Spatial and Temporal Colocalization of the Golgi Apparatus and Microtubules Rich in Detyrosinated Tubulin

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Abstract. The integrity and intracellular distribution of the Golgi apparatus appear to depend upon microtubules. We have found that the microtubules rich in detyrosinated tubulin are located preferentially in the vicinity of the Golgi. Cells were double-stained with antibodies specific for either tyrosinated or detyrosinated tubulin and an antibody to prolactin or wheat germ agglutinin (Golgi markers). Microtubules rich in detyrosinated tubulin showed a close codistribution with the Golgi in three different cultured cell lines GH3, BS-C-1, and AtT20. Disruption of microtubules with nocodazole in GH3 cells resulted in fragmentation and dispersal of the Golgi apparatus as reported previously. During recovery of the microtubules and the Golgi complex after removal of the nocodazole, there was a spatial and temporal colocalization of the Golgi apparatus and microtubules rich in detyrosinated tubulin. Our results suggest that a functional relationship may exist between the structure and organization of the Golgi complex and the detyrosination of α-tubulin in microtubules.

THE importance of the microtubule cytoskeleton in the organization, position, and trafficking of intracellular organelles is well established (Dustin, 1984; Roberts and Hyams, 1979). One example of the role microtubules play in the proper organization of intracellular organelles is in determining the integrity and spatial distribution of the Golgi apparatus. In most animal cells, the Golgi complex is closely associated with the microtubule-organizing centers (MTOCs),[1] usually compact structures located near the nuclei (Thyberg and Moskalewski, 1985). The Golgi complex associates closely with MTOCs, even when the MTOCs are dispersed in the cytoplasm, such as in skeletal myotubes, in which the MTOCs have a circumnuclear organization (Tassin et al., 1985). Thus, it has been suggested that the MTOCs interact with the Golgi complex in a manner that determines the intracellular distribution of the Golgi apparatus (Kronebush and Singer, 1987).

Although interactions between the Golgi apparatus and MTOC may play such a role, other studies suggest that the microtubules rather than the MTOCs are responsible for organizing and determining the position of the Golgi apparatus. For example, the Golgi apparatus assumes a microtule-dependent and MTOC-independent distribution in cells exposed to conditions at which microtubules are organized independently of the MTOC (e.g., in the presence of taxol; Rogalski and Singer, 1984; Sandoval et al., 1984). Furthermore, as cells enter mitosis they reorganize their interphase microtubule networks into mitotic spindles, and the Golgi complex becomes fragmented and distributed randomly throughout the cytoplasm (Burke et al., 1982; Hiller and Weber, 1982; Lucocq and Warren, 1987; Lucocq et al., 1989). A similar fragmentation and dispersal of the Golgi apparatus takes place when interphase cells are exposed to microtubule-disrupting drugs, such as nocodazole (Rogalski and Singer, 1984). After completion of mitosis and cell division or removal of the drug, the Golgi complex recovers its structural integrity and normal location as the interphase microtubule network returns. These observations suggest that the integrity and position of the Golgi complex are dependent on the integrity and organization of microtubules.

Several steps involved in the dispersal of the Golgi apparatus have been described recently and include microtubule depolymerization, Golgi fragmentation, and fragment dispersal (Turner and Tartakoff, 1989). Similarly, a number of steps have been described in recovery of the Golgi apparatus, including microtubule repolymerization, centralization of Golgi fragments, fragment coalescence, and Golgi positioning (Turner and Tartakoff, 1989). Although dispersal and reclustering of Golgi fragments depend on microtubules, these processes are not determined simply by binding of microtubules to the Golgi complex, since dispersal and recovery of the Golgi apparatus take place in an energy- and temperature-dependent manner (Turner and Tartakoff, 1989). In addition, molecular motors have been implicated in the reclustering of Golgi complex elements (Ho et al., 1989); it

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1. *Abbreviation used in this paper*: MTOC, microtubule-organizing center.

was shown that microtubules serve as tracks along which Golgi apparatus fragments move in a retrograde fashion toward the nucleus (minus end-directed).

Recent evidence has suggested that a specific subpopulation of microtubules plays a role in the structural organization of the Golgi apparatus (Thyberg and Moskalewski, 1989). The Golgi apparatus was found to be associated with microtubules which were less sensitive to nocodazole than the majority of microtubules in the cytoplasm. We reasoned that the drug-stable, Golgi apparatus–associated microtubules could be rich in detyrosinated α-tubulin. Two well-characterized microtubule populations result from the cyclic detyrosination/tyrosination posttranslational modification of the carboxy terminus of α-tubulin (Barra et al., 1974; Raybin and Flavin, 1977; Argarana et al., 1978). Electron microscopic immunolocalization studies have revealed that individual tyrosinated or detyrosinated microtubules are in fact copolymers of the two posttranslationally modified tubulins (Geuens et al., 1986), being rich either in tyrosinated or in detyrosinated α-tubulin (referred to henceforth as detyrosinated or tyrosinated microtubules). Immunofluorescence microscopy with antibodies that specifically recognize each form of tubulin has shown that tyrosinated and detyrosinated microtubules are distributed differently in cells (Gundersen et al., 1984). In addition, microtubules composed of the two different posttranslationally modified tubulins exhibit different stabilities (Kreis, 1987; Webster et al., 1987*a*,*b*); detyrosinated microtubules exchange their tubulin subunits more slowly and are less sensitive to depolymerizing agents such as nocodazole than tyrosinated microtubules. The functional roles of the differential distribution and stability of these two microtubule populations are not understood.

In this report we investigated whether tyrosinated or detyrosinated microtubules associate selectively with the Golgi complex. We used double-label immunofluorescence microscopy with antibodies recognizing total tubulin, tyrosinated α-tubulin, or detyrosinated α-tubulin, and compared the tubulin staining to the location of the Golgi complex in three different cell lines (GH3, AtT20, and BS-C-1 cells). The Golgi complex was localized by staining with an antibody to prolactin or with fluorescently labeled wheat germ agglutinin. Our results indicate that detyrosinated microtubules codistribute with the Golgi apparatus. In addition, during recovery from nocodazole-induced microtubule depolymerization, we observed a spatial and temporal correlation between the reappearance of detyrosinated microtubules and the recovery of a completely reclustered and asymmetrically positioned Golgi apparatus in GH3 cells. The results are consistent with the hypothesis that there is a functional interdependence between detyrosinated microtubules and the structural integrity and position of the Golgi apparatus.

Materials and Methods

Cell Culture

GH3 cells, originally derived from prolactin-secreting rat pituitary tumors, (Tashjian et al., 1968; Tashjian, 1979) were grown in monolayers at 37°C in an atmosphere of 10% CO_2, in a 1:1 mixture of DME and Ham's F-10 medium (Sigma Chemical Co., St. Louis, MO), supplemented with 15% horse serum and penicillin/streptomycin. AtT20/D-16v cells, derived from a mouse pituitary tumor, (Furth et al., 1953) and BS-C-1 cells, derived from monkey kidney, (Hopps et al., 1963) were grown in DME supplemented with 10% defined bovine calf serum (Hyclone Labs, Inc., Logan, UT) and penicillin/streptomycin at 37°C in 10% CO_2. Where indicated 4×10^{-4} M nocodazole (Janssen Pharmaceutica, Piscataway, NJ), in 100% DMSO was added to complete culture media at a final drug concentration of 4×10^{-6} M (1% final concentration of DMSO). After 4 h of incubation at 37°C, cells either were immediately fixed (see below) or were rinsed three times with drug-free culture medium, and incubated with complete medium for an additional 1 or 4 h.

Immunofluorescence

Cells were plated onto poly-L-lysine-coated coverslips and grown for 2 d under normal culture conditions. Cells were then incubated with or without nocodazole as indicated in the text and figure legends. At the end of the incubation cells were rinsed once with PBS (2.9 mM KH_2PO_4, 14.1 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl, pH 7.4) at 37°C and fixed at room temperature with 10% formalin in PBS for 10 min followed immediately by treatment with 100% methanol/2 mM EGTA (10 min at −20°C). Cells were rinsed twice with PBS and nonspecific binding sites were blocked by sequential 10-min incubations with 1% BSA in PBS and normal goat serum, diluted 1:4 with PBS. Primary antibodies were diluted (see below) into PBS containing 0.1% BSA and were incubated with fixed and blocked cells for 1 h at 37°C in a humidified chamber. After two 5-min rinses with PBS/0.1% BSA cells were incubated with the appropriate secondary antibody (see below) and for Figs. 2–4, simultaneously with rhodamine-labeled wheat germ agglutinin (E-Y Laboratories, Inc., San Mateo, CA), diluted 1:500 in PBS/0.1% BSA, for 30 min at 37°C. Cells were washed twice with PBS/0.1% BSA for 5 min and rinsed once with PBS. Coverslips were drained, mounted on slides with 95% glycerol containing 1 mg/ml *p*-phenylenediamine to retard photobleaching (Sigma Chemical Co., St. Louis, MO) and sealed with nail polish. Indirect immunofluorescence microscopy was carried out with a Zeiss Photomicroscope III using epi-fluorescence optics. Images were photographed using T-MAX (ASA 400) or Technical Pan 2415 film (Kodak, Rochester, NY).

Antibodies

Total tubulin was analyzed with a β-tubulin-specific mAb (a generous gift from M. Klymkowsky, University of Colorado, Boulder, CO; Chu and Klymkowsky, 1987) diluted 1:200. A rhodamine-conjugated goat anti-mouse secondary antibody (1:200) (Organon Teknika-Cappel, Malvern, PA) was used to visualize the β-tubulin/anti-β-tubulin complex by epi-fluorescence microscopy. Tyrosinated or detyrosinated α-tubulin was analyzed with specific polyclonal rabbit antibodies (generous gifts from C. Bulinski and G. Gundersen, Columbia University; Gundersen et al., 1984). Prolactin antigen was analyzed with a rabbit antiserum (1:200) provided as a gift to T. L. Burgess from the National Hormone and Pituitary Program of the National Institute of Diabetes and Diseases of the Kidney. The rabbit antibody/antigen complexes were detected with a fluorescein-conjugated goat anti–rabbit secondary antibody (1:200) (Organon Teknika Cappel).

Quantitation of Data

The location of the Golgi apparatus was determined with antiprolactin antibodies or with rhodamine-labeled wheat germ agglutinin. Cells stained with wheat germ agglutinin were also stained with anti-detyrosinated α-tubulin antibodies. Every cell in a field was categorized as showing either juxtanuclear, circumnuclear, or dispersed staining. Mitotic cells and very small round cells were not included (~21% of all cells examined) as their staining patterns could not be categorized accurately. At least 87 cells with discernible staining were scored for each condition/time point.

Results

Distributions of Microtubules Rich in Tyrosinated and Detyrosinated Tubulin in Relation to the Location of the Golgi Apparatus

The distributions of detyrosinated and tyrosinated microtubules in GH3 cells (derived from rat pituitary) were determined by double-label immunofluorescence microscopy. We

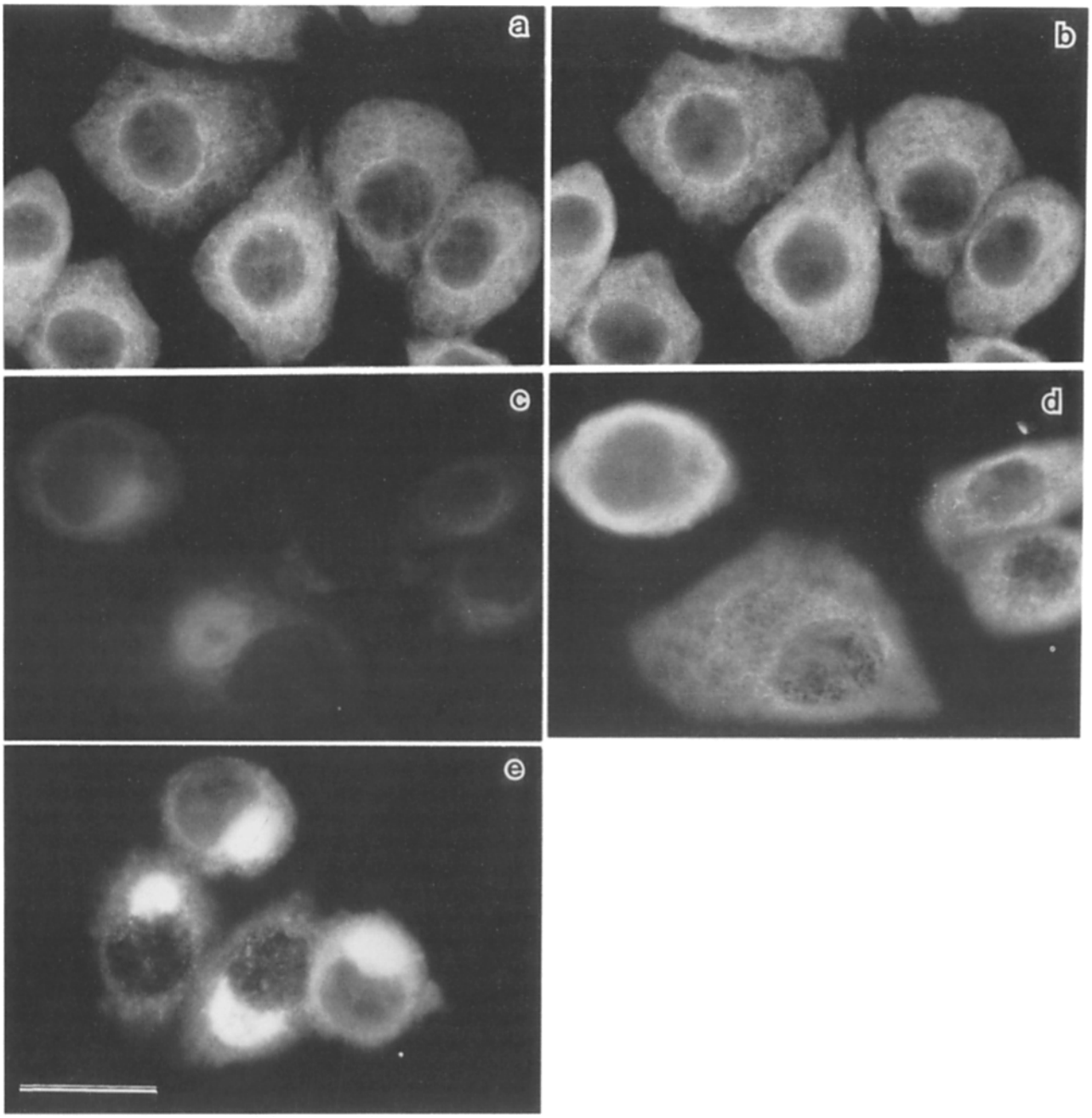
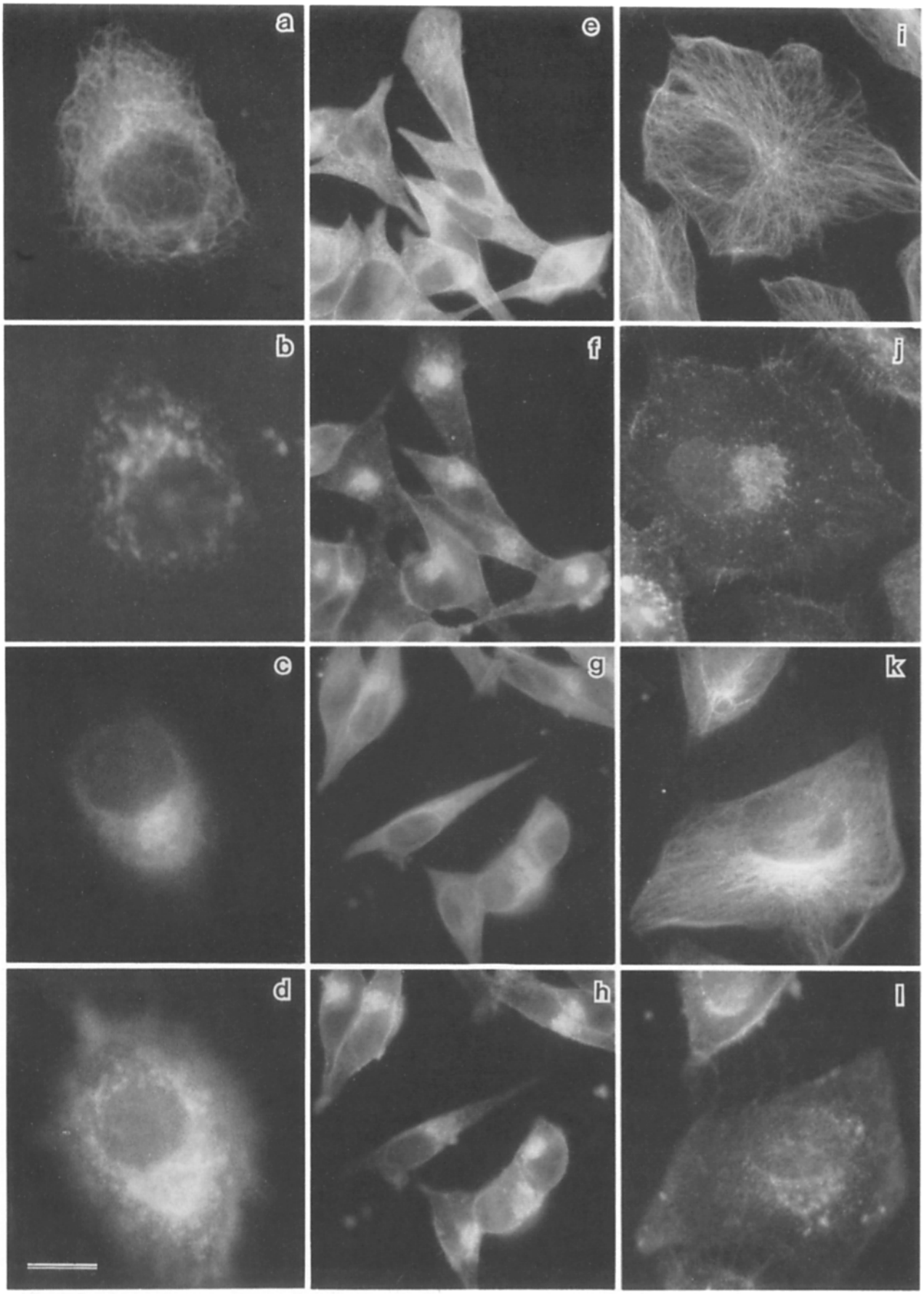


Figure 1. Localization of tubulin and prolactin in GH3 cells by indirect immunofluorescence. Cells double-stained for (*a*) tyrosinated tubulin and (*b*) total tubulin. Cells double stained for (*c*) detyrosinated tubulin and (*d*) total tubulin. Cells stained for prolactin (*e*). Bar, 20 μm.

used antibodies that specifically recognized either the tyrosinated or detyrosinated forms of α-tubulin, and compared the distributions of tyrosinated and detyrosinated microtubules with the distribution of the entire microtubule network using an antibody to β-tubulin. The distribution of tyrosinated microtubules was indistinguishable from the distribution of the entire microtubule network (Fig. 1, *a* and *b*). However, the detyrosinated microtubules comprised only a subpopulation of the entire microtubule network as reported by other investigators (Gundersen et al., 1984). Since GH3 cells were relatively small and round, the microtubule staining was less distinct than in large and flat cells. In GH3 cells, the detyrosinated microtubules were restricted to a region near the nucleus and appeared to emanate from the MTOC (Fig. 1, *c* and *d*). By focusing through the cells, it was apparent that the detyrosinated microtubules had a sinuous appearance as described previously by Gundersen et al. (1984). They also appeared to be organized into a hollow basket-like structure on one side of the nucleus (Fig. 1 *c*) close to where the Golgi apparatus is normally situated. GH3 cells were stained with an antibody to prolactin (the major secreted protein made by GH3 cells) to visualize the Golgi apparatus. The Golgi apparatus was localized in asymmetric fashion near the nucleus (Fig. 1 *e*), and appeared to have a distribu-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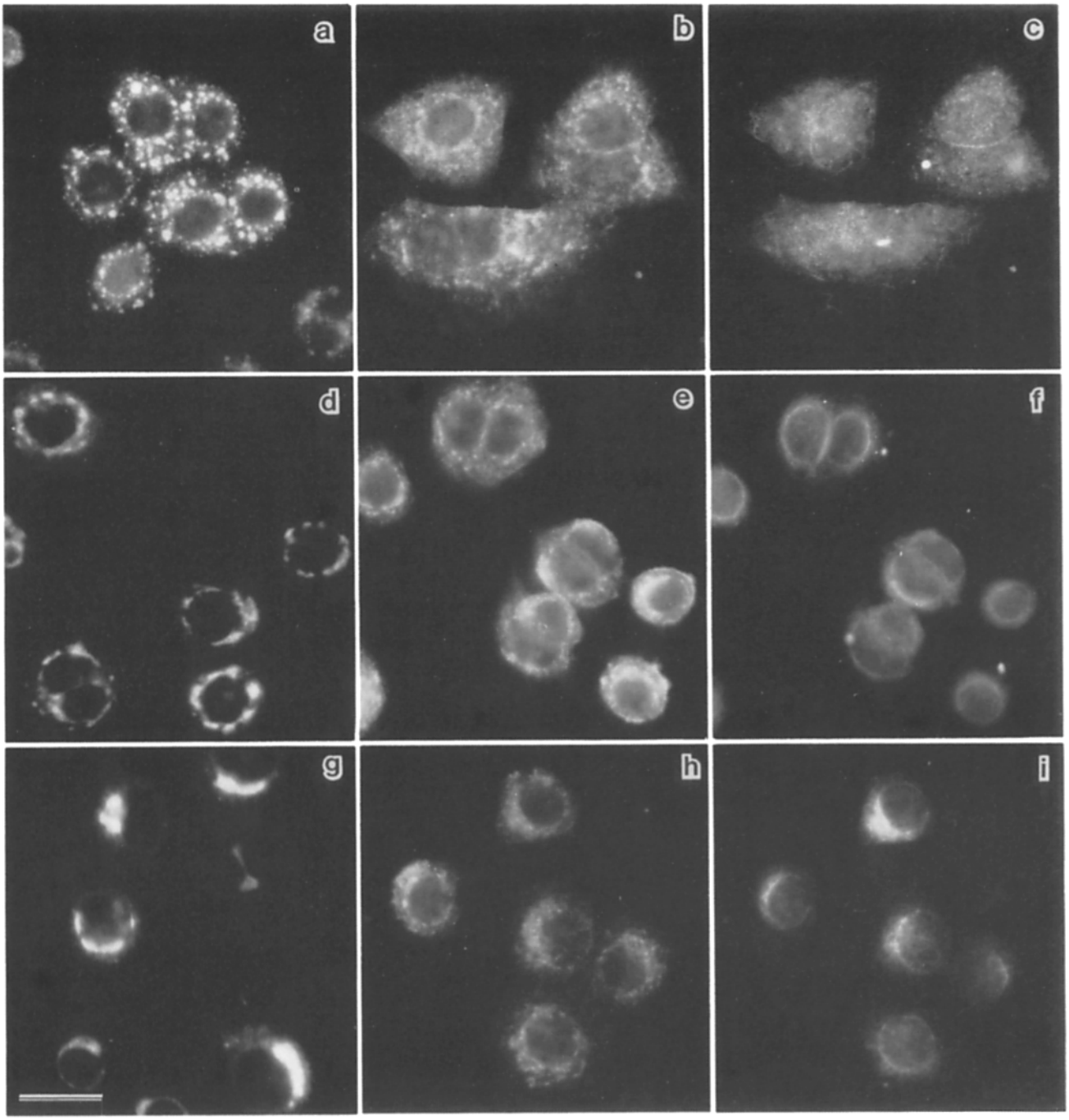

Figure 3. Localization of detyrosinated tubulin and the Golgi complex in GH3 cells treated with nocodazole. All cells were treated with 4 μM nocodazole for 4 h. No washout in drug-free media (*a–c*). 1 h after washout in drug-free media (*d–f*). 4 h after washout in drug-free media (*g–i*). Prolactin staining by indirect immunofluorescence (*a*, *d*, and *g*). Cells double-stained for Golgi complex with rhodamine-conjugated wheat germ agglutinin (*b*, *e*, and *h*) and for detyrosinated tubulin by indirect immunofluorescence (*c*, *f*, and *i*). Bar, 20 μm.

tion similar to the detyrosinated microtubules (compare Fig. 1, *c* and *e*).

To investigate further the possibility that the detyrosinated microtubules colocalized with the Golgi apparatus in GH3 cells, the cells were double-stained with an antibody either against tyrosinated or detyrosinated tubulin, and with wheat germ agglutinin; a lectin that binds to N-acetyl glucosamine and N-acetyl neuraminic acid residues found predominantly in the *trans*-Golgi apparatus (Tartakoff and Vassalli, 1983). In agreement with the staining patterns in Fig. 1, the anti-

Figure 2. Localization of tubulin and the Golgi complex in GH3 (*a–d*), AtT20 (*e–h*), and BS-C-1 (*i–l*) cells by indirect immunofluorescence and rhodamine-conjugated wheat germ agglutinin staining. Cells double-stained for tyrosinated tubulin (*a*, *e*, and *i*); and Golgi apparatus (*b*, *f*, and *j*). Cells double-stained for detyrosinated tubulin (*c*, *g*, and *k*) and Golgi apparatus (*d*, *h*, and *l*). Bar: (*a–d*) 8 μm; (*e–l*) 20 μm.

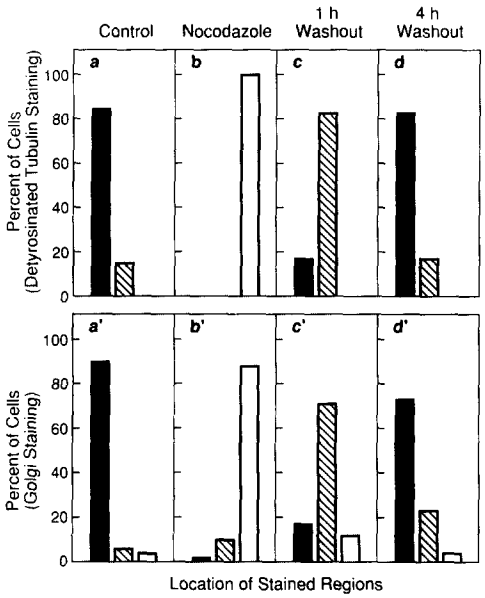

Figure 4. Quantitation of the location of the Golgi and detyrosinated tubulin staining in GH3 cells treated with nocodazole. Data are presented as percent of total cells counted ($n > 86$ cells). Location of detyrosinated tubulin staining (*a–d*). Location of Golgi staining as determined by anti-prolactin immunofluorescence or rhodamine-conjugated wheat germ agglutinin staining (*a'–d'*). (*Solid bars*) Asymmetric, juxtanuclear staining; (*hatched bars*) circumnuclear staining; (*open bars*) dispersed (Golgi complex) or diffuse (detyrosinated tubulin) staining.

body against the tyrosinated tubulin stained the entire microtubule network (Fig. 2 *a*), whereas the antibody against the detyrosinated tubulin stained only an asymmetric juxtanuclear subset of microtubules (Fig. 2 *c*). Golgi apparatus staining with wheat germ agglutinin produced a punctate, predominantly juxtanuclear staining pattern (Fig. 2, *b* and *d*) that appeared to colocalize with staining observed with the antibody to detyrosinated tubulin (compare Fig. 2, *c* and *d*).

The same experimental protocol was followed with two additional cell lines: AtT20, derived from mouse pituitary, and BS-C-1, derived from monkey kidney, to determine whether or not the Golgi apparatus and detyrosinated microtubules colocalized in cells other than GH3. Similar to the results obtained with GH3 cells, the antibody to tyrosinated tubulin stained the entire microtubule network (Fig. 2, *e* and *i*) whereas the antibody against detyrosinated tubulin stained a subpopulation of microtubules that was concentrated asymmetrically near the nucleus (Fig. 2, *g* and *k*). In contrast to the results obtained with GH3 cells, the detyrosinated microtubules in AtT20 and BS-C-1 cells were not restricted to the juxtanuclear location, but extended beyond the vicinity of the Golgi apparatus. The highest density of detyrosinated microtubules, however, also colocalized with the Golgi apparatus in both cell lines (compare *g* and *h*, and *k* and *l* in Fig. 2). Thus, the Golgi apparatus and detyrosinated microtubules codistributed in all three cell lines examined.

Reorganization of the Golgi Apparatus and the Reappearance of Detyrosinated Microtubules after Microtubule Disruption by Nocodazole

The observation that detyrosinated microtubules appear to be associated with the Golgi apparatus suggested that a possible functional interaction may exist between the two organelles. To test this possibility we examined the time course for reassembly of the Golgi apparatus in relation to the reappearance of detyrosinated microtubules after disrupting the entire microtubule network with nocodazole (Figs. 3 and 4). The rabbit antibody to prolactin is a more specific stain for Golgi apparatus in GH3 cells than is rhodamine-conjugated wheat germ agglutinin, which also stains secretory vesicles and the cell surface. However, because the antibody to detyrosinated tubulin was also from rabbits, it could not be easily used with the rabbit prolactin antibody for double-label immunofluorescence. Thus we used rhodamine-conjugated wheat germ agglutinin and the antibody to detyrosinated tubulin on the same cells, but also show typical cells stained with anti-prolactin antibody for comparison of the Golgi apparatus staining patterns.

GH3 cells were incubated with 4 μM nocodazole for 4 h and were allowed to recover their normal microtubule network and Golgi apparatus organization for 4 h in the absence of drug. Cells were then stained to localize both the Golgi apparatus and detyrosinated microtubules. Typical examples are shown in Fig. 3, and the data from two separate experiments are quantified in Fig. 4. After 4 h of nocodazole treatment, cells stained either with an antibody to prolactin (Fig. 3 *a*) or with wheat germ agglutinin (Fig. 3 *b*) displayed a dispersed and punctate pattern of staining, typical of fragmented Golgi apparatus (Fig. 4 *b'*). All microtubules, including the detyrosinated microtubules, were completely depolymerized by the nocodazole (Fig. 3 *c* and 4 *b*). The normal microtubule network reappeared within 30 min to 1 h after removing the nocodazole (data not shown). However, the reappearance of the detyrosinated microtubules was delayed, and occurred simultaneously with reorganization of the Golgi apparatus. 1 h after removal of the nocodazole, fragments of the Golgi apparatus had partially coalesced around the nucleus (Figs. 3, *d* and *e* and 4 *c'*). This distribution paralleled the circumnuclear distribution of detyrosinated microtubules at the same time (compare Fig. 3, *e* and *f*; and Fig. 4, *c* and *c'*). 4 h after the removal of nocodazole, the Golgi apparatus had completely reclustered and assumed its normal asymmetric, juxtanuclear position (Figs. 3, *g* and *h* and 4 *d'*). Again, the juxtanuclear distribution of the detyrosinated microtubules coincided with the distribution of the Golgi apparatus in the fully recovered cells (compare Fig. 3, *h* and *i*; and Fig. 4, *d* and *d'*).

Discussion

The cyclic detyrosination and retyrosination of tubulin was discovered >15 yr ago (Barra et al., 1974; Argarana et al., 1978), and despite considerable effort in many laboratories, the functional significance of this unique posttranslational modification remains obscure. In the present study we found that microtubules rich in detyrosinated tubulin colocalized with the Golgi apparatus in three different cell lines, GH3, AtT20, and BS-C-1 (Figs. 1 and 2). Furthermore, using a nocodazole washout protocol we observed a spatial and temporal colocalization of the recovering Golgi and the detyrosinated microtubules (Figs. 3 and 4). Previous studies have shown that the location and integrity of the Golgi apparatus depends upon microtubules (reviewed by Thyberg and Maskolewski, 1985). Recently Thyberg and Maskolewski (1989) demonstrated that the microtubules associated with

the Golgi apparatus represent a subset of the total cytoplasmic microtubule population that is relatively resistant to microtubule-depolymerizing drugs. One of the most significant clues to date regarding the potential function of the cyclic tyrosination and detyrosination of α-tubulin was the observation that microtubules rich in detyrosinated tubulin comprise a subset of the total microtubule population in cultured cells that is relatively drug resistant and thus, relatively more stable than the majority of the microtubules (Khawaja et al., 1988; Kreis, 1987). The results of the present study combined with the recent work of Thyberg and Maskolewski (1989), suggest that the stable microtubule subpopulation associated with the Golgi apparatus is rich in detyrosinated tubulin and further that a functional relationship may exist between the Golgi apparatus and the cyclic tyrosination and detyrosination of α-tubulin. Because detyrosinated microtubules extend beyond the Golgi apparatus in AtT20 and BS-C-1 cells, and since it is clear that detyrosinated microtubules are widely distributed in the cytoplasm of a number of other kinds of cells (Gundersen and Bulinski, 1986; Wehland and Weber, 1987), it seems reasonable to assume that the functions of detyrosinated microtubules are not restricted to the Golgi apparatus. It is perhaps fortuitous that GH3 cells lack extensive extra-Golgi apparatus detyrosinated microtubules, thus making the observation of the correlation between the Golgi apparatus and detyrosinated microtubules possible.

Interestingly, in vitro studies on the dynamics of microtubules rich in either detyrosinated tubulin or tyrosinated tubulin have not revealed any detectable differences (Kumar and Flavin, 1982; Paturle et al., 1989; Skoufias, D., B. Matsumoto, K. Farrell, and L. Wilson, unpublished data). Therefore, the differences in the stabilities of tyrosinated and detyrosinated microtubules in vivo appear to be due to intracellular factors that selectively interact with and stabilize detyrosinated microtubules (Khawaja et al., 1988). Such a stabilizing factor may be provided through association of detyrosinated microtubules with the Golgi apparatus.

We observed that the subset of detyrosinated microtubules in GH3 cells formed a basket-like structure that was coincident with the juxtanuclear location of the Golgi apparatus. A similar microtubule structure was described surrounding the Golgi in L929 mouse fibroblasts by Thyberg and Moskalewski (1989), but it was not determined if this structure was enriched in detyrosinated α-tubulin. We did not observe any basket-like microtubule structures associated with the Golgi apparatus in AtT20 or BS-C-1 cell lines, however, the majority of the detyrosinated microtubules did colocalize with the Golgi in these cells. The lack of basket-like structures may be a consequence of the flattened nature of these two cell lines relative to GH3 cells which are more spherical.

Previous studies have shown that individual Golgi apparatus elements travel along microtubules toward the center of cells during recovery from treatment with nocodazole (Ho et al., 1989). This movement appears to take place in the absence of detyrosinated microtubules because it occurs before the microtubules become detyrosinated (Kreis, 1987). Our results are in agreement; we found that during the movement of Golgi apparatus fragments from the cell periphery to the circumnuclear location in GH3 cells (15–30 min after removal of nocodazole) no detyrosinated microtubules could be detected. Thus, the first step in reformation of the Golgi apparatus (i.e., fragment centralization) occurs independently of detyrosinated microtubules. However, our results indicate that once the Golgi fragments have centralized to a circumnuclear location, there is a striking spatial and temporal colocalization of the detyrosinated microtubules and the coalescence and localization of Golgi fragments. The detyrosinated microtubules reappeared in a circumnuclear location 1 h after removal of nocodazole, concomitant with coalescence of the Golgi fragments around the nucleus. Recovery continued so that 4 h after removal of the nocodazole both the Golgi apparatus and detyrosinated microtubules had attained coincident, compact, juxtanuclear locations. Although it is difficult to make a strong argument that colocalization by immunofluorescence microscopy is synonymous with an actual physical interaction between the Golgi apparatus and detyrosinated microtubules, that these two structures codistribute during recovery from nocodazole favors this interpretation.

Microtubules rich in detyrosinated tubulin may play an active role in coalescence of the Golgi fragments and/or in the positioning of the Golgi apparatus near the MTOC and the nucleus. These roles could be related to the slower dynamics of detyrosinated microtubules relative to the dynamics of tyrosinated microtubules. The greater stability of detyrosinated microtubules may enable the Golgi elements to be maintained in a centralized position. Alternatively, the interaction of the Golgi membranes with the microtubules may stabilize the microtubules and allow their posttranslational detyrosination. Thus, the detyrosination of tubulin may be a consequence of selective stabilization by the Golgi apparatus. Our present data do not distinguish between these possibilities.

Although it is clear that the integrity, structure and intracellular position of the Golgi apparatus are dependent on microtubules, little is known about the nature of the interaction between the microtubules and the Golgi apparatus or the identity of the factors involved in the interaction. If such factors exist they should bind both to microtubules and to the Golgi membranes. A 110-kD peripheral membrane protein associated with the Golgi apparatus has been proposed to play a role in the interactions of the Golgi apparatus with microtubules (Allan and Kreis, 1986). The protein shares antigenic epitopes with MAP-2 and can bind to polymerized tubulin in vitro. Recently, another peripheral Golgi membrane protein of 58 kD has been identified, which binds tubulin stoichiometrically in microtubules in vitro (Bloom and Brashear, 1989). The 58-kD protein is immunologically and biochemically distinct from tau and tubulin. Conceivably, one or both of these peripheral membrane proteins of the Golgi apparatus may have a higher affinity for detyrosinated microtubules than tyrosinated microtubules. Alternatively, these proteins may stabilize microtubules which in turn may result in detyrosination of the tubulin. These possibilities can be tested because of the 58- and 110-kD proteins have been purified and antibodies to them have been raised (Allan and Kreis, 1986; Bloom and Brashear, 1989).

The location of the Golgi apparatus in the cytoplasm has been implicated as having a role in directing cell movement. For example at the edge of an experimentally induced wound in a monolayer of fibroblasts, there is coordinate reorientation of the Golgi apparatus and the MTOCs toward the leading edge of the cells (Kupfer et al., 1982). In addition, recent studies on the organization of the microtubule cytoskeleton

during cell migration induced by experimental wounding have revealed that there is selective stabilization of detyrosinated microtubules in the direction of migration (Gundersen and Bulinski, 1988). These observations support the hypothesis that selective stabilization of microtubules may generate cellular asymmetry such as in the repositioning of the MTOC and Golgi apparatus during migration (Kirschner and Mitchison, 1986). The spatial and temporal correlation we observed between the reappearance of detyrosinated microtubules and reassembly of the asymmetrically positioned Golgi after nocodazole disruption also supports this hypothesis.

Our conclusion that there may be a functional relationship between detyrosinated microtubules and the Golgi apparatus contradicts previous studies suggesting that tyrosinated microtubules play a role in the structural integrity and intracellular positioning of the Golgi apparatus (Sandoval et al., 1984; Wehland et al., 1983; Wehland and Willingham, 1983). In those studies microinjection into cells of high concentrations of antibodies against tyrosinated α-tubulin resulted in disruption of the Golgi apparatus in a manner similar to that which occurred when cells were exposed to nocodazole. However, it is not known whether detyrosination can take place in microtubules that are decorated with antibodies. Thus, the observed dispersal of the Golgi apparatus after microinjection of antibodies against tyrosinated tubulin could be due to inhibition of detyrosination along the microtubules. Furthermore, microtubules containing detyrosinated α-tubulin are actually copolymers containing both tyrosinated and detyrosinated α-tubulin (Geuens et al., 1986). Thus, the high antibody concentration could have disrupted the detyrosinated microtubules by binding to the tyrosinated tubulin present in the copolymers.

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Note Added in Proof. Jasmin et al. (Jasmin, B. J., J.-P. Changeux, and J. Cartaud. 1990. *Nature [Lond.].* 344:673–675) recently reported that cold-stable and acetylated microtubules are localized in the areas underlying motor endplates of chick skeletal muscle fibers together with the Golgi apparatus. These investigators hypothesize that such stable microtubules together with the Golgi apparatus may be involved in the transport of vesicles and organelles at the endplate level.

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