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Assembly and Disassembly of the Golgi Complex: Two Processes Arranged in a *cis-trans* Direction

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Abstract. We have studied the disassembly and assembly of two morphologically and functionally distinct parts of the Golgi complex, the *cis*/middle and *trans* cisterna/*trans* network compartments. For this purpose we have followed the redistribution of three *cis*/middle- (GMP_{c-1}, GMP_{c-2}, MG 160) and two *trans*- (GMP_{t-1} and GMP_{t-2}) Golgi membrane proteins during and after treatment of normal rat kidney (NRK) cells with brefeldin A (BFA). BFA induced complete disassembly of the *cis*/middle- and *trans*-Golgi complex and translocation of GMP_c and GMP_t to the ER. Cells treated for short times (3 min) with BFA showed extensive disorganization of both *cis*/middle- and *trans*-Golgi complexes. However, complete disorganization of the *trans* part required much longer incubations with the drug. Upon removal of BFA the Golgi complex was reassembled by a process consisting of three steps: (*a*) exit of *cis*/middle proteins from the ER and their accumulation into vesicular structures scattered throughout the cytoplasm; (*b*) gradual relocation and accumulation of the *trans* proteins in the vesicles containing the *cis*/middle proteins; and (*c*) assembly of the cisternae, and reconstruction of the Golgi complex within an area located in the vicinity of the centrosome from which the ER was excluded. Reconstruction of the *cis*/middle-Golgi complex occurred under temperature conditions inhibitory of the reorganization of the *trans*-Golgi complex, and was dependent on microtubules. Reconstruction of the *trans*-Golgi complex, disrupted with nocodazole after selective fusion of the *cis*/middle-Golgi complex with the ER, occurred after the release of *cis*/middle-Golgi proteins from the ER and the assembly of the *cis*/middle cisternae.

THE Golgi complex plays an important role in the posttranslational modifications and sorting of proteins transported from the ER, recycling of receptors involved in endocytosis and transport of proteins to organelles, and in the resorting of ligand–receptor complexes internalized by transcytosis (Palade, 1975; Farquhar, 1985; Pfeffer and Rothman, 1987). The organelle is constituted by two morphologically and functionally distinct parts: a stack of cisternae (Morré and Ovtracht, 1977; Farquhar and Palade 1981), and a network of tubules called the *trans* network (Novikoff et al., 1971; Novikoff, 1977; Griffiths and Simons, 1986). A striking feature of the Golgi complex is the functional compartmentation of its components and the arrangement of the compartments (i.e., cisternae, *trans* network) in a functional order (Dunphy et al., 1981, 1983; Rothman et al., 1984*a*,*b*; Orci et al., 1987; Tartakoff, 1983; Kornfeld and Kornfeld, 1985). By acting synchronously, the enzymes contained in different compartments introduce a variety of posttranslational modifications into the proteins transported through the organelle (reviewed in Tartakoff, 1983; Dunphy and Rothman, 1985; Kornfeld and Kornfeld 1985). Transport of molecules to, through, and out of the Golgi complex is probably mediated by specific populations of vesicles (Rothman et al., 1984*a*,*b*; Balch et al., 1984*a*,*b*; Dunphy and Rothman, 1985; Orci et al., 1986, 1989; Pfeffer and Rothman, 1987). Sorting of the proteins occurs in the *trans*-Golgi network (Novikoff and Novikoff, 1977; Habener et al., 1979; Griffiths et al., 1985, 1989; Snider and Rogers, 1985; Griffiths and Simons, 1986; Sossin et al., 1990).

The Golgi complex is an organelle that through the cell cycle, undergoes dramatic changes in morphology, localization, and function. Throughout interphase the functionally active Golgi displays a characteristic organization in parallel cisternae juxtaposed to the *trans* network (Morré and Ovtracht, 1977; Farquhar and Palade, 1981), and is located in the vicinity of the centrosome (Kupfer et al., 1982; Wehland and Sandoval, 1983; Sandoval et al., 1984; Tassin et al., 1985). Furthermore, Golgi and centrosome show synchronous and superimposed displacements in moving cells (Kupfer et al., 1982). At the onset of mitosis the whole Golgi is disrupted, becomes inactive, and the dispersed fragments are excluded from the area containing the mitotic spindle (Robbins and Gonatas, 1964; Maul and Brinkley, 1970; Zeiligs and Wollman, 1979). Reconstruction of the organelle is initiated during cytokinesis and completed before separation of the two daughter cells (Robbins and Gonatas, 1964; Maul

and Brinkley, 1970; Zeiligs and Wollman, 1979; Warren et al., 1983, 1984; Lucocq and Warren, 1987; Lucocq et al., 1987; Gaspar et al., 1988).

Recent studies have shown that brefeldin A (BFA)[1], a fungal-antiviral antibiotic (Harri et al., 1963), can be used as a useful tool to study the function and organization of the Golgi complex. Minutes after treatment with BFA, proteins normally transported through the ER-Golgi complex pathway are retained in the ER (Takatsuki and Tamura, 1985; Misumi et al., 1986; Oda et al., 1987; Magner and Papagiannes, 1988). Furthermore, it has been reported that BFA induces the selective fusion of parts of the Golgi with the ER (Lippincott-Schwartz, 1989; Doms et al., 1989), and promotes the release from the Golgi or β-Coat protein (Donaldson et al., 1990), a protein associated with nonclathrin-coated vesicles (Orci et al., 1986; Malhotra et al., 1989; Serafini et al., 1991; Duden et al., 1991) acting as bulk carriers (Wieland et al., 1987; Orci et al., 1989; Karrenbauer et al., 1990).

Here we have studied the disassembly and reassembly of the *cis*/middle and *trans* (i.e., *trans* most cisterna and *trans* network) parts of the Golgi complex in cells treated with BFA by following the redistribution of resident membrane proteins of these compartments. Our results show that both processes occur in an orderly fashion: the *cis*/middle- precedes the *trans*-Golgi in the fusion with the ER and in the reassembly that follows the removal of BFA. Moreover, the *cis*/middle-Golgi is reconstructed under conditions preventing the reorganization of the *trans*-Golgi. In contrast conditions inhibiting the reconstruction of the *cis*/middle-Golgi also block the reorganization of the *trans*-Golgi. The significance of these results is discussed within the context of the anterograde and retrograde mechanisms involved in the transport of molecules between the ER and compartments of the Golgi complex, and of the mechanisms operating in the disruption and reconstruction of the Golgi complex during the cell cycle.

Materials and Methods

Cell Culture

Normal rat kidney (NRK) cells were grown on plastic bottles or glass coverslips, in 90% DME, 10% FCS, 10 mM morpholinoethane-sulfonic acid, 2 mM glutamine, penicillin (50 U/ml), streptomycin (50 μg/ml) (normal medium) at 37°C, or 23°C, in an atmosphere of 93% air, 7% CO_2, and 85% humidity.

Antibodies

All the mAbs against rat Golgi complex and lysosomal membrane proteins were raised in mouse. Monoclonal antibodies IgG 15C8 and IgM 20.1 reacted with membrane proteins of 130 kD (Golgi integral membrane protein [GMP]; GMP_{c-1}) (Yuan et al., 1987) and 180 kD (GMP_{c-2}), respectively, contained in the *cis*- and middle-Golgi cisternae. Monoclonal antibodies IgG 18B11 and IgM 21.1 reacted with proteins of 100 kD (GMP_{t-1}) (Yuan et al., 1987) and 200 kD (GMP_{t-2}), respectively, contained in the *trans*-most Golgi cisternae and *trans* network. Polyclonal antibodies against GMP_{c-1} and GMP_{t-1} were raised by injecting the proteins purified by monoclonal antibody affinity chromatography into rabbits. Monoclonal antibodies IgG 38C7 and 29G10 reacted with lysosomal integral membrane protein (LIMP) III and LIMP II, two LIMPs of 100 kD and 74 kD, respectively (Barriocanal et al., 1986). Other antibodies used were the rat antitubulin antibody YL 1/2 (Kilmartin et al., 1982; Wehland et al., 1983b), the rabbit polyclonal antibody against rat protein disulphide isomerase (PDI), and the anti-MG-160 antibody, which recognizes a 160-kD protein located in the *cis*/middle-Golgi (Croul et al., 1990; Gonatas et al., 1989). Fluorescein-, rhodamine-, and peroxidase-conjugated antibodies were from Cooper-Biomedical Inc. (Malvern, PA). Peroxidase conjugated protein A was from Boehringer Mannheim Diagnostics, Inc. (Houston, TX).

Light Microscopy

The morphological changes of the Golgi complex and the redistribution of GMPs in cells treated with BFA were studied by immunofluorescence and immunoperoxidase microscopy. The strong signal produced by the accumulation of the product of the peroxidase reaction made the staining of cells with peroxidase the method of choice to study the morphology of protein-depleted Golgi cisternae, and to detect the presence of GMPs in the ER. Cells studied by immunofluorescence were fixed-permeabilized with cold (−20°C) methanol for 2 min. Cells studied by immunoperoxidase staining were prepared by the modification of the PLP/saponin procedure (McLean and Nakane, 1974) described before (Yuan et al., 1987). The cellular distribution of individual Golgi membrane proteins was studied by simple immunofluorescence microscopy as described (Yuan et al., 1987). Double-immunofluorescence microscopy was performed by the four-step procedure described before (Barriocanal et al., 1986).

Other Methods

Metabolic labeling, immunoprecipitation, treatment with neuraminidase, and analysis of LIMPs by two-dimensional IEF/SDS-PAGE were performed as described (Barriocanal et al., 1986).

Results

Disassembly of the cis/middle- and trans-Golgi Network after BFA Treatment

To study the disassembly of the Golgi complex we have analyzed the model of NRK cells treated with BFA.

The drug promotes the rapid disassembly of the Golgi complex (Fujiwara et al., 1988), and quick translocation of the *cis*/middle-Golgi marker, mannosidase II, and middle/*trans* marker, β-galactosyltransferase (Lippincott-Schwartz et al., 1989, 1990) to the ER. However, from studies performed with the markers of the *trans*-most cisterna and *trans*-Golgi network, antigen GMP_{t-1} (Lippincott-Schwartz et al., 1989), and sialyltransferase (Chege and Pfeffer, 1990), it has been concluded that the *trans*-most Golgi cisternae is not fused with the ER.

A study of both the disassembly and assembly of the Golgi complex in cells treated with BFA requires an exact definition of which parts of the Golgi complex fuse with the ER in response to the drug. For this purpose, we have reexamined the effects of the drug on the distribution of three membrane proteins resident in the *cis*/middle-Golgi, antigens GMP_{c-1}, GMP_{c-2}, and MG 160 (Yuan et al., 1987; Gonatas et al., 1989), and two located in the *trans*-most cisterna and *trans*-Golgi network, GMP_{t-1} and GMP_{t-2} (Yuan et al., 1987). The results are shown in Fig. 1. In contrast to previous studies we observe that cells treated with 1–10 μg/ml BFA and stained for GMP_{t-1} with antibody 18B11, displayed marked changes in the morphology of the *trans*-Golgi. Identical changes were observed when the cells were stained for GMP_{t-2} with antibody 21.1. The changes were already observed 3 min after addition of the drug (Fig. 1, *C* and *D*), and consisted in extensive disorganization of the

1. *Abbreviations used in this paper*: BFA, brefeldin A; GMP, Golgi integral membrane protein; LIMP, lysosomal integral membrane protein; NEM, *N*-ethylmaleimide; NRK, normal rat kidney; PDI, protein disulphide isomerase.

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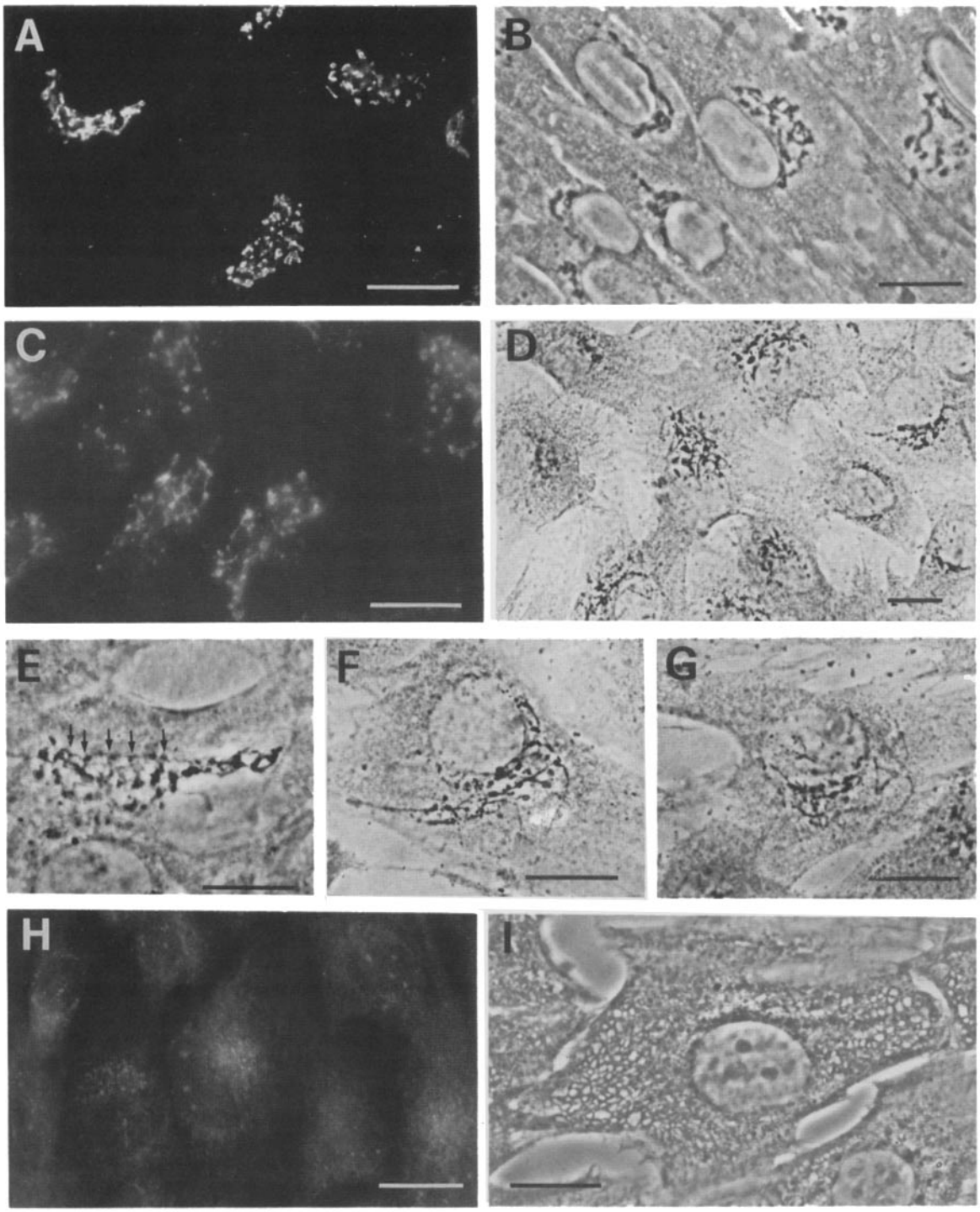

Figure 1. Effects of BFA on the morphology and organization of the Golgi complex. NRK cells were incubated with normal medium (*A* and *B*), or 10 μg/ml BFA for 3 min (*C* and *D*), 15 min (*E* and *F*), or 60 min (*G* and *H*). The distribution of GMP_{t-1} was studied by light microscopy using the monoclonal antibody 18B11, and fluoresceine-(*A*, *C*, and *H*), or peroxidase-(*B*, *D–G*, and *I*) conjugated goat anti-mouse antibodies. Comparable results were obtained with the anti-GMP_{t-2} antibody 21.1. Note the rapid disorganization of the *trans*-Golgi complex in response to BFA. Bars, 15 μm.

trans-Golgi, which acquired a necklace morphology (i.e., strings of beads connected by fibers) already described for the *cis*/middle-Golgi complex (Lippincott-Schwartz et al., 1989). Longer incubations resulted in progressive loss of the reticular structure (Fig. 1, *E–G*), its substitution by small vesicles clustered in the vicinity of the nucleus (Fig. 1 *H*), and the appearance of faintly stained fibers extended throughout the cytoplasm (Fig. 1 *H*). Staining with peroxidase revealed that the fibers corresponded to a polygonal network of tubules extending throughout the cytoplasm (Fig. 1 *I*). The tubular network was identified as the ER by its staining with DiOC6 (2), and with peroxidase using an antibody

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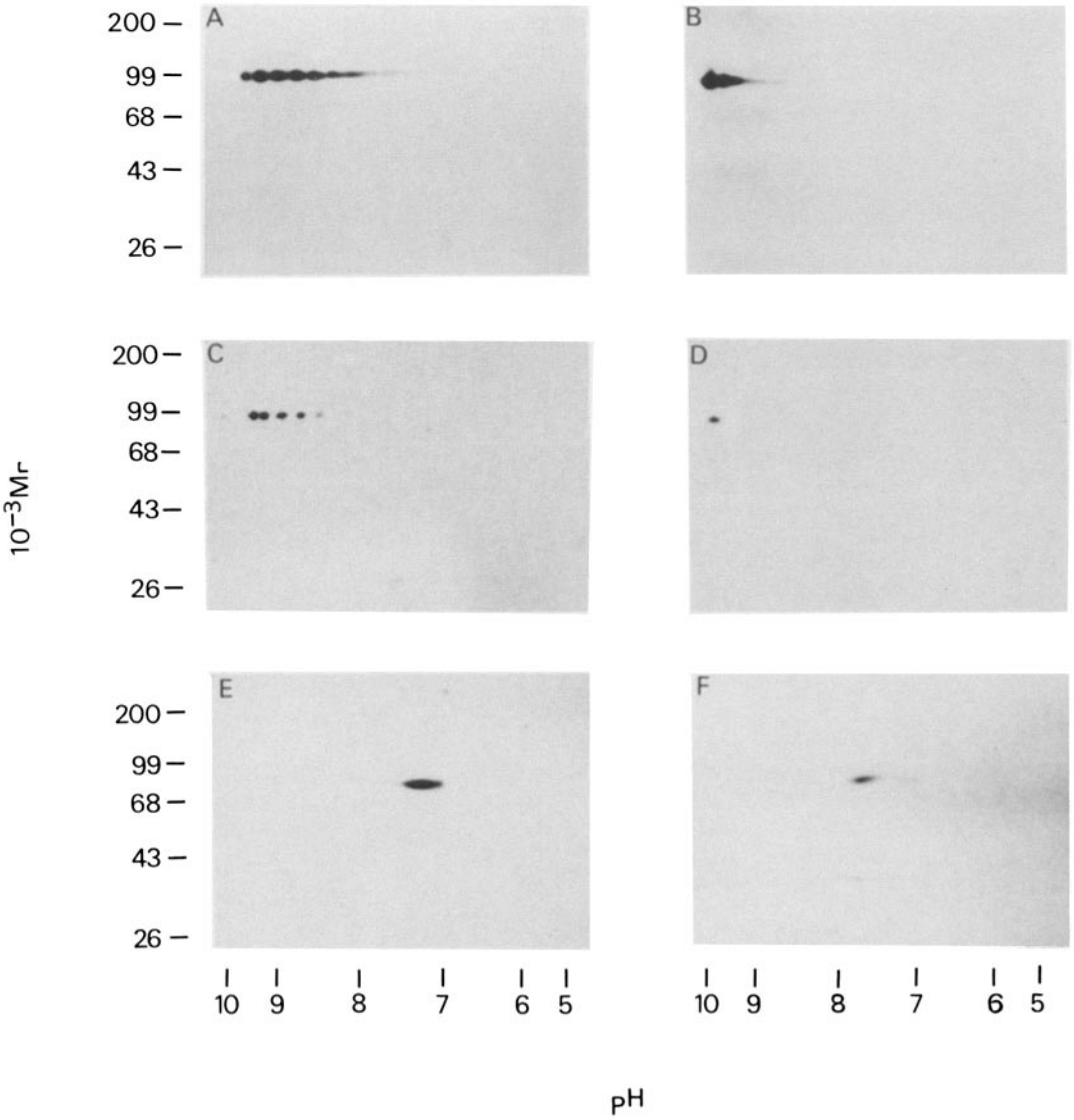

Figure 2. Translocation of enzymes involved in the sialylation of N-linked carbohydrates from the *trans*-Golgi complex to the endoplasmic reticulum in BFA-treated cells. LIMP III (*A* and *B*) immunoprecipitated from NRK cells treated for 2 h with 10 μg/ml BFA and then labeled for 3 h with [^{35}S]methionine in the presence of 10 μg/ml BFA. LIMP III (*C–F*) and LIMP II (*E* and *F*) immunoprecipitated from NRK cells pulse-labeled for 15 min with [^{35}S]methionine, and then incubated for 2 h with 100 μM cycloheximide and 10 μg/ml BFA. The immunoprecipitated proteins were incubated without (*A*, *C*, and *E*) and with (*B*, *D*, and *F*) neuraminidase, and resolved by two-dimensional SDS-PAGE to analyze their content in sialic acid.

against the ER resident enzyme, PDI (not shown). Finally, after 75 min incubation with BFA, the vesicles disappeared and the staining was spread throughout the cytoplasm (Fig. 4 *B*). These results strongly suggested that BFA promoted the translocation of proteins resident in the trans most cisternae and *trans*-Golgi network to the ER.

To further test that suggestion, we studied whether the drug promoted the translocation from the trans most cisternae and *trans*-Golgi network (Bennett and O'Shaughnessy, 1981; Roth et al., 1985) to the ER of the sialyltransferases involved in transferring sialic acid to N-linked carbohydrates. For this purpose, we studied the acquisition of sialic acid by the lysosomal membrane proteins LIMP II and LIMP III (Barriocanal et al., 1986). Both proteins were shown to contain sialylated N-linked oligosaccharides and to lack O-linked carbohydrates (Barriocanal et al., 1986). As shown in Fig. 2 *A*, LIMP III retained for 3 h in the ER of cells pretreated with BFA displayed four major forms with pI between 8.2 and 9.2. Treatment of the protein with neuraminidase (Fig. 2 *B*) produced three forms with pI between 9.0 and 9.5. The shift in pI strongly suggested that the molecules of LIMP III retained in the ER acquired sialic acid. To exclude the possibility that the protein was sialylated by sialyltransferases synthesized and retained in the ER during the 3-h period of protein labeling, LIMP III was pulse labeled for 15 min (a lapse of time during which the protein synthesized in normal NRK cells remained in the ER and displayed only high mannose carbohydrates) (Morales et al., 1989) in the presence of 100 μM cycloheximide (concentration of antibiotic that caused an immediate inhibition of protein synthesis but had no effect on the BFA induced fusion of the *trans*-Golgi with the ER [data not shown]). Under the later

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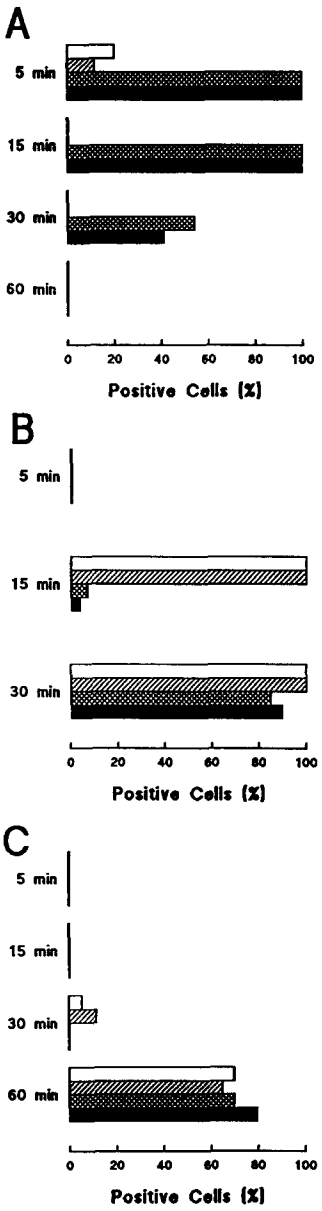

Figure 3. Time courses of disassembly and assembly of the *cis*/middle and *trans*-Golgi in cells treated with BFA. (*A*) Cells were treated with 10 μg/ml BFA for the indicated times and then studied by immunofluorescence microscopy using monoclonal antibodies against GMP_{c-1} (□), GMP_{c-2} (▨), GMP_{t-1} (⊠), and GMP_{t-2} (■). Bars indicate the percent of cells containing identifiable Golgi complexes (i.e., normal, beaded, and fibrous Golgi complexes) in two separate experiments in which 200 cells were counted. (*B* and *C*) Cells were treated for 75 min with 10 μg/ml BFA, washed rapidly with DME, and incubated with normal medium for the indicated times. They were then studied by immunofluorescence microscopy using monoclonal antibodies against GMP_{c-1} (□), GMP_{c-2} (▨), GMP_{t-1} (⊠), and GMP_{t-2} (■). Bars in *B* indicate the percent of cells displaying the GMP loaded vesicles described in Fig. 4, *C–H*, and in *C* the percent of cells exhibiting fully reconstructed Golgi complexes as shown also in Fig. 4 *I* and *J*. The values were obtained by studying 200 cells in two separate experiments. Studies performed with the antibody anti-MG-160 produced results similar to those obtained with the anti-GMP_c antibodies.

conditions LIMP III showed the four major forms with pI between 8.0 and 9.2 observed after the 3-h labeling in the absence of antibiotic (compare Fig. 2, *A* with *C*). Furthermore, the shift in pI showed by the protein, after treatment with neuraminidase (compare Fig. 2, *C* with *D*) indicated that it acquired sialic acid in the absence of new synthesis of sialyltransferase. Similar results were obtained with LIMP II (Fig. 2, *E* and *F*). These results agreed with those of recent studies on the processing of alkaline phosphatase (Takami et al., 1990).

Taken together, the translocation of GMP_{t-1}, GMP_{t-2}, and sialyltransferase activity to the ER of cells treated with BFA indicated that the drug promoted the fusion of the *trans*-Golgi and *trans*-Golgi network with the ER.

With respect to the effects of BFA on the organization of the *cis*/middle-Golgi stained with anti-GMP_c antibodies, our results were identical to those described using antibodies against the *cis*/middle marker mannosidase II and the itinerant G protein of vesicular stomatitis virus (Lippincott-Schwartz et al., 1989, 1990; Doms et al., 1989).

Comparison between the temporal responses to the BFA revealed that the *cis*/middle-Golgi became disorganized much faster than the *trans*-Golgi complex. As can be seen in Fig. 3 *A*, after 5 min incubation with 10 μg/ml BFA 75 and 80% of the cells stained with anti-GMP_{c-1} and anti-GMP_{c-2} antibodies, respectively, did not show staining of the *cis*/middle-Golgi complex. In contrast, after identical treatment all the cells stained with anti-GMP_{t-1} and anti-GMP_{t-2} antibodies showed recognizable *trans*-Golgi elements. Moreover, whereas the *cis*/middle-Golgi complex was completely disorganized after 15 min of treatment with BFA, nearly half of the cells incubated for 30 min with the drug still showed fibrous *trans*-Golgi elements.

Exit of cis/middle- and trans-Golgi Proteins from the ER and Reconstruction of the Golgi Complex

Previous studies have demonstrated the reversibility of the effects of BFA on the Golgi complex (Fujiwara et al., 1988; Lippincott-Schwartz et al., 1989; Doms et al., 1989; Lippincott-Schwartz et al., 1990). This provided us with a system to study the assembly of the *cis*/middle and *trans* parts of the organelle. The studies were performed by double immunofluorescence microscopy. 10 min after removal of BFA the distribution of the *cis*/middle-Golgi markers, GMP_{c-1}, GMP_{c-2}, and MG 160 were dramatically changed. The proteins were found in discrete vesicular structures (Fig. 4, *C* and *D*). By comparison, the relocation of the *trans*-Golgi proteins from the ER proceeded significantly more slowly, and no change in their distribution was observed 10–12 min after removal of the drug (Figs. 3 *B* and 4 *E*). It was only after 15 min that half of the cells began to show redistribution of the *trans* proteins (Figs. 3 *B* and 4 *G*). Interestingly, the redistributed *trans* proteins were found to colocalize with the *cis*/middle proteins in the same structures (Fig. 4, *F* and *G*). The process of colocalization increased with time and was complete 15 min after the exit of the first *trans* molecules from the ER (Fig. 4, *H* and *I*). Finally, the reassembly of the Golgi complex (i.e., cisternae) (Fig. 4, *J* and *K*) was slow as compared to the rapidity with which the Golgi proteins exit the ER (Fig. 3, compare *B* with *C*).

Further analysis of the process of Golgi assembly revealed that the organelle was reconstructed in an area from which the ER marker PDI was excluded and that remained visible after complete disassembly of the Golgi complex (Fig. 5, *A* and *B*). The vesicular structures that became loaded with GMPs shortly after removal of the drug were found scattered throughout the cytoplasm (Fig. 5, *C* and *D*). Later the GMPs residing at tubular structures were concentrated at the periphery of the area of ER exclusion (Fig. 5, *E* and *F*), before entering (Fig. 5, *G* and *H*) and forming the reticular structure characteristic of the Golgi complex (Fig. 5, *I* and *J*).

In 90% of the cells studied, the centrosome (organelle that regulates the assembly and organization of the cytoplasmic microtubules and whose function has been associated with the assembly of the Golgi complex [Kupfer et al., 1982; Wheland and Sandoval, 1983]) was located at the edge of the area of ER exclusion closer to the nucleus (Fig. 5, *K* and *L*). In the remaining 10% of the cells, the centrosome was found within that area.

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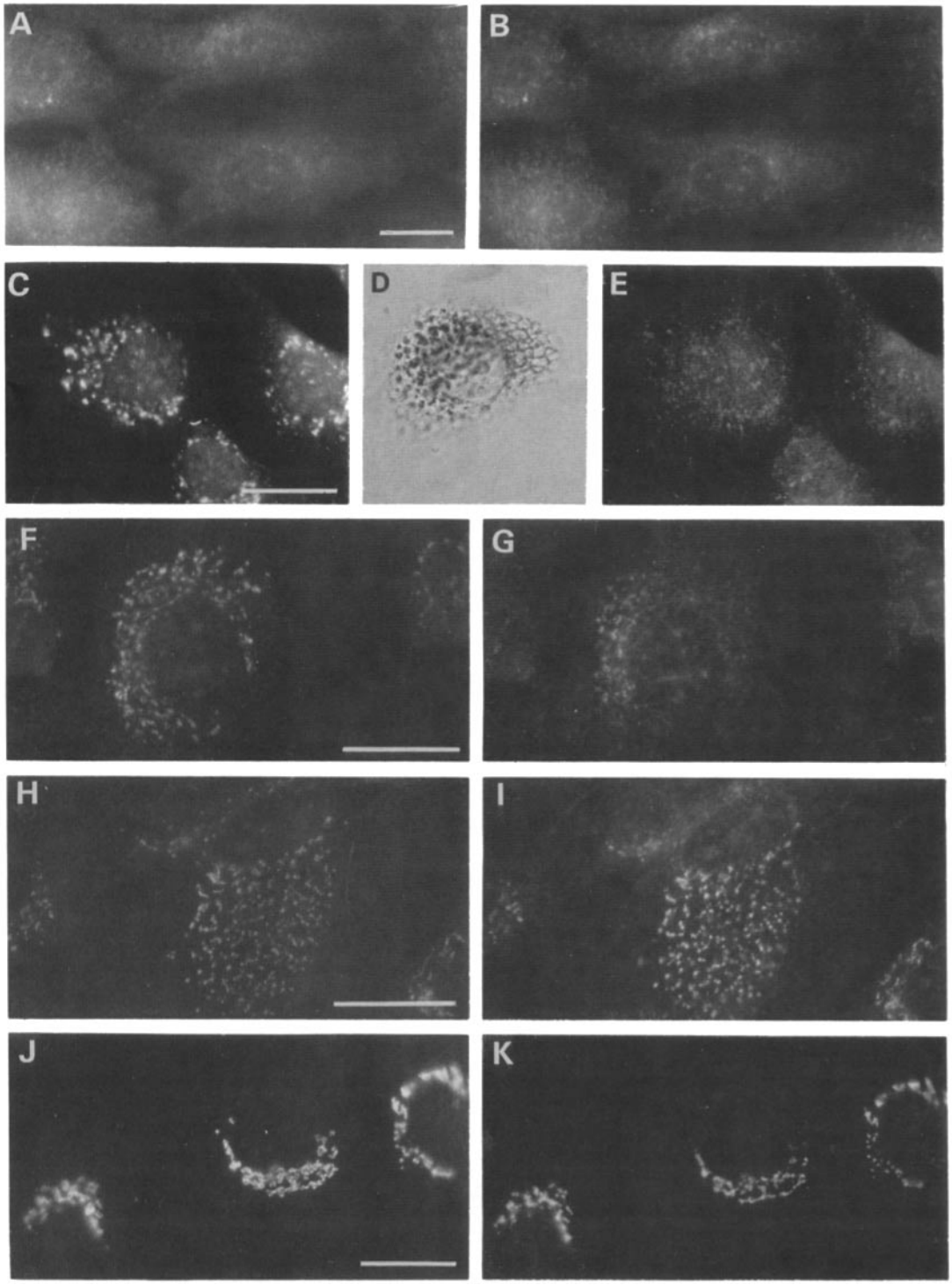

Figure 4. Exit of *cis*/middle- and *trans*-proteins from the ER and reconstruction of the Golgi complex. NRK cells were treated for 75 min with 10 μg/ml BFA (*A* and *B*) and then incubated in drug-free medium for 10 min (*C*, *D*, and *E*), 15 min (*F* and *G*), 30 min (*H* and *I*), and 1 h (*J* and *K*). The distribution of *cis*/middle- and *trans*-Golgi proteins was studied by double immunofluorescence microscopy using the mouse monoclonal anti-GMP_{c-1} antibody 15C8 (fluorescein channel; *A*, *C*, *F*, *H*, and *J*) and a rabbit polyclonal anti-GMP_{t-1} antibody (rhodamine channel; *B*, *E*, *G*, *I*, and *K*). The distribution of GMP_{c-1} between the endoplasmic reticulum and vesicles after removal of BFA was studied with peroxidase staining (*D*). Note the faster exclusion of GMP_{c-1} as compared with GMP_{t-1} from the ER, and the gradual incorporation of the *trans* protein into vesicular structures loaded with the *cis*/middle protein. Similar results were obtained with cells stained with anti-GMP_{c-2} and anti-GMP_{t-2}. Bars, 15 μm.

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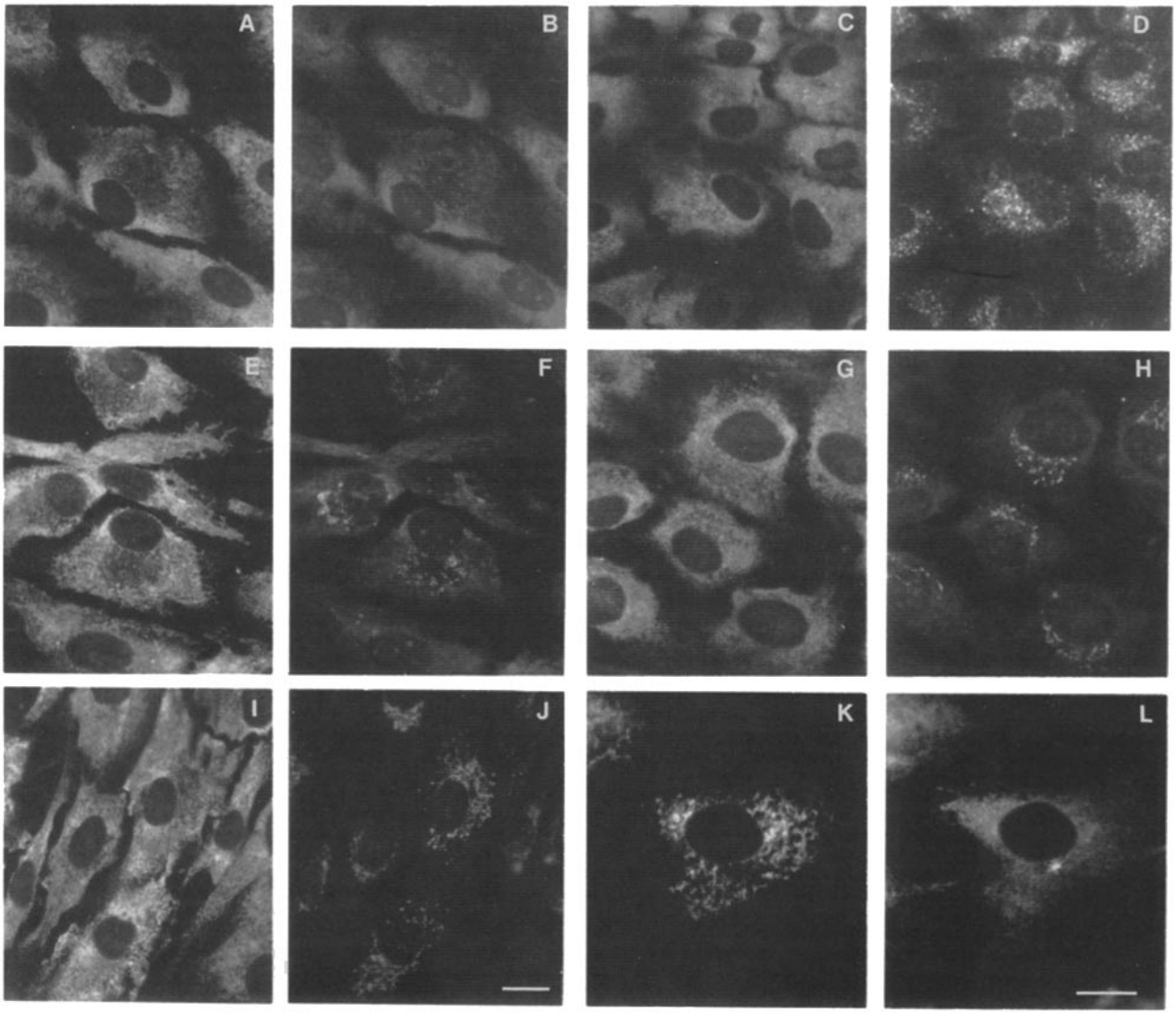

Figure 5. Reconstruction of the Golgi complex occurs in an area devoid of ER located in the vicinity of the centrosome. NRK cells treated 60 min with 10 μg/ml BFA and simultaneously stained with a rabbit polyclonal antibody against the ER marker PDI (*A*; rhodamine channel) and the anti-GMP_{c-1} antibody (*B*; fluoresceine channel). Note that the PDI negative area remains intact after the disassembly of the *cis*-Golgi complex. Identical results were obtained in studies of the *trans*-Golgi. Cells treated 60 min with 10 μg/ml BFA and incubated for 10 min (*C* and *D*), 30 min (*E* and *F*), 45 min (*G* and *H*), and 75 min (*I* and *J*) in drug-free medium, simultaneously stained with the anti-PDI (*C*, *E*, *G*, and *I*; rhodamine channel) and the mouse monoclonal anti-GMP_{t-1} (*D*, *F*, *H*, and *J*; fluorescein channel) antibodies. Note the dotty fluorescence throughout the cytoplasm 10 min after removal of BFA, and later the increasing formation of tubular structures, first around and then inside the area of ER exclusion, before formation of the reticular structure characteristic of the Golgi complex. Similar results were obtained with the anti-GMP_{c-1} antibody. Cells treated for 45 min with 20 μM nocodazol stained for PDI (*K*; rhodamine channel) and for microtubules with the anti-tubulin antibody YL 1/2 (*L*; fluorescein channel). Observe the location of the centrosome at the edge of the area of ER exclusion and in the vicinity of the nucleus. Bars, 15 μm.

Back Transport of cis/middle- and trans-Proteins from Vesicles to the ER

The accumulation of GMPs in vesicular structures upon removal of BFA raised the question whether they were part of the ER or constituted a separate compartment. To answer that question the following experiment was performed: cells were incubated for 75 min with 100 μg/ml BFA to induce the complete fusion of the Golgi with the ER, then for 20 or 40 min in the absence of the drug to release the GMPs from the ER, and again with 10 μg/ml BFA for periods of time between 10 and 60 min to induce their relocation into the ER. It was reasoned that if the vesicles loaded with GMPs were part of the ER, upon addition of BFA the *cis*/middle and *trans* proteins would diffuse with the same rates through the ER cisternae. On the contrary, accumulation of GMPs into vesicles independent from the ER would result in a pattern of ordered transport similar to that observed in the transport of GMPs from the Golgi complex. The results are shown in Fig. 6. It can be observed that the *cis*/middle-Golgi proteins entered the ER faster than the *trans*-Golgi proteins. Furthermore, the translocation of GMPs from the vesicles to the ER occurred considerably faster than from the Golgi complex (compare Fig. 3 with Fig. 6), suggesting that they were in a compartment close to the ER. It was noteworthy that during the process of transport, we never noted the fibrous struc-

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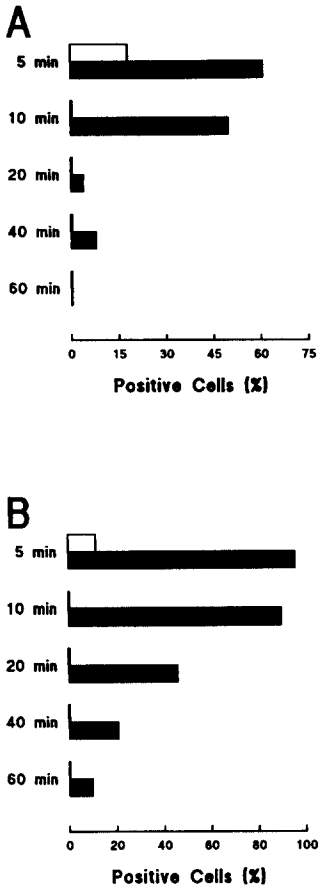

Figure 6. Back transport of Golgi proteins to the ER before assembly of the Golgi complex. Cells were treated for 75 min with 10 μg/ml BFA, washed rapidly with DME, incubated for 20 min (*A*) and 40 min (*B*) at 37°C with normal medium, and again treated with 10 μg/ml BFA for the indicated times. The cellular localization of the proteins was studied by immunofluorescence microscopy using monoclonal antibodies against GMP_{c-1} and GMP_{t-1}. Bars indicate the percent of cells displaying vesicular and tubular structures loaded with GMP_{c-1} (□) and GMP_{t-1} (■). The values were obtained by studying 200 cells in two separate experiments.

tures visible during the disassembly of the Golgi complex. This observation suggested that the fibers could correspond to cisternae depleted of proteins and lipids.

Inhibition of the Disassembly and Assembly of the Golgi Complex by N-ethylmaleimide (NEM) and Low Temperatures

The fusion of the Golgi complex with the ER upon addition of BFA and the substitution of cisternae for vesiculotubular elements after removal of the drug strongly suggested the occurrence of fusion events mediated by vesicles. The recent discovery that an NEM-sensitive fusion protein, or fusogen, is involved in the traffic of vesicles moving from the ER to and through the Golgi complex (Malhotra et al., 1989; Wilson et al., 1989; Beckers et al., 1989; Díaz et al., 1989), prompted us to study whether NEM could inhibit the disassembly and reassembly of the Golgi complex. The studies were performed under conditions that caused no cell mortality (see Fig. 7, *A* and *B*). It was noted that incubation of cells with 200 μM NEM for 1 min resulted in immediate inhibition of the BFA-induced disassembly of the Golgi complex (Fig. 7, *C–F*). The rapidity of the inhibition permitted the observation that swelling and disruption of the Golgi cisternae into vesicles were events preceding the fusion of the Golgi membranes with the ER. Moreover, NEM also caused the immediate inhibition of the transport of Golgi proteins from the ER (in Fig. 7, compare *G* with *H*).

In separate experiments we also studied the effects of low temperatures on the disassembly and assembly of the Golgi complex. Briefly, only temperatures below 17°C inhibited completely the disassembly of the Golgi complex induced by BFA. Transport of Golgi proteins from the ER was completely blocked at 17°C and markedly slowed down at 23°C, a temperature that also decreased the rate of assembly of the cisternae. As described below, the use of low temperatures was a strong tool to study the separate assembly of Golgi compartments.

Assembly of the cis/middle-Golgi Complex under Conditions Inhibiting the Reconstruction of the trans-Golgi Complex

To gain further insight into the reconstruction of the Golgi complex we examined if the *cis*/middle and *trans* parts of the organelle could be assembled separately. For this purpose, cells were incubated at 37°C for 90 min with 10 μg/ml BFA to induce the complete resorption of the whole Golgi complex into the ER; then, for 10–12 min at 37°C with drug-free medium to allow the selective transport of *cis*/middle proteins from the ER; and finally, at 23°C for 40–60 min to study the reconstruction of the *cis*/middle-Golgi complex under conditions inhibiting the transport of *trans* proteins out from the ER. The results are shown in Fig. 8. 90% of the cells double stained for *trans*- (*A*) and *cis*/middle-Golgi (*B*) proteins showed the diffuse staining of fibers characteristic of the ER and strong staining of a reticular organelle forming a cap near the nucleus, respectively. Only five percent of the cells showed a few GMP_t-positive vesicles scattered throughout the cytoplasm and incorporation of GMP_c Golgi proteins into the retiform organelle. A study of the organelle stained with anti-*cis* antibodies using peroxidase produced the following results. In 70% of the cells the *trans* proteins were located in elements displaying a beaded or tubular shape, arranged in two or three rows of strings juxtaposed to the nucleus (Fig. 8, *E–G*). The structure of the organelle housing the *cis* proteins was comparable to that of the *cis*-Golgi complex in normal cells incubated for 40 min at 23°C (Fig. 8, *C* and *D*), with the difference that the elements constituting the later were significantly longer. The remaining 30% of the cells showed lower forms of organization, often consisting in independent vesicular elements clustered in the vicinity of the nucleus (Fig. 8 *H*). The staining patterns suggested that the *cis*/middle-Golgi complex was assembled under conditions preventing the reconstruction of the *trans*-Golgi complex.

When the 37°C and 23°C incubations were done in the presence of 20 μM nocodazol, it was observed that disruption of microtubules by nocodazol (Fig. 8 *I*) produced the scattering of vesicles loaded with *cis* proteins throughout the cytoplasm and prevented the assembly of the *cis*/middle-Golgi complex in the vicinity of the nucleus (Fig. 8 *J*). This result suggested that the network of cytoplasmic microtubules played an important role in the reorganization and relocalization of the *cis*/middle-Golgi complex (see Discussion).

Reconstruction of the trans-Golgi Complex Is Preceded by the Assembly of the cis/middle-Golgi Complex

The reassembly of the *cis*/middle-Golgi complex under conditions preventing the assembly of the *trans*-Golgi complex was compatible with two models of Golgi reorganization:

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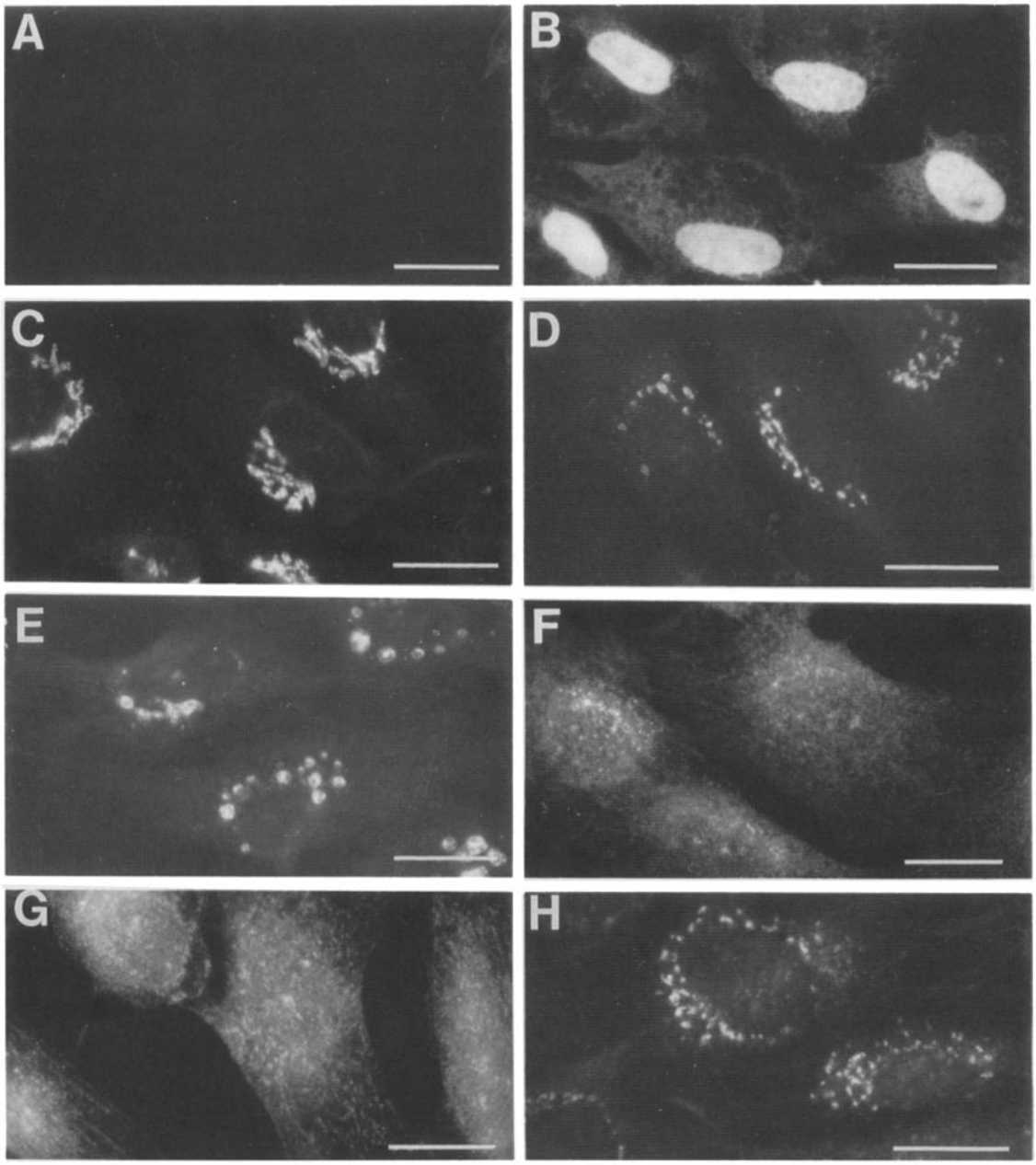

Figure 7. Inhibition of the disassembly and assembly of the Golgi complex by *N*-ethylmaleimide. Cells incubated 1 min with 0.2 mM NEM, and then for 25 (*A*), or 60 (*B*) min in drug-free medium, were tested for viability by propidium iodide staining. It can be seen that NEM-treated cells were fully viable within the first 25 min after treatment with the drug. Cells before (*C*) and after treatment for 20 min with 10 μg/ml BFA (*F*). Cells incubated for 3 min (*D*) or 5 min (*E*) with 10 μg/ml BFA, and then treated for 1 min with 0.2 mM NEM before incubation with 10 μg/ml BFA for 16 and 14 min, respectively. Cells incubated 60 min with 10 μg/ml BFA, 5 min in drug-free medium, 1 min with 0.2 mM NEM and again 14 min without drugs (*G*). Control cells (*H*) incubated 60 min with 10 μg/ml BFA and then for 20 min in drug-free medium. All the studies were performed using the monoclonal anti-GMP_{c-1} antibody. Comparable results were obtained with the other anti-Golgi antibodies. Bars, 15 μm.

first, a reorganization preceded by the independent assembly of the different compartments constituting the organelle; second, a reorganization based on the early assembly of the *cis*/middle compartments. To distinguish between these two possibilities the reconstruction of the *trans*-Golgi complex disrupted with nocodazol under conditions interfering with the reorganization of the *cis*/middle-Golgi complex previously fused with the ER was studied. Two different protocols were used. In both, the cells were incubated for 10 min with 1 μg/ml BFA to induce the fusion of the *cis*/middle-Golgi complex with the ER and to limit the effects of the drug on the *trans*-Golgi complex, and then for 40 min at 23°C with 20 μM nocodazol and 0.1 μg/ml BFA to disrupt the *trans*-Golgi located in the cytoplasm and to slow the release of *cis*/middle proteins from the ER (Fig. 9, *A* and *B*). In the first protocol, the reconstruction of the *trans*- and *cis*/middle-Golgi was studied after incubation of the cells without drugs for periods between 1 and 2 h at 23°C. In the second, the study was performed after incubating the cells at 37°C for 40 min in the presence of 0.1 μg/ml BFA. Both treatments

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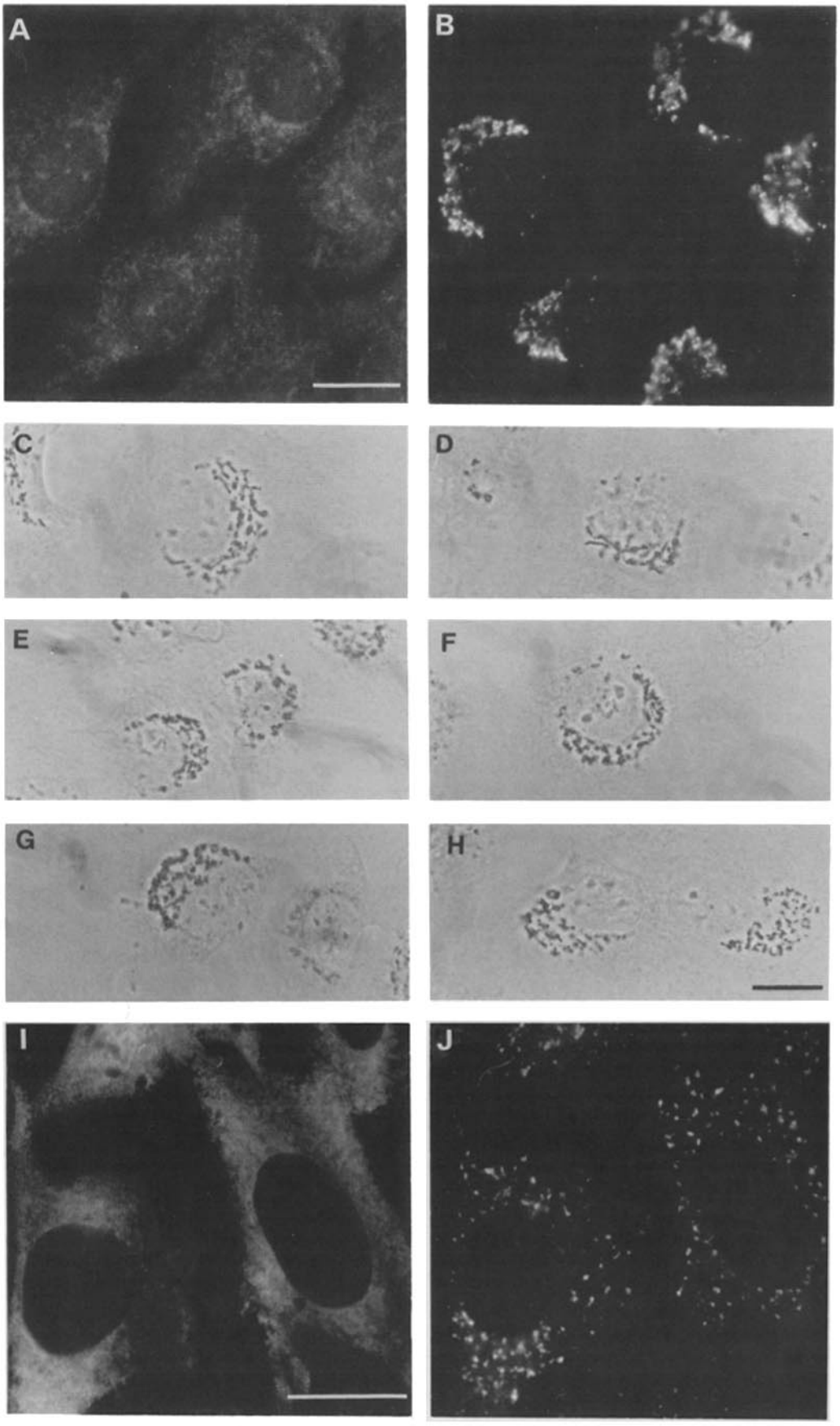

slowed drastically the transport of *cis*/middle proteins out from the ER, and permitted the reorganization of the cytoplasmic network of microtubules disrupted by nocodazol. A significant difference between them was that cells incubated at 37°C with 0.1 μg/ml BFA showed more extensive incorporation of *trans*-Golgi proteins into the ER. Study of the cells by double immunofluorescence microscopy, using anti-GMP_c and anti-GMP_t antibodies, revealed that 80–90% of them displayed no reorganization of the Golgi compartments. This population of cells showed large *trans*-Golgi–derived vesicles clustered in the vicinity of the centrosome (Fig. 9 *B*), many small vesicles loaded with *cis*/middle proteins translocated from the ER (Fig. 9 *A*), and significant amounts of *cis*/middle proteins in the ER, as shown by the diffuse staining of the cytoplasm (Fig. 9 *A*). The rest of the cells displayed Golgi complexes in different stages of reorganization, from the very poorly to the completely reconstructed. Further analysis revealed that the *trans*-Golgi was never reorganized in cells showing no reconstruction of the *cis*/middle-Golgi complex. Moreover, study of partly organized Golgi complexes showed that in the process of reorganization the assembly of the *cis*/middle-Golgi complex preceded the assembly of the *trans*-Golgi complex (Fig. 9, *C–J*) (see Discussion).

Discussion

In this study we have examined the disassembly and assembly of the *cis*/middle and *trans* parts (i.e., *trans*-most cisterna and *trans* network) of the Golgi complex in NRK cells treated with BFA. The approach to examine these problems has been to analyze in parallel the changes in localization undergone by *cis*/middle- and *trans*-Golgi membrane proteins in response to BFA. We have assumed that these changes are the result of changes in the organization and integrity of the Golgi compartments in which the proteins reside.

Disorganization of the Golgi Complex and Vesicle-mediated Retrograde Transport

The effects of BFA on the *cis*- and middle-Golgi are well established (Lippincott-Schwartz et al., 1989; Doms et al., 1989). In contrast, studies on the response of the *trans* parts (i.e., *trans* cisternae and *trans* network) of the Golgi to the drug have produced contradictory results (Lippincott-Schwartz et al., 1989, 1990; Doms et al., 1989; Nuchtern et al., 1989; Ulmer et al., 1989; Chege and Pfeffer, 1990). The later studies were performed by examining the effects of the drug on the morphology of the *trans* compartments and on the relocation of galactosyltransferase and sialyltransferases resident in them. From immunofluorescence microscopy studies of NRK cells treated with BFA and stained either with the monoclonal anti-GMP_{t-1} antibody or wheat germ lectin, it was concluded that the *trans*-Golgi was insensitive to the drug (Lippincott-Schwartz et al., 1989). This is in contrast to the results of our studies of NRK cells studied with the antibodies against GMP_{t-1} and GMP_{t-2}: we observed a dramatic and rapid effect of BFA on the organization of the *trans*-Golgi complex (3 min) and its complete disassembly after long incubations (75–90 min) with the drug. The reason for the discrepancy between the results of Lippincott-Schwartz et al. (1989) and ours is not clear. Our results are in agreement with the observations made by Ulmer and Palade (1989) who noted the complete disappearance of the cisternae stack in transformed erythroleukemia cells treated with BFA. Also a recent study performed with an antibody against the middle/*trans*-resident enzyme β-galactosyltransferase (Roth et al. 1986) in human M_1 cells has shown that BFA promotes the disorganization of the cisternae containing the enzyme (Lippincott-Schwartz et al., 1990). Transfer of sialyltransferase to the ER has been also observed in rat liver H4-II-E-C3 cells treated with BFA and studied by immunofluorescence microscopy (Berger, E. G., personal communication). Biochemical studies on the transport of enzymes from the *trans*-Golgi to the ER in cells treated with BFA have produced contradictory results. Analysis of the oligosaccharide chains of the α subunit of the T-cell receptor (Lippincott-Schwartz et al., 1989) and the G protein of vesicular stomatitis virus (Doms et al., 1989), extracted from cells treated with BFA did not reveal the presence of sialic acid. In the same line of results the N-linked carbohydrates of the CI-M6P retained into the ER of cells incubated with BFA were found to acquire galactose but only trace amounts of sialic acid (Chege and Pfeffer, 1990). In contrast, analysis of the carbohydrates of glycophorins and of alkaline phosphatase in cells treated with BFA showed high levels of sialic acid (Ulmer and Palade 1989; Takami et al., 1990). The finding that LIMPs II and III are sialylated while retained in the ER of cells treated with BFA is in agreement with the later results. The discrepant results obtained in the analysis of the translocation of sialyltransferase to the ER could be explained by disparities in the processing of different substrates under the abnormal and different conditions that may exist in the ER of the different cell lines studied. The effect of ER conditions on the activity of sialyltransferases is clearly shown by the differences in pI observed between the forms of LIMP III isolated from normal (pI 4.5) (Barriocanal et al., 1986) and BFA-treated (pI 7.9–8.8) NRK

Figure 8. Microtubule dependent reorganization of the *cis*/middle-Golgi complex in the absence of *trans*-Golgi complex reconstruction. Cells were incubated for 90 min at 37°C with 10 μg/ml BFA, then for 10 min at 37°C without drug, and finally for 40 min at 23°C also without drug. The reconstruction of the *cis*/middle- and *trans*-Golgi was studied by double immunofluorescence using the rabbit polyclonal anti-GMP_{t-1} (*A*, rhodamine channel) and mouse monoclonal anti-GMP_{c-1} (*B*, fluorescein channel) antibodies. Note the assembly of the *cis*/middle-Golgi complex near the nucleus in the absence of *trans*-Golgi reorganization. *Cis*-Golgi complex of cells incubated for 40 min at 23°C (*C* and *D*) and cells treated as described in *A* and *B* (*E–H*) stained with peroxidase-conjugated protein A. The dependence of the reorganization of the *cis*/middle-Golgi complex on microtubules was studied under the conditions described in *A* and *B*, but with the difference that the 10-min incubation at 37°C and the 40-min incubation at 23°C were performed in the presence of 20 μM nocodazol. The cells were stained simultaneously with the rat monoclonal antitubulin antibody YL 1/2 (*I*) and the mouse monoclonal anti-GMP_{c-1} (*J*). Note how the disruption of microtubules with nocodazol prevents the reorganization and relocalization of the *cis*/middle-Golgi complex near the nucleus. Bars, 15 μm.

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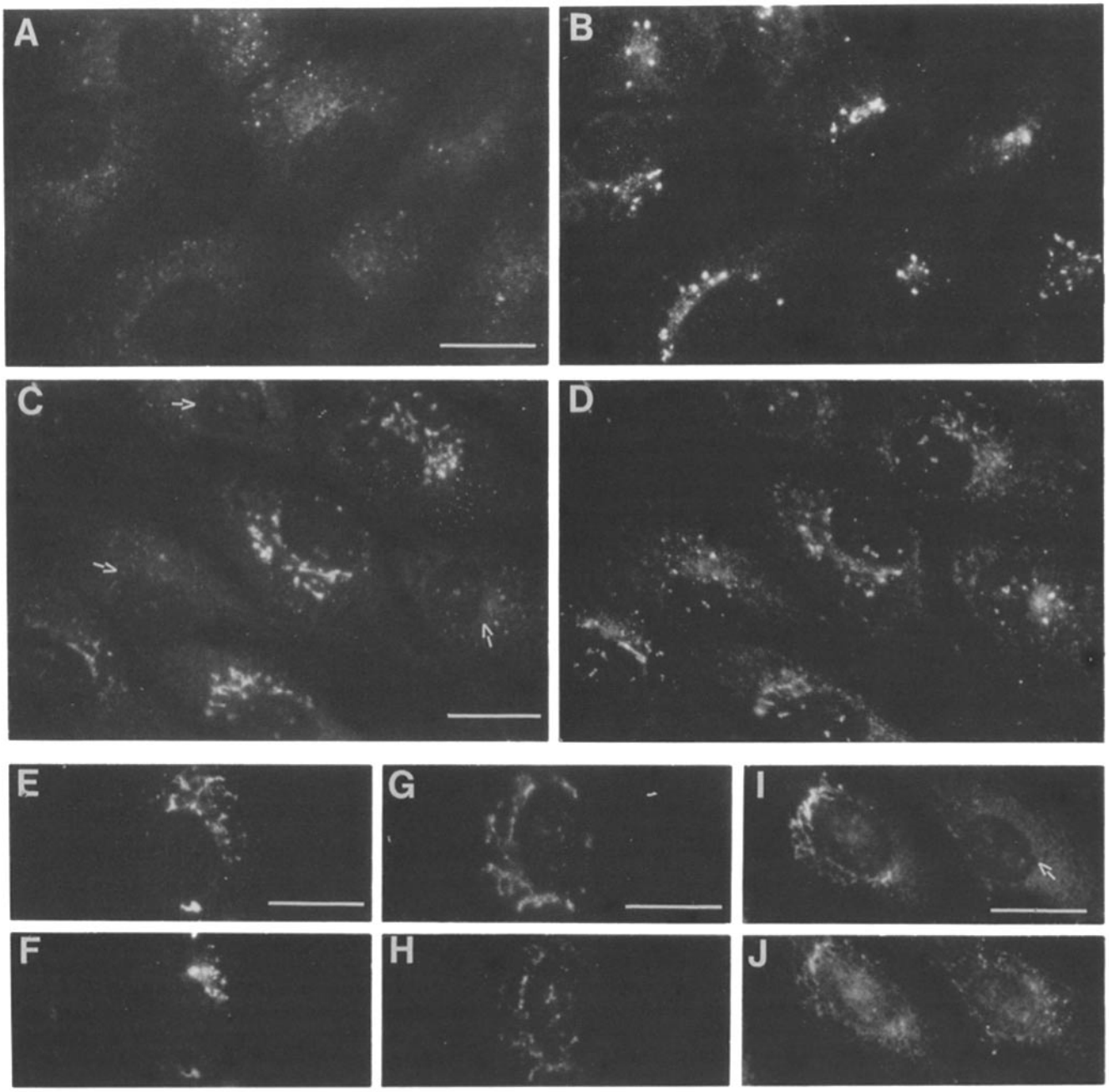

Figure 9. Assembly of the *trans*-Golgi complex follows the reconstruction of the *cis*/middle-Golgi complex. Cells were incubated at 37°C for 10 min with 1 μg/ml BFA, and then at 23°C for 40 min with 20 μM nocodazol and 0.1 μg/ml BFA (*A* and *B*) before incubation for 90 min at 23°C in drug-free medium (*C–J*). The cells were stained with the mouse monoclonal anti-GMP_{c-1} antibody (*A*, *C*, *E*, *G*, and *I*) and rabbit polyclonal anti-GMP_{t-1} antibody (*B*, *D*, *F*, *H*, and *J*) and studied by double-immunofluorescence microscopy. It can be seen that before the removal of nocodazol and BFA, GMP_{c-1} was located to small vesicles scattered throughout the cytoplasm and to the ER (diffuse fluorescence), whereas GMP_{t-1} was found in comparatively large vesicles retained in the vicinity of the nucleus. Removal of both drugs resulted in slow reconstruction of the Golgi complex, as shown by the presence of partially reorganized Golgi complexes in 10–20% of the cells (*C–J*). It is important that inhibition of the reconstruction of the *cis*/middle-Golgi complex (cells marked with *white arrows* in *C* and *I*) resulted in failure of the cells to organize the *trans* part. The examination of incompletely reconstructed Golgi complexes reveals that in the process of reconstruction the assembly of the *cis*/middle-Golgi complex always precedes the assembly of the *trans*-Golgi complex (*E–J*). Bars, 15 μm.

cells (see Fig. 2). In conclusion, so far most of the results from morphological and biochemical studies indicate that BFA promotes the fusion of the *trans*-most cisternae and *trans* network with the ER, and therefore the drug can not act selectively on the *cis*/middle parts of the Golgi complex (Lippincott-Schwartz et al., 1989, 1991; Doms et al., 1989; Chege and Pfeffer, 1990).

The need for a system of retrograde transport that could recover the membranes of the vesicles involved in the transport of molecules from the ER to the Golgi complex, and

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help to maintain constant the difference between the surface areas of both organelles, was postulated several years ago (Warren, 1985). More recently, the observation made by Pelham (1988) that soluble ER proteins escaped from the ER to the Golgi complex are recovered from the *cis*-Golgi, suggests that the system of retrograde transport could be involved in the recovery of proteins. Lippincott-Schwartz et al. (1989) have recently suggested that both the shuttling of transporting vesicles between the *cis*-Golgi and the ER, and the prevalence of the retrograde transport in cells treated with BFA, might explain the fusion of the *cis*-Golgi with the ER in these cells. However, the involvement of vesicles in the anterograde transport of proteins and lipids through the entire Golgi complex (Rothman et al., 1984*a*,*b*; Balch et al., 1984*a*,*b*; Dunphy and Rothman, 1985; Orci et al., 1986, 1989; Pfeffer and Rothman, 1987) extends the recovering problem to the middle and *trans* parts of the Golgi complex. The existence of a vesicle-mediated, or as recently proposed tubular-mediated (Orci et al., 1991) transport of molecules from the *trans*- and through the middle- to the *cis*-Golgi could solve the problem. The existence of such transport could explain the presence of sialic acid in the carbohydrates of GMP_{c-1} (Yuan et al., 1987) and MG 160 (Gonatas et al., 1989). Moreover, it could also explain the observation that in cells treated with BFA the *cis*/middle-Golgi is disorganized and fuses with the ER faster than the *trans*-Golgi. In the presence of BFA the fusion of the *trans*-Golgi with the ER could be carried out by vesicles or tubules specialized in transporting molecules from the *trans*- to the middle-Golgi. This fusion could require the expression on the surface of the ER of medial Golgi proteins specialized in the recognition of such vesicles or tubules. According to this model, the fusion of the Golgi complex with the ER would proceed in a *cis-trans* direction.

Characterizing the Pathway Followed by the Golgi Membrane Proteins in the Reconstruction of the Golgi Complex

The study by double immunofluorescence microscopy of the redistribution of *cis*/middle and *trans* proteins occurring after removal of BFA, had the limitation that the organelles involved in the putative transport of Golgi proteins as well as the Golgi precursors could not be characterized. Such characterization should await double label–gold immuno-EM experiments using antibodies against GMPs and markers of the organelles suspected of being involved in those processes. However, the study has disclosed some interesting features of the process of Golgi reconstruction (Fig. 3). The reassembly of the Golgi complex appears to proceed in at least two steps. The first consists in the accumulation of the Golgi proteins into distinct vesicular structures scattered throughout the cytoplasm. During the second step the vesicles are replaced by tubular structures, the long cisternae are assembled, and the Golgi complex is organized. With respect to the first step it is clear that the *cis*/middle proteins contained in the ER are more rapidly mobilized than the *trans* proteins after removal of BFA. Furthermore, the ordered back transport of GMPs from the vesicles to the ER suggests that the former are entities distinct from the ER. The earliest vesicles could be transporting vesicles (i.e., transitional) (Palade, 1975; Beckers et al., 1987, 1989) or belong to the intermediate compartment that mediates the transport of vesicles between the ER and Golgi complex (Saraste and Kuismanen, 1984; Schweizer et al., 1988, 1990). Whether *cis*/middle and *trans* proteins are transported in the same or in separate closely juxtaposed vesicles, organized in clusters as observed in mitosis (Lucocq and Warren, 1987), or kept in register, as seen in cells treated with taxol (Wehland et al., 1983*a*), will have to be determined by EM.

With respect to the assembly of the Golgi cisternae, we have noted that it occurs together with a parallel decrease in the number of vesicles containing the GMPs. Whether the latter fuse directly with each other to produce the cisternae we do not know. The compartments forming the Golgi complex (i.e., cisternae, *trans* network) are probably highly dynamic structures (Griffiths et al., 1989; Kreis et al., 1990) formed and stabilized by the simultaneous operation of segregating mechanisms (involved in separating resident from itinerant molecules and components of neighboring Golgi compartments as well as functionally linked compartments [i.e., ER, intermediate compartment, PLC]), and salvage devices specialized in recovering the molecules missorted during the processes of segregation (Warren, 1985; Pelham, 1988). With respect to this it is possible that the vesicle-mediated systems of anterograde and retrograde transport that function throughout the ER and Golgi pathway might play an important role in the ordered and gradual (see below) formation of Golgi compartments.

An interesting aspect of the reconstruction of the Golgi complex is that the assembly and reorganization of the cisternae always occurs in a discrete area located in the vicinity of the nucleus. The more relevant characteristics of the assembly area are the almost complete exclusion of the ER, as judged by the absence of PDI and the presence of the centrosome. This area probably corresponds to the "zone of exclusion" described by Morré (reviewed in Morré and Ovtracht, 1978) and the one stained with antibodies against the KDEL binding protein (Vaux et al., 1990). The observation that the assembly area remains intact after the complete disassembly of the Golgi complex with the ER raises the possibility that it is permanently occupied by a structural scaffold or by organelles functionally related to the Golgi complex (i.e., prelysosomal compartment). The presence of the centrosome in the vicinity of the assembly area is consistent with evidence that microtubules play an important role in maintaining the location and organization of the Golgi complex (Kupfer et al., 1982, 1983; Wehland and Sandoval, 1983; Sandoval et al., 1984; Turner and Tartakoff, 1989), and of the *cis*/middle-Golgi complex when assembled under conditions inhibiting the reorganization of the *trans* elements.

Mechanisms that May Operate in Assembling the Golgi Compartments in a Functional Order

The Golgi complex disrupted at the onset of mitosis is reassembled during cytokinesis. How the organelle is organized with the different compartments arranged in a functional order (Dunphy and Rothman, 1985; Kornfeld and Kornfeld, 1985; Griffiths and Simons, 1986) is an important question that we are still far from understanding. We do not know if formation and arrangement of the compartments occur simultaneously. In either case the compartments could be formed from the same or separate (Lucocq and Warren, 1987; Lucocq et al., 1987) precursors. The arrangement of the compartments in a functional order might require that the

precursor(s), or one of the mature compartments, acts as a primer and template, and that the casting role is assumed in an ordered fashion by the rest of the precursors, or compartments, until the organelle is completed. The mechanism operating in this process could be based on the mutual and specific recognition between molecules expressed on the surface of contiguous compartments. Our results indicate that the *cis* and middle compartments can be assembled under conditions in which the reconstruction of the *trans*-Golgi complex is inhibited. They also suggest that the reorganization of the *trans*-Golgi complex requires the previous reconstruction of the *cis*/middle-Golgi complex. We would like to speculate that the *cis*, or less likely the middle compartment, could play the role of primer and first template in the process of organization. Implicit in this model is that the assembly of the Golgi complex would proceed in a *cis* to *trans* direction.

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