Journal of Cell Science 110, 2495-2505 (1997)
Printed in Great Britain © The Company of Biologists Limited 1997
JCS8152

Dispersal of Golgi apparatus in nocodazole-treated fibroblasts is a kinesin-driven process

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SUMMARY

The morphology and location of the Golgi apparatus (GA) has been shown to change upon microtubule (Mt) depolymerization. The GA in different cell types undergoes fragmentation and dispersal throughout the cytoplasm upon treatment with nocodazole. In this study experiments were performed on human skin fibroblasts (HSFs) and rat fibroblasts (REF 52) to determine whether the dispersal of GA in HSFs treated with nocodazole is dependent on Mts that show the higher resistance to this Mt-depolymerizing drug. It is shown here that nocodazole at concentrations as low as 100 nM caused the GA to disperse in treated fibroblasts that still contained a fairly high amount of Mts. Antibody-blocking analysis of Mts after injection of biotin-tubulin into the HSFs was used to show that nocodazole at low concentrations induced the stabilization of the remaining Mts. The complete disruption of Mts by the incubation of HSFs at 0°C prevented the dispersal of GA from the perinuclear area when the cells were subsequently warmed to 37°C in the presence of nocodazole. Microinjection of the well-characterized HD antibody against kinesin but not the preimmune IgG caused inhibition of GA dispersal in HSFs by nocodazole. These data demonstrate that the dispersal of GA in the cytoplasm of nocodazole-treated HSFs is a kinesin-driven process with stable Mts serving as tracks.

Key words: Golgi apparatus, Kinesin, Stable microtubule, Nocodazole

INTRODUCTION

The Golgi apparatus (GA) plays the principal role in the transport of secretory and membrane proteins between various cytoplasmic membrane compartments (Farquhar, 1985; Dunphy and Rothman, 1985). Newly synthesized membrane and secretory proteins undergo modification and sorting en route from endoplasmic reticulum to their final destination when passing through the GA.

A large body of evidence indicates that the structure of the GA as well as its multiple functions are strongly dependent on the microtubule (Mt) network (for reviews see Thyberg and Moskalewski, 1985; Kreis, 1990; Cole and Lippincott-Schwartz, 1995). The involvement of Mts in vesicle transport to and from the GA is well documented (Rindler et al., 1987; Rogalski et al., 1984; Matteoni and Kreis, 1987; Herman and Albertini, 1984; Lippincott-Schwartz et al., 1990, 1995; Cooper et al., 1990; Saraste and Svensson, 1991; Mizuno and Singer, 1994).

The perinuclear location and the compactness of this organelle is lost as a consequence of Mt depolymerization during mitosis (Lucocq and Warren, 1987; Lucocq et al., 1987). The drugs that disrupt or alter Mt structure (such as nocodazole or taxol) also cause fragmentation and dispersion of GA throughout the cytoplasm (Rogalski and Singer, 1984). However, the dispersed clusters maintain the properties of premitotic organelles and continue to carry out at least some of their functions (Boyd et al., 1982; Rogalski et al., 1984; Salas et al., 1986). This model of the reversible dispersion of GA resulting from the Mt disruption has been utilized in numerous studies to better understand the interaction of these two structures.

Turner and Tartakoff (1989) have reported that GA dispersion caused by Mt depolymerization as well as its reclustering after drug removal can be blocked by metabolic inhibitors indicating the energy dependence of both processes and making the participation of translocator proteins very likely. Ho and coworkers (Ho et al., 1989) presented clear evidence that the reclustering of Golgi elements near the centrosomes upon removal of the nocodazole occurs along recovered Mts. In good agreement with these results are data obtained using the antibody against minus-end directed Mt motor cytoplasmic dynein (Vaisberg et al., 1996). The enrichment of cytoplasmic dynein on the GA membranes was revealed by indirect immunofluorescence. In addition, microinjection of this antibody into normal rat kidney (NRK) cells led to the GA dispersal.

The plus-end directed motor kinesin was shown to be associated with the GA in rat hepatocytes (Marks et al., 1994) and NRK cells (Lippincott-Schwartz et al., 1995). This translocator protein is suggested to be responsible for the extension of the tubulovesicular trans-Golgi network (Feiguin et al., 1994; Lippincott-Schwartz et al., 1995), the recycling of membrane back to ER (Lippincott-Schwartz et al., 1995) and the transport of secretory vesicles to plasma membrane. Thus, the central localization of GA is most likely a result of interaction of this

organelle with oppositely directed Mt-dependent translocator proteins.

Though the dispersal of GA to the periphery of the cells treated with nocodazole was shown to be an energy-dependent process, the involvement of the Mts as well as other cytoskeletal components has not been demonstrated (Turner and Tartakoff, 1989). The authors suggested that only the fragmentation of GA is the energy-dependent stage but the dispersal of fragments occurs by the diffusion. However, this could not explain the progressive inhibition of the scattering of Golgi elements below 34°C (Turner and Tartakoff, 1989). The recent study of Cole and coworkers (Cole et al., 1996) has shown that Mt disruption only slightly inhibits retrograde traffic from Golgi to ER, while anterograde transport of pre-Golgi intermediates into the centrosomal region is blocked. However, these data on Golgi dispersal in cells treated with nocodazole contradict earlier findings that inhibition of microtubule motor kinesin impedes the Golgi-to-ER transport (Lippincott-Schwartz et al., 1995).

These discrepancies may be reconciled if the possible involvement of the stable Mts is taken into the consideration. In contrast to the preponderant dynamically unstable Mts in the most eukaryotic cells, this minor subpopulation of Mts does not undergo rapid depolymerization in the presence of disrupting agents, but instead can persist for hours (Kreis, 1987; Webster et al., 1990). The stable Mts could serve as tracks for the active Golgi-to-ER transport in the course of the Golgi dispersal. This hypothesis is supported by the data of Mizuno and Singer (1994) demonstrating the involvement of stable Mts in the ER-to-GA transport. They have shown with immunoelectron microscopy the specific association of the transition vesicles with the stable, but not the dynamic subclass of Mts.

This study addresses the question of the role that Mt-dependent motor kinesin plays in the dispersal of GA caused by nocodazole. The results show that GA dispersal occurs when dynamic Mts are disrupted and only stable Mts are present in cytoplasm. Microinjection of the antibody inhibiting kinesin translocator activity in vitro (antibody HD) (Rodionov et al., 1991) into fibroblasts before nocodazole treatment provided a method for determining the role of this motor protein in the process of GA dispersion caused by Mts depolymerization. HD antibody was produced against the motor portion of recombinant heavy chain of *Drosophila* kinesin which was characterized earlier (Rodionov et al., 1991). The microinjection of this antibody has already been shown to interfere with centrifugal transport of pigment granules in fish melanophores (Rodionov et al., 1991) and mitochondria distribution in fibroblasts (Rodionov et al., 1993b). It is demonstrated here that the injected HD antibody but not the preimmune IgG inhibits dispersal of GA when cells are treated with nocodazole. The results presented in this paper suggest that the nocodazole-induced dispersal of the GA depends on stable Mts and the activity of the plus-end directed motor kinesin.

MATERIALS AND METHODS

Materials

N-Hydroxysuccinimidyl biotin was obtained from Polysciences, Inc. (Warrington, PA, USA); [7-nitro-2,1,3-benzoxadiazol-4-yl]-aminocaproyl sphingosine (C_6-NBD-ceramide) was from Molecular Probes, Inc., (Eugene, OR, USA). Unless otherwise indicated all materials were purchased from the Sigma Chemical Company, St Louis, MO, USA.

Cell culture

Normal human skin diploid fibroblasts (HSFs), line 1036, were kindly provided by Dr V. Kuharenko (Institute of Medical Genetics, Russian Academy of Medical Sciences, Moscow). Cells between 15 and 25 passages were cultured in Dulbecco's modified Eagle's medium (Sigma) supplemented with 5% fetal bovine serum and 5% postnatal human serum. Rat embryo fibroblasts (REF 52) were obtained from Dr P. Chumakov (Institute of Molecular Biology, Moscow) and were cultured in the same medium supplemented with 10% fetal bovine serum. The antibiotics penicillin (100u/ml) and streptomycin (100 μg/ml) were also added to the media, and cells were incubated at 37°C in a water-saturated atmosphere of 5% CO_2.

For experiments, cells were plated onto sterile glass coverslips in 35 mm diameter culture dishes at a density 6×10^4 per cm^2 and were incubated for 16-20 hours. To aid location of injected cells, coverslips with photoetched locator grids (Bellco Biotechnology, Vineland, NJ) were used.

Before incubation of cells at 0°C they were shifted into the same culture medium containing 14 mM Hepes. Nocodazole (Calbiochem-Behring, La Jolla, CA, USA) dissolved in DMSO was added to the culture medium to a final concentration of either 5.0 μM or 100 nM for the times indicated below.

Preparation of biotinylated tubulin

Phosphocellulose-purified tubulin was prepared from bovine brains by a modification of the procedure of Weingarten et al. (1975) as described by Mitchison and Kirschner (1984). Biotinylation of tubulin was performed as described by Mitchinson and Kirschner (1985) and the final pellet was resuspended in microinjection buffer (50 mM potassium glutamate, 1 mM $MgCl_2$, pH 6.8), centrifuged, frozen in aliquots in liquid nitrogen, and stored at −75°C until use as in Schulze and Kirschner (1986).

Microinjection

Microinjections were performed as described previously (Rodionov et al., 1991) using the ICM inverted microscope (Zeiss) with a ×32 objective lens equipped with micromanipulator (Zeiss) and 5242 Eppendorf microinjector. Cells were injected using glass micropipettes made from 1.2 mm o.d. capillaries containing internal filaments to aid loading needles (World Precision Instruments). High concentrations of antibody HD and preimmune IgG (approx. 20 mg/ml) and biotin-tubulin (16.5 mg/ml) were used to perform gentle injections which did not exceed 5% of cell volume. The temperature was kept at 37°C throughout microinjection experiments. Cells were transferred into an atmosphere of 5% CO_2 and incubated until fixation or GA labeling were performed.

Fluorescence microscopy

For immunostaining of Mts in HSFs, cells were washed with PBS, permeabilized with 1% Triton X-100 in 50 mM imidazole-HCl buffer pH 6.8, supplemented with 50 mM KCl, 0.5 mM $MgCl_2$, 0.1 mM EDTA, 1 mM EGTA, 1 mM β-mercaptoethanol and 4% polyethylene glycol 40,000 for 3 minutes, fixed with 1% glutaraldehyde and, after reduction with $NaBH_4$, incubated with the monoclonal antibody DM1A against α-tubulin (Sigma) and TRITC-conjugated goat anti-mouse antibody (Sigma). The Mts containing Glu-tubulin in REF 52 were immunostained after fixation in cold methanol (−20°C) for 6 minutes using rabbit polyclonal antibody kindly provided by Dr J. C. Bulinski.

Stable Mts in cells injected with biotin-tubulin were revealed using the antibody-blocking technique as described by Schulze and Kirschner (1987) except that unlabeled sheep anti-rabbit, rabbit anti-

sheep and fluorescein goat anti-rabbit antibodies were used to block and to visualize the dynamic biotin-containing Mts. Rabbit anti-biotin antibody was from Sigma.

GA was fluorescently labeled either in methanol-fixed REFs by FITC-labeled lentil lectin (Sigma) or in living HSFs using C_6-NBD-ceramide (Lipski and Pagano, 1983). For staining the living cells, dye dissolved in the ethanol was added to the culture medium to the final concentration of 0.5 μg/ml for 10 minutes. The cells were then washed with dye-free medium and incubated for 30 minutes prior to microscopy in chambers filled with growth medium. Immunostained and C_6-NBD-ceramide-stained cells were analyzed and photographed with a Photomicroscope III (Zeiss) equipped with a condenser IIIRS.

Quantitative analysis of GA dispersion

The degree of GA dispersion in nocodazole-treated cells was estimated as the ratio of the cell area occupied by Golgi elements to the area of the whole cell. Briefly, cells were stained with C_6-NBD-ceramide and photographed with a Photomicroscope III (Zeiss) using epifluorescence and phase contrast optics. The outlines of cells and the areas containing fluorescently labeled particles were then drawn with the aid of a photo enlarger and entered into a PC AT computer by tracing on a digitizing tablet (Summasketch II, Summagraphics, UK). TRACER V1.0 software (Copyright Dr A. Brown) was used both for entering, storage of outlines and for calculation of the areas. Each outline consisted of approximately 100-200 coordinate pairs.

RESULTS

Relationship of GA dispersal to Mts depolymerization in fibroblasts treated with nocodazole

The depolymerization of Mts in cells by nocodazole at concentrations ranging from 1 μM to 10 μM leads to the dispersal of Golgi elements throughout the cytoplasm (Rogalski and Singer, 1984; Ho et al., 1989). However, it was not clear if the partial disruption of the Mt system was sufficient for GA dispersal or if the complete depolymerization of Mts was necessary. To clarify this question the kinetics of Mt depolymerization and the GA dispersal in HSFs and REF 52 cells treated with 5 μM nocodazole was compared.

In good agreement with the earlier studies (Kreis, 1987; Cambray-Deakin et al., 1988; Khawaja et al., 1988) the obtained data show that the entire process of Mt depolymerization includes roughly two stages: the rapid disruption of the major part of Mts which are more sensitive to the drug, and the considerably slower step – the depolymerization of stable subpopulation of Mts less sensitive to nocodazole. Fig. 1 shows the immunofluorescent staining of Mts in HSFs that were treated with 5 μM nocodazole for various times. After 30 minutes of incubation with nocodazole, most of the Mts were depolymerized (Fig. 1B). The remaining Mts had a sinuous shape typical of the stable subclass. The depolymerization of the remaining Mts took another 2.5 hours (Fig. 1B-F). The kinetics of depolymerization of stable Mt stained with antibody against Glu-tubulin in REF 52 is shown in Fig. 3. It is seen that the complete disruption of Mts in this type of cells takes approximately 2 hours.

The staining of GA with fluorescent dye C_6-NBD-ceramide (Lipsky and Pagano, 1983, 1985) in living HSFs (Fig. 2) and with FITC-conjugated lentil lectin in fixed REF 52, at the same time points after addition of nocodazole, shows that GA dispersal in the cytoplasm takes place long before the complete Mt depolymerization. It can be seen also (Figs 2B, 3C) that the integrity of GA is lost at the initial stages of Mt depolymerization though the Golgi membranes still form a compact group at the cell center. Thus, the loss of structural integrity of the GA coincides with the disruption of the dynamic Mts that are more sensitive to the nocodazole. The dispersal of the GA can be seen after 1 hour of nocodazole treatment of HSFs (Fig. 2C) and 30 minutes of REF 52 (Fig. 3E) and it is fully dispersed after 2 hours (Figs 2E, 3I). The double staining of the stable Mts containing the detyrosinated tubulin and the GA in REF 52 cells treated with 5 μM nocodazole also shows that the patterns of stable Mts coincide with the areas of dispersed GA.

So the dispersal of the GA to the periphery of the cells treated with nocodazole occurs when stable Mts are still present in the cytoplasm.

Stabilization of Mts and GA dispersal in fibroblasts by 100 nM nocodazole

To study the possible involvement of the stable Mts in GA dispersal, the cells were treated with 100 nM nocodazole. Such treatment was shown to partially disrupt the Mt system and to stabilize the remaining polymers (Vasquez et al., 1995). Fig. 4 shows the dispersed GA in the HSFs incubated for 5 hours (A)

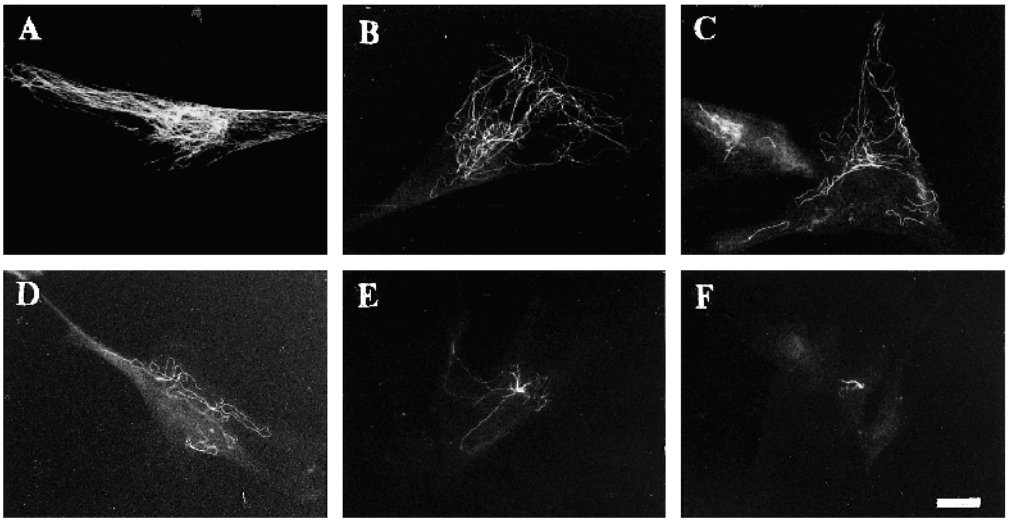

Fig. 1. Time course of Mt depolymerization by nocodazole. HSFs were extracted with Triton X-100 and fixed (see Materials and Methods) before (A) or 30 minutes (B), 1 hour (C), 1.5 hours (D), 2 hours (E) and 3 hours (F) after addition of 5 μM nocodazole, and Mts were visualized by indirect immunofluorescence. Bar, 10 μm.

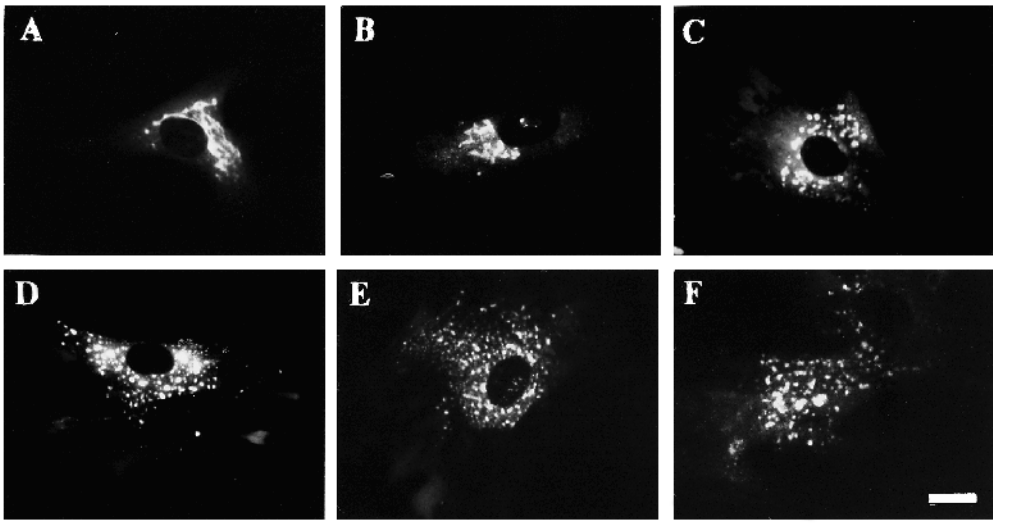

Fig. 2. Time course of GA dispersal in HSFs by nocodazole. The GA was visualized by fluorescence microscopy of living cells stained with NBD-C_6-ceramide (see Materials and Methods) at the same time points after addition of 5 μM nocodazole as in Fig. 1. Bar, 10 μm.

and REF 52 incubated for 2 hours (C) with 100 nM of nocodazole and immunofluorescent staining of the Mts in the same cells after extraction with Triton X-100 and fixation (B) or by double labeling (D). The effect of such treatment on GA is similar to that of 5 μM of the drug causing the complete depolymerization of Mts in HSFs (Fig. 2F and Fig. 3I). Thus, the integrity of the GA is not maintained in cells treated with low-dose nocodazole though they contain a relatively high amount of Mts.

The Mts in these cells look essentially disordered and twisted showing the characteristic morphology of stable Mts. In contrast to the partial disruption of Mts by 5 μM nocodazole within 30 minutes that reveals stable subpopulation of Mts (Fig. 1B), incubation with 100 nM of the drug caused a greater number of Mts to remain in the cytoplasm. An incubation time as long as 2 days did not cause additional loss of polymer (not shown). Moreover, these Mts showed the increased resistance to the higher (5 μM) concentration of nocodazole typical of the stable Mts (not shown).

To test the subunit/polymer exchange in these Mts the approach suggested by Schulze and Kirschner (1987) was used. This method is based on the fact that tagged tubulin microinjected into the cell incorporates rapidly in the dynamic Mts, though the stable ones remain unlabeled. Fig. 5 shows the results of experiments with biotin-labeled tubulin which was microinjected into the control and nocodazole-treated cells. Staining with anti-biotin antibodies revealed many Mts that were formed in the control cells during 25 minutes after the injection (Fig. 5C). In contrast, there was no incorporation of biotin-tubulin into the Mts in cells treated with 100 nM nocodazole (Fig. 5A). The stable Mts in the injected cells could be revealed with anti-tubulin antibodies after blocking of biotin-labeled Mts by several layers of secondary antibodies (Fig. 5B,D). These Mts had the same curly shape as in nocodazole-treated non-injected cells (Fig. 5B, arrow, and Fig. 4A). Taken together these data indicate that low-dose nocodazole treatment causes the stabilization of the remaining Mts.

GA dispersal arrest by low-temperature disruption of Mts

To examine the necessity of stable Mts for nocodazole-induced dispersal of GA the approach suggested by Turner and Tartakoff (1989) was used with special attention to the completeness of Mt depolymerization by cold. After the incubation of the cells at low temperature, 5 μM nocodazole was added to the culture medium to avoid Mt repolymerization when the cells were warmed to 37°C. The preincubation of the HSFs on ice (at 0°C) for 3 hours was enough to depolymerize all Mts (Fig. 6C). It was shown earlier that the disruption of Mts by cold does not lead to the GA dispersal until the cells are warmed to 37°C (Moskalewski et al., 1980). It can be seen in Fig. 6A,B that although the GA lost its integrity in cold-treated HSFs after 2.0 hours of incubation at 37°C in the presence of the nocodazole, it stayed in the perinuclear region.

When Mts were depolymerized by incubation of the HSFs at 0°C for 1 hour, some polymers persisted (Fig. 6F). The surviving Mts had the characteristic morphology of stable polymers (compare with Fig. 1B,C and D) and in fact they showed the reduced sensitivity to nocodazole (not shown). The further incubation of so treated HSFs at 37°C led to the dispersal of GA in the cytoplasm (Fig. 6D,E). The quantitative data in Table 1 demonstrates that the presence of remaining Mts after incomplete depolymerization caused the significant dispersal of GA even after 1 hour incubation at 37°C. In the contrast, the complete disruption of Mts in the HSFs prior to the temperature elevation arrested the GA elements in a perinuclear region. The same results were obtained for REF 52

Table 1. Effect of the Mt disruption by cold on the GA dispersal in the presence of nocodazole

Duration of treatment of HSFs at 0°C (hours)	1 hour incubation at 37°C	2 hours incubation at 37°C
1	18.1±3.7	26.8±5.1
3	6.9±1.2	8.0±1.7

The cells were incubated in an ice bath for the times indicated (last 30 minutes with added 5 μM nocodazole) and then warmed to 37°C. GA was labeled in cells with NBD-C_6-ceramide (see Materials and Methods) during the incubation time and visualized in living cells. Values are expressed as the ratios of the areas containing GA elements to the areas of the whole cell and represent means ± s.e.m. of determinations made for 20-30 cells of each type.

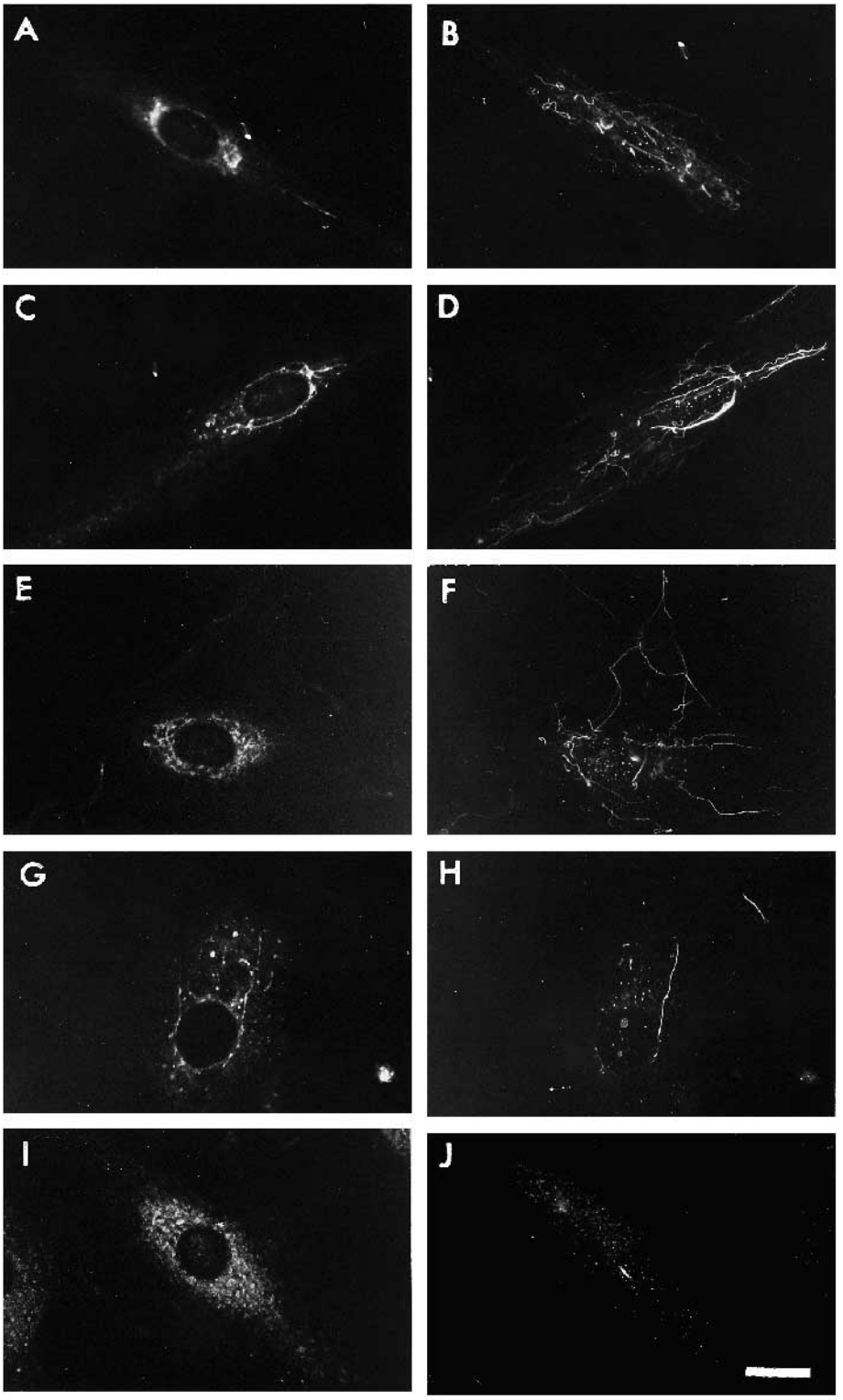

Fig. 3. Time course of GA dispersal and Glu-Mt depolymerization in REF 52 cells by nocodazole. REF 52 cells were fixed with cold methanol (see Materials and Methods) before (A,B) or 15 minutes (C,D), 30 minutes (E,F), 1 hour (G,H), 2 hours (I,J) after addition of 5 μM nocodazole. GA was stained with FITC-conjugated lentil lectin and Mts were visualized by indirect immunofluorescence using anti-Glu-tubulin antibody and TRITC-labeled goat anti-rabbit IgG antibody. Bar, 10 μm.

though the Mts in that cell type were more cold-stable and the 4-hour treatment was necessary for the complete Mt disruption (not shown).

To control the viability of the cells after 3 hours at 0°C the reduced concentration of the nocodazole (100 nM) was added to the culture medium before warming to 37°C. Fig. 6I shows that the Mts recovered in such conditions and had the morphology of stable polymeres (compare with Fig. 4A). The Mt recovery was also accompanied by GA dispersal (Fig. 5G,H). Thus, the presence of stable Mts is an obligatory condition for the GA dispersal.

Antibody HD inhibits the GA dispersal

The results presented above show that the dispersal of the GA

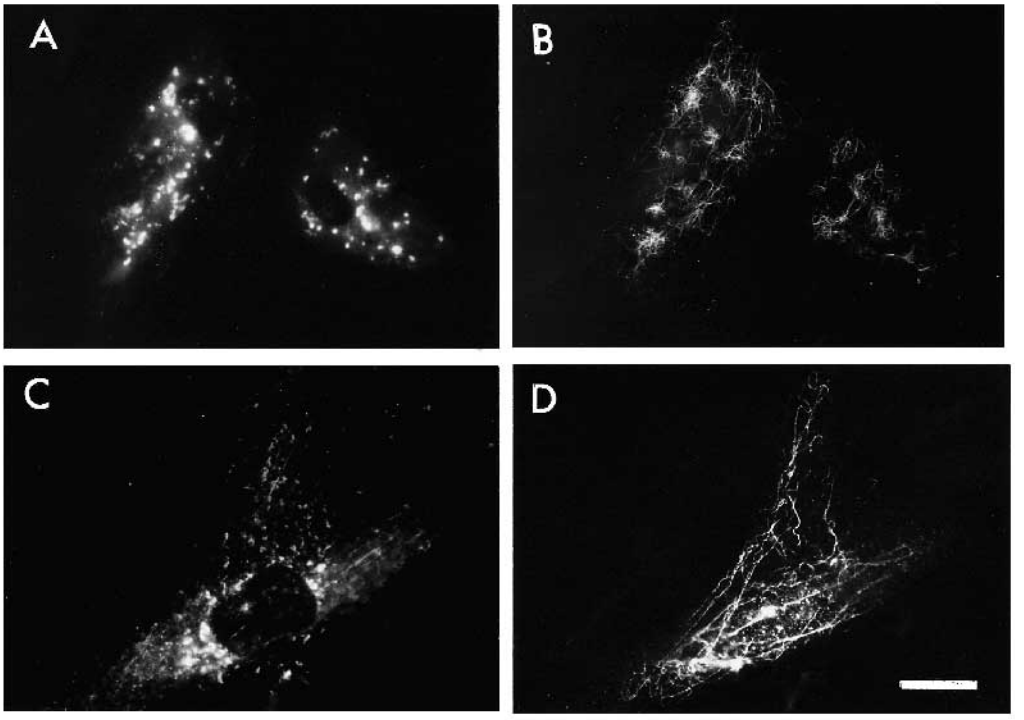

Fig. 4. The effect of low concentration of nocodazole on the GA and the Mt system in fibroblasts. HSFs were incubated for 5 hours (A,B) and REF 52 cells for 2 hour (C,D) with 100 nM of nocodazole before revealing of GA and Mts. HSFs were stained with NBD-C_6-ceramide (A), photographed and immediately removed for extraction, fixation and immunofluorescent staining of Mts with anti-tubulin antibodies (B). The same cells were found with the help of locator grids. The GA (C) and Glu-tubulin containing Mts (D) in REF 52 cells were double labeled after methanol fixation with FITC-conjugated lentil lectin and anti-Glu-tubulin antibody and TRITC-labeled goat anti-rabbit IgG antibody as described in Materials and Methods. Bar, 10 μm.

in cytoplasm takes place when the dynamic Mts are disrupted by high concentration of nocodazole or turned to the stable state by low-dose of this drug. To examine the possibility that these Mts are involved in the dispersal of the GA as the tracks for plus-end directed transport, the antibody against kinesin (antibody HD) was microinjected into the HSFs prior to the nocodazole treatment.

It can be seen in Fig. 7A,B that the GA in cells injected with HD antibody maintained a central location after 5 hours of incubation with 100 nM nocodazole. In contrast, the control cells injected with preimmune IgG (Fig. 7C,D) showed the scattered distribution of GA elements similar to that in non-injected cells. It is notable also, that although the scattering of GA was suppressed by HD antibody its integrity was disturbed. To quantify the degree of the inhibition of the GA dispersal by injected antibodies, the ratios of the cell areas occupied by Golgi fragments to the whole cell areas was measured (see Materials and Methods). A comparison of the

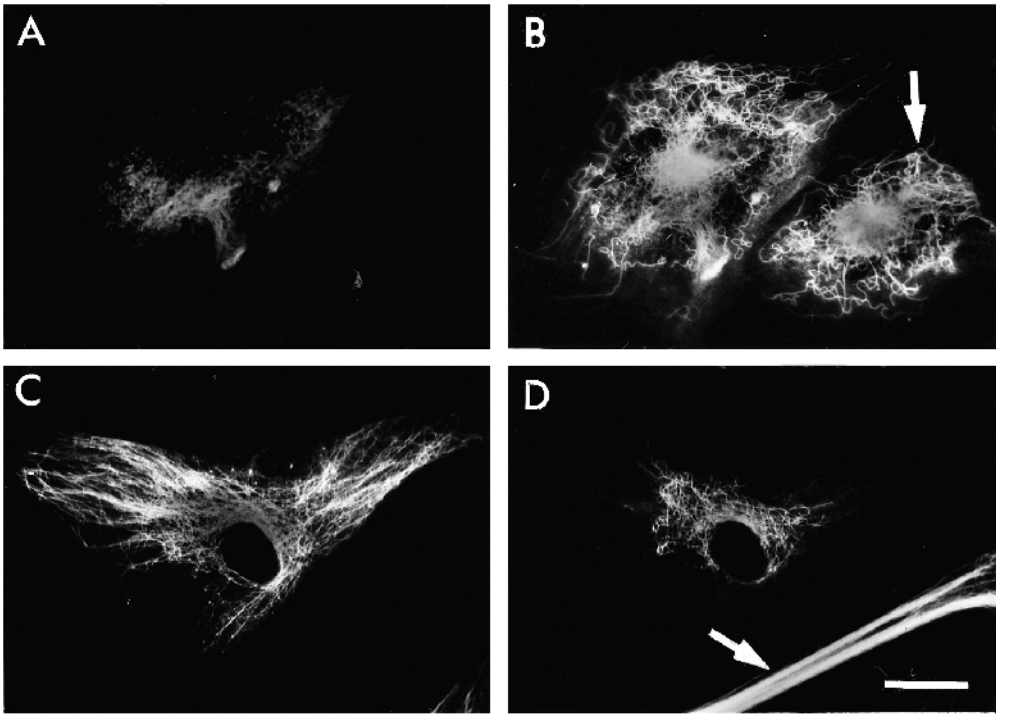

Fig. 5. The visualisation of stable Mts in HSFs using the antibody-blocking technique. Biotin-tubulin was microinjected into cells treated with 100 nM nocodazole for 5 hours (A,B) and control cells (C,D). 25 minutes later the cells were fixed and immunostained as described in Materials and Methods. (A,C) Anti-biotin staining showing the dynamic Mts. (B,D) Anti-tubulin staining revealing the stable Mts. Arrows point the neighboring uninjected cells. Bar, 10 μm.

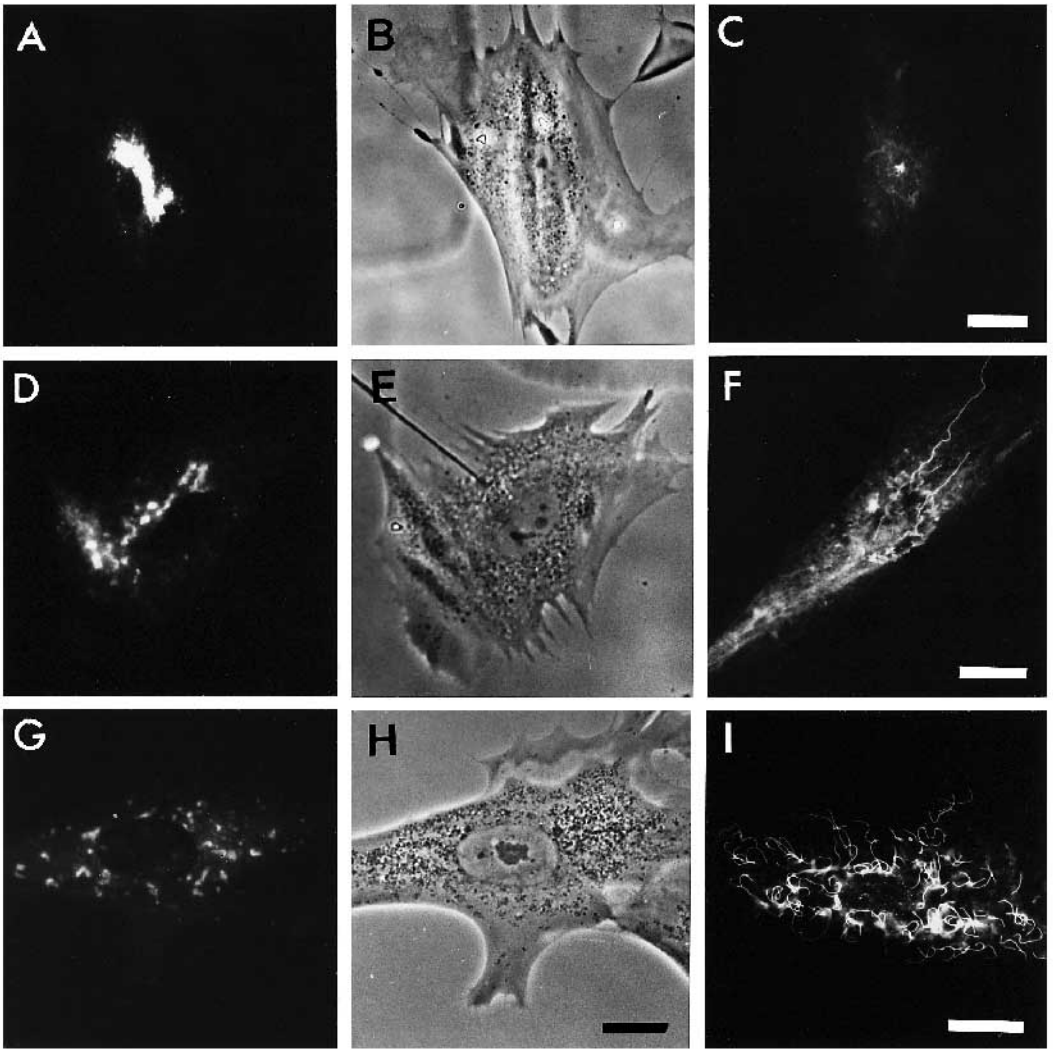

Fig. 6. The disruption of Mts by low temperature inhibits GA dispersion. Cells were incubated for 2.5 (A,B,C) or 0.5 hours (D,E,F) at 0°C in medium containing HEPES (see Materials and Methods). Then the nocodazole was added to a final concentration of 5 μM (A-F) or 100 nM (G,H,I) and 30 minutes later the cells were warmed to 37°C. Some cells were fixed just after cold treatment (C,F) or after 3 hours at 37°C (I) for Mt immunostaining (C,F) and others were incubated for 2 hours before labeling with NBD-C_6-ceramide to visualize Golgi elements (A,D,G). (B,E,H) Phase contrast images of the cells in A, D and G. Bar, 10 μm.

levels of GA dispersal by 100 nM nocodazole in the HSFs injected with HD antibody and preimmune IgG (Table 2) demonstrates the three-fold inhibitory effect. The quantitative data also shows that the mean degree of GA dispersal in cells injected with preimmune IgG was similar to that in noninjected ones.

The injection of HD antibody into the HSFs prior to the treatment with 5 μM of nocodazole had the same effect on GA dispersal. Fig. 8 demonstrates the distribution of Golgi elements after 3 hours incubation with 5 μM of nocodazole in cells injected with antibody HD (A,B) and preimmune IgG (C,D). These data show that central localization of the Golgi elements was maintained in the cells injected with anti-kinesin antibody even after complete disruption of Mts. The mean levels of GA dispersal in the HSFs treated with high nocodazole concentration were similar to those in cells subjected to the low-dose drug (Table 2). It was shown previously (Rodionov et al., 1993b) that the HD antibody did not cause any visible alterations of Mt pattern in the injected HSFs. Fig. 9 shows that the subset of stable Mts was also unchanged in these cells (compare with Fig. 1B). Thus the suppression of the GA dispersal by HD antibody was not the consequence of Mt changes but the inhibition of kinesin.

Table 2. Effect of microinjections of HD antibody on the GA dispersal by nocodazole

Concentration of nocodazole, μM	Control noninjected	Preimmune IgG	HD antibody
0.1	33.7±7.9	30.5±9.2	10.3±2.5
5.0	26.3±7.1	31.3±11.1	10.0±4.6

The cells were injected with either HD antibody or preimmune IgG. 3 hours later 5 μM or 100 nM nocodazole was added for another 3 hours. GA was visualized in living cells after labeling with NBD-C_6-ceramide (see Materials and Methods). Values are expressed as the ratios of the area containing GA particles to the area of the whole cell and represent means ± s.e.m. of determinations made for 20-30 cells of each type.

DISCUSSION

In this paper, data is presented showing that the dispersal of Golgi membranes from the perinuclear region of cells exposed to the Mt-disrupting drug nocodazole is a kinesin-driven process and depends on the stable Mts. These Mts which are less sensitive to depolymerizing drug nocodazole (Kreis, 1987)

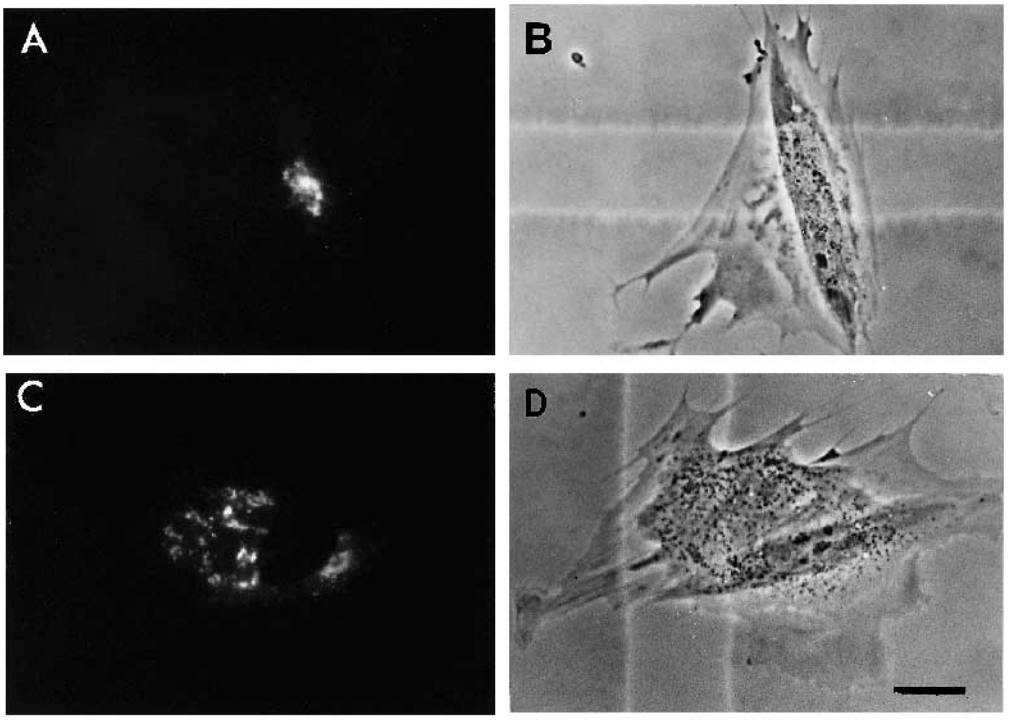

Fig. 7. The effect of HD antibody injections on the GA dispersal by 100 nM nocodazole. Cells were microinjected with HD antibody (A,B) or preimmune IgG (C,D). 1 hour later 100 nM nocodazole was added for 5 hours. GA was visualized in living cells (A,C) after labelling with NBD-C_6-ceramide (see Materials and Methods). (B,D) Phase contrast images. Bar, 10 μm.

could serve as tracks for the plus-end directed transport of the Golgi elements, causing their redistribution in cytoplasm. The data presented here could reconcile the apparent contradictory results demonstrating energy dependence of GA dispersal (Turner and Tartakoff, 1989) with the observations showing that Mts are not involved in this process (Turner and Tartakoff, 1989; Cole et al., 1996).

The fluorescent probe C_6-NBD-ceramide was used to label GA in this study. It was shown earlier (Lipski and Pagano, 1983, 1985; Van Meer et al., 1987) that the metabolites of this lipid analog accumulated in GA membranes and could be utilized as a specific dye for this organelle. The C_6-NBD-ceramide fluorescence in living cells corresponded to the patterns obtained for the same cells after fixation when the other probes specific to GA were used (Lipski and Pagano, 1985; Ho et al.,1989). The advantages of this probe in studies of the nocodazole-induced GA dispersion was also demonstrated (Ho et al., 1989).

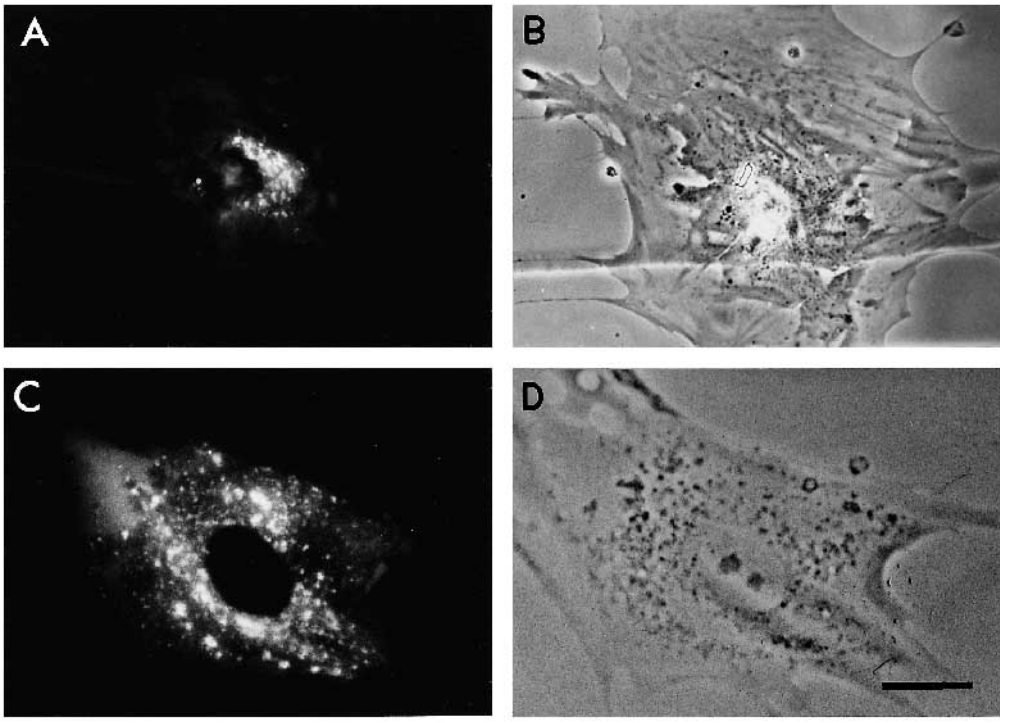

Fig. 8. The effect of HD antibody injections on the GA dispersal by 5 μM nocodazole. Cells were microinjected with HD antibody (A,B) or preimmune IgG (C,D). 3 hours later 5 μM nocodazole was added for another 3 hours. GA was visualized in living cells (A,C) after labelling with NBD-C_6-ceramide (see Materials and Methods). (B,D) Phase contrast images. Bar, 10 μm.

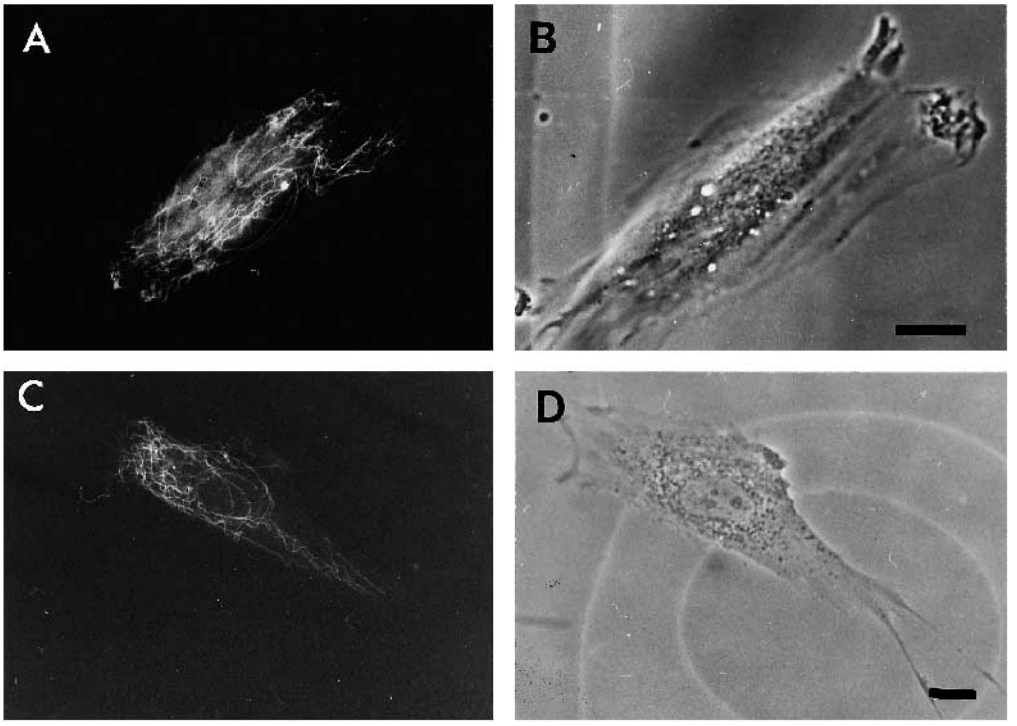

Fig. 9. The effect of HD antibody injections on stable Mts. Cells were microinjected with HD antibody (A,B) or with preimmune IgG (C,D) and 3 hours later 5 μM nocodazole was added for 30 minutes. Cells were then fixed for indirect immunofluorescence. Mts staining (A,C) and phase contrast image (B,D) of injected cell. Bar, 10 μm.

The results presented here show that GA scattering in HSFs and REF 52 cells caused by nocodazole is a relatively rapid process that occurs well before the complete disruption of Mts. The comparison of the time course of the Mt depolymerization (Fig. 1 and Fig. 3B,D,F,H,J) with that of the GA dispersal (Fig. 2 and Fig. 3A,C,E,G,I) shows that redistribution of Golgi elements takes place after the disruption of dynamic subpopulation of Mts. At the same time the stable Mts, which are less sensitive to the drug, are still present during the entire process. If the Mt depolymerization is achieved by the incubation of the cells at 0°C the subsequent warming to 37°C in the presence of nocodazole does not cause the dispersal of GA (Fig. 6A,B). The latter is observed only if some stable Mts have survived after the cold treatment (Fig.6D-F) or if partial restoration of the Mt system occurred when the lower concentration of nocodazole was added before the warming (Fig. 6G,H). Thus, the temporary presence of stable Mts before the complete disruption seems to be required for GA dispersal.

When the partial disruption of Mts was achieved by the treatment of the cells with the low concentration of the nocodazole (100 nM) GA dispersal took place as well (Fig. 4). In contrast to the gradual depolymerization of Mts in the presence of 5 μM of nocodazole incubation with the low concentration resulted in a relatively high amount of Mts in the cells. These remaining Mts showed a slow dynamics when probed by antibody-blocking technique (Schulze and Kirschner, 1987). Rogalski and Singer (1984) have shown that the Mt-stabilizing drug taxol disperses the GA in cells though the density of Mts is very high. So, the reason for the GA dispersal is not the decrease of Mt density. These findings, together with the data of Rogalski and Singer (1984) showing that Mt-stabilizing drug taxol disperses the GA, indicate that the main reason for GA dispersal by nocodazole is the removal of the dynamic Mts at the initial stages of the depolymerization process and the continued presence of stable Mts.

The reclustering of the dispersed GA upon nocodazole removal and the restoration of Mts was shown to occur along the Mts at their minus ends (Ho et al., 1989). Similarly, the results presented here demonstrate that dispersal of the GA could be a Mt-dependent process involving plus-end directed motor protein such as kinesin. In fact, it is shown here that antibody HD against kinesin, but not preimmune IgG, inhibits the GA dispersal when injected into cells before nocodazole treatment (Figs 7, 8 and Table 2). The data of Turner and Tartakoff (1989) demonstrating the energy dependence of the GA dispersal are in good agreement with these results. However, the use of HD antibody does not identify which member(s) of kinesin superfamily is(are) involved, as it cross-reacts with different kinesin-related proteins (Rodionov et al., 1993a). At the same time, the involvement of kinesin in plus-end directed Golgi-to-ER transport was demonstrated earlier using another antibody against this motor protein (Lippincott-Schwartz et al., 1995).

It is still unknown why the stable Mts do not maintain the central location of Golgi elements. It could be suggested that when both kinesin and dynein are bound to the same element of the GA the former is dominant in the absence of the dynamic Mts with stable ones serving as a tracks. Conversely, when the dense array of the dynamic Mts is present at the cell center the dynein ‘out-drives’ the kinesin and localizes the GA near the centrosome. However, it is not known yet if motor proteins can distinguish between two dynamic states of Mts.

Thus, the removal of the dynamic subpopulation of Mts by nocodazole starts the scattering of the GA throughout the cell, and kinesin (or another kinesin-like motor) is involved in this process. However, double staining of Golgi membranes and stable Mts in REF 52 cells treated with nocodazole (Fig. 3 and Fig. 4C,D) shows that although stable Mt patterns coincide with dispersed GA only some Golgi elements colocalize with Mts. These results mean that Golgi fragments large enough to

be visible with the light microscope are not transported along Mts.

The recently proposed (Cole et al., 1996) explanation of GA dispersal involves the disturbance of the recycling of membranes between the GA and the ER. The nocodazole treatment in this model impedes only the pathway from ER to GA and does not affect the oppositely directed transport. However, our confirmation that the Golgi-to-ER pathway survives in the absense of Mts support other data (Lippincott-Schwartz et al., 1995) that show the involvement of Mt motor kinesin in this transport. Our results demonstrating the participation of the stable Mts in GA dispersal offer a good explanation of this contradiction. If the partial depolymerization of the Mt system disturbs the pathway from the ER to the GA and does not affect the movement in the opposite direction, it could lead to the gradual disappearance of the Golgi material at the center and its accumulation in the exit sites of the ER. This could occur if the remaining stable Mts could serve as tracks only for plus-end directed vesicle transport. However, the association of the stable Mts with ER-to-GA transport inter-mediates, which was demonstrated earlier by Mizuno and Singer (1994), makes the possible mechanism much more complicated.

Finally, the specific role of the dynamic Mts in GA localization could be emphasized in view of these data. Our data indicate that the removal of the dynamic Mts causes the fragmentation of the GA even in the conditions when its dispersal is impeded either by HD antibody (Figs 7A, 8A) or by cold disruption of stable Mts (Fig. 6A). There might be some mechanisms by which the dense array of these polymers maintain the GA integrity near the Mts organizing center. The data obtained recently showing the ability of depolymerizing Mts to move particles and kinetochores (Coue et al., 1991; Lombillo et al., 1993) coupled to their plus ends in the direction of their minus ends could be relevant. These and others questions concerning the interactions of GA and Mts remain to be answered.

I am grateful to Prof. V. Gelfand and Dr E. Vaisberg for valuable discussions and constructive criticism of the manuscript, to Dr F. Gyoeva, Dr E. Nadezhdina, Prof. G. Langford, Dr S. Kuznetzov and Amy Reilein for the critical reading and helpful advice in the manuscript preparation, to Mrs O. Derjabina and Mrs N. Minina for excellent technical assistance. This work was supported by Russian Academy of Sciences.

REFERENCES

Boyd, A. E. III, Bolton, W. E. and Brinkley, B. R. (1982). Microtubules and beta cell function: effect of colchicine on microtubules and insulin secretion in vitro by mouse beta cells. *J. Cell Biol*. **92**, 425-434.

Cambray-Deakin, M., Robson, S. J. and Burgoyne, R. D. (1988). Colocalisation of acetylated microtubules, glial filaments and mitochondria in astrocytes in vitro. *Cell Motil.* **10**, 438-449.

Cole, N. B. and Lippincott-Schwartz, J. (1995). Organization of organelles and membrane traffic by microtubules. *Curr. Opin. Cell Biol.* **7**, 55-64.

Cole, N. B., Sciaky, N., Marotta, A., Song, J. and Lippincott-Schwartz, J. (1996). Golgi dispersal during microtubule disruption: Regeneration of Golgi stacks at peripheral endoplasmic reticulum exit sites. *Mol. Biol. Cell* **7**, 631-650.

Cooper, M. S., Cornell-Bell, A. H., Chernjavsky, A., Dani, J. W. and Smith, S. J. (1990). Tubulovesicular processes emerge from trans-Golgi cisternae, extend along microtubules and interlink adjacent trans-Golgi elements into a reticulum. *Cell* **61**, 135-145.

Coue, M., Lombillo, V. A. and McIntosh, J. R. (1991). Microtubule depolymerization promotes particle and chromosome movement in vitro. *J. Cell Biol.* **112**, 1165-1175.

Dunphy, W. G. and Rothman, J. E. (1985). Compartmental organization of the Golgi stack. *Cell* **42**, 13-21.

Farquhar, M. G. (1985). Progress in unraveling pathways of Golgi traffic. *Annu. Rev. Cell Biol.* **1**, 447-488.

Feiguin, F., Ferreira, A., Kosik, K. S. and Caceres, A. (1994). Kinesin-mediated organelle translocation revealed by specific cellular manipulations. *J. Cell Biol.* **127**, 1021-1039.

Herman, B. and Albertini, D. F. (1984). A time-lapse video image intensification analysis of cytoplasmic organelle movements during endosome translocation. *J. Cell Biol.* **98**, 565-576.

Ho, W. C., Alan, V. J., VonMeer, G., Berger, E. G. and Kreis, T. E. (1989). Reclustering of scattered Golgi elements occurs along microtubules. *Eur. J. Cell Biol*. **48**, 250-263.

Kreis, T. E. (1987). Microtubules containing detyrosinated tubulin are less dynamic. *EMBO J.* **6**, 2597-2606.

Kreis, T. E. (1990). Role of microtubules in the organization of the Golgi apparatus. *Cell Motil. Cytoskel*. **15**, 67-70.

Khawaja, S., Gundersen, G. G. and Bulinski, J. C. (1988). Enhanced stability of microtubules enriched in detyrosinated tubulin is not a function of detyrosination level. *J. Cell Biol.* **106**, 141-150.

Lippincott-Schwartz, J., Donaldson, J. G., Schweizer, A., Berger, E. G., Hauri, H. P., Yuan, L. C. and Klausner, R. D. (1990). Microtubule-dependent retrograde transport of proteins into the ER in the presence of brefeldin A suggests an ER recycling pathway. *Cell* **60**, 821-836.

Lippincott-Schwartz, J., Cole, N. B., Marotta, A., Conrad, P. A. and Bloom, G. S. (1995). Kinesin is the motor for microtubule-mediated Golgi-to-ER membrane traffic. *J. Cell. Biol.* **128**, 293-306.

Lipsky, N. G. and Pagano, R. E. (1983). Sphingolipid metabolism in cultured fibroblasts: Microscopic and biochemical studies employing a fluorescent ceramide analog. *Proc. Nat. Acad. Sci. USA* **80**, 2608-2612.

Lipsky, N. G. and Pagano, R. E. (1985). A vital stain for the Golgi apparatus. *Science* **228**, 745-747.

Lombillo, V. A., Coue, M. and McIntosh, J. R. (1993). In vitro motility assay using microtubules tethered to Tetrahymena pellicles. *Meth. Cell Biol.* **39**, 149-165.

Lucocq, J. M., Pryde, J. G., Berger, E. G. and Warren, G. (1987). A mitotic form of the Golgi apparatus in HeLa cells. *J. Cell Biol.* **104**, 865-874.

Lucocq, J. M. and Warren, G. (1987). Fragmentation and partitioning of the Golgi apparatus during mitosis in HeLa cells. *EMBO J.* **6**, 3239-3246.

Marks, D. L., Larkin, J. M. and McNiven, M. A. (1994). Association of kinesin with Golgi apparatus in rat hepatocytes. *J. Cell Sci.* **107**, 2417-2426.

Matteoni, R. and Kreis, T. E. (1987). Translocation and clustering of endosomes and lysosomes depends on microtubules. *J. Cell Biol.* **105**, 1253-1265.

Mitchinson, T. J. and Kirschner, M. W. (1984). Microtubule assembly nucleated by isolated centrosomes. *Nature* **312**, 232-236.

Mitchinson, T. J. and Kirschner, M. W. (1985). Properties of the kinetochore in vitro. I. Microtubule nucleation and tubulin binding. *J. Cell Biol.* **101**, 755-765.

Mizuno, M. and Singer, S. J. (1994). A possible role for stable microtubules in intracellular transport from the endoplasmic reticulum to the Golgi apparatus. *J. Cell Sci.* **107**, 1321-1331.

Moskalewski, S., Thyberg, J. and Friberg, U. (1980). Cold and metabolic inhibitor effects on cytoplasmic microtubules and Golgi complex in rat epiphyseal chondrocytes. *Cell. Tissue Res.* **210**, 403-415.

Rindler, M. J., Ivanov, I. E. and Sabatini, D. D. (1987). Microtubule-acting drugs lead to nonpolarized delivery of the influenza hemagglutinin to the cell surface of polarized Madin-Darby canine kidney cells. *J. Cell Biol.* **104**, 231-241.

Rodionov, V. I., Gyoeva, F. K. and Gelfand, V. I. (1991). Kinesin is responsible for centrifugal movement of pigment granules in melanophores. *Proc. Nat. Acad. Sci. USA* **88**, 4956-4960.

Rodionov, V. I., Gelfand, V. I. and Borisy, G. G. (1993a). Kinesin-like molecules involved in spindle formation. *J. Cell Sci.* **106**, 1179-1188.

Rodionov, V. I., Gyoeva, F. K., Tanaka, E., Bershadsky, A. D., Vasiliev, J. M. and Gelfand, V. I. (1993b). Microtubule-dependent control of cell shape and pseudopodial activity is inhibited by the antibody to kinesin motor domain. *J. Cell Biol.* **123**, 1811-1820.

Rogalski, A. A., Bergman, J. E. and Singer, S. J. (1984). Effect of microtubule assembly status on the intracellular processing and surface expression of an integral protein of the plasma membrane. *J. Cell Biol.* **99**, 1101-1109.

Rogalski, A. A. and Singer, S. J. (1984). Associations of elements of the Golgi apparatus with microtubules. *J. Cell Biol.* **99**, 1092-1100.

Salas, P. J., Misek, E., Vega-Salas, D., Gunderson, D., Cereijido, M. and Rodrigues-Boulan, E. (1986). Microtubules and actin filaments are not critically involved in the biogenesis of epithelial cell surface polarity. *J. Cell Biol.* **102**, 1853-1867.

Saraste, J. and Svensson, K. (1991). Distribution of the intermediate elements operating in ER to Golgi transport. *J. Cell Sci.* **100**, 415-430.

Schulze, E. and Kirschner, M. W. (1986). Microtubule dynamics in interphase cells. *J. Cell Biol.* **102**, 1020-1031.

Schulze, E. and Kirschner, M. W. (1987). Dynamic and stable populations of microtubules in cells. *J. Cell Biol*. **104**, 277-288.

Thyberg, J. and Moskalewski, S. (1985). Microtubules and the organization of the Golgi complex. *Exp. Cell Res*. **159**, 1-16.

Turner, J. R. and Tartakoff, A. M. (1989). The response of the Golgi complex to microtubule alterations: The roles of metabolic energy and membrane traffic in Golgi complex organization. *J. Cell Biol*. **109**, 2081-2088.

Vaisberg, E. A., Grissom, P. M. and McIntosh, J. R. (1996). Mammalian cells express three distinct dynein heavy chains that are localized to different cytoplasmic organelles. *J. Cell Biol*. **133**, 831-842.

Van Meer, G., Stelzer, E. H. K., Wijnaendts-van-Resandt, R. W., Simons, K. (1987). Sorting of sphingolipids in epithelial (MDCK) cells. *J. Cell Biol.* **105**, 1623-1635.

Vasquez, R. J., Howell, B., Yvon, A., Wadsworth, P. and Cassimeris, L. (1995). Nanomolar nocodazole puts the brakes on microtubule dynamic instability in vivo. *Mol. Biol. Cell (Suppl.)* **6**, 259a, Abstr. 1505.

Webster, D. R., Wehland, J., Weber, K. and Borisy, G. G. (1990). Detyrosination of alfa tubulin does not stabilize microtubules in vivo. *J. Cell Biol.* **111**, 113-122.

Weingarten, M. D., Lockwood, A. H., Hwo, S. and Kirschner, M. W. (1975). A protein factor essential for microtubule assembly. *Proc. Nat. Acad. Sci. USA* **72**, 1858-1862.

(Received 27 December 1996 – Accepted 3 June 1997)