DIRECTIONALITY OF MICROTUBULE ASSEMBLY: AN IN VIVO STUDY WITH THE CILIATE TETRAHYMENA

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SUMMARY
A temperature-sensitive mutant homozygous for the recessive gene mo1b in Tetrahymena thermophila offers opportunity for studying the direction of microtubule assembly in vivo. At 39 °C the mutant fails to divide properly; the 2 daughter animals remain attached and bend over each other. As revealed by protargol staining, the bending results in acute turning and breaking of some of the longitudinal microtubular bands close and parallel to the surface. Hence, 2 broken microtubular ends are available for study of the problem of directionality of microtubule assembly, by assessing which of the 2 ends regenerates. In most cases the posterior portion of the longitudinal microtubular band regenerates. The present study hence supports the conclusion based on in vitro observation in other systems that microtubule assembly is predominantly unidirectional.

INTRODUCTION
Previous in vitro studies have indicated mostly uni-directionality of tubulin assembly on microtubular seeds (Borisy, Olmsted, Marcum & Allen, 1974; Rosenbaum et al. 1975). In some of these demonstrations, the labelled (with radioisotope or DEAE) microtubular seed contains 2 opposite broken ends. The directionality of assembly was assessed by measuring the distance of the label from the 2 ends of the microtubular piece at the end of the regeneration period. The direction of assembly with respect to the topography of the living cell cannot, however, be assessed in such experiments. Other in vitro studies have employed isolated basal bodies (Chlamydomonas) and flagellar axonemes (Chlamydomonas, sea-urchin sperm tail). There, the organellar microtubules under study are ordinarily oriented perpendicular to the cell surface and their direction of extension is normally towards the outside of the cell (i.e. at the distal ends of the tubules). These studies, suggesting that intussusceptive assembly of tubulin subunits on such microtubules is unlikely, do not address the general problem of the control of the direction of assembly for those cytoplasmic microtubules oriented more or less parallel to the cell surface. One wants to know whether, when such microtubules are broken in a living cell, both broken ends contribute equally to regeneration in acting as sites of assembly. In the in vivo study of Witman (1975) on regenerating flagella of Chlamydomonas, the flagellar stub remaining on the body was the only broken end studied; regeneration of the axoneme detached from the body during deflagellation was not compared. The present paper reports observation on in vivo regeneration of both broken ends.
of microtubular bands running parallel and close to the cell surface of Tetrahymena, and shows that microtubule assembly is mostly in a particular direction with respect to the cellular topography; however, strikingly, in a minority of cases the reverse direction is the predominant mode of assembly.

MATERIALS AND METHODS

Animals

All experimental animals are of a derivative of strain B of Tetrahymena thermophila which is homozygous for the recessive gene molB (Frankel, Jenkins, Doerder & Nelsen, 1976).

Culture

The animals were normally kept in 1 % proteose peptone – 0.1 % yeast extract medium at about 25 °C.

Heteropolar doublets were induced when log-phase cells were transferred to 39 °C. They were then sampled for staining (see below) after 3.5 and 5 h.

Staining

The major cortical microtubular bands around the basal bodies can be demonstrated by using an improved version of the protargol staining method (Ng & Nelsen, 1977).

Figs. 1, 3 and 5 show singlets oriented with the anterior end toward the top of the page; Figs. 4 and 6–16 show doublets with the dorsal (concave) side toward the top of the page. The positions of the oral apparatuses of heteropolar doublets are indicated, though they are out of focus in some pictures. Magnifications: Fig. 2, x 90000; Figs. 9, 10, x 4000; all others, x 1400.

Fig. 1. Distribution of microtubular bands in the cortex.

Fig. 2. EM section across the longitudinal band.

Fig. 3. Two ciliary rows are inverted (arrows) in an animal. Note the 180-degree opposite orientation of the microtubular bands on the inverted rows.

Fig. 4. Heteropolar doublet sampled after 3.5 h in 39 °C. The anterior and posterior daughters bend over each other. No sign of extension of lm into the developing right juncture is yet seen.

Fig. 5. An animal bearing 2 inverted ciliary rows (arrows) and an extra longitudinal microtubular band in the right juncture.

Figs. 6–16. Heteropolar doublets showing the developing right juncture and extension of a lm into the right juncture at the point of bending.

Fig. 6. After 5 h at 39 °C. The lm at the point of bending extends into the developing right juncture to form the extra longitudinal microtubular band seen in singlets subsequently derived (see Fig. 5). In this heteropolar doublet the direction of microtubule assembly cannot be assessed.

Fig. 7. Clear separation between the anterior and posterior portions of the lm showing that the structure is broken at the point of bending. The posterior portion has extended for a short distance (arrow).
Direction of microtubule assembly in vivo
Abbreviations on figures

- **a**: anterior portion of longitudinal band
- **ad**: anterior daughter
- **bb**: basal body
- **c**: cilium
- **e**: epiplasm
- **elm**: extra longitudinal microtubular band
- **lm**: longitudinal microtubular band
- **m**: inner alveolar membrane
- **oa**: oral apparatus
- **p**: posterior portion of longitudinal band
- **pd**: posterior daughter
- **pm**: postciliary microtubular band
- **rj**: right juncture
- **tm**: transverse microtubular band

RESULTS

**Cortical microtubular bands**

The most prominent is the longitudinal band (**lm**) running close to one side of each ciliary row (Fig. 1). EM observations (Allen, 1967; Ng & Williams, 1977) indicated that the **lm** consists of 7–12 tubules parallel to, and sandwiched between the inner alveolar membrane above and the epiplasm below (Fig. 2). In addition, 2 other major microtubular bands exist: the transverse band (**tm**) originates anterior to each basal body and extends laterally between 2 ciliary rows; the small postciliary band (**pm**) arises from the posterior face of each basal body and extends posteriorly at an angle.
The extra longitudinal microtubular band

As in Paramecium (Beisson & Sonneborn, 1965), the ciliary rows in Tetrahymena can be rotated 180° (Ng & Frankel, 1977). Such inverted rows are propagated, with all the microtubular bands around the basal bodies oriented at 180° from their normal positions (Fig. 3). The ciliary row rotation is accomplished here by making use of a temperature-sensitive mutant molb (Frankel et al. 1976). Such animals at 39 °C form an incomplete mid-body fission zone during an abortive process of cell division; the anterior and posterior daughters thus remain attached and bend over to form a heteropolar doublet (Figs. 4, 8). When returned to 28 °C, the doublet gives rise by growth and division to singlets, some of which have incorporated ciliary rows of opposite polarity. Interestingly, in some of the singlet cell lines bearing inverted rows, the juncture between the normal and inverted rows on one particular side of the inversion (the ‘right’ juncture) carries an inheritable extra band of longitudinal microtubules (emb, Fig. 5). The microtubular nature of this extra structure has been confirmed by electron microscopy (Ng & Williams, 1977).

The origin of the extra band of longitudinal microtubules has been traced to an extension of a longitudinal band into the developing right juncture in the heteropolar doublet (Fig. 6). It is believed that this extension is subsequently incorporated, together with inverted ciliary rows and the right juncture, into singlets (Ng & Frankel, 1977); evidence in support of this notion is detailed elsewhere (Ng, 1978). An early stage of doublet formation shows ciliary rows looping along the concave side of the doublet, with the longitudinal band following the contour of the ciliary row facing the right juncture (Figs. 4, 8). Later, at the base of the loop the longitudinal band is apparently broken. This is especially evident in some cases where there is a clear separation between the anterior (a) and the posterior (p) portions of the band (Fig. 7). Since both broken ends are present, this offers an opportunity of studying the direction of tubulin assembly on the regenerating band, by assessing which (a or p) portion of the band extends into the right juncture (Figs. 8–10).

Of 129 heteropolar duplexes clearly bearing longitudinal band extensions, there were 83 showing extension from the posterior end (Figs. 7, 11, 12), 8 from the anterior end (Figs. 13, 14), and 7 exhibiting an X-shaped structure (Figs. 15, 16), indicating that the a and p ends have extended about equally. The remaining 31 cases showing longer extensions cannot be assessed with certainty, perhaps because the longitudinal band extension shifts position during subsequent development to align itself more in parallel with the right juncture (Fig. 6). Thus, the ratio of p- to a-extension is (83 + 7) : (8 + 7), or 6:1. Clearly, the p-extension is the preferred mode of assembly of tubulin on the regenerating microtubular ends.

DISCUSSION

The present study shows that microtubule assembly in the longitudinal microtubular band of Tetrahymena is mainly unidirectional. When the band is broken, the posterior (p) end is the most favoured site for assembly; it is inferred that the direction of extension in regenerating longitudinal microtubular bands in singlets is from posterior to anterior of the animal. However, a small amount of assembly on
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the disfavoured end (anterior, $a$) in each case of clear extension of the posterior end cannot be excluded. In vitro studies in other systems have indicated that at high temperature (30°C, 37°C) bidirectional assembly is favoured (Olmsted et al. 1974); heteropolar doublets in the present study have been harvested at a comparably high temperature, 39°C. Moreover, the X-structures seen in 7 heteropolar doublets demonstrate that assembly did take place at both the $a$ and $p$ ends simultaneously in the same cell. Hence, it is unlikely that there is an absolute determination of assembly on only one of the broken ends. More likely, there is a difference in the rate of assembly on the 2 broken ends; in most cases assembly is more rapid at the $p$ end. It is conceivable that once assembly is under way at the $p$ end, the assembled tubules lying directly across the $a$ end would interfere with any possible elongation of the latter. Elongating tubules at the $a$ end obviously will have to cross over the $p$ tubules (unless they change directions), as evidenced by the X-structures. However, the rarity of the X-structures, and the fact that the tubules lie in a narrow space between the inner alveolar membrane and the rather rigid epiplasm (Vaudaux, 1976; Williams, 1977) (Fig. 2), together suggest that crossing over of the tubules is not executed without difficulty.

The 8 cases of preferred extension of the $a$ end are enigmatic. In previous assembly studies (Borisy et al. 1974; Rosenbaum et al. 1975; Witman, 1975) involving flagellar axonemes and basal bodies in which the favoured end of the microtubular seed can be morphologically distinguished from the disfavoured end, the disfavoured end never exhibited an assembly rate more rapid than that of the favoured end under conditions allowing bidirectional assembly. Such studies demonstrate that there is a reluctance to assemble tubulin units on to the disfavoured end and that this reluctance may be partially overcome at high temperature or high tubulin concentration to allow a slow rate (or limited amount) of assembly. The nature of the constraints at microtubular ends restricting assembly is poorly understood (Roth & Pihlaja, 1977). The present 8 cases may be due to an occasional lead in the elongation of the $a$ end which thus interferes with the extension at the $p$ end. Alternatively, these may indicate that the molecular constraints at the $a$ end may not be just partially overcome, but, in fact reversed to produce a favoured end; this would then suggest a certain plasticity in the conformational changes of the microtubular end molecules in governing assembly.

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Figs. 11, 12. Extension (arrow) from the posterior portion of the lm. Two focal levels with the posterior portion in focus in Fig. 11 and the anterior portion in Fig. 12.

Figs. 13, 14. One of the 8 rare cases of heteropolar doublet showing an extension (arrow) from the anterior portion of the lm. Two focal levels with the anterior portion in focus in Fig. 13 and the posterior portion in Fig. 14. Inset drawing shows a cilium lying across the posterior portion of the lm.

Figs. 15, 16. Extensions (arrows) from both the anterior and posterior portions of the lm (2 focal levels).
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REFERENCES


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