Posttranslational Modifications of α Tubulin: Detyrosination and Acetylation Differentiate Populations of Interphase Microtubules in Cultured Cells

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Abstract. Subsets of microtubules enriched in posttranslationally detyrosinated (Gundersen, G. G., M. H. Kalnoski, and J. C. Bulinski. 1984. *Cell.* 38: 779) or acetylated (Piperno, G., M. Le Dizet, and X. Chang. 1987. *J. Cell Biol.* 104:298), α tubulin have previously been described in interphase cultured cells. In this study an immunofluorescence comparison of these minor populations of microtubules revealed that, in African green monkey kidney epithelial cells (TC-7 line), the population of microtubules enriched in detyrosinated tubulin was virtually coincident with the population enriched in acetylated α tubulin. In some cell types, however, such as human HeLa or marsupial PtK-2 cells, only one posttranslationally modified form of tubulin, i.e., acetylated or detyrosinated, respectively, was detectable in microtubules. In TC-7 cells, although both modifications were present, dissimilar patterns and kinetics of reappearance of microtubules enriched in detyrosinated and acetylated tubulin were observed after recovery of cells from microtubule-depolymerizing treatments or from mitosis. Thus, a minor population of microtubules exists in cultured cells that contains an elevated level of tubulin modified in either one or two ways. While these two modifications occur primarily on the same subset of microtubules, they differ in their patterns of formation in vivo.

Dramatic alterations in the structure and function of the microtubule cytoskeleton have been demonstrated in a variety of cellular morphogenetic events. Modulation of microtubule function could involve differential expression of functionally distinct isoforms of α or β tubulin (Fulton and Simpson, 1976; Cleveland and Sullivan, 1985), local or transient changes in the complement of assembly-promoting microtubule-associated proteins (Vallee, 1984), or posttranslational modification of tubulin subunits. However, the extremely rapid dynamics of microtubules in vivo (Schulze and Kirschner, 1986, 1987) suggest the involvement of posttranslational modifications of tubulin subunits as rapid and reversible modulators of function and organization of subsets of cellular microtubules. Three posttranslational modifications of tubulin have been described: a tyrosination (Barra et al., 1974) and an acetylation (L'Hernault and Rosenbaum, 1985) of α tubulin, and a phosphorylation of β tubulin (Eipper, 1972; Sandoval and Cuatrecasas, 1976; Gard and Kirschner, 1985).

Recent studies have demonstrated that both tyrosinated and acetylated α tubulin species are differentially distributed in the interphase microtubule arrays of cultured cells. Immunofluorescence and immunoelectron microscopy with antibodies specifically reactive with tyrosinated (Tyr)[1] and detyrosinated (Glu) α tubulin demonstrated that cellular microtubules are heterogeneous in their content of these two species; most microtubules contain predominantly Tyr tubulin, while a small number contain largely Glu tubulin (Gundersen et al., 1984; Geuens et al., 1986). In similar studies using immunofluorescence, an antibody specifically reactive with acetylated (Ac) α tubulin labeled a small subset of the total microtubule array. Both the minor populations of microtubules enriched in Glu α tubulin and those enriched in Ac α tubulin exhibited a characteristic morphology and a similar recovery after release of cells from treatments that depolymerized cellular microtubules; reappearance of microtubules enriched in either Glu (Gundersen et al., 1987*a*) or Ac (Piperno et al., 1987) tubulin was delayed relative to the bulk of the microtubule array. In addition, in separate studies, both Glu and Ac tubulin were found to be enriched in stable microtubules such as those in cilia and flagella (Piperno and Fuller, 1985; Gundersen and Bulinski, 1986), in taxol-treated cells (Gundersen et al., 1987*a*; Piperno et al., 1987), and in cells treated with microtubule-depolymerizing drugs (LeDizet and Piperno, 1986; Piperno et al., 1987; Khawaja et al., 1988).

In this study, we compared the distributions of Glu and Ac tubulin in the microtubules of cultured cells in order to answer the following questions: Do the two posttranslational modifications, detyrosination and acetylation, demarcate the same or different populations of microtubules? Is the tem-

1. *Abbreviations used in this paper:* Ac, acetylated; Glu, detyrosinated; Tyr, tyrosinated.

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poral occurrence of each form of modified tubulin consistent with a common mechanism by which both acetylation and detyrosination are formed concomitantly on the same subset of cellular microtubules? In addition, localization of Glu and Ac tubulin in the same cells might provide circumstantial evidence that would be useful in determining the function of each posttranslational modification.

Materials and Methods

Cell Culture

African green monkey kidney cells (TC-7) were grown on glass coverslips as described in Gundersen et al. (1984). PtK-2 cells, a gift of Michael Berns, University of California at Irvine, were cultured on coverslips in F-10 medium containing 10% FBS, and HeLa cells were cultured in MEM containing 10% FCS. TC-7 cells were fixed in cold methanol (−20°C, 5 min), PtK-2 cells were fixed and extracted as described by Geuens et al. (1986), and HeLa cells were fixed and extracted according to Piperno et al. (1987); however, differences in fixation techniques appeared to make no qualitative difference in the results obtained.

Drug Treatments

TC-7 cells were grown on glass coverslips (for immunofluorescence) or plastic petri dishes (for Western blots) for 2-3 d, then treated with 4 μM nocodazole for 4 h as described previously (Gundersen et al., 1987*a*). At appropriate times after release from the drug treatment, cells were methanol fixed for immunofluorescence or prepared for immunoblot analysis as described below.

Immunofluorescence

Immunofluorescence was performed as described in Gundersen et al. (1984), except that the following antibodies were used. For double immunofluorescence of Ac and Glu α tubulin, the primary antibody solution contained 1% (vol/vol) rabbit antibody specifically reactive with Glu tubulin (Gundersen et al., 1984), 1% normal goat serum, and 98% hybridoma supernatant specifically reactive with Ac α tubulin (6-11B-1; Piperno and Fuller, 1985). For immunofluorescence of total α tubulin in TC-7 and PtK-2 cells, primary antibody solution consisted of 1% (vol/vol) Glu antibody, 1% rabbit antibody specifically reactive with Tyr α tubulin (Gundersen et al., 1984), 1% normal goat serum, and 97% PBS. HeLa cells were stained for total tubulin with B-5-1-2, a mouse monoclonal antibody specifically reactive with α tubulin (Piperno et al., 1987). Secondary antibody solution consisted of PBS containing a 1:50 dilution of fluorescein-conjugated goat anti-rabbit IgG (CooperBiomedical, Inc., Malvern, PA), and a 1:25 dilution of biotin-conjugated goat anti-mouse IgG (Amersham Corp., Arlington Heights, IL), followed by a 1:25 dilution of Texas Red-labeled streptavidin (also from Amersham Corp.). Microscopy and photography were performed exactly as described earlier, except that a Texas Red filter (Omega Optical, Inc., Brattleboro, VT) was used to visualize staining of Ac tubulin.

Western Blot Analysis

Cells were grown and nocodazole-blocked in 100-mm plastic tissue culture dishes. A coverslip included in each dish was fixed for immunofluorescence at the time of drug release in order to ascertain that microtubule depolymerization was virtually complete. Whole cell samples of TC-7 cells were prepared as described previously (Gundersen et al., 1987*a*) except that cells were solubilized from nearly confluent dishes into 1 ml of SDS sample buffer (2% SDS, 100 mM Tris-HCl [pH 6.8], 30% glycerol, 50 mM dithiothreitol, and 0.2 mM phenylmethylsulfonyl fluoride) and boiled 5 min. Samples were subjected to electrophoresis and transferred to nitrocellulose, essentially as described elsewhere (Gundersen et al., 1987*a*). Blots were reacted with Glu rabbit antibody (diluted 1:5,000) or Ac mouse hybridoma supernatant (diluted 1:4) followed by horseradish peroxidase-conjugated goat anti-rabbit or goat anti-mouse IgG (both from CooperBiomedical, Inc.; diluted 1:1,000). Dried blots were analyzed with a Video Densitometer (model 620; Bio-Rad Laboratories, Richmond, CA) in the Reflectance Mode.

Results

Colocalization of Ac and Glu Tubulin in Cultured Cells

We used double-indirect immunofluorescence to colocalize Glu and Ac tubulin in TC-7 cells, making use of two previously characterized antibodies: a rabbit peptide antibody, specifically reactive with Glu α tubulin (Gundersen et al., 1984) and a mouse monoclonal antibody, specifically reactive with Ac α tubulin (Piperno and Fuller, 1985). Fig. 1, *a* and *b*, demonstrates that in TC-7 cells, the microtubules enriched in Glu tubulin (Glu microtubules) essentially coincide with those enriched in Ac tubulin (Ac microtubules). Fig. 1 *c* shows the complete microtubule staining pattern of a TC-7 cell for comparison. As observed in other cell types (Gundersen and Bulinski, 1986; Piperno et al., 1987), the Glu and Ac antibodies label only a minor population of the total microtubules in this cell line, thus making the coincidence of the Glu and Ac tubulin patterns striking. The staining along individual fibers appears more continuous with the Glu than with the Ac antibody. This apparent heterogeneity in the level of Ac tubulin along a microtubule was consistently seen in TC-7 cells, regardless of fixation or staining conditions. Also, the labeling of some microtubules or bundles of microtubules showed differences in intensity of Glu and Ac antibody labeling; microtubules, or entire cellular arrays of microtubules, that were moderately stained in one image sometimes appeared quite dim in the other image. In other cell lines in which both Glu and Ac microtubules were observed (for example, mouse 3T3 cells), the patterns were always nearly coincident, except for the noticeably segmented appearance of the Ac microtubules. In this cell type, differences in staining intensity were often apparent between cells: in some cells, all Glu microtubules were more brightly labeled than all Ac microtubules, while in adjacent cells the converse was true (data not shown).

We also compared the distribution of Glu and Ac tubulin in cell types whose microtubules possessed disparate levels of the two posttranslationally modified tubulin forms. For example, we examined HeLa cells, since most HeLa cells (excepting some multinucleate or "giant" cells) lack microtubules detectably stained with Glu antibody (Fig. 1 *d*). Although the staining of HeLa cells with Glu antibody is grainy, these cells do contain a low amount of polymeric Glu tubulin; this level, ∼3%, is detectable by Western blots (data not shown), but is not sufficiently concentrated in fibers to permit distinct Glu staining of individual microtubules. HeLa cells do contain a number of fibers that can be visualized with Ac antibody (Fig. 1 *e*), many of which appear to be continuously labeled. Thus, in this cell line a similar distribution of the Glu and Ac tubulin is not observed. Fig. 1 *f* shows the pattern obtained when immunofluorescence was used to visualize the complete microtubule network; as in other cell types (e.g., Fig. 1 *c* and *i*, also see Piperno et al., 1987), the Ac microtubules in HeLa cells constitute a minor subset of the total microtubule complement. Treatment of HeLa cells with the microtubule-stabilizing drug, taxol (1 μM, 4 h), resulted in a dramatic increase in the level of both Glu and Ac tubulin. In fact, taxol-treated cells not only contained Glu microtubules, but both Ac and Glu patterns in drug-treated cells were indistinguishable from the total microtubule pattern (data not shown). A comparison of Glu

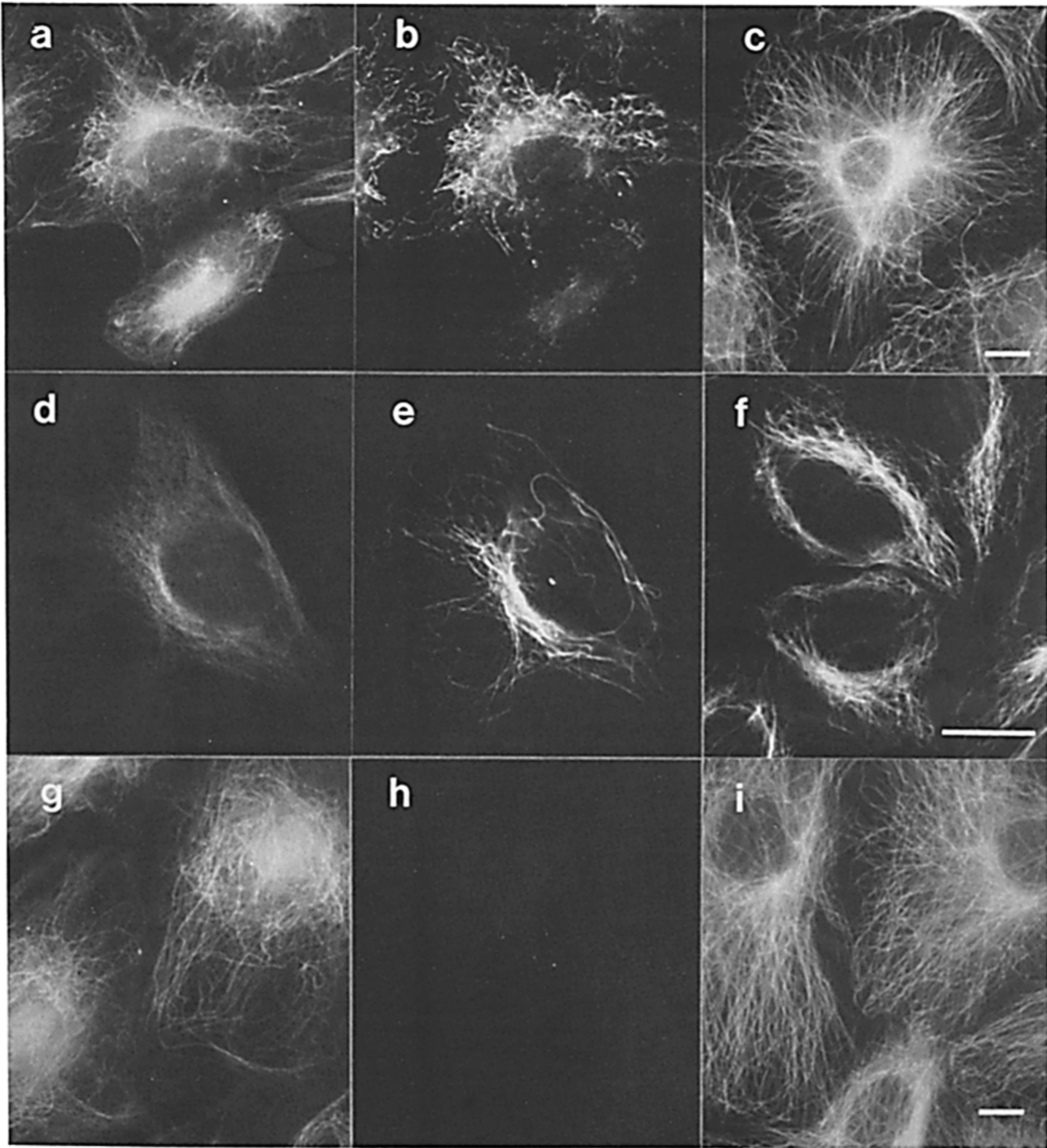
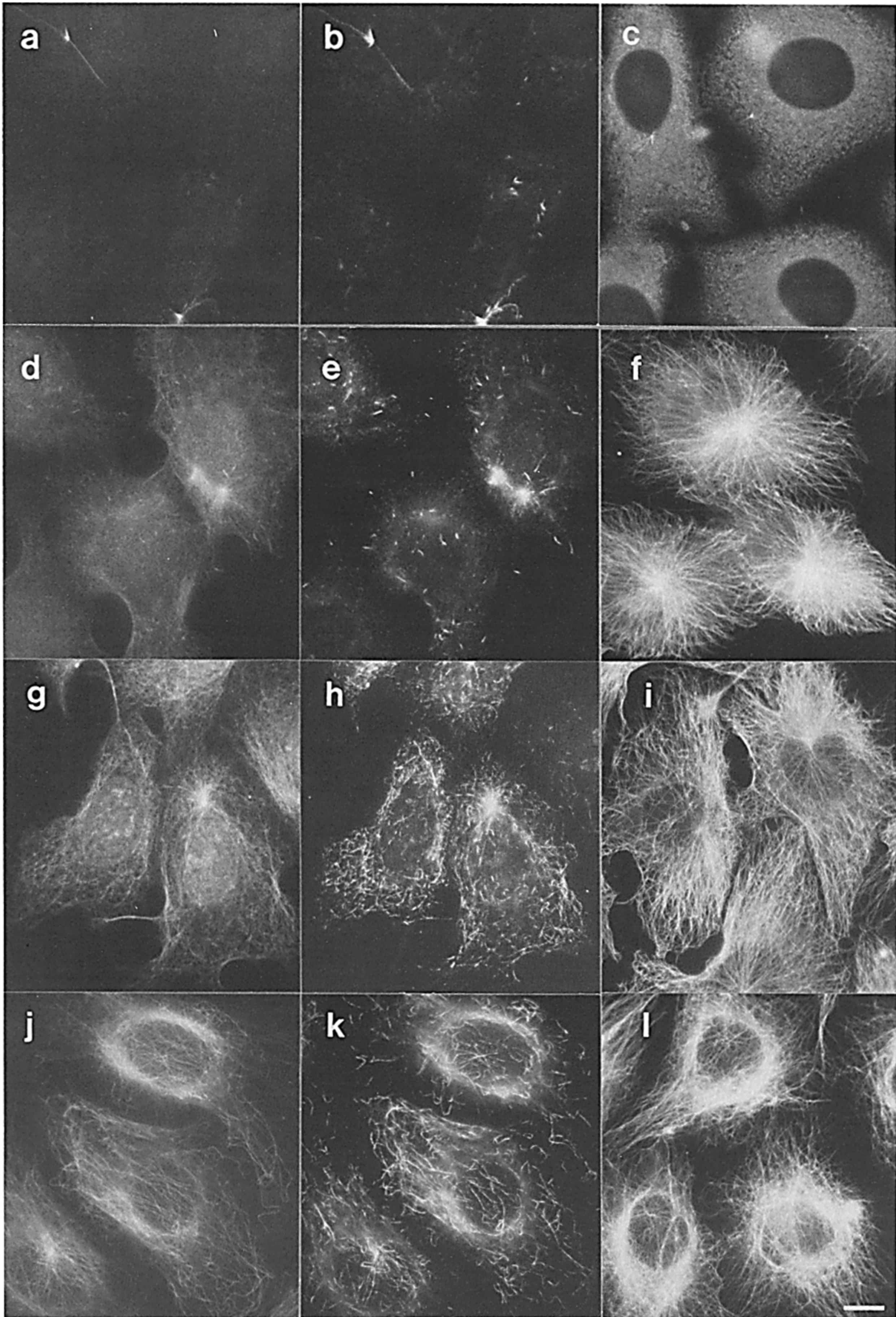


Figure 1. Distribution of Glu, Ac, and total tubulin in vivo. TC-7 (*a–c*), HeLa (*d–f*), and PtK-2 (*g–i*) cells were fixed and stained as described in Materials and Methods. Double-indirect immunofluorescence was used to obtain images of Glu (*a*, *d*, and *g*) and Ac (*b*, *e*, and *h*) tubulin: single immunofluorescence of comparable cells yielded images of total tubulin (*c*, *f*, and *i*). Bar, 10 μm.

and Ac patterns in human or chick embryo fibroblasts gave results identical to those obtained for HeLa cells.

Glu and Ac tubulin distributions were also compared in rat kangaroo (PtK-2) cells. As shown in Fig. 1, *g–i*, our results confirmed those obtained earlier with another subculture of this cell line: the cells contained a population of microtubules stained with Glu antibody (Gundersen and Bulinski, 1986), but they were completely devoid of Ac staining, either within the extensive microtubule network or in other structures such as the midbodies of postmitotic cells (Piperno et al., 1987). In addition, both the primary cilia and the centrioles lacked detectable Ac staining in the PtK-2 cells we examined. Taxol-treated PtK-2 cells gave results that differed from those obtained with similarly treated HeLa cells: although taxol increased the number of Glu microtubules and the level of Glu tubulin, no Ac tubulin or Ac microtubules could be detected after taxol treatment (data not shown; Piperno et al., 1987). Thus, cells in which either Glu or Ac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staining is not detectable demonstrate that the presence of arrays of both Glu and Ac microtubules is not an obligate feature of the interphase microtubule network of cultured cells.

Reformation of Ac and Glu Microtubules after Release from Drug-induced Depolymerization

Previous reports indicated that, when cells were released from cold or drug treatments that depolymerized cellular microtubules, the timing of reappearance of both Glu and Ac microtubules lagged behind the timing of the repolymerization of the total microtubule network (in Gundersen et al., 1987*a*, and Piperno et al., 1987, respectively). To determine the temporal relationship between the accumulation of the two posttranslationally modified forms of tubulin in cellular microtubules, we monitored the reappearance of Glu and Ac microtubules after release of TC-7 cells from nocodazole-induced depolymerization. Fig. 2 shows that before drug release (T = 0; Fig. 2, *a–c*), virtually no microtubules could be visualized with Glu, Ac, or tubulin-specific antibodies. Glu staining (Fig. 2 *a*) was limited to approximately two drug-resistant microtubules in each cell, as well as a bright dot adjacent to the nucleus, which corresponds to staining of the centrioles (Gundersen and Bulinski, 1986). Bright dots of centriolar staining were also observed in the Ac staining pattern; however, unlike the Glu-stained image, the Ac image contained short microtubule segments (Fig. 2 *b*). In the total tubulin staining pattern, the bright diffuse staining of monomeric tubulin was also obvious (Fig. 2 *c*). After release from nocodazole, microtubules reformed rapidly as an aster; neither Ac nor Glu microtubules were detectable for ~3 min (not shown). Shortly thereafter (T = 5 min) the patterns of Glu and Ac tubulin staining began to differ markedly: Glu staining appeared only dim and diffuse except for brighter staining in the centrosomal region (Fig. 2 *d*) while multiple dots and small segments of Ac staining could be seen (Fig. 2 *e*). At this time the array of cellular microtubules (see antitubulin image; Fig. 2 *f*) was already extensive. By 15 min after release from nocodazole, significant staining for each posttranslationally modified form was detectable (Fig. 2, *g* and *h*). However, the patterns observed were markedly different: the Ac pattern again consisted of bright segments, in this case more and longer than those seen at 5 min (compare Fig. 2, *e* and *h*). Glu tubulin immunofluorescence was first detected as hazy staining (T = 15 min; Fig. 2 *g*), in contrast to the discrete pattern of Ac microtubule segments observed at this time. Glu microtubules became increasingly distinct during the time course until, eventually (T = 30 min, Fig. 2, *j* and *k*), the staining of Glu and Ac microtubules began to resemble those seen in untreated cells (see Fig. 1, *a* and *b*). By 60 min after nocodazole release (data not shown), the arrays of Glu, Ac, and total tubulin were more focused to the centrosome and were indistinguishable from those in untreated cells. Thus, under our staining conditions, a significant level of Ac staining of spotty segments could be detected at earlier time points than could the Glu staining of either hazy or distinct microtubules (Fig. 2). We determined that the staining patterns represented a real difference in the distributions of Ac and Glu tubulin. By using either higher levels of primary antibody or the enhanced sensitivity of the Texas Red fluorochrome, Glu staining appeared brighter but the pattern of Glu staining (grainy or hazy, without distinct segments or spots) was unchanged. Conversely, use of a less sensitive fluorochrome (fluorescein, rather than Texas Red) to detect Ac staining altered only the brightness but not the pattern of Ac microtubules, spots, and segments.

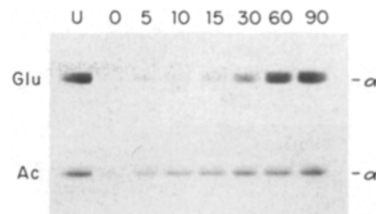

Figure 3. Immunoblot analysis of Glu and Ac tubulin in extracts of TC-7 cells recovering from nocodazole treatment. Immunoblot samples were prepared from TC-7 cells and probed with Glu and Ac antibodies as described in Materials and Methods. Sample from an equal number of cells was loaded onto each lane; that these contained a constant amount of protein and tubulin was determined by staining the blots with Naphthol blue-black and with tubulin antibody. *U*, untreated cells; numbers given above each lane indicate time (in minutes) after release from nocodazole treatment. Blots were immunostained with Glu and Ac antibodies. α, the α tubulin band.

Recovery of Steady-State Levels of Ac and Glu Tubulin after Nocodazole Release

In addition to examining the immunofluorescence patterns of Glu and Ac microtubules, we also examined the level of each posttranslationally modified tubulin accumulated at intervals after release from nocodazole. The results of Western blot analysis of extracts of TC-7 cells are shown in Fig. 3. As compared to the levels of Glu and Ac tubulin in untreated TC-7 cells (lanes *U*), in nocodazole-treated cells (lanes *0*), Glu and Ac levels were greatly reduced. In most experiments a faint band for each was barely detectable on the original blot. After nocodazole release, both Ac and Glu tubulin staining could be visualized at T = 5 min (lanes *5*). At longer times after recovery (30–90 min after nocodazole release; lanes *30*, *60*, and *90*) both Ac and Glu tubulin amounts increased such that the levels appeared similar to those in untreated cells. The timing of recovery of the steady-state level of each posttranslationally modified form of tubulin was quantified from densitometric scans of Western blots, as shown in Fig. 4, in which the recovery from 0 (not detectable by our scanner) to >100% (the level of modified tubulin in untreated cells) is plotted as a function of time. Fig. 4 shows that the recovery of the steady-state level of Ac tubulin is significantly

Figure 2. Microtubule regrowth after release from nocodazole treatment in TC-7 cells. Cells were treated with 4 μm nocodazole for 4 h to depolymerize microtubules; then cells were released by incubation in drug-free medium for the following intervals before methanol fixation: 0 min (*a–c*); 5 min (*d–f*); 15 min (*g–i*); 30 min (*j–l*). Cells were stained as described in Materials and Methods to visualize the patterns of Glu (*a*, *d*, *g*, and *j*) and Ac (*b*, *e*, *h*, and *k*) in the same cells, and the total tubulin network (*c*, *f*, *i*, and *l*) in comparable cells. Bar, 10 μm.

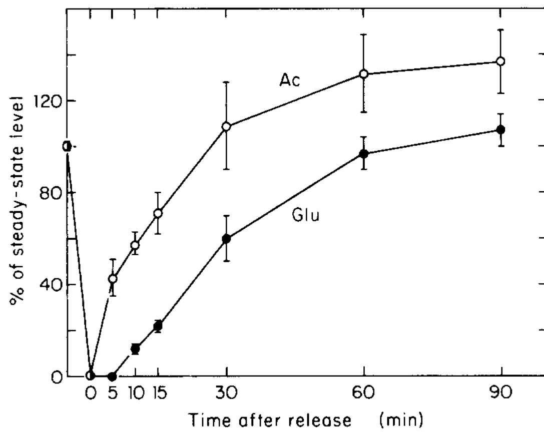

Figure 4. Recovery to steady-state levels of Glu and Ac α tubulin in TC-7 cells after release from nocodazole treatment. Immunoblots such as the ones in Fig. 3 were analyzed by densitometry, and the level of immunoreactivity in each sample of drug-released cells was expressed as the percentage of the level measured in untreated cells from the same experiment. 100% level was defined as the level in untreated cells; 0% indicates a level below the detection limit in our scanning assay. Each point on the graph represents the mean ± SEM of two blots each of two experiments.

faster than the recovery of Glu. While the Glu level barely reached its steady-state level within 90 min, the Ac tubulin level had reverted to that in untreated cells by 30 min following nocodazole release and continued to increase during the next 60 min. The "overshoot" in the level of Ac tubulin is probably analogous to a similar overshoot reported for Glu tubulin levels in other drug recovery experiments (Gundersen et al., 1987*a*); however, we do not yet understand the nature of this overshoot phenomenon. In analysis of Ac and Glu tubulin levels in monomeric and polymeric fractions (Caron et al., 1985; Gundersen et al., 1987*a*) obtained from cells released from nocodazole treatment, both modified forms of tubulin were found primarily in the polymeric fractions at each time point, and the appearance of each lagged behind the appearance of total tubulin polymer (data not shown). Therefore, the examination of cells allowed to regenerate normal levels of Ac and Glu tubulin after release from nocodazole revealed spatially distinct patterns by immunofluorescence assay and different timing of recovery by quantitative immunoblots.

Recovery of Ac and Glu Tubulin after Mitosis

It is conceivable that microtubule regrowth in cells released from a drug treatment such as nocodazole would not reflect the microtubule behavior typical of untreated cells. For this reason, we examined the regrowth of microtubules after mitosis. Cells with persistent midbodies were identified in log phase cultures of TC-7 cells, and their Ac, Glu, or total microtubule networks were examined. Since these cells were not as synchronous as cells released from nocodazole, we could only evaluate the timing of appearance of Ac and Glu microtubules statistically; we could not measure their cellular level. We scored pairs of daughter cells joined by a midbody and we found that many cells possessed distinct Ac microtubule segments, with no distinct Glu microtubules (32:100 pairs scored), while the remainder consisted of cells that contained neither Ac nor Glu microtubules (8:100), and cells with both Ac and Glu microtubule arrays (60:100). No cells (0:100) were found that contained an array of Glu microtubules but no array of Ac microtubules. Fig. 5 shows that, as observed in the nocodazole release experiment, the pattern of Ac and Glu reappearance differed. Results from this "physiological microtubule regrowth," then, indicate that the more detailed information obtained from the nocodazole experiment does not represent some unforeseen artifact that resulted from the drug treatment.

Discussion

In this study we have explored the relationship between two posttranslational modifications of tubulin in cultured mammalian cells. We have determined that, in cells in which both modifications are detectable, such as 3T3 (data not shown) and TC-7, both Ac and Glu antibodies label the same subset of cellular microtubules. Previous studies domonstrated that microtubules that were visibly stained with Glu antibody at the light microscopic level also contained quantitatively high

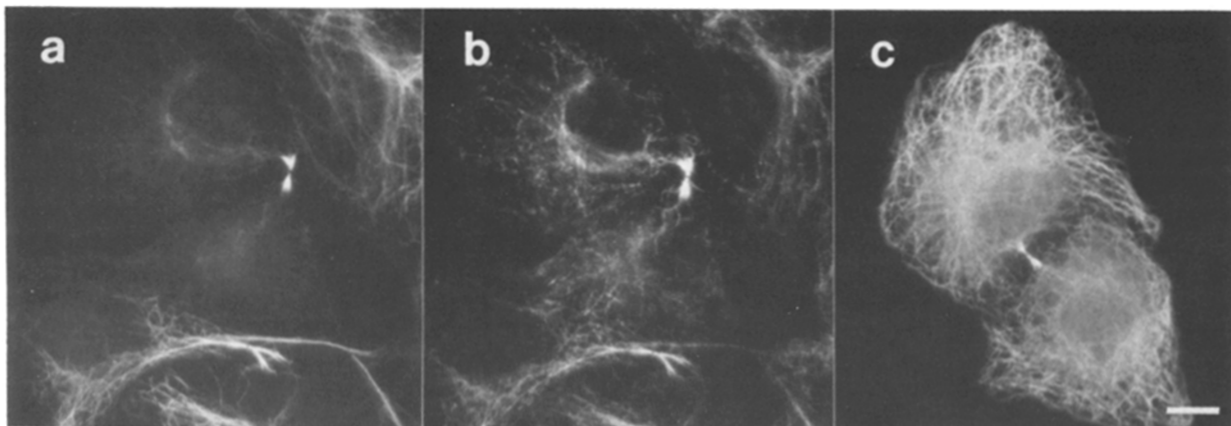

Figure 5. Distribution of Glu, Ac, and total tubulin in TC-7 cells after mitosis. Cells that were in the G_1 phase of the cell cycle, as judged by the midbodies connecting them, were double stained to localize Glu (*a*) and Ac (*b*) tubulin, and comparable postmitotic cells were single stained to visualize total tubulin (*c*). Bar, 10 μm.

immunoreactivity with Glu antibody at the electron microscopic level (Geuens et al., 1986). In addition, increases in the number of microtubules labeled with the Glu antibody correspond to increases in the level of total cellular Glu tubulin in Western blots (Gundersen et al., 1987*a*), and in one case, this has also been demonstrated for Ac tubulin (Piperno et al., 1987). Thus, results from this study, combined with previous results, indicate that in several cell types, a subset of cellular microtubules is enriched in both Ac and Glu tubulin.

Results presented in this paper are consistent with the suggestion of Piperno et al. (1987), that Ac microtubules arise by postpolymerization modification of unacetylated tubulin. Ac tubulin was primarily detectable in polymeric tubulin, and its appearance in polymer lagged behind the appearance of total microtubule polymer in cells released from drug-induced microtubule depolymerization. Glu microtubules were previously shown to arise by postpolymerization modification of Tyr tubulin (Gundersen et al., 1987*a*). Although coincident populations of microtubules are modified and a similar postpolymerization modification is implicated in each case, differences in the pattern of accumulation of each modified α tubulin form suggest that Ac and Glu microtubules are generated by two independent mechanisms. In the drug release experiment, which was performed with two cell types (African green monkey kidney [see Fig. 2] and mouse 3T3 fibroblasts [not shown]), Ac microtubules could be detected sooner than Glu microtubules. The difference in appearance of nascent Ac and Glu microtubules was striking: Ac microtubules appeared as discrete small segments that became longer at longer times after drug release, while Glu microtubules appeared to be more uniformly converted from Tyr microtubules, with no discrete Glu segments observed at any time after nocodazole release. This pattern was echoed in untreated cells of these two lines: Ac staining along the length of a microtubule usually appeared discontinuous and sometimes punctate, while Glu staining almost always appeared to be fairly uniform along the entire length of a microtubule. This uniformity of Glu staining was previously examined quantitatively at the ultrastructural level (Geuens et al., 1986). These observations suggest that particular domains of a microtubule, either at ends or along the length of a fiber, may favor binding and acetylation by the tubulin acetylating enzyme, although ultrastructural localization of Ac tubulin has not yet been carried out to verify this.

Two cell types discussed, HeLa and PtK-2, did not exhibit coincident populations of Ac and Glu microtubules. All HeLa cells contained Ac microtubules, but virtually all lacked Glu microtubules, although they contained Glu-staining centrosomes and a low, but detectable, level of Glu tubulin as determined by Western blot analysis. This lack of detectable Glu microtubules could result from a low level of the detyrosinating enzyme, tubulin carboxypeptidase, or from a rate of microtubule turnover too rapid to permit accumulation of Glu protomers by postpolymerization modification. Either explanation is supported by our ability to induce Glu microtubules, which are coincident with Ac (and, indeed, with the total microtubule network), in cells treated with the microtubule-stabilizing drug, taxol. The situation in PtK-2 cells, which contain Glu microtubules but no Ac microtubules, is apparently not a consequence of rapid turnover of cellular microtubules that fail to accumulate detectable levels of Ac tubulin. Examination of centrioles, primary cilia, midbodies, and cytoplasmic microtubules stabilized by taxol suggest that the lack of Ac microtubules can more readily be explained by a lack (or a very low level) of activity of the tubulin acetylase. To date, this enzyme has been assayed only in *Chlamydomonas* material (Greer et al., 1985; Maruta et al., 1986); its presence in cultured cell lines has only been presumed by antibody detection of its presumptive product, Ac tubulin.

Properties that have previously been demonstrated for either Ac or Glu microtubules can now be ascribed to both, at least in cell types such as TC-7 and 3T3, in which the two populations are coincident. For example, because Glu microtubules were shown to arise by a postpolymerization mechanism in vivo (Gundersen et al., 1987*a*), microtubules enriched in Glu tubulin represent "old" microtubules, whose elapsed time since polymerization is greater than that of adjacent Tyr microtubules. Coincidence of Ac and Glu microtubules indicates that an enrichment in Ac tubulin is another characteristic of old microtubules. In addition, LeDizet and Piperno (1986) and Piperno et al. (1987) demonstrated that microtubules that remain after treatments of many cell types with drugs, that incompletely depolymerize cellular microtubules were Ac microtubules. Similarly Khawaja et al. (1988) monitored the kinetics of the rapid breakdown of cellular microtubules in response to depolymerizing treatments; in this case, microtubules enriched in Glu tubulin were found to be significantly more stable against depolymerization than those enriched in Tyr tubulin, although increased stability could not be induced by enzymatically detyrosinating microtubules in cytoskeletons. It can therefore be concluded that the subpopulation of microtubules enriched in Ac and Glu protomers is more resistant to drug-induced depolymerization. However, it is likely that at least detyrosination, and possibly acetylation, occur on microtubules already differentiated by another mechanism, such as capping of their ends (Khawaja et al., 1988).

A final property that can now be ascribed to microtubules enriched in both Ac and Glu tubulin is their decreased dynamics. Both have been found in flagella and other nondynamic microtubule arrays (Piperno and Fuller, 1985; Piperno et al., 1987; Gundersen and Bulinski, 1986). In TC-7 cells, it has recently been demonstrated that Glu microtubules are nongrowing; i.e., they are not undergoing addition of tubulin subunits (Gundersen et al., 1987*b*; Webster et al. 1987). Since these microtubules coincide with Ac microtubules in TC-7, we can now conclude that, at least in this cell line, Ac microtubules are also nongrowing. Neither the role of detyrosination and/or acetylation in preventing subunit addition nor the fate of these nongrowing microtubules, either depolymerization or enhanced longevity, has yet been addressed. It is interesting to note that, to date, all of the more than 25 cell lines we have examined have been found to possess a subset of microtubules enriched in either Glu or Ac tubulin or both. While many unusual properties have been ascribed to the subset of cellular microtubules enriched in Ac and/or Glu tubulin, the functional significance of these microtubules remains to be discovered.

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