Development 111, 337-356 (1991)
Printed in Great Britain © The Company of Biologists Limited 1991

Microsurgically generated discontinuities provoke heritable changes in cellular handedness of a ciliate, *Stylonychia mytilus*

XINBAI SHI[1,*], LI LU[1], ZIJIAN QIU[1], WEI HE[1] and JOSEPH FRANKEL[2,†]

[1]*Department of Biology, Harbin Normal University, Harbin 150080, China*
[2]*Department of Biology, University of Iowa, Iowa City, IA 52242, USA*

* To whom reprint requests from China should be sent
† To whom reprint requests from other countries should be sent

Summary

***Stylonychia mytilus* is a dorsoventrally flattened ciliate with compound ciliary structures arranged in a specific manner on the cell surface. In mirror-image (MI) doublets of this ciliate, two nearly complete sets of ciliary structures are arrayed side-by-side, one in a normal or 'right-handed' (RH) arrangement, the other in a reversed or 'left-handed' (LH) arrangement. MI-doublets exist in two forms, one with the RH component on the right, the LH component on the left, and feeding structures near the center ('buccal-adjoining MI-doublet'); the other with the RH component on the left, the LH component on the right, and feeding structures on the lateral edges ('buccal-opposing MI-doublet').**

We describe an operation that can generate either type of MI-doublet. This operation interchanges large anterior and posterior regions of the cell, transposing the original posterior region anteriorly (P→A) and the original anterior region posteriorly (A→P), while retaining the original anteroposterior polarity of each region. Two sets of new ciliary structures then are formed in mirror-image arrangement, with the set in the P→A region oriented normally and the set in the A→P region undergoing a reversal of polarity along its anteroposterior axis. This sometimes creates end-to-end MI forms, but more commonly produces side-by-side MI-doublets through a folding together of the P→A and A→P regions. This folding occurs because one lateral edge of the cell had been removed during the operation; if the left edge was removed, the complex folds to the left and forms a buccal-adjoining MI-doublet, whereas if the right edge was removed, the complex folds to the right and forms a buccal-opposing MI-doublet. Both types can reorganize and later divide true-to-type, although the 'buccal-opposing' type is by far the more stable of the two.

The generation of mirror-image forms is dependent on the prior abnormal juxtaposition of regions from opposite ends of the cell, and involves a coordinated respecification of large-scale organization. We interpret this response to be a consequence of intercalation of missing intervening positional values in the zone of posterior–anterior abutment.

Key words: *Stylonychia mytilus*, mirror-image, doublets, pattern formation, positional information.

Introduction

Ciliates are distinctive among organisms not only in the complexity of the structural order that is expressed within the limits of a single cell, but also in the pervasive asymmetry of that order. The asymmetry is apparent not only in the internal organization of the basic building block of ciliate organization, the ciliary unit, but also in the arrangement of these units over the ciliate surface. One therefore can imagine reversals of structural handedness at each of several levels of intracellular organization.

Detection of pattern reversals in ciliates is facilitated by a geometry that allows amplification of pre-existing structural order. This unique ciliate geometry, visualized as a 'clonal cylinder' by Tartar (1962), is one in which growth is longitudinal and fission transverse; this permits any structure or arrangement that can grow longitudinally without spreading laterally to propagate its pre-existing order across cell generations. The classic demonstration of such propagation of pre-existing order is the perpetuation of inversions of one or more longitudinal rows of closely spaced ciliary units (Beisson and Sonneborn, 1965; Ng and Frankel, 1977). In this case, however, the units have undergone a 180° rotational permutation without any change in their intrinsic coordinates. The failure to observe individual ciliary units with reversed asymmetry even though such a reversal could be perpetuated suggests that reversal at this level is difficult to achieve.

Reversals of asymmetry of global arrangements of ciliary units have, however, been observed repeatedly in ciliates, beginning with the discovery by Fauré-Fremiet (1945) of a self-propagating back-to-back mirror-image doublet of the hypotrich *Urostyla trichogaster*. Although one example of such a phenotype, janus in *Tetrahymena thermophila* (Jerka-Dziadosz and Frankel, 1979; Frankel *et al.* 1984), is dependent on

mutant-gene expression (Frankel and Jenkins, 1979; Frankel *et al.* 1987), most or all of the other known cases probably do not involve genetic changes. An outstanding example is the vegetatively inherited mirror-image doublet configuration of the hypotrich ciliate *Stylonychia mytilus*, generated from completely normal cells by regulation following a microsurgical operation (Tchang *et al.* 1964; Tchang and Pang, 1965).

How can a form with normal asymmetry switch to its mirror-image? In principle, all that is required is the reversal of a single morphological axis. It was proposed earlier that nongenic pattern reversals in *Tetrahymena* come about through reverse intercalation along the circumferential (transverse) axis, stimulated by an abnormal distribution of positional values in Siamese-twin doublet cells (Nelsen and Frankel, 1986; Frankel and Nelsen, 1986; Frankel, 1989 chapter 11). However, this pathway of regulation was inferred from observations on populations of cells. What is required for a more direct demonstration is a ciliate that is large enough to be readily operated upon and that has a morphological pattern sufficiently complex so that intermediate stages in pattern-reversal can be followed in individual cells examined over a short time period. These requirements are fulfilled by hypotrich ciliates, notably *Stylonychia mytilus*.

We describe here the morphogenetic regulation of microsurgically rearranged *Stylonychia* cells, emphasizing the outcome of one specific type of operation that frequently generated mirror-image (MI) doublets. This outcome has shown that a clonally heritable change of large-scale intracellular handedness can be obtained from rearranged normal components. It also has shown that this change in handedness is a consequence of a reversal of the anteroposterior axis that is dependent on a prior abnormal juxtaposition of anterior and posterior cellular positions.

Materials and methods

Four stocks (1,6,7, and 10) of *Stylonychia mytilus* collected in 1980 from ponds located near a suburb of Harbin were used in this work. The photographs shown in this paper were all of cells of stock 7. Cells were cultured at room temperature (18–20°C) with Pringsheim's solution and wheat infusion, using the flagellate *Chilomonas paramecium* as food.

Operations were performed with two fine steel microneedles on cells that were immobilized in a drop of methyl cellulose on a glass slide. The two principal types of operation are illustrated in Figs 1 and 2, using schematic diagrams of the ventral surface in which only the membranelle band (MB), left-marginal cirri (LM), and right-marginal cirri (RM) are illustrated (see Results for a more detailed description of this cell). These operations were performed on nondividing cells of unknown cell-cycle stage.

In one variant of the operation, the cytoplasm of the cell's left border, including most or all of the left-marginal cirri, was removed (Fig. 1A, cut 1). Immediately afterwards, the remainder of the cell was cut transversely into two equal parts (Fig. 1A, cut 2), and the posterior half was folded up against the anterior half along the wounded left edge (Fig. 1B). Then the front edge of this complex was removed (Fig. 1B, cut 3), after which the original posterior end was folded over onto the anterior end. The end result was a tandem complex in which most of the former posterior half of the cell was in front of the former anterior half, with an intact cell border on its right margin and wounded edges on the anterior, posterior and left margins (Fig. 1C). We call this a minus-left (−L) complex.

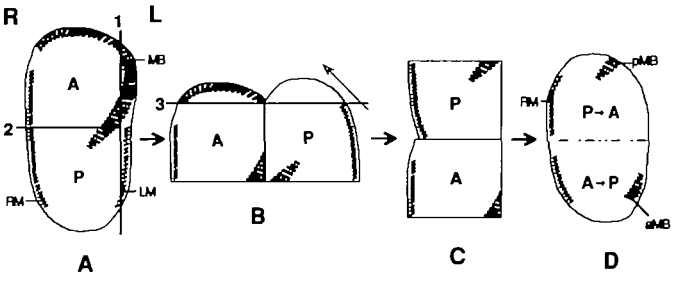


Fig. 1. Microsurgical generation of a 'minus-left' (−L) tandem complex. The cells are viewed from the ventral surface, with the cell's right (R) at the observer's left, the cell's left (L) at the observer's right. The numbers indicate the sequence of cuts, and the open-headed arrows show the direction of folding of parts by the operator. (A) The first cut (1) removes the left edge and a second cut (2) separates the posterior (P) and anterior (A) halves. (B) The P half then is folded 180° counterclockwise to abut side-by-side against the A half. Thereafter, the anterior and posterior extremities are removed by cut 3, and (C) a second 180° fold places the P region anterior to the A region. After wound-healing, (D) the border (dashed line) between the anteriorly transposed posterior (P→A) region and posteriorly transposed anterior (A→P) region no longer is visible. The posterior portion (pMB) of the original membranelle-band (MB) ends up at the anterior end of the complex, and a more anterior portion of the membranelle band (aMB) usually is present near the posterior end of the complex [sometimes it is missing, when cut 2 is more anterior than shown in A]. The right-marginal cirral row (RM) remains at the intact right edge, whereas the left-marginal cirral row (LM) was removed by cut 1, leaving the wounded left edge bare of ciliature.

The other variant of the operation, illustrated in Fig. 2, was similar to that shown in Fig. 1, except that in this case the cell's right edge was removed (Fig. 2A, cut 1), followed by the transverse cut (Fig. 2A, cut 2) and the folding of the posterior half along the right border (Fig. 2B) to generate a tandem complex with an intact cell border on its left margin and wounded edges elsewhere (Fig. 2C). This is named a minus-right (−R) complex.

In both of the illustrated operations, the two halves of the cell were of opposite anteroposterior orientation (heteropolar) when side-by-side in the intermediate stage of the operation (Fig. 1B and 2B), but ended up with the same orientation (homopolar) at the conclusion of the operation (Figs 1C and 2C). One other experiment (not illustrated) was performed that placed a posterior half-cell in front of an anterior half-cell, but with an inverted orientation (heteropolar). This was accomplished by carrying out the operation shown in Fig. 2 after first rotating the posterior half-cell in place, 180° counterclockwise, around its own longitudinal axis.

In all of these operations, parts of cells were kept in continuous contact along wounded edges during relocation. True grafting of separated parts is virtually impossible in this ciliate, owing to nearly-immediate healing, with loss of 'stickiness', of wounded edges.

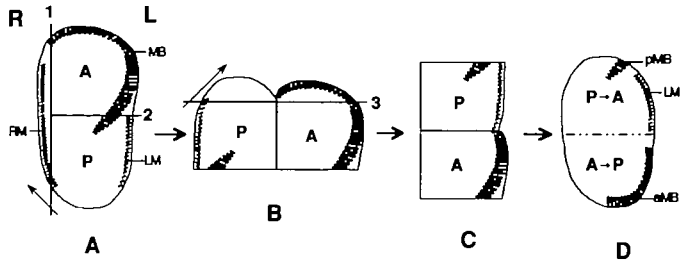


Fig. 2. Microsurgical generation of a 'minus-right' (−R) tandem complex. The operation is similar to that shown in Fig. 1, except that (A) cut 1 removes the right edge, (B,C) the folding of the P region is in a clockwise direction both times, and (D) the left edge remains intact, with the LM cirral row and a large aMB fragment remaining, whereas the wounded right edge lacks ciliary structures. All abbreviations are the same as in Fig. 1.

The operated cells were washed thoroughly and allowed to regenerate in culture medium. Soon after the operation, the cells became ellipsoidal (Figs 1D and 2D), and initiated regenerative development. These cells were fixed at various times after the operation for staining using a modified protargol technique, described previously (Shi and Frankel, 1990).

Results

(A) *Normal cortical anatomy and development*

(1) *Cortical anatomy*

Stylonychia mytilus is a dorsoventrally flattened ciliate with a highly specialized dorsal and ventral ciliature. The ventral surface (Figs 3A, 5) bears compound ciliary units of three kinds: membranelles, undulating (paroral) membranes and cirri. The membranelles are stacked horizontally along the left side of the oral (buccal) cavity to form a membranelle band (MB), whereas the two undulating membranes (UM) are aligned vertically along the right side of the buccal cavity (in viewing the ventral surface, the cell's right corresponds to the viewer's left). Each membranelle consists of three long rows of closely spaced basal bodies, with a very short fourth row on the anterior right end, visible in Fig. 11F as a short anterior protrusion of each membranelle at the cell's right side of the membranelle band [see also Shi and Frankel (1990), Fig. 19]. The cell-mouth, or buccal opening (Fig. 5, B), is located near the point where the MB and UMs converge.

The cirri are compound ciliary structures used in 'walking' and swimming, and include eighteen large frontal-ventral-transverse (Figs 3A, 5: F, V, T) cirri flanked by rows of left-marginal cirri (LM) and right-marginal cirri (RM). LM and RM cirri have similar anterior and posterior longitudinal rootlet fibers (drawn in open outline in the enlargements at the bottom of Fig. 3A, and lightly stained in Fig. 5), whereas the transverse rootlets (filled in Fig. 3A and darkly stained in Fig. 5) differ (Grim, 1972; Shi and Frankel, 1990). Each LM cirrus has two transverse rootlets, a short diagonal one to the left and a long horizontal one to the right, whereas each RM cirrus has only one such rootlet, a long diagonal one to the left. In diagrams of experimental material, only the transverse rootlets are shown. Nomenclature will always be based on organization rather than location, so that, for example, cirri of the right-marginal structural type will be called right-marginal (RM) cirri no matter where they are found.

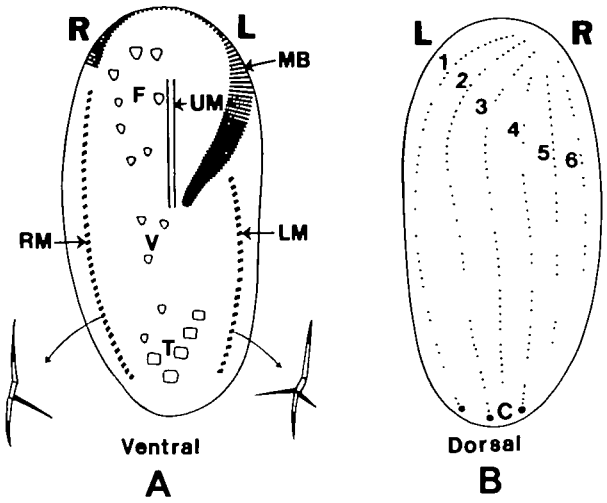


Fig. 3. Diagrams of ciliary organization of the (A) ventral and (B) dorsal surfaces of *Stylonychia mytilus*. The cell's left (L) and right (R) are opposite to the left and right of an observer who is viewing the ventral surface, and the same as the left and right of an observer who is viewing the dorsal surface. (A) The ciliature of the ventral surface includes a membranelle band (MB), two parallel undulating membranes (UM), the frontal (F), ventral (V), transverse (T), right-marginal (RM) and left-marginal (LM) cirri. Details of the rootlet structures of the two types of marginal cirri are shown as insets (see text for description). (B) The ciliature of the dorsal surface consists of six rows of ciliary units, numbered from left to right, as well as caudal cirri at the posterior ends of rows 1, 2, and 4.

The ciliature of the dorsal surface is considerably simpler than that of the ventral surface (Fig. 3B). It consists of six longitudinal rows of ciliary units, each unit made up of a pair of basal bodies and accessory structures (Görtz, 1982). The four rows on the left (nos. 1 to 4) extend from pole to pole, whereas the two on the right (nos. 5 and 6) are shorter (for the dorsal surface, viewer's and cell's right and left coincide). Caudal cirri (C) are located at the posterior ends of rows 1, 2, and 4 respectively.

(2) *Cortical development*

Cortical development in *S. mytilus* involves the successive appearance and differentiation of ciliary primordia of the membranelle band (MB), the undulating membranes (UM), the frontal-ventral-transverse (FVT) cirral group, the marginal cirri and the dorsal ciliary rows. Essentially the same developmental processes occur under three different circumstances: binary fission, postoperative regeneration and 'physiological regeneration', also known as cortical reorganization (Jerka-Dziadosz, 1963; Frankel, 1989, pp. 37–38). Cortical reorganization is shown diagrammatically in

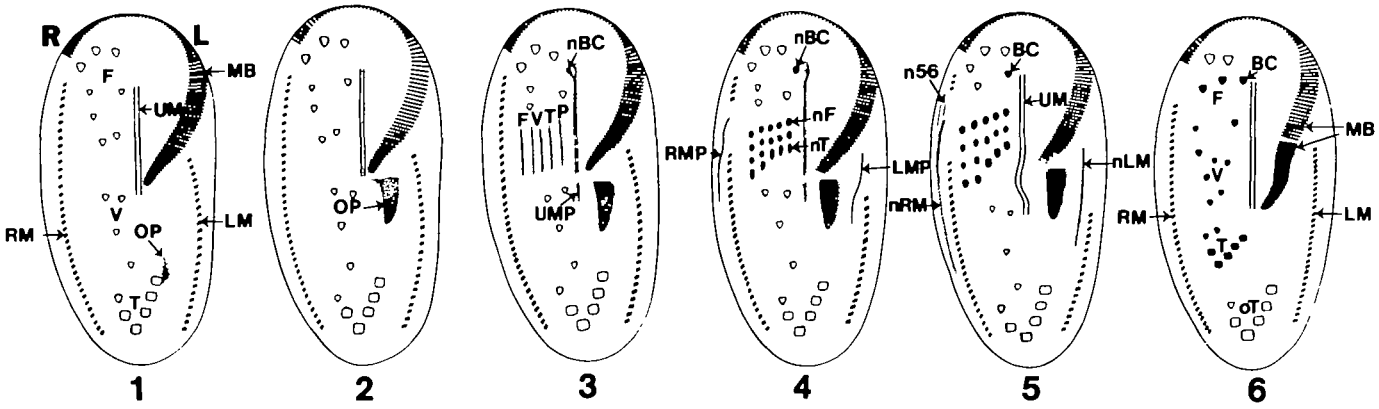


Fig. 4. Stages of development during reorganization of *S. mytilus*. Only the ventral surface is shown. Mature ciliary structures are labelled as in Fig. 3. Old cirri are shown in outline, newly developing cirri are filled in. Stage 1: An oral primordium (OP) appears as a small cluster of basal bodies to the anterior-left of the left-most transverse cirrus. Stage 2: The OP has become enlarged by addition of new basal bodies and has migrated anteriorly to a position just posterior to the old MB. Stage 3: Membranelles begin to develop at the anterior-right edge of the OP, and a small undulating-membrane primordium (UMP) has split off from the right edge of the OP. The ordered ciliary rows of the pre-existing UMs partially disaggregate, and a new buccal cirrus (nBC) begins to develop near the anterior end of the partially dedifferentiated UM complex. Five rows of ciliary streaks appear to the right of the UMs, collectively making up the frontal-ventral-transverse cirral primordium (FVTP). Stage 4: Membranelles now occupy the majority of the OP. The nBC detaches from the partially dedifferentiated UMs. The ciliary streaks of the FVTP have widened by lateral addition of new basal bodies, and break up into separate new cirri, with 3 cirri formed from each of the three streaks nearer the UMs and 4 cirri formed from each of the two remaining streaks. The most anterior cirri become new frontal (nF) cirri, the most posterior become new transverse (nT) cirri. Right-marginal cirral primordia (RMP) and left-marginal cirral primordia (LMP) develop as streaks of ciliary units derived from certain old marginal cirri. These streaks extend to the right of the marginal cirral rows. Stage 5: Formation of membranelles within the OP is completed, and the OP begins to move anteriorly while the most posterior membranelles of the old MB are being resorbed. The UMP has fused with the reorganized old UMs, and these together form the two new UMs. The new frontal-ventral-transverse cirri spread anteriorly and posteriorly. The RMP and LMP respectively segment into new right-marginal (nRM) and new left-marginal (nLM) cirri. The anteriormost portion of the RMP does not form cirri, but instead breaks off and then segments into two short ciliary rows, the new 5th and 6th dorsal ciliary rows (n56). Stage 6: The new membranelles of the OP join with the remaining membranelles of the old MB to form a composite new MB. The new frontal (F), ventral (V), transverse (T) and marginal (LM, RM) cirri are close to their final positions, and most of the old cirri are resorbed, though some, especially old transverse cirri (oT), will be resorbed later. The new dorsal rows 5 and 6 have migrated to their final positions on the dorsal surface (see Fig. 3B).

Fig. 4 [for details of this and the closely related binary fission process, see Shi and Frankel (1990)]. The oral primordium (OP), which will generate the MB and part of the UM, appears first (Fig. 4, stage 1) and then migrates anteriorly (Stage 2). Assembly of new membranelles then begins at its anterior-right corner (stage 3). Additional membranelles are added posterior to old ones, and each individual membranelle develops from right to left. As this process nears completion (stage 4), the OP migrates anteriorly and replaces the posterior portion of the old oral apparatus (stages 5–6). A short undulating-membrane primordium (UMP) forms (stage 3) and later (stage 5) joins with the reorganized anterior UMs to form two composite UMs.

The FVT cirral primordia (FVTP) appear at stage 3 as five elongated ciliary streaks to the right of the UMs. These streaks develop in part from disaggregated basal bodies derived from old frontal cirri, and in part *de novo*. These streaks later proliferate laterally and segment (stage 4) into new cirri. This segmentation proceeds rapidly from anterior to posterior, with the posteriormost (future transverse) cirri initially more elongated and later somewhat larger than the others. These 17 new FVT cirri then spread over the ventral surface (stages 5–6), replacing the old cirri, thereby producing all of the new cirri of the ventral surface except for frontal cirrus no. 1. The new frontal cirrus no. 1, also known as the buccal cirrus (BC), originates differently, developing from a thickening at the anterior end of one of the reorganizing undulating membranes (stages 3–4, nBC).

Right- and left-marginal cirral primordia (RMP, LMP) originate during stage 4 from disaggregated basal bodies of some of the old marginal cirri and extend to the right of the old marginal cirral rows. The marginal cirral primordia differentiate into the new marginal cirral rows of the product of reorganization, replacing the old marginal cirri.

On the dorsal surface, two groups of new dorsal ciliary rows originate from two distinct sources. The new rows 1 through 4 develop during stage 4 from ciliary primordia that arise by proliferation of certain basal bodies of the old rows 1 through 3, and new caudal cirri then form at the posterior ends of new rows 1, 2 and 4 (not shown here; see Shi and Frankel, 1990). New rows 5 and 6 (n56) arise during stage 5 from the anterior portion of the RMP and then migrate to the dorsal surface. The two groups of new dorsal bristle

rows and caudal cirri replace their old counterparts during reorganization.

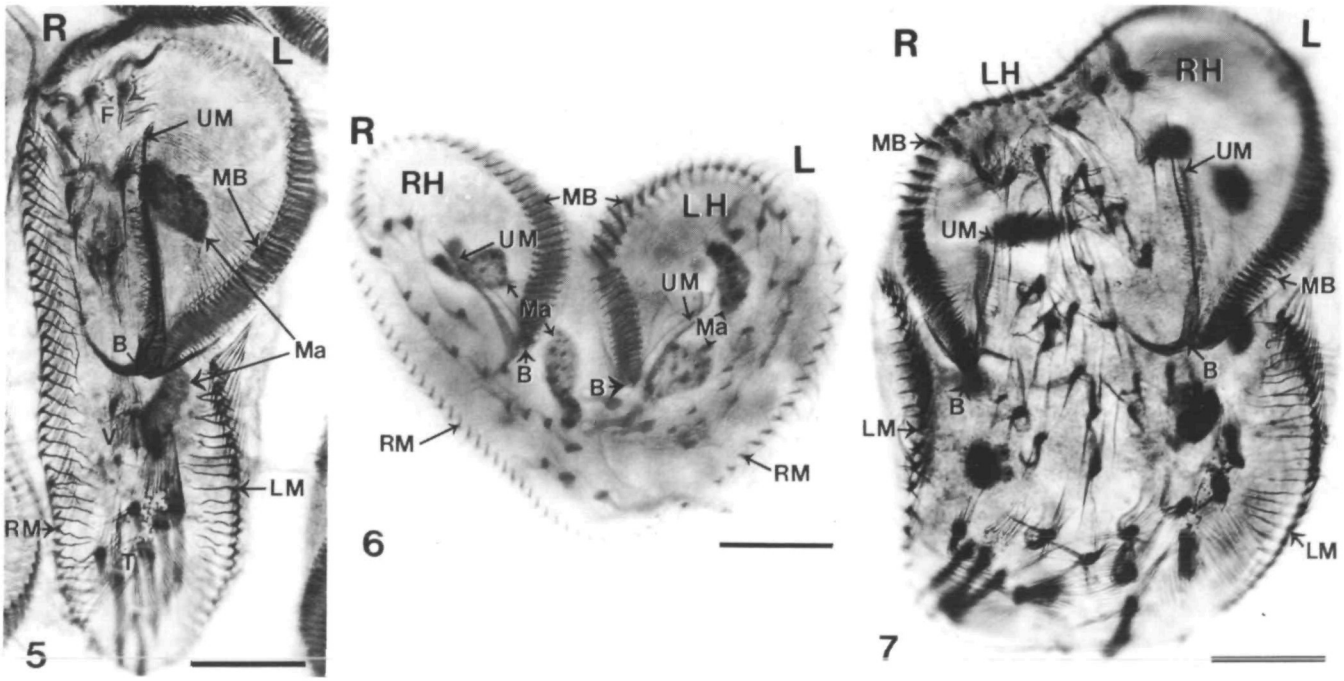
The formation of ciliary primordia typically is highly coordinated; if one ciliary primordium forms, so do most or all of the others, in a stereotyped temporal sequence. The organism, however, is flexible in the number of sets of ciliary primordia produced and to some degree in their location. During division, for example, one large OP generates the oral apparatus of the posterior division product (the anterior OA remains intact and is passed on to the anterior division product), whereas *two* tandem sets of all other ciliary primordia generate the remainder of the ciliature of the two division products (Grimes, 1972; Wirnsberger *et al.* 1986; Shi and Frankel, 1990). The cortical development in operated cells (section C) manifests aspects of both reorganization and division plus certain unique features.

(B) *The organization of mirror-image doublets*

In Figs 5 to 7, the organization of the ventral surface of a normal *S. mytilus* cell is contrasted to that of the two types of mirror-image (MI) doublets. In both types of MI-doublets, the two components are arranged side-by-side. In one type (Fig. 6), the component on the right side of the doublet is of normal asymmetry, or 'right-handed' (RH), and the component on the left side is of reversed asymmetry, or 'left-handed' (LH). In the complementary type (Fig. 7), the component on the left side of the doublet is of normal asymmetry (RH) and that on the right side is of reversed asymmetry (LH).

In the RH component of MI-doublets, the membranelle band (MB), buccal opening (B) and undulating membranes (UM) together form a lopsided 'V' similar to that of normal singlets, whereas in the LH component the configuration of the 'V' is reversed, with the MB on the right rather than the left side of the 'V'. We designate the type of MI-doublet shown in Fig. 6 as the 'buccal-adjoining' form because the two Vs are adjacent to one another in the center of the complex, whereas we call the type of MI-doublet shown in Fig. 7 the 'buccal-opposing' form, because the two Vs are near the edge of the cell. These two types of MI-doublets have the same geometrical relation to each other as the two mirror-image configurations one can form by placing one's two hands side-by-side, with the thumbs either adjacent in the center or opposite at the edges.

The FVT cirri are close to double the normal number, in a roughly mirror-image arrangement in buccal-adjoining MI-doublets (Fig. 6) and in a more disordered arrangement in buccal-opposing MI-doub-



Figs 5–7. Protargol preparations of the ventral ciliature of the three self-reproducing forms of *Stylonychia mytilus*. Scale bars represent 50 μM.

Fig. 5. A normal (right-handed) singlet cell. The labelled structures are the same as in the diagram (Fig. 3A), plus the buccal opening (B) and the bilobed macronucleus (Ma).

Fig. 6. A buccal-adjoining mirror-image (MI) doublet. The 'right-handed' (RH) component, located on the right (R) (viewer's left) side of the MI-doublet, has an arrangement of ciliary structures similar to that of the normal cell in Fig. 5, whereas in the 'left-handed' (LH) component, located on the left (L) side of the MI-doublet, these structures are arranged with reversed asymmetry. Both margins of the doublet have cirri of the right-marginal (RM) type, although the ones on the left margin are inverted. Each component has its own bilobed macronucleus (Ma).

Fig. 7. A buccal-opposing MI-doublet. The RH component is located on the *left* (viewer's right) side of the MI-doublet, whereas the LH component is situated on the *right* side. Both margins of the doublet have normally-oriented cirri of the left-marginal (LM) type.

lets (Fig. 7). Cirri of the right-marginal type are present at both margins of the buccal-adjoining MI-doublets (Fig. 6 – in this case inverted on the LH side), whereas cirri of the left-marginal type are present at both margins of buccal-opposing MI-doublets (Fig. 7). Dorsally, 8 to 12 rows of cilia are present in a near mirror-image arrangement in both types of MI-doublets (not shown; see Shi and Frankel, 1990 for the buccal-opposing type).

The LH and RH components of MI-doublets are mirror-images only with regard to the *arrangement* of ciliary structures. The internal organization of each individual ciliary unit is always the same in both components, although certain structures, notably membranelles but sometimes also marginal cirri, are inverted in the LH component (cf. Grimes *et al.* 1980; Jerka-Dziadosz, 1983, 1985; Shi and Frankel, 1990).

Whereas the buccal-opposing MI-doublets (Fig. 7) are stable and self-propagating, and have been studied for nearly 30 years (references in Shi and Frankel, 1990), the buccal-adjoining MI-doublets (Fig. 6) can be propagated for several fissions at most, and are new to this study, except for the comparable but incomplete (and nondividing) buccal-adjoining MI-doublets described by Grimes and L'Hernault (1979).

(C) *The origin of mirror-image doublets*

(1) *Overview*

Fig. 8 provides a schematic overview of the major experimental results. The two types of operations (see Figs 1 and 2) both bring about an interchange of large anterior and posterior regions of the cell, with the original posterior region displaced anteriorly (P→A), and the original anterior region displaced posteriorly (A→P); a consequence of this interchange is that a zone originally near the posterior pole of the cell (labelled F) becomes juxtaposed to a zone formerly near the anterior pole of the cell (labelled A) (Fig. 8B).

The typical cellular response to this interchange is the formation of one of three types of mirror-image (MI) forms. In all three types, two sets of new ciliature differentiate, with a reversal of anteroposterior orientation (polarity) in the set within the A→P region. The simplest consequence is an end-to-end MI form (Fig. 8F). However, the more common outcome is a MI-doublet, in which the RH and LH components end up side-by side (Fig. 8E,G). This can be visualized as a consequence of folding of an end-to-end MI form, either bringing together the left sides of the P→A and A→P regions to form a buccal-adjoining MI-doublet (Fig. 8E) or bringing together the right sides of these regions to form a buccal-opposing MI-doublet (Fig. 8G). Buccal-adjoining MI-doublets are produced only from 'minus-left' (−L) complexes created when the left edge has been removed (Fig. 8C), and buccal-opposing MI-doublets are produced only from the 'minus-right' (−R) complexes created when the right edge has been removed (Fig. 8D).

In the sections to follow, we describe the development of buccal-adjoining MI-doublets first, then that of buccal-opposing MI-doublets, and finally the formation of the end-to-end MI forms. Only the most essential events will be described in the text, with further details in the legends to the photographs. The examples that involve development of MI forms are selected from a larger body of operated complexes, many of which formed normal or reversed singlets (see section C5).

(2) *Formation of buccal-adjoining mirror-image doublets*

Buccal-adjoining MI-doublets arose from −L complexes, with intact marginal cirri at the right but not the

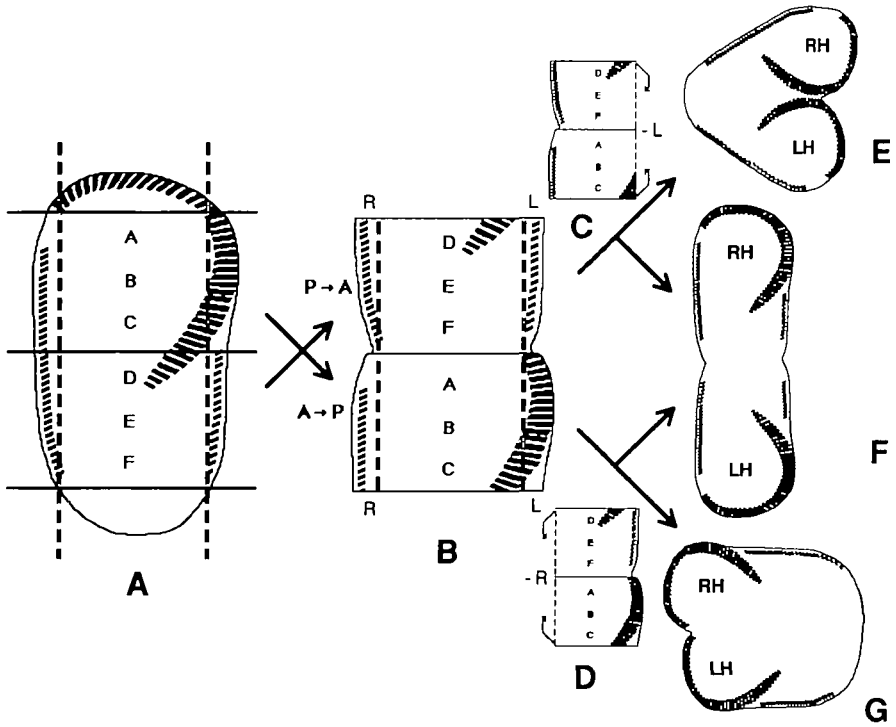


Fig. 8. A simplified schematic summary of the operations and major results of this study. A and B show the features common to the two major operative protocols (Figs 1 and 2), with the letters inside the cell regions marking cell positions in an anteroposterior order. The horizontal solid lines indicate cuts made in all operations, whereas the vertical double-dashed lines indicate *alternative* cuts, removing the left edge (L) *or* the right edge (R). C shows the minus-L (−L) complex, and D shows the minus-R (−R) complex. The curved arrows at the ends of vertical cut edges give the direction of the ensuing unilateral shortening and folding. E, F, and G indicate the alternative outcomes, a buccal-adjoining MI-doublet (E), an end-to-end MI-form (F), or a buccal-opposing MI- doublet (G). RH stands for the 'right-handed' (normal asymmetry) component, LH for the 'left-handed' (reversed asymmetry) component. For further description, see the text.

left edge (compare Fig. 9A to Figs 1D and 8C). The P→A region (Fig. 9A, top) included the original posterior ends of the MB (pMB) and UMs (pUM) as well as a few transverse cirri (T) and the original posterior half of the right-marginal cirral row (pRM); the A→P region (Fig. 9A, bottom) contained what was originally a more anterior portion of the MB (aMB) and UMs (aUM), the buccal cirrus (BC), some frontal cirri (F) and the former anterior half of the right-marginal cirral row (aRM). Each transposed region included one lobe of the original bilobed macronucleus (Fig. 9D, Ma).

An oral primordium (OP) appeared by about 1.5 h after the operation and occupied a position just behind the posterior end of the pMB of the P→A region (Fig. 9B). One set of new membranelles (Fig. 9C, nM) developed at the anterior end of the OP, and membranelles were added successively more posteriorly along its right margin, as in typical cortical development (Fig. 4). What was not typical was the formation of a second set of membranelles at the posterior end of the OP (Fig. 9C, nm) [ciliary primordia of the P→A region are labelled in upper case; those of the A→P region are labelled in lower-case]. There, new membranelles probably were added from posterior to anterior along the *left* margin of the OP (cf. Section C3), a sequence that is rotationally symmetrical with respect to the nM set. The two new membranelle sets remained separate as they completed their development (Fig. 9D–F).

Two simultaneous morphogenetic processes brought the new membranelle sets together with the remaining portions of the old membranelles. First, a shortening of the naked left edge of the cell brought the pMB and aMB fragments closer to each other and to the newly developing membranelle sets (Fig. 9D–F); second, the nM set (open arrow) maneuvered into a seamless union with the pMB (sometimes with counterclockwise rotation), while a clockwise rotation of the nm set (closed arrow) led to its more imperfect juncture with the aMB (Fig. 9D–F). The nm set underwent a clockwise rotation even when the aMB was absent (Fig. 9E), and then formed an anteriorly truncated membranelle band (Fig. 9I).

The formation of two composite membranelle bands included a process of regulation in which the original global cell polarity was retained in the P→A region, but was reversed in the A→P region (Fig. 10). Consider first the membranelles of the P→A region marked U, V and W in Fig. 10B. Membranelle U, which had been located in the posterior portion of its MB before the operation (Figs 1, 8), became the anterior end of the reconstructed U-V-W MB. Membranelle V, at the posterior end of the original MB, became the middle of this reconstructed MB. Membranelle W, the posteriormost membranelle of the nM set, became the posterior end of this reconstructed MB. The original anteroposterior positional order of the U-V-W MB was retained, although pre-existing membranelles of the pMB took on more anterior identities. The situation was different for the membranelles of the A→P region, marked X, Y, and Z in Fig. 10B. Membranelle Z, which before the operation had been a posterior membranelle located close to membranelle U (Figs 1, 8), similarly became the most anterior membranelle of the reconstructed Z-Y-X MB. However, membranelle Y, which had been *anterior* to membranelle Z in the original MB, was respecified to become *posterior* to membranelle Z in this reconstructed MB. Finally, membranelle X, which by its internal organization ought to have been the most anterior membranelle of the nm set, instead became the most posterior membranelle of the Z-Y-X MB. Thus, the Z-Y-X sequence, inherently a posterior-to-anterior sequence, became respecified to its opposite, an anterior-to-posterior sequence. This respecification determines the overall form of the membranelle band and its geographical relation to other structures such as the undulating membranes, but does not alter the intrinsic conformation of each individual membranelle. This is why the individual membranelles of the LH component of a MI-doublet became 180° inverted relative to those of the RH component.

The remaining ciliary structures developed within two separate sets of primordia, one in each of the two transposed regions. The structures in the P→A region differentiated nearly normally, almost as they would have if the region had remained in its normal posterior location and were reorganizing or preparing to divide (compare to Fig. 4 and Section A2). In the A→P region, the undulating-membrane and frontal-ventral-transverse ciliary primordia (ump, fvtp) differentiated somewhat belatedly and much more abnormally. Their spatial arrangement started out irregular (Fig. 9D,E) and ended up globally reversed (Fig. 9F). Fig. 9D–F can be interpreted as depicting stages of a 180° counterclockwise rotation of the ump and fvtp. A reversal also occurred in the location (but not the orientation) of the right-marginal cirral primordia (rmp) of the A→P region (Fig. 9D,E) and in the location of the primordia of the new dorsal ciliary rows 5 and 6 (Figs 9F and 13B: n56). Thus, by the stage shown in Fig. 9F, the ciliary arrangement of the A→P region had become a mirror-image of that of the P→A region.

At the same time, a reorientation of cell axes was taking place. The P→A and A→P regions folded together on the left side, so that the major ciliary systems, originally deployed end-to-end, became more and more parallel; this readjustment was approximately halfway completed in the cell shown in Fig. 9F.

By 6 h after the operation, the remodelling of the nascent MI-doublet has been completed (Fig. 9G), and the components derived from the two regions became aligned nearly parallel to one another, in a 'V' configuration. The new anteroposterior axis of the doublet thus had been shifted 90° clockwise. The original P→A region had rotated 90° clockwise to become the RH component of the MI-doublet, whereas the original A→P region had rotated 90° counterclockwise and simultaneously undergone a reversal of its anteroposterior axis to become the LH component of the new MI-doublet. As a consequence of this reorientation, the *right*-marginal cirri that ended up on the *left* margin of the LH component became inverted

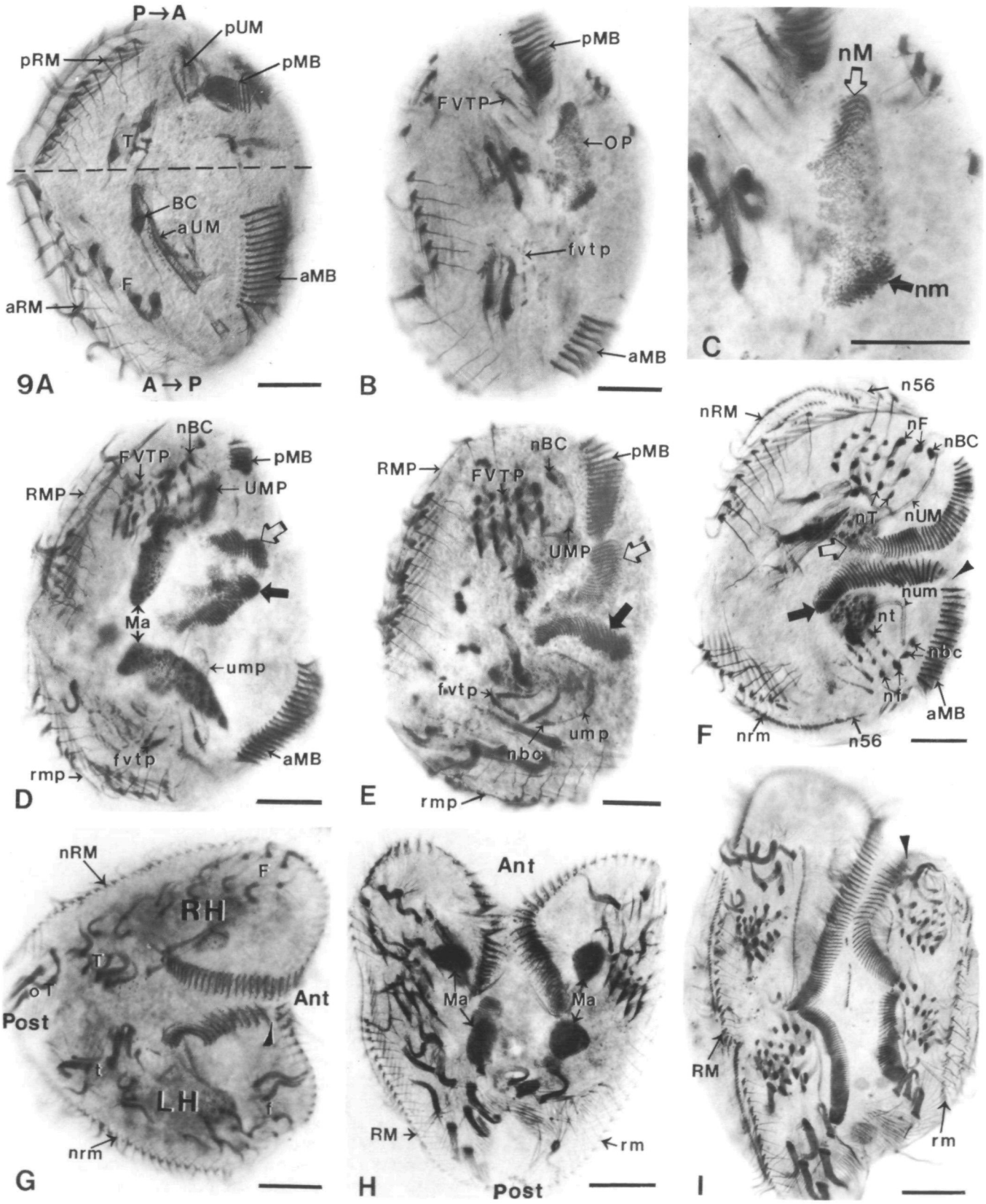

P→A
pUM
pRM
pMB
T
BC
aUM
F
aMB
aRM
A→P
9A
pMB
FVTP
OP
fvtp
aMB
B
nM
nm
C
nBC
FVTP
pMB
RMP
UMP
Ma
ump
fvtp
aMB
rmp
D
nBC
pMB
RMP
FVTP
UMP
fvtp
ump
nbc
rmp
E
n56
nRM
nF
nBC
nT
nUM
num
nt
nbc
nf
aMB
nrm
n56
F
nRM
F
RH
T
oT
Post
Ant
t
LH
f
nrm
G
Ant
Ma
Ma
RM
rm
Post
H
RM
rm
I

Fig. 9. Protargol preparations illustrating the development of buccal-adjoining MI-doublets from 'minus-left' (−L) complexes. A to G are oriented with the *original* anteroposterior axis vertical on the page, whereas H and I are oriented so that the *new* anteroposterior axis of the MI-doublet is vertical. Ciliary primordia of the P→A region are labelled with capital letters, those of the A→P region in lowercase. Scale bars indicate 25 μM in A to G, 50 μM in H and I. (A) A −L complex fixed 1 h after the operation, before new cortical development has begun. The horizontal dashed line indicates the approximate border between the anteriorly transposed posterior region (P→A) and the posteriorly transposed anterior region (A→P). The P→A region includes the original posterior end of the membranelle band (pMB) and undulating membranes (pUM) as well as a few transverse cirri (T) plus the posterior half of the original right-marginal cirral row (pRM). The A→P region includes a more anterior portion of the membranelle band (aMB), the anterior part of the undulating membranes (aUM), the buccal cirrus (BC), a few additional frontal cirri (F), plus the anterior half of the original right-marginal cirral row (aRM). (B) A −L complex at stage 3, fixed 2 h after the operation. A large oral primordium (OP) is located posterior to the pMB; frontal-ventral-transverse cirral primordia are well developed in the P→A region (FVTP), and just beginning to form in the A→P region (fvtp). (C) An enlargement of part of (B), showing the two sets of new membranelles appearing within the OP, one (nM) at the normal location at the original anterior end of the OP, the other (nm) ectopically situated at the original posterior end of the OP. (D) A −L complex midway between stages 3 and 4, fixed 2.5 h after the operation. Both sets of new membranelles (large arrows) are well developed. In the P→A region, a new buccal cirrus (nBC) has formed at the anterior end of the UM-primordium (UMP). New cirri are emerging in the FVTP, and a long right-marginal primordium (RMP) has become positioned to the right of the old pRM cirral row. In the A→P region, the long ump is partly obscured by the posterior lobe of the macronucleus (Ma), ciliary streaks of the fvtp are variously oriented, and the rmp has just begun to develop at the expense of three old marginal cirri. (E) A −L complex at stage 4, fixed 3 h after the operation; it differs from (D) in having a larger pMB and in lacking an aMB. The nM set (open arrow) approaches the pMB, whereas the nm set (closed arrow) has rotated in a clockwise direction. A new buccal cirrus (nbc) has begun to develop at the *posterior* end of the curved ump. The ciliary streaks of the fvtp have no uniform orientation. (F) A −L complex at stage 5, fixed 3.5 h after the operation. The bare left edge is fully contracted and the two regions are reorienting from tandem to parallel. The seamless membranelle band of the P→A component probably is derived from a merger of the nM set (open arrow) with the pMB, whereas the nm set (closed arrow) is separated from the aMB by a gap (arrowhead). The remaining new structures are arranged in opposite anteroposterior order: the new buccal cirri (nBC, nbc) are at the outer ends whereas the more elongated new transverse cirri (nT, nt) are at the inner ends of the new frontal-ventral-transverse sets; new dorsal rows 5 and 6 (n56) are situated near or at the outer ends of the new right-marginal cirral rows (nRM, nrm) (in the A→P region, the n56 primordium has not yet split into separate rows 5 and 6 but is distinguishable because it is thinner than the nrm cirri). (G) A −L complex at stage 6, fixed 6 h after the operation. The P→A region has become the right-handed (RH) component and the A→P region has become the left-handed (LH) component of a buccal-adjoining MI-doublet. The new anterior (Ant)–posterior (Post) axis is oriented 90° clockwise relative to the original anteroposterior axis. Each component has a complete new ciliature, although some old transverse cirri (oT) have not yet been resorbed. The membranelle band of the LH component still has a suture (arrowhead) indicating incomplete juncture of the new membranelle set with the old aMB. The arrangement of frontal (F,f) and transverse (T,t) cirri is imperfectly mirror-imaged in the two components. New marginal cirri of the right-marginal type are present in both components, those of the LH component (nrm) being inverted relative to those of the RH component (nRM). (H) A completed buccal-adjoining MI-doublet in stage 3 of a renewed reorganization, fixed 24 h after the operation. The two oral primordia (not labelled) are largely obscured by the posterior lobes of the two bilobed macronuclei (Ma). The individual frontal, ventral and transverse cirri are similarly oriented in the two components, whereas the rm cirri of the LH component are inverted relative to the RM cirri of the RH component. The frontal-ventral-transverse primordia (not labelled) are arranged as mirror-images in the two components. (I) A dividing buccal- adjoining MI-doublet in stage 5 of cell division, fixed 48 h after the operation. The membranelle band of the LH component of this MI-doublet ends short of the anterior end of the cell (arrowhead), indicating that this doublet originated from a complex without an aMB, like the one shown in E. In this cell, the rm cirri of the LH component have the same orientation as the RM cirri of the RH component.

with respect to their usual orientation (Fig. 9G,H). Frontal-ventral-transverse cirri were everywhere normal (not inverted) in orientation, possibly because of an earlier 180° rotation of the fvtp in the A→P region (see Discussion).

One of the two original macronuclear lobes occupied each component, and became bilobed (Fig. 9H, Ma), just as at the end of a normal cell division process. Thus the two laterally fused components of the MI-doublet each appear to have their own complete macronucleus.

Buccal-adjoining MI-doublets can undergo subsequent reorganization and up to three divisions. A reorganizing MI-doublet is shown in Fig. 9H, and a dividing MI-doublet in Fig. 9I. Normal sets of ciliary structures developed in RH components, and sets with reversed arrangement formed in LH components. Development in the LH component was synchronous with that in the RH component and was orderly at all stages, indicating that the lag and early disorder in the initial round of development of the A→P region was not inherent to cortical development in LH regions.

During further cortical reorganization, the nature of the marginal cirri (structurally *right*-marginal cirri on both margins) was maintained, but the initial inversion of orientation at the left margin often was rectified (Fig. 9I). This rectification probably involved a reorganizational event in which a new right-marginal cirral primordium was formed without direct continuity with the old marginal cirral row (see Discussion).

Buccal-adjoining MI-doublets have one of four fates:

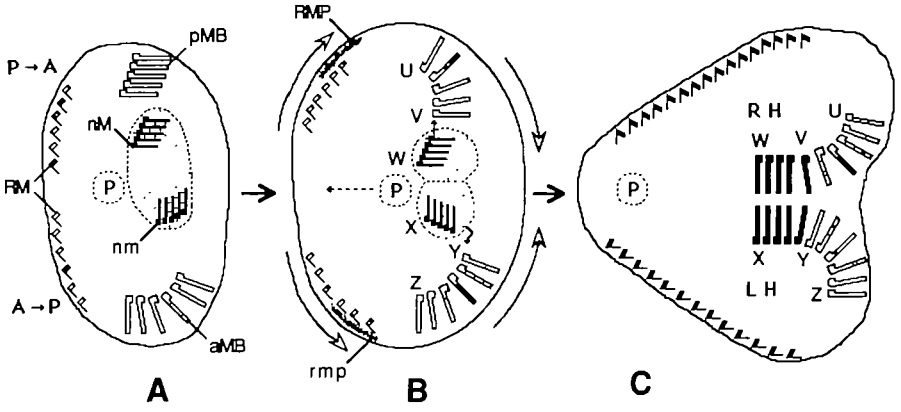


Fig. 10. A diagrammatic summary of key events in the development of a buccal-adjoining MI-doublet from a −L complex. The stages shown in A, B, and C correspond to those of Fig. 9B–C, 9D–E, and 9G, respectively. Only the membranelles, marginal cirri and corresponding primordia are shown. The new membranelles (shaded) are drawn to exaggerate their asymmetry (see Fig. 11F). Abbreviations are the same as in Fig. 9, with the addition of P (circled) for the presumptive posterior end of the MI-doublet, and of the letters U-V-W-X-Y-Z to label particular membranelles. The open-ended arrows indicate directions of cortical shortening (on the left side) and of expansion or envelopment (on the right). See text for further explanation.

(a) death, presumably of starvation, following progressive loss of oral components at the center [similar to the incomplete buccal-adjoining MI-doublets generated by Grimes and L'Hernault (1979)]; (b) separation into RH and LH cells; (c) reversion to viable RH singlets by resorption of the LH component; (d) reversion to inviable LH singlets by resorption of the RH component.

(3) *The formation of buccal-opposing mirror-image doublets*

Buccal-opposing MI-doublets arose from −R complexes, in which intact marginal cirri were at the left rather than the right edge (compare Fig. 11A to Figs 2D and 8D). Development largely resembled that observed in the −L complexes, with differences related to the different locus of cortical shortening. A single OP became positioned just behind the original pMB (Fig. 11A), and one new membranelle set developed at each end of this OP (Fig. 11B: nM, nm). Since in this case the left edge of the cell, close to the OP, was uninjured, the pMB and aMB remained far apart. The OP split into two, each with one new membranelle set (Fig. 11C,D). The nM set (open arrows) linked up with the pMB (Fig. 11E,G), exactly as in normal reorganization (Fig. 4), while the nm set (closed arrows) migrated toward the aMB (Fig. 11C,D), and then underwent a clockwise rotation of 90° or more to become seated on the aMB (Fig. 11D–I). This rotation assured geometrical conformity of the old and new membranelles (Fig. 12).

The process that generates new membranelle bands in buccal-opposing MI-doublets is illustrated schematically in Fig. 12. The argument made above (Results, Section C2) for a respecification of global polarity of the new MB of the LH component in buccal-adjoining MI-doublets applies equally to these nascent buccal-opposing MI-doublets.

The development of frontal-ventral-transverse primordia in the A→P region was more complex in the −R than in the −L complexes. It appears as if two sets of these primordia had first formed (Fig. 11A,C: fvtp1, fvtp2) and later merged (Fig. 11C–G), and that the reorganizing ump and possibly also the fvtp underwent a 180° counterclockwise rotation in the plane of the cell surface (Fig. 11A,C,D,E). At subsequent stages, the arrangement of nascent cirri in the A→P region sometimes was a fair mirror-image of that in the normally developing P→A region (Fig. 11E), and sometimes was chaotic (Fig. 11G). The reason for this large variation is unknown; it could be due to prior variation in the location of the equatorial transection and/or in the cell-cycle stage at which the operation was performed. Despite this variation, the posterior location of the large transverse cirri (T,t) at the end of this process (Fig. 11H,I) indicates that a reversal of anteroposterior order (and therefore of right-left order after folding) had occurred during development of the fvtp of the A→P region.

As only the left-marginal cirral row remained intact, the two cirral primordia that developed from it were both *left*-marginal cirral primordia (Fig. 11E,G), with no adjacent dorsal-row primordia. In some complexes, separate marginal cirral primordia also developed at the original *right* edge (Fig. 11D); these cases probably led to the production of end-to-end MI forms (Section C4).

Reorientation in the −R complexes occurred in a direction opposite to that of the −L fragments, but was similar in that the wounded edge underwent shortening. The shortening of the right edge brought together parts of the original membranelle bands that originally were located at the extreme ends of the complex while the new structures formed at the intact left edge of the complex spread out to envelop most of the complex (Fig. 11G–I). As a result, the original P→A region was reoriented 90° counterclockwise to become the RH component of the MI-doublet, whereas the original A→P region was reoriented 90° clockwise while

undergoing reversal of its anteroposterior axis, to become the LH component. The new *left*-marginal cirri that ended up on the *right* margin of the LH component therefore became inverted (photographs not shown; cf. Fig. 12).

The provisional MI-doublet formed at the conclusion of these events (Fig. 11I) lacked the final contour of buccal-opposing MI-doublets (Fig. 7), but possessed all of the basic elements of the 'body-plan' of such doublets. Two or more successive rounds of further cortical reorganization converted this provisional MI-doublet into a definitive buccal-opposing MI-doublet similar to the one shown in Fig. 7. These reorganizations modified cell shape but did not alter ciliary organization, except for the replacement of the inverted left-marginal cirri located on the right margin of the LH component by normally oriented left-marginal cirri. The membranelle band of the LH component maintained its inverted configuration through the indefinite number of fissions that this stable type of MI-doublet can undergo (Shi and Frankel, 1990).

(4) *The formation of end-to-end mirror-image forms*

End-to-end MI forms developed from complexes of both −L and −R types that failed to fold (Fig. 8). In such complexes, sets of new marginal cirral rows formed at *both* edges of the cell, including the edge from which most or all old marginal cirri had been removed (Fig. 11D, 13A,B). In other respects, development of ciliary primordia was similar to that in the folding −L complexes described previously. Both the arrangement and the orientation of the structures formed from the frontal-ventral-transverse and undulating-membrane primordia of the A→P region were reversed: frontal and buccal cirri developed near the poles and transverse cirri near the center, and individual cirri of this group were inverted (Fig. 13A–C). On the dorsal surface, new caudal cirri developed near the equator of the complex (not shown).

The end-to-end MI forms eventually pulled apart into two macronucleated cells: one was a normal RH singlet capable of propagating itself, whereas the other was an LH singlet that ultimately died, almost certainly because it could not feed (cf. Shi *et al.* 1990). To visualize the geometry of such an LH cell, one must rotate Fig. 13C through 180° and then examine the LH component, imagining that it had detached itself at the dotted line. Immediately after separation, such a LH singlet had normally oriented frontal (f) and transverse cirri (t), an inverted right-marginal cirral row on its *left* side (Fig. 13C, rm), and an inverted left-marginal cirral row on its *right* side (Fig. 13C, lm). Before such a LH singlet died, it corrected the inverted orientation of these marginal rows during reorganization, forming typical rows of right-marginal cirri on its left margin and of left-marginal cirri on its right margin (cf. Shi *et al.* 1990).

(5) *Variations in outcome of operations*

The 'standard' operations shown in Figs 1 and 2 did not yield mirror-image doublets every time that they were attempted. Operations normally were performed in batches of ten cells, and the proportions of the types described above varied from zero to ten. In the best batch, operations of the type illustrated in Fig. 2 yielded six buccal-opposing MI-doublets and four end-to-end MI forms from ten complexes; the most common yield of both types of operations was one or two MI-doublets out of ten attempts. The average yield of MI-doublets roughly conformed to the proportion of linear complexes of equal-sized components, corresponding to the idealized results depicted in Figs 1C and 2C. In the majority of actual operations, either cut no. 2 was not truly equatorial, or the two regions became misaligned as a consequence of uneven fusion and shortening during the course of the operation. In these cases, either fewer or more than two sets of ciliary primordia were produced, and the complex eventually regulated either to an RH- or an LH-singlet. For example, when cut 2 was too far posterior, the P→A region was smaller than the A→P region, and ciliary primordia developed successfully only in the latter region, leading to production of an inviable LH singlet. These 'unsuccessful' cases probably underwent the same intracellular interactions and developmental processes as the 'canonical' examples considered in Sections C2 and C3, but failed to form MI-doublets because of an imbalance in formation of oral primordia and/or in the deformations at wounded edges.

(D) *Operations that failed to generate mirror-image forms*

If the two cell-halves were allowed to remain side-by-side while still in a heteropolar orientation (Fig. 14A; compare to Fig. 2B), each half retained its original anteroposterior axis during the production of ciliary primordia, and then restored structures characteristic of the missing region (circled letters in Fig. 14), as if the halves were physically separate. A heteropolar doublet (two RH cells fused together in head-to-tail orientation) resulted (Fig. 14B). Mirror-image forms or LH singlets never emerged from this type of operation.

Mirror-image complexes also were not produced from an operation that was similar to the type shown in Fig. 2 except that the P→A region was *inverted* before being transposed to its anterior position, giving it a polarity opposite to that of the A→P region (Fig. 14C). In these cases, one set of ciliary primordia always was produced at the appropriate locations in the P→A region (e.g. an oral primordium appeared near the 'E' in Fig. 14C), and another set sometimes appeared near the posterior end of the A→P region (posterior to C). The P→A region completed its development successfully, whereas the new ciliary structures, as well as the aMB, of the A→P region eventually were resorbed. The result was a normal singlet whose ciliary organization was derived entirely from the original P→A region (Fig. 14D). Although this was accomplished without any reversal of the anteroposterior order of retained ciliary structures, it did involve extensive regulation. Since the A→P region became occupied by structures of opposite orientation derived from the

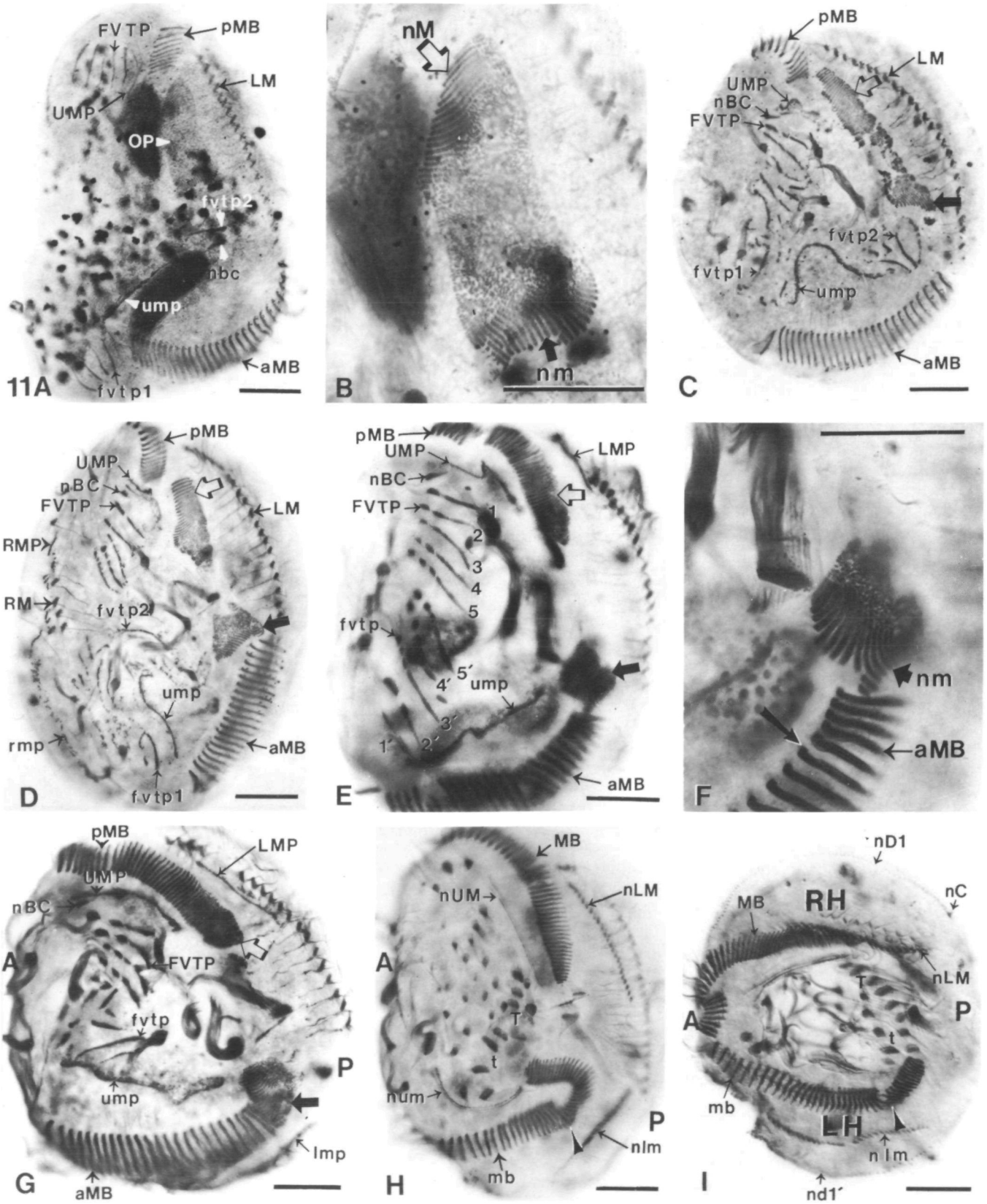

FVTP
pMB
LM
UMP
OP
fvtp2
nbc
ump
aMB
fvtp1
11A
nM
nm
B
pMB
LM
UMP
nBC
FVTP
fvtp2
fvtp1
ump
aMB
C
pMB
UMP
nBC
FVTP
LM
RMP
RM
fvtp2
ump
rmp
aMB
fvtp1
D
pMB
UMP
nBC
FVTP
LMP
1
2
3
4
5
fvtp
4′
5′
ump
3′
1′
2′
aMB
E
nm
aMB
F
pMB
UMP
nBC
LMP
FVTP
A
fvtp
P
ump
lmp
aMB
G
MB
nUM
nLM
A
T
t
num
P
nlm
mb
H
nD1
MB
RH
nC
nLM
T
A
P
t
mb
LH
n lm
nd1′
I

Fig. 11. Protargol preparations illustrating the development of buccal-opposing MI-doublets from 'minus-right' (−R) complexes. All photographs are oriented with the original anteroposterior axis vertical on the page. Labelling conventions are the same as in Fig. 9. Scale bars represent 25 μm. (A) A −R complex in stage 3, fixed 1 h after the operation. The original posterior portion of the membranelle band (pMB) as well as the intact left-marginal cirral row are located in the P→A region, whereas a larger anterior section of the membranelle band (aMB) is in the A→P region. An oral primordium (OP) is located posterior to the pMB, and frontal-ventral-transverse cirral primordia have formed to the right of the pMB (FVTP) and of the posterior end of the aMB (fvtp1). Undulating-membrane primordia (UMP, ump) are present in both regions, with a new buccal cirrus (nbc) visible near the anterior end of the ump. Immediately adjacent to the nbc, a single cirral streak may represent a second frontal-ventral-transverse cirral primordium (fvtp2). (B) An enlargement of part of A, with two sets of membranelles appearing within the OP, one (nM) at the normal location at the original anterior end of the OP (open arrow), the other (nm) ectopically situated at the original posterior end of the OP (closed arrow). The organization of these two membranelle sets is rotationally symmetric. (C) A −R complex in late stage 2, fixed 1.5 h after the operation. The process of shortening by the right side and envelopment by the left has begun. The oral primordium has split into two, each bearing one of the two sets of new membranelles (arrows). In the P→A region, a new buccal cirrus (nBC) is present near the anterior end of the undulating membrane primordium (UMP), and the ciliary streaks of the FVTP are breaking up into anlagen of the individual cirri. In the A→P region, no buccal cirrus can be seen at either end of the curved ump, and fvtp2 is better developed than fvtp1. (D) A −R complex in late stage 3, fixed 2 h after the operation. This complex is not bent, and a few right-marginal cirri (RM) plus right-marginal cirral primordia (RMP, rmp) are present at the right edge in both P→A and A→P regions. The two oral primordia (arrows) have separated further. The UMP, nBC, and FVTP of the P→A region appear as in C. In the A→P region, the ump seems to be caught midway in a counterclockwise rotation, there is no nbc, and fvtp are scattered over most of the region, possibly representing two sets (fvtp1, fvtp2). (E) A −R complex in early stage 4, fixed 2.5 h after the operation. The nM set (open arrow) has almost merged with the pMB, whereas the nm set (closed arrow) is close to the aMB (see F). The UMP, nBC, and FVTP of the P→A region have developed normally. In the A→P region, the ump now lies parallel to the aMB, possibly having completed a 180° counterclockwise rotation. The pattern of cirral subdivision of the two sets of frontal-ventral-transverse streaks (1–5 of the P→A region, 1′–5′ of the A→P region) is mirror-imaged. (F) An enlargement of E, focused on the nm set, which is undergoing a clockwise rotation. At this magnification, the protrusion at one end of each mature membranelle resulting from the presence of a short fourth row of basal bodies is apparent (arrow); such protrusions are just beginning to be visible in the nm set. (G) A −R complex in stage 4, fixed 2.5 h after the operation. The right margin of the original complex is becoming the anterior end (A) of the nascent MI-doublet, whereas the center of the original left margin is becoming the posterior end (P). Both new membranelle sets (arrows) have merged with their respective old membranelle segments (aMB, pMB), but the nm set (closed arrow) has not completed either its differentiation or its clockwise rotation. In this cell, the FVTP is properly oriented and subdivided into cirral rudiments, whereas the ciliary streaks of the fvtp are chaotically arranged and have not yet formed cirri. New left-marginal cirral primordia (LMP, lmp) have formed at the two margins of the nascent MI-doublet. (H) A −R complex in stage 6, fixed 3 h after the operation. Composite membranelle bands (MB, mb) have been assembled; the line of juncture of old and new membranelle sets is clear in the A→P region (arrowhead). New undulating membranes (nUM, num), new left-marginal cirri (nLM, nlm), and new frontal-ventral-transverse cirri have differentiated, with the cirral complement nearly normal in the P→A region and severely deficient in the A→P region; however, the large transverse cirri (T,t) are the posteriormost of the new cirri in both components. (I) A −R complex in late stage 6, fixed 4 h after the operation. The former right margin (now anterior) is fully contracted and enveloped by ciliature derived from the former left margin (now posterior and lateral). The former P→A region now is the right-handed (RH) component of the provisional mirror-image doublet, whereas the former A→P region has become the left-handed (LH) component. The ventral ciliature is similar to that in (H), with the nLM cirri optically juxtaposed on the MB. The composite membranelle band (mb) of the LH component still shows its dual origin, with a suture (arrowhead) marking the imperfect juncture between the former nm set and the aMB. One dorsal cirral row is visible at each edge (nD1, nd1′), and a new caudal cirrus (nC) is seen at the posterior end of nD1.

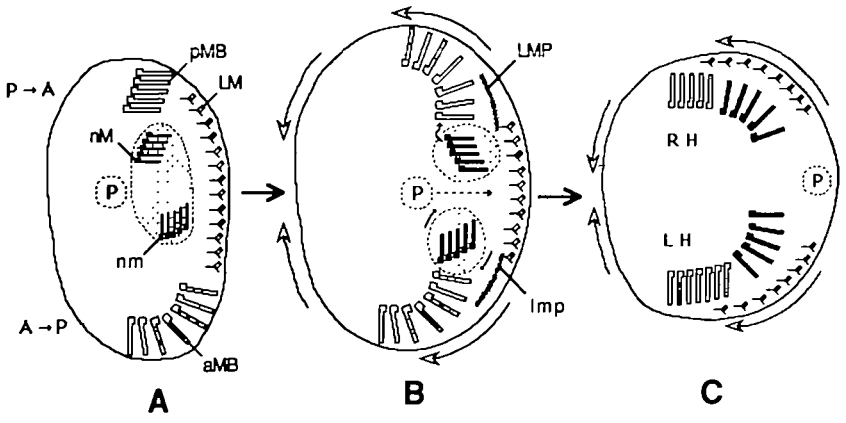


Fig. 12. A diagrammatic summary of key events in the development of a buccal-opposing MI-doublet from a −R complex. The stages shown in A, B, and C correspond to those of Fig. 11A, 11E, and 11G–H respectively. The abbreviations and conventions are the same as in Fig. 10. The open-ended arrows indicate directions of cortical shortening (on the right side) and of expansion or envelopment (on the left). See text for further explanation.

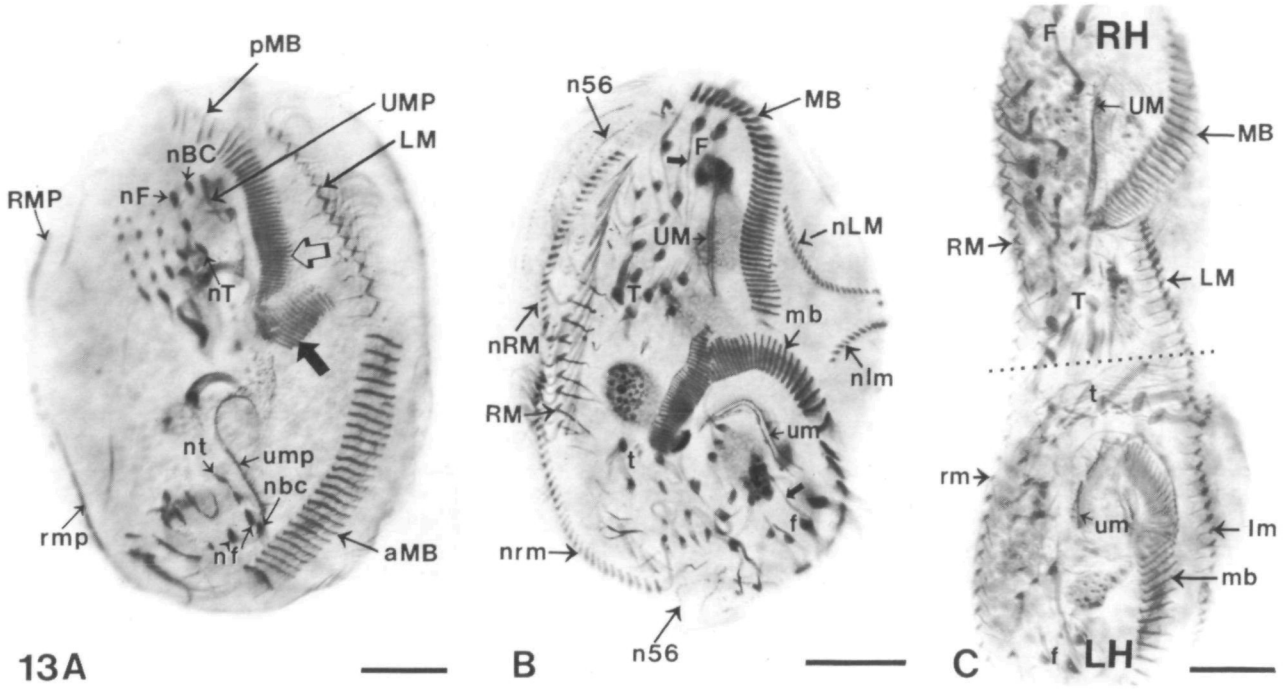


Fig. 13. Protargol preparations illustrating the development of end-to-end MI-doublets. Conventions of labelling are the same as in Figs 9 and 11. Scale bars represent 25 μm. (A) A −R complex in stage 4, fixed 3 h after the operation. The nM set (open arrow) has already fused with the pMB, whereas the nm set (closed arrow) is still separate. The two sets of other ciliary primordia are arranged as mirror images: the new buccal cirri (nBC, nbc) are at outer ends of the undulating membrane primordia (UMP, ump); the compact new frontal cirri (nF, nf) are at the outer ends whereas the more elongated new transverse cirri (nT, nt) are at the inner ends of the new frontal-ventral-transverse cirral sets. The cirral set of the A→P region is incomplete. Two new right-marginal cirral primordia (RMP, rmp) are visible at the bare right edge, whereas left-marginal cirral primordia have not yet begun to form [they normally appear slightly later than right-marginal primordia]. (B) A −L complex in early stage 6, fixed 3.5 h after the operation. Except for the curvature of the mb and um of the A→P region, the two regions have adopted an end-to-end mirror-image configuration. The new frontal cirri (F,f) are located near the poles of the complex, whereas new transverse cirri (T,t) are near the center. The cirral arrangement in the P→A region is typical of reorganizing cells, whereas in the A→P region new transverse cirri are poorly developed and abnormally arranged. The long posterior rootlets of the new frontal cirri (arrows) make it easy to see that cirri of the A→P region are 180° rotationally permuted relative to the corresponding cirri of the P→A region. The primordia of the new 5th and 6th dorsal ciliary rows (n56) are located at opposite ends of the new right-marginal cirral rows (nRM, nrm). Old right marginal cirri (RM) are undergoing resorption. The new left-marginal cirral rows (nLM, nlm) are found near the formerly bare left edge. (C) A −R complex in late stage 6, fixed 4 h after the operation. All cortical components are arranged in a mirror image around an imaginary plane indicated by the dotted line. The membranelle band and undulating membranes both are less regular in the LH component (mb, um) derived from the A→P region, than in the RH component (MB, UM) derived from the P→A region. Transverse cirri (T,t) of both components are located near the mirror plane, frontal cirri (F,f) near the poles. The marginal cirri on the left side of the complex are of the left-marginal (Lm, lm) type, those on the right side of the complex are of the right-marginal (RM, rm) type.

P→A region, one can think of the A→P region as undergoing a cryptic reversal of polarity (circled letters in Fig. 14D; see Discussion).

Discussion

(1) *A heritable reversal of intracellular organization is brought about by a developmental interaction*

We have described here the consequences of an operation that can, within a few hours, convert a normal *Stylonychia mytilus* cell into a mirror-image (MI) doublet. The operation involved an interchange of posterior (P→A) and anterior (A→P) cell regions, bringing about a juxtaposition of cell extremities (Fig. 8). The process that converted this tandem arrangement into a mirror-image organization was a reversal of the anteroposterior order and orientation of developing cortical structures in a zone near the line of juxtaposition. This zone of reversal then spread posteriorly (Fig. 15A, dashed vertical line), eventually including the entire A→P region. However, the polarity reversal in itself could only create a tail-to-tail mirror-image form. A MI-doublet arose because, during the same period, a shortening or contraction of the originally wounded edge caused the two regions to fold relative to each other (Fig. 15); the two serial and oppositely oriented anteroposterior axes therefore became parallel, whereas the originally parallel right–left axes in the two regions became serial and opposed. A MI-doublet thus was generated. The P→A region of

the original complex became the normal or 'right handed' (RH) component of the MI-doublet, whereas the A→P region, in which the anteroposterior reversal had taken place, became the reversed or 'left-handed' (LH) component.

The type of mirror-image doublet produced depended upon which edge was wounded, and on the direction of subsequent folding. If the original left (viewer's right) edge of the cell was removed (−L), folding was toward the left side of the complex, the P→A region bent clockwise while the A→P region bent counterclockwise, and a 'buccal-adjoining' MI-doublet with the RH component on the right and the LH component on the left resulted (Fig. 15, A to B). If the right (viewer's left) edge was removed (−R), folding was toward the right side of the complex, the P→A region bent counterclockwise and the A→P region bent clockwise, and a 'buccal-opposing' MI-doublet with the RH component on the left and the LH component on the right was generated (Fig. 15, A to C). End-to-end MI forms were produced in the exceptional cases in which the ciliary structures characteristic of the ablated edge regenerated and maintained the integrity of that edge.

The RH-to-LH conversion is reversible. Whereas the operations described here converted RH cells into MI (i.e. RH-LH) doublets, thereby creating new LH components, the same type of operation carried out on LH cells (obtained from longitudinally transected MI-doublets) also produced MI-doublets, thereby re-creating RH components from LH cells (Shi *et al.* 1987).

An operation that frequently changes the handedness of a cellular domain is unlikely to bring about its phenotypic effects though genic mutations. Mirror-image doublets of *S. mytilus* retain their character after

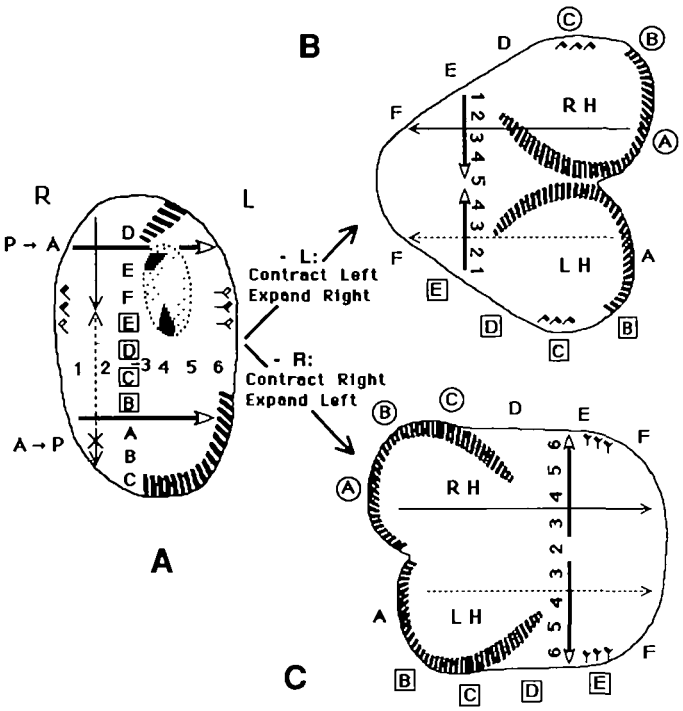


Fig. 15. How a reversal of the anteroposterior axis generates mirror image symmetry. In all diagrams, the cell's right is at the observer's left, the cell's left at the observer's right. (A) A generalized complex at stage 3, with old membranelles, oral primordium, and three left and three right-marginal cirri shown (any actual complex will have *either* one of these two types of cirri). The transverse axes are represented by double arrows and by the 1-2-3-4-5-6 sequence of numbers. The original anteroposterior axis is represented by the vertical continuous arrows and the letters D-E-F and A-B-C, whereas the reversed axis is represented by the dashed arrow and the boxed sequence of letters (E-D-C-B). (B) The reorientation of axes in a buccal-adjoining MI-doublet, formed from −L complexes (in which the edge marked by the '6' was removed). (C) The reorientation of axes in a buccal-opposing MI-doublet, formed from −R complexes (in which the edge marked by the '1' was removed). In both B and C, the axial reorientation is accompanied by the regeneration of new A-B-C values (circled) at the anterior end of the complex, and by the loss of the original (A)-B-C sequence at the posterior end. These mirror-image doublets are shown in their final form, after subsequent cortical reorganizations have modified their shape [particularly in C] and replaced the inverted marginal cirri on the edge of the LH component with normal marginal cirri of the same type.

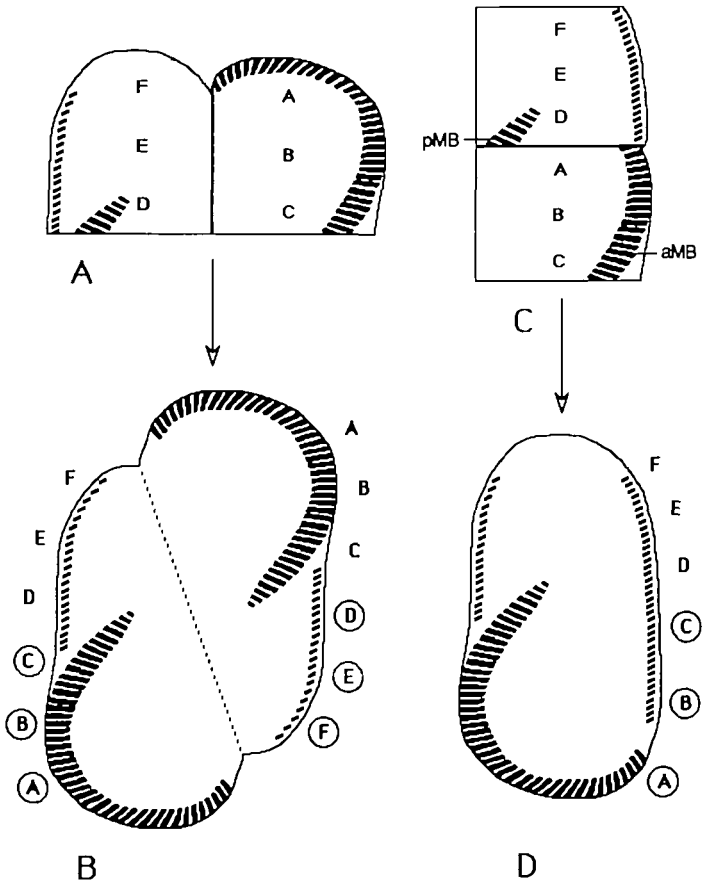


Fig. 14. Two operations that did not produce mirror-image doublets. The style of illustration is the same as in Fig. 8; the additional circled letters in the lower diagrams represent new positional values added during cortical reorganization. For further explanation, see the text.

conjugation with normal singlets (Shi and Qiu, 1989), but no detailed genetic analysis was carried out. In another ciliate, *Tetrahymena thermophila*, the difference between heritable LH and RH singlet cells was shown by genetic analysis to be *not* based on differences in nuclear genes (Nelsen *et al.* 1989*a*). The pathway of the transformation could not be ascertained for *Tetrahymena* in the precise manner accomplished for *Stylonychia*, although an unstable MI-doublet intermediate is strongly suspected (Nelsen and Frankel, 1989). In *Stylonychia*, unlike *T. thermophila* and the related *Glaucoma scintillans* (Suhama, 1985), an LH component must be supported nutritionally by a laterally attached RH partner (Grimes, 1990; Shi *et al.* 1990). Nonetheless, the organization of an LH component of a MI-doublet resembles that of an LH singlet cell; thus a fundamental generalization that applies to all three of these ciliates is that in an appropriate cellular setting a vegetatively generated LH cell-domain can propagate its organization indefinitely.

(2) *How is the anteroposterior reversal accomplished?* The operation that triggered a reversal of anteroposterior organization in the A→P region of *Stylonychia mytilus* involved a drastic rearrangement of cell components. The first step in seeking a mechanism for this reversal is to find out which aspect of this rearrangement is most relevant to the outcome. The most promising candidates are the relocation of the ciliary structures, especially oral structures, and the repositioning of former cell extremities.

We have formulated three hypotheses associated with these two possibilities (Fig. 16). The first hypothesis, of 'oral induction' (Fig. 16A), assigns a principal role to the relocated oral structures. In normal development (Fig. 4), membranelles first appear within that portion of the oral primordium that is closest to the pre-existing oral apparatus. Therefore, proximity to pre-existing oral structures might be sufficient to induce the formation of new membranelles within a neighboring oral primordium (Fig. 16A, open arrow). If so, the abnormal presence of pre-existing membranelles posterior to the oral primordium might induce the formation of a second set of membranelles in the portion of the oral primordium nearest the displaced membranelle set (Fig. 16A, closed arrow). Once this secondary center of membranelle development becomes established, that center might induce formation of further reversed ciliary structures in a step-by-step manner (see Frankel, 1989, pp. 205–206).

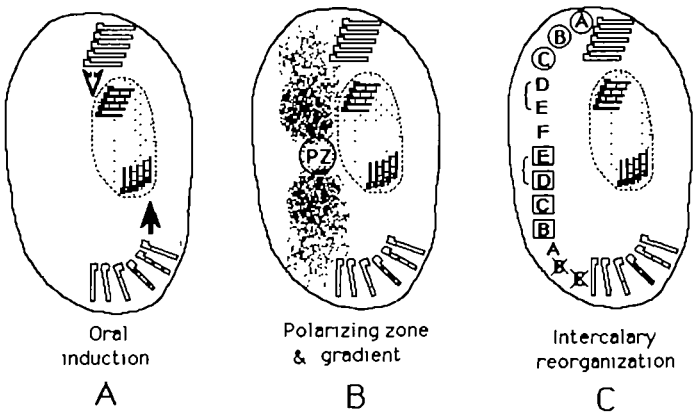


Fig. 16. Three hypotheses of reversal of the anteroposterior axis. The general illustrative conventions are similar to those of Figs 10 and 12, except that marginal cirri are omitted. In A, the large vertical arrows show the putative direction of induction of new membranelle sets. In B, the polarizing zone (PZ) is shown as a source of a gradient of some substance (or condition). In C, intercalated values are indicated by boxed letters, latitudes of active cortical development ('proliferative zones') by braces. Values newly generated at the anterior end are circled and values lost at the posterior end are crossed out. For further explanation, see the text.

We find this hypothesis unsatisfactory. It has been shown earlier that the old membranelle band is *not* necessary for the formation of new membranelles because complete sets of new ciliary structures are produced in the absence of *all* pre-existing ciliary structures in excysting cells of *Oxytricha fallax*, a close relative of *S. mytilus* (Grimes, 1973). In the present study, the developing posterior (nm) membranelle set is distant from the remnants of the old membranelle band (aMB) (Figs 9B,C; 11A,B), and the reversal of anteroposterior organization of developing ciliary structures in an A→P region takes place even when pre-existing membranelles are absent from that region (Fig. 9E,I). Therefore, the presence of nearby old membranelles is not a necessary condition for induction of formation of new oral membranelles.

The other two hypotheses assume that the crucial relocation is that of the end(s) of the cell. In the 'polarizing zone hypothesis' (Fig. 16B), a posterior 'zone of polarizing activity' (cf. Tickle *et al.* 1975) organizes a posterior-to-anterior sequence of structures in the remainder of the cell. Such a center at its natural position, the posterior end of the cell, would specify other regions as being progressively more anterior *via* a positional signalling system (Wolpert, 1971, 1989). When misplaced in the center of a nondividing cell, the posterior polarizing center would signal in both directions, thus reversing the anteroposterior organization of the region posterior to it in a manner analogous to the anteroposterior reversals following transplantation of the zone of polarizing activity in chick embryos (Tickle *et al.* 1975) or the displacement of the posterior polarizing region in certain insect embryos (Sander, 1960).

This polarizing-zone hypothesis is consistent with most of our observations. The earliest structural reversal, the formation of a new posterior set of membranelles in the bipolar oral primordium, happens closest to the relocated posterior end. The subsequent posterior migration of the oral primordium in the A→P region (in complexes whose left edge remained undamaged) probably is geometrically the reverse of the anterior migration of a normal oral primordium (Fig. 4). The initially delayed and disorderly development of the other ciliary primordia in the A→P region can be accounted for by time required for respecification of the anteroposterior axis, as no such delay or

disorder were found during subsequent rounds of development in LH components of MI-doublets; exactly the same had been observed by Grimes and L'Hernault (1979) and Grimes *et al.* (1981) in folded regions of longitudinal fragments of *S. mytilus*.

This polarizing-zone hypothesis can be applied to other experimental situations as well. In the rotated and transposed P→A fragment (Fig. 14C), the posterior organizing region is relocated to the 'top' of the complex instead of the center, and thus organizes an 'upside-down' singlet cell of normal asymmetry (Fig. 14D). In the side-by-side heteropolar complex (Fig. 14A,B), the A-B-C half lacks a polarizing zone and must be assumed to be capable of regenerating rapidly, as must also happen in totally separated half-cells (Hashimoto, 1961). This entails the subsidiary postulate that the polarizing zone is capable of rapid reconstruction in a manner expected of dynamic gradient systems (Meinhardt, 1982; Slack, 1983).

The polarizing-zone hypothesis is in part a revival of Uhlig's concept of a basal–apical gradient, for which substantial evidence was obtained in Stentor (Uhlig, 1960; Tartar, 1964) and *Blepharisma* (Eberhardt, 1962) (reviewed in Frankel, 1989, pp. 131–134, 155–158).

The third, 'intercalary reorganization' hypothesis (Fig. 16C) is that the necessary and sufficient condition for reversal of the anteroposterior axis is an abnormal juxtaposition of distant regions of the cell. This view presumes that different regions of the cell surface are positionally nonequivalent (Lewis and Wolpert, 1976), and that juxtaposition of regions that are not normally neighboring stimulates the intercalation of intervening positional values by the shortest permitted route (cf. French *et al.* 1976; Mittenthal, 1981). This hypothesis views the anteroposterior reversal of in the A→P region as a consequence of reverse-intercalation of the positions (the boxed sequence E, D, C, and B in Fig. 16C) located between the juxtaposed posterior (F) and anterior (A) positional values. Intercalation in this case would have to proceed through intracellular and morphallactic reorganization rather than through localized cell division and growth (cf. Frankel, 1989, Chapter 10).

The intercalary reorganization hypothesis, being more explicit in labelling cell regions than the polarizing-zone hypothesis, forces us to take into account certain spatial peculiarities of ciliary development in *Stylonychia*. Ciliary primordia develop in restricted latitudinal 'proliferative zones' (Grimes, 1976), corresponding roughly to regions D and E in Fig. 16C; the primordia formed there generate virtually the total ciliature of the cell or cellular domain, while the old ciliature is resorbed (Fig. 4). Thus, at every morphogenetic episode all latitudinal values of the cell appear to be generated anew from a restricted subset of values. New C-B-A values therefore are produced at the anterior end (circled in Fig. 16C), and old B and C values are deleted at the posterior end (crossed out in Fig. 16C). This combination of restricted sites for initiation of development and subsequent spread of ciliary primordia applies only to latitudes and not to longitudes, as development of ciliary organelles takes place along all longitudes and produces new structures that retain their original longitudinal positions while spreading latitudinally (Fig. 4).

The spatial restriction of development to particular latitudes makes it impossible to perform the crucial test of seeking to elicit different intercalated structures (or sets of structures) when different regions of the anteroposterior axis are juxtaposed (see Frankel, 1989, chapter 10, for the successful application of this test to the transverse axis). Nonetheless, there is one other testable difference between the polarizing-zone and intercalary reorganization hypothesis, which depends on the reciprocity of posterior and anterior extremities in the latter hypothesis, and the absence of such reciprocity in the former. If the polarizing-zone hypothesis is correct, anteroposterior reversal should occur only in the A→P region under the influence of the transposed polarizing zone of the P→A region. A retrograde reversal, with the P→A region becoming reversed while the A→P region retained its original polarity, would not be expected, since then the former anterior extremity (value A) rather than the former posterior extremity (value F) would have had to act as a 'polarizing zone'. On the intercalary reorganization hypothesis, 'A' and 'F' have no special properties except that they mark nonequivalent positions and the active primordium site happens to be positionally between the two. Therefore, if the intercalary reorganization hypothesis is correct, a retrograde reversal might be possible in an exchange-operation following a subequatorial transection in *Stylonychia* or following an equatorial transection in another ciliate in which the primordium site is located more anteriorly than in *Stylonychia*. For example, if the primordium site were at C, a DEF/ABC* complex could be converted to F*EDC**BA*/ABC*DEF (intercalated values italicized, primordium site starred). This was observed in operated complexes of the hypotrich *Paraurostyla weissei*, in which the MB is smaller than in *Stylonychia* and the ciliary primordia develop more anteriorly (Lu and Shi, 1987), and occasionally was seen in operated LH cells of *Stylonychia* (Shi *et al.* 1987).

The experiment shown in Fig. 14A provides an apparent counter-example; F and A were laterally juxtaposed yet no additional polar axes developed. However, comparable juxtapositions following the folding of surgically generated lateral half-cells did bring about the formation of a second reversed polar axis in *Blepharisma* (Suzuki, 1957) and *Stylonychia* (Grimes and L'Hernault, 1979) (summarized in Frankel, 1989, pp. 198–199). Indeed, the operation that generated the first mirror-image doublets in *Stylonychia*, the isolation of a central disc from a dividing cell (Tchang *et al.* 1964; Tchang and Pang, 1965), produced mirror-image doublets when (and only when) the anterior and posterior ends had rotated within the plane of the disc so that their posterior and anterior regions came into close juxtaposition (Shi and Qiu, 1985; summarized in Frankel, 1989, pp. 199–200). The failure to develop additional reversed axes in the experiment shown in Fig. 14A therefore may be due to features specific to the geometry of this complex, in which

longitudinal regeneration may be favored over lateral interaction across an artificial juncture. In a more readily operable ciliate, *Blepharisma japonicus*, heteropolar complexes resembling those of Fig. 14B could be stimulated to form mirror-image doublets by certain further deletions that modified the geometry of the complexes (Suzuki, 1957, pp. 169–173 and Fig. 47).

Any uncertainty in finding the best language to describe the reversal of global polarity in *Stylonychia* should not be allowed to obscure the major properties that clearly are inherent in the cortex of *Stylonychia*: a positional system that operates over large intracellular distances, that coordinately specifies the locations and migrations of separate ciliary primordia, that is itself capable of large-scale reorganization under suitable conditions, and that can be dissociated from the local processes that control the internal organization of individual ciliary organelles. All of these characteristics are much more reminiscent of certain positional information systems in multicellular organisms than of the local assembly-mediated processes that normally are thought of as dominating intracellular and some intercellular development.

(3) *The orientation of ciliary structures*

The individual ciliary organelles that are found in LH components of MI-doublets of ciliates never are genuinely reversed in their internal organization (Grimes *et al.* 1980; Frankel *et al.* 1984; Jerka-Dziadosz, 1983, 1985; Nelsen *et al.* 1989*b*; Shi and Frankel, 1990; Suhama, 1982, 1985). However, the spatial orientation of these structures may vary, being either normal or rotationally permuted by 90° or 180° relative to their counterparts in RH components. In MI-doublets of *Stylonychia mytilus* and related hypotrich ciliates, the orientation of the frontal-ventral-transverse cirri in LH components always is normal, that of oral structures always is 180°-permuted (inverted), and that of marginal cirri usually is normal but sometimes is inverted (Grimes *et al.* 1980; Grimes, 1990; Jerka-Dziadosz, 1983, 1985; Lu and Shi, 1987; Shi and Frankel, 1990).

The way in which MI-doublets arise might help us to understand how these varying orientations come about. The simplest hypothesis assumes that differentiating structures orient along one of the two orthogonal cell-surface axes (anteroposterior and transverse). If this is so, the reversal of the anteroposterior axis in the A→P region (Fig. 15A) should bring about an inversion of any ciliary structure that aligns along that axis, in the same way that the reversal of a magnetic field will cause a magnetized needle to swing through 180°. The oppositely directed folding of the A→P and P→A regions (Fig. 15B,C) then would cause these initially opposed ciliary structures to assume similar, parallel, orientations in the two components. Conversely, since the transverse axis is initially the same throughout the complex, any new ciliary structure that orients along that axis should be similarly oriented in both regions (Fig. 15A), but after folding (Fig. 15B,C) should become inverted in the LH region relative to its orientation in the RH region.

The frontal-ventral-transverse cirri obey this hypothesis perfectly. As shown schematically in Fig. 17, when these cirri first appeared they were inverted in the A→P region (Fig. 17A, arrows marked by F), possibly as a consequence of a 180° rotation of the early primordia (see Results). Then, after folding, these cirri became normally oriented everywhere in the provisional MI-doublet (Fig. 17B), and remained so during subsequent reorganizations and divisions (Fig. 17C).

The marginal cirri violate this hypothesis. Although the *location* of formation of structures was reversed in the A→P region (e.g. the primordia of the new 5th and

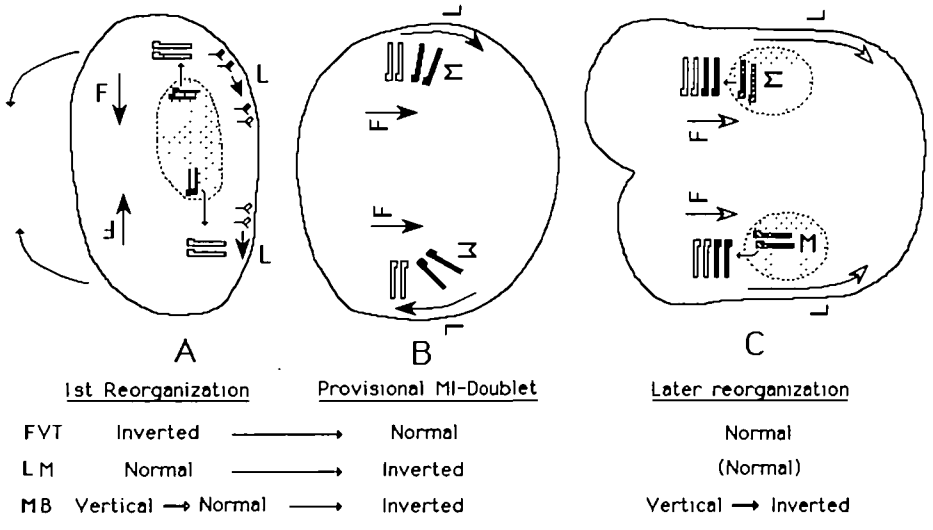


Fig. 17. The orientation of ciliary structures during formation of a buccal-opposing MI-doublet from a −R complex. The stages shown are (A) a −R complex during formation of ventral ciliary primordia, before substantial folding has taken place, (B) a provisional MI-doublet after the end of the first round of cortical development and of folding, (C) a definitive buccal-opposing MI-doublet after the completion of subsequent cortical reorganizations. A few left-marginal cirri and membranelles are illustrated in the same style as in Fig. 12. Membranelles produced in the first reorganization are shaded, whereas those formed in subsequent reorganization are cross-hatched. Large-headed arrows indicate the orientation of ciliary primordia, with the heads representing the ends that are posterior in normal cells. Filled heads indicate the first and open heads subsequent primordia. The letters stand for ciliary primordia (F=frontal-ventral-transverse cirral primordia; L=left-marginal primordia; and M=new membranelle bands), and their orientation symbolizes the spatial configuration of these primordia. Small-headed arrows indicate the direction of movement of new membranelle bands (in A and C) and the bending of regions (in A). See text for further explanation.

6th dorsal ciliary rows formed at opposite ends of the right-marginal cirral primordia), the *orientation* of the new cirral structures remained uniform throughout the complex (Fig. 17A). Then, after the P→A and A→P regions folded together to form the provisional MI-doublet, the same marginal cirri now appeared inverted in the LH component (Fig. 17B, arrows marked by Ls). Only later, in subsequent reorganizations, were the inverted marginal cirri of the LH component replaced by normally oriented cirri of the same type (Fig. 17C).

This behavior can be understood as the consequence of a constraint that prevents reorientation in response to a reversal of the anteroposterior axis. The most obvious possibility is that cirri of pre-existing old marginal cirral rows, from which the new marginal cirri develop, could control the orientation of new cirri (Grimes and L'Hernault, 1979; Grimes *et al.* 1981) in the same way that pre-existing ciliary organization maintains inversions of ciliary rows in *Paramecium* (Sonneborn, 1970). However, the same uniform orientation was observed after a marginal cirral row developed along a formerly bare edge (Fig. 13). Possibly the geometry of the cell margin itself restricts free rotation of ciliary primordia, whereas rotation might be possible at a different, non-marginal site. However, this line of reasoning fails to explain the *de novo* formation of *inverted* marginal cirral rows near normally oriented rows in LH components of MI-doublets of the hypotrich *Paraurostyla weissei* (Jerka-Dziadosz, 1985).

The orientation of membranelles is more complex yet. The formation of a membranelle band has both transverse features (the order of addition of ciliary units to each growing promembranelle) and longitudinal aspects (the stacking of separate membranelles). Developing membranelles in the A→P region gave the appearance of striking a compromise, as membranelles were oriented approximately vertically in the A→P region compared to the horizontal orientation typically observed in normal cells and in the P→A region (Fig. 17A). This peculiar orthogonal orientation of developing membranelles of the two oral primordia was carried over to the two components of a mature MI-doublet (Fig. 17C; Shi and Frankel, 1990). The orientation of mature membranelles of the LH component, inverted after folding (Fig. 17B,C), is most easily attributed to a requirement to achieve a compatible fit adjacent to pre-existing membranelles; this requirement does not, however, explain a similar reorientation that takes place in −L fragments that lack pre-existing membranelles in the A→P region (Fig. 9E,I).

The incomplete agreement between our observations and the hypothesis of orientation of ciliary structures along separate transverse and anteroposterior axes is not surprising, as this hypothesis oversimplifies the true geometrical situation. Ciliary units are both polar and asymmetrical (see Lynn, 1981; Frankel, 1989; Chapter 2). Therefore, if they rotate 180° in the plane of the membrane to adjust to the reversal of one axis, they will automatically become misaligned relative to the other, non-reversed axis. Therefore, the hypothesis can be correct only if ciliary units respond to a single axis when they align to form compound ciliary organelles. Whenever they are sensitive to both at once, a perfect adjustment of local organization to global axes becomes geometrically impossible. As was documented in detail in another example of such a conflict (Nelsen *et al.* 1989*b*), the consequence can be far greater variability in ciliary organization than normally is observed. The existence of this organizational dilemma for the cell and not just for the observer implies a superposition of at least two distinct patterning systems in the ciliate surface layer.

The authors gratefully acknowledge the scientific ambassadorship of Dr Stephen F. Ng of the University of Hong Kong, who originally introduced two of the authors (X.-b.S. and J.F.) to each other and who assisted in the discussions that planned this paper. They would also like to thank Dr E. Marlo Nelsen for his skill in producing Figs 1, 2, 8 10, 12, and 14–17. Dr Nelsen as well as Drs Eric S. Cole, Anne W. Koopmans Frankel, Gary W. Grimes, and Norman E. Williams read the manuscript and provided helpful comments. This project was supported by the funds of the Educational Commission of Heilongjang Province and partially by the National Science Foundation of China to X.-b.S., and by US National Institutes of Health Grant HD-08485 to J.F. The results of some of these experiments, including versions of Figs 5, and 11a,b,d, and g, were described briefly by Frankel (1989), pp. 201–206, and also have been presented in several published abstracts in Chinese as well as in one in English.

References

BEISSON, J. AND SONNEBORN, T. M. (1965). Cytoplasmic inheritance of the organization of the cell cortex of *Paramecium aurelia*. *Proc. natn. Acad. Sci. U.S.A.* **53**, 275–282.

EBERHARDT, R. (1962). Untersuchungen zur morphogenese von *Blepharisma* und *Spirostomum*. *Arch. Protistenk.* **106**, 241–341.

FAURÉ-FREMIET, E. (1945). Symétrie et polarité chez les Ciliés bi- ou multicomposites. *Bull. Biol. France Belg.* **79**, 106–150.

FRANKEL, J. (1989). *Pattern Formation: Ciliate Studies and Models*. Oxford University Press, New York.

FRANKEL, J. AND JENKINS, L. M. (1979). A mutant of *Tetrahymena thermophila* with a partial mirror-image duplication of cell surface pattern. II. Nature of genic control. *J. Embryol. exp. Morph.* **49**, 203–227.

FRANKEL, J., JENKINS, L. M. AND BAKOWSKA, J. (1984). Selective mirror-image reversal of ciliary patterns in *Tetrahymena thermophila* homozygous for a *janus* mutation. *Wilhelm Roux's Arch. devl Biol.* **194**, 107–120.

FRANKEL, J. AND NELSEN, E. M. (1986). Intracellular pattern reversal in *Tetrahymena thermophila*. II. Transient expression of a *janus* phenocopy in balanced doublets. *Devl Biol.* **114**, 72–86.

FRANKEL, J., NELSEN, E. M. AND JENKINS, L. M. (1987). Intracellular pattern reversal in *Tetrahymena thermophila*: *janus* mutants and their geometrical phenocopies. In W. F. Loomis (ed.), *Genetic Regulation of Development (Society for Developmental Biology Symposium* **45**), Alan R. Liss, New York, pp. 219–244.

FRENCH, V., BRYANT, P. J. AND BRYANT, S. V. (1976). Pattern regulation in epimorphic fields. *Science* **193**, 969–981.

GÖRTZ, H.-D. (1982). The behavior and fine structure of the dorsal bristles of *Euplotes minuta*, *E. aediculatus*, and *Stylonychia mytilus*. *J. Protozool.* **29**, 353–359.

GRIM, J. N. (1972). Fine structure of the surface and infraciliature of *Gastrostyla steini*. *J. Protozool.* **19**, 113–126.

GRIMES, G. W. (1972). Cortical structure in nondividing and cortical morphogenesis in dividing *Oxytricha fallax*. *J. Protozool.* **19**, 428–445.

GRIMES, G. W. (1973). Morphological discontinuity of kinetosomes during the life cycle of *Oxytricha fallax*. *J. Cell Biol.* **57**, 229–232.

GRIMES, G. W. (1976). Laser microbeam induction of incomplete doublets of *Oxytricha fallax*. *Genet. Res., Camb.* **27**, 213–226.

GRIMES, G. W. (1990). Inheritance of cortical patterns in ciliated protozoa. In G. M. Malacinski (ed.), *Cytoplasmic Information Systems, Primers in Developmental Biology*, Volume IV. Macmillan, New York, (in press).

GRIMES, G. W., KNAUPP-WALDVOGEL, E. A. AND GOLDSMITH-SPOEGLER, C. M. (1981). Cytogeometrical determination of ciliary pattern formation in the hypotrich ciliate *Stylonychia mytilus*. II. Stability and field regulation. *Devl Biol.* **84**, 477–480.

GRIMES, G. W. AND L'HERNAULT, S. W. (1979). Cytogeometrical determination of ciliary pattern formation in the hypotrich ciliate *Stylonychia mytilus*. *Devl Biol.* **70**, 372–395.

GRIMES, G. W., MCKENNA, M. E., GOLDSMITH-SPOEGLER, C. M. AND KNAUPP, E. A. (1980). Patterning and assembly of ciliature are independent processes in hypotrich ciliates. *Science* **209**, 281–283.

HASHIMOTO, K. (1961). Stomatogenesis and formation of cirri in fragments of *Oxytricha fallax* Stein. *J. Protozool.* **8**, 433–442.

JERKA-DZIADOSZ, M. (1963). Morphogenesis in division and regeneration of *Urostyla grandis* Ehrbg. *Acta Protozoologica* **1**, 43–54.

JERKA-DZIADOSZ, M. (1983). The origin of mirror-image symmetry doublet cells in the hypotrich ciliate *Paraurostyla weissei*. *Wilhelm Roux's Arch. devl Biol.* **192**, 179–188.

JERKA-DZIADOSZ, M. (1985). Mirror-image configuration in the cortical pattern causes modifications in propagation of microtubular structures in the hypotrich ciliate *Paraurostyla weissei*. *Roux's Arch. devl Biol.* **194**, 311–324.

JERKA-DZIADOSZ, M. AND FRANKEL, J. (1979). A mutant of *Tetrahymena thermophila* with a partial mirror-image duplication of cell surface pattern. I. Analysis of the phenotype. *J. Embryol. exp. Morph.* **49**, 167–202.

LEWIS, J. H. AND WOLPERT, L. (1976). The principle of non-equivalence in development. *J. theor. Biol.* **62**, 479–490.

LU, L. AND SHI, X. B. (1987). The grafting method applied to five species of hypotrich ciliates for creating mirror-imaged doublets. *Symp. Chinese Protozool. Soc.* **4**, 43–44 (Abstract, in Chinese).

LYNN, D. H. (1981). The organization and evolution of microtubular organelles in ciliated protozoa. *Biol. Rev.* **56**, 243–292.

MEINHARDT, H. (1982). *Models of Biological Pattern Formation*. Academic Press, London.

MITTENTHAL, J. E. (1981). The rule of normal neighbors: A hypothesis for morphogenetic pattern regulation. *Devl Biol.* **88**, 15–26.

NELSEN, E. M. AND FRANKEL, J. (1986). Intracellular pattern reversal in *Tetrahymena thermophila*. I. Evidence for reverse intercalation in unbalanced doublets. *Devl Biol.* **114**, 53–71.

NELSEN, E. M. AND FRANKEL, J. (1989). Maintenance and regulation of cellular handedness in *Tetrahymena*. *Development* **105**, 457–471.

NELSEN, E. M., FRANKEL, J. AND JENKINS, L. M. (1989*a*). Non-genic inheritance of cellular handedness. *Development* **105**, 447–456.

NELSEN, E. M., FRANKEL, J. AND WILLIAMS, N. E. (1989*b*). Oral assembly in left-handed *Tetrahymena thermophila*. *J. Protozool* **36**, 582–596.

NG, S. F. AND FRANKEL, J. (1977). 180° rotation of ciliary rows and its morphogenetic implications in *Tetrahymena pyriformis*. *Proc. natn. Acad. Sci. U.S.A.* **74**, 1115–1119.

SANDER, K. (1960). Analyse des ooplasmatischen Reaktionssystems von *Euscelis plejibus* Fall (Cicadina) durch Isolieren und Kombinieren von Keimteilen. II. Mitt.: Die Differenzierungsleistungen nach verlagerung von Hinterpolmaterial. *Wilhelm Roux's Arch. EntwMech. Org.* **151**, 660–707.

SHI, X.-B. AND FRANKEL, J. (1990). Morphology and development of mirror-image doublets of *Stylonychia mytilus*. *J. Protozool.* **37**, 1–13.

SHI, X.-B., LU, L., QIU, Z. AND FRANKEL, J. (1990). Morphology and development of left-handed singlets derived from mirror-image doublets of *Stylonychia mytilus*. *J. Protozool.* **37**, 14–19.

SHI, X.-B. AND QIU, Z. (1985). A tracing observation of the course of jumelle formation of *Stylonychia mytilus*. *Symp. Chinese Protozool. Soc.* **3**, 29–30 (Abstract, in Chinese).

SHI, X.-B. AND QIU, Z. (1989). Induction of amicronucleate mirror-imaged doublet in the ciliate *Stylonychia mytilus* and observation of its reproductive behavior. *Acta Zool. Sinica* **35**, 364–369 (In Chinese, with English summary).

SHI, X.-B., QIU, Z. AND LU, L. (1987). The correction of the reversed half in mirror-imaged doublet of *Stylonychia mytilus* and its genetic implications. *Symp. Chinese Protozool. Soc.* **4**, 38–39 (Abstract, in Chinese).

SLACK, J. M. W. (1983). *From Egg to Embryo: Determinative Events in Early Development*. Cambridge University Press, Cambridge.

SONNEBORN, T. M. (1970). Gene action in development. *Proc. R. Soc. Lond.* B **176**, 347–366.

SUHAMA, M. (1982). Homopolar doublets of the ciliate *Glaucoma scintillans* with a reversed oral apparatus. I. Development of the oral primordium. *J. Sci. Hiroshima Univ., Ser. B, Div. 1* **30**, 51–65.

SUHAMA, M. (1985). Reproducing singlets with an inverted oral apparatus in *Glaucoma scintillans* (Ciliophora, Hymenostomatida). *J. Protozool.* **32**, 454–459.

SUZUKI, S. (1957). Morphogenesis in the regeneration of *Blepharisma undulans japonicus* Suzuki. *Bull Yamagata Univ. Nat. Sci.* **4**, 85–192.

TARTAR, V. (1962). Morphogenesis in *Stentor*. *Adv. Morphogen.* **2**, 1–26.

TARTAR, V. (1964). Morphogenesis in homopolar tandem grafted *Stentor coeruleus*. *J. exp. Zool.* **156**, 243–252.

TCHANG, T.-R. AND PANG, Y.-B. (1965). Conditions for the artificial induction of monster jumelles of *Stylonychia mytilus* which are capable of reproduction. *Scientia Sinica* **14**, 1331–1338.

TCHANG, T.-R., SHI, X.-B. AND PANG, Y.-B. (1964). An induced monster ciliate transmitted through three hundred and more generations. *Scientia Sinica* **13**, 850–853.

TICKLE, C., SUMMERBELL, D. AND WOLPERT, L. (1975). Positional signalling and specification of digits in chick limb morphogenesis. *Nature* **254**, 199–202.

UHLIG, G. (1960). Entwicklungsphysiologische Untersuchungen zur Morphogenese von *Stentor coeruleus* Ehrbg. *Arch. Protistenk.* **105**, 1–109.

WIRNSBERGER, E., FOISSNER, W. AND ADAM, H. (1986). Biometric and morphogenetic comparison of the sibling species *Stylonychia mytilus* and *S. lemnae*, including a phylogenetic system for the Oxytrichids. *Arch. Protistenk.* **133**, 167–185.

WOLPERT, L. (1971). Positional information and pattern formation. *Curr. Top. devl Biol.* **6**, 183–224.

WOLPERT, L. (1989). Positional information revisited. In *The Molecular Basis of Positional Signalling* (ed. R. Kay and J. Smith), pp. 3–12.

(Accepted 29 October 1990)