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Distribution of Microtubules Containing Post-Translationally Modified α-Tubulin During *Drosophila* Embryogenesis

R.M. Warn, A. Harrison, V. Planques, N. Robert-Nicoud, and J. Wehland

School of Biological Sciences, University of East Anglia, Norwich, England (R.M.W., A.H., V.P.); Max-Planck Institute for Biophysical Chemistry, Gottingen, Federal Republic of Germany (N.R.-N., J.W.)

The distribution of microtubules (MTs) enriched in detyrosinated α-tubulin (Glu-tubulin) was studied in *Drosophila* embryos by immunofluorescence microscopy by using a monoclonal antibody (ID5) which was raised against a 14-residue synthetic peptide spanning the carboxyterminal sequence of Glu-tubulin (Wehland and Weber: *J. Cell Sci.* 88:185–203, 1987). While all MT arrays contained tyrosinated α-tubulin (Tyr-tubulin), MTs rich in Glu-tubulin were not found during early stages of development even by using an image intensification camera. Elevated levels of microtubular Glu-tubulin were first detected after CNS condensation in neurone processes. In addition, sperm tails, which remained remarkably stable inside the embryo until late stages of development, were decorated by ID5. This was in marked contrast to the distribution of microtubule arrays containing acetylated α-tubulin, which could already be detected during the cellular blastoderm stage. Additional experiments with taxol suggested that the absence of MTs rich in Glu-tubulin during early stages of development was not due to the rapid turnover rate of MTs, which would be too fast for α-tubulin to be detyrosinated. The possible significance of the differential detyrosination and acetylation of microtubules during development is discussed.

Key words: detyrosinated α-tubulin, *Drosophila* embryo, confocal microscopy

INTRODUCTION

Because microtubules are involved in a wide variety of cellular activities, specific mechanisms have been suggested for regulating and controlling these functions. The apparent structural and functional MT diversity may arise from a heterogeneous α- and β-tubulin polypeptide composition and/or from the binding of different MT-associated proteins (MAPs) [Cleveland and Sullivan, 1985]. In addition post-translational modifications of tubulin polypeptides could result in the modulation of MT functions in vivo.

Two different reversible post-translational modifications of α-tubulin have been studied in detail: the removal of the carboxy-terminal tyrosine [for references see Barra et al., 1988] and the acetylation of the ε-amino group of a lysine residue [L'Hernault and Rosenbaum, 1983; Greer et al., 1985]. Both of these modifications occur primarily on assembled microtubules and their reversal on unpolymerized α-tubulin (detyrosination: Kumar and Flavin [1981]; acetylation: Piperno et al. [1987]). Sub-populations of microtubules have been identified with specific monoclonal antibodies as containing predominantly the detyrosinated [Gundersen et al., 1984; Gundersen and Bulinski, 1986; Wehland and

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Address reprint requests to Dr. R.M. Warn, School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, U.K.

A. Harrison's current address is Department of Biology, Darwin Building, University College, Gower Street, London WC1E 6BT, England.

J. Wehland's current address is G.B.F., Division of Microbiology, Mascheroder Weg I, 3300 Braunschweig, F.R.G.

Weber, 1987; Kreis, 1987] or the acetylated [Piperno et al., 1987; Sasse et al., 1987; Diggins and Dove, 1987] forms of α-tubulin. Some cell types contain microtubules staining for only one post-translational modification whilst others have both [Bulinski et al., 1988].

Studies of microtubule kinetics in vivo using labelled brain tubulin [Soltys and Borisy, 1985; Schulze and Kirschner, 1986, 1987, 1988; Sammak and Borisy, 1988], *Physarum* tubulin [Prescott et al., 1989], and *Paramecium* axonemal tubulin [Geuens et al., 1989] injected into cultured mammalian cells have also demonstrated the presence of two populations of microtubules. There is a rapidly turning-over population which constitutes roughly 80–90% of the total number of microtubules with an average half-life of 5–10 minutes, and a second, much more stable type of microtubule which persists for one hour or more. For a number of cultured cell types the stable microtubules have been found to correspond to those showing post-translational modifications [Schulze et al., 1987; Webster and Borisy, 1989]. The functions of these modified forms of α-tubulin in microtubules are presently unclear. Although there is little doubt that a correlation exists between their presence and microtubule stability, the current evidence strongly argues against a causal relationship [Webster et al., 1987, 1988]. Rather it appears that the post-translational modifications occur after the microtubules have been stabilized by as yet uncharacterized mechanisms.

One approach to examining the roles of microtubules rich in post-translationally modified α-tubulin is to investigate changes in the distribution of the different kinds of microtubules as cell differentiation and organogenesis proceeds. The *Drosophila* embryo is a very suitable material for such studies. It is quite small (approximately 450 μm in length by 150 μm in diameter) and develops very rapidly, hatching after only 22 hours. Particularly because the mitoses at syncytial blastoderm occur close to the surface, immunofluorescence studies of both fixed [Warn 1986; Warn and Warn, 1986; Karr and Alberts 1986] and living [Warn et al., 1987; Kellogg et al., 1988] material and electron microscopy studies [Fullilove and Jacobson, 1971; Stafstrom and Staehelin, 1984; Callaini and Anselmi, 1988] have described the microtubule organization during the early stages.

Wolf et al. [1988] have recently investigated the distribution of microtubules rich in acetylated α-tubulin during *Drosophila* embryogenesis. They found that such microtubules were not present during the syncytial blastoderm stages but appeared during the process of cellularization when the mitotic cycles slow down and cells form simultaneously over the whole surface [Zalokar and Erk, 1976; Foe and Alberts, 1983]. Because interphase is extended during cellularization the acetylated microtubules may represent a sub-population of more stable microtubules. After cytokinesis has been completed most of the cells contain microtubules rich in acetylated α-tubulin during interphase but not in their mitotic spindles.

Here we describe the distribution of Glu-tubulin of various MT arrays in different cell types during the embryonic development of *Drosophila* and compare it with that of acetylated α-tubulin. Our results show that, in contrast to acetylated α-tubulin, Glu-tubulin appears only at late stages of development, in particular during neurone differentiation.

MATERIALS AND METHODS

Antibodies

The mouse monoclonal antibody ID5 was used to recognise detyrosinated α-tubulin [Wehland and Weber, 1987]. This antibody was prepared against a 14-residue peptide corresponding to the C-terminus of mammalian detyrosinated α-tubulin and has been found to recognise specifically this form of α-tubulin in cultured cells. Tyrosinated α-tubulin was recognised using the antibody YL 1/2 [Kilmartin et al., 1982; Wehland et al., 1983], purchased from Sera-Lab (Crawley Down, Sussex). To identify acetylated α-tubulin the mouse monoclonal antibody 6-11B-1 was used. This has been previously characterised and described in detail [Piperno and Fuller, 1985; Wolf et al., 1988]. Anti-β-tubulin was purchased from Amersham.

Electrophoresis and Immunoblotting

Extracts enriched in MT protein were obtained from embryos aged for 16–22 hours; 0.5–2.0 gm of embryos were homogenized in a small glass homogenizer in twice the volume of a buffer containing 0.1M Pipes pH 6.9, 4M glycerol, 2 mM EGTA, 1 mM GTP plus 1 mM dithiothreitol, and DNase and RNase both at 2 mg/ml. Also added was a cocktail of protease inhibitors including phenylmethylsulphonyl fluoride (PMSF), leupeptin, aprotinin, and pepstatin A, all at 10 μg/ml, 10 μM benzamidine hydrochloride, and 1 μg/ml phenanthroline. To maximise the yield of pelleted microtubules 10 μg/ml taxol was added. The homogenate was then filtered twice through fine muslin and spun at 100,000*g* for one hour at 25°C in a Beckman Ti 70.1 rotor. Samples were then reduced and alkylated following the method of Lane [1978] and run on SDS-acrylamide gels (10% acrylamide) with 3.5M added urea. SDS, highly purified for electrophoresis, (B.D.H., Poole), was used for these gels. Gels were run on an Atto mini-gel apparatus (Tokyo, Japan). Transfer to nitrocellulose was carried out by using the method of Towbin et al. [1979] with an LKB multiphor apparatus (model 2117). Blocking of non-specific staining was done for one hour in Tris-buffered saline (TBS) containing 2% Marvel powdered milk

(Cadbury's, Birmingham), 3 mg/ml bovine serum albumin and 0.1% Tween 20. Strips of nitrocellulose were incubated in the primary antibody (in all cases ascites fluid diluted 1:1,000) for one hour at room temperature. They were then washed for 30 minutes in five changes of TBS as before and incubated with peroxidase-conjugated anti-mouse IgG, (ID-5, 6-11B-1, 3A5) or anti-rat IgG (YL 1/2) (Bio-Makor, P & S Biochemicals, Liverpool) for one hour and the bands were visualized with 4-chloro-1-napthol.

Fixation and Immunofluorescence Microscopy

Embryos of Oregon-R strain *Drosophila melanogaster* were obtained from mass cultures. When necessary they were allowed to develop to the desired stages at 25°C. Embryos were dechorionated in 30% sodium hypochlorite and thoroughly washed in phosphate-buffered saline (P.B.S.). They were then fixed either in 90% methanol + 50 mM EGTA [after Warn and Warn, 1986] or 3.3% paraformaldehyde + 0.5 μM taxol [after Karr and Alberts, 1986]. The vitelline membanes were removed following the method of Mitchison and Sedat [1983]. The embryos were stained with the above antibodies as described in Warn and Warn [1986]. For double staining ID5 or 6-11B-1 was used first, followed by rhodamine-conjugated anti-mouse IgG (Dako, High Wycombe), YL 1/2, and finally FITC-conjugated anti-rat IgG (Dako). Prior to use second-stage antibodies were incubated for two hours at 37°C with fixed embryos to remove non-specific binding. Incubation with taxol (a kind gift of the Drug Synthesis & Chemistry Branch, National Institutes of Health, Bethesda, USA) was done as part of the method of Karr and Alberts [1986] and extended when necessary. To test for binding of ID5 after removal of the c-terminal tyrosine residue of α-tubulin methanol-fixed embryos were treated with 20 μg/ml pancreatic carboxypeptidase A (Sigma) in PBS for 20 minutes at 37°C and then double stained with ID5 and YL 1/2.

Sperm were obtained by dissecting testes from 3–4-day-old males, gently mashing them on acid-washed slides, and air drying. They were fixed for 10 minutes at room temperature in 4% paraformaldehyde in P.B.S., washed briefly in P.B.S., and then post-fixed with methanol at −20°C for 5 minutes. Staining for detyrosinated and tyrosinated α-tubulin was carried out as before except that each step was done for one hour at 37°C. Both sperm and embryos were finally incubated with the nuclear stain diamidine-2-phenylindole dihydrochloride (DAPI) at 0.5 μg/ml for 10 minutes at 25°C. They were then mounted and observations made with epifluorescence optics as previously described [Warn and Warn, 1986]. Photographs were taken with T-MAX 400 (Kodak) push developed to 1,600 A.S.A. To visualize FITC-stained sperm tails and DAPI-stained nuclei simultaneously a wide-band excitation filter (LP 515) was used and the structures observed in the green emission range.

Image Intensification and Processing

In order to investigate the patterns of post-translational modification after very short incubations with taxol, when only very weak fluorescence is present after 6-11B-1 staining, an intensified silicon-intensified target (ISIT) camera (model TC 1040/H Burle Industries, Lancaster, USA) was used mounted on a standard R microscope (Zeiss). The primary image was processed through a "Crystal" image processor (Quantel, Newbury) and recorded on a "U-matic" video tape recorder (Sony). Photographs were taken directly from the monitor by using Tri-X film (Ilford).

Confocal Laser-Scanning Microscopy

The confocal laser-scanning microscope used in this study has been described previously [Robert-Nicoud et al., 1989]. Briefly, it is equipped with three laser excitation beams and a high-precision mechanical scanning stage (0.25 μm in the X-Y plane and 50 nm in the z axis) operated by an MPC controller through an IEEE interface. The acquisition of data is through an 8-bit, 512-512 frame buffer coupled by an 1EEE interface to a central DEC MicroVax II processing system. Image processing was carried out by using TCL-image (Multihouse, TSI Amsterdam), an image analysis software developed at the Delft Center for Image Processing.

RESULTS

Lack of Staining of Interphase Arrays and Spindles in Early Stage Embryos With Antibodies to Detyrosinated α-Tubulin

Staining of preblastoderm- and syncytial blastoderm-stage embryos with antibodies against detyrosinated α-tubulin has revealed only one structure visible with epifluorescence optics—the sperm tail. This is in marked contrast to the structures revealed by using antibodies against tyrosinated α-tubulin (compare Fig. 1a and b). Interphase arrays, spindles, centrosomes, and mid-bodies, all of which have been previously found to stain strongly with YL1/2 [Warn and Warn, 1986], do not show staining with ID5. Up to gastrulation the sperm tail is seen as a long, tangled fibre located just below the micropyle where it enters. In every embryo examined only one sperm tail has been seen, with one exception where seemingly two were present. The pattern of coiling of the sperm tail is quite variable but it is always located in the same area of the embryo and is clearly visible. Incubation with 6-11-B1, to detect acetylated

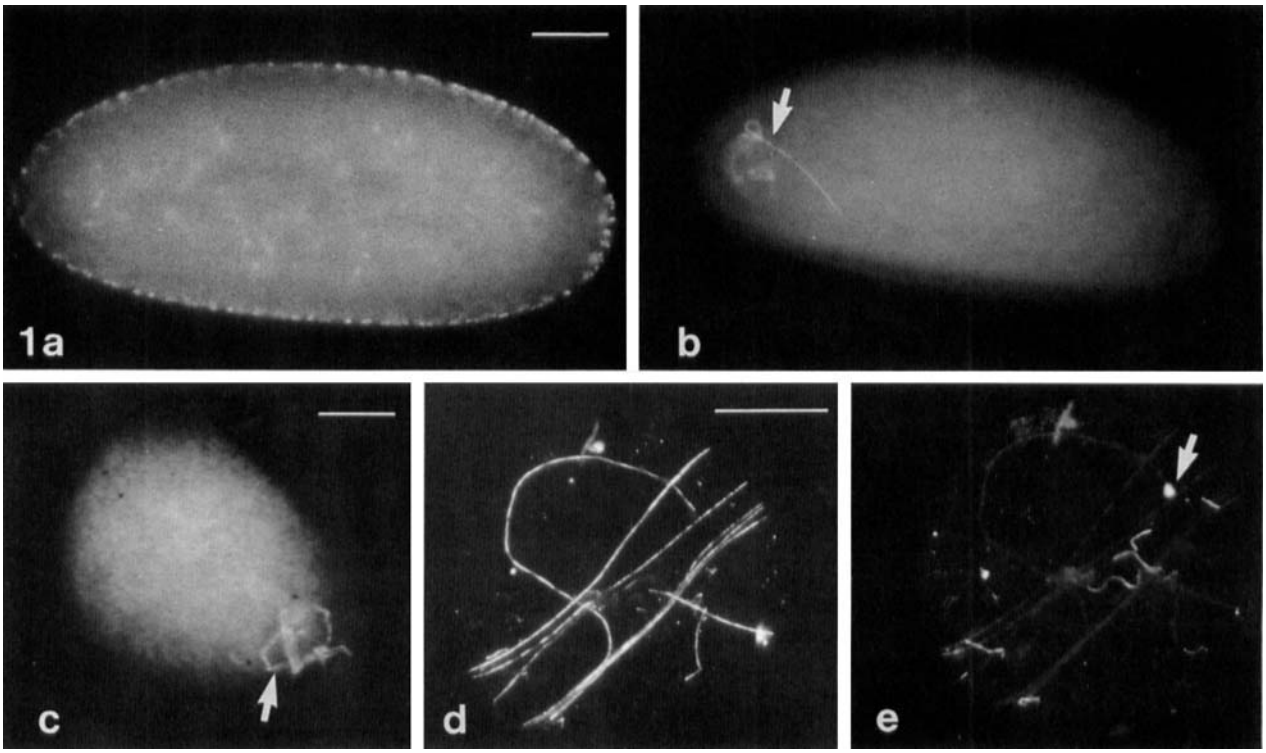

Fig. 1. Distribution of Tyr-tubulin, Glu-tubulin, and acetylated α-tubulin in a cycle 11 embryo and in sperm isolated from testes. **a** and **b:** Double immunofluorescence at low magnification of embryos fixed with 90% methanol and labelled with YL 1/2 (a) and ID5 (b), micrographs taken at the same section plane in the embryo interior. Arrow in b indicates sperm tail. **c:** Cycle 11 embryo labelled with 6-11B-1 antibody and viewed at medium magnification; arrow indicates sperm tail. **d** and **e:** Double immunofluorescence of isolated sperm labelled with ID5 (d) and YL 1/2 (e); arrow in e indicates sperm nucleus stained with DAPI and visible due to the use of LP filter 515. Bars: (a,b) 50 μm, (c) 30 μm, (d,e) 20 μm.

α-tubulin, also identifies the sperm tail (Fig. 1c). However, the tail is not visible after YL 1/2 staining for tyrosinated α-tubulin. This could be due to the absence of tyrosinated α-tubulin in *Drosophila* sperm tails or to a masking by the intense cortical fluorescence. Possible lack of staining was investigated by double-staining sperm from testes with antibodies against detyrosinated and tyrosinated α-tubulin (Fig. 1d,e). The tails stained strongly with ID5 antibodies (Fig. 1d) but not with YL 1/2 (Fig. 1e). Sperm from testes also stained strongly with antibodies against acetylated α-tubulin (not shown).

To check that spindles and other microtubule-containing structures within early stage embryos entirely lacked detyrosinated α-tubulin an ISIT camera and image processor was used to detect low levels of staining. No staining of microtubules associated with cortical buds or yolk islands was discernable with ID5 (compare Fig. 2a and b). To confirm that the lack of staining was due to absence of the detyrosinated α-tubulin isoform fixed embryos were treated with carboxypeptidase A to remove the carboxyterminal tyrosine residue of α-tubulin. After this treatment YL 1/2 did not show any labelling (Fig. 2d) whilst ID5 now clearly labelled the spindles and all other structures previously recognised by YL 1/2 (Fig. 2e).

Assuming that low levels of tubulin carboxypeptidase were present during the early developmental stages, the microtubules might turn over too fast for the α-tubulin to be detyrosinated. To exclude such a possibility syncytial-stage embryos were permeabilized, treated with taxol for periods up to 30 minutes, and stained with antibodies against either detyrosinated or acetylated α-tubulin and counterstained with antibodies against tyrosinated α-tubulin. Even after long periods of incubation with taxol, when significant reorganization and growth of tyrosinated microtubules had occurred (Fig. 2g) no staining at all was seen with the antibodies against detyrosinated α-tubulin when viewed with the ISIT camera (Fig. 2h). This result was markedly different from the results obtained by using anti-acetylated α-tubulin where

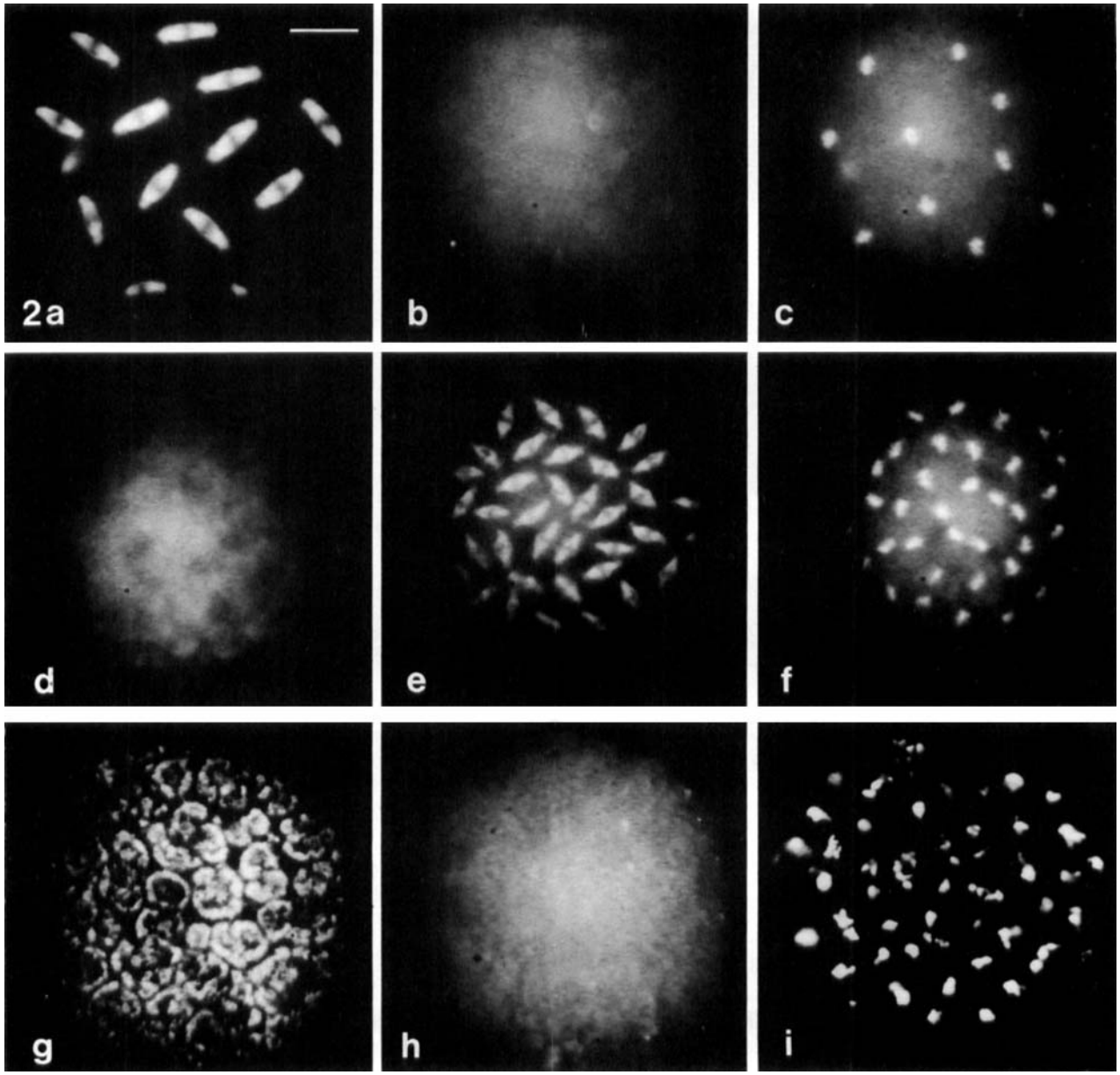

Fig. 2. Distribution of Tyr- and Glu-tubulin in cycle 10 and 11 embryos. Embryos were labelled with YL 1/2 and ID5 antibodies and counterstained with DAPI. **a, d, g:** Distribution of Tyr-tubulin. **b, e, h:** Distribution of Glu-tubulin. **c, f, i:** The corresponding DAPI images. Cycle 10 (a–c) and cycle 11 (d–f) (g–i) embryos were methanol fixed; the embryo in (d–f) was treated with carboxypeptidase A after fixation: embryo in (g–i) was permeabilised and treated with 0.5 μM taxol for 30 minutes before fixation with formaldehyde. Note the absence of Glu-tubulin in (b) which is detectable when fixed embryos were treated with carboxypeptidase A (compare b and e). Note also that Glu-tubulin does not appear even when massive stabilization and rearrangement of MTs occurred upon taxol stabilization (compare b and h). All micrographs were taken at higher magnification by using an ISIT video camera. Bar: 20 μm.

staining was conspicuous after only 30 seconds of treatment with taxol prior to the addition of fixative. Interphase arrays, spindles, asters, and mid-bodies were all stained (Fig. 3b,e,h,k). Thus acetylation of microtubule α-tubulin occurred at all stages of the cell cycle after taxol treatment. When compared with YL 1/2 staining the microtubule ends were frequently only weakly or not stained at all by 6.11B-1 (e.g., Fig. 3e,h) and the staining in general was rather punctate, particularly of the asters and interphase arrays. With shorter incubation times in the presence of taxol even as brief as 5 seconds some antibody staining could be detected (not shown). However, after such brief taxol treatments, preservation of microtubules was much reduced due to lack of time

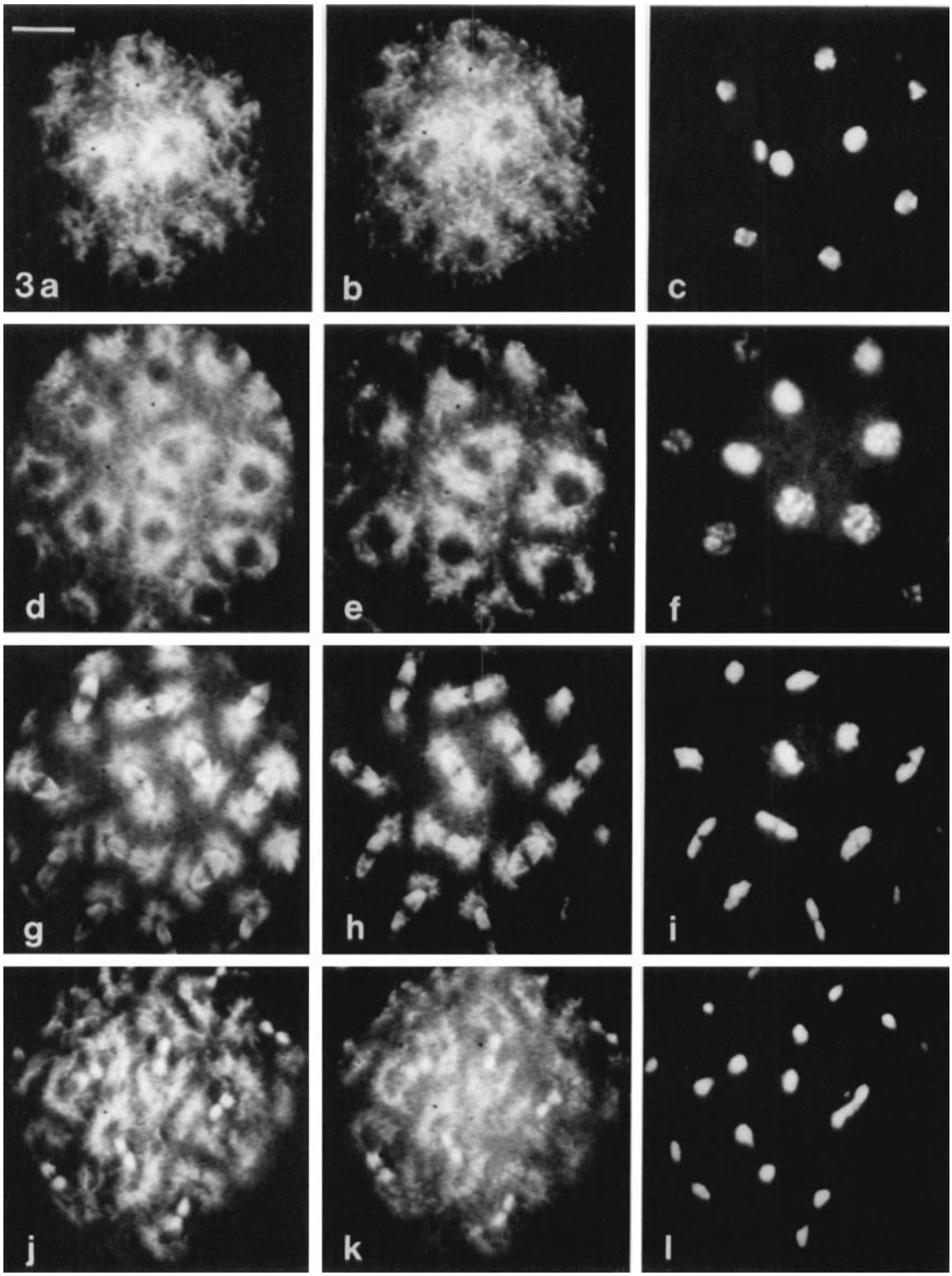

Fig. 3. Distribution of tyrosinated and acetylated α-tubulin in cycle 10 embryos fixed at different times of the cell cycle and examined by double immunofluorescence. Embryos were treated with 0.5 μm taxol for 30 seconds before formaldehyde fixation. **a, d, g, j:** Distribution of tyrosinated α-tubulin. **b, e, h, k:** Corresponding fields showing the distribution of acetylated α-tubulin. **c, f, i, l:** Corresponding DAPI images; a–c, interphase; d–f, prophase; g–i, metaphase and anaphase; j–l; telophase. All micrographs were taken at higher magnification by using an ISIT video camera. Bar: 20 μm.

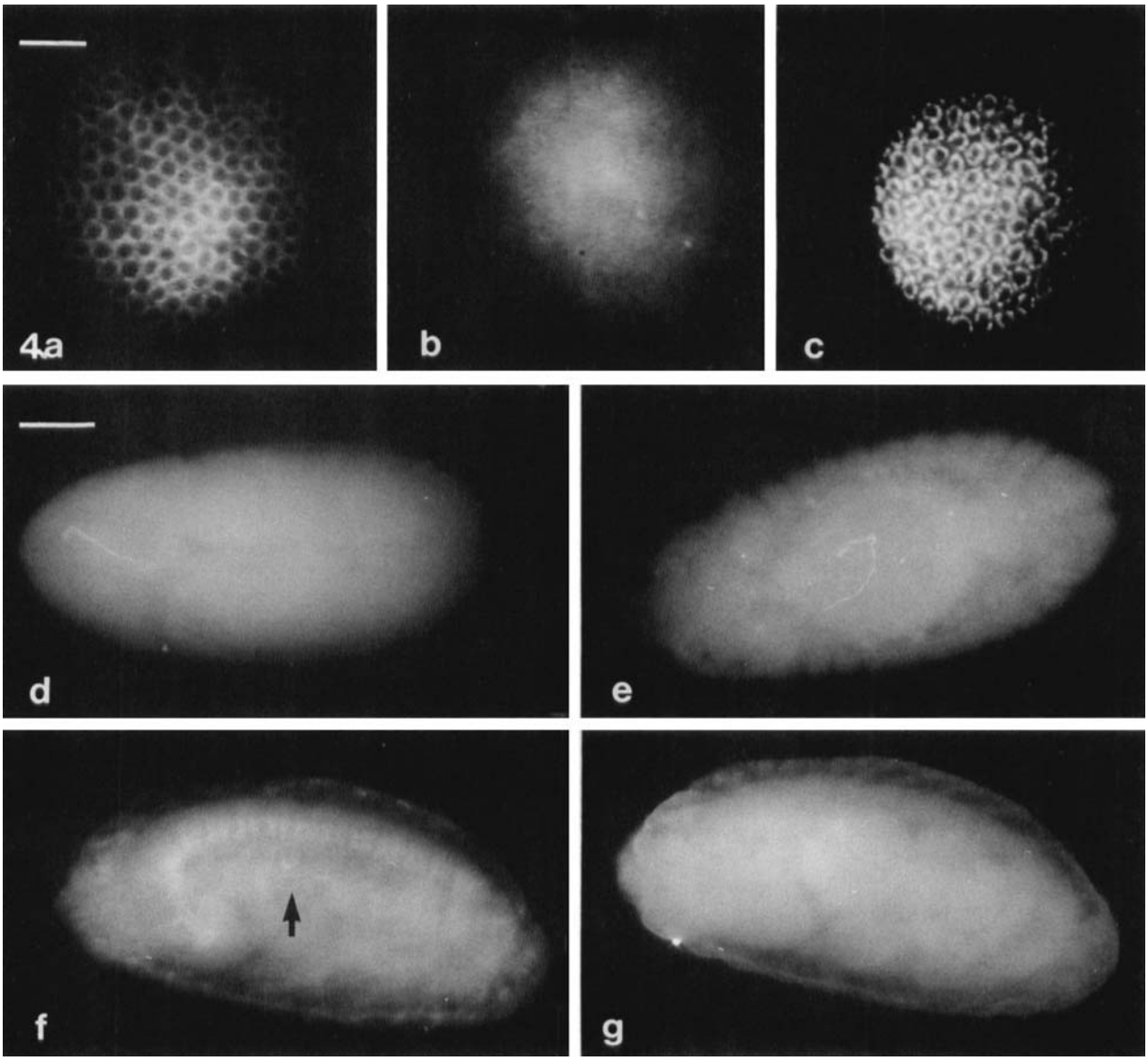

Fig. 4. Distribution of tyrosinated, detyrosinated, and acetylated α-tubulin in different stages of development from cellular blastoderm to CNS condensation. **a, b:** High magnification of cellular blastoderm labelled with YL 1/2 (a) and ID5 (b). **c:** Another embryo at a slightly later stage labelled with 6-11B-1. Images were taken by using an ISIT video camera. **d–g:** Later stages labelled with ID5 (d–f) or YL 1/2 (g); (d) gastrula; (e) after gut dorsal closure; (f) after completion of CNS condensation. f and g show the same field examined by double immunofluorescence; arrow in f indicates sperm tail. Bars: (a–c) 20 μm, (d–g) 50 μm.

for sufficient stabilization prior to formaldehyde fixation. Without prolonged treatment of permeabilized embryos with taxol, acetylated α-tubulin is first detectable during cellular blastoderm (Fig. 4c) as already described by Wolf et al. [1988]. In contrast to the appearance of acetylated α-tubulin, staining for Glu-tubulin is absent during these early stages of development, except for the labelling of the sperm tail (Fig. 4a,b).

Changes in Sperm Tail Position During Embryogenesis

Up to cellular blastoderm the sperm tail was seen to remain located in the anterior part of the embryo not far from the micropyle. By gastrulation, after the completion of cellularization, the sperm tail was found to be situated in the yolk which had concentrated in the more

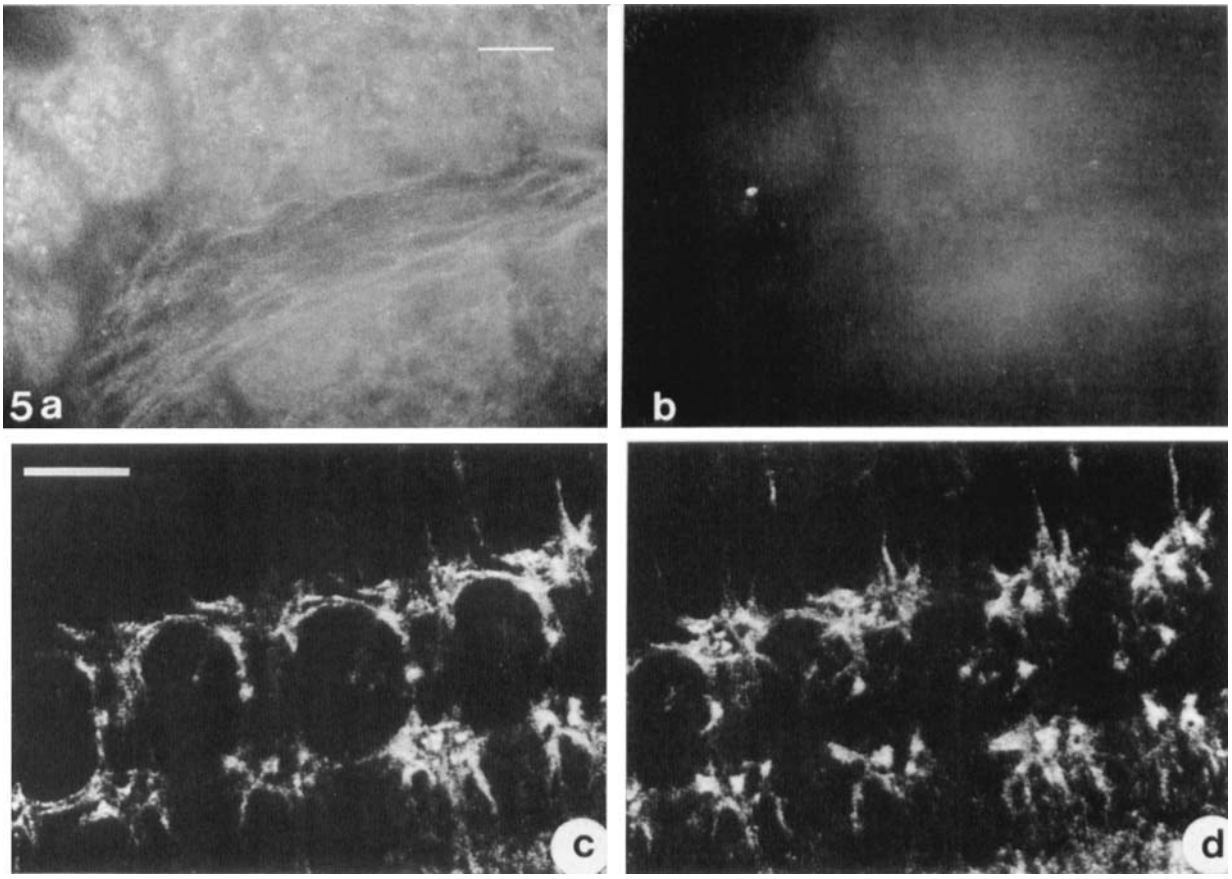

Fig. 5. **a** and **b**: High-power view of amnioserosa of embryo during germ band elongation to show microtubule bundles within the cells (a) YL 1/2 (b) ID5 counter-staining. Bar: 10 μm. **c** and **d:** Two optical sections through the CNS taken at an optical separation of 2 μm. Bar: 20 μm.

central portion of the embryo (Fig. 4d). It appeared somewhat shortened as though degradation had occurred at the ends. After closure of the mid-gut at stage 14 [Campos-Ortega and Hartenstein, 1985] the sperm tails were located within this structure (Fig. 4e). The tails were completely degraded within the gut but even in quite late stages where significant differentiation had taken place and condensation of the central nervous system had occurred, short tail pieces were occasionally seen within the gut (Fig. 4f).

Presence of Detyrosinated α-Tubulin in Later Embryonic Stages

During and after gastrulation quite complex patterns of staining with antibodies to acetylated α-tubulin have been previously reported for the interphase arrays [Wolf et al., 1988]. However, no staining at all was seen of cellular structures for detyrosinated α-tubulin (Fig. 4d). Also during the following germ band extension the amnioserosa, which forms the dorsal cover of the embryo, showed strong staining of conspicuous bundles of microtubules with YL 1/2 but no staining of these structures was found with ID5 (Fig. 5a,b). It was only after differentiation of the nervous system that the first labelling of embryonic MT structures with anti-detyrosinated α-tubulin became apparent.

In these later developmental stages, the central nervous system was found to be strongly stained with ID5 (Fig. 4f) while after labelling with YL 1/2 for tyrosinated α-tubulin it could not be distinguished from other tissues in whole mounts (Fig. 4g). The strong staining of the CNS with ID5 could be particularly well seen using a confocal microscope which allows optical sectioning of the embryo and can reveal fine details of internal structures in spite of a high background fluorescence. Images obtained with a confocal microscope are shown in Figure 5c and d where the ladder-like arrangement of commissures, connectives, and axons of the central nervous system are clearly stained with antibodies against detyrosinated α-tubulin.

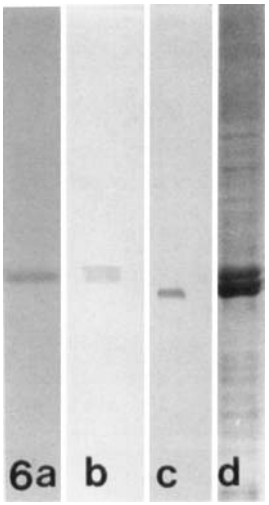

Fig. 6. Immunoblots of 16–22 hour embryo extracts enriched in MT-protein. Lane (**a**) labelled with ID5, lane (**b**) with YL 1/2, lane (**c**) with anti-β-tubulin antibodies. **d:** The corresponding SDS-acrylamide gel slice after staining with Coomassie blue.

Immunoblot Analysis of Embryo Extracts

To confirm the presence of Glu-tubulin in late-stage embryos cell extracts enriched in MT protein were analyzed by immunoblotting. What appeared to be a single band was detected on Western blots of SDS-urea polyacrylamide gels by using the ID5 antibody for Glu-tubulin (Fig. 6, lane a). This corresponded to the position of the band found with YL 1/2 (lane b) and ran above that for β-tubulin (lane c). With YL 1/2 labelling a slight separation was occasionally seen of the α-band on the blots (lane b). This split was not present when urea was omitted. It cannot be ruled out that some reactivity with ID5 could be due to remainders of the sperm tails, which were occasionally seen in the gut of the late-stage embryos. However, immunoblots of 0–10 hour embryos failed to show the presence of Glu-tubulin (not shown) and it is concluded that the ID5-stained band in Figure 6 lane a is principally due to Glu-tubulin derived from the central nervous system.

DISCUSSION

The Presence of Detyrosinated α-Tubulin in *Drosophila* Embryos

The antibody ID5 was made against a carboxy-terminal sequence of detyrosinated α-tubulin ending with a glutamic acid residue [Wehland and Weber, 1987]. ID5 does not react with the corresponding sequence, having in addition a carboxy-terminal tyrosine. However, some α-tubulins are more varied in sequence, terminating with other c-terminal residues, and the question must be asked as to whether the c-terminal region of the *Drosophila* α-tubulins can be detyrosinated. Four tubulin genes have been identified in *Drosophila* so far, all of which have been sequenced and characterized [Theurkauf et al., 1986]. Three of them (α1, 2, and 3) have conserved sequences which are similar to those of other groups and possess the expected tripeptide Glu-Glu-Tyr at the c-terminus. The fourth isotype (α4) is highly divergent at the c-terminus and has the terminal tripeptide sequence of Asp-Glu-Phe. However, α4 is not present in either testis or nervous tissue whilst α1 and 3 are ubiquitious in their distribution and α2 may be present in testis in small amounts and is present in later-stage embryos [Matthews et al., 1989]. Thus ID5 most likely identifies one or more of the α1, 2, and 3 tubulin isotypes when detyrosinated. Matthews et al. [1989] also find a number of modified α-tubulins present in both testis and C.N.S. tissues and speculate that detyrosinated α-tubulin may be one of these.

This is the first report of α-tubulin detyrosination in insects. Although quite a variety of mammalian cells and those of some other vertebrates have been found to contain microtubules rich in tubulin thus modified, its distribution in the rest of the animal kingdom has been only sporadically described. However, sea urchin egg and sperm tubulin can be tyrosinated in vitro [Kobayashi and Flavin, 1981] and their sperm tails can be decorated by antibodies against detyrosinated α-tubulin [Gundersen and Bulinski, 1986]. Furthermore, Gabius et al., [1983] and Sherwin et al. [1987] have found that tyrosination of nematode and trypanosome α-tubulin occurs. Thus it seems likely that the post-translational loss and addition of a carboxy-terminal tyrosine will prove to be a quite widespread feature of tubulin metabolism among different groups.

Microtubule Dynamics in Early Stage *Drosophila* Embryos

During the 14 cleavages which precede cellularization mitosis is extremely rapid, occurring approximately every 10 minutes [Zalokar and Erk, 1976], which is possibly the fastest recorded rate for a higher eucaryote. Microtubules might be expected to turn over rapidly under these circumstances. The data from staining these stages with anti-acetylated α-tubulin after a very brief taxol treatment demonstrates that they indeed turn over quickly, i.e., over less than 30 seconds at 25°C for both interphase arrays and mitotic spindles. The interphase microtubules are much smaller than those of cultured mammalian cells, being 5 μm in length or less, and thus the turnover rate is, in fact, not significantly different. Polar microtubules within mammalian spindles turn over with similar fast kinetics to those reported here for early stage *Drosophila* embryos [Saxton et al., 1984; Mitchison et al., 1986]. Thus it is not surprising that such

rapidly turning-over microtubules do not show post-translational modifications.

Structures Stained by Antibodies to Detyrosinated α-Tubulin

Immunofluorescence staining of embryos has revealed two microtubule-rich structures which stain strongly for detyrosinated α-tubulin: sperm tails, which enter the egg during fertilization [Hildreth and Lucchesi, 1963], and neurone processes present after CNS condensation [Campos-Ortega and Hartenstein, 1985]. In both cases the α-tubulin is also strongly acetylated as was previously recorded by Piperno and Fuller [1985] and Wolf et al. [1988]. The sperm tails of several groups, including man and sea urchin, have also been found to stain both for detyrosinated [Gundersen and Bulinski, 1986] and acetylated α-tubulin [Piperno and Fuller, 1985]. Similarly neurones from a number of sources contain microtubules which stain for detyrosinated [Gundersen and Bulinski, 1986; Wehland and Weber, 1987; Cambray-Deakin and Burgoyne, 1987] and acetylated [Cambray-Deakin and Burgoyne, 1987] α-tubulin. Although detyrosination does not in itself confer stability [Webster et al., 1987, 1988] it may be required for the maintenance of stable microtubule arrays when they have formed. *Drosophila* sperm tails do indeed demonstrate considerable stability because they remain within the gut for 15 hours or longer after fertilization, becoming part of the gut contents for some while before disappearing.

The surprising finding that detyrosinated α-tubulin did not appear in microtubule arrays until a late stage of development, and then seemingly only or mainly in the differentiated CNS, raises the questions as to why it does not appear together with acetylated α-tubulin and what the significance of the difference is. As already shown by Wolf et al. [1988], microtubules rich in acetylated α-tubulin first occur at cellular blastoderm, about three hours after fertilization, and such acetylated microtubules are frequently found in interphase arrays after that time. That the absence of detectable acetylated α-tubulin in earlier stages of development is not due to the lack or inactivity of the acetylase could readily be demonstrated by treatment of early embryos with taxol. However, Glu-tubulin does not appear upon taxol treatment in early stages of development (i.e., during syncytial and cellular blastoderm), suggesting that the level of carboxypeptidase is too low to generate detectable Glu-tubulin, or more likely entirely absent. Previous results on cultured mammalian cells support these findings. Certain cell types such as CHO and neuroblastoma cells lack Glu-rich MTs as long as these cells are proliferating [Wehland and Weber, 1987]. In contrast to other cultured cell lines, such as mouse 3T3 cells, taxol treatment did not increase the level of detyrosinated tubulin in CHO cells. Only when differentiation was induced by raising the intracellular cAMP level were extended arrays of Glu-rich MTs detected in both cell types. Thus the appearance of Glu-rich MTs during neurone differentiation in *Drosophila* embryos suggests that specific differentiation events may control the detyrosination/tyrosination cycle of α-tubulin at the level of the carboxypeptidase similarly to the situation found in the above-mentioned mammalian cell lines. As it is now clear that the function of tubulin detyrosination is not to stabilize MTs directly [Webster et al., 1987, 1988] this modification may specify stable MTs for specific activities or for very stable microtubules. Such modified MTs could be the preferred target for certain MAPs, since other results indicated that the carboxytermini of both α- and β-tubulin are involved in MAP binding [Serrano et al., 1984; Sackett et al., 1985].

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