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Patterning and Assembly of Ciliature Are Independent Processes in Hypotrich Ciliates

Abstract. *Mirror-imaged doublets of the hypotrich ciliate* Pleurotricha lanceolata *were induced and analyzed with respect to the overall patterning (structural asymmetry and polarity) of the individual components of the ciliature. The overall pattern is arranged as a mirror image, but the individual components in the two halves of the doublet show the same organizational asymmetry. These data demonstrate the independence of the mechanisms for this kind of large-scale (global) patterning and control of assembly of the individual ciliary components.*

Among unicells, the mechanism of cell patterning has been studied most extensively in the ciliated protozoa in which complex arrays of cilia form the cell pattern. The demonstration by Sonneborn of the lack of genic differences, genic activity differences, or differences in the fluid cytoplasm in cells possessing different cortical phenotypes (*1*, *2*) emphasizes the value of these organisms in studies of cell patterning and intracellular localizations. A question remaining from such studies is whether or not the overall pattern of the ciliature is exclusively a reflection of the assembly processes of the individual components of the ciliature. We report that the overall pattern of the ciliature is determined independently from the detailed structure and assembly of the component ciliature.

The hypotrich ciliates, including *Pleurotricha lanceolata*, are well suited for studies of cell patterning because of the specific localization of ciliary units, structural polarity and asymmetry of each ciliary unit, overall cellular polarity and asymmetry, and developmental flexibility (*3*-*5*). The typical morphostatic cell (Fig. 1a) has an oral apparatus composed of parallel arrays of rectangularly packed cilia (each array is a membranelle) occupying approximately the antero left cell quadrant. Furthermore, clusters of hexagonally packed cilia are located regularly elsewhere on the ventral surface (the ventral ciliature and the marginal rows of ciliature, one on the left side, and two on the right). In addition to this overall polarization and asymmetry of the cell, each component of the ciliature likewise is polarized and asymmetric. Each membranelle is composed of four rows of cilia; the two postero-most the longest, the antero-most composed of only three cilia at the antero right edge of each membranelle (Figs. 1e and 2b).

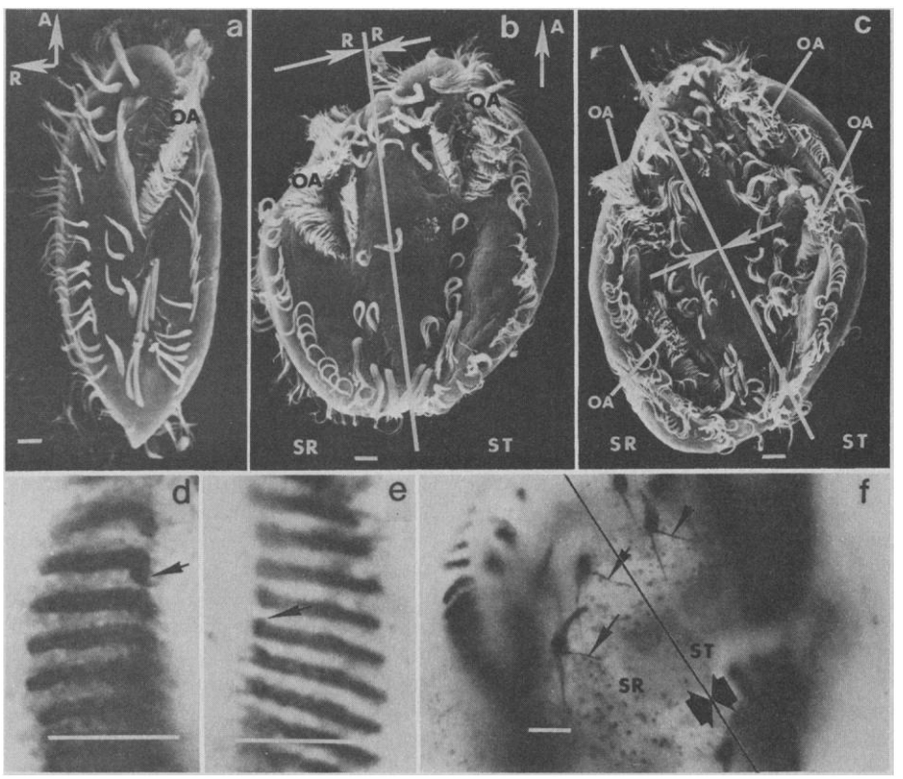

Fig. 1. Micrographs of the ventral surface of *Pleurotricha lanceolata* visualized from outside the cells. (a to c) Scanning electron micrographs; (d to f) light micrographs of silver-stained preparations. A-P, cellular polarity; L-R, axis of asymmetry of the entire cell or halves of the cell; *OA*, oral apparatus. Each bar represents 10 micrometers. (a) Typical morphostatic singlet cell illustrating the standard asymmetric array of ventral ciliature as well as the position and curvature of the oral apparatus (*OA*) in the antero left quadrant. (b) Morphostatic mirror-imaged doublet, the common polarity of both halves (approximate line of bilateral symmetry marked by vertical line) and mirrored patterning of ciliature, including curvature of the oral apparatus are shown. The lateral arrows indicate L-R asymmetry of each half. *ST*, standard symmetry half of the doublet [see (a)]; *SR*, symmetry reversal half of the doublet. (c) Predivision mirror-imaged doublet. Lateral arrows indicate L-R asymmetry of each half as above. There are four oral apparatuses (*OA*), and ventral ciliature is present on both sides of the line of bilateral symmetry (vertical line). (d) Organization of membranelles within the symmetry reversed oral apparatus (*OA* of *SR*) [see (b)]. The short fourth row (at arrow) is on the postero left margin of each membranelle; that is, it is an *inversion* of the standard arrangement [*OA* of *ST* illustrated in (e)] rather than a *mirror image* of the standard arrangement (Fig. 2). (e) Structure of membranelles in the oral apparatus of typical singlet cells and the standard symmetry half of mirror-imaged doublets. The short fourth row (arrow) located on the antero right margin of each membranelle (see also Fig. 2). (f) Morphostatic mirror-imaged doublet illustrating the same position of the lateral fiber bundles (at narrow arrows) on the ventral ciliature of both the standard symmetry half of the cell (*ST*) as well as on the symmetry reversed half of the cell (*SR*).

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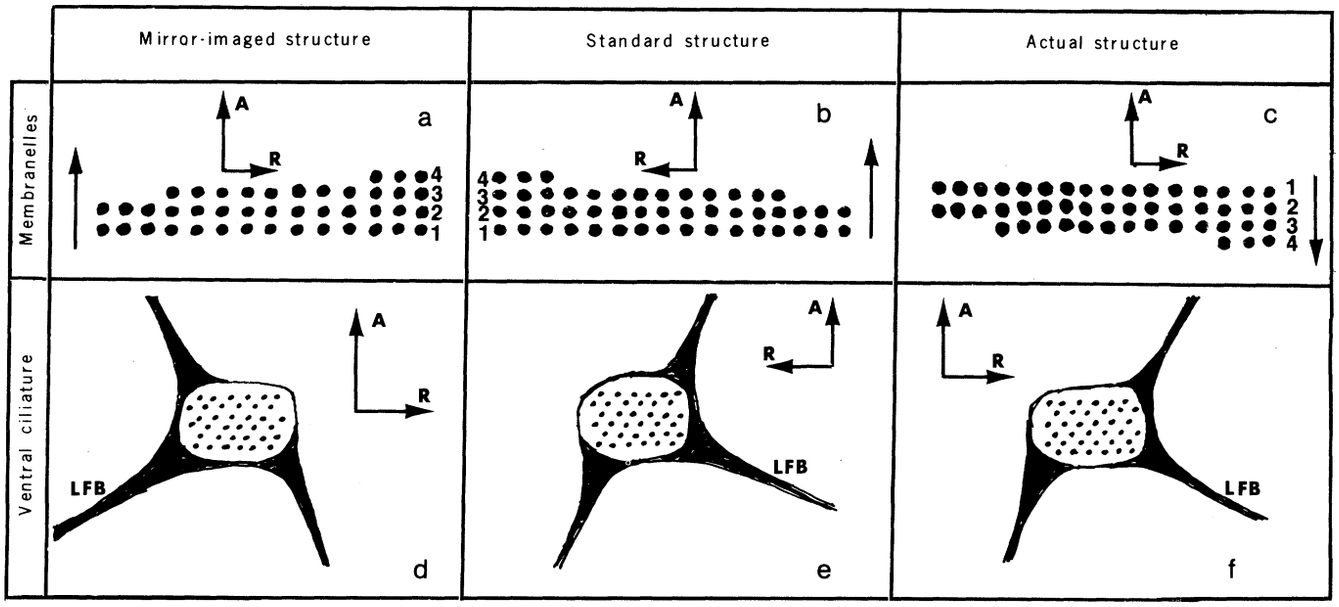

Fig. 2. Representations of the polarity and asymmetry of the individual components of the ciliature of mirror-imaged doublets. Intersecting arrows indicate polarity and asymmetry of the overall ciliary pattern of the halves of the cell on which the structures are located. (a to c) Single arrows indicate structural polarity of individual membranelles; each membranelle row is correspondingly numbered (*LFB*, lateral fiber bundle). (b and e) The standard organization of components of ciliary organelles typical of singlet cells and the standard symmetry half of mirror-imaged doublets. Row 4 is on the anatomical antero right of each membranelle and the lateral fiber bundle is on the left side of the ventral ciliary aggregates. (a and d) The expected organization of ciliary organelles in the symmetry reversal half of mirror-imaged doublets if patterning and assembly were coordinately determined. The polarity of individual membranelles would be typical but with reversed asymmetry (a). Likewise, the lateral fiber bundle would originate on the opposite side of the ventral ciliature (d). (c and f) The actual organization of structures in the symmetry reversal half of mirror-imaged doublets. The membranelle (c) is organized as an inversion of the standard arrangement and the ventral ciliature (f) is identical to the standard ventral ciliature.

Each ventral cluster of cilia also has an asymmetric array of associated microtubular bundles (Figs. 1f and 2e). Thus from observation of an individual ciliary organelle in a typical morphostatic cell, the cellular axes and the overall orientation of organellar pattern can be ascertained.

To investigate the relation of assembly processes and patterning, an axial conflict was created in the organism which yielded asexually propagative mirror-imaged doublets. Two lines of these cells were isolated from independent experiments under two different experimental conditions (*6*). However, the cells from both lines are similar.

The mirror-imaged doublet (Fig. 1b) has two oral apparatuses and approximately a double complement of ventral ciliature. The polarity of each half is normal; however, the asymmetry of the right half is reversed (the symmetry reversal half); thus the overall pattern of the right half is a mirror image of the left half. The two distinct and independent developmental fields that are expressed during prefission morphogenesis are arranged as mirror images (Fig. 1c).

If pattern and assembly are coordinately determined, then the detailed structure of individual components of the ventral ciliature on the symmetry reversal half would also be arranged in mirror-imaged symmetry as predicted in Fig. 2d. The fibers attached to the ventral clumps of cilia would be the mirror image of those of the typical half; that is, the lateral fiber would be attached to the opposite side of the ciliary aggregate. The membranellar structure in the symmetry reversal half would be a mirror image (Fig. 2a). Stained preparations of these cells, however, reveal that none of these structures is a mirror image with respect to details of their individual structural components (Figs. 1, d to f, and 2, c and f). The ventral ciliary aggregates would have the lateral microtubular bundles on the anatomical right if complete mirror imagery existed, but the lateral bundles are on the left side (as in the typical morphostatic singlet cell) (Figs. 1f and 2f). Furthermore, the structure of each membranelle in the symmetry reversal half is not mirror-imaged, but rather inverted (the short row of cilia is on the postero left of each membranelle instead of the antero left, Figs. 1d and 2c). The assembly of membranelles within the primordium in the symmetry reversal half occurs from anterior to posterior, the same as in the typical symmetry half of the mirror-imaged doublet. However, assembly occurs from left to right instead of the typical right to left (*3*); thus the pattern of development is also mirror-imaged. Nevertheless, each basal body is positioned typically relative to its adjacent one, thus constructing a membranelle whose internal organization is normal. The overall mirror-imaged pattern plus the typical internal assembly mechanism result in membranelles in the symmetry reversed half that are inverted relative to cellular polarity. These data show that the patterning of ciliary organelles as well as the pattern of development of these organelles on the ventral surface of hypotrich ciliates does not represent the sum of the individual assembly events of those organelles.

Other types of asexually propagated cortical anomalies have been analyzed with respect to the control of their inheritance. Previous accounts of homopolar doublets have demonstrated that the inheritability of the doublet phenotype is independent of changes in nuclear genotype or fluid cytoplasm (*1, 2, 7, 8*). In these studies doublets in which both halves showed a common polarity and asymmetry were used. Another extensively investigated cortical anomaly is the inverted ciliary row, which is propagated in the inverted fashion during both sexual and asexual reproduction of the cell, and the individual components of the rows have the same asymmetry (*2, 7*). Common to both of these experimentally derived anomalies is the typical asymmetry of overall pattern and of the

component ciliature, as well as the propagation of those anomalies under the direct influence of the existing ciliature. In *Pleurotricha*, most primordial fields develop without direct structural continuity with ciliature of a like kind. Indeed, when these hypotrichs encyst, all ciliature is broken down and an entirely new set is formed during excystment (*9*).

The mirror-imaged doublets reported here also are capable of encystment and excystment, and they do so true to type. Thus, redevelopment of the entire mirror-imaged pattern occurs without the direction of any existing ciliature, and illustrates that information for pattern asymmetry is retained in the cyst even in the absence of ciliature. If the overall pattern of the ciliature were the sum of individual events of the assembly of the ciliary components and there were no directive influence of existing ciliature, then the prediction would be that mirror-imaged doublets would not pass through the cyst true to type but rather revert to a typical symmetry. The fact that mirror-imagery is retained upon excystment further substantiates the conclusion that the mechanism of global patterning of the ciliature is independent of the mechanism of assembly of the individual ciliary components.

Although Tchang Tso-run and coworkers (*10*) have studied this type of mirror-imaged doublet and we have studied a different type of mirror-imaged doublet (*11*), none of those studies presented data on the internal organization of the ciliary components. Presumably, this is why the conclusions that we present have not been reached previously.

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Prolongation of Islet Xenograft Survival Without Continuous Immunosuppression

Abstract. *The survival of isolated rat islets transplanted into diabetic mice was prolonged markedly by maintaining the rat islets in vitro at 24°C for 7 days before transplantation and administering to the recipients a single injection of antiserum to mouse and rat lymphocytes shortly before transplantation.*

Prolonged survival of thyroid allografts was obtained by Lafferty *et al.* (*1*) when they cultured donor thyroids in vitro for several weeks before transplantation. The thyroids were maintained in an atmosphere of 95 percent O_2 during the culture period. The cultured thyroids were rejected if donor peritoneal exudate cells were injected into the recipients at the time of transplantation (*2*). The authors suggested that passenger leukocytes in the thyroid had been destroyed by the culture conditions and that these lymphoid cells were required to initiate rejection of the thyroid. The method of organ culture used by Lafferty *et al.* (*1*) has also produced prolonged survival of thyroid xenografts (rat to mouse) (*3*).

Recently, we reported that islet allografts survived for prolonged periods (> 100 days) across a major histocompatibility barrier in rats when the islets were cultured in vitro at 24°C for 7 days before transplantation and the recipients were given a single injection of rabbit antiserum to rat lymphocytes (RALS) shortly before transplantation (*4*). Low-temperature culture of the islets for 1 to 4 weeks without the single injection of RALS did not produce a marked prolongation of allograft survival. Injection of donor peritoneal exudate cells produced prompt rejection of successfully established islet allografts in histoincompatible hosts at 100 to 220 days after islet transplantation. Since culture of the islets in vitro at low temperature and a single injection of RALS were effective in prolonging islet allograft survival, we sought to determine whether this simple procedure could be used to prolong the survival of rat islets transplanted into diabetic mice. Previous studies had shown that transplants of rat islets induced normoglycemia in diabetic mice for 1 to 5 days before rejection (*5*) and that normoglycemia could be maintained for 9 to 21 days by continuous immunosuppression of the recipients with antiserum to lymphocytes (*6*).

Male BALB/c mice were made diabetic by the intravenous injection of streptozocin (220 mg per kilogram of body weight). Concentration of plasma glucose was determined in the nonfasting mouse on blood obtained from the orbital sinus; only mice with plasma glucose concentrations > 400 mg/100 ml were used as recipients. Plasma glucose concentrations were determined three times weekly before and after transplantation, and the animals were weighed daily. The transplant was considered to be rejected when the plasma glucose level of the nonfasting animal exceeded 200 mg/100 ml.

Islets were isolated from male Wistar Furth rats by the collagenase technique (*7*) and separated on a Ficoll gradient (*8*). Islet tissue was removed from the gradient, and with the aid of a dissecting microscope, islets free from attached vascular and ductal tissue were selected and removed with a Pasteur pipette. The selected islets were then examined again under a dissecting microscope with a reflected green light for illumination, since this procedure permitted the identification and removal of small lymph nodes that are occasionally present in the preparation (*9*). The isolated rat islets were maintained in vitro in tissue culture medium (CMRL-1066) containing fetal calf serum (10 percent), penicillin (100 U/ml), streptomycin sulfate (100 μg/ml), and D-glucose (1.5 mg/ml). The islets were incubated in untreated plastic culture dishes and were maintained in an atmosphere of air and 5 percent CO_2 at 24°C for 7 days.

We found that the portal vein technique (*10*) could be used for transplanting islets into diabetic mice. Isografts of 650 BALB/c islets transplanted via the portal vein produced normoglycemia in diabetic recipients within 2 to 4 days. Five isografts (BALB/c) were still maintaining normoglycemia in the recipients 125 to 150 days after transplantation. Rat islets are slightly larger than mouse islets, and we found that transplants of 450 rat islets produced normoglycemia in

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