Reconstitution of the Golgi Apparatus After Microinjection of Rat Liver Golgi Fragments into *Xenopus* Oocytes

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Abstract. We have studied the reconstitution of the Golgi apparatus in vivo using an heterologous membrane transplant system. Endogenous glycopeptides of rat hepatic Golgi fragments were radiolabeled in vitro with [*H]*sialic acid using detergent-free conditions. The Golgi fragments consisting of dispersed vesicles and tubules with intraluminal lipoprotein-like particles were then microinjected into *Xenopus* oocytes and their fate studied by light (LM) and electron microscope (EM) radioautography. 3 h after microinjection, radiolabel was observed by LM radioautography over yolk platelet-free cytoplasmic regions near the injection site. EM radioautography revealed label over Golgi stacked saccules containing the hepatic marker of intraluminal lipoprotein-like particles. At 14 h after injection, LM radioautographs revealed label in the superficial cortex of the oocytes between the yolk platelets and at the oocyte surface. EM radioautography identified the labeled structures as the stacked saccules of the Golgi apparatus, the oocyte cortical granules, and the plasmalemma, indicating that a proportion of microinjected material was transferred to the surface via the secretion pathway of the oocyte. The efficiency of transport was low, however, as biochemical studies failed to show extensive secretion of radiolabel into the extracellular medium by 14 h with approximately half the microinjected radiolabeled constituents degraded. Vinblastine (50 μM) administered to oocytes led to the formation of tubulin paracrystals. Although microinjected Golgi fragments were able to effect the formation of stacked saccules in vinblastine-treated oocytes, negligible transfer of heterologous material to the oocyte surface could be detected by radioautography. The data demonstrate that dispersed fragments of the rat liver Golgi complex (i.e., unstacked vesicles and tubules) reconstitute into stacked saccules when microinjected into *Xenopus* cytoplasm. After the formation of stacked saccules, reconstituted Golgi fragments transport constituents into a portion of the exocytic pathway of the host cell by a microtubule-regulated process.

The cellular and molecular requirements for Golgi apparatus stack formation are poorly understood. To define whether such a reconstructive process may be carried out with a heterologous system and using cytoplasmic components from cells of animals representing a considerable gap in vertebrate species, we have microinjected rat liver Golgi fragments into *Xenopus* oocytes.

Forbes et al. (11) showed that DNA provoked the reconstitution of nuclear envelopes after microinjection into *Xenopus* eggs, hence, that the subcellular constituents required for the reconstitution of cellular organelles may be available in the amphibian oocyte cytoplasm. Consistent with this finding was the observation of apparent Golgi “stack” reformation after microinjection into oocytes of rat liver Golgi fragments (24) and the demonstration of reconstitution of ER after microinjection of rat liver microsomes into similar oocytes (25). ER reconstitution was observed to be membrane specific since the microinjection of rough microsomes yielded parallel, flattened cisternae and the microinjection of smooth microsomes led to the formation of cytoplasmic regions filled with an interconnecting meshwork of tubules (25). In the present study we have used a radiolabeled marker ([*H]*sialoglycoproteins prepared via in vitro glycosylation [4]) and EM radioautography to enable identification and determination of the fate of rat hepatic Golgi fragments after cellular transplantation. Furthermore, the use of the labeled marker as well as the microtubule-disrupting agent vinblastine has enabled an assessment of the transfer of microinjected Golgi molecular constituents into the *Xenopus* secretory pathway and the role of microtubules thereon.

Materials and Methods

Subcellular Fractionation

The Golgi intermediate (Gi) I fraction was prepared from rat liver homogenates as described previously (3). Nondisruptive radiolabeling of the endogenous glycopeptides of the Gi fraction was carried out with CMP-
[\textsuperscript{3}H]sialic acid as described (4). Briefly, freshly prepared Gi fractions (1.9 mg) were incubated with 100 \muCi CMP-[\textsuperscript{3}H]sialic acid (sp act 11.5 Ci/mmol), 60 mM Na-cacodylate buffer, pH 6.5, and 4 mM ATP in a final volume of 6 ml, and incubated at 37°C for 15 min at which time the tubes containing the incubation mixtures were placed on ice. Portions of the incubation mixture (25 \mul) were evaluated for their content of total and acid-insoluble radioactivity by precipitation with ice-cold 10% trichloroacetic acid.

Separation of free CMP-[\textsuperscript{3}H]sialic acid from incorporated radioactivity was carried out by centrifugation. To minimize damage to the Golgi fractions induced by pelleting, fractions were washed by flotation in sucrose gradients. Hence, after incubation of Gi fractions with CMP-[\textsuperscript{3}H]sialic acid, the incubation mixture was adjusted to 1.18 M sucrose and recentrifuged in the identical sucrose step gradient. The Golgi fraction was recovered at the 0.25 M/1.0 M sucrose interface and evaluated for its content of acid-soluble and acid-insoluble radioactivity as well as by EM radioautography and fluorography.

For SDS-PAGE analysis of the radiolabeled products, separate incubations were carried out with 2 mg Gi fraction protein, 25 \muCi CMP-[\textsuperscript{3}H]sialic acid (sp act 30.7 Ci/mmol) in the same buffer as described above but in a final volume of 5.5 ml. After incubation for 15 min at 37°C, an aliquot representing \sim 10^6 dpm of TCA-precipitable radioactivity (300 \mul) was placed on ice and, after the addition of Laemmli (19) sample preparation buffer, boiled for 5 min. The remaining material (5.2 ml) was subjected to the double flotation protocol as described above and aliquots representing \sim 10^6 dpm of TCA-precipitable radioactivity were removed from the first and second flotation wash and were likewise prepared for SDS-PAGE. SDS-PAGE and fluorography were as described previously (5). The proportion of radiolabel associated with the membrane and content of the flotation-washed Golgi fraction was assessed by NaC\textsubscript{3}0\textsubscript{4} extraction as described by Howell and Palade (12).

**Microinjections and In Vivo Incubations**

Golgi fractions (Gi) along with equivalent volumes of liquid paraffin were microinjected into Xenopus oocytes exactly as described previously (24). Incubation of oocytes was done at ambient temperature for 3 and 14 h. Vinblastine sulfate (Sigma Chemical Co., St. Louis, MO) was made up as a stock solution (50 mM in dimethylsulfoxide) and diluted to 50 \muM in modified Barth's medium before use. For treatment of the oocytes with vinblastine, they were incubated in this solution 7 h before injection with Golgi fragments. After microinjection, the oocytes were incubated in the same medium for 3 h and then processed for light microscopy (LM) and EM. Other oocytes were microinjected and incubated afterward identically, but they were incubated for an additional 14 h in the absence of vinblastine.

The extent of secretion was evaluated biochemically by microinjecting radiolabeled Gi fraction (~6,000 dpm) into a total of 10 oocytes. At 3 and 14 h after injection into oocytes, from which follicular cells had been removed (21), the extent of acid-soluble and acid-insoluble radioactivity secreted into the medium and retained within cells was evaluated. Control experiments were also done in which CMP-[\textsuperscript{3}H]sialic acid (~12,000 dpm) was microinjected into a total of 10 oocytes with acid-soluble and acid-insoluble radioactivity in cells and medium determined at 3 and 14 h after microinjection. For the biochemical experiments medium was supplemented with 1 \mug/ml of pepstatin, leupeptin, aprotinin, antipain, and phenanthroline to inhibit extracellular protease activity.

**Morphological Procedures**

Membrane subfractions were fixed with or without prior incubation and collected onto Millipore Corp. (Bedford, MA) membranes as previously outlined (23) and processed for EM radioautography as described previously (16, 17). For routine EM, oocytes were fixed and processed using the procedure of Kalt and Tandler (13).

The injection of cytoplasmic membranes along with equivalent volumes of liquid paraffin into the equatorial region of stage V and VI Xenopus oocytes leads to the formation of large cytoplasmic droplets composed of liquid paraffin which can easily be recognized as vacuoles (to be referred to as injection vacuoles) by LM at low magnification (24). The injection of Golgi fragments also leads to the appearance of yolk platelet-free regions in the cytoplasm of the oocyte (24). Such regions were mostly found within 200–300 \mu m distance from the injection vacuoles. Thus for LM and EM radioautography, the injection site of the oocyte cytoplasm was found and semithin and thin sections were prepared which contained the injection vacuole, the adjacent cytoplasm, and the nearest portion of the oocyte cortex. Such sections were then coated with photographic emulsion and processed for radioautographic analysis (16-18). Quantitation of radioautographs was as previously described (27).

**Results**

Endogenous glycoprotein acceptors in rat hepatic Gi fractions were sialylated via CMP-[\textsuperscript{3}H]sialic acid in a nondisruptive manner. Free CMP-[\textsuperscript{3}H]sialic acid was removed by a two-step flotation protocol to minimize organelle damage. The results (Figs. 1 and 2) demonstrated removal of free nucleotide sugar with acceptable retention of fraction morphology. Radiolabeled acceptors were identified by SDS-PAGE (Fig. 3) with a similar spectrum of peptide acceptors as identified previously for the intact Golgi fraction (stacked saccular Golgi fraction [4]). No selective loss of radiolabeled peptide acceptors was observed by the washing protocol which was used (Fig. 3). Separation of membrane from content of the fractions according to the NaC\textsubscript{3}0\textsubscript{4} washing method of Howell and Palade (12) revealed 45.5 ± 4.5% (n = 2 ± 0.5) variation of macromolecular radioactivity in the soluble content and 54.5 ± 4.5% in the membrane.

After microinjection of such radiolabeled hepatic Golgi fragments into Xenopus oocytes, LM radioautography was carried out (Fig. 4). Silver grains were observed over yolk platelet-free regions of the oocyte near the injection vacuole at 3 h after injection. At this time interval the majority of label was restricted to regions near the injection vacuole. By 14 h, label was also observed over the oocyte cortex nearest the injection sites (Fig. 5).

EM radioautography revealed the majority of label at 3 h over disrupted hepatic Golgi fragments. However, significant label was observed over reconstituted stacked Golgi saccules (Fig. 6, Table I). These saccules retained the hepatic marker,
Figure 2. EM radioautographs of Gi fraction prepared immediately after incubation with CMP-[3H]sialic acid (A) or after recentrifugation by double flotation (B). Large endosome-like structures (e) are unlabeled. Silver grains (arrows) are found over tubules (t), annulus-like structures (a), and other membraneous components (m). Exposure, 77 d. Bars, 0.5 μm.
intraluminal lipoprotein particles. Golgi apparati of oocyte origin could be distinguished from reconstituted stacked Golgi saccules based on the following observations made in control (uninjected) oocytes. Oocyte Golgi apparatus never showed intraluminal lipoprotein particles (Fig. 7). The oocyte Golgi apparatus were often observed as large cup-shaped structures near yolk platelets and the saccules tended to be longer and often flattened (Fig. 7). In oocytes microinjected with radiolabeled hepatic Golgi fragments no silver

grains were observed over oocyte secretory components in the cortex of the oocyte at 3 h after injection. By 14 h, label was associated with disrupted Golgi fragments as well as with stacked Golgi saccules in yolk platelet-free regions (Fig. 8). Furthermore, grains were observed over cortical Golgi fragments, cortical granules, and the cell surface of the oocyte (Fig. 9). Silver grains were not observed over yolk platelets or pigment granules.

Two quantitative studies were carried out. In one analysis (Tables I and II) only cytoplasmic structures restricted to the yolk platelet-free regions near the injection site were evaluated. In Table I the relative proportion of label directly associated (24.3%) with the Golgi apparatus is indicated. Because of radiation scatter, the true value may be as high as 44%. In Table II Golgi apparati were scored for the number of saccules per Golgi apparatus and the presence of the intraluminal lipoprotein particles and the [3H]sialic acid radio-label. The results showed the majority of Golgi apparatus analyzed in these regions at 3 h after injection to be marked with intraluminal lipoprotein particles. Furthermore, the Golgi apparatus were overlaid by silver grains. At 14 h after injection, stacking of the saccules was more polydisperse and the proportion of Golgi apparatus fragments containing intraluminal lipoprotein particles decreased. Radiolabeling of the stacked Golgi apparatus was maintained presumably due to the continued formation of stacked saccules from dispersed radiolabeled vesicles and tubules.

A second quantitative analysis was carried out to determine the distribution of silver grains over the cortical region of the oocyte at 14 h after injection (Table III). Here, the

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**Figure 3.** Fluorography of radiolabeled sialoglycoproteins after incubation of the Gi fraction (2 mg cell fraction protein) with radiolabeled CMP-[3H]sialic acid (25 μCi, sp act 307 Ci/mmol) for 10 min at 37°C as described in Materials and Methods. Subsequent to incubation, equal aliquots of acid-precipitable radioactivity (~10^6 dpm) were processed directly for SDS-PAGE (lane 1) or after a single (lane 2) or double flotation wash (lane 3) as described in Materials and Methods. Radioactivity is associated with several Golgi peptides with the relative mobilities of protein standards indicated on the extreme left in kD. Exposure was for 2 wk.

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**Figure 4.** LM radioautograph of oocyte cytoplasm at 3 h after injection of radiolabeled hepatic Golgi fragments. Silver grains (arrows) are over yolk platelet-free regions near the injection vacuole (iv). yp, yolk platelets. Exposure, 120 d. Bar, 10 μm.
majority of silver grains were observed over the plasmalemma and cortical granules of the oocyte.

Since the microinjection of Golgi fragments led to the formation of yolk platelet-free regions (reference 24; Figs. 4 and 5) we reasoned that this might be related to the interaction of microinjected elements with the oocyte cytoskeleton and therefore tested the effect of vinblastine on the organization of microinjected Golgi fragments and on the exit of rat hepatic molecules to the oocyte surface.

Incubation of oocytes with 50 μM vinblastine led to distinct changes in oocyte organization. Low magnification LM indicated a displacement of oocyte nuclei and microinjection vacuoles towards the surface of the animal pole of the oocyte (Fig. 10 A). Higher magnification LM revealed pigment granule dispersion in the animal pole cortex as well as the presence of large pale-staining crystalloids (Fig. 10 B). Often the crystalloids were surrounded by pigment granules and were found just beneath the cortical granules which were aligned at the oocyte surface (Fig. 10 B). The crystalloids were shown by electron microscopy to be composed of tightly bound tubular paracrystals (Fig. 10 C).

The morphology of the yolk platelet-free regions produced
Figure 6. EM radioautographic detection of [3H]sialic acid label in reconstituted stacked Golgi apparatus at 3 h after injection. Both intraluminal lipoprotein particles (arrowheads) and silver grains (arrows) are observed associated with reconstituted Golgi apparatus (G). Mitochondria (m) and lysosome-like components (X) are unlabeled. Exposure, 77 d. Bar, 0.5 μm.

Table I. Silver Grain Distribution Over Cytoplasmic Structures in Yolk Platelet-free Regions Near the Injection Sites at 3 h After Microinjection of [3H]Sialo-labeled Rat Liver Golgi Fragments

| Vesicles with lipoprotein particles | Stacked saccular Golgi apparatus | Others | Total |
|------------------------------------|---------------------------------|--------|-------|
| Number of grains (n)               | 110                             | 73     | 117   | 300   |
| Number of grains (%)               | 36.6                            | 24.3   | 39    |

Quantitation was done using 23 electron microscope photomicrographs printed at a final magnification of 45,000. Silver grains were scored as belonging to one of the identified compartments when a grain was observed directly over the structure. Grains scored in Others compartment represent grain scatter and were found in the cytosol in the immediate vicinity of the above labeled compartments. Yolk platelet-free regions near the injection sites are cytoplasmic regions devoid of yolk platelets showing radioactive labeling and are normally within 200–300 μm from an injection vacuole.

in vinblastine-treated oocytes after microinjection with radiolabeled rat liver Golgi fragments was examined. Stacked saccules were often observed associated with numerous lipoprotein particle-filled vesicles and variable numbers of lysosome-like structures (Fig. 11). Colocalization of the morphological marker for rat liver components (the lipoprotein particle), and radiolabel (hepatic Golgi marker) was often observed in stacked saccules (Fig. 11). Neither marker was observed near or within oocyte cortical structures, including cortical granules and plasmalemma, 14 h after the microinjection of radiolabeled Golgi fragments (not shown). Radiolabeling of the cortex in similarly treated oocytes was not observed by light or electron microscope radioautography even after prolonged exposures (>10 mo) of the radioautographs.

When secretion was evaluated biochemically, i.e., after in-
Table II. Distribution of Silver Grains Over Golgi Apparatus in Yolk Platelet-free Regions Near the Injection Sites at 3 and 14 h After Microinjection of \[\text{SHlSialo-labeled Rat Liver Golgi Fragments}\]

| In vivo incubation (h) | Number of Golgi apparatus examined | Number of saccules per Golgi apparatus (mean ± SD) | Number of Golgi apparatus with intraluminal lipoprotein particles (mean ± SD) | Number of silver grains per Golgi apparatus (mean ± SD) |
|-----------------------|-----------------------------------|--------------------------------------------------|--------------------------------------------------------------------------|------------------------------------------------------|
| 3                     | 20                                | 3.4 ± 0.8                                        | 18                                                                       | 3.7 ± 2.3                                             |
| 14                    | 17                                | 5.7 ± 2.5                                        | 12                                                                       | 4.2 ± 2.6                                             |

Quantitation was as described for Table I. 23 and 35 electron microscope photomicrographs were used for the analysis of the 3- and 14-h time points, respectively. Yolk platelet-free regions are defined as in the legend to Table I.

Microinjection of the radiolabeled Gi fraction into four groups of 10 oocytes, only 5.6 ± 1.6% (mean ± SD) of injected radioactivity was secreted with little difference seen with time of incubation or incubation in the presence of vinblastine (data not shown). Indeed, by 3 h 29% of injected Gi radiolabel was degraded. This value rose to 45.7% by 14 h. By contrast, microinjection of CMP-[3H]sialic acid showed at 3 h only 9.9% incorporated into macromolecules with a slight increase to 11.5% by 14 h. Less than 1% of the radiolabel was found in the extracellular medium after microinjection with CMP-[3H]sialic acid.

Discussion

The Golgi fraction chosen for microinjection has been well characterized morphologically and biochemically (3). For the purposes of the present study, the most noteworthy feature of the fraction was the absence of stacked saccules (Fig. 2; see also references 3 and 9). The intraluminal lipoprotein-like particles were originally thought to be an exclusive Golgi apparatus marker (9). However, particles with a similar morphology have been identified in endosomal contaminants of the fraction (5, 14, 15). Hence a second, less ambiguous marker was chosen, namely, in vitro sialylated glycopeptide acceptors in Golgi fractions via endogenous sialyl transferase (4). The endogenous sialylation reaction has been characterized previously for an intact, stacked-saccule Golgi fraction with a spectrophotometric assay occurring as a consequence of the in vitro sialylation

**Figure 7.** EM of normal *Xenopus* oocyte Golgi complex. Electron micrographs of control endogenous Golgi apparatus observed in three different cytoplasmic regions of the animal pole of uninjected oocytes. (A) A cortical Golgi apparatus is observed near a pigment granule (pg). The saccules are devoid of content, as are the numerous small vesicles at the periphery. (B) A Golgi apparatus of the deep cortex of the oocyte. A large cup-shaped stack of saccules is prominent in a cytoplasm enriched in ribosomes (r). er, endoplasmic reticulum. (C) A Golgi apparatus observed in the perinuclear cytoplasm. The bulbous ends of several tubules appear to be coated (arrows). er, endoplasmic reticulum. Bars, 0.5 μm.
Figure 8. EM radioautographic detection of [3H]sialic acid label in reconstituted stacked Golgi apparatus at 14 h after injection. Silver grains (arrows) are observed over lipoprotein particle–containing vesicles as well as over a Golgi stack (G) in the yolk platelet-free region. A large lysosome-like structure filled with lipoprotein-particle content is also indicated (x). Tubules and vesicles with lipoprotein particle content are indicated by arrowheads. Exposure, 175 d. Bar, 0.5 μm.

Table III. Silver Grain Distribution Over the Cortex of Xenopus Oocytes 14 h After Microinjection of [3H]Sialo-labeled Rat Liver Golgi Fragments

|                        | Plasma membrane | Cortical granules | Vesicles with lipoprotein particles | Stacked saccular Golgi apparatus | Others | Total |
|------------------------|-----------------|-------------------|-------------------------------------|----------------------------------|--------|-------|
| Number of grains (n)   | 89              | 52                | 25                                  | 13                               | 59     | 238   |
| Number of grains (%)   | 37              | 22                | 11                                  | 6                                | 24     | –     |
| Number of structures identified (n) | 75        | 119               | 8                                   | 4                                | –      | –     |

Quantitation was done using 75 electron microscope photomicrographs printed at a final magnification of 45,000. Silver grains were scored as belonging to one of the identified compartments when a grain was observed directly over the structure. Grains scored in Others compartment represent grain scatter and were found in the cytosol in the immediate vicinity of the above-labeled compartments as well as in the extracellular space close to the plasmalemma.
Figure 9. EM radioautographs of cortical regions of *Xenopus* oocytes at 14 h after injection of [3H]sialo-labeled hepatic Golgi fragments. In A, silver grains (arrows) are found over stacked sacculles of a cortical Golgi apparatus. Grains (arrows) are also observed in B over the plasmalemma (pm) of the oocyte as well as over a cortical granule (cg). In C, silver grains (arrows) are found over the periphery of a cortical granule (cg) in proximity to the plasmalemma (pm). pg, pigment granules. Exposure, 175 d. Bars, 0.5 μm.

reaction (4). Similar findings were observed here for the Gi fraction. As pelleting of Golgi fractions leads to the extensive loss of structure and the majority of intraluminal proteins (data not shown), then separation of free CMP-[3H]sialic acid was effected by flotation. This yielded hepatic Golgi fractions with only a slightly altered ultrastructure (Fig. 2) and containing negligible amounts of free nucleotide sugar. The intraluminal lipoprotein particles evident in the cytoplasmic fragments thus were used as a hepatic marker and the radiolabel as the hepatic Golgi marker.

The radioautographic studies demonstrated the association of silver grains with the intraluminal lipoprotein particles enabling ready identification of microinjected hepatic Golgi fragments. Both these markers were observed associated with organized stacked sacculles in oocyte cytoplasm after microinjection of the hepatic Golgi fragments. The
Figure 10. Light and electron microscopy of sections from oocytes after pretreatment with 50 μM vinblastine for 7 h, followed by microinjection with Golgi fragments and further incubation with vinblastine for 3 h. In A, the injection vacuole (iv) and the nucleus (n) are observed unusually close to the oocyte surface. The vegetal pole (vg) can be recognized on the basis of the larger darkly staining yolk platelets. (B) Higher power LM at the oocyte surface shows three light-staining crystalloids (asterisks) surrounded by numerous small round pigment granules. Regions (r) devoid of recognizable structures subdivide the heavily pigmented cortex and the deeper cortex containing numerous yolk platelets. Cortical granules appear as unstained spheres aligned beneath the oocyte surface (os). (C) EM of the cortical region shows a large crystalloid of microtubules (xt). Pigment granules (pg) segregate the crystalloid from a cortical granule (cg) observed just beneath the oocyte plasmalemma (arrow). rer, rough endoplasmic reticulum. Bars: (A) 100 μm; (B) 5 μm; (C) 0.5 μm.

results suggest that the Golgi fragments retain sufficient molecular characteristics after isolation to permit reconstitution into defined Golgi structures when required extrinsic (presumably cytosolic) factors are added. The membrane constituents of the organelle fragments and the cytosolic factors required for reconstitution have yet to be better defined, although ATP, Mn++, and GTP are potential candidates since these have been shown to promote Golgi-specific membrane interaction in vitro (1, 4, 22, 26).

Reconstitution of injected Golgi fragments into stacks of Golgi saccules showed between three to five saccules per stack. Although some stacks contained >10 saccules (data not shown), this was observed infrequently. The Golgi fragments were either transported and incorporated within pre-
Figure 11. EM radioautograph of a yolk platelet-free region observed in an oocyte after pretreatment with vinblastine for 7 h followed by microinjection with Golgi fragments and further incubation with vinblastine for 3 h. The cytoplasm shows a normal content of rat liver vesicles and saccules many of which contain recognizable lipoprotein particles. The arrowheads indicate stacked saccules with cisternae which exhibit variable dilations. Silver grains indicative of the sites of location of radiolabeled heterologous Golgi elements are also evident (arrows) throughout the yolk platelet-free region. Numerous lysosome-like (L) structures are also evident in this area. Exposure, 70 d. Bar, 0.5 μm.
existing host Golgi apparatus, or the injected fragments fused amongst themselves and/or with host membranes to reconstitute Golgi apparatus. Consistent with the former proposal is the observation of the hepatic Golgi marker (radiolabel) in association with oocyte cortical Golgi apparatus (Table III and Fig. 9). This observation is also in agreement with the previous demonstration of Rothman et al., (30) of inter-Golgi complex transport. Whether hybrid Golgi stacks were formed remains to be determined, for example by double-labeling procedures capable of distinguishing both host and foreign Golgi-specific membrane constituents.

The radioautographic studies showed labeling first over reconstituted stacks of Golgi saccules and only later (14 h) over cortical granules and the cell surface suggesting a transport of heterologous molecules along the oocyte secretion pathway. However, extensive exocytosis of secretory contents could not be demonstrated by biochemical assay of the extracellular medium. Thus, either soluble content was selectively degraded with selected membrane proteins allowed to integrate in the oocyte secretory pathway, or the biochemical assay was too insensitive to pick up the limited amount of functioning reconstituted Golgi apparatus. The slow transport kinetics may be related to the low efficiency of stack formation in our system, thus implying that the presence of stacks is a prerequisite for transport, as has been suggested previously by studies showing the lack of transport during disruption of the Golgi apparatus at cell division (7, 10, 33).

Alternatively, slow transport kinetics may be a consequence of altered secretory transport routes in the cytoplasm arising from microinjection of foreign membranes. After microinjection of mRNA into oocytes, several groups have demonstrated secretion by 14 h (e.g., references 6 and 31).

Two trivial explanations of the results with radiolabeled Golgi apparatus may be ruled out. In the first, the possibility that radiolabel is due to free CMP-[3H]sialic acid was considered unlikely as >99% of free CMP-[3H]sialic acid was removed from the parent microinjected hepatic Golgi fractions by the double flotation protocol (Fig. 1). Furthermore, the microinjected radiolabel was maintained in discrete patches as determined by LM radioautography, i.e., restricted to the sites of injection 3 h after insertion (small molecules such as free nucleotide sugars are expected to diffuse throughout the oocyte in this time frame, reference 8). Also, subsequent transport to the cortical granules and cell surface was restricted to a cone from the injection site to the cortex of the oocyte (i.e., not dispersed throughout the oocyte cytoplasm as expected for a soluble radiolabeled precursor). These arguments also apply to the possibility that sialic acid was metabolized from the injected glycoproteins of the hepatic Golgi fragments and reused. When CMP-[3H]sialic acid was injected, only 9–12% of the injected nucleotide sugar was incorporated, thereby providing an upper limit for its reuse. In this context it is noteworthy that pretreatment of oocytes with vinblastine prevented the appearance of radiolabel and lipoprotein particle-containing elements in the oocyte cortex even at 14 h after injection. This is as would be predicted from the known effects of microtubule-disrupting agents on secretion of plasma proteins by liver parenchyma (2, 20, 28, 29). Vinblastine treatment did, however, permit the formation of stacked saccules of microinjected hepatic Golgi fragments. Other microtubule-disrupting agents (i.e., colchicine or nocodazole) have resulted in Golgi apparatus fragmentation but with the retention of stacked saccules (28, 29, 32).

The microinjection of radioactive Golgi fragments from rat liver into Xenopus oocytes led to a considerable amount of degradation (45.7% of injected radiolabeled Golgi fragments) as assessed by radioactivity measurements 14 h after injection. Despite this, morphology and radioautography indicated that at least some components of the Golgi fraction were able to reconstitute into recognizable Golgi elements after insertion into oocyte cytoplasm. The reconstitution of ER fragments in oocyte cytoplasm was also observed after microinjection and this occurred in the absence of degradation as judged by the lack of change in lysosomal activity in oocyte homogenates 21 h after injection (25). Thus, there appears to be selectivity in degradation of specific membrane components microinjected into oocytes. The factors regulating such selectivity remain to be determined.

These studies extend the prior initial observation on hepatic Golgi apparatus reformation after microinjection of Golgi fragments into oocytes when evaluated by purely morphologic criteria and at a single time point (24). Although still based on relatively few time points (3 and 14 h) and with a more rigorous hepatic Golgi marker ([3H]sialo-labeled endogenous acceptors labeled by the Golgi apparatus marker, sialyltransferase) and a single microtubule inhibitor (vinblastine), the following sequence of events may be deduced. Microinjected disrupted hepatic Golgi fragments remain in yolk platelet-free regions where they acquire cytoplasmic coats (Figs. 5 and 7 of reference 24), then form stacks and fenestrae presumably by membrane fusion. By 3 h, stacking has not sufficed to allow for exit from the stack but has by 14 h. Transport of some constituents occurs to the oocyte cortical granules and cell surface via a microtubule-dependent process as evaluated from radioautographs; Golgi apparatus stack formation appears to be microtubule independent.

The ability of dispersed hepatic Golgi saccules, vesicles, and tubules to reconstitute into a stack after microinjection into Xenopus oocytes points to the feasibility of identifying the factors regulating Golgi stack formation in a cell-free system.

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