Golgi/CV + Golgi/ER + Golgi/C = Golgi total), post-Golgi vesicles (POG/VIN and POG/OUT = POG total), and secretory granules (ZG/IN and ZG/OUT + ZG total) permitted an adequate quantitative comparison of GEF and GDF cells.

**Morphometric Analysis to Determine Areas Occupied by Various Compartments**

The point counting method of Weibel et al. (45) was employed to determine the percent area occupied by each compartment. For 950 THE JOURNAL OF CELL BIOLOGY. VOLUME 96, 1983
this study a transparent overlay was placed over each micrograph and points by intersection overlying the various subcellular compartments were scored using a lattice where \( d \) equaled 1 cm. The percent area was determined as previously described (3). The consistency of the determinations at various chase intervals took into consideration the factors outlined by Weibel et al. (45). Within experiment groups there was no significant difference in the analysis of variance for the intra group volume density data.

**RESULTS**

**Morphology of Pancreatic Acinar Carcinoma Cells**

Two different preparations of neoplastic acinar cells were investigated by high resolution electron microscopic autoradiography: GEF cells consisting of neoplastic cells that show abundant secretory granules and prominent Golgi apparatus and closely resemble normal pancreatic acinar cells; and GDF cells consisting of morphologically undifferentiated carcinoma cells that are devoid of zymogen granules. The GDF cells differ from GEF cells in nuclear position, nuclear/cytoplasmic ratio, and amount of stored secretory product in the form of mature zymogen granules (3). Both GEF and GDF neoplastic cell subpopulations have been isolated by subjecting the freshly dissociated neoplastic cell population of Percoll gradient centrifugation (3).

**Differentiation of Cells Sampled**

The cellular differentiation and degree of zymogen maturation of the component cells of the two different preparations used for autoradiographic study were investigated by morphology and morphometry. Table I shows both the number of granule enriched and granule-deficient cells contained in GEF and the number contained in GDF cells. GEF cells show a high degree of zymogen maturation and >90% of the cells sampled in this experimental group were highly differentiated granule-enriched cells. GDF cells show a much lower level of phenotypic zymogen maturation as evidenced by the virtual absence of secretory granules. This preparation of carcinoma cells consists of >85% granule-deficient carcinoma cells and the <15% granule containing cells included in GDF reveal few, small secretory granules.

**Area Percent Values**

The area of the two enriched subpopulations occupied by each of the nine organelle compartments defined in Materials and Methods is given in Table II. The percentage of total cell area occupied by various cell organelles depicted in Table II represents an average volume density of the nine time points analyzed for each group. Actual grain density determinations were however, based on the area percent distribution of a given cell organelle at the individual time points. The variations in area percent determinations observed at the individual time points are represented statistically in Table II.

**Electron Microscopic Autoradiographic Analysis of Intracellular Transport**

**INCORPORATION OF [\(^3\)H]LEUCINE IN GEF AND GDF SUBPOPULATIONS SEPARATED ON PERCOLL:** As previously described (3), both GEF and GDF cells incorporate [\(^3\)H]leucine to a comparable extent as visualized by light microscopic autoradiography (Fig. 1a and b). At the end of the pulse period, autoradiographic grains appear randomly distributed over the nucleus and cytoplasm in both GEF and GDF cells (Fig. 1a and b). At the electron microscope level, immediately postpulse, the majority (~47% for GEF and ~37% for GDF) of autoradiographic grain centers was localized directly over the nucleus and 2-HD of the plasma membrane in GDF cells appears higher than that in GEF cells, but a significant grain density difference was noted only for the plasma membrane compartment (\( P < 0.01 \)).

**INTRACELLULAR TRANSPORT IN GEF SUBPOPULATION:** The GEF neoplastic pancreatic acinar cells transport newly synthesized proteins similar to that observed in dissociated normal pancreatic acinar cells (Table III; Fig. 2). Results of grain counts clearly demonstrated the peak specific activity of the isotope in the RER at 0 and 5 min of chase incubation ( grain percent 46.6 and 43.6; and grain density 1.7 and 1.5, respectively). With increasing periods of chase incubation the level of specific activity in RER fell to basal level by 30 and 60 min ( grain density 0.2–0.5). The grain density in the Golgi complex increased to a maximum of 8.2 at 20 min of chase incubation ( grain percent 35.3) with the peak of activity probably occurring between 10 and 20 min postpulse.

Fig. 3 shows transport of radio-labeled proteins in GEF at 10, 20, and 30 min of chase incubation. At 10 min of chase (Fig. 3a) autoradiographic grains are seen near transitional elements of RER and have begun to concentrate in the Golgi complex. By 20 min (Fig. 3b) the majority of label in GEF cells is over the cisternae of the Golgi complex. Transfer of radio-labeled proteins to the trans face of the Golgi to post-

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**Table I**

| Cell group* studied | Highly differentiated granule-enriched cells (%) | Poorly differentiated granule-deficient cells (%) | Total number of cells |
|---------------------|-----------------------------------------------|-----------------------------------------------|----------------------|
| GDF                 | 47 (11.2)                                     | 450 (88.8)                                    | 507                  |
| GEF                 | 470 (93.8)                                    | 31 (6.2)                                      | 501                  |

* The GDF and GEF subpopulations of acinar carcinoma cells isolated on Percoll gradients were classified according to the extent of cytodifferentiation as described by Becich and Reddy (3).
Golgi vesicles is seen at 30 min postpulse (Fig. 3c). Little retention of radioactivity is seen in the Golgi complex after 60 min of chase incubation (Fig. 4a) indicating efficient transport of newly synthesized protein. Concentration of grains over post-Golgi vesicles begins at 10 min postpulse and reaches a maximum at 60 min (Fig. 4a) (grain percent 38.2, grain density 4.02 at 60-min chase). The label over post-Golgi vesicles does not return to basal levels (0.2 at 5 min) even by 240 min postpulse incubation indicating retention of radioactivity in these vesicles. Concentration of label in secretory granules was first evident at 60 min of chase incubation (Fig. 4a), reaching a level of 41% at 240 min at chase. GEF cells contain a large amount of pre-existing secretory granules; the incorporation of newly synthesized secretory proteins into this pool of stored protein led to labeling of several, but not all secretory granules (Fig. 4b). At all time points analyzed mitochondrial and nuclear labeling remained relatively constant, but the observed grains were higher than those derived from studies using normal pancreatic acinar epithelium (2). Plasma membrane labeling remained constant through 60 min of chase; thereafter there was a slight increase in grain density that coincided with a peak in transport to post-Golgi vesicles and zymogen granule compartments.

INTRACELLULAR TRANSPORT IN GDF SUBPOPULATION: At the end of a 3-min pulse, the majority of (~75% of the radioautographic grains) were associated with cytoplasm of these granule-deficient pancreatic acinar carcinoma cells (Fig. 5 and Table IV). The results of grain counts expressed as the percentage of total grains over a given compartment at each time point shown in Table IV and Fig. 5 demonstrate the transport of proteins from RER to the Golgi complex. Concentration of radioactivity in the Golgi complex of GDF occurs at levels comparable to those of GEF cells (grain density of 10.2 at 10 min and 8.3 at 20 min), with the peak activity occurring closer to 10 min of chase incubation (Figs. 5 and 6a). This is somewhat earlier than that observed for GEF cells. By 30-min chase (Fig. 6b) transport of newly synthesized protein to post-Golgi vesicles is evident and by 60 min (Fig. 7) the grain density in the Golgi complex dropped to 1.7 from its peak level of 10.2. Because these neoplastic cells are devoid of secretory granules the resolution of the post-Golgi transport, i.e., concentration and storage steps of the secretory process of label, was not possible, although there was a rise in the number of autoradiographic grains in post-Golgi vesicles at 30- and 60-min postpulse. By 240 min, a considerable number of autoradiographic grains appeared over the nucleus and the remainder over profiles of RER, mitochondria, and plasma membrane (Table IV). At this interval, only a small percentage of autoradiographic grains were associated with secretory elements of these cells (the Golgi complex, post-Golgi vesicles, etc.). The
### Table III

**Intracellular Distribution of Newly Synthesized Proteins as Visualized by Electron Microscope Autoradiography in Isolated Granule-enriched Cells from Pancreatic Acinar Carcinoma**

| Compartment                | 0 min | 5 min | 10 min | 20 min | 30 min | 60 min | 120 min | 180 min | 240 min |
|----------------------------|-------|-------|--------|--------|--------|--------|---------|---------|---------|
| RER                        |       |       |        |        |        |        |         |         |         |
| Distribution of autoradiographic grains at incubation times post-pulse* |
| Golgi complex              | 46.6 (1.7) | 43.6 (1.5) | 24.9 (1.1) | 19.2 (0.7) | 12.8 (0.5) | 5.9 (0.2) | 5.0 (0.2) | 5.0 (0.2) | 5.2 (0.2) |
| Post-Golgi vesicles        | 7.9 (1.7) | 12.1 (3.9) | 25.7 (6.7) | 35.3 (6.2) | 19.5 (4.6) | 8.7 (2.1) | 3.6 (0.7) | 5.9 (0.9) | 2.2 (0.6) |
| Secretory granules         | 0.9 (0.1) | 1.8 (0.2) | 6.5 (0.7) | 4.2 (0.5) | 25.4 (2.7) | 38.2 (4.0) | 20.3 (1.9) | 14.8 (1.6) | 12.7 (1.3) |
| Plasma membrane zone       | 6.8 (0.6) | 4.2 (0.3) | 8.1 (0.5) | 14.2 (0.8) | 12.6 (0.8) | 17.1 (1.1) | 35.4 (2.1) | 37.2 (2.4) | 41.0 (2.7) |
| Nuclear                    | 96.1 (1.1) | 10.5 (1.4) | 11.6 (1.7) | 9.5 (1.3) | 11.7 (1.5) | 11.8 (1.5) | 17.1 (1.8) | 15.3 (2.0) | 15.2 (2.0) |
| Cytoplasmic matrix         | 20.4 (0.8) | 16.8 (0.8) | 14.9 (0.6) | 10.6 (0.3) | 13.3 (0.6) | 12.0 (0.5) | 11.2 (0.6) | 14.9 (0.6) | 13.3 (0.5) |
| Mitochondria               | 4.6 (0.6) | 6.3 (0.7) | 4.7 (0.6) | 3.7 (0.5) | 3.4 (0.4) | 4.6 (0.6) | 4.3 (0.5) | 3.2 (0.4) | 6.0 (0.7) |
| Miscellaneous + lysosomes  | 0.5 (1.0) | 0.9 (1.8) | 0.7 (1.6) | 0.4 (3.5) | 0.4 (0.7) | 0.0 (0.0) | 0.2 (0.3) | 1.4 (2.8) | 1.6 (1.0) |
| * Values in parentheses are grain density data. Underlined values represent maximum concentrations of radio-label in that compartment.

### Table IV

**Intracellular Distribution of Newly Synthesized Proteins as Visualized by Electron Microscope Autoradiography in Isolated Granule-deficient Cells from Pancreatic Acinar Carcinoma**

| Compartment                | 0 min | 5 min | 10 min | 20 min | 30 min | 60 min | 120 min | 180 min | 240 min |
|----------------------------|-------|-------|--------|--------|--------|--------|---------|---------|---------|
| RER                        |       |       |        |        |        |        |         |         |         |
| Distribution of autoradiographic grains at incubation times post-pulse* |
| Golgi complex              | 37.6 (1.3) | 36.5 (1.3) | 28.3 (1.0) | 22.6 (0.7) | 18.0 (0.6) | 16.3 (0.6) | 13.0 (0.5) | 10.4 (0.4) | 9.6 (0.4) |
| Post-Golgi vesicles        | 7.0 (4.0) | 10.8 (4.4) | 20.0 (10.2) | 16.0 (8.3) | 15.0 (6.9) | 4.3 (1.7) | 3.6 (1.5) | 3.2 (1.2) | 1.5 (0.7) |
| Secretory granules         | 2.3 (0.3) | 2.6 (0.3) | 3.3 (0.5) | 2.5 (0.4) | 16.6 (2.3) | 22.6 (3.3) | 21.5 (3.0) | 20.7 (2.4) | 12.9 (1.8) |
| Plasma membrane zone       | 17.2 (2.4) | 18.2 (2.1) | 13.0 (2.0) | 13.9 (2.4) | 12.7 (2.0) | 14.8 (2.0) | 15.2 (2.2) | 19.6 (2.8) | 15.3 (2.1) |
| Nucleus                    | 24.6 (0.6) | 20.9 (0.5) | 26.4 (0.6) | 36.2 (0.9) | 30.3 (0.7) | 34.7 (0.8) | 36.2 (0.8) | 38.7 (0.9) | 50.2 (1.2)‡ |
| Cytoplasmic matrix         | 4.1 (0.6) | 2.8 (0.6) | 3.0 (0.6) | 2.5 (0.4) | 1.5 (0.3) | 0.8 (0.2) | 3.0 (0.5) | 0.9 (0.2) | 1.2 (0.2) |
| Mitochondria               | 6.2 (0.9) | 6.9 (0.9) | 5.3 (0.7) | 5.5 (0.7) | 5.4 (0.8) | 5.7 (0.9) | 6.8 (1.0) | 5.7 (0.8) | 6.9 (1.0) |
| Miscellaneous + lysosomes  | 0.4 (1.0) | 0.5 (1.4) | 0.8 (1.9) | 0.3 (0.6) | 0.1 (0.2) | 0.2 (0.8) | 0.3 (0.7) | 0.4 (0.7) | 0.8 (1.8) |
| * Values in parentheses are grain density data. Underlined values represent maximum concentrations of radio-label in that compartment.
| ‡ Significantly different from 0 min (P < 0.01).
Mitotic profiles were also seen in some (0.5) and GDF (1.2) at 240 postpulse, however, was statistically
the grain density of the nucleus of GEF
cells did not show a significant change at any time point (0.8-
shrinkage. In the mitotic cells of both GDF and GEF there
several autoradiographic grains are discerned over secre-
postpulse, ~26% of the total grains is associated with the Golgi
apparatus and from there to the post-Golgi vesicles
(vacuoles or immature granules) and eventually to mature
secretory granules as in a variety of other cells that store their
secretory product (5, 6, 8, 15). Transfer of label to the Golgi
apparatus occurs very rapidly in these GEF cells; by 10-min
postpulse, ~26% of the total grains is associated with the Golgi
apparatus, reaching a peak of ~35% by 20-min postpulse. The
kinetic data demonstrate that movement of secretory proteins
into the post-Golgi vesicles commences rapidly because by 10-
min postpulse ~6% of the label is in association with this
compartment. Movement of label into secretory granules be-
comes evident by ~20-min postpulse and continues even up to
4-h postpulse. The persistence of high residual levels of radio-
activity in the post-Golgi vesicles at 4-h postpulse suggests a
possible defect involving condensation of secretory proteins in
this region. The possibility that this residual label might rep-
resent a nonsecretory protein, however, cannot be excluded.
The majority of label associated with RER moves very
rapidly (i.e., drained within 30 min) in these GEF neoplastic
acinar cells. Our results further indicate that ~40% of the label
at 4-h postpulse is in association with secretory granules. This
value is comparable to that observed by Amsterdam and
zymes in all secretory granules and the Golgi apparatus of the acinar cells, suggesting that these well-differentiated GEF cells retain the essential regulatory controls involved in the secretory process (3). Similar to that seen in normal pancreas (23), further suggests that these well-differentiated neoplastic cells retain the ability to express pancreatic specific gene function. This level of synthesis of exportable proteins is remarkable considering the fact that GEF cells are neoplastic and that a sizable proportion of this subpopulation is in DNA-replicative phase at any given period (3, 31). The presence of several of these cells in mitosis at 4-h postpulse and the autoradiographic evidence of label in secretory granules of such mitotic cells observed in the present study suggest that the processing of exportable proteins is not interrupted during cell division.

In the GDF neoplastic pancreatic acinar cells, secretory granules are virtually absent. The Golgi apparatus in these cells is less developed when compared to that present in GEF cells (3, 31). At the end of the 3-min pulse ~38% of the label is seen over the RER in these GDF cells. Transport to the Golgi apparatus takes place relatively rapidly because by 10-min postpulse ~20% of the label is seen in association with this organelle. The data also indicate that the drainage of RER is slower in GDF cells; however, the drainage of the Golgi apparatus is essentially complete by 60-min postpulse as in GEF cells. Our results also indicate that the relative amount of the peak label in the Golgi and the post-Golgi vesicles in GDF cells is less than that present at these intracellular sites in GEF cells. The present autoradiographic results do not provide additional insight into the fate of proteins transported into the post-Golgi vesicles through the Golgi apparatus because of the absence of typical condensing vacuoles and secretory granules in these GDF cells.

The RER synthesizes a variety of proteins (bonefide secretory or exportable proteins, lysosomal enzymes, and membrane proteins) that have different intracellular destinations (18). The plasma membrane glycoproteins synthesized in the RER pass through the Golgi apparatus and transported to the cell periphery for insertion with kinetics similar to those reported for secretory proteins (18). The present autoradiographic study of GDF neoplastic cells does not, however, distinguish between secretory and nonsecretory component of the label seen in the Golgi and post-Golgi vesicles. The relatively marginal immunocytochemical labeling of secretory enzymes in the Golgi apparatus of these GDF cells (Bendayan, M., M. J. Becich, and J. K. Reddy, manuscript in preparation) provides only an equivocal support for the assumption that at least part of the label is destined for export. Accordingly, on the basis of the present autoradiographic data and somewhat limited immunocytochemical evidence, we tentatively conclude that GDF cells synthesize secretory proteins which move from the RER → the Golgi apparatus → the post-Golgi vesicles but do not store the secretory product in recognizable mature granules.

The autoradiographic data also demonstrate that at 4-h postpulse only ~15% of the label in GDF cells is in association with the Golgi, the post-Golgi vesicles, and secretory granules, whereas these three compartments in GEF cells account for ~55% of the label. Calculation of total grain density per cell as a function of incubation time postpulse in GEF and GDF revealed that these subpopulations retain the same proportion (i.e., ~72% and ~80%, respectively) of the labeled macromolecules at 4-h postpulse. These data suggest that GDF cells may at least secrete constitutively a portion of the labeled macromolecules. At present, the reasons for the inability of these GDF cells to concentrate and store secretory proteins are not readily apparent, though two possibilities can be considered.

(a) There is a defect in the synthesis, either qualitative or quantitative, and/or packaging of secretory proteins in these GDF cells. Recently, Iwanj and Jamieson (16, 17) compared the secretory proteins synthesized and discharged by the heterogeneous population of neoplastic pancreatic acinar cells with that of adult rat pancreas, as well as pancreatic rudiments of embryonic rat. They noted that a group of basic polypeptides, such as proelastase, basic chymotrypsinogen, and ribonuclease, were greatly reduced or absent in tumor cell secretion and concluded that the secretory protein patterns of the acinar tumor cells most closely resembles that of day-19 embryonic rat pancreas (17). However, it is appropriate to note that secretory protein pattern of a highly purified fraction of secretory granules from the pancreatic acinar carcinoma appears essentially similar to that of secretory granules isolated from adult rat pancreas (Hansen, L. J., M. K. Reddy, and J. K. Reddy, manuscript in preparation). These studies suggest that

![Figure 5](image-url)

**Figure 5** Intracellular transport of pulse-labeled secretory proteins in secretory granule-deficient neoplastic pancreatic acinar cell subpopulation (based on data presented in Table IV). Wavelike movement of label from RER (O) → the Golgi complex (●) → post-Golgi (▲) vesicles is evident. The absence or paucity of ultrastructurally identifiable secretory granules (■) precludes the delineation of the concentration and storage steps of the secretory process at this stage of cytodifferentiation.

![Figure 4](image-url)

**Figure 4** Autoradiograms of secretory granule-enriched neoplastic acinar cells after 60 (a) and 240 min (b) of chase incubation. At 60 min, the stack of Golgi cisternae no longer contains appreciable quantity of the label, but the small vesicles in the inner face of the Golgi complex are labeled (arrows). Some label is seen associated with secretory granules (5). By 240 min of chase incubation, the label is concentrated over secretory granules. Very little label is found over the Golgi complex. a, × 20,000; b, × 18,000.
secretory protein expression in individual tumor cell subpopulations (e.g., GEF and GDF) may vary considerably. The GDF cells contain substantially lower amounts of secretory product when compared to GEF cells as judged by the amylase/DNA ratio of 0.45 and 3.3, respectively (3). The absence of secretory granules in GDF cells, therefore, may be due to substantially lower levels of synthesis of the secretory proteins and/or to a defect in the yet to be elucidated concentration mechanism(s) in the Golgi or the post-Golgi vesicles (13, 18, 27). For example, differences in the synthesis of sulfate-containing macromolecules (33, 34, 46) in the Golgi elements of the GDF and GEF neoplastic cells might play a significant role.

(b) The newly synthesized secretory proteins in these GDF cells bypass the typical storage step and are rapidly discharged due to a functional defect. It is pertinent to note that Jamieson and Palade (22) demonstrated that in secretagogue-stimulated normal pancreatic acinar cells the secretory proteins are concentrated and packaged in numerous small storage vesicles in a normal fashion, but they are rapidly discharged. Preferential release of newly synthesized prolactin in mammtrophs has been described (43).

Additional studies, therefore, are necessary to determine whether one or more of the possibilities considered above contribute to the difference in the secretory process between GDF and GEF neoplastic pancreatic acinar cells. Analysis by two-dimensional PAGE of the [35S]methionine-labeled total (9) and secretory proteins (38) synthesized by these two subpopulations will be of value in this regard.

The pancreatic acinar carcinoma cells and fragments have been shown to respond to several secretagogues and discharge protein at approximately one-fifth the rate determined for the normal pancreas lobules (16, 44). The decreased responsiveness of acinar carcinoma cells is attributable to cellular heterogeneity and to the higher levels of nonexportable protein synthesis, particularly in GDF cells. The reduced secretory response may be due to differences in cell surface receptors for secretagogues in this heterogeneous tumor cell population. In conclusion, this transplantable pancreatic acinar carcinoma provides an important model system for the investigation of factors that modulate cytodifferentiation and gene expression (16, 17, 19, 32). Study of differentiation in these neoplastic cells may provide a better understanding of both abnormal and normal differentiation.

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