

# The Golgi Stack Reassembles during Telophase before Arrival of Proteins Transported from the Endoplasmic Reticulum

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**Abstract.** HeLa cells arrested in prometaphase were pulse-labeled with [<sup>35</sup>S]methionine and chased in the absence of nocodazole to allow passage through mitosis and into G1. Transport of histocompatibility antigen (HLA) molecules to the *medial*- and *trans*-Golgi cisternae was measured by monitoring the resistance to endoglycosidase H and the acquisition of sialic acid residues, respectively. Transport to the plasma membrane was measured using neuraminidase to remove sialic acid residues on surface HLA molecules. The half-time for transport to each of these compartments was about 65-min longer in cells progressing out of

mitosis than in G1 cells. This delay was only 5-min longer than the half-time for the fall in histone H1 kinase activity suggesting that inactivation of the mitotic kinase triggers the resumption of protein transport. The half-time for reassembly of the Golgi stack, measured using stereological procedures, was also 65 min, suggesting that both transport and reassembly are triggered at the same time. However, since reassembly was complete within 5 min, whereas HLA took 25 min to reach the *medial*-cisterna, we can conclude that the Golgi stack has reassembled by the time HLA reaches it.

**T**HE Golgi apparatus in animal cells fragments and vesiculates at the onset of mitosis (Warren, 1993). The disassembly pathway involves the generation of several hundred Golgi clusters as a result of vesiculation (Lucocq et al., 1987) which subsequently shed thousands of vesicles into the mitotic cell cytoplasm (Lucocq et al., 1989). A random distribution of these vesicles ensures nearly equal partitioning of Golgi membranes by a stochastic process (Birky, 1983) provided the mother cell is divided into two equally sized daughters (Rappaport, 1986). Reassembly occurs during telophase by essentially the reverse process. Clusters grow by accretion of free vesicles which then fuse to form several hundred discrete Golgi stacks. These congregate in the centriolar region where they fuse to form one or a few copies of the interphase Golgi apparatus (Lucocq et al., 1989).

Disassembly of the Golgi apparatus during mitosis is accompanied by a general cessation of membrane traffic (Warren, 1985, 1993). Viral membrane proteins no longer reach the cell surface (Warren et al., 1983) because they are trapped after synthesis in the ER (Featherstone et al., 1985). Transport of glycosphingolipids between Golgi cisternae (Collins and Warren, 1992; Kobayashi and Pagano, 1989) also ceases as does fusion of secretory granules with the plasma membrane (Hesketh et al., 1984). The only step of vesicular protein transport that does not appear to be in-

hibited is that from the *trans*-Golgi network (TGN)<sup>1</sup> to the plasma membrane (Kreiner and Moore, 1990).

Protein transport resumes during telophase/G1 (Warren et al., 1983) though the timing of this resumption relative to Golgi reassembly has not been determined. Glycosphingolipid (GSL) transport resumes at the anaphase to telophase transition (Collins and Warren, 1992). Since reassembly of the Golgi apparatus is known to occur in telophase (Lucocq et al., 1989), this strongly suggests that GSL transport through the Golgi occurs while the stack is reassembling.

In this study, we have looked at the resumption of protein transport during telophase/G1 and correlated it with the reassembly of the Golgi stack. To avoid the earlier technical problems caused by the use of virally infected mitotic cells (Featherstone et al., 1985), we have used class I molecules of the major histocompatibility complex (HLA), well-characterized plasma membrane proteins which provide suitable markers for protein transport along the secretory pathway in HeLa cells. We find that resumption of protein traffic through the Golgi apparatus occurs after the stack is fully reassembled.

## Materials and Methods

### Media and Reagents

Unless otherwise stated, all reagents were from Sigma Chemical Company (Poole, England), BDH Laboratory Supplies (Lutterworth, England), or

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1. *Abbreviations used in this paper:* GM, growth medium; GSL, glycosphingolipid; HLA, (heavy chain of the class I major) histocompatibility complex; IEF, isoelectric focusing; TGN, *trans*-Golgi network.

Boehringer Mannheim Biochemicals (Lewes, England). Cell culture reagents came from Northumbria Biologicals Ltd. (Cramlington, England), Flow Laboratories (High Wycombe, England), or GIBCO-BRL (Uxbridge, England).

## Cell Culture

Monolayer HeLa cells (ATCC CCL 185; American Type Culture Collection, Rockville, MD) were grown at 37°C in an atmosphere of 95% air/5% CO<sub>2</sub> in growth medium (GM) comprising MEM supplemented with 10% FCS, penicillin, streptomycin, and nonessential amino acids.

## Production of Prometaphase and G1 HeLa Cells

The method for generating synchronous HeLa cells followed essentially that of Zieve et al. (1980) except that the cells were incubated for 9.5 h before the addition of nocodazole. Prometaphase HeLa cells were harvested from roller bottles by rotation at 200 rpm for 5 min, generating a 15–20 × 10<sup>6</sup> cells per bottle with a mitotic index in excess of 97%. A G1 population was generated by washing twice in GM comprising MEM modified for suspension cultures supplemented with 10% FCS, penicillin, streptomycin, nonessential amino acids and 10 mM Hepes, pH 7.2 (chase medium), and incubating in chase medium for 2 h at 37°C at a density of 10<sup>6</sup>/ml. The mitotic index of the population was then typically <10%.

## Labeling Cells

Cells were washed twice in ice-cold methionine-free RPMI containing 0.2% dialysed FCS, nocodazole at 40 ng/ml and 10 mM Hepes, pH 7.2, (labeling medium), resuspended in pre-warmed labeling medium containing 5 mCi of [<sup>35</sup>S]methionine (1,300 Ci/mmol sp act) and pulsed for 5 min at 37°C. After two washes in ice-cold E4 medium containing 10% FCS, 10 mM Hepes, pH 7.2, and 10 μg/ml cycloheximide, the cells were resuspended in chase medium containing cycloheximide at a cell density of 10<sup>6</sup>/ml, and incubated at 37°C.

## Cell Lysis and Immunoprecipitation

Cell lysis and immunoprecipitation were carried out as described by Yang (1989). 50 μl of pre-spun (5 min, 50,000 rpm, 4°C) W6/32 hybridoma supernatant or 1.5 μl affinity-purified OKT9 were used for the immunoprecipitation of HLA and the transferrin receptor, respectively.

## Enzyme Digestions

For cell surface neuraminidase digestion, the cells were washed once in 1 ml of ice-cold PBS, resuspending in PBS containing 1 mM CaCl<sub>2</sub> with or without 85 mU neuraminidase (type VIII; Sigma Chemical Company), left on ice for 30 min, washed four times in ice-cold PBS, and lysed in 1 ml of lysis buffer. Endo H digestion was carried out as described by Sege et al. (1981). For neuraminidase digestion of immunoprecipitated HLA, the *Staphylococcus aureus* pellets were washed once in 1 ml of ice-cold digestion buffer (300 mM NaCl, 100 mM NaAcetate, 14 mM CaCl<sub>2</sub>, pH 5.5), resuspended in digestion buffer containing 1 mM PMSF with or without 50 mU neuraminidase, incubated for 4 h at 37°C, washed in TNEN, and resuspended in 40 μl of isoelectric focusing (IEF) sample buffer.

## Electrophoretic Separation

SDS-PAGE analysis of endoglycosidase H-treated samples was carried out as described by Blobel and Dobberstein (1975). The gels were then fixed, treated with Amplify (Amersham International, Amersham, UK), dried, and fluorographed. The method of IEF followed the protocol described in Yang (1989). Gel fixation and fluorography followed the procedure used for SDS-PAGE gels.

## Laser Densitometry

Fluorographs were scanned using an Ultrascan laser densitometer (LKB Instruments, Inc., Bromma, Sweden). Protein bands were quantified by generating a printed profile of track intensities followed by cutting out and weighing the relevant peaks. The extent of transport to the medial-Golgi was measured as the proportion of HLA present in the endo H-resistant band relative to total HLA at each time point (or transferrin receptor). The extent of transport to the trans-Golgi was measured as the proportion of HLA-A

containing either one or two sialic acid residues relative to the total HLA-A. Transport to the plasma membrane was measured as the proportion of total HLA-A sensitive to exogenous neuraminidase. In each case, the results were plotted as the percentage of the maximum value over the chase period.

## Mitotic Index Scoring and Histone H1 Kinase Assay

Mitotic indices were measured by bis-benzamide staining (Hoechst 33258; Berlin et al., 1978). Histone kinase activities were assayed by breaking cells open by rapid freeze-thawing in liquid nitrogen and following the protocol described by Felix et al. (1989) except that incubations were carried out at 37°C.

## Electron Microscopy and Stereology

Non-radioactive mitotic cells were treated in parallel with the radioactively labeled cells and chased for up to 180 min at 37°C. Samples were prepared for EM following the method of Lucocq et al. (1989).

For each of the time points, 10 fields containing cells were sampled at random and photographed at ×11,500. These fields were then scanned at higher magnification and every Golgi stack or cluster was photographed at ×28,500. Golgi clusters were defined as groups of at least five vesicles (Lucocq et al., 1989). Photographs were printed at a final magnification of ×102,560.

Surface densities of Golgi membrane per cell volume ( $S_{go}/V_{cell}$ ) were calculated using the following equation:

$$S_{go}/V_{cell} = S_{go}/V_{go} \times V_{go}/V_{cell} \quad (1)$$

where  $S_{go}/V_{go}$  is the surface density of the Golgi membrane within individual Golgi stacks and  $V_{go}/V_{cell}$  is the fraction of cell volume occupied by Golgi stacks.

$S_{go}/V_{go}$  was estimated from the high-magnification pictures using a square lattice grid with 20-mm spacing using the following equation:

$$S_{go}/V_{go} = \Sigma I/\Sigma P \times d \quad (2)$$

where  $\Sigma I$  is the sum of vertical and horizontal intercepts of the grid with Golgi membranes,  $\Sigma P$  is the sum of the points lying on Golgi cisternae, associated vesicles and intercisternal space, and  $d$  is the distance separating the vertical and horizontal lines of the grid.

A cisterna was defined as any structure within the Golgi region of which the length was at least four times the width (Lucocq et al., 1989). A cisterna was further defined as stacked if a line perpendicular to the cisterna at the point of lattice intersection also intersected an adjacent parallel cisterna within 1-cisternal width. The number of cisternae within a stack was defined as the number of these adjacent parallel cisternae.

$V_{go}/V_{cell}$  was estimated directly from the lower magnification negatives using a double-square lattice grid (16- and 2-mm spacings). Points lying over the cell were counted using the 16-mm grid lines and those over the Golgi using 2-mm grid lines.  $V_{go}/V_{cell}$  was then given by the ratio of the number of points over the Golgi to the points over the cell, multiplied by 64 to allow for the different line spacings.

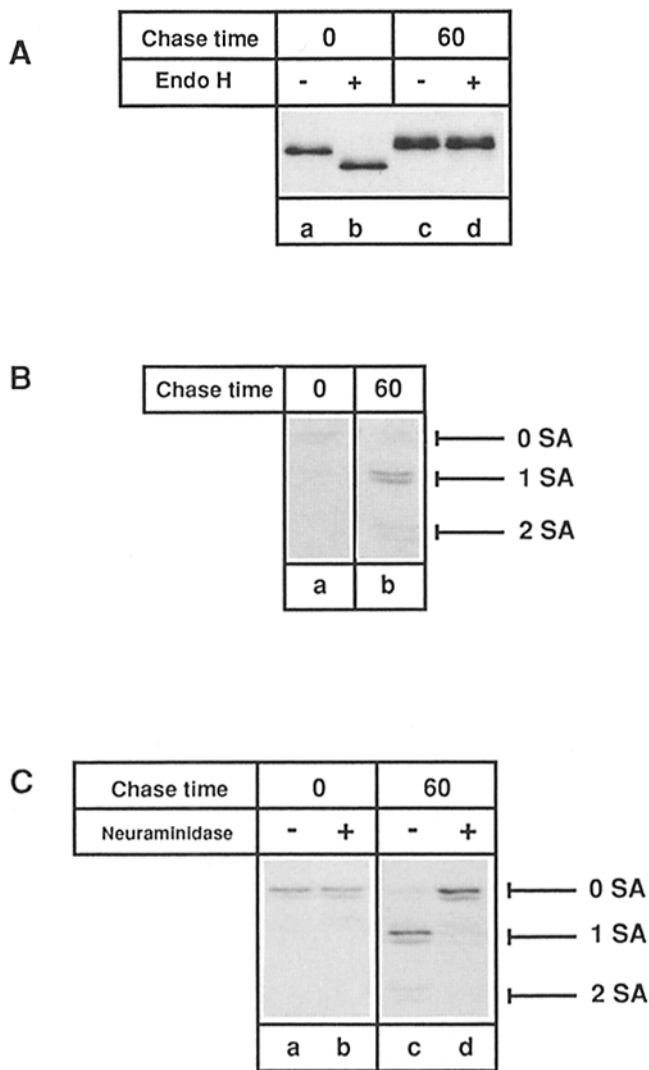
Cisternal length was measured by counting intersections of a grid with 13-mm spacings with cisternal membranes of Golgi stacks sampled at random, photographed, and printed at a final magnification of ×102,560. The total length of cisternae for each Golgi was calculated, and this was then divided by the number of cisternae in the stack.

## Results

### Transport of HLA in G1 Cells

Prometaphase HeLa cells, isolated by mechanical shake-off, were washed free of nocodazole, and incubated for 2 h so that the entire population entered G1. Cells were then pulsed for 5 min with [<sup>35</sup>S]methionine and chased for up to 90 min. HLA-A, -B, and -C antigens were extracted using Triton X-114, immunoprecipitated using the W6/32 mAb (Barnstable et al., 1978), and fractionated by SDS-PAGE followed by fluorography.

Transport to the medial-Golgi cisterna was monitored by the acquisition of resistance to endoglycosidase H (endo H).



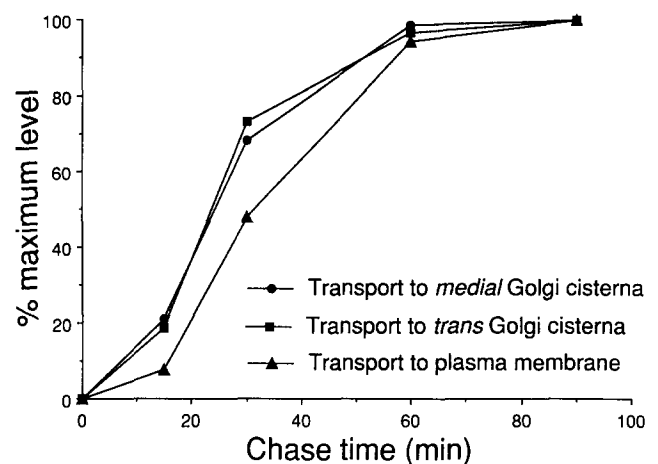
**Figure 1.** Transport of HLA in G1 cells. Cells were pulsed for 5 min with [<sup>35</sup>S]methionine and chased for 0 or 60 min. HLA-A, -B, and -C were immunoprecipitated from lysed cells using the W6/32 mAb, fractionated by SDS-PAGE (A) or IEF (B and C) and fluorographed. (A) HLA was treated (lanes b and d) or mock-treated (lanes a and c) with endo H. (B) IEF shows HLA-A containing 0 (0 SA), 1 (1 SA), or 2 (2 SA) sialic acid residues. (C) Labeled cells were treated (lanes b and d) or mock-treated (lanes a and c) with neuraminidase before lysis. IEF of HLA-A is shown containing 0 (0 SA), 1 (1 SA), or 2 (2 SA) sialic acid residues.

Two time points are shown as examples in Fig. 1 A. In the absence of a chase, all of the HLA was sensitive to endo H; after 60 min of chase, all of it was resistant. The minor protein band, most easily seen in lane b and even more clearly in Figure 3 A, is likely to be HLA-C. This band remains sensitive to endo H throughout the chase period consistent with the slower transport rate previously observed for this form of HLA (Neefjes and Ploegh, 1988; Stam et al., 1986). The relative amounts of the sensitive and resistant forms of HLA-A and -B were quantitated by densitometry and the results from two experiments were averaged and are plotted in Fig. 2. The half-time for acquisition of resistance to endo H was 24 min.

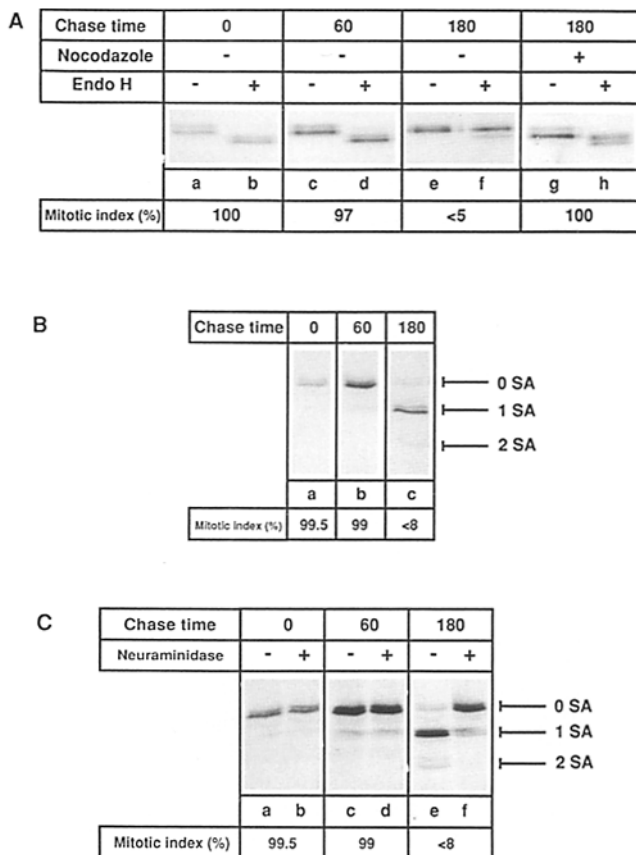
Transport to *trans*-Golgi cisternae and the *trans*-Golgi network was monitored by the acquisition of sialic acid residues (Roth et al., 1985). Pulse-chased samples were fractionated by IEF and examples of two time points are given in Fig. 1 B for HLA-A. HLA-B, and -C migrated further down the gel and were too close together for densitometric analysis. In the absence of a chase, a single doublet of HLA-A was observed. After a 60-min chase two more doublets appeared which collapsed to a single band after digestion with neuraminidase (data not shown) confirming their identity as HLA-A bearing either one or two sialic acid residues. The structure of HLA has been well-documented (reviewed by Bjorkman and Parham, 1990) and this pattern of sialylation is consistent with the presence of a single N-linked bi-antennary oligosaccharide on the protein. The reason for the doublet is unclear. It is due to neither glycosylation nor phosphorylation (Hidde Ploegh, personal communication). The extent of sialylation at different times of chase was quantitated by densitometry and the results are plotted in Fig. 2. The half time for acquisition of sialic acid residues was also 24 min, indicating rapid protein transfer from the *medial*- to the *trans*-Golgi cisternae.

Transport to the plasma membrane was monitored using exogenous neuraminidase. This will only remove sialic acid from HLA that has reached the cell surface. The example in Fig. 1 C shows that, after 60 min of chase, most of the sialylated HLA-A was accessible to neuraminidase, which collapsed the pattern down to the non-sialylated form. Quantitation at different chase times showed that the half-time for transport to the plasma membrane was 31 min showing that it takes ~7 min for transport from the *trans*-Golgi cisternae.

Experiments carried out using unsynchronised interphase HeLa cells gave very similar kinetics of HLA transport to the *medial*-cisterna ( $t_{1/2} = 20$  min). Addition of nocodazole during the pulse-chase period in G1 cells was also found to have no effect on the kinetics of transport (data not shown).



**Figure 2.** Kinetics of HLA transport in G1 cells. Cells were pulsed for 5 min with [<sup>35</sup>S]methionine, chased for varying times, and HLA analyzed for resistance to endo H (arrival in *medial*-Golgi cisterna), acquisition of sialic acid residues (arrival in *trans*-Golgi cisternae), and sensitivity to exogenous neuraminidase (arrival at the plasma membrane). Fluorographs were scanned by laser densitometry and the results presented are the average of two experiments.



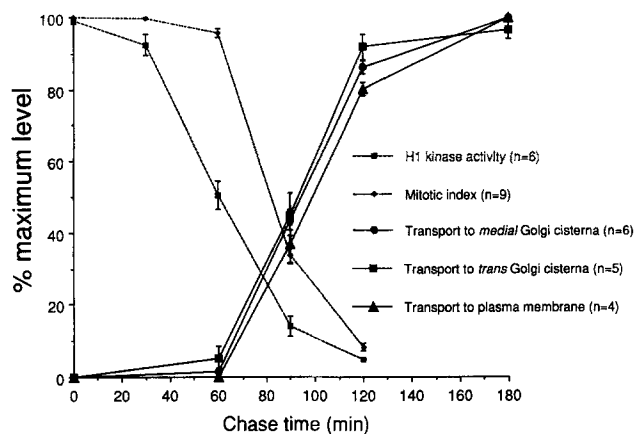
**Figure 3.** Transport of HLA in mitotic cells. Prometaphase cells were pulsed for 5 min with [<sup>35</sup>S]methionine and chased for 0, 60, or 180 min in the absence of nocodazole or for 180 min in its presence (A, lanes g and h). HLA-A, -B, and -C were immunoprecipitated from lysed cells using the W6/32 mAb, fractionated by SDS-PAGE (A) or IEF (B and C) and fluorographed. (A) HLA was treated (lanes b, d, f, and h) or mock-treated (lanes a, c, e, and g) with endo H. The minor band is most likely HLA-C and remains endo H sensitive. (B) IEF shows HLA-A containing 0 (0 SA), 1 (1 SA), or 2 (2 SA) sialic acid residues (C) Labeled cells were treated (lanes b, d, and f) or mock-treated (lanes a, c, and e) with neuraminidase before lysis. IEF of HLA-A is shown containing 0 (0 SA), 1 (1 SA), or 2 (2 SA) sialic acid residues. Cells were scored for mitotic index using Hoechst 33258.

The half times for transport of HLA through the Golgi to the cell surface were similar to those obtained for other mammalian cell lines (Owen et al., 1980; Williams et al., 1988; Neefjes and Ploegh, 1988).

### Transport of HLA in Mitotic Cells

Prometaphase HeLa cells were isolated by mechanical shake-off, pulsed for 5 min with [<sup>35</sup>S]methionine, and chased either in the absence or presence of nocodazole for up to 180 min. In the presence of nocodazole, cells remain in prometaphase and, as shown in Fig. 3 A (lanes g and h), HLA-A and B remained sensitive to endo H even after 180 min of chase, consistent with the proteins remaining in the ER.

In the absence of nocodazole, cells left mitosis and both HLA-A and B became resistant to endo H (Fig. 3 A) though the time taken was much longer than in G1 cells (Fig. 1 A).

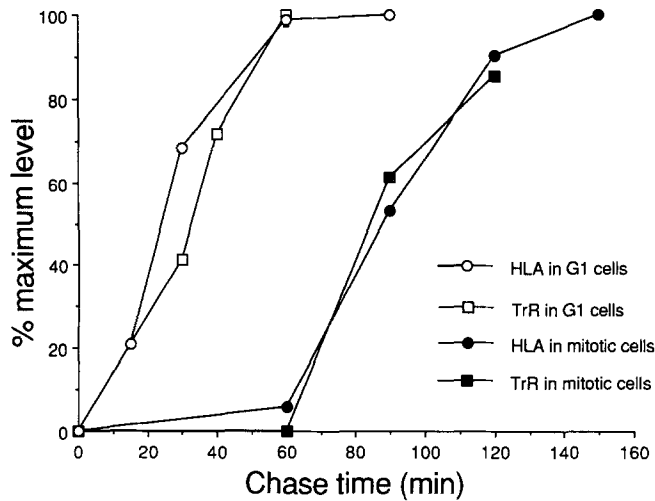


**Figure 4.** Kinetics of HLA transport in mitotic cells. Prometaphase cells were pulsed for 5 min with [<sup>35</sup>S]methionine, chased for varying times in the absence of nocodazole, and HLA analyzed for resistance to endo H (arrival in *medial*-Golgi cisterna), acquisition of sialic acid residues (arrival in *trans*-Golgi cisternae) and sensitivity to exogenous neuraminidase (arrival at the plasma membrane). Fluorographs were scanned by laser densitometry and the results are presented as the average of "n" experiments  $\pm$  SEM. Cells were also scored for mitotic index using Hoechst 33258 and assayed for the H1 kinase activity of mitotic kinase.

The same was true for both the acquisition of sialic acid (Fig. 3 B), and appearance at the plasma membrane (Fig. 3 C). After a chase of 60 min in G1 cells, HLA had reached the cell surface having passed through the *medial*- and *trans*-Golgi cisternae (Fig. 1). At the same time in cells leaving mitosis, no transport had occurred, and similar results to G1 cells were only obtained after a chase of 180 min when the cells had left mitosis (Fig. 3).

These observations were confirmed by quantitating the results as shown in Fig. 4. The form and pattern of the kinetic profiles were very similar to those of G1 cells in that the half-time for acquisition of resistance to endo H was very similar to that for acquisition of sialic acid residues whereas both were about 5 min less than the half-time for arrival at the plasma membrane. The major difference was that the half-times were all delayed by  $\sim$ 65 min when compared to G1 cells. The H1 kinase activity of the mitotic kinase was also measured in these experiments. The fall in mitotic kinase activity, most easily monitored as a fall in histone H1 kinase activity, usually occurs shortly before the metaphase to anaphase transition (Hunt et al., 1992). We have used this fall as a means of comparing the kinetics of protein transport with the deactivation of mitotic kinases which cause cells to exit mitosis. The measured half-time for the fall in H1 kinase activity was 60 min, 5 min before cells resume intracellular protein transport and therefore kinetically consistent with protein transport resuming as a consequence of the loss of mitotic kinase activity.

In the experiments described so far, the pulse labeling was carried out immediately before the release from the nocodazole block. To show that the results were not dependent on the time of the pulse, we varied the pulse time to either 30 min before or 30 min after the release from the nocodazole block. Analysis of the kinetics of HLA transport demonstrated that despite a 60-min interval between pulse times,



**Figure 5.** Kinetics of transport of both HLA and the transferrin receptor in mitotic and G1 cells. Prometaphase and G1 cells were pulsed for 10 min with [<sup>35</sup>S]methionine, chased for varying times in the absence of nocodazole, and both HLA and the transferrin receptor (*TrR*) analyzed for resistance to endo H (arrival in *medial*-Golgi cisterna). The  $t_{1/2}$  values for transport of HLA and *TrR* to the *medial*-cisterna were respectively 24 and 33 min in G1 cells and 88 and 85 min in cells leaving mitosis.

there was only about a 5-min difference in the time of arrival of labeled HLA at the *medial*-Golgi. This result also demonstrated that there was no preferential transport of any particular protein pool once protein transport resumed at the end of mitosis; protein synthesized 30 min before the release from nocodazole was transported at almost the same rate as protein synthesized 30 min after this release.

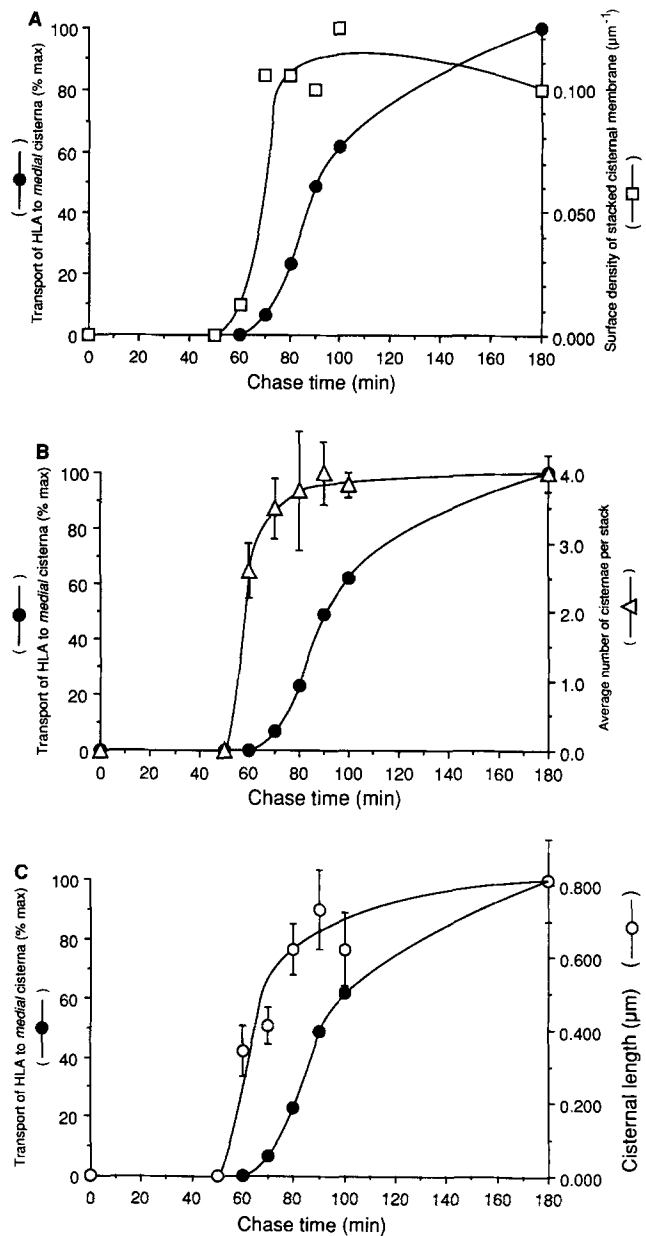
#### Transport of the Transferrin Receptor in Mitotic and G1 Cells

It was important to show that the observed delay in transport was a general feature of newly synthesized proteins and not restricted to HLA. This was tested by following the kinetics of transport of the transferrin receptor, which was immunoprecipitated from the same cell lysates using the OKT9 mAb (Omary et al., 1980). As shown in Fig. 5, the half-times for acquisition of resistance to endo H were very similar for both HLA and the transferrin receptor in cells leaving mitosis. In G1 cells the half-time for the transferrin receptor was ~10-min longer than for HLA.

#### Reassembly of the Golgi Apparatus

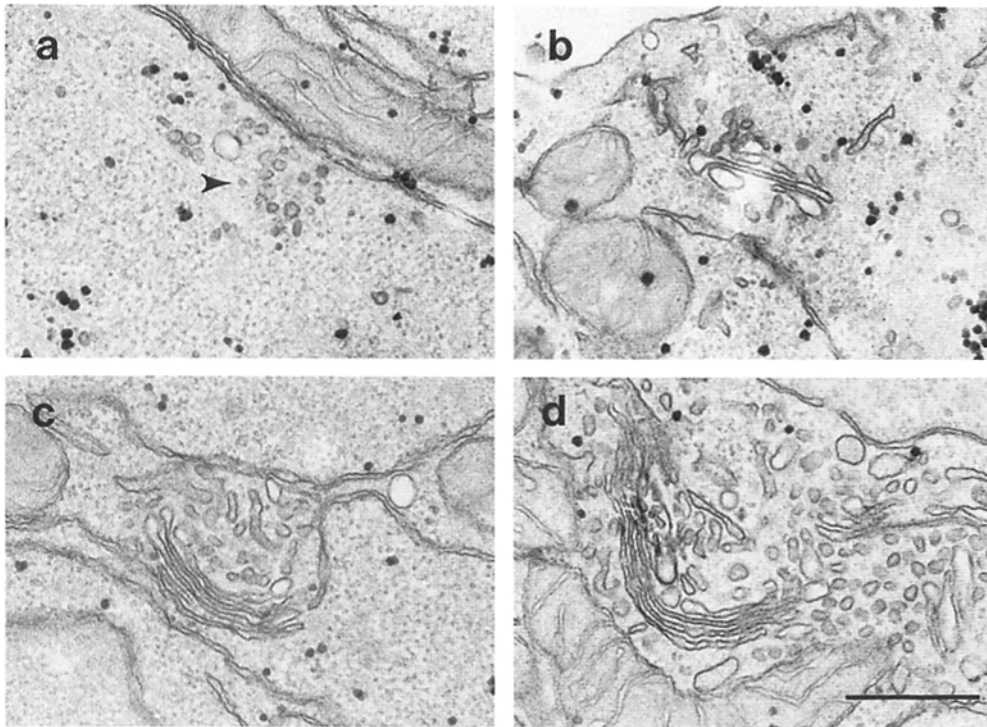
The mitotic index measures the transition between telophase and G1 and the half-time for this transition was shorter than that for arrival of HLA at the *medial*-cisterna (Fig. 4). In other words, protein transport through the Golgi resumes during G1. Since Golgi reassembly occurs during telophase (Luocq et al., 1989) this raised the possibility that reassembly precedes the onset of transport of newly synthesized protein through the organelle.

In preliminary experiments, parallel samples of cells leaving mitosis were assayed for endo H-resistant HLA, and the percentage of stacked cisternal membrane. At 60 min, the percentage of stacked cisternal membrane was 0 and 8.3 for



**Figure 6.** Kinetics of transport of HLA and reassembly of Golgi stacks. Prometaphase cells were pulsed for 5 min with [<sup>35</sup>S]methionine, chased for varying times in the absence of nocodazole, and HLA analyzed for resistance to endo H. Parallel samples were prepared for EM and stereological procedures were used to measure: (A) The surface density of stacked cisternal membrane ( $t_{1/2} = 65$  min); (B) the average number of cisternae per stack  $\pm$  SEM ( $t_{1/2} = 60$  min); and (C) the average cisternal length  $\pm$  SEM ( $t_{1/2} = 65$  min). The  $t_{1/2}$  for endo H resistance was 91 min and for the mitotic index, 77 min.

two experiments. At 90 min, it was 97.2 and 100 showing that reassembly had been completed within 30 min. More time points were taken and the results are presented in Fig. 6. Fig. 6 A shows that reassembly of stacked cisternae occurs essentially within a 10-min interval (60 to 70 min), giving a half-time of 65 min. The average number of cisternae in the stack rose even faster (Fig. 6 B) suggesting that stacks form and then grow laterally rather than one cisterna grow-



**Figure 7.** Reassembly of Golgi stacks. Representative micrographs from the samples used for stereological analysis in Fig. 6. Prometaphase cells were incubated in the absence of nocodazole for (a) 50 min, (b) 60 min, (c) 70 min, and (d) 180 min. Note the Golgi clusters (a, arrowhead), the early Golgi stacks (b), and the fully reassembled Golgi stacks (c and d). The cisternal length increased from c to d. Bar, 0.5  $\mu\text{m}$ .

ing, followed by the next and so on. The cisternal length (Fig. 6 C) continued to grow after 70 min consistent with lateral fusion of Golgi stacks that occurs once they have congregated in the peri-centriolar region (Lucocq et al., 1989). Measurement of mitotic phases showed that telophase cells peaked between 60 and 70 min. This confirms earlier work (Lucocq et al., 1989) showing that reassembly occurs during telophase.

Fig. 7 presents representative micrographs at some of these time points. At 50 min only Golgi clusters were observed (Fig. 7 a) and 10 min later the first Golgi stacks appeared (Fig. 7 b). After a further 10 min, the Golgi stacks were fully formed (Fig. 7 c) and indistinguishable, apart from the length of cisternae, from Golgi stacks after several more hours of incubation (Fig. 7 d).

## Discussion

We have compared the kinetics of transport of newly synthesized HLA along the secretory pathway in G1 HeLa cells and in cells leaving mitosis. We find that the same sequence and timings of HLA transport were observed except that it now took about 90 min for HLA to reach the *medial*-Golgi in mitotic cells in contrast to the value of around 25 min in G1 cells. This 65-min delay was not the consequence of using nocodazole to arrest cells in prometaphase; treatment of interphase cells with nocodazole during the pulse-chase period had no effect on the kinetics of transport to the Golgi. It was also not caused by a delay in forming the complex of HLA heavy chain,  $\beta_2$ -microglobulin and peptide which alone can be transported out of the ER since the mAb W6/32 only recognizes fully assembled class I complexes. Moreover, coprecipitation of HLA and  $\beta_2$ -microglobulin using anti-

$\beta_2$ -microglobulin antibodies gave the same half-time for transport to the *medial*-Golgi cisterna ( $t_{1/2} = 89$  min) showing that the results were independent of the antibody used.

Passage out of mitosis is signaled by the fall in activity of the mitotic kinase which can be assayed most easily as a fall in histone H1 kinase activity (Hunt et al., 1992). The half-time for this fall was 60 min so the simplest explanation for the observed delay in HLA transport is that proteins cannot leave the ER during mitosis but do so about 5 min after the H1 kinase activity falls.

When prometaphase cells were held in mitosis during the pulse and chase, the HLA remained sensitive to endo H, consistent with it remaining in the ER. Earlier experiments using viral proteins gave similar results (Featherstone et al., 1985). Because of the abundance of viral protein present during infection, it was also possible to show that the oligosaccharides bound to the viral proteins were those characteristic of proteins exposed only to enzymes in the ER, and that the viral proteins could not be chased out of the ER in the presence of cycloheximide using quantitative immunoelectron microscopy. There is, however, a suggestion from this earlier work that the proteins accumulate in a part of the ER distal to the site of their synthesis. Different proteins have varying rates of exit from the ER (Fries et al., 1984) and we have confirmed this by showing that it takes the transferrin receptor 10-min longer to reach the Golgi cisterna in G1 cells than does HLA. In cells leaving mitosis, however, the half-times were the same, suggesting that these two proteins accumulate during mitosis past the point which normally determines the rate of transport. If this rate-limiting step were the correct folding and oligomerization of proteins to achieve transport competency (reviewed by Hurtley and Helenius, 1989), then the implication of these data is that these assem-

bly processes continue during M phase. Alternatively, folding and oligomerization could be rapid and the rate-limiting step the delivery to a distal site. This site cannot be the *cis*-Golgi cisterna because HLA was insensitive to endoglycosidase D (data not shown). Endoglycosidase D acts on the product provided by mannosidase I, present in *cis*-Golgi cisternae (Balch and Keller, 1986). The site could be earlier than the *cis*-cisternae, perhaps the intermediate compartment or transitional element region. This will need to be studied using immunoelectron microscopy.

The half time for reassembly of the Golgi stack was 65 min, 5 min later than the fall in H1 kinase activity. This suggests that both transport and reassembly are triggered at the same time but the Golgi reassembles so rapidly that, by the time the HLA reaches the Golgi, reassembly is complete. At 70 min, when >85% of the stack was complete, only 5% of the HLA had reached the *medial*-cisterna. The absence of newly synthesized HLA in the reassembling Golgi apparatus does not mean that *intra*-Golgi transport is not occurring. Vesicle-mediated transport of GSLs occurs during reassembly (Collins and Warren, 1992) as measured by the conversion of lactosylceramide, made in an early Golgi compartment, to GA2, made in a late Golgi compartment. This suggests that vesicle-mediated transport at all levels of the secretory pathway is triggered at the same time as fusion of vesicles to form the Golgi stack. The trigger is probably different for the different steps. The ER to Golgi step is sensitive to micromolar levels of Ca<sup>2+</sup> (Beckers and Balch, 1989). Ca<sup>2+</sup> plays important roles during mitosis particularly in the assembly and disassembly of the mitotic spindle (reviewed by Whitaker and Patel, 1990) and could also be used to trigger this step at the appropriate time. It is interesting to note that ER to Golgi transport continues up to the TGN throughout mitosis and meiosis in *Xenopus* (Ceriotti and Colman, 1989) which might be explained by the lack of Ca<sup>2+</sup> transients in amphibia (Cork et al., 1987). *Intra*-Golgi transport is not sensitive to Ca<sup>2+</sup> (Fries and Rothman, 1980) so a different trigger would be needed, perhaps a change in the phosphorylation state of one of the components needed for vesicle-mediated transport (Stuart et al., 1993).

The reason for transporting components as the stack is being re-assembled is unclear but one possibility is that it offers the opportunity to re-model the stack. Animal cells are unique in that the Golgi apparatus undergoes complete vesiculation during mitosis. The Golgi apparatus in plants and fungi do not, existing throughout the cell cycle as discrete, dispersed stacks which must somehow divide to keep pace with cell growth (Warren, 1993). Vesiculation offers the possibility of re-building the Golgi every cell cycle from its basic subunit, the vesicle. Fusion coupled to transport could change the organization of resident enzymes and help explain the diversity of form and function found in the many differentiated cells of multicellular animals.

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