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180° rotation of ciliary rows and its morphogenetic implications in *Tetrahymena pyriformis*

(morphogenesis/microtubular assembly)

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ABSTRACT With quasi-surgical techniques, longitudinal somatic ciliary rows in *Tetrahymena pyriformis* have been rotated 180°. New structures formed in the rotated ciliary rows during growth and reproduction are disposed 180° opposite to their normal positions or orientations, confirming the earlier findings of Beisson and Sonneborn on *Paramecium*. However, during cell fission the rotated ciliary rows exhibit abnormality in orientation along the fission zone; the configuration of these rows near the anterior end of the posterior product of fission is consequently affected. Rotated ciliary rows have been employed as a tool in the analysis of morphogenetic problems: (*a*) The contractile vacuole pore is normally located on the left side of a ciliary row; but it is on the right of inverted rows. Hence, the morphogenetic properties of the two sides of the ciliary row associated with the contractile vacuole pore are different and this difference is the sole determinative factor as to the side of the ciliary row on which the contractile vacuole pore is located. (*b*) The process that generates the rotated ciliary rows frequently also brings about the implantation of an extra band of longitudinal microtubules at a specific site on the cell surface. This extra structure is inheritable, which opens up opportunities for the study of microtubular assembly *in vivo*.

Beisson and Sonneborn (1) showed that ciliary rows in *Paramecium* can be experimentally rotated 180° ("inverted rows," or IRs). The IRs are propagated in successive fissions: new structures arising in the IRs in the progeny animals are positioned or orientated 180° opposite to the normal position or orientation. This provides the most rigorous and clear-cut demonstration of the notion of cytotaxis, i.e., that preformed or preexisting structures impose a restraint on the development and orientation of newly formed structures (1–3).

There are good reasons for obtaining and studying IRs in another ciliate, namely *Tetrahymena pyriformis*. The total number of ciliary rows and units is considerably fewer in *Tetrahymena* than in *Paramecium*, thus facilitating study of the details of the ultrastructure and propagation of the IRs. In addition, *Tetrahymena* exhibits distinct differences from *Paramecium* in morphology (4, 5) and morphogenesis (6), which allow for analysis of certain morphogenetic patterns apart from the orientation and propagation of the IRs themselves. Most strikingly, the generation of IRs in *Tetrahymena* opens up new possibilities in the study of microtubular assembly in living systems. The present communication gives a brief account of the mode of obtaining and identifying IRs in *Tetrahymena pyriformis* syngen 1, and also introduces some of the major known properties of the IRs and associated morphogenetic phenomena in this organism.

Abbreviations: IR, inverted ciliary row; CVP, contractile vacuole pore; ELM, extra longitudinal microtubular band; lm, longitudinal microtubular band; tm, transverse microtubular band; pm, post-ciliary microtubular band.

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MATERIALS AND METHOD

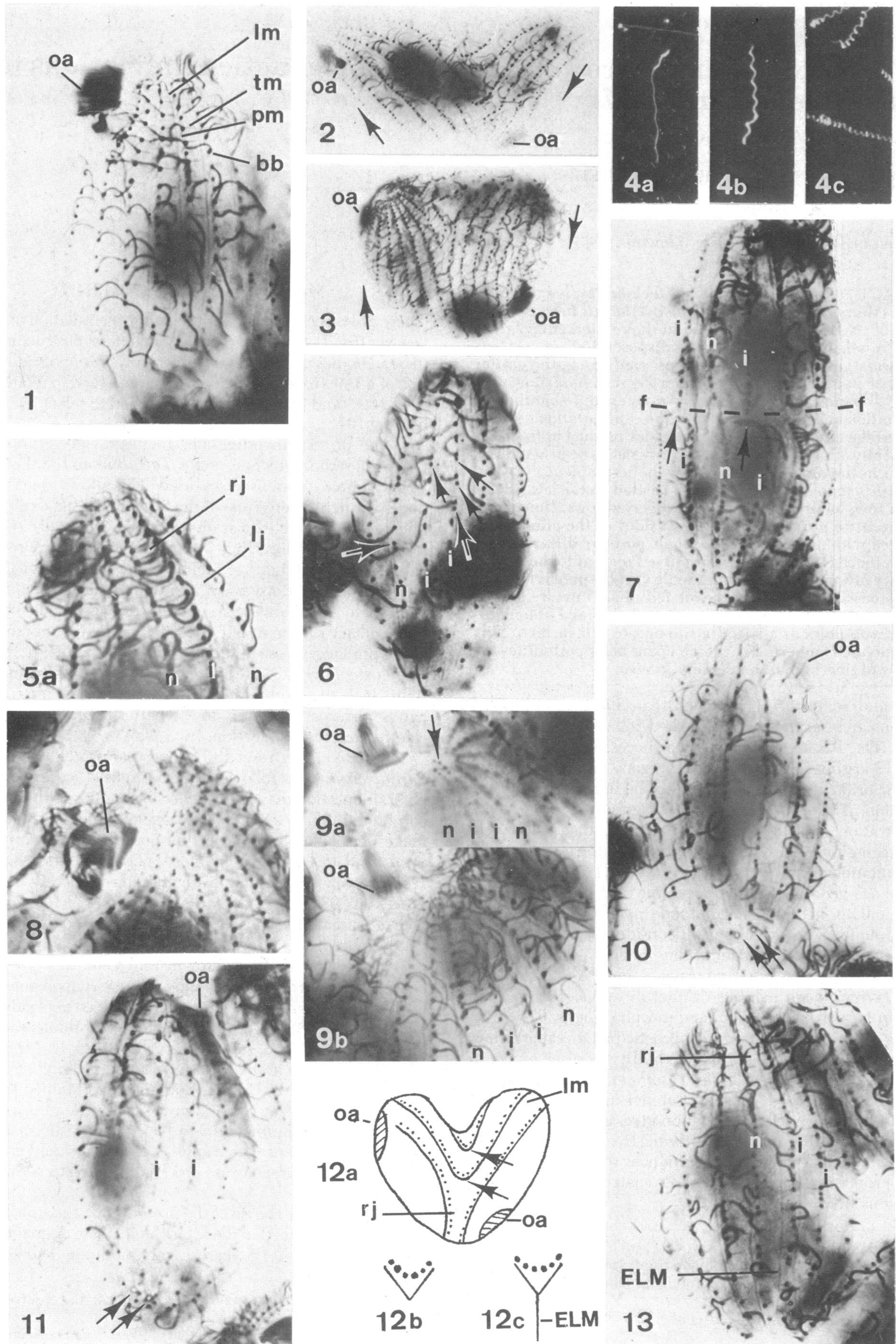
There are three basic requirements for the isolation of ciliates bearing IRs: (*i*) an asymmetrical arrangement of structures close to basal bodies in the somatic ciliary rows to enable identification of a 180°-rotated ciliary row; (*ii*) a method to rotate ciliary rows 180°; and (*iii*) a convenient method to select for animals bearing IRs.

In the present investigation, an improved version of the protargol staining technique for *Tetrahymena* (S. F. Ng and E. M. Nelsen, unpublished) is used. This allows identification under the light microscope of the three major bands of cortical microtubules, which are asymmetrically arranged close to the somatic basal bodies (Fig. 1): the longitudinal microtubular band (lm) on the right‡ of each row of basal bodies runs from pole to pole; the transverse microtubular band (tm) arises just anterior to each basal body, extends leftward and stops short of the ciliary row on its left; the small postciliary microtubular band (pm) arises close to the posterior-right corner of the basal body, extends right-posteriorly and stops short of the lm on the right. Such an asymmetrical arrangement of the microtubular bands corresponds exactly to the pattern reconstructed from electron microscope sections by Allen (7).

To rotate ciliary rows 180°, the basic principle applied in *Paramecium* (1) is followed: two animals attached in heteropolar orientation may give rise to single animals that have incorporated ciliary rows in 180° orientation. In practice, we make use of homozygotes of temperature-sensitive mutants (*mo1^b* and, to a lesser extent, *mo8^a*, both from strain B, syngen 1; see ref. 8) which exhibit difficulties in completing the fission process at 39°C. Frequently the ciliary rows on the dorsal surface of animals dividing at 39°C fail to develop the equatorial discontinuities that indicate a fission zone. Probably as a result, the two daughters often fail to separate from each other. Later on they bend over each other and form a V-shaped heteropolar duplex, with their dorsal surfaces on the concave side of the duplex (Figs. 2, 3, and 12*a*). Such heteropolar duplexes isolated after 7–10 hr at 39°C are then returned to 25°C. After 15–20 hr at 25°C, single animals, some of which exhibit a "twisty" swimming behavior (Fig. 4*a*, *b*, and *c*), are obtained from the heteropolar duplexes. Such "twisty" swimming behavior has been shown in *Paramecium* to be characteristic of animals bearing IRs (9). Hence, such animals are isolated and grown for 3 days, and their progeny are protargol-stained to ascertain the presence of IRs.

Throughout the course of the present investigation, the animals were maintained in the logarithmic phase of growth in 1% proteose peptone/0.1% yeast extract medium. Tube cultures

‡ Throughout this report, *right* and *left* refer to the observer's right and left, assuming that he stands inside the animal so that his anterior-posterior axis coincides with that of the animal, and keeps turning around his longitudinal axis to face the surface of the animal.



FIGS. 1–13. *(Legend appears at bottom of the following page.)*

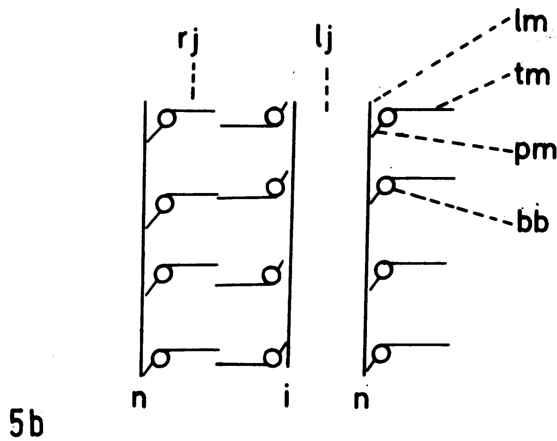


FIG. 5b. Diagrammatic representation of part of the cortex showing an inverted ciliary row between two normal ciliary rows. Abbreviations are as in Figs. 1 and 5a.

at 25°C were transferred in drops (every day) or in wire loops (every 1½ days) to new culture tubes containing 5 ml fresh medium each. Depression cultures kept at 16°C were reisolated once every week.

RESULTS

Recognition of IRs

An IR can be cytologically recognized by the arrangement of the three major cortical microtubular bands (lm, tm, and pm) disposed 180° opposite to their normal positions and orientations around the basal bodies (Fig. 5a, and b). Purely as a result of a 180° rotation, two types of junctures between normal and inverted (or group of inverted) ciliary rows can be identified on the two sides of the inversion. The one on the right side of the inversion ("right juncture") is characterized by two lms "bracketing" two rows of basal bodies and by tms interdigitating with each other in the juncture. The "left juncture" is characterized by two lms being bracketed by two rows of basal bodies and by the absence of any tm in the juncture.

The 180° rotation of the position and orientation of the three major cortical microtubular bands in IRs has been confirmed by electron microscopy (S. F. Ng and R. J. Williams, unpublished). In addition, the ultrastructural study demonstrates that cortical structures not revealed by protargol staining, such as the kinetodesmal fibers normally found on the right of the basal body rows, and the parasomal sacs normally located anterior to basal bodies, are also rotated 180° in the IRs.

Propagation and maintenance of IR-cell lines

Altogether, 20 lines (line = descendants from a single cell isolate) bearing IRs were obtained independently in four separate experiments, from 20 heteropolar duplexes (16 from $mo1^b$ and 4 from $mo8^a$ mutants). One to five IRs per animal are found in these lines and they are found on the dorsal or lateral surfaces of the animal. A few animals do bear IRs close to the mid-ventral surface; preliminary observations suggest that such animals apparently encounter difficulties in maintaining the IRs, which soon disappear in their descendants.

Of the 16 lines from the $mo1^b$ mutant, the IRs in 14 of them have been maintained (until the termination of the investigation) for over 200 fissions at 16°C, in one line for over 500 fissions at 25°C, and in one line for about 1500 fissions at 25°C. The IRs disappear in some descendants, irrespective of their location in the animal. Thus, in order to maintain the IRs in the lines, periodic selection for animals exhibiting the most extreme twisty-swimming behavior is carried out, once every week at 16°C, and once every 10–14 days at 25°C.

The first conspicuous step in the propagation of a ciliary row, the formation of new basal bodies, is inverted in IRs. In normal ciliary rows, new basal bodies appear on the cell surface just *anterior* to old ones and remain unciliated for some time (10, 11). In IRs, new basal bodies appear just *posterior* to old ones (Fig. 6). This clearly shows that the restriction of formation of basal bodies to the microenvironment on only one side of the preexisting basal bodies is solely determined by the orientation of the preexisting basal bodies and/or their immediate vicinities. Exceptions to this rule are only occasionally encountered: in normal ciliary rows one in 1015 and in IRs one in 894 new basal bodies are found on the "wrong" side (observation based on "pairs" of basal bodies in which one is ciliated while the other is not and the two in a pair are not more than the diameter of a basal body apart).

Partitioning of inverted ciliary rows during cell fission

Ciliary rows become partitioned to daughter animals by the development of an equatorial fission zone perpendicular to the ciliary rows during cell fission. The anterior and posterior halves of the ciliary rows are then inherited by the anterior and pos-

FIGS. 1–13 (*on preceding page*). Light micrographs and drawings of *Tetrahymena*. Abbreviations: n, normal ciliary row; i, inverted ciliary row; oa, oral apparatus (out of focus in some cases) to indicate orientation of animals in the figures. Except in Figs. 2, 3, 4, and 12a, b, and c, the anterior end of the animal in the figures points toward the top of the page. *Fig. 1.* A normal animal showing asymmetrical arrangement of the three major cortical microtubular bands (longitudinal, lm; transverse, tm; and post-ciliary, pm) close to basal bodies (bb). ×1400. *Figs. 2 and 3.* Heteropolar duplex at 3½ hr (Fig. 2) and 5 hr (Fig. 3) at 39°C. Arrows indicate the orientations of the two attached daughter animals. ×700. *Fig. 4.* Dark-field tracing of the forward swimming paths of normal animals (a), an animal having two out of its 19 ciliary rows inverted (b), and animals having three out of their 16 ciliary rows inverted (c). *Fig. 5a.* Anterior part of an animal bearing one inverted ciliary row. rj = right juncture; lj = left juncture. ×1800. *Fig. 6.* An animal showing appearance of new basal bodies (arrows) posterior to old basal bodies (ciliated) in inverted ciliary rows, but anterior to old basal bodies in normal ciliary rows. ×1400. *Fig. 7.* A dividing animal showing the bending toward the right of the anterior part of inverted ciliary rows in the prospective posterior daughter animal. The normal ciliary rows (notice especially the one between the two inverted ciliary rows) do not exhibit such abnormality. f—f = fission zone. ×1400. *Fig. 8.* Anterior pole region of an animal, showing "couplet" organization of basal bodies at the anterior ends of normal ciliary meridians on the left of the oral apparatus and on the dorsal surface. ×1600. *Fig. 9.* Two focal levels of the anterior pole region of the same animal showing basal bodies from the anterior ends of two inverted ciliary rows (b) "intruding" into the pole region and failing to organize into couplets (a). ×1600. *Fig. 10.* A normal animal having two contractile vacuole pores (arrows), each located to the immediate left of a normal ciliary row. ×1400. *Fig. 11.* An animal having two contractile vacuole pores (arrows) each located to the immediate right of an inverted ciliary row. ×1400. *Fig. 12.* Drawings to illustrate implantation of an extra longitudinal microtubular band into the right juncture (rj). Ciliary rows that fail to form a transverse zone of discontinuity during cell fission make loops (arrows) on the concave side of the V-shaped heteropolar duplex (a). Later on the longitudinal microtubular band (lm) normally associated with the looping ciliary row abutting the prospective right juncture becomes remote from the basal bodies and develops into a V-tip (b). From the V-tip an extra microtubular band appears and extends into the right juncture, giving a Y-shaped configuration (c). The extra longitudinal microtubular band (ELM) found in the right juncture of single animals cut off from the heteropolar duplex (see Fig. 13) results from incorporation of the long arm of the Y-structure into the right juncture. *Fig. 13.* An animal bearing an extra longitudinal microtubular band (ELM) in the right juncture (rj). ×1400.

terior daughter animals, respectively. Preliminary observation of dividing animals indicates that IRs behave abnormally at this stage: a fission zone develops across the IRs only at a late stage; the portion of the IR just posterior to the fission zone makes a right-angle turn toward the right, parallel to the fission zone (Fig. 7) and frequently meets the normal ciliary row to its right. Such abnormality implies that a normal pattern of partitioning of a ciliary row during cell fission is attained only if the ciliary row has a normal anterior–posterior orientation with respect to some other parameter, possibly the polarity of the animal or that of the fission zone.

The abnormality described above does not hinder subsequent separation of the two daughter animals, at least in cases when only a few IRs are present. The abnormal partitioning of the IRs, however, appears to leave a "mark" on the anterior end of the IRs in the posterior daughter. The anterior ends of the normal ciliary rows to the left of the oral apparatus ending on the anterior pole and of those on the dorsal surface characteristically describe an arc around the anterior pole (Fig. 8); the first two basal bodies of each of these ciliary rows are close to each other [hence called "couplets" (5)]. The IRs deviate from this pattern: the first two basal bodies are not organized into couplets, and the anterior ends of IRs often extend beyond, or fall short of, the anterior arc of couplets of the neighboring normal rows (Fig. 9*a*, and *b*). The normal organization of the anterior end of a ciliary row therefore appears to depend on a correct alignment of the ciliary row during cell fission.

The common transgression of IRs into the polar region of the animal is reminiscent of the extension of IRs of *Paramecium* into the anterior suture (12), which is comparable to the apical arc of *T. pyriformis*.

Ciliary row inversions as a tool in the study of problems in subcellular morphogenesis

Because preexisting patterns or structures in some systems definitely play an essential role in the development of certain structures that are to be formed, an obvious approach to further our understanding of morphogenesis in such systems is by surgical alteration of preexisting patterns or structures and assessment of whether the development of any newly formed structures is affected, and, if so, in what ways. Furthermore, if the surgical alteration can be propagated from generation to generation, then, with little work, the sample size of surgically altered animals can be greatly increased and experimental analysis much facilitated. The IRs, as an inheritable, altered pattern, provide exactly this kind of convenience, as will be exemplified by the following two cases:

Fine Positioning of Contractile Vacuole Pores (CVPs). Each animal possesses usually two CVPs found posteriorly at about a quadrant to the right of the mid-ventral surface (Fig. 10). Each CVP is almost invariably positioned close to the *left* of a ciliary row (a ciliary row associated with a CVP is commonly referred to as a CVP meridian). During cell fission, the posterior daughter appears to inherit the old CVPs, whereas new CVPs are developed at the posterior end of the anterior daughter. The question of interest in this regard is whether the fine positioning of the CVP is determined solely by the CVP meridian or by other factors extrinsic to the CVP meridian. It is possible to choose between these two alternatives by rotating the CVP meridian 180° and then observing on which side of the rotated CVP meridian the CVP is located. When this is done, the CVP is now found to the *right* of the rotated CVP meridian (Fig. 11). This clearly shows that the fine positioning of the CVP with respect to left-right asymmetry is determined solely by the CVP meridian, suggesting that the morphogenetic properties of the two sides of the ciliary row are different with regard to CVP development. Details on this and additional effects of 180° rotation of CVP meridians on CVP morphogenesis will be discussed elsewhere (Ng, unpublished).

Extra Longitudinal Microtubular Band (ELM). During the generation of the IRs in heteropolar duplexes, a fragment of longitudinal microtubular band is sometimes implanted in the right juncture between the normal and inverted ciliary rows (Fig. 12*a*, *b*, and *c*). Together with the IRs and the right juncture, the fragment is incorporated into single animals arising subsequently from the heteropolar duplex (Fig. 13). The implanted fragment (ELM) may be propagated indefinitely in the progeny, but it can also be lost from some of them. The latter, when isolated, give rise to descendants that do not possess the ELM, except occasionally by a secondary mode of implantation which is in principle similar to that occurring in heteropolar duplexes (Ng, unpublished). This provides a clear-cut *in vivo* demonstration of the principle of "seeding": the extra microtubular band acts as a center of growth or further assembly of microtubular subunits in successive cell generations.

The extent to which the IRs or the right juncture participates in the maintenance of the ELM awaits further investigation. Nonetheless, the close association of the ELM with the IRs and the right juncture facilitates the selection of cell lines bearing the ELM and provides a favorable system for the investigation of microtubular assembly *in vivo*.

DISCUSSION

The present investigation on inverted ciliary rows in *Tetrahymena pyriformis* supports the main conclusion from the pioneering study in *Paramecium tetraurelia* (1). Preexisting ciliary rows in the cortex provide information for the formation, positioning, and orientation of new structures arising within the rows, such as basal bodies, microtubular bands, kinetodesmal fibers, and parasomal sacs. Furthermore, the ciliary rows also provide information for the positioning of structures or organelles which are not part of the ciliary rows but are found in their immediate neighborhood, such as is the case with the fine positioning of the contractile vacuole pores.

The cell as an architect thus not only makes use of the genomic information to produce the appropriate building blocks, but, in addition, also arranges the building blocks according to the blueprints as defined in the preexisting architecture. Such blueprints in different parts of the preexisting architecture enjoy a certain amount of autonomy in directing the assembly and arrangement of new structures. Hence, when the blueprint in one part of the architecture becomes misaligned or misplaced in relationship to its neighbors, it can still maintain its own informational function, in spite of the fact that the newly assembled structures become likewise misaligned or misplaced. The propagation of the extra longitudinal microtubular band by further assembly of microtubular subunits on a preexisting "seed" illustrates how such autonomy can be attained in a relatively simple way in some cases.

However, the inverted ciliary rows and also the extra longitudinal microtubular bands are not as stable as their normal counterparts. This suggests a second level of control to guarantee faithful maintenance of the normal pattern and organization by the elimination of abnormalities, though this is not done instantaneously. We are not sure yet how this is achieved.

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