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Fragmentation and dispersal of Golgi proteins and redistribution of glycoproteins and glycolipids processed through the Golgi apparatus after infection with herpes simplex virus 1

(cell type dependence/Golgi enzymes/lectins/viral glycoproteins)

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ABSTRACT **In Vero monkey cells and HEp-2 human epidermoid carcinoma cells infected with herpes simplex virus 1 the proteins β-COP, galactosyltransferase, and α-mannosidase II associated with the Golgi apparatus appear to be associated with numerous smaller structures dispersed throughout the cytoplasm. Concomitantly, the intracytoplasmic ligands of lectins normally associated wholly (*Helix pomatia* or *Ricinus communis* agglutinin) or in part (wheat germ agglutinin) with the Golgi apparatus increased in amount and became dispersed. This phenomenon was seen in some of the baby hamster kidney cells analyzed but not in others and not in the human 143TK⁻ cells. The fragmentation and dispersal of the Golgi apparatus was a late event in the reproductive cycle coinciding with virion assembly, processing of viral glycoproteins, and exocytosis from infected cells. The fragmentation of the Golgi apparatus is morphologically different from that seen with brefeldin A and may reflect disequilibration between the anterograde and retrograde Golgi transport caused by the huge influx of viral glycoproteins contained in virions and membranes flowing through the exocytic pathway.**

This report shows that in some cell lines infected with herpes simplex virus 1 (HSV-1), the Golgi apparatus becomes fragmented and dispersed. These studies were prompted by observations that morphologically recognizable Golgi structures could be identified by electron microscopy in infected baby hamster kidney (BHK) (1) or human (2) fibroblasts but not in Vero cells. To determine whether functions associated with the Golgi apparatus are carried out in infected cells by morphologically identifiable structures and whether these modifications are cell-line dependent, we undertook a detailed analysis of the African green monkey cell line Vero, the human epidermoid carcinoma line HEp-2, the human thymidine kinase-negative cell line designated as 143TK⁻, and BHK cell lines infected with HSV-1. The objectives of these studies were to (*i*) trace the distribution in cells of two Golgi resident enzymes, α-mannosidase II (medium Golgi) (3) and galactosyltransferase (trans-Golgi) (4) and of β-COP, a Golgi coat protein of non-clathrin-coated vesicles (5), and (*ii*) trace the distribution of glycoproteins and glycolipids normally processed by the Golgi apparatus by their reactivity with the lectins *Helix pomatia* agglutinin (HPA), *Ricinus communis* agglutinin (RCA), and wheat germ agglutinin (WGA). HPA, RCA, and WGA are specific for and bind terminal *N*-acetylgalactosamine (6), galactose (7), and sialic acid (8) residues, respectively. *N*-acetylgalactosamine is the first sugar in O-linked chains (see ref. 9); in HSV glycoproteins, *N*-acetylgalactosamine is generally replaced with galactose and sialic acid (10, 11). Galactose is an intermediate sugar in N-linked complex-type chains (9). Sialic acid residues are the terminal unsubstituted sugars in both N- and O-linked oligosaccharides (9–12), and therefore WGA detects fully processed glycoproteins in the Golgi apparatus as well as at the plasma membranes.

MATERIALS AND METHODS

Cells and Viruses. Vero, HEp-2, and 143TK⁻ cells were grown in Dulbecco's modified Eagle's medium supplemented with 5% fetal bovine serum. BHK 21/c113 cells were grown in the same medium containing 10% newborn calf serum. The cells were exposed to HSV-1 strain F [HSV-1(F)] (13) or R7032, a genetically engineered recombinant virus lacking glycoprotein E (14), at 10 plaque-forming units per cell.

Antibodies and Lectins. Antibodies to human galactosyltransferase (4), rabbit liver α-mannosidase II (3), and β-COP (5) were obtained from E. G. Berger, K. W. Moremen, and T. Kreis, respectively. Fluorescein-labeled HPA, RCA, WGA, and anti-rabbit and anti-mouse IgG antibodies were from Sigma.

RESULTS

Electron Microscopic Observations. Electron microscopic studies were done on Vero, HEp-2, BHK, and 143TK⁻ cells harvested 15 hr after infection with HSV-1(F) and processed as described in the legend to Fig. 1.

In HSV-1(F)-infected Vero (Fig. 1 *A* and *B*) and HEp-2 (Fig. 1 *C* and *D*) cells, typical Golgi structures were rarely observed, although they were readily seen in uninfected cells (data not shown). A common feature of infected cells were numerous perinuclear vacuoles about 500 nm in diameter in close juxtaposition and often containing two or more enveloped capsids. Clusters of short dilated cisternae present in the same cytoplasmic location were often found (Fig. 1 *A* and *C*, arrows), and because of their morphology and location they may represent remnants of Golgi structures. In contrast, in HSV-1(F)-infected BHK (Fig. 1*E*) and 143TK⁻ (Fig. 1*F*) cells, Golgi structures virtually indistinguishable from those in the corresponding uninfected cells (data not shown), consisting of several stacked cisternae and showing a clear polarity, were readily detected. Additional differential characteristics of the cell lines studied to date were the structure surrounding the enveloped capsids in transit to the extracellular space. In Vero and HEp-2 cells the enveloped capsids were most frequently contained inside the large vacuoles described above. In BHK and 143TK⁻ cells the common

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Abbreviations: HPA, *Helix pomatia* agglutinin; HSV-1, herpes simplex virus; RCA, *Ricinus communis* agglutinin; WGA, wheat germ agglutinin.

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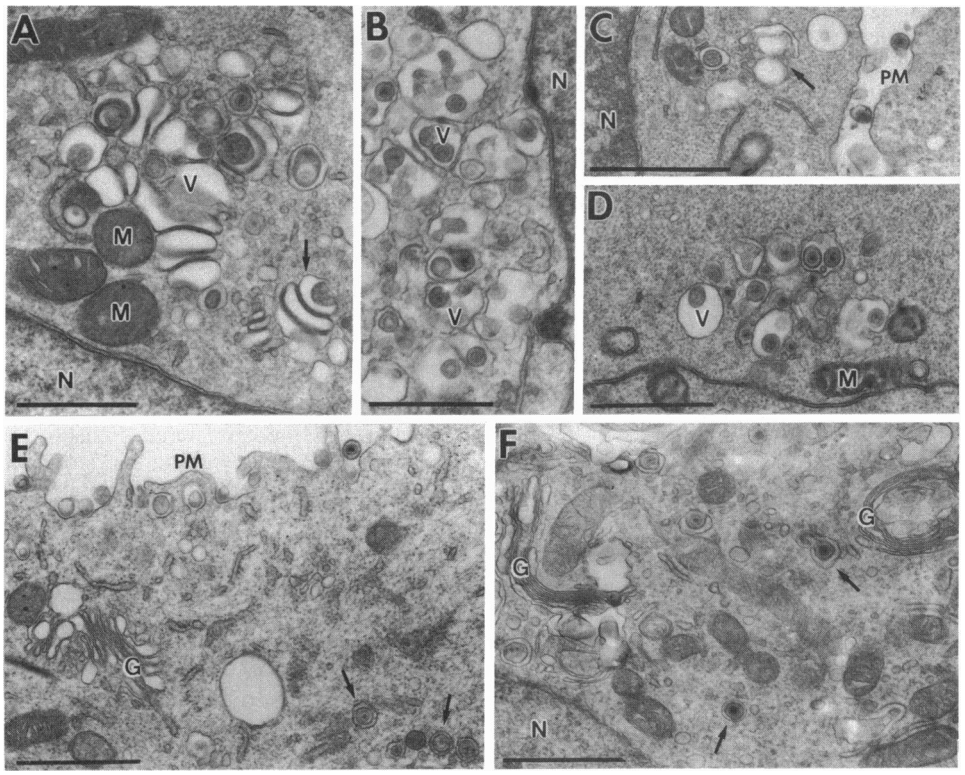

FIG. 1. Ultrastructural appearance of cells infected with HSV-1(F): Vero cells (*A* and *B*), HEp-2 cells (*C* and *D*), BHK cells (*E*), and 143TK$^-$ cells (*F*). Uninfected and HSV-1(F)-infected cells were fixed in glutaraldehyde (2% in phosphate-buffered saline, 60 min at 4°C), postfixed in 1% osmium tetroxide in Veronal/acetate buffer (pH 7.4) for 2 hr at 4°C, stained with uranyl acetate (5 mg/ml), dehydrated in acetone, and embedded in Epon 812. Thin sections were examined after poststaining with uranyl acetate and lead hydroxide. In Vero and HEp-2 cells, vacuoles (V) in close apposition were found in juxtanuclear position probably derived from Golgi cisternae. Enveloped virions were frequently observed within the vacuoles. In *A* and *C* arrows point to small dilated cisternae, very likely Golgi-derived. In BHK and 143TK$^-$ cells well-developed Golgi complexes (G) were clearly visible. Arrows point to enveloped virions inside vesicles. In *C* and *E* extracellular virions are present. N, nucleus; V, vacuoles; G, Golgi complex; M, mitochondria; PM, plasma membrane. (Bar = 1 μm.)

transit vehicles were vesicles ≈250 nm in diameter, each tightly surrounding a single virion.

Localization of Golgi Markers in HSV-Infected Cells. These experiments were done with R7032, from which the gene specifying glycoprotein E (gE) had been deleted (14). HSV-1 gE has the ability to bind the Fc portion of IgG (15) and therefore the use of a gE$^-$ mutant virtually eliminated the nonspecific binding of IgG to infected cells. In preliminary experiments Vero and HEp-2 cells infected with HSV-1(F) or with R7032 could not be differentiated either with respect to visualization of Golgi markers or with respect to their reactivity with Golgi-specific reagents used in these studies (data not shown). In this series of experiments, mock-infected and 15-hr-infected cells were processed for indirect immunofluorescence studies. The times at which the cells were fixed were chosen on the basis of time-course studies described below. For each cell line we selected the antibodies which reacted with greatest specificity. The results were as follows.

(*i*) As could be expected (5), antibodies to β-COP in uninfected Vero cells yielded a punctate staining clustered in a typical Golgi region (Fig. 2*A*). In R7032-infected Vero cells the fluorescence pattern was drastically altered to that of a punctate staining scattered throughout the cytoplasm (Fig. 2*B*). Similar patterns, respectively, were observed in uninfected and R7032-infected HEp-2 cells probed with anti-β-COP antibodies (Fig. 2 *D* and *E*).

(*ii*) In uninfected HEp-2 cells, antibody to galactosyltransferase reacted with structures confined to the Golgi region (Fig. 2*F*). In R7032-infected HEp-2 cells the dots stained with antibody to galactosyltransferase were dispersed throughout the cytoplasm (Fig. 2*G*). These dots were more heterogeneous in size than those observed in cells stained with the anti-β-COP antibody.

(*iii*) The distribution of α-mannosidase II in R7032-infected BHK cells varied from cell to cell. In some cells the staining was clustered to structures associated with Golgi apparatus; in others it appeared as partially dispersed dots (Fig. 2*I*).

(*iv*) Mock-infected and infected 143TK$^-$ cells were stained with antibodies to β-COP, galactosyltransferase, and α-mannosidase II. The fluorescence patterns observed with these markers in infected cells (Fig. 2 *K*, *M*, and *O*) were not significantly different from those of uninfected cells (Fig. 2 *J*, *L*, and *N*).

Lectin Staining in HSV-Infected Cells. The objective of these experiments was to determine whether the distribution of the glycoproteins and glycolipids processed by Golgi enzymes reflected the dispersal of the Golgi enzymes and proteins observed in infected Vero, HEp-2, and, to a lesser extent, BHK cells. The results were as follows.

(*i*) In uninfected Vero (Fig. 3 *A*, *C*, and *E*) and HEp-2 (Fig. 3 *G*, *I*, and *K*) cells the lectins illuminated perinuclear structures typical of Golgi apparatus. In R7032-infected Vero (Fig. 3 *B*, *D*, and *F*) and HEp-2 (Fig. 3 *H*, *J*, and *L*) cells HPA, RCA, and WGA labeled fragmented elements heterogeneous in size and scattered throughout the cytoplasm.

(*ii*) In infected BHK cells the staining pattern showed some variation from cell to cell. In some cells the distribution was similar to that of uninfected cells, whereas in others the labeling was localized to punctate structures scattered throughout the cytoplasm.

(*iii*) As could be expected from the results presented in the preceding section, in both infected and uninfected 143TK$^-$ cells the three lectins illuminated typical Golgi structures.

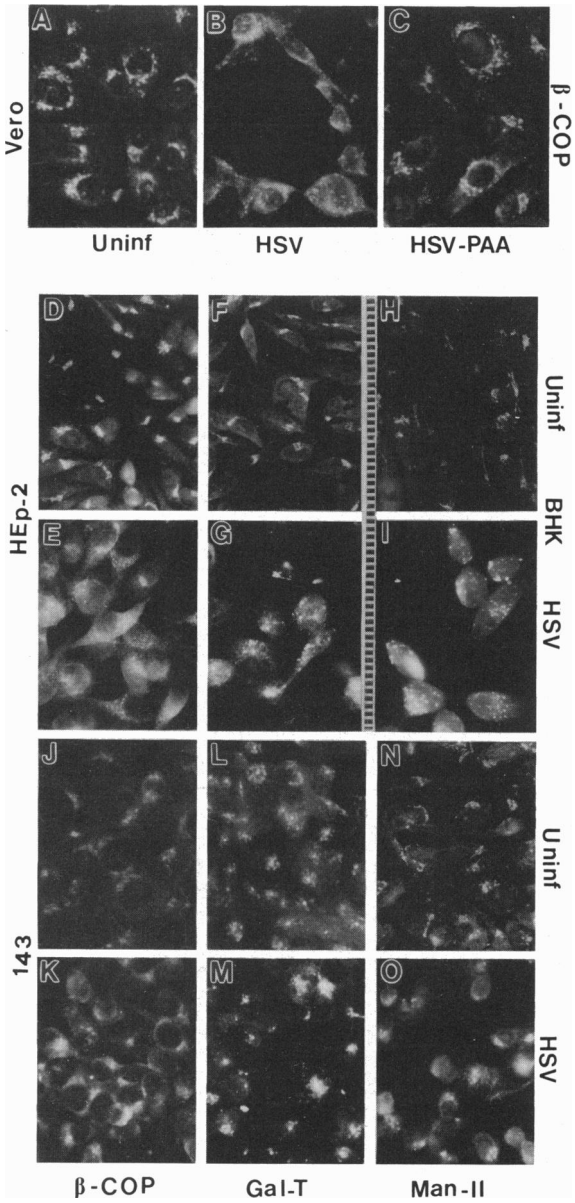

FIG. 2. Fluorescence staining with antibodies to β-COP, galactosyltransferase (Gal-T) and α-mannosidase II (Man-II) of uninfected (Uninf) and R7032-infected (HSV) cells. Fixed cells were incubated for 1 hr at room temperature with the appropriate antibodies, rinsed, and incubated with fluorescein-labeled goat anti-rabbit or anti-mouse IgG from Sigma. (*A–C*) Vero cells, uninfected (*A*) R7032-infected (*B*), or phosphonoacetate (PAA)-treated R7032-infected (*C*). (*D–G*) HEp-2 cells. (*H* and *I*) BHK cells. (*J–O*) 143TK$^-$ cells. Uninfected and infected cells were fixed in methanol at −20°C for 4 min.

(*iv*) WGA also stained the plasma membranes of uninfected cells and both the plasma membranes and structures more peripheral to the Golgi apparatus in the four uninfected cell lines.

(*v*) The overwhelming increase in the substrates reacting with the three lectins in infected as compared with the uninfected cells was one of the most striking features of the photomicrographs obtained in these studies. We should note that HSV-1 specifies 11 glycoproteins (16, 18) and that these are processed by Golgi enzymes as components of the virions or of intracellular membranes as they transit from the perinuclear space or rough endoplasmic reticulum, respectively, to the surface of the infected cell. The huge amount of glycoproteins made within a few hours in the infected cell undoubtedly forms a large fraction of the ligands which bound the lectins.

Time Course of the Redistribution of the Proteins Associated with Golgi Apparatus. The HSV-1 genes form several groups (α, β, γ_1, and γ_2) whose expression is coordinately regulated and sequentially ordered (16, 17). Two series of experiments were done to determine the time course of the dispersal of proteins associated with the Golgi apparatus. In the first, R7032-infected Vero cells were fixed at various times after infection. HPA-specific ligands were localized to a compact perinuclear element at 3 hr after infection, became partially dispersed at 6 hr, and were totally dispersed between 8 and 12 hr after infection (data not shown). In the second series, R7032-infected Vero cells were exposed to phosphonoacetate (300 μg/ml), an inhibitor of HSV DNA synthesis, from the time of virus adsorption until the cells were fixed and stained at 15 hr postinfection. Phosphonoacetate reduces the synthesis of γ_1 proteins and totally prevents the synthesis of γ_2 proteins. The pattern of staining with the anti-β-COP antibody (Fig. 2*C*) or HPA (data not shown) of Vero cells infected with R7032 and treated with phonoacetate could not be differentiated from that of uninfected cells. Similar results, respectively, were obtained with uninfected and infected HEp-2 cells treated with phosphonoacetate. The results indicate that the dispersal of the markers associated with Golgi structures coincides with the maximum rate of synthesis of viral glycoproteins, virion assembly, and extracellular transport.

DISCUSSION

HSV-1 infection of Vero and HEp-2 cells causes a dispersal of the glycoproteins and glycolipids processed by Golgi enzymes along with a redistribution of the Golgi resident enzymes and of a Golgi coat protein. The phenomenon is cell type-dependent. Whereas in Vero and HEp-2 cells intact Golgi could not be detected by any of the methods employed in this study, no significant redistribution of Golgi markers or of substrates of Golgi enzymes was demonstrable in infected 143TK$^-$ cells. That dispersal of Golgi markers and substrates is not a universal feature of HSV-1-infected cells is also reflected in reports of the presence of structures associated with Golgi apparatus in a variety of cell lines infected with HSV-1 (1, 2) and pseudorabies virus (23). Inasmuch as phosphonoacetate blocks the process in the cells in which it occurs, the results suggest that while a viral gene product triggers the fragmentation and dispersal of the Golgi apparatus, the process is cell- rather than virus-dependent.

The redistribution of Golgi markers in Vero and HEp-2 cells is associated with late events of viral reproductive cycle characterized by the maturation, processing, and sorting of virions. A plausible explanation of the phenomenon is that it arises from a huge influx of glycoproteins that reach the Golgi apparatus concurrent with the onset of viral DNA synthesis. The hypothesis that the Golgi apparatus breaks down when confronted with a huge amount of substrate for its enzymes rests on several observations that the changes in the dynamic equilibrium of anterograde and retrograde Golgi transport readily perturb its structure. Thus, perturbation of the equilibrium between the anterograde transport from rough endoplasmic reticulum to Golgi and the retrograde from Golgi to rough endoplasmic reticulum, induced for example by brefeldin A (19) or by overexpression of the human homolog of the ERD-2 rough endoplasmic reticulum salvage receptor (20), results in dramatic alterations in the structure of the Golgi apparatus. The hypothesis predicts that the breakdown of Golgi structures following infection with HSV may depend on the cell-specific features of the dynamics of Golgi function. In our studies, HSV-1 infection resulted in the alteration of HEp-2 and Vero cell Golgi but not those of 143TK$^-$ cells.

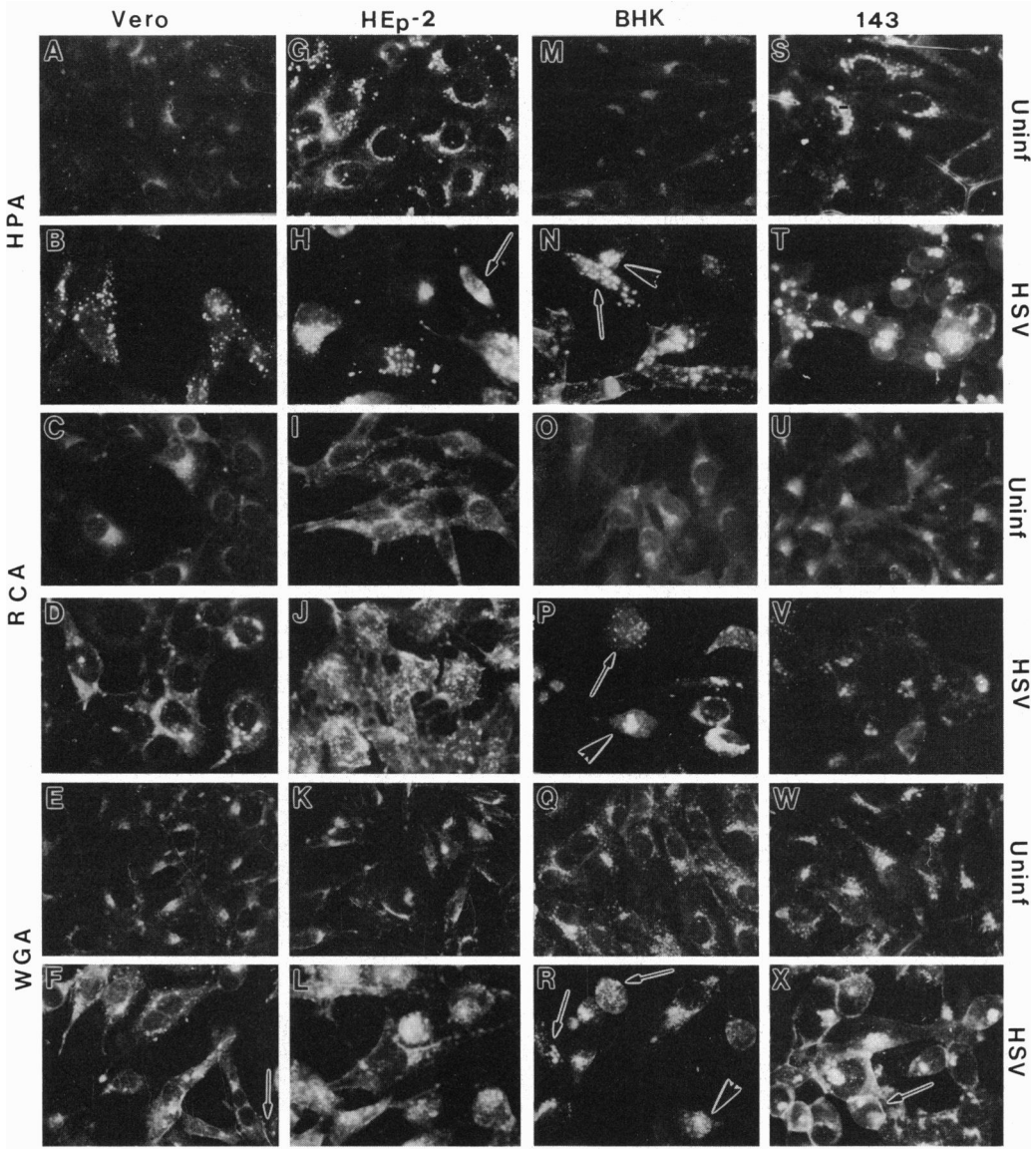

FIG. 3. Fluorescence staining of lectin binding sites in uninfected (Uninf) and HSV-1(F)-infected Vero (*A–F*), HEp-2 (*G–L*), BHK (*M–R*), and 143TK$^-$ (*S–X*) cells. In *H*, an arrow points to an uninfected cell. In *N*, *P*, and *R*, arrows point to cells with dispersed staining, while arrowheads point to cells with compact Golgi-like staining. In *F* and *X*, arrows point to plasma membrane labeling. For lectin labeling, methanol-fixed cells were incubated with fluorescein-conjugated HPA (top two rows), RCA (middle two rows), or WGA (bottom two rows) for 1 hr at room temperature.

BHK cells may be heterogeneous with respect to this function. It is noteworthy that some of the effects of brefeldin A vary depending on the cell line (21).

Studies on cell mutants defective in Golgi enzymes (22, 24) and on cells exposed to monensin (25) indicated that the Golgi are required for herpes simplex virion egress and for the maturation of viral glycoproteins. Subsequent studies on the effects of brefeldin A reinforced the view that HSV exploits the exocytic pathway for egress from infected cells (2, 24, 26). The four cell lines analyzed in this study are all highly permissive for HSV replication and release virions into the extracellular medium. Therefore, Vero and HEp-2 cells can sustain HSV egress notwithstanding the dispersal of Golgi apparatus. Whether these cells release HSV virions into the extracellular space with a lower efficiency as compared with cells whose Golgi apparatus is morphologically intact remains to be determined. A similar reasoning applies to viral glycoprotein maturation. All four cell lines have the ability to process HSV glycoproteins to mature forms. Studies on the rate of maturation of HSV glycoproteins showed that BHK cells display a higher maturation rate of gD and gC relative to HEp-2 (27). Furthermore for the same cell line the rate of maturation was higher at 7 hr than at 15 hr after infection (27). Whether a cell with a fragmented Golgi apparatus has an impaired ability to process glycoproteins as compared with a cell whose Golgi apparatus remains morphologically intact also remains to be assessed.

The effect of HSV-1 infection on the Golgi apparatus of Vero and HEp-2 cells is morphologically different from that of brefeldin A. In HSV-1-infected Vero and HEp-2 cells, discrete elements, very likely fragments derived from Golgi apparatus, were detectable. By contrast, brefeldin A induces a complete dissolution of Golgi apparatus and concomitantly a redistribution of Golgi markers into rough endoplasmic reticulum (19). The lack of analogy suggests that the molecular basis of fragmentation of the Golgi apparatus during HSV infection should differ from that of cells treated with brefeldin A, whose first known effect is the release of the coat protein β-COP (30–32). Morphologically the breakdown of Golgi apparatus observed in infected Vero and HEp-2 cells

resembles superficially that observed during mitosis (28, 29). It is currently thought that in this case breakdown of Golgi ensures that each daughter cell inherits remnants of Golgi apparatus, which serve as a template for Golgi reassembly.

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