Microtubules are acetylated in domains that turn over slowly

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Summary

Tubulin is subject to a post-translational acetylation reaction that is thought to be correlated with increased stability of the modified microtubules (MTs). We sought to test directly the stability of acetylated MTs by determining their specific rate of turnover. We used human fibroblasts, which contain a subset of MTs that display terminal and internal domains of acetylation. The turnover of acetylated domains was analysed by microinjecting cells with biotinylated brain tubulin and determining, by triple-label immunofluorescence, the progress of incorporation of biotinylated tubulin into acetylated and non-acetylated domains. Within two minutes after injection, biotinylated domains were contiguous with virtually all observed non-acetylated MT ends but were not contiguous with terminal acetylated domains, demonstrating that the former were growing while the latter were not. Ten minutes after injection, many MTs lacking acetylated domains had incorporated biotinylated subunits uniformly while most MTs containing acetylated domains remained unlabelled, indicating that non-acetylated MTs were turning over while most acetylated domains were not. One hour after injection, virtually all non-acetylated MTs were labelled with biotin whereas approximately half of the acetylated domains contained biotin, demonstrating that acetylated domains turned over much more slowly than the non-acetylated, bulk array. Non-acetylated MT regions flanking acetylated domains also lacked hapten, indicating that acetylation modified discrete regions along stable MTs. Sixteen hours after injection, cells that had not entered mitosis still retained acetylated domains that had not turned over (13% of all acetylated domains), indicating that acetylated domains can be extremely long-lived. Prophase cells displayed no acetylated tubulin staining, indicating that the maximum lifetime of cytoplasmic acetylated domains was one cell cycle. The results show that in this cell line only limited domains of stable MTs usually become acetylated, and that the turnover of these domains and the MTs that bear them is much slower than that of the bulk array.

Key words: acetylated tubulin, post-translational modification, microtubule turnover, acetylation, triple-label immunofluorescence.

Introduction

The cytoplasmic microtubule (MT) network has recently been recognized to be a highly dynamic entity, turning over with a half-time of minutes (Saxton *et al.* 1984; Soltys & Borisy, 1985; Schulze & Kirschner, 1986; Sammak *et al.* 1987). The plasticity of the cytoskeleton offers a way of understanding how motile and differentiating cells might accommodate changes in shape. However, the maintenance of asymmetric cell forms would also seem to imply a need for mechanisms of selective stabilization (Kirschner & Mitchison, 1986). The concept of a uniform, dynamic MT cytoskeleton was modified by the identification of MT subsets that turn over more slowly than the bulk array (Schultze & Kirschner, 1987; Kreis, 1987; Webster *et al.* 1987*b*). In two of these studies (Kreis, 1987; Webster *et al.* 1987*b*) the composition of this stable MT subset was found to include an elevated level of tubulin subunits that were post-translationally modified by detyrosination, a unique process whereby the carboxyl-terminal tyrosine of alpha tubulin is reversibly removed (Barra *et al.* 1974). Although this finding offered a potential mechanism for selectively stabilizing individual MTs for specific functions, results from other studies (Wehland & Weber, 1987; Bré *et al.* 1987; Khawaja *et al.* 1988) suggested that detyrosination alone does not generate MT stability but instead occurs on MTs stabilized by another mechanism.

Alpha tubulin can also be post-translationally modified by the reversible addition of an acetyl group to Lys40 (L'Hernault & Rosenbaum, 1983, 1985; LeDizet & Piperno, 1987), and MT subsets have been identified by virtue of their reactivity with antibodies specific for acetylated (Ac) alpha tubulin (Piperno *et al.* 1987; Schulze *et al.* 1987; Black & Keyser, 1987; Sale *et al.* 1988; Bulinski *et al.* 1988). In some cases the MT staining appeared fragmented or segmental (Piperno *et al.* 1987; Bulinski *et al.* 1988), in contrast to the uniform

staining detected using antibodies to detyrosinated (Glu) tubulin (Geuens *et al.* 1986). However, the studies of Ac tubulin staining did not offer sufficient resolution to determine whether the segments comprised domains within longer MTs or stained short MTs uniformly. Ac MTs were also found to resist drug-induced depolymerization (Black & Keyser, 1987; Sale *et al.* 1988) but were as labile to cold treatments as the bulk, dynamic array (Piperno *et al.* 1987).

We have investigated the distribution and turnover of Ac MTs in a human cell line that contains few or no MTs enriched in Glu subunits (Glu MTs) (Webster *et al.* 1987*a*), using the techniques of hapten-mediated immunocytochemistry and triple-label immunofluorescence as described (Webster *et al.* 1987*b*). We found that Ac tubulin antibodies stained terminal and internal domains along a subset of MTs and a limited number of MTs along their entire lengths. Terminal Ac domains were not efficient substrates for end-growth of injected subunits, and the subset as a whole turned over much more slowly than the bulk array. Non-Ac flanking regions bordering these domains also did not turn over with the bulk array, suggesting that Ac domains occupy regions along stable MTs.

Materials and methods

Preparation of biotinylated tubulin

MT protein was isolated from porcine brain as described (Borisy *et al.* 1975) and tubulin was separated from MT-associated proteins (MAPs) by DEAE-Sephadex chromatography (Murphy, 1982). Tubulin was biotinylated essentially as described by Kristofferson *et al.* (1986), except that, after incubation with *N*-hydroxysuccinimidyl biotin (Sigma Chemical Co., St Louis, MO, USA), the MTs were centrifuged and subjected to two rounds of temperature-induced assembly and disassembly using 5 % (v/v) dimethylsulphoxide (Himes *et al.* 1976). Samples of this material were frozen in liquid nitrogen until use.

Cells and microinjection

Human foreskin fibroblasts (type 356) were a gift from the laboratory of Dr Robert DeMars, University of Wisconsin, Madison, WI, and were maintained in Ham's F-10 medium supplemented with 15 % foetal bovine serum. Cells were microinjected with biotinylated tubulin at room temperature as described (Soltys & Borisy, 1985). We injected approximately 10 % of the cell volume with 4 mg ml^{-1} tubulin to achieve a final intracellular ratio of approximately 1 biotinylated subunit to 5 underivatized subunits (calculated from data of Hiller & Weber, 1978). Cells were incubated at 37 °C for established times before being given a rapid wash in PHEM buffer containing 60 mM-Pipes, pH 6·95, 25 mM-Hepes, 10 mM-EGTA, 2 mM-$MgCl_2$ (Schliwa *et al.* 1981), followed by lysis in PHEM containing 0·1 % Triton X-100 for 1 min. Lysed cells were fixed for 20 min with 5 mM-ethylene glycol bis-(succinic acid *N*-hydroxysuccinimide ester) (Sigma Chemical Co., St Louis, MO, USA). All procedures through fixation were performed at 37 °C.

Triple-label immunofluorescence

Injected, fixed cells were first incubated with Texas Red-conjugated streptavidin (Jackson Immunoresearch, Avondale, PA, USA) to detect injected, biotinylated subunits that had become incorporated into MTs. Cells were next incubated simultaneously with a hybridoma supernatant containing a mouse monoclonal antibody (IgG) to Ac tubulin (a gift from Dr G. Piperno, Rockefeller University, New York, NY, USA) and a peptide-specific, rabbit polyclonal antiserum (IgG) to tyrosinated tubulin (a gift from Drs Jeannette Bulinski and Gregg Gundersen, Columbia University, New York, NY, USA). The antibody to Tyr (tyrosinated) tubulin was used in this study because it labels virtually all MTs in this cell line (Webster *et al.* 1987*a*), it produces a strong immunofluorescence signal, and it was produced in a rabbit, thereby precluding any cross-reactivity with the mouse antibody to Ac tubulin. The cells were next incubated with fluorescein-conjugated, goat anti-mouse antibodies to detect Ac subunits. Coverslips were then mounted in a solution containing 10 % (w/v) polyvinyl alcohol and 2–4 mg ml^{-1} *p*-phenylenediamine (Sammak *et al.* 1987), and photographed using a Zeiss Universal microscope (Carl Zeiss, Inc., Thornwood, NY, USA) equipped with epifluorescence and a Planapochromat 63× (1·4 NA) objective. MT patterns were photographed with Technical Pan film (type 2415) that was hypersensitized using the method described by Lumicon, Inc. (Livermore, CA, USA) and developed with HC-110 developer (Dilution A). After the biotinylated and Ac MT patterns were photographed, the coverslips were demounted and washed by sequential 2–4 h incubations in distilled water and mounting medium minus the polyvinyl alcohol. Cells were then stained with Texas Red-conjugated, goat anti-rabbit antibodies to detect the bulk array, remounted and photographed as before. Coverslips containing uninjected cells were immunolabelled with the Tyr and Ac tubulin antibodies, followed by Texas Red-conjugated, goat anti-rabbit and fluorescein-conjugated, goat anti-mouse antibodies.

Quantification of MT turnover

Ac MTs from uninjected and injected cells were traced onto clear acetate sheets as described (Webster *et al.* 1987*a*). Next, the bulk MT pattern of uninjected cells or the biotin MT pattern of injected cells was aligned with the Ac MT pattern by the use of MTs staining with both labels or by the use of other cellular landmarks, and the second pattern was then also traced. Finally, the bulk MT pattern in injected cells was aligned with the first two patterns and similarly traced to classify Ac domains as either terminal, internal or uniform with the bulk pattern. Ac domains that also contained biotin (or were contiguous with biotin segments after incubations of 2 or 10 min) were scored as having turned over or grown, whereas those Ac domains that lacked hapten staining were scored as not having turned over or grown over that particular interval. The mean proportions of all Ac domains tabulated for each category (terminal, internal, uniform and lacking biotin) were weighted according to the general formula:

$$St_w = \frac{\sum^{n} w_i St_i}{\sum^{n} w_i},$$

as described by Sokal & Rohlf (1987), where St_w = weighted statistic, w_i = individual weighting factor, and St_i = individual statistic to be weighted. The weighting factor assigned to each cell analysed was calculated as the number of Ac domains traced in that cell divided by the average number of Ac domains traced per cell for that time point. This weighting factor was designed to give more significance to those cells containing an Ac MT distribution yielding increased numbers of traceable domains. However, these cells displayed no obvious increase in the total number of Ac domains as compared with the other cells analysed. Further, many Ac domains traced were located close to the centrosome, thereby minimizing any bias toward periph-

eral Ac domains. The standard error of each proportion was calculated using the same weighting factor for the quantity $(x-\bar{x})^2$.

Results

A subset of MTs contains discrete domains of acetylation

We reported previously (Webster *et al.* 1987*b*) that an MT subset displaying an elevated level of Glu subunits turned over much more slowly than the bulk array. In this study, we wished to examine the turnover of an MT subset post-translationally modified with an acetyl group. Therefore, we chose a cell line that contained few or no Glu MTs, in order to study MT acetylation independently of the tyrosination cycle.

MTs in human fibroblasts stained heterogeneously with an antibody specific for Ac alpha tubulin (Fig. 1). As seen at low magnification (A,B), staining was most concentrated at the centrosomal focus, though Ac domains of varying lengths and intensities were also present in peripheral regions of the cytoplasm. In addition, many MTs lacked Ac domains along their entire observable lengths. Further details were revealed at higher magnification (C–F): domains of acetylation occurred at the ends of MTs (terminal domains, curved arrows in C–F) and along their lengths (internal domains, small arrows in E,F). Further, some MTs contained multiple domains of acetylation interspersed with non-Ac regions (arrowheads in C and D). A few MTs were stained uniformly with the Ac tubulin antibody, along their entire observable lengths, while many were not stained (asterisks in C and D) or were close to the threshold of detection under our staining conditions. The proportions of each type of Ac domain are presented in Table 1. Internal domains comprised the largest subgroup, accounting for 77% of the total. Terminal domains comprised 16% of the population, while 5% of the Ac domains extended the entire observable MT length. A small proportion (2%) of the Ac MTs were not located in the corresponding micrographs of the bulk array; this may have been either the result of blocking due to the previous antibody applications (see Materials and methods) or due to the presence of a small proportion of Glu MTs. However, these MTs represented an insignificant proportion in this study and were not analysed in greater detail. Further staining heterogeneity was observed within Ac domains. For example, some MTs displayed regions continuously stained with the Ac tubulin antibody, but with variations in intensity along those regions. Thus, the degree of acetylation of MTs in human fibroblasts varied considerably, both in the proportions of the MTs visibly Ac and in staining intensity.

Experimental approach

Following the rationale of our previous study (Webster *et al.* 1987*b*), MT turnover was evaluated by assaying incorporation of hapten-labelled tubulin following microinjection. Consequently, Ac domains that had or had not incorporated biotinylated subunits at various times following microinjection were scored as having or not having turned over within that time.

The tubulin used for biotinylation and microinjection was purified without specifically considering its degree of acetylation. Bovine brain extracts contain a small proportion of Ac subunits, and at least a portion remains through five cycles of assembly and disassembly (Sale *et al.* 1988). If our preparations contained a significant proportion of Ac subunits that assembled *in vivo* and were observable in MTs by immunofluorescence, then all

Table 1. *Turnover of acetylated microtubule domains*

	% Ac internal domains	% Ac terminal domains	% Uniform Ac domains	% Ac other*
Uninjected cells $\left(\frac{574}{14}\right)$	77 ± 3	16 ± 3	5 ± 2	2 ± 1
	% Biotinylated Ac internal domains	% Biotinylated Ac terminal domains	% Uniform biotin/Ac MTs	% Ac domains without biotin
Injected cells				
Incubation time				
2 min $\left(\frac{123}{11}\right)$	0	3 ± 1	0	97 ± 1
10 min $\left(\frac{410}{12}\right)$	9 ± 4	0	0	91 ± 4
1 h $\left(\frac{348}{11}\right)$	41 ± 3	5 ± 2	4 ± 2	49 ± 5
16 h $\left(\frac{415}{10}\right)$	74 ± 4	10 ± 2	4 ± 1	13 ± 2

Ac and total MT immunostaining patterns were traced onto clear acetate (see Materials and methods) and Ac domains were classified as either internal, terminal or comprising the entire MT. The proportions of each domain type containing biotin were scored for each time interval by tracing the biotinylated tubulin staining pattern onto the same acetate sheet. The mean values ± S.E. were weighted as described in Materials and methods. n = the number of MTs traced per time point/the number of cells traced per time point.

* A small proportion of Ac MTs stained weakly or not at all with the antibody used to detect the bulk array.

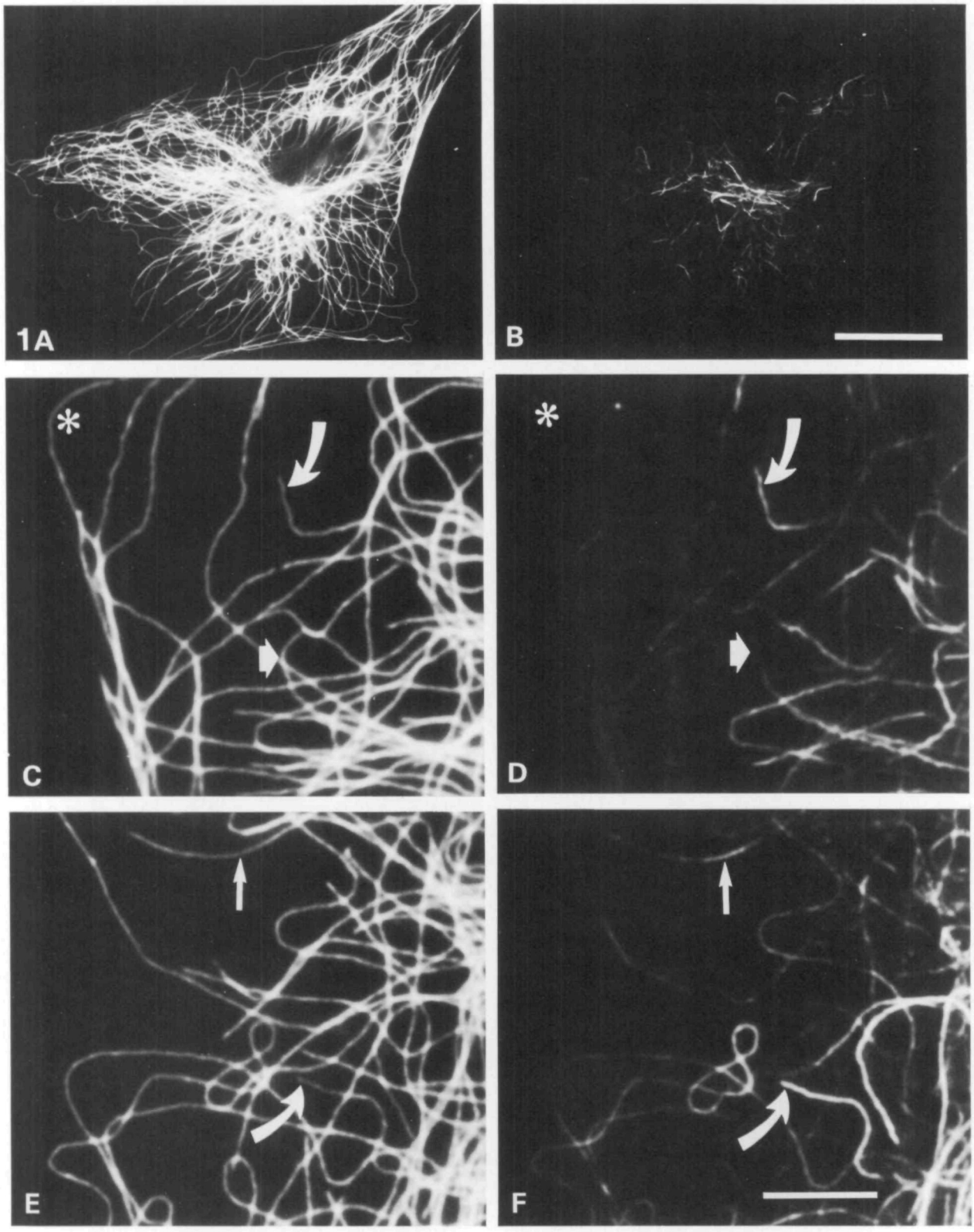

Fig. 1. MTs are acetylated in terminal and internal domains. Human fibroblasts displayed an extensive array of MTs (A), some of which contained Ac domains (B). These domains were often centrosomally focused but were also observed at peripheral locations. At higher magnification (C–F) more detail was revealed: Ac domains on individual MTs occupied terminal (curved arrows) or internal (straight arrows) positions. Some MTs contained multiple Ac domains, which were interspersed with non-Ac regions (arrowheads). In addition, some MTs were not visibly Ac (asterisks in C and D). Bars: A,B, 20 μm; C–F, 5 μm.

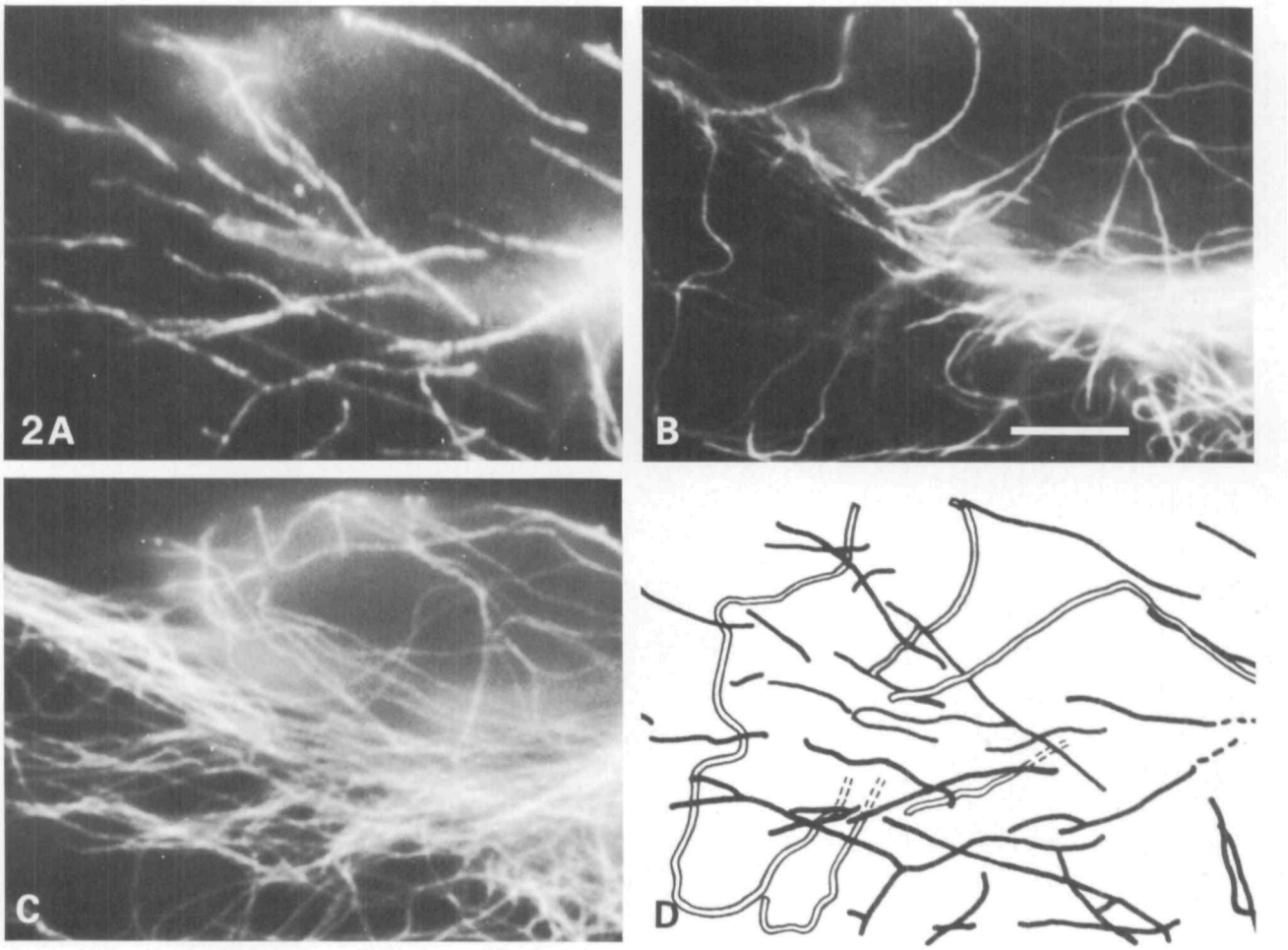

Fig. 2. Terminal Ac domains do not grow. Cells lysed 2 min after injection displayed biotin segments (A) that were rarely contiguous with terminal Ac domains (B) but were usually contiguous with non-Ac, MT ends (C). Ac domains were compared with the bulk staining pattern to distinguish terminal domains, then drawn together with the biotin staining pattern in D to illustrate the lack of correlation between biotin ends and terminal Ac domains. Biotin segments are represented by the filled MTs and Ac domains by the open MTs. Ends of biotin segments that become obscured are depicted as broken extensions of filled MTs, and Ac MTs that fade or become obscured as broken extensions of open MTs. Bar, 5 μm.

domains of incorporation would be Ac and would not be distinguishable from endogenous domains. However, this was not the case. Domains of biotin incorporation were initially distinct from endogenous Ac domains, and became concordant with them only after the cells had entered mitosis (usually 8–24 h after injection). Therefore, we conclude that the level of Ac subunits in the injectant was low, and certainly below our threshold of detection.

Terminal Ac domains are not growing

Fibroblasts were lysed and fixed within 2 min after injection of hapten-labelled tubulin. These cells displayed an array of biotinylated segments (Fig. 2A) that were contiguous with non-Ac MT ends (Fig. 2C) but were not contiguous with terminal Ac domains (Fig. 2B). Fig. 2D compares the distribution of terminal Ac domains and biotin segments, and demonstrates that the terminal Ac domains are not contiguous with injected subunit domains and therefore the MTs with these domains were not growing. Of the MTs that could be unambiguously traced, only 3 % of Ac ends (Table 1), compared with virtually all of the non-Ac ends, were contiguous with biotinylated domains, suggesting that only a few Ac ends but most non-Ac MT ends were growing. The small proportion of growing Ac ends might be attributable either to perturbation caused by the injection of tubulin subunits or to the existence of a subclass of Ac ends more closely resembling non-Ac MT ends.

Turnover of Ac domains is much slower than the bulk array

The estimated half-time for turnover of the entire MT cytoskeleton has been estimated to be 5–20 min, depending on the cell type studied and the method used for its determination (Saxton *et al.* 1984; Schulze & Kirschner, 1986; Sammak *et al.* 1987; Webster *et al.* 1987*a*,*b*). For the purposes of this study, we assumed a half-time of 10 min. Cells incubated for 10 min after injection showed

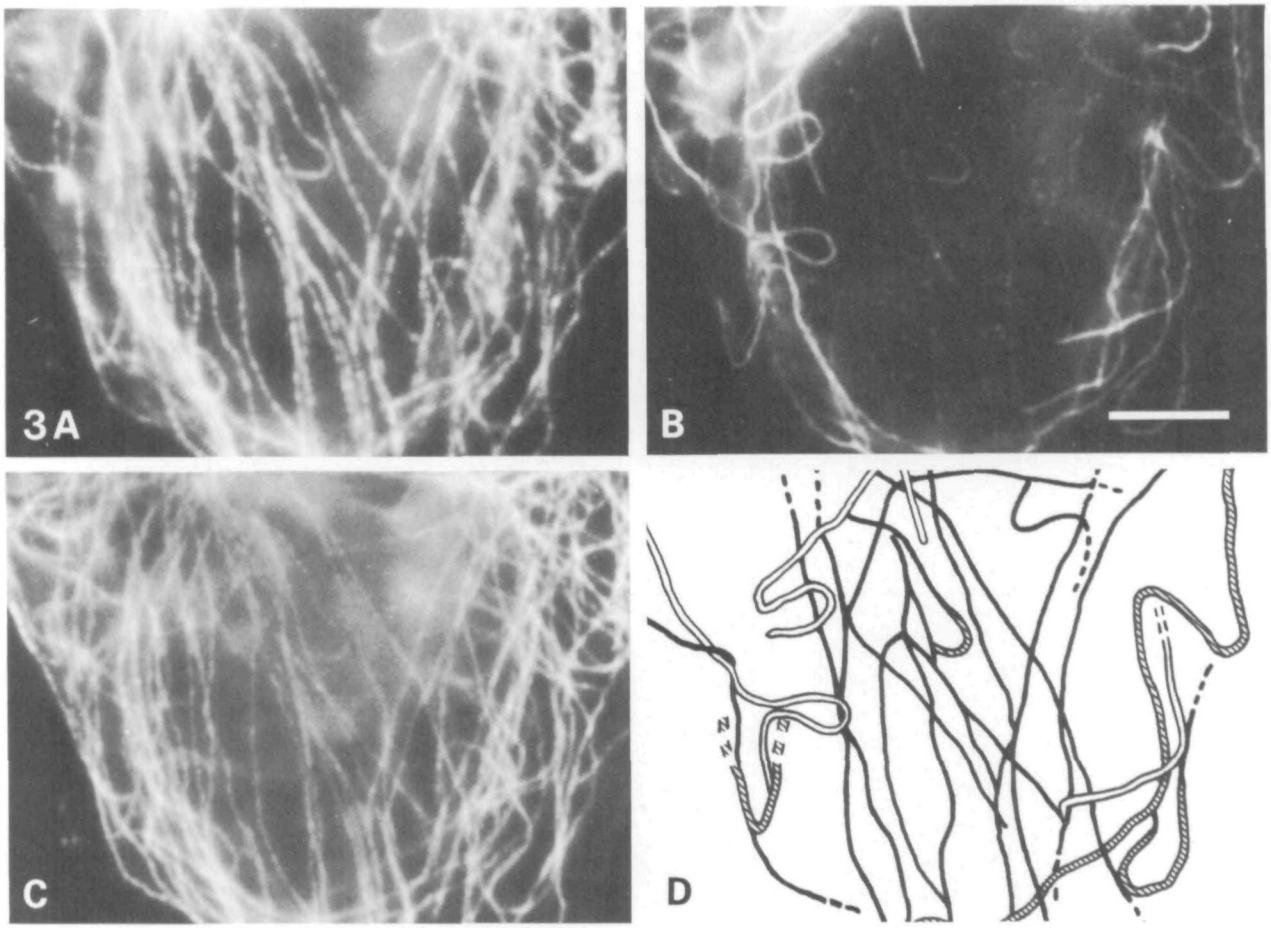

Fig. 3. Ac MT domains turn over slowly. One hour after injection, virtually all non-Ac MTs (C) also stain for biotin (A), while approximately half of the Ac population remains unlabelled for biotin (B). The diagram in D illustrates Ac domains that have turned over (hatched MTs) and some that have not (open MTs). Non-Ac MTs are shown as filled (many have been omitted for clarity). Bar, 5 μm.

biotin staining in roughly half of the MTs in the bulk array (data not shown), consistent with the half-times determined in the previous studies. However, only a few Ac domains (9 %) were also stained for hapten (Table 1), indicating that only a small proportion had turned over. In addition, the ends of terminal Ac domains still lacked hapten, minimizing the possibility that they were growing, but more slowly than their non-Ac counterparts.

Assuming 10 min as the half-time for bulk turnover, greater than 98 % of the bulk array should turn over within one hour (six half-times). Injected cells fixed after one hour of incubation showed that virtually all MTs not containing Ac domains were copolymers with hapten-labelled subunits (Fig. 3A,C). In contrast, these cells displayed a heterogeneous population of Ac domains, some of which also stained for hapten and some that did not (Fig. 3B,D; Table 1). Approximately 51 % of the Ac domains contained hapten and most likely represented MTs that polymerized after the injection and then became Ac. However, 49 % of the Ac domains still lacked hapten, suggesting that this MT population, similarly to the Glu MT subset examined in monkey kidney fibroblasts (Webster *et al.* 1987*b*), turned over much more slowly than the bulk array.

Examination of injected cells 16 h later revealed that 13 % of the Ac domains still lacked hapten (Table 1), demonstrating the presence of an MT subset that is extremely long-lived. At this time most of the Ac domains (87 %) had turned over and the proportion of each domain category containing biotin approached the steady-state proportions, suggesting that Ac domains at all sites along MTs turned over equivalently and no domain category was preferentially disassembled, consistent with a tubule by tubule turnover mechanism. This result was confirmed by calculating the proportions of each domain category at each time point, but using only the values derived from the population of domains that had turned over. If Ac MT turnover progressed independently of the site of acetylation, then these proportions should not vary over time and should approximate the values for uninjected cells. Although after 10 min of incubation the few hapten-labelled Ac MTs contained only internal domains, after 1 h 79 ± 6 % of the internal domains, 11 ± 4 % of the terminal domains and 9 ± 4 % of the uniform domains contained hapten, which closely reflected the steady-state proportions. After 16 h, 84 ± 3 % of the internal domains, 11 ± 2 % of the terminal domains, and 4 ± 2 % of the uniform domains

contained hapten, confirming that the rate of Ac MT turnover does not depend on the site of acetylation.

Acetylation differentiates discrete domains along stable MTs

Initially, we limited our analysis of turnover to Ac domains only. To evaluate the turnover of the non-Ac regions of these MTs, we analysed the non-Ac regions that flanked stable Ac domains (lacked hapten) in cells incubated for 1 h after microinjection. Although the flanking MT regions of many internal Ac domains were obscured due to MT congestion, some (47, from 4 cells) were scored for the presence or absence of hapten. Of these internal Ac domains, over 60 % displayed discernible flanking regions at both Ac/non-Ac MT boundaries and 40 % displayed one discernible boundary. Overall, only 4 % (2/47) of the flanking domains continued as biotinylated polymer. Further, biotinylated flanking domains were never observed on both sides of an Ac domain lacking hapten. The polarity of these particular MTs could not be determined, yet the paucity of overall biotin staining at the boundaries of acetylation suggested that at least those regions and possibly the entire MTs were also stable to exchange.

Cells subjected to nocodazole (25 μM) for 30 min, or a combination of nocodazole and cold treatment (4 °C), retained a small number of MTs (roughly six per cell), most of which demonstrated no apparent contiguity with the centrosome (data not shown). If Ac domains represented stable regions within labile MTs, then the depolymerizing conditions employed here would be expected to disassemble the labile portions back to the stable Ac domains, leaving only stable MT segments bearing Ac ends. The MTs from 20 drug- and cold-treated cells were examined for their content and distribution of Ac domains. Approximately 30 % of these remaining MTs lacked Ac domains altogether, while 56 % of the total bore Ac domains at one or both ends and 14 % contained only internal Ac domains. These results indicated that acetylation was not required to stabilize MTs and that acetylation arose independently of stabilization.

Ac domains are depolymerized during prophase of mitosis

Some injected cells that were examined 16–24 h later had entered or completed mitosis (Fig. 4). The cell in A–C had entered prophase at the time of cell lysis (the nuclear envelope was still intact), and displayed growing mitotic asters. In this cell no staining for Ac tubulin was observed, indicating that all interphase Ac MTs had depolymerized. Ac domains became apparent again in early G_1 cells (Fig. 4D–F), most often located close to the nucleus, preventing an analysis of biotin staining due to MT congestion around the centrosome. Thus, as for the stable Glu MT subset examined previously (Webster *et al.* 1987*b*), the maximum longevity of Ac domains in human fibroblasts was one cell cycle. Further, no Ac domains survived the interphase–mitosis transition, when the bulk array disassembles.

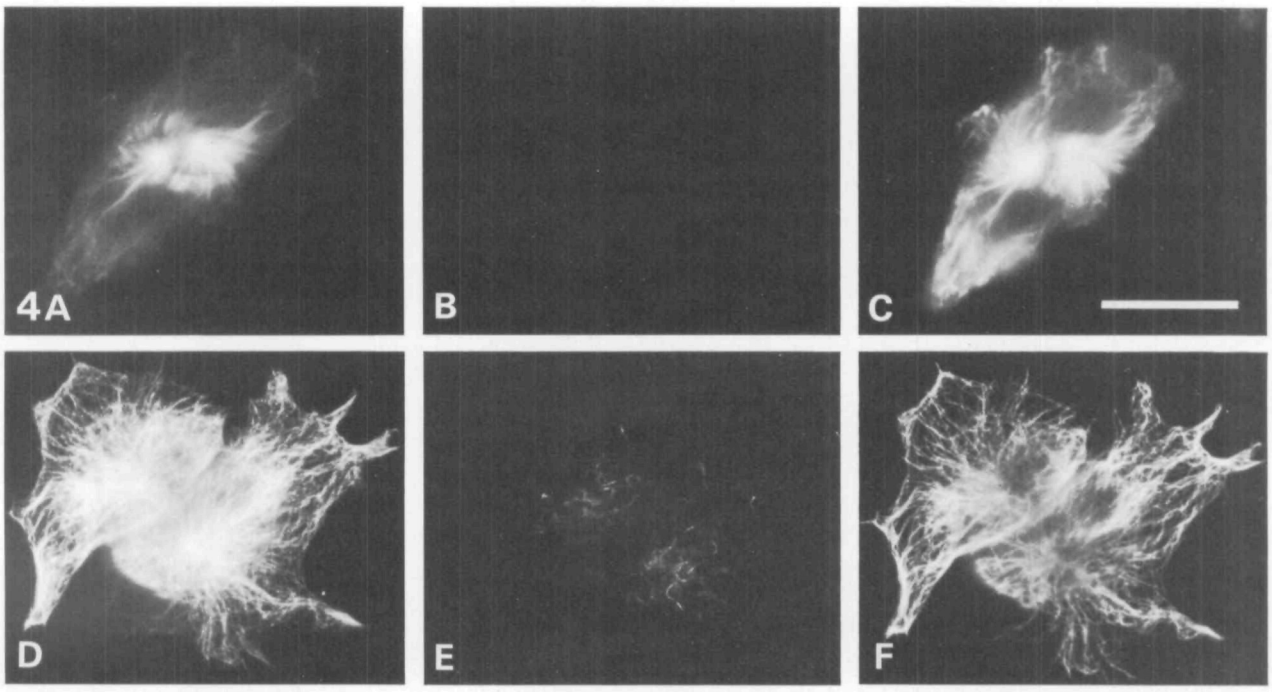

Fig. 4. Ac domains disassemble during prophase of mitosis. Some cells injected in interphase and incubated for 16–24 h before lysis and fixation had entered mitosis, showing that the injection had not severely affected the cells and that the injected subunits were used to make the mitotic asters (biotin, A; total MTs, C). At this stage, no Ac tubulin staining was observed (B), indicating that all cytoplasmic Ac domains had disassembled and that few, if any, subunits had become reacetylated. Cells that had completed mitosis (D–F) displayed indistinguishable biotin (D) and total MT (F) tubulin patterns and demonstrated the reemergence of Ac domains (E) around the centrosomal focus. Bar, 20 μm.

Discussion

Ac tubulin staining in human fibroblasts was observed on a stable subset of MTs, often restricted to discrete domains along their lengths. Non-uniform MT staining for Ac subunits has been noted in other fibroblastic cell types (Piperno *et al.* 1987; Cambray-Deakin & Burgoyne, 1987; Bulinski *et al.* 1988), and after release from nocodazole, Ac staining first appears as short cytoplasmic segments (Piperno *et al.* 1987; Bulinski *et al.* 1988; and our unpublished observations). In contrast, Glu MTs display uniform antibody labelling along their entire lengths (Geuens *et al.* 1986) and Glu staining does not appear as segments after nocodazole release (Gundersen *et al.* 1987), suggesting that acetylation and detyrosination may be regulated differently and that the staining heterogeneity observed for Ac tubulin may reflect tubulin acetyltransferase (TAT) or tubulin deacetylase activity restricted to particular MT domains.

Ac MT domains, like Glu MTs in other cell types, were more stable to exchange with soluble subunits than the non-Ac MT network. The half-time for turnover of Ac domains was measured to be approximately 1 h, as compared with a half-time of 5–20 min for the dynamic, bulk array. Within the constraints of a simple kinetic model for turnover, this value predicts that after 6 h (six half-times) less than 2% of Ac domains would not have turned over and would therefore lack hapten. After 16 h, virtually all Ac domains would be expected to have turned over, yet we observed that 13% of Ac domains lacked hapten after this time, a result that is incompatible with the simple kinetic model. This result suggests that an additional, stabilizing process may contribute towards the slow turnover observed for Ac domains.

Do these domains represent 'islands' of stable polymer within dynamic, non-Ac MTs? The absence of hapten staining on non-Ac flanking MT regions and the lack of correlation between cold- and nocodazole-resistant MTs and terminal Ac domains suggested that MT stability arose independently of acetylation. The soluble subunit pool contains very little Ac tubulin (L'Hernault & Rosenbaum, 1983; Piperno & Fuller, 1985) and MT acetylation increases markedly upon exposure to the MT-stabilizing drug taxol (Piperno *et al.* 1987), suggesting that this modification occurs on polymer, and might be a visible consequence of MT stabilization.

MTs with Ac ends identified in this study and MTs with Glu ends studied previously (Webster *et al.* 1987*b*; Gundersen *et al.* 1988) were not growing, suggesting that they might be capped or otherwise rendered inefficient as growth substrates. Capping of the (+) end would presumably result in MTs resistant to end-mediated modes of depolymerization, and would then allow further, polymer-specific post-translational modification to occur, including uniform detyrosination, non-uniform acetylation, or both.

The loss over time of Ac domains lacking hapten and the corresponding increase in hapten-labelled domains suggested that a steady-state level of acetylation is maintained throughout the cell cycle until mitosis, when all Ac domains are disassembled. Early G_1 cells acquire Ac domains rapidly, which presumably disassemble as the MTs of which they are a part turn over. Turnover of Ac domains may be accomplished passively as the MT network turns over, maintaining a relatively constant level corresponding to the amount of stable polymeric substrate available.

Why is the pattern of acetylation non-uniform? A simple reaction scheme involving a modifying enzyme that randomly binds to and releases from a homogeneous, polymeric substrate would predict a uniform development of acetylation. This simple type of interaction may describe MT detyrosination, which is uniform as assayed by immunofluorescence and immunoelectron microscopy (Geuens *et al.* 1986). Domain acetylation of MTs implies the presence of an additional factor(s) that limits enzyme interaction to particular regions of MTs and perhaps to a limited subset of MTs. Specifically, domain acetylation would result if a factor that either facilitated or inhibited TAT activity was distributed non-homogeneously along the length of MTs. TAT binding factors have not been identified, but could conceivably include MAPs, other cytoskeletal fibres, or cellular organelles that interact with MTs. For example, Job *et al.* (1985) induced MAPs to distribute non-homogeneously along MTs *in vitro*. A similar non-uniform distribution of a TAT-binding MAP *in vivo* could explain the observed patterns of acetylation. Conversely, domain acetylation generated by a different mechanism might facilitate MAP binding to that particular MT region. In addition, contacts between MTs and actin microfilaments, intermediate filaments or other MTs could provide a structural interface required for TAT activity, thus limiting acetylation to those regions of overlap. Organelles that interact with MTs (and possibly MAPS), either statically or as a prelude to translocation, could similarly provide a structural interface necessary for acetylation to occur. MT acetylation might even continue as organelle translocation progressed, resulting in Ac domains corresponding in size to the excursion length of the organelle along one MT. These possible mechanisms have not been tested directly, but are compatible with known characteristics of the enzyme and functions of MTs.

The possible functions for such localized subunit modification on MTs remain unresolved. Since Ac domains occupy specific sites within stable MTs, acetylation presumably does not generate MT stability. However, acetylation of discrete domains may confer functional specificity to particular regions of stable MTs, allowing further interaction between these domains and other fibre systems, structures or organelles. Definitive answers to these questions await fuller understanding of MT-requiring processes and the ability to disrupt acetylation or deacetylation *in vivo*.

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