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γ-Tubulin Distribution in Interphase and Mitotic Cells upon Stabilization and Depolymerization of Microtubules

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Indirect immunofluorescence and digital videomicroscopy were used to study γ-tubulin distribution in normal mitotic and interphase HeLa cells and after their treatment with microtubule-stabilizing (taxol) and depolymerizing (nocodazole) drugs. In interphase HeLa cells, the affinity-purified antibodies against γ-tubulin and monoclonal antibodies against acetylated tubulin stain one or two neighboring dots, centrioles. The γ-tubulin content in two centrioles from the same cell differs insignificantly. Mitotic poles contain fourfold amount of γ-tubulin as compared with the centrioles in interphase.

The effect of nocodazole (5 (μg/ml) on interphase cells resulted in lowering the amount of γ-tubulin in the centrosome, and in 24 h it was reduced by half. Treatment with nocodazole for 2 h caused a fourfold decrease in the γ-tubulin content in mitotic poles. Besides, the mitotic poles were unevenly stained, the fluorescence intensity in the center was lower than at the periphery. Upon treatment with taxol (10 μg/ml), the γ-tubulin content in the interphase cell centrosome first decreased, then increased, and in 24 h it doubled as compared with control. In the latter case, bright dots appeared in the cell cytoplasm along the microtubule bundles. However, after 24 h treatment with taxol, the total amount of intracellular γ-tubulin did not change. Treatment with taxol for 2-4 h halved the γ-tubulin content in the centrosome as compared with normal mitosis. In some cells, antibodies against γ-tubulin revealed up to four microtubule convergence foci. Other numerous microtubule convergence foci were not stained.

Thus, the existence of at least three γ-tubulin pools is suggested: (1) constitutive γ-tubulin permanently associated with centrioles irrespective of the cell cycle stage and of their ability to serve as microtubule organizing centers; (2) γ-tubulin unstably associated with the centrosome only during mitosis; *(3)* cytoplasmic γ-tubulin that can bind to stable microtobules.

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INTRODUCTION

The centrosome in a living cell is recognized as the microtubule network organizer [1, 2], but the molecular aspects of function of the microtubule organizing centers (MTOC) are still unclear. γ-Tubulin is a centrosome-associated minor cell protein playing an important role in microtubule nucleation [3-8]. Microinjection of anti-γ-tubulin antibodies into living cells inhibits the centrosome ability to initiate microtubule polymerization during mitosis [9]. According to the generally accepted hypothesis by Oakley [10], γ-tubulin molecules form rings on the surface of MTOC surrounding the centrioles and bind to the β-tubulin molecules of α-β-tubulin dimers; microtubules grow on this template.

Anti-γ-tubulin antibodies stain the centrosome in the cells of different tissues both in mitosis and in interphase [11-14], and the stain survives the microtubule depolymerization [15]. The presence of γ-tubulin in the centrosome after depolymerization of interphase microtubules was explained by the fact that γ-tubulin is an integral part of centrioles [11, 16]. However, the immunoelectron investigation of the centrosome in mitosis revealed γ-tubulin in a cloud of pericentriolar material rather than in the centrioles [11]. In addition, basal bodies of *Xenopus* sperm do not contain γ-tubulin; they acquire it together with some other proteins from the ooplasm before formation of microtubule asters [17, 18], whereas basal bodies in the ciliated epithelium of the eye retina contain γ-tubulin [19]. Thus, the above-mentioned results allow one just to state definitely that γ-tubulin is a component of the microtubule organizing center.

Besides, γ-tubulin was also identified within cytoplasmic microtubules [20], where it was bound directly to the microtubule minus-ends at a ratio of 12-13 γ-tubulin molecules per microtubule [21-24].

Our previous results confirmed that in interphase cells, antibodies against γ-tubulin often stained the centrosome as a double spot [13, 14]. The centrosome in cells of higher animals consists of a maternal and daughter centrioles, which are morphologically and functionally different: many more microtubules radiate from the active centriole than from the inactive one [25-28]. Owing to this, one could expect different intensity of staining of the spots corresponding to two centrioles in the same cell. Moreover, in mitosis significantly more microtubules are associated with the active centrosome as compared with the interphase [29], whereas according to our data, the staining intensity remained at the same level [14]. The data obtained put several questions. First, is γ-tubulin associated with microtubules or the centrosomes? Will staining of mitotic poles survive degradation of mitotic spindle microtubules and the subsequent removal of γ-tubulin associated with

the microtubule minus-ends, or the stained spots will become equal in size to the interphase ones?

In this study, we used quantitative immunofluorescence to follow changes in the γ-tubulin content in the centrosomes during normal mitosis and interphase, as well as upon alteration of normal relationships between the centrosome and microtubules. For this aim, microtubules were stabilized with taxol or degraded with nocodazole.

EXPERIMENTAL

Cell cultures. HeLa cell cultures were grown at 37°C in culture medium 199 supplemented with 10% fetal calf serum and gentamycin. Cells were treated with nocodazole (5 μg/ml) or taxol (10 μg/ml) for 2,4, and 24 h.

Antibodies. Monoclonal antibodies against acetylated tubulin were the courtesy of Prof. K. Gull (Manchester, UK). Monoclonal antibodies against oc-tubulin (DM-1A) and FITC-conjugated second antibodies were purchased from Sigma. The Texas-Red-conjugated antibodies were the courtesy of Prof. B. Breton (Ren, France).

To obtain polyclonal antibodies against γ-tubulin, a recombinant protein consisting of 134 amino acid residues corresponding to the C-terminal part of human γ-tubulin (aa residues 318-451) (GeneBank M61764) was used. The chosen part of γ-tubulin is highly immunogenic, and its sequence differs from corresponding regions of oc-tubulin and β-tubulin (GeneBank M61764) [14].

Purification of polyclonal antibodies against γ-tubulin. The affinity purification of polyclonal antibodies against γ-tubulin was carried out using nitrocellulose membranes Hybond C-extra (Amersham) as follows.

The chromatographically purified recombinant protein was separated by electrophoresis in 10% PAAG as described by Laemmli [30]. Then, the protein was transferred onto nitrocellulose membrane Hybond C-extra which was followed by blocking of nonspecific binding sites with 3% bovine serum albumin (BSA). The band containing 17 kDa recombinant protein was cut out and incubated with polyclonal immune serum. Antibodies were eluted with 200 mM glycine-HCl buffer, pH 2.8. The eluate was neutralized with 1 M Tris-HCl buffer, pH 8.0, then dialyzed against phosphate saline (physiological) buffer, pH 7.2. The affinity-purified antibodies were used in a dilution 1 : 20 for immunofluorescent staining and in a dilution 1: 100 for immunoblotting.

Immunoblotting. Western immunoblotting was carried out using a standard technique [31]. The cytoskeleton fraction isolated from the cells was used for immunoblotting. To obtain this fraction, cells grown in a culture flask were lyzed in Triton X-100-containing microtubule-stabilizing buffer: 50 mM imidazole buffer, pH 6.8, 5 mM $MgCl_2$, 1 mM EGTA, 0.1 mM EDTA, 4% polyethylene glycol (PEG) m. w. 1500,1% Triton X-100 (Sigma), and 1 mM

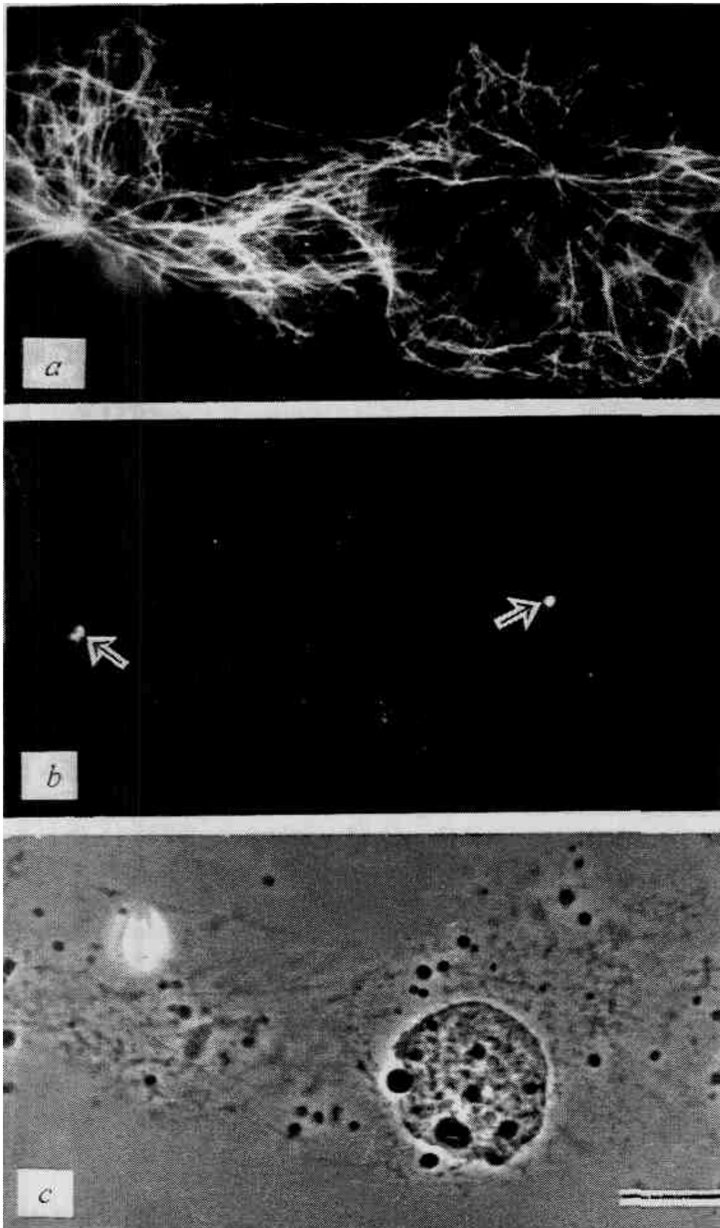

Figure 1. Immunofluorscence *(a, b)* and phase contrast *(c)* microphoto-graphs of interphase HeLa cells. Bar, 10 μm. (a) Microtubules stained with antibodies against α-tubulin; *(b)* the centriole stained with antibodies against γ-tubulin (arrows); *(c)* phase contrast.

PMSF. Then, the material was treated with 8 M urea solution, dialyzed, and concentrated by centrifuging through Ultracent-30 Ultrafilters (Bio-Rad). The concentrated material was dissolved in SDS buffer. Proteins were separated by electrophoresis in 10% PAAG according to Laemmli [30].

The cytoskeleton fraction from the cells incubated for 24 h in the presence of taxol (10 μg/ml) was obtained as described above.

Before obtaining the protein fraction soluble in SDS-buffer, cells were counted in Goryaev chamber and equal amounts of cells were applied onto each lane.

The antibody binding sites were revealed using the peroxidase-conjugated second antibodies (Sigma). o-Dianisidine was used as the peroxidase substrate.

Immunofluorescence. For immunofluorescent analysis, cells were lyzed in advance under microtubule-stabilizing conditions. Coverslips with cells were washed several times with PBS at 37°C, then lyzed for 5 min in a solution containing 50 mM imidazole, pH 6.8, 5 mM $MgCl_2$, 1 mM EGTA, 0.1 mM EDTA, 4% polyethylene glycol (PEG) m. w. 1500, and 1% Triton X-100. Then, the cells were fixed with 1% glutaraldehyde (Merck) in phosphate buffer for 30 min at room temperature with subsequent triple (10 min each) treatment with $NaBH_4$ solution (2 mg/ml), and underwent sequential immunocytochemical staining with anti-γ-tubulin and anti-α-tubulin antibodies (Sigma). To prevent the background fluorescence, 1% BSA solution in phosphate buffer was used upon staining. Preparations were embedded in 2.5% solution of 1,4-diazabicyclo-[2,2,2]octane (DABCO) (Sigma) in glycerol.

Specimens were viewed in aphotomicroscope Opton-3 (Opton) and photographed using RF-3 film (Tasma, Russia). The same cells were photographed in parallel under phase contrast on Mikrat-300 film (Tasma, Russia). Quantitative analysis was carried out using a cooled CCD camera MicroMax (Princeton Instruments). The image scale with an objective Plan lOOx/1.25 was equal to 13.2 pixel/μm. All images were obtained at 2 s exposure. The 8-bit images were analyzed in Adobe Photoshop. The spot diameters and fluorescence intensities were measured. To do this, the arithmetic mean of two maximal values within a fluorescent spot was taken and the arithmetic mean of the background measured in three pixels near the centrosome was subtracted from it. The γ-tubulin amount (in arbitrary units) on the centrosome was estimated by multiplying the fluorescence intensity by spot area.

RESULTS

In many interphase HeLa cells, a fraction of microtubules converge to a single center near the cell nucleus (Fig. 1). These cells are almost free of stable (acetylated) microtubules (Fig. 2). Polyclonal antibodies against the

C-tenninal fragment of human γ-tubulin, obtained in our laboratory [13, 14], in most cases revealed two adjacent spots of 0.82+0.12 μm (n = 31) in diameter (in one fourth of cells, a single spot is visible) in the microtubule convergence center (Fig. 2). The same spots were also stained with antibodies against acetylated tubulin (Fig. 2), giving evidence that the centrioles were stained in this case [32, 33]. The diameter and the fluorescence intensity of two spots in the cell had close values. In the cells with a single stained spot, its intensity was 30% higher as compared with that for each of the two spots in other cells.

In mitotic cells, staining with γ-tubulin antibodies revealed two bright spots of 1.35 ±0.21 μm *(n* = 17) in diameter corresponding to two mitotic poles. In mitosis, the amount of γ-tubulin in the spindle poles was four times higher than in interphase (Table). In telophase, it decreases and becomes equal to that in the interphase cells (data not shown).

Table. γ-Tubulin content in the spindle poles.

Agent	Treatment time, h	Protein amount, relative units
Control		52±3.9
Nocodazole	2	15.1 ±2.7
Taxol	2	19.1±1.3
Taxol	4	24.2±6.2

To estimate the experimental error, we compared (a) the γ-tubulin amount on two centrioles in interphase cells and (b) that in a pair of mitotic poles. Comparison of two centrioles in an interphase cell by the γ-tubulin content shows that the difference of staining reaches 40%. The difference between two poles in mitosis was 30%, which is probably an experimental error. Based on these data, it was concluded that two centrioles in an interphase cell contained approximately the same amounts of γ-tubulin and less than 1.5-fold differences in its contents were further considered as insignificant.

γ-Tubulin distribution after nocodazole treatment. After 2 h treatment of interphase cells with nocodazole, single microtubules remained in the cytoplasm. As a rule, they were 10-20 μm and more in length and bent. The centrosome could be associated with no microtubules or with 1-2 long or several short microtubules (Fig. *3, a*). In four hours after the start of the nocodazole treatment, no microtubules were found in the cytoplasm of the interphase cells. The character of distribution of the spots stained with anti-γ-tubulin antibodies after the cell incubation with nocodazole for 1 -4 h did not differ from control (Fig. 3, *b).* In 2-4 h after nocodazole introduction, two separate centrioles were seen in about 40% of cells. The nocodazole effect on the interphase cells resulted in a gradual decrease in the γ-tubulin content of

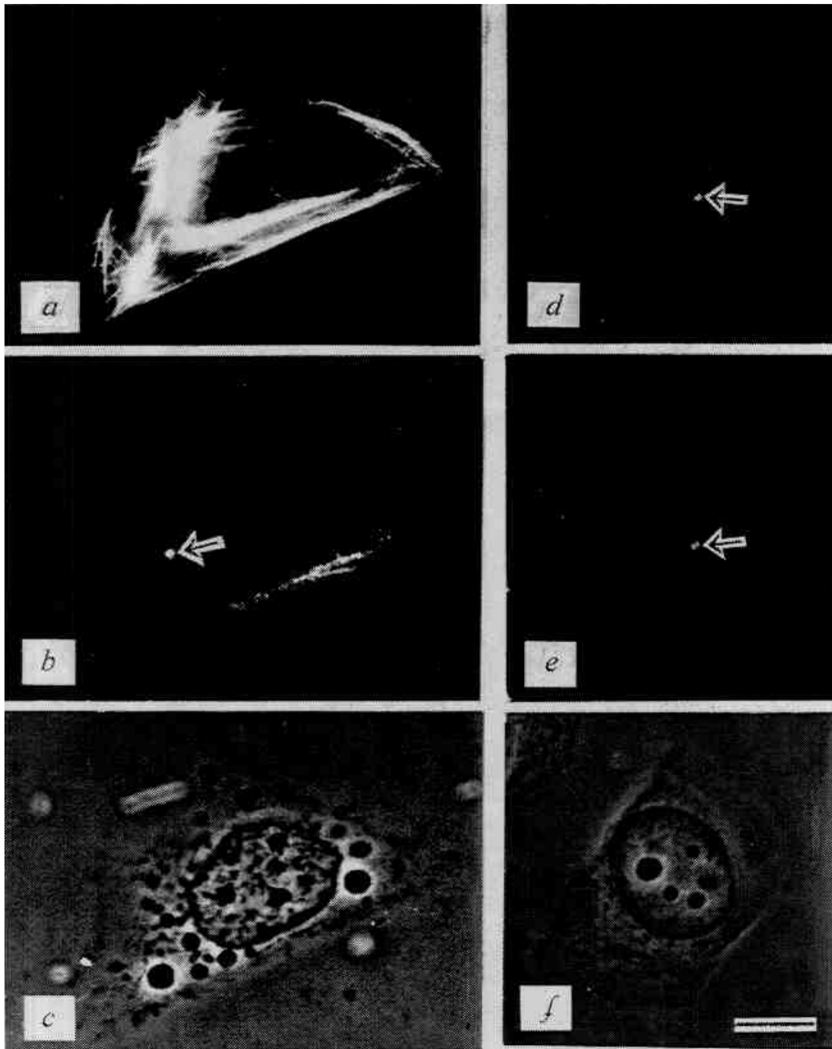

Figure 2. Immunofluorescence *(a, b, d, e)* and phase contrast *(c, f)* microphotographs of interphase HeLa cells: *a, b, c* - after treatment with taxol (10 μg/ml, 24 h); *d, e,f*- a control untreated cell. The centrioles are shown by the arrows. Bar - 10 μm. *a, d,* microtubules stained with antibodies against acetylated tubulin; *b, e,* the centriole stained with antibodies against γ-tubulin; *c,f,* phase contrast.

the centrosome (Fig. 4). In two hours after nocodazole addition to the culture medium, the amount of γ-tubulin on the centrosome decreased by 20%, and it halved in 24 h after the cytostatic effect.

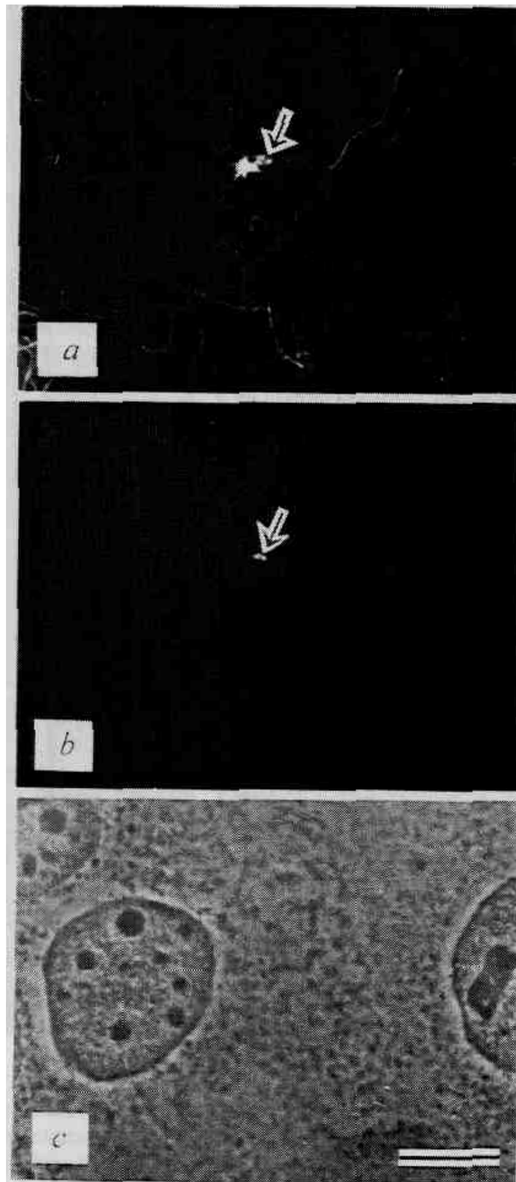

Figure 3. Immunofluorescence *(a, b)* and phase contrast *(c)* microphotographs of interphase HeLa cells after treatment with nocodazole (5 μg/ml, 1 h). Bar, 10 μm. *(a)* Microtubules stained with antibodies against α-tubulin (arrow shows the microtubule convergence center); *(b)* the centriole stained with antibodies against γ-tubulin (arrow); (c) phase contrast.

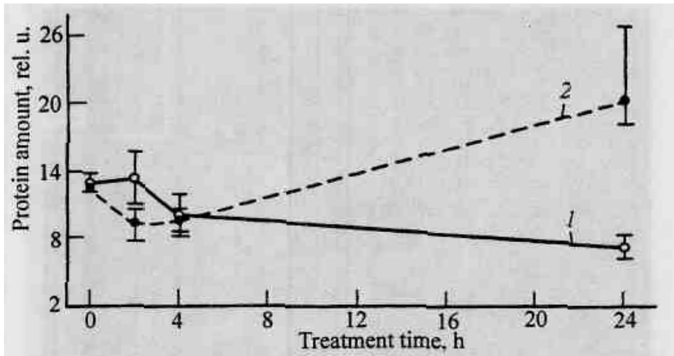

Figure 4. The amount of γ-tubulin on the centrosome in interphase HeLa cells after treatment with nocodazole *(1)* and taxol *(2)*.

In mitotic cells (C-mitoses), virtually no microtubules were found already in an hour after incubation with nocodazole. The distance between the mitotic poles located at the opposite sides of the chromosomal plate decreased against control and was 6-8 μm on average (Fig. 5). The spots corresponding to the mitotic spindle poles were stained unevenly, the fluorescence intensity in their middle part was lower than at the periphery. Due to this, they were ring-like in shape (Fig. 4). The fluorescence intensity at the pole periphery was 20% higher than at its periphery and it was comparable with that in control preparations. Treatment with nocodazole for 2 h resulted in a fourfold reduction of the γ-tubulin content in the cleavage spindle poles as compared with control. In this case, the spot diameter was 40% smaller and became closer to that of the interphase spots in control.

γ-Tubulin distribution after taxol treatment. The microtubule system was significantly altered by taxol. In two hours, the network was formed by shorter and straighter microtubules as compared with control. After 4 h incubation in the presence of taxol, microtubules were packed in short bundles at the cell periphery, and the radial microtubule array disappeared (Fig. 6, *a).* After 24 h taxol effect, all microtubules were associated in thick rather extended bundles whose length exceeded that of a half of the cell length. Most microtubules in the bundles were acetylated (Fig. 2, a).

Treatment with taxol for 1-4 h did not change the character of the interphase cell staining with anti-γ-tubulin antibodies (Fig. 6, *b).* The separated centrioles were more rare as compared with control, they were found in less than half of the cells. Quantitative estimation of γ-tubulin showed a certain decrease (40%) in the protein content near the centrosome during the first two hours and then its increase (Fig. 4). After 24 h taxol effect, the γ-tubulin amount doubled as compared with control (Fig. 2, *b, e).* The spot became larger in diameter, but the mean fluorescence intensity in it did not change. In

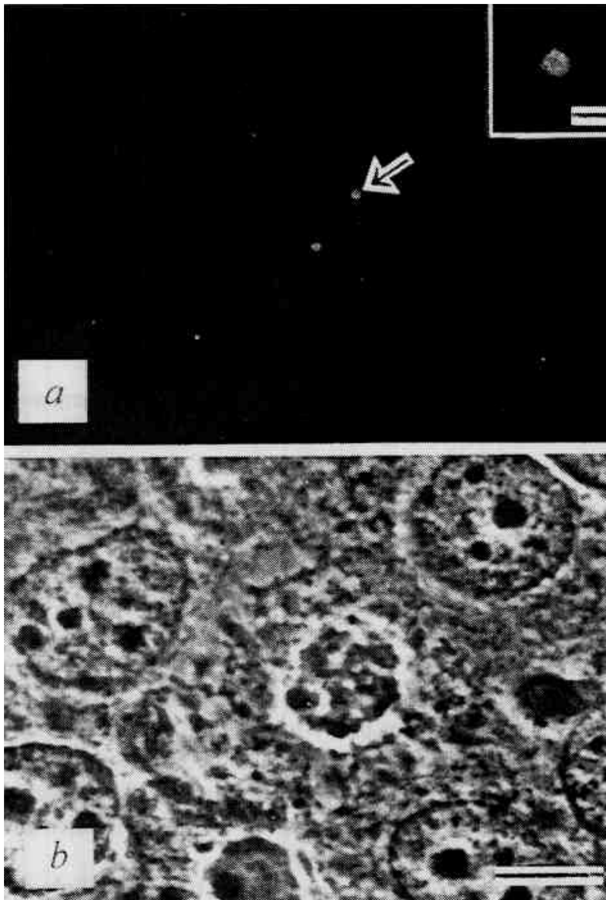

Figure 5. Immunofluorescence *(a)* and phase contrast *(b)* microphotographs of mitotic HeLa cells after treatment with nocodazole (5 μg/ml, 1 h). Bar, 10 μm. Bar in the inset, 1 μm. (a) The centriole stained with antibodies against γ-tubulin (arrow).

addition, numerous small bright dots emerged in the cell cytoplasm along the bundles of acetylated microtubules (Fig. 2, *a, b).*

Within 1 h after the beginning of treatment with taxol, there still could be found bipolar mitoses with their poles stained with anti-γ-tubulin antibodies like in control (data not shown). Characteristic features of mitotic cells treated with taxol for 2—4 h were random positions of chromosomes in the central part of the cell and the presence of several (from 5 to 25) microtubule convergence foci (Fig. 7, *a).* As a rule, antibodies against γ-tubulin stained

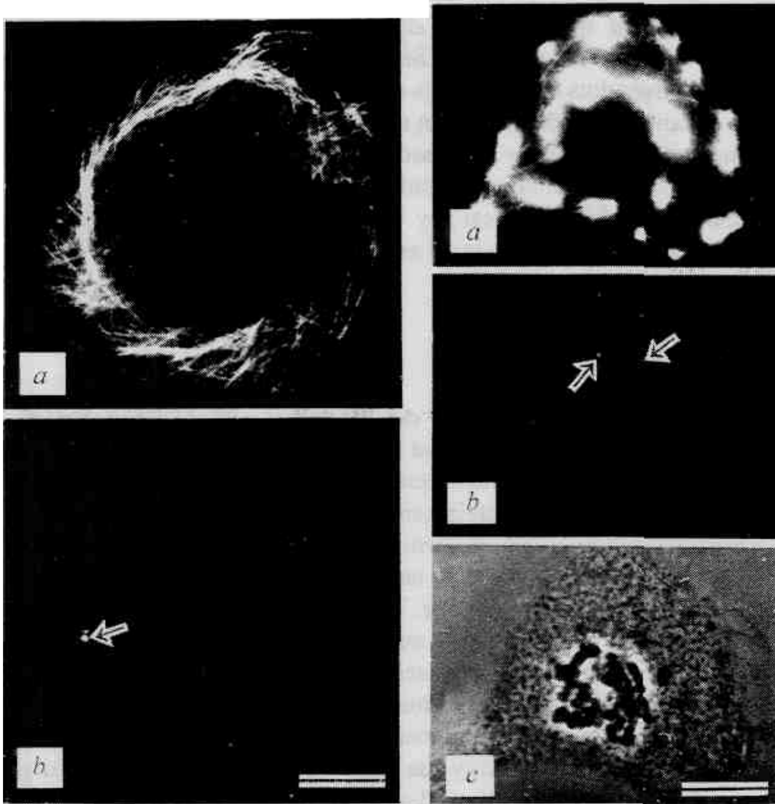

Fig. 6 Fig. 7

Figure 6. Immunofluorescence *(a, b)* microphotographs of interphase HeLa cells after treatment with taxol (10 μg/ml, 4 h). Bar, 10 μm. (a) Microtubules stained with antibodies against ac-tubulin; *(b)* the centriole stained with antibodies against γ-tubulin (arrow).

Figure 7. Immunofluorscence *(a, b)* and phase contrast *(c)* microphotographs of mitotic HeLa cells after treatment with taxol (10 μg/ml, 4 h). Bar, 10 μm. *(a)* Microtubules stained with antibodies against α-tubulin; *(b)* the centriole stained with antibodies against γ-tubulin (arrows); (c) phase contrast.

only two foci (Fig. 7, *b).* The amount of γ-tubulin in these foci was halved as compared with normal mitosis. The spot diameter was the same as in control (about 1.3 μm), but the staining intensity decreased. In some cells, antibodies against γ-tubulin stained up to four microtubule convergence foci. However, in these cases all the other microtubule convergence foci were not stained at all.

The prolonged effect of taxol does not result in increased amount of γ-tubulin in interphase cells. Since anti-γ-tubulin antibodies stained numerous bright dots along bundles of acetylated microtubules in cell preparations obtained after the long-term taxol effect (Fig. 2, *a, b*), and the protein content in the centrosome increased, it is reasonable to suggest that these changes are due to γ-tubulin accumulation in the presence of taxol. However, immunoblotting did not reveal any visible increase in the γ-tubulin content after the cell incubation with taxol as compared with control (Fig. 8, bands 1 and 2).

DISCUSSION

The amount of γ-tubulin on the centrosome is variable. The finding of γ-tubulin prompted suggestions that the key protein in the initiation of polymerization of microtubules had been finally revealed [10, 23]. In contrast to the previously described protein centriole markers, γ-tubulin is involved in polymerization of microtubules, which was confirmed in experiments in the cell-free system [34]. However, until now it has only been proven that γ-tubulin is the centrosome marker. This stimulated us to carry out a quantitative analysis and not to confine ourselves, as in the previous work, to the qualitative investigation of the fluorescence intensity of the spots stained with anti-γ-tubulin antibodies. The estimate made in the previous work was so subjective that the earlier visual evaluation of the fluorescence intensity of mitotic poles [14] was exactly the opposite of the results obtained upon quantitative analysis. The above data show that the amount of γ-tubulin in mitotic poles in metaphase is four times higher that in interphase centrosomes. They correspond to the recent observations that the amount of γ-tubulin on the centrosome in mitosis is 5-7 times higher as compared with the G_1 stage [35]. These changes are in accord with the fact that mitotic centrosomes isolated from the metaphase cells are able to initiate approximately five times more microtubules than interphase centrosomes [29]. The next question is whemer the change in the γ-tubulin content on the centrosome is related to the microtubules radiating from it.

There are data in the literature on the possible association of γ-tubulin molecules to minus-ends of cytoplasmic microtubules [20, 35, 36]. From those data, it was reasonable to expect the decreased intensity of the centrosome staining upon decrease in the amount of associated microtubules. The loss of the microtubule-associated γ-tubulin could be visible first of all in mitotic cells. Indeed, already 2 h later, a marked (fourfold) decrease in the γ-tubulin content in the centrosome to the level characteristic of interphase cells is determined in nocodazole-induced C-mitoses. Taxol also induced a decrease in the γ-tubulin content in the mitotic cell centrosome, but it was only twofold.

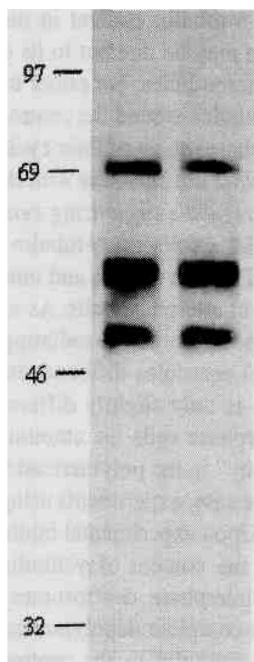

Figure 8. Western immunoblotting with antibodies against γ-tubulin after 24 h treatment with taxol (samples obtained from equal amounts of cells were applied on lanes 1 and 2). (1) proteins of HeLa cell cytoskeleton fraction obtained in conditions of microtubule stabilization; (2) proteins of cytoskeleton fraction of HeLa cells incubated for 24 h before fixation in the presence of taxol (10 μg/ml).

After a 2 h effect of taxol, numerous foci consisting of radiating bundles of microtubules ("asters") were formed. The minus-ends of microtubules faced the centers of the asters [37, 38]. Thus, as in mitosis, the effect of taxol led to the concentration of the microtubule minus-ends in the local cytoplasm regions. Comparison of mitotic and interphase centrosomes staining suggests that half or more γ-tubulin in a mitotic centrosome is bound to the minus-ends of the microtubules, which allows one to expect that all aster centers should be stained with anti-γ-tubulin antibodies. However, under such conditions, γ-tubulin was revealed, as a rule, only in two centers located in the microtubule convergence foci, whereas no γ-tubulin was found in other asters. Thus, the amount of γ-tubulin at the minus-ends of the microtubules is not sufficient to be detected by immunofluorescence. Four hours after taxol was introduced, anti-γ-tubulin antibodies revealed in mitotic cells up to four spots of a similar diameter. This may be explained by the centriole splitting from the diplosomes taking place in the cells that were blocked in mitosis [39].

Thus, the decrease in γ-tubulin content in the mitotic poles of the cells blocked with nocodazole may be due not to its disappearance together with the minus-ends of the microtubules, but rather to the fact that, upon depolymerization of the microtubules around the centrosome, the centrioles quickly pass into the next (interphase) stage of their cycle [38].

The γ-tubulin amount does not correlate with the level of the in vivo *centrosome activity as the microtubule organizing center (MTOC).* Our data have shown that in normal HeLa cells the γ-tubulin content correlates with the centrosome activity as MTOC in mitosis and interphase. However, this is not the case in the centrioles of interphase cells. As we mentioned above, in interphase cells the amounts of microtubules radiating from the maternal (mature) and daughter (immature) centrioles differ several times [25, 40]. However, the amount of γ-tubulin is only slightly different (within the experimental error). Thus, in the interphase cells the amount of γ-tubulin is inconsistent with the centriole "activity" in the polymerization of microtubules.

To further elucidate the case, experiments using nocodazole and taxol treatments were performed. Upon experimental inhibition of the centrosome activity as MTOC (taxol), the content of γ-tubulin in mitotic centrosomes is decreased, whereas in interphase centrosomes it first decreases and then increases. In the case of complete depolymerization of microtubules (nocodazole), the content of γ-tubulin in the centrosomes is decreased both in mitosis and in interphase.

As was shown previously [41, 42], the cell incubation in the presence of taxol results in a significant decrease of the number of microtubules bound to the centrosome. The decrease in the γ-tubulin amount was observed upon the short-term taxol effects, which may be explained by the disappearance of microtubules around the centrosome [37]. However, later the protein amount was gradually increased and in 24 h it was double that in control. This apparent paradox may be explained if we take into account that, as the other authors [43], we observed the γ-tubulin binding along the taxol-induced stable microtubules. One may suggest that the prolonged treatment of cells with taxol results both in γ-tubulin association with stable cytoplasmic microtubules and its additional association with the centrioles. The same can also explain the fact that after incubation for four hours in the presence of taxol, the amount of γ-tubulin is only halved in the mitotic centrosomes, which do not induce polymerization of microtubules any more, whereas nocodazole causes the fourfold decrease of the γ-tubulin amount compared to control.

Summing up, we can conclude that the amount of centrosome-associated γ-tubulin is variable and is probably independent of the total content of this protein in the cell. We suggest that there are several intracellular pools of γ-tubulin. Our observations give evidence that intracellular γ-tubulin is not able to bind in any detectable amounts to the minus-ends of the newly-formed

microtubules, but it is capable of association with the long-lived stable, and possibly modified microtubules. To check this hypothesis, the time of incubation with taxol was increased, which resulted in gradual modification (acetylation) of most cytoplasmic microtubules. In a previous paper we showed [13] that after a prolonged incubation of cells in the presence of taxol, antibodies revealed γ-tubulin both in the centrosome and associated with bundles of stable microtubules. An increased total amount of intracellular γ-tubulin could be expected in this case. However, it was not the case. This means that the appearance of γ-tubulin along the bundles of stable microtubules is due to the redistribution of the pool of this protein not associated with the centrosomes rather than to its synthesis *de novo.*

Thus, the existence of three intracellular pools of γ-tubulin is suggested, which may be associated with different proteins. One of them is a constitutive component of centrioles both in mitosis and in interphase. The centrioles remain "naked" upon full depolymerization of microtubules, and in this case only a minimal amount of γ-tubulin is associated with them. This γ-tubulin pool is probably associated with basal bodies of ciliated epithelium [19], not inducing the growth of cytoplasmic microtubules, and with "inactive" centrosomes in polarized MDCK [44]. The second γ-tubulin pool forms complexes with pericentrin and plays the role of primers for microtubule polymerization, possibly by binding to their minus-ends. Most likely, just this form is associated with the centrosome activity as MTOC. This pool is recruited in mitosis and, according to our data, it is released as the cells are blocked for a long time in mitosis. In addition, as was shown before [43], up to 80% of γ-tubulin in cell cultures are in the cytoplasm. Probably, in the presence of taxol, cytoplasmic γ-tubulin may be associated with stabilized microtubules (including the microtubules of the centrioles), but it does not specifically associate with the centrosome.

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