

A Mitotic Form of the Golgi Apparatus in HeLa Cells

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Abstract. Galactosyltransferase, a marker for *trans*-Golgi cisternae in interphase cells, was localized in mitotic HeLa cells embedded in Lowicryl K4M by immunoelectron microscopy. Specific labeling was found only over multivesicular structures that we term Golgi clusters. Unlike Golgi stacks in interphase cells, these clusters lacked elongated cisternae and ordered stacking of their components but did comprise two distinct regions, one containing electron-lucent vesicles and the other, smaller, vesiculo-tubular structures. Labeling for galactosyltransferase was found predominantly over the latter region. Both structures were embedded in a

dense matrix that excluded ribosomes and the cluster was often bounded by cisternae of the rough endoplasmic reticulum, sometimes on all sides. Clusters were present at all stages of mitosis examined, which included prometaphase, metaphase, and telophase. They were also identified in conventionally processed mitotic cells and shown to contain another *trans*-Golgi marker, thiamine pyrophosphatase. Serial sectioning showed that clusters were discrete and globular and multiple copies appeared to be dispersed in the cytoplasm. Their possible role in the division of the Golgi apparatus is discussed.

DURING normal division of an animal cell, the chromosomes segregate and all other intracellular organelles appear to be equally distributed between the two daughter cells (2). For organelles such as mitochondria that exist in multiple copies, random diffusion alone should ensure that each daughter cell receives approximately equal numbers. For organelles that exist as a single copy in interphase cells, a mechanism for division must exist.

The Golgi apparatus appears to be a single-copy organelle in animal cells (23, 27–29) and a series of studies starting at the beginning of this century have provided an overview of the division process at the light microscopic level (4, 11, 13, 15, 25, 41). At the onset of mitosis the Golgi apparatus begins to fragment and loses its pericentriolar location. Fragmentation continues through prometaphase, and in metaphase and anaphase the fragments appear to be dispersed throughout the cytoplasm of the mitotic cell. During telophase, cytokinesis separates the two daughter cells and reassembly of the Golgi apparatus occurs in the centrosomal region of each daughter cell. Electron microscopic studies have confirmed the fragmentation and reassembly of this organelle by documenting the disappearance and reappearance of stacks of Golgi cisternae (17, 30, 42). The difficulty has been to identify the fragmentation intermediates and products. Most authors favor small vesicles as the end product (see reference 42 for a list of references) but formal proof requires that these vesicles be shown to contain a Golgi marker.

There have been many attempts to stain Golgi membranes in mitotic cells using specific cytochemical markers such as thiamine pyrophosphatase (TPPase),¹ but these attempts

have either met with no success (30) or very limited success (21, 24, 36). In addition, a proper analysis of the division process requires that all Golgi membranes be accounted for throughout the fragmentation and reassembly process. We have therefore used a quantitative immunocytochemical method to localize a Golgi protein in thin sections of mitotic cells. We chose galactosyltransferase because it is a resident membrane protein of the Golgi apparatus (39) and using immunocytochemistry it is found to be restricted to the *trans*-cisternae (32). It should, therefore, serve as a marker for at least some of the intermediates and products of Golgi fragmentation. Using this technique we have been able to identify a structure in mitotic HeLa cells that appears to contain most of the galactosyltransferase. Here we provide the first detailed description of this structure.

Materials and Methods

Cells

HeLa cells were grown at 37°C in minimal essential medium (Gibco Europe, Glasgow) supplemented with 10% (vol/vol) FCS, nonessential amino acids, and 100 U/ml each of penicillin and streptomycin in an atmosphere of 5% CO₂/95% air.

Antiserum to Galactosyltransferase

Human milk galactosyltransferase was purified as described previously (8), deglycosylated using hydrogen fluoride (20), and injected into rabbits (32).

Immunoelectron Microscopy

Mitotic cells were isolated from subconfluent (80–90%) monolayer cultures in 850 cm² plastic roller bottles (Falcon Labware, Oxnard, CA). After rapid rotation for 3 min to remove debris and a 1-h incubation at 37°C, mi-

1. *Abbreviations used in this paper:* NBCS, newborn calf serum, TPPase, thiamine pyrophosphatase.

totic cells were selectively detached by shake-off (12). The cells were collected by centrifugation at 400 g_{av} for 5 min at 4°C. Greater than 90% of the cells were mitotic as determined by staining with bis-Benzamide (Hoechst dye 33258; see reference 1). The pellets were fixed by overlaying them with 0.5% glutaraldehyde in Pipes buffer (100 or 200 mM Pipes-NaOH, pH 7.2) for 30 min at room temperature. The pellets were then washed three times in the same buffer over a 15-min period and embedded in Lowicryl K4M as described previously (5,33). Some pellets were embedded in 2% agar before dehydration.

Interphase cells were either fixed *in situ* (see above) and removed from the culture dish using a rubber policeman, or removed by treatment with proteinase K (50 μ g/ml) on ice (9), collected by centrifugation, and then fixed and processed as for the mitotic cells.

Ultrathin sections with silver or yellow interference colors were mounted on parlodion/carbon coated grids. Sections mounted on grids were labeled at room temperature (20°C) as follows. After 5–10 min on drops of 0.1 M NH_4Cl in PBS (150 mM NaCl, 10 mM sodium phosphate, pH 7.4) and 10-min preincubation on 3% newborn calf serum (NBCS; Gibco) in PBS, the grids were transferred onto drops of anti-galactosyltransferase antiserum (1:10 dilution in 3% NBCS-PBS) for 2 h. The sections were then washed three times in PBS over a 15-min period and incubated with protein A-gold diluted in 3% NBCS-PBS. Colloidal gold (7 nm diameter; 38) was complexed to protein A (14 or 31) and used at the minimum dilution giving lowest background in the absence of antiserum. After three final rinses in PBS (over a 15-min period) and three rinses in distilled water (over a 3-min period) the sections were air-dried and contrasted with uranyl acetate and lead acetate.

Observations were made using a Jeol 1200 EX electron microscope. For quantification of gold particle labeling, negatives were taken at 15,000 \times (Golgi clusters) or 10,000 \times (other cellular structures). Organelle areas were estimated by point counting methods (6) on prints of negatives enlarged 3.3 \times . Quantification was carried out by a person with no knowledge of the experimental protocol but familiar with the cellular structures being measured. The nonparametric test of Mann and Whitney (16, see also reference 37) was used to compare immunolabeling since it avoids restrictive assumptions and requirements made by parametric tests such as the *t* test.

Controls were carried out by replacing the antiserum with (a) 3% NBCS in PBS; (b) nonimmune rabbit serum diluted to the same extent as the antibody in 3% NBCS-PBS; or (c) antiserum depleted of antibodies against galactosyltransferase by passage down a column of purified human milk galactosyltransferase coupled to Sepharose. This depleted serum was used at a protein concentration at least as high as that of the diluted antiserum.

Labeling with antiserum against galactosyltransferase was considered specific once all three of the above controls gave the same acceptably low level of labeling over mitochondria, nuclei, and the Golgi clusters. In practice this meant ≤ 3 gold particles/ μm^2 .

Conventional Electron Microscopy

Cell pellets fixed as described above were rinsed in Pipes buffer, and then in cacodylate buffer (100 mM cacodylate-HCl, pH 7.4). After postfixation in 1% osmium tetroxide/1.5% potassium ferrocyanide (for 1 or 15 h) the pellets were washed briefly in cacodylate buffer and distilled water, and then treated with 0.5% magnesium uranyl acetate in distilled water for 1 h. After washing in distilled water, dehydration in graded ethanols was followed by embedding in Epon 812 or Transmit EM resin (TAAB Laboratories, Berkshire, UK).

Cytochemistry

TPPase was localized by the method of Novikoff and Goldfisher (22) using the modification of Griffiths et al. (10). Briefly, mitotic cells were fixed by dispersion of the cell pellets in 0.5% glutaraldehyde in Pipes buffer for 1 h at 20°C. Cells were then washed three times in Pipes buffer and then soaked for 48 h in 10% DMSO in Pipes buffer at 4°C. After three washes in Tris-maleate buffer (100 mM Tris-maleate buffer, pH 7.2) the pellet was chopped with a razor blade until staining of a sample with bis-benzamide showed that >50% of the nuclei were brightly stained. Chopping made the interior of the cells more accessible to the reaction mixture. The cytochemical reaction was performed at 37°C for 1 h using the reaction mixture described by Griffiths et al. (10). Controls lacked substrate. After further washes in Tris-maleate buffer and cacodylate buffer the cells were post-fixed in 1% osmium tetroxide in cacodylate buffer containing 1.5% potassium ferrocyanide (1 h at 20°C). Dehydration started in 70% ethanol and embedding was performed as described above. Interphase controls were fixed as above and processed as described by Griffiths et al. (10).

Galactosyltransferase Assay

Mitotic cells were prepared as described above except that, after the spin to remove debris, nocodazole (0.04 μ g/ml) was added to arrest cells in prometaphase and so improve the yield (43). At 1-h intervals, over a 4-h period, the mitotic cells were harvested by shake-off (12) and the cells, recovered by centrifugation, were kept on ice. Interphase controls comprised the cells left behind on the roller bottle after shake-off. They were released either by using a rubber policeman or trypsin which was subsequently quenched using HeLa growth medium. Both interphase (>96% pure) and mitotic (>93% pure) cells were washed in PBS and the pellets solubilized at 0°C in 50 mM Hepes-NaOH, pH 7.0, containing 0.5% (wt/vol) Triton X-100, 10 mM DDT, and 10 mM MgCl_2 , to a final protein concentration of 15–20 mg/ml. After centrifugation at 2,600 g_{av} for 10 min at 4°C, 150–500 μ g of the supernatant protein was assayed for galactosyltransferase activity as described by Bretz and Stäubli (3). Protein was measured by the method of Peterson (26).

Results

Galactosyltransferase in Interphase Cells

Antibodies against human milk galactosyltransferase label one, perhaps two, cisternae on the *trans* side of the Golgi stack in HeLa cells (32). Labeling is absent from all other parts of the cell, validating the use of this protein as a marker enzyme for the Golgi apparatus (35). Unfortunately, in other cell types the same antibody labels other membranes such as the plasma membrane (34) and there is now a suspicion that this is because the antibody recognizes oligosaccharide structures that are found on proteins other than galactosyltransferase. In HeLa cells these oligosaccharides are either absent from other proteins or are not accessible under the labeling conditions. We had to guard against this last possibility because the dramatic reorganizations that take place during mitosis might well expose them and this would have led us to a false conclusion. To prevent this from happening we decided to use a deglycosylated form of the enzyme to prepare antibodies.

Antibodies to the deglycosylated enzyme had exactly the same biochemical characteristics as the original antiserum and these studies will be published elsewhere. In Lowicryl K4M thin sections of HeLa cells, labeling was found only over the Golgi apparatus with most of the labeling present over the *trans*-most cisterna (Fig. 1). Careful electron microscopic observations using this new antiserum confirmed the results of Roth and Berger (32) that used antibodies to the glycosylated protein, thus validating its use as a marker for Golgi membranes in HeLa cells.

Staging of Mitotic Cells

Mitotic cells were isolated by shake-off (12) and all stages were abundantly present except for prophase and anaphase. Mitosis continues as the cells are shaken off, which accounts for the relative lack of prophase cells, whereas the difficulty in finding anaphase cells reflects the fact that anaphase is the shortest stage of mitosis (see reference 43). Our studies were therefore carried out on a cell population containing mainly prometaphase, metaphase, telophase, and telophase- G_1 cells.

At the electron microscopic level some ambiguity may occur if cells are staged purely on the position of the chromosomes, especially at the end of mitosis. We therefore added criteria that refined the classification using the state of the nuclear envelope and the shape of the nuclear profile. Thus we defined mitotic stages as follows: (a) prophase: condensing

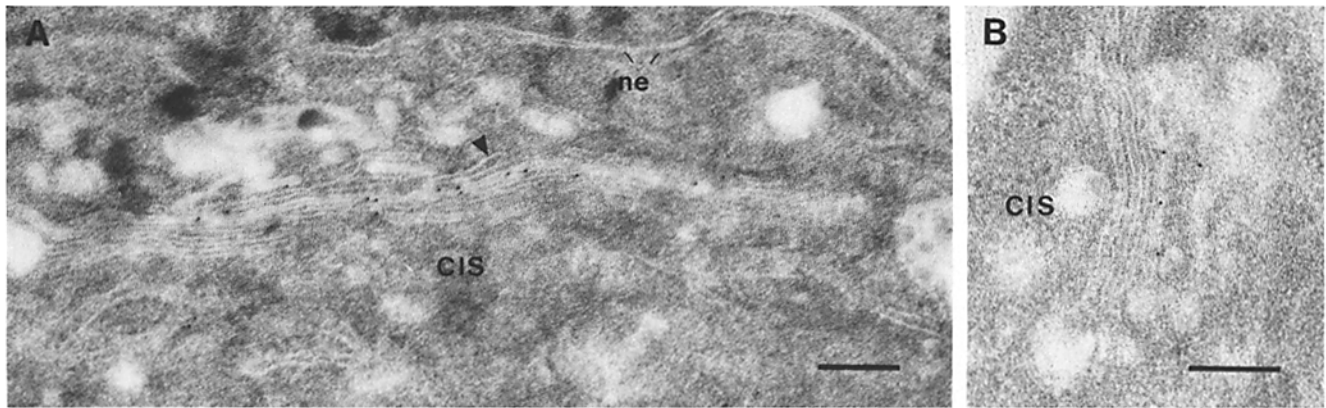


Figure 1. Galactosyltransferase in interphase cells. Labeling for galactosyltransferase was found over the *trans*-most cisterna of the Golgi apparatus with little or no labeling on the *cis* side. The *trans*-most cisterna had indistinct membranes and a content that was less electron dense than other cisternae. The element close to the *trans* side of the stack in *A* (arrowhead) is probably rough endoplasmic reticulum since its content is similar in density to that of the nuclear envelope (*ne*). Bars, 0.2 μm .

chromosomes attached to nuclear envelope that is either intact or in the process of breaking down; (*b*) prometaphase: condensed chromosomes dispersed in the cytoplasm, nuclear envelope absent (note that, due to sectioning effects, a small number of equatorially sectioned metaphase cells may be included in this population); (*c*) metaphase: equatorial chromosomes, nuclear envelope absent; (*d*) anaphase: polar chromosomes, nuclear envelope absent; (*e*) telophase: polar chromosomes with a re-forming nuclear envelope; (*f*) telophase- G_1 : complete nuclear envelope with an elongated nucleus and decondensing chromosomes; and (*g*) G_1 (interphase): complete nuclear envelope, no evidence of an elongated nucleus and decondensed chromosomes.

Galactosyltransferase in Mitotic Cells

To use galactosyltransferase as a marker for mitotic Golgi membranes we had to establish the presence of this enzyme in mitotic HeLa cells. To get enough material for assay we used nocodazole to arrest cells in prometaphase, before their isolation. The cells that remained on the roller bottle provided the interphase controls (see Materials and Methods). After extraction with Triton X-100, the specific activity in interphase and mitotic supernatants was almost identical. A typical result gave 113 and 105 nmol $^{[3}\text{H}]$ galactose incorporated/h/mg total cell protein for interphase and mitotic cells, respectively.

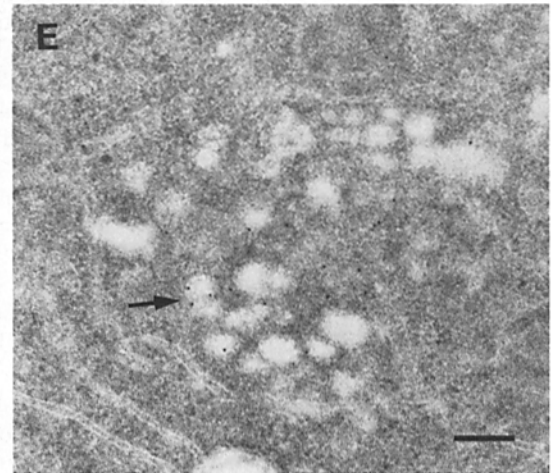
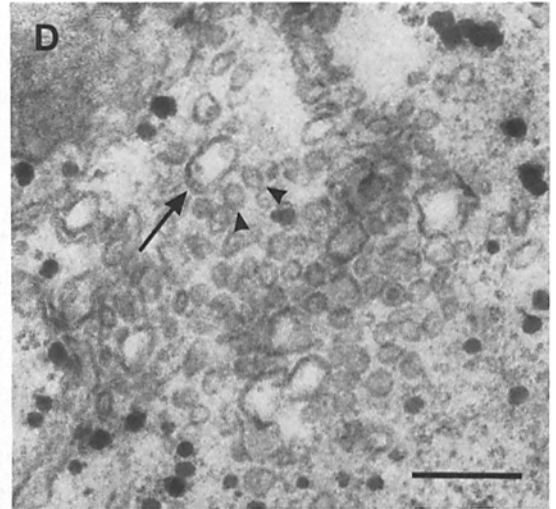
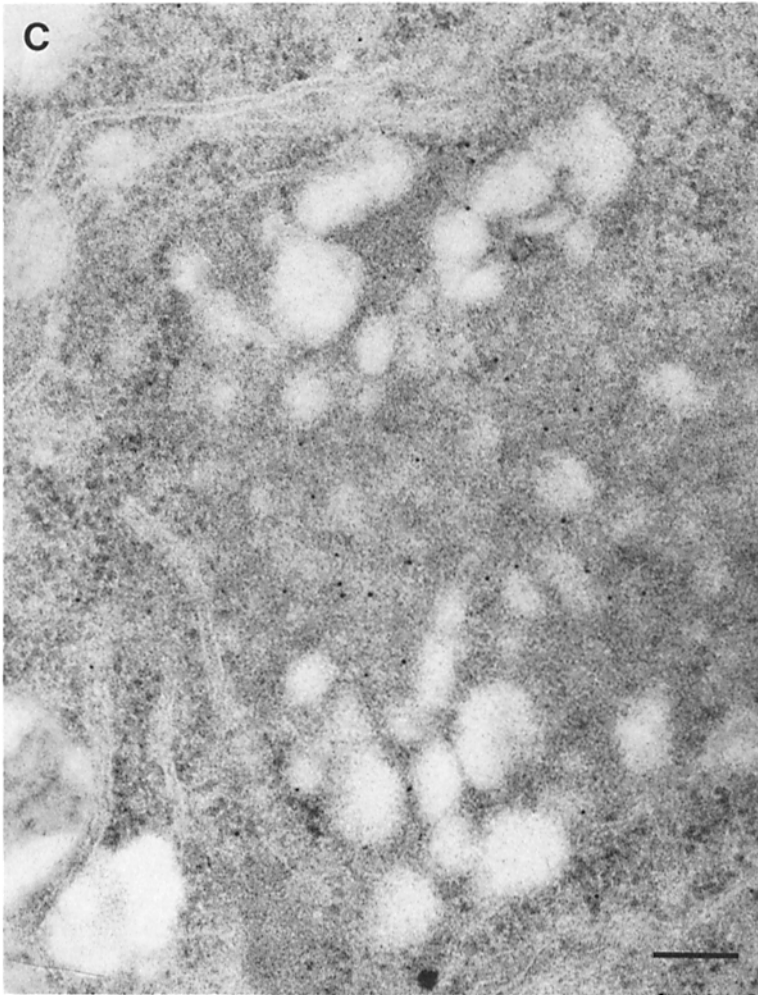
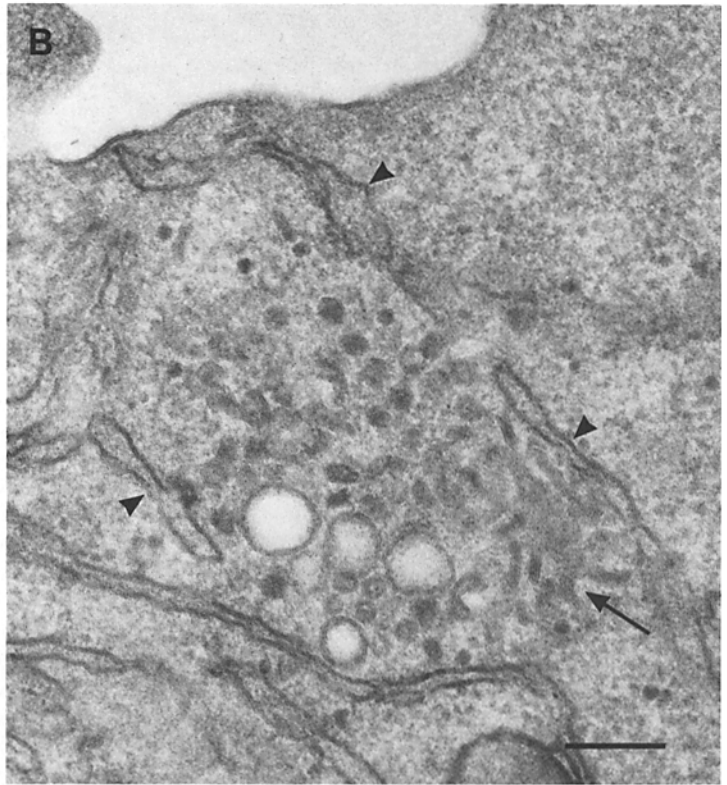
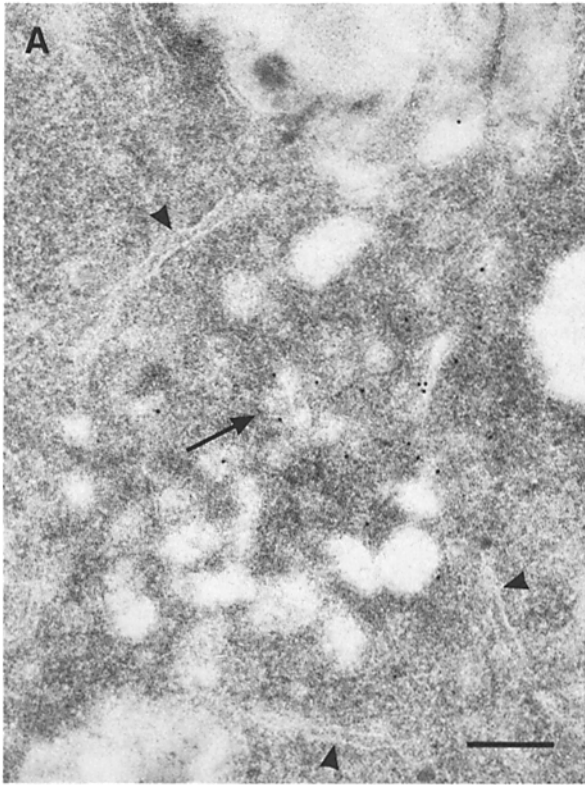
In thin sections of Lowicryl K4M, galactosyltransferase was localized to distinct clusters of membranous elements that we term Golgi clusters (Fig. 2). These contained at least two types of structures, identified on the basis of size and appearance. The larger structures appeared as vesicular profiles that were electron-lucent (Fig. 2, *A* and *C*) and were usually grouped together in one region of the cluster. The other region was occupied by the smaller structures that appeared as vesiculo-tubular profiles and were less well-defined than the larger structures (Fig. 2 *A*). Labeling for galactosyltransferase was found over the smaller structures (Fig. 2, *A* and *C*) even when they appeared to be intermingled with the larger ones (data not shown). Only rarely was labeling also found over a few of the larger structures (Fig. 2 *E*). Both of these structures were embedded in a matrix

that was more electron dense than the rest of the cytoplasm and did not contain cytoplasmic components such as ribosomes. This matrix resembles the zone of exclusion that surrounds the interphase Golgi stack (19) and is of unknown function. Cisternae of the rough endoplasmic reticulum were commonly aligned along the periphery of these clusters, sometimes surrounding it on all sides (Fig. 2 *A*).

These qualitative observations of specific labeling were confirmed by the quantitative data shown in Fig. 3. Clusters sequentially encountered on each tissue section were photographed and the micrographs given to a person who had no knowledge of the experimental conditions. This person then counted the number of gold particles over the Golgi clusters that were defined as a group ($n \geq 3$) of lucent vesicles closely associated with a group ($n \geq 3$) of smaller, indistinct structures of higher density. Both groups were embedded in a dense matrix that excluded ribosomes. This dense matrix was used to define the border of the cluster either alone or in conjunction with the closely apposed rough endoplasmic reticulum, when present. Since the extent of the labeled, smaller structures was often difficult to define, the number of gold particles over the whole Golgi cluster was counted. The degree of labeling is therefore an underestimate. Labeling over the Golgi cluster was fivefold higher than over any other identifiable organelle (Fig. 3 *A*), which is highly significant by the Mann Whitney test ($P < 0.002$). When the antiserum was depleted by passage over immobilized galactosyltransferase, labeling was reduced to the level of nuclear labeling (Fig. 3 *B*). Labeling over the cytoplasm (Fig. 3 *A*), a possible site for other labeled structures such as small vesicles, did not differ significantly from the nuclear labeling ($P > 0.05$) and was, in fact, significantly lower than the labeling over mitochondria ($P < 0.036$).

Cytochemistry and Conventional Electron Microscopy

Membranes in Golgi clusters were ill-defined in thin sections of Lowicryl K4M and this made further structural observations difficult. However, the criteria used above to quantitate the level of labeling were sufficient to identify probable Golgi clusters in thin sections of mitotic cells post-fixed in osmium tetroxide and embedded in epoxy resin. Examples are given



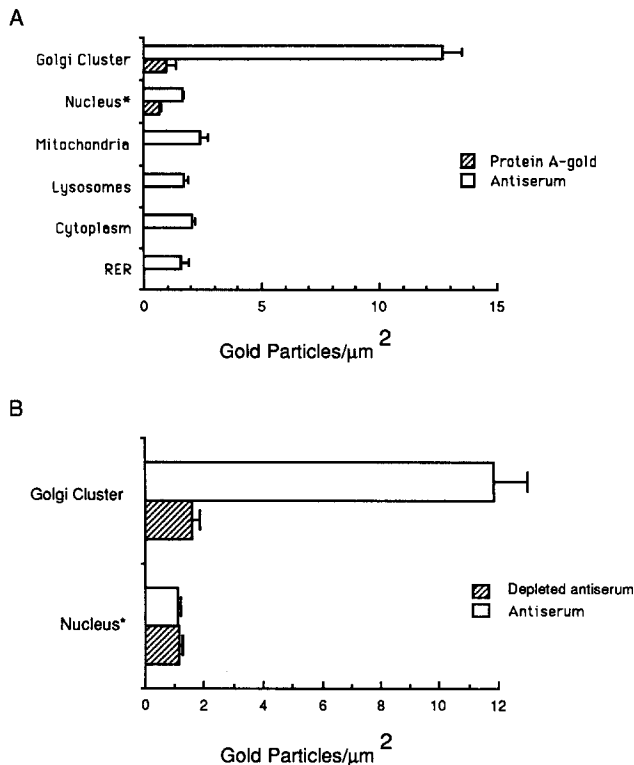


Figure 3. Quantification of immunolabeling in mitotic HeLa cells. (A) Lowicryl sections were incubated with antiserum to galactosyltransferase followed by protein A-gold or incubated with protein A-gold alone. In B the sections were incubated with antiserum before or after removal of anti-galactosyltransferase antibodies. (*) The nuclei used were from G₁ cells present in the sections of mitotic cells. Data in A are from two pooled experiments, and in B from a single experiment.

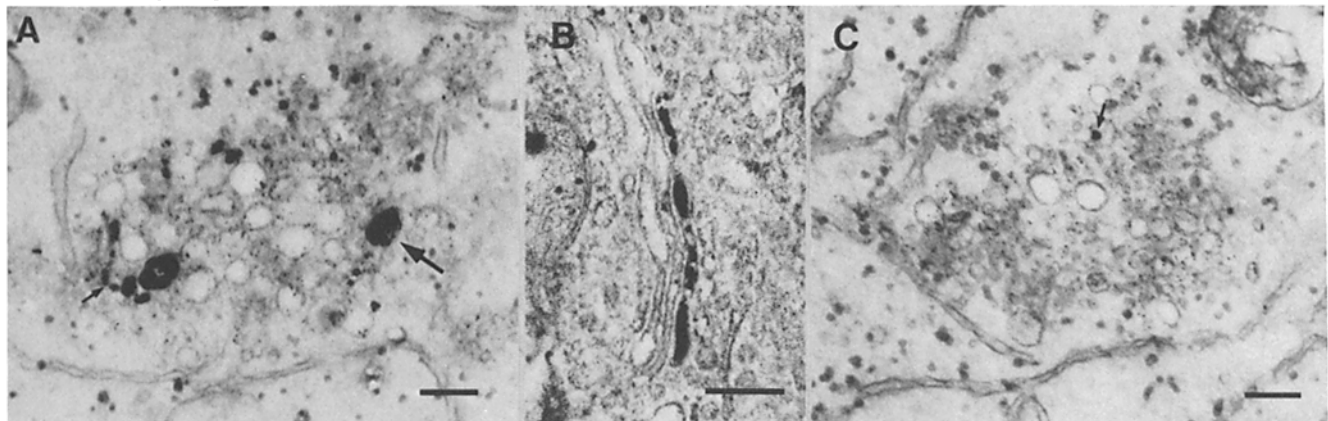
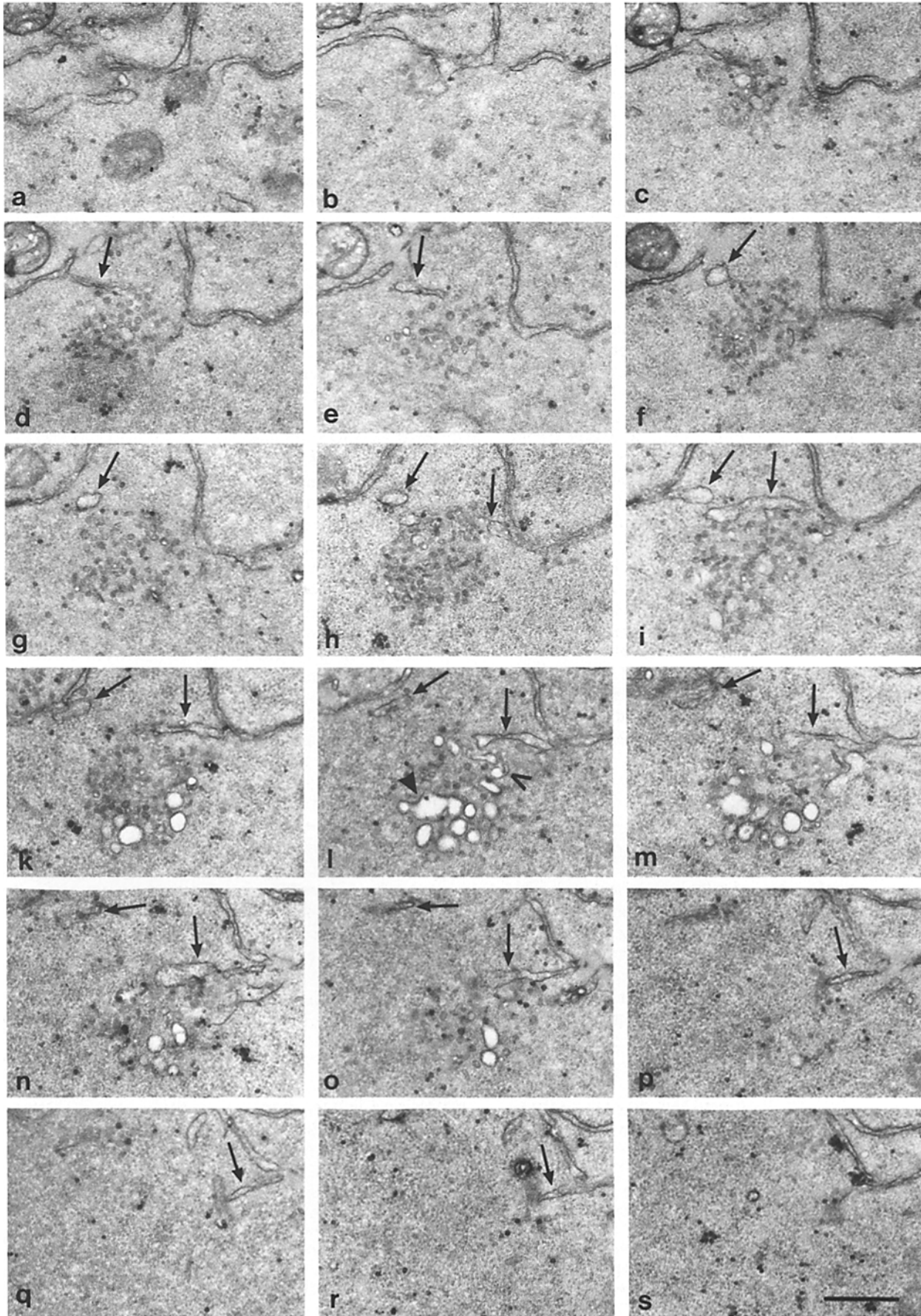


Figure 4. TPPase in mitotic and interphase HeLa cells. The reaction product was found only in Golgi clusters, in some of the large vesicular structures (large arrow in A), and in some of the smaller ones (small arrow in A). Similar staining was absent from Golgi clusters in controls incubated without substrate (C). However, electron-dense spots were often seen (arrow in C) but they were smaller in area and lacked the fine granular appearance of the specific staining. In interphase HeLa cells, staining for TPPase was limited to the *trans*-cisternae of the Golgi apparatus (B). Both A and C are parts of either prometaphase or metaphase cells. Bars, 0.2 μm .

Figure 2. Golgi clusters in mitotic HeLa cells. In Lowicryl K4M sections (A, C, and E) both structures have indistinct membranes but the grouping into regions is clearly seen. The large lucent structures appear to occupy one region in A and two in C and E. Labeling for galactosyltransferase was found mostly over the small vesiculo-tubular structures (A, C, and E); an example with clear membranes is indicated with an arrow in A. On occasion, the larger structures are labeled (arrow in E). In cells postfixated with osmium tetroxide (B and D) the membranes are very clearly seen, the smaller structures having vesicular and tubular profiles (arrow in B) and a denser content than the larger ones. Sometimes the smaller structures (arrowheads in D) appear to be connected to the larger ones (arrow in D). Elements of the rough endoplasmic reticulum are closely apposed to the clusters illustrated by arrowheads in A and B. The dense matrix in which the cluster components are embedded is more clearly seen in the Lowicryl K4M sections (A) than in sections of cells postfixated with osmium tetroxide (B). C is part of a prometaphase cell; the rest are either prometaphase or metaphase cells. Bars, 0.2 μm .

in Fig. 2, B and D. Additional evidence that these Golgi clusters were the same as those observed in cells embedded in Lowicryl K4M was obtained by localizing TPPase in mitotic HeLa cells. The product of this staining reaction is a marker for *trans* cisternae in interphase Golgi stacks (22, 23; Fig. 4 B) and clusters identified using morphological criteria also stained for TPPase. The reaction product was found mostly in the smaller vesiculo-tubular structures though some of the larger lucent vesicles were stained. Overall, 47% ($n = 36$) of the identifiable clusters were positive for TPPase whereas none of the controls had the same type of staining. Some of the controls (6%; $n = 37$) showed spots of stain but these were readily distinguishable from the positives (see legend to Fig. 4).

Having firmly established the link between clusters in Lowicryl K4M and in conventionally processed cells, we were then able to extend our knowledge of the components within this structure. We started by trying to find structures reminiscent of interphase Golgi stacks to assess the extent of fragmentation during mitosis. These attempts proved fruitless despite the improved visibility of the membranes. Apart from the division into two regions there was no obvious ordered stacking of components or elongated cisternae. The larger, electron-lucent structures in one region had clearly defined membranes and measured up to 250 nm in diameter (Fig. 2 B). Examination of serial sections showed that most of these structures appeared roughly spherical and vesicular (Fig. 5). Some profiles bore depressions on one side that would make the vesicles cup-shaped (data not shown) or were irregular in form with tubular or vesicular evaginations (arrowhead in Fig. 5 I). The smaller structures in the other region had predominantly circular profiles measuring 47 ± 2.3 nm ($n = 11$), although tubular structures (Fig. 2 B, ar-



row), sometimes interconnected, were observed. The dense matrix was not seen in conventionally processed cells but the apposed elements of the rough endoplasmic reticulum were clearly visible. By serial sectioning these elements appear as branches which appose themselves to the cluster (Fig. 5).

The first indication that Golgi clusters were discrete came from measurements on Lowicryl K4M sections of cells in prometaphase and metaphase. The maximum ratio of caliper diameters for an individual cluster was 2.2 and this low value suggested that the Golgi clusters were not continuous throughout the mitotic cell cytoplasm. This was confirmed using conventionally processed cells by serial sectioning through three Golgi clusters. One example is presented in Fig. 5. In all cases sections taken above and below the Golgi cluster lacked recognizable cluster structures. In addition, assuming a section thickness of between 60 and 100 nm, the clusters were globular in shape. This was supported by measurements of the mean caliper diameters for a series of clusters in Lowicryl K4M. A typical cluster was $1.2 \pm 0.1 \mu\text{m}$ long (maximum diameter) and $0.8 \pm 0.09 \mu\text{m}$ ($n = 12$) wide (minimum diameter). This estimate must be biased toward larger clusters since only those containing both regions of large and small structures, and positive for galactosyltransferase, were selected. Since clusters were found to be discrete and many cluster profiles could be observed in sections of single mitotic cells (e.g., Fig. 6), we deduce that there are many copies in each mitotic cell. Clusters could be found in any part of the mitotic cell cytoplasm in prometaphase, metaphase, and telophase cells. Most were peripheral in location (Fig. 6), though in telophase-G₁ cells there was some grouping of the clusters close to the centrioles.

The appearance of the clusters changed as the cells progressed through mitosis. The largest clusters were present during prometaphase and metaphase, and in telophase the large lucent vesicles became less prominent so that the smaller structures appeared to predominate. At the boundary of telophase and G₁, the first recognizable stacks of cisternae appeared.

Discussion

Using immunocytochemical and cytochemical techniques we have been able to identify a structure containing *trans*-Golgi markers in mitotic HeLa cells. We term these structures Golgi clusters because each contains several hundred closely associated vesicles, all embedded in a dense matrix and often bounded by elements of the rough endoplasmic reticulum. They are globular in shape with a diameter of $\sim 1 \mu\text{m}$ and preliminary data suggest that there are around a score of them dispersed, apparently at random, in the cytoplasm of each mitotic HeLa cell.

The Golgi clusters were discovered because an immunocytochemical technique was used. Only by using antibodies to membrane proteins specific for a particular organelle is it

possible to follow the fate of an organelle that radically changes its characteristic morphology. There is even an additional advantage in looking at organelles in mitotic cells because the absence of vesicular traffic (40) should prevent artifactual transfer of the marker protein to other organelles. It is worth noting that Golgi clusters have been described before in other cell types (18, 42) but they could not be identified as such. There is even one clear example of a cluster in an anaphase cell (Fig. 20 in reference 30) which was not commented upon by the authors. The importance of using an immunocytochemical technique cannot therefore be overstated.

The most striking difference between Golgi clusters and the interphase Golgi stacks from which they are presumably derived is that the clusters lack recognizable cisternae at all stages of mitosis up to and including early telophase. Only at the telophase-G₁ boundary does one begin to see small stacks appearing within the clusters, and these probably represent the first step on the reassembly pathway. Despite the absence of polarized stacks of cisternae, the clusters do exhibit polarity. This is because there are at least two types of structure that self-associate, thereby creating two regions within the cluster. Galactosyltransferase was found mostly in the smaller vesiculo-tubular structures but there was occasional labeling of some larger lucent vesicles. The distribution of TPPase was similar, suggesting that the *trans*-cisternae break down to give both types of structure. Not all of the vesiculo-tubular structures contain galactosyltransferase or TPPase, suggesting that the *cis*- and *medial*-cisternae may also contribute to one or both of these regions. Their precise contribution must, however, await immunocytochemical studies using antibodies against proteins specific for these cisternae.

As far as one can tell from thin sections and serial sectioning, there are few, if any, connections between the large lucent vesicles in the cluster; those that exist between the smaller vesiculo-tubular structures appear to be infrequent. The formation of clusters from stacks would therefore involve extensive conversion of cisternae into vesicles. The mechanism is unknown but may result from an imbalance in transport through the Golgi stack. Newly synthesized proteins move from cisterna to cisterna, undergoing modifications in each. They appear to be carried in vesicles that bud from the dilated cisternal rims and fuse with the next cisterna in the stack toward the *trans* side (7). If, at the onset of mitosis, budding were to continue but fusion with the next cisterna were to stop, then each cisterna would be rapidly converted into small vesicles. At the end of mitosis, fusion would resume leading to spontaneous reassembly of the stack. The evidence obtained so far is indirect (see reference 40) but is consistent with this interpretation. The difficulty is that the hypothesis predicts the formation of vesicles and not clusters of vesicles. The hypothesis was based on earlier work at the light microscopic level, which led us to expect complete

Figure 5. Serial sections of a Golgi cluster. Recognizable cluster components are not visible at either end of the series, suggesting the cluster is discrete. Sectioning passes first into the region of small vesiculo-tubular structures (*d-h*) and then into the region of large lucent vesicles (*k-o*). The latter were mostly circular in profile though *l* shows an irregular profile with membrane-bound evaginations (*arrowhead*). This same section shows a few structures, small and irregular in shape (*open arrowhead*), which are close to an element of rough endoplasmic reticulum. This element is closely related to the cluster in later sections (*arrows* on the right-hand side in *h-r*) in contrast to another element which is near to the cluster in earlier sections (*arrows* on the left-hand side in *d-o*). Note that one section is missing from the series (*j*). Bar, 0.5 μm .

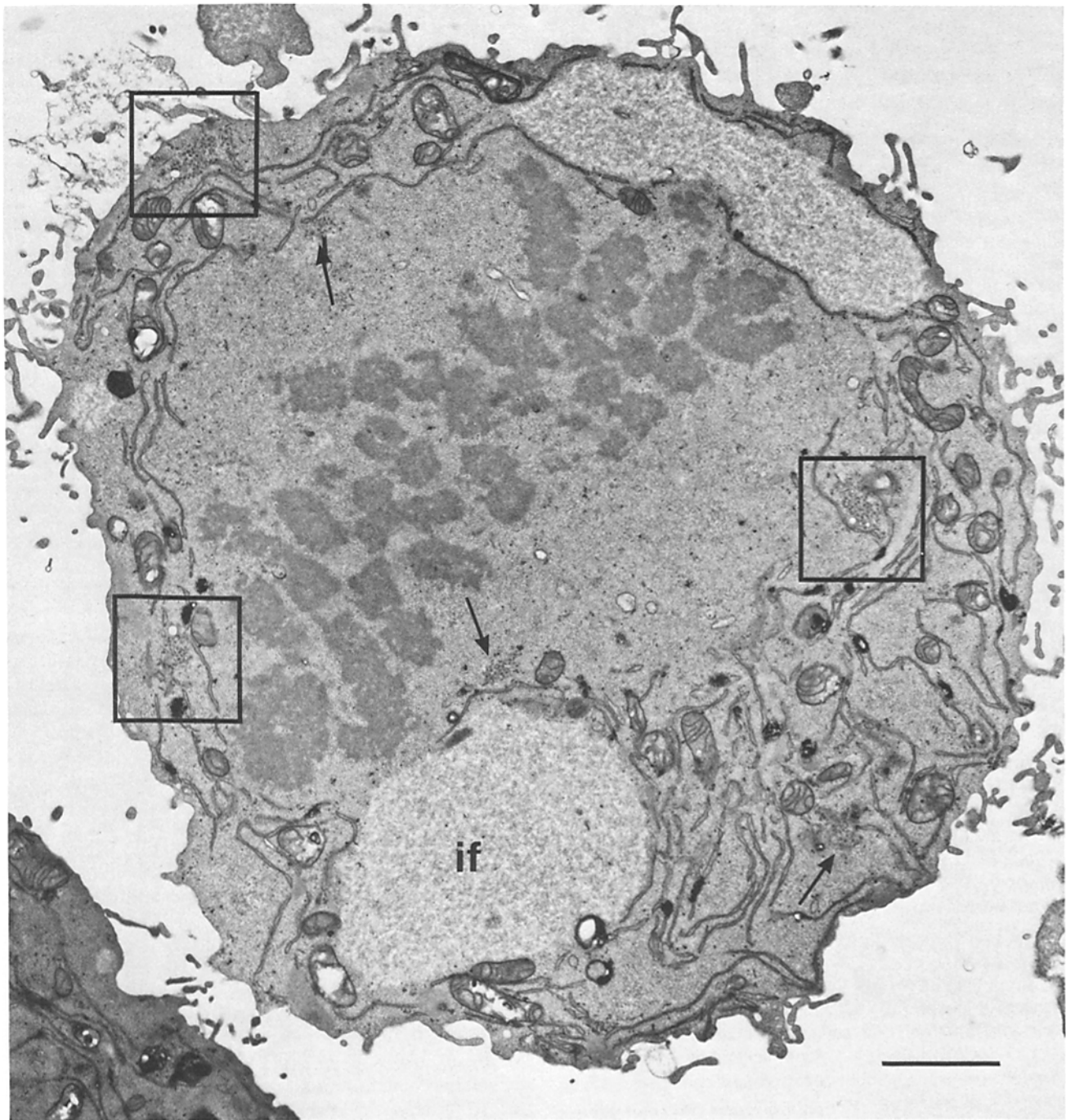


Figure 6. Distribution of Golgi clusters in a mitotic HeLa cell. This low power cross-section of a selected metaphase cell shows three definite clusters (boxed), two of which are located in the peripheral cytoplasm. Three probable clusters showing only smaller structures are indicated by arrows. Definite identification would require serial sectioning. Two large bundles of intermediate filaments (*if*) are seen in the cytoplasm. Bar, 2 μ m.

fragmentation of the Golgi apparatus, an expectation reinforced by the available electron microscopic data, which, though not definitive, did suggest that the disappearance of the Golgi apparatus was accompanied by the appearance of thousands of small vesicles. The finding of Golgi clusters poses the question as to their role in the division process. Three possibilities can be envisaged.

The first is that the clusters are intermediates on the fragmentation pathway and are not the end product. The single

copy of the Golgi apparatus found in animal cells and described by Rambourg (27-29) comprises a series of discrete stacks linked by tubules. Vesiculation of these tubules would separate these stacks from each other and continued vesiculation would convert the cisternae in each stack into vesicles, thereby generating Golgi clusters. Further breakdown of these clusters would generate thousands of single vesicles. This interpretation does not conflict with our immunocytochemical data because we do not know how much of the total

cellular galactosyltransferase is in the clusters, and any that was present in single vesicles would raise the labeling over the cytoplasm only slightly and insignificantly above background levels. Against this interpretation is the stability of the clusters which can still be found in mitotic HeLa cells arrested for up to 26 h in prometaphase by using nocodazole (unpublished data).

The second possibility is to imagine that the clusters are the end product of the fragmentation process and that the single vesicles observed previously have nothing to do with the fragmentation of the Golgi apparatus. Implicit in this possibility is the assumption that the small number of clusters generated are sufficient to ensure equal partitioning of Golgi membrane between the daughter cells. But this does not explain why the cisternae are converted into vesicles unless one argues that it is the inevitable consequence of the vesiculation needed to break the tubules binding the Golgi stacks together. It also does not explain our preliminary observation that the percentage of the mitotic cell volume occupied by the clusters is <5% of that occupied by the Golgi stacks in interphase cells.

The third and most attractive possibility is that the products of fragmentation are very heterogeneous, ranging in size from single vesicles to clusters, and that clusters represent the only fragment that can be unambiguously identified by labeling for galactosyltransferase. Smaller clusters and vesicles would not be identified as such, both because of the low level of labeling and the difficulty of identifying membranes in Lowicryl sections. Frozen thin sections offer better visualization of membrane structures which should allow us, in the future, to look for and characterize these smaller structures should they exist.

Irrespective of the position occupied by the Golgi cluster in the fragmentation process it is clear that the process can break down the Golgi apparatus to the extent of making it morphologically unrecognizable and then rebuild it in each daughter cell. This means that a detailed study of the fragmentation process will not only lead to the elucidation of the division mechanism but should also lend insight into the subunit structure of the Golgi apparatus in animal cells.

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