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Discontinuities and Overlaps in Patterning Within Single Cells

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Discontinuities and overlaps in patterning within single cells

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Ciliates such as *Tetrahymena* manifest longitudinal vegetative growth and periodic equatorial subdivision. Evidence is presented suggesting that this subdivision involves the formation of discontinuities associated with the fission zone that closely resemble expressions of the segment border in multicellular organisms. Unlike latitudes, cellular longitudes can potentially maintain clonal continuity. Features of the system of longitudinal positioning of contractile vacuole pores (c.v.ps) in wild-type cells are suggestive of a circular positional system wrapped around the cell circumference, with a reference border coinciding with the axis of oral development. This border marks a discontinuity that, unlike the fission zone, can be clonally propagated. A recessive mutant, *janus* (*jan*), brings about alterations in c.v.p. positioning that suggest that a second longitudinal reference border is located about 45% of the cell circumference to the cell's right of the first. This second border, along which abnormal oral structures sporadically appear, seems to maintain a positional system oriented in a direction opposite to the primary system. When *jan* first comes to expression in cells previously jan^+, the pattern of c.v.p. longitudes changes gradually from that characteristic of wild-type cells to the *jan* pattern; this change begins before abnormal oral structures first appear along the second reference border. We suggest that the two reference borders, and the positional systems that they control, might be present in wild-type as well as *jan* cells. The oppositely directed positional systems are likely to overlap. A simple model is proposed to illustrate how overlapping positional systems might cooperate to generate patterns such as those observed.

Introduction

The major objective of this study is to explore the field properties of the ciliate surface, with the aim of learning more about those general properties of developmental fields that might transcend cellularity. Investigations conducted over the span of nearly a century have indicated that the basic properties of developmental fields are not markedly different in ciliates and multicellular animals (see, for example, Whitman 1893; Frankel 1974). The really important differences in patterning mechanisms between ciliates and most multicellular organisms may lie not so much in the absence or presence of cellular subdivision as in the mode of reproduction. The typical manner of reproduction of multicellular organisms, with their specialized gametic cells, probably precludes any *direct* continuity of developmental fields over generations. In ciliates, sexual acts are dissociated in time from growth and cell division, and all occur in the same cell. Hence, ciliates can maintain direct continuity of developmental fields across generations.

This continuity exists along one dimension only. The reason why is illustrated schematically in figure 1. Growth is mainly longitudinal and cell division is tandem, yielding daughter cells of identical polarity. A ciliate clone may thus be thought of as a continuously elongating cylinder (figure 1*b*, inspired by fig. 12 of Tartar (1962)). This mode of growth implies that distinctive features of cell longitudes can maintain their continuity, while developmental and functional properties of latitudes must be changing continuously as the clone undergoes its growth and

subdivision. The longitudinal continuity which allows the clonal propagation of differences in number and organization of ciliary rows (see, for example, Beisson & Sonneborn 1965) also permits large-scale field systems to be clonally inherited (reviewed by Aufderheide *et al.* 1981).

This paper presents an analysis of expresssions of these two complementary geometrical aspects of the large-scale patterning of the ciliate surface. We shall here restrict ourselves to one

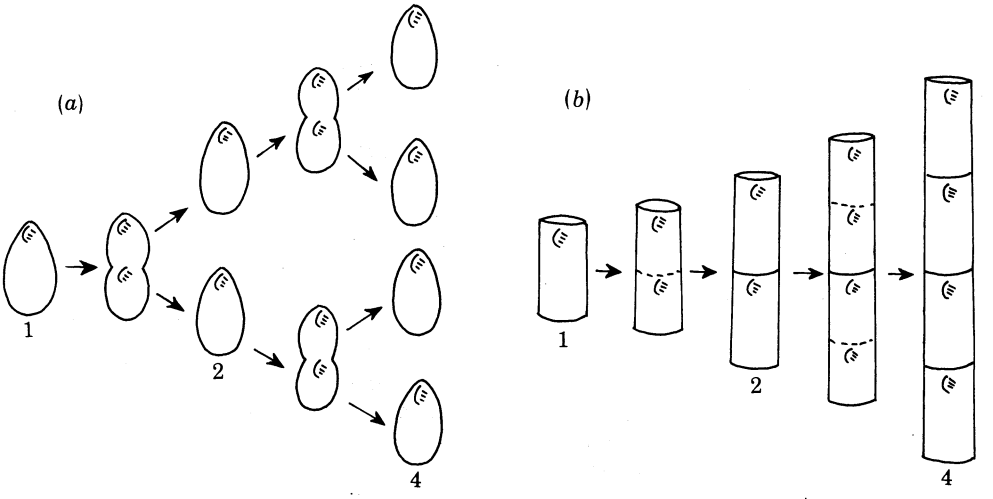


FIGURE 1. A schematic of clonal growth in ciliates. (*a*) Two division cycles of *Tetrahymena*. (*b*) The same visualized as a longitudinally growing cylinder that undergoes sequential segmentation. Such 'chain' formation is actually observed in mutants undergoing fission arrest at late stages, e.g. '*mo*3' (Frankel *et al.* 1977).

ciliate species, *Tetrahymena thermophila*. We consider, first, how this ciliate can subdivide itself into two, and argue that a major pattern-discontinuity analogous to the segment border of a worm or an insect is generated in every cell cycle. We shall then turn to the longitudinal clonal axis, and present evidence for the existence of clonally propagated discontinuities that function as field boundaries governing the proportional positioning of certain landmarks of the ciliate surface. Finally, consideration of the properties of the longitudinal positioning of these landmarks in wild-type and mutant cells will lead us to the notion of a dual system of spatial determination with overlapping expression, and to a tentative model that makes use of this overlap.

CELL SURFACE PATTERNS IN *TETRAHYMENA*

Tetrahymena thermophila is a small ciliate averaging 50 μm in length. Its major surface features are indicated schematically in figure 2. The cell typically possesses 18–21 longitudinal ciliary rows. An oral apparatus (o.a.), located near the anterior end of the cell, includes four compound ciliary structures (three membranelles and one undulating membrane). Most ciliary rows extend from the anterior to posterior pole, but two, known as the postoral rows, have their anterior terminations near the posterior margin of the o.a. The cell's right postoral ciliary row is by convention numbered 1, and enumeration proceeds clockwise around the cell as viewed from the anterior pole; the highest-numbered ciliary row ('*n*') is thus the left-postoral row.

In growing cells, not all basal bodies are ciliated. The basal bodies of the ciliary rows are uniformly spaced and almost all ciliated in the anterior third of the cell, whereas on the remainder of the cell surface the spacing is more irregular and roughly half of the basal bodies

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

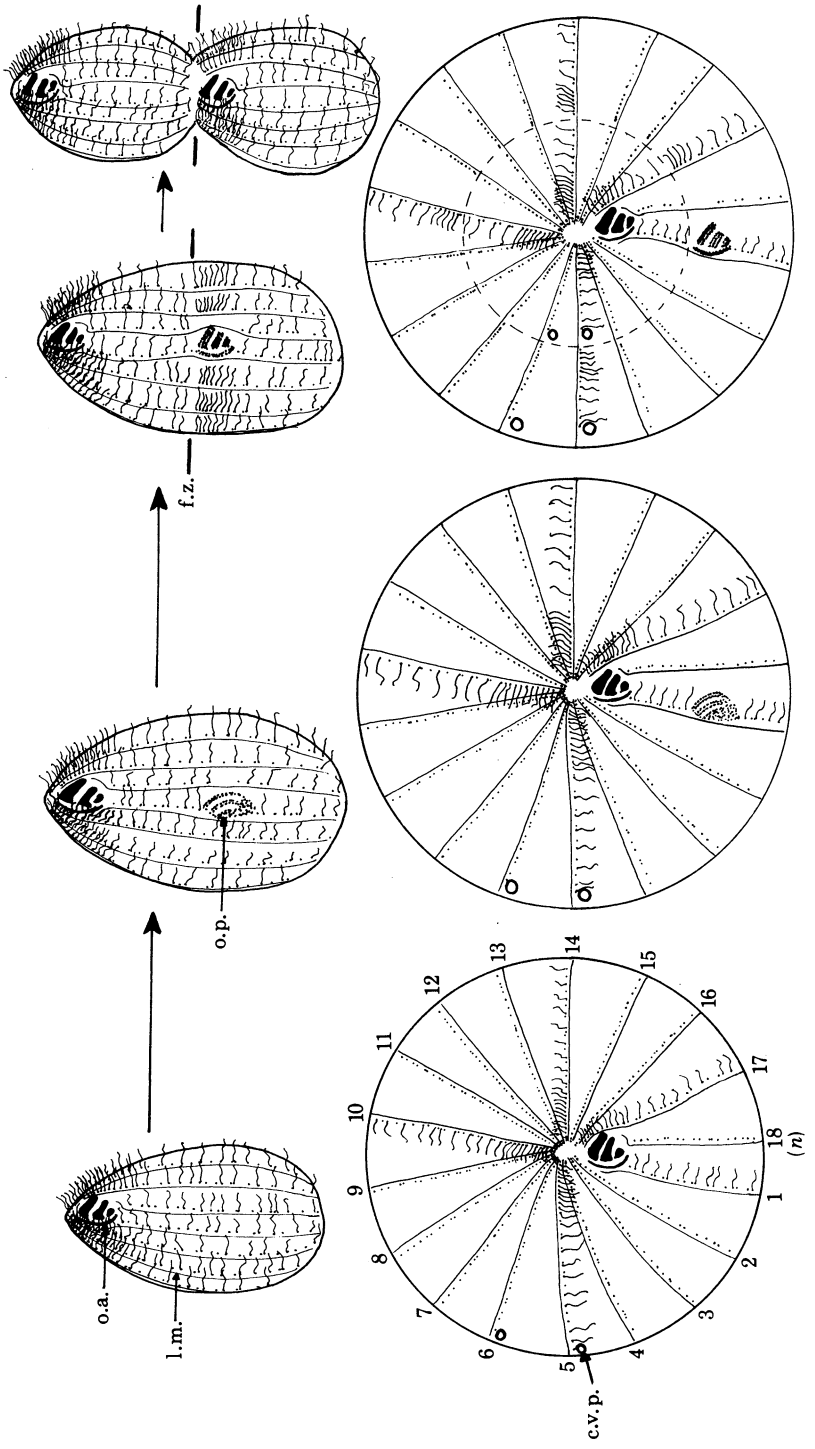


FIGURE 2. Diagrams of the oral (ventral) surface (above) and polar projections (below) of wild-type *T. thermophila* before the onset of oral development (left), during oral development before formation of the fission zone (centre) and after formation of the fission zone (f.z.) (right). A ventral view of a dividing cell is also shown. The ventral views show the oral apparatus (o.a.), oral primordium (o.p.) as well as basal bodies (dots), cilia, and longitudinal microtubule bands (l.m.) of seven ventral ciliary rows. The polar projections show the contractile vacuole pores (c.v.p.). Cilia are drawn in only in rows 1, 5, 10, 14, and 17 in these projections. On the right, the circular dashed line indicates the fission zone.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

lack cilia. In addition, specialized basal body couplets are located at the anterior ends of ciliary rows 5 to $n-2$; the anterior basal body of each of these couplets is unciliated. These couplets are in register laterally, and jointly make up a 'crown' at the anterior cell apex (indicated diagrammatically in the polar views in figure 2).

The contractile vacuole pores (c.v.ps) are among the most prominent of the various types of cell surface structures that are organized in definite spatial relations to the ciliary rows. These pores, which are complex structures (Elliott & Bak 1964), are located immediately to the cell's left of the posterior ends of one, two, or three adjacent ciliary rows. These 'c.v.p. rows' are located several rows to the cell's right of the right-postoral ciliary row.

Differentiation of latitudes: segmentation in ciliates

In ciliates, the cell surface pattern duplicates before the onset of cytokinesis. This duplication begins with the formation of a primordium for the new o.a., which is destined for the posterior division product. The oral primordium (o.p.) is typically located to the cell's left of the right postoral ciliary row (no. 1), approximately at the cell equator. The o.p. appears initially as an 'anarchic field' of disordered proliferating basal bodies (not shown), which then acquire cilia and become organized into the membranelles and undulating membrane (figure 2), eventually occupying a site in the posterior half of the cell that is equivalent to that of the pre-existing o.a. in the anterior half.

In exponentially growing cultures, most of the increase in number of basal bodies in ciliary rows takes place during oral development (Nanney 1975), and the two processes appear to be temporally coordinated (Nelsen *et al.* 1981). During most of the cell cycle, formation of cilia fails to keep up with appearance of new basal bodies, so the proportion of basal bodies that lack cilia increases (Nanney 1975; Nelsen *et al.* 1981). The general pattern of ciliation does not change, however, until a presumptive fission zone appears at a late stage of oral development (figure 2). At this stage the basal bodies *posterior* to the fission zone become ciliated in a way that suggests a wave of ciliation progressing posteriorly from the presumptive fission site (Frankel *et al.* 1981). The basal body couplets that will make up the 'crown' of the future posterior daughter cell develop at the same time. In sharp contrast, the pattern of ciliation of basal bodies situated *anterior* to the fission zone remains unchanged. In addition, new c.v.ps appear near certain ciliary rows, immediately adjacent to basal bodies located just anterior to the fission zone (Ng 1977, 1979*a*). These events are all illustrated schematically in figure 2.

Examination of cell division in cells bearing inverted (180° rotated) ciliary rows (Ng & Frankel 1977) indicates that the spatial orientation of the ciliation wave posterior to the fission zone, and the appearance of new c.v.ps anterior to the zone, are unaffected by the inversion; hence the geometry of these events is globally controlled. However, when a temperature-sensitive mutant (*cdaA*1) that conditionally prevents formation of the fission zone is incompletely expressed such that the fission zone develops in some ciliary rows but not in others, the ciliation wave and new c.v.ps appear in those ciliary rows that manifest fission zones and do not appear in those ciliary rows that fail to form fission zones. Hence, while the determination of the cellular subdivision that precedes fission is global, its expression is autonomous within cellular longitudes. The formation of the fission zone is not itself the direct cause of this coordinated expression, as under certain circumstances the passage of the ciliation wave and formation of new c.v.ps can temporally precede the overt appearance of the fission zone (Ng 1979*b*; Frankel *et al.* 1981). Thus we are observing various manifestations of the coordinated formation, within each of the

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

ciliary rows, of a new latitudinal boundary that separates newly developing structures characteristic of the two polar extremes of the ciliate body.

We believe that this formation of new anterior and posterior cell-ends in immediate juxtaposition to one another, in cells that have not yet begun cytokinesis, is fundamentally a process of segmentation, homologous to that found in the segmental divisions of certain flatworms (Child 1903) and perhaps even to insect segmentation. This perspective focuses attention on the fission zone, which we consider to be an expression of a newly generated segmental border. This zone is recognized only by a subtle change in the spacing of basal bodies within ciliary rows. A relatively wide gap appears just anterior to the closely spaced basal bodies that are undergoing ciliation, and as this gap is typically at the same latitude in all ciliary rows, the fission zone can be recognized as an equatorial zone of gaps in the ciliary rows. However, the longitudinal microtubule bands extend uninterrupted across these gaps, and only become broken much later, during cytokinesis (Frankel *et al.* 1981). Hence the cell becomes morphogenetically subdivided while the structural continuity of at least some cell surface fibrillar systems is maintained.

The evidence for resemblance between this subdivision of ciliate organization and segmentation in a multicellular organism such as an insect will now be summarized: in both, similar and repeated organizational patterns appear where there was formerly one. In both, spatially extreme elements of these patterns develop in close juxtaposition, but without any change in the polarity of the whole system. In both, analysis of phenotypic mosaics indicates that the formation of the 'segment border' and the expression of the organizational subdivision are spatially associated (cf. Tokunaga & Gerhart 1976). In both, there are few, if any, cytologically unique features detectable at the 'segment border' at the time of its formation (cf. Lawrence & Green 1975). In addition, the insect segment border (Wright & Lawrence 1981) and the site of cleavage in the ciliate *Stentor* (Tartar 1967) can both be regenerated following total removal, further supporting the notion that the visible border is the expression rather than the cause of the underlying discontinuity in pattern.

In insects, there is abundant evidence for a repeating segmental gradient with abrupt discontinuities ('cliffs') at the segment borders (Lawrence 1970). While there is no equivalent body of evidence for sequential *repeated* gradients in dividing ciliates, there is substantial evidence for the existence of an antero-posterior gradient of developmental properties in ciliates that are not actually dividing (Uhlig 1960; Tartar 1964; Frankel 1974). It is reasonable to suppose that when the fission zone develops, this gradient becomes subdivided, with the fission zone marking the site of the 'cliff' (Tartar 1968; Frankel *et al.* 1981). What is then most remarkable in ciliates is the ability to develop juxtaposed extremes of this gradient before cellular or even fibrillar subdivision.

On the basis of our anatomical observations we would predict that the major difference in distribution of ion-specific mechanoreceptor channels known to exist at the two ends of ciliate cells (Naitoh & Eckert 1969; Ogura & Machemer 1980) would appear before cell division, on the two sides of the fission zone.

Continuity of longitudes and the dual specification of c.v.p. positions

(*a*) *C.v.p. positioning in wild-type cells*

The relational system of specification of longitudes at which c.v.ps develop is one of the more striking manifestations of global patterning in *Tetrahymena*. We have already seen that c.v.ps

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

develop anterior to the fission zone, adjacent to certain ciliary rows. These are specific 'c.v.p. rows' that are located several rows to the cell's right of the 'oral axis', defined by the right postoral ciliary row (no. 1). Nanney (1966*a*) first recognized that the number of ciliary rows between the oral axis and the c.v.p. rows is proportional to the total number of ciliary rows in the cell, or more accurately, to the number of ciliary rows between two oral axes (figure 3).

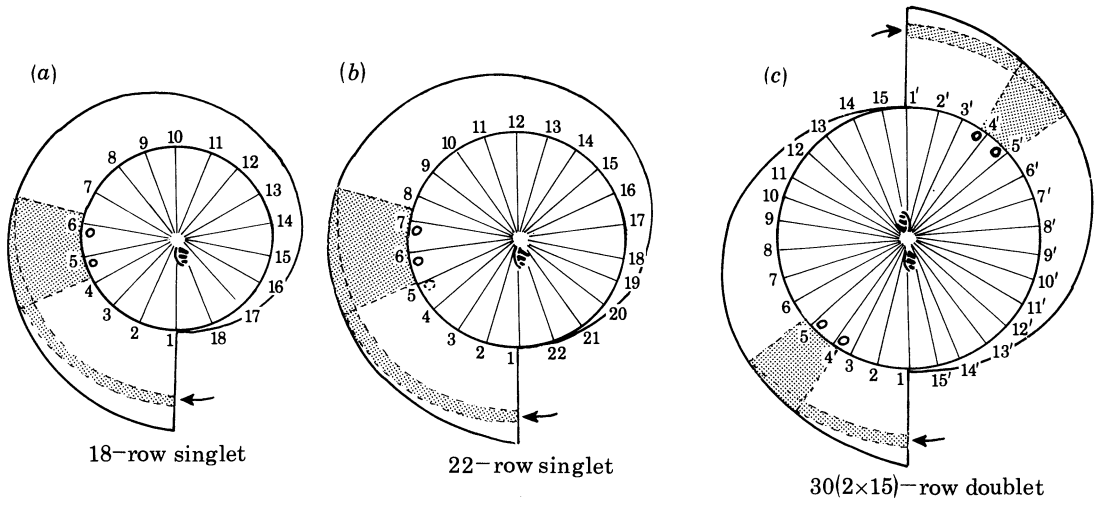


FIGURE 3. A schematic view of c.v.p. cytogeometry in wild-type cells, including singlets with differing numbers of ciliary rows (left and centre) and a parabiotic doublet (right). Ciliary rows are numbered as in figure 2, with the parabiotic doublet treated as two cells fused side by side. The representation of the constant relative position of the c.v.p. arc (shaded) is taken from Nanney (1966*a*, figs. 2 and 4B), but modified by replacing Nanney's 'inductive angle' with a specified level (arrows) in Uhlig's 'circular gradient' (Uhlig 1959).

Nanney also showed that the oral axis itself appears to serve as a reference point in this system. This is suggested by the subdivision of c.v.p. longitudes in parabiotic doublets (figure 3*c*) created by the lateral fusion of two similar cells (Nanney 1966*a*; Nanney *et al.* 1975). The fact that a lateral shift in the location of the new o.a. ('cortical slippage') brings about an equivalent shift in the longitudes at which the c.v.ps develop (Nanney 1967*a*) argues most convincingly that the oral axis is a crucial morphogenetic boundary. While Nanney (1966*a*) described the mode of determination of c.v.p. longitudes in terms of a 'central angle', he explicitly recognized this as artificial and proposed that the cell actually measures a relative surface distance between oral axes. We shall represent this in the form of a system in which a 'circular gradient' (Uhlig 1959) provides the relevant positional values. This gradient model places a single major discontinuity at the oral-axis reference boundary. This discontinuity, like that which develops at the fission zone, involves the juxtaposition of extreme positional values without a reversal of polarity. However, unlike the fission zone, the oral-axis reference boundary is longitudinally oriented and so can be clonally propagated, and thus need not be formed anew in every cell cycle.

There is one additional aspect of 'c.v.p. cytogeometry' that is of some importance in wild-type cells, but becomes critical when we consider the evidence offered by *janus* cells (see below) for the participation of a second reference boundary in the determination of c.v.p. longitudes. This is the internal organization of the region in which c.v.ps can form. To make the presentation clear we shall need some explicit definitions. First, we have already called a ciliary row

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

next to which a c.v.p. develops a *c.v.p. row*. Secondly, we shall define the width of a group of neighbouring c.v.p. rows as the *c.v.p. arc*; the c.v.p. arc is bounded by the outermost c.v.p. rows of the group. Thirdly, we shall designate the longitude of a c.v.p. by the number of its c.v.p. row.† Finally, we define the *c.v.p. midpoint* as the mean of the longitudes of all of the ciliary rows located within the c.v.p. arc.

Using this language, we can state that wild-type cells have one to three c.v.p. rows within a c.v.p. arc and that the c.v.p. midpoint is a roughly constant fraction, slightly less than 25%, of the distance (viewed clockwise from the anterior pole) from one oral axis to the next. The width of the c.v.p. arc is variable, but is roughly proportional to the total number of ciliary rows from one oral axis to the next (see Nanney (1966, 1967*b*) for more detailed information). There is, however, one complexity in the application of this language that poses an interpretive problem in analysis of the *janus* cells that we will discuss below. Consider cells in which the c.v.p. arc comprises three ciliary rows. Either all three may bear c.v.ps, or a central row lacking a c.v.p. may be flanked by two rows that possess c.v.ps. Nanney (1967*b*) recorded both types as possessing three c.v.p. rows, remarking also that the latter type, with a 'skipped' row, is extremely rare in wild-type cells. But when 'skipped' rows become much more common and more than one row may be 'skipped', as in *janus* cells, it becomes tempting to represent this as a subdivision of one 'c.v.p. set' into two. This was actually done in an earlier publication (Jerka-Dziadosz & Frankel 1979). Here, however, we shall view the c.v.p. arc as unitary even when there are as many as three or four 'skipped' rows within it. The reasons for this will become more apparent as we consider the *janus* c.v.p. configurations.

(*b*) *C.v.p. positioning in established* janus *clones*

A recessive allele known as *janus* (*jan*), when homozygous, brings about a substantial change in oral and c.v.p. cytogeometry. As described in more detail elsewhere (Jerka-Dziadosz & Frankel 1979; Frankel & Jenkins 1979), cells expressing *jan* produce one set of basically normal oral structures and a second set that is highly abnormal. The normal o.a. is located along one longitudinal axis of the cell, the primary (1°) oral axis, along which new, normal oral structures are generated much as in wild-type cells. The abnormal o.a., when present, is located along a secondary (2°) oral axis that is located approximately 45% of the cell circumference clockwise from the primary oral axis (figure 4*b*). New o.a.s may or may not be produced along the 2° oral axis, but when produced they are always abnormal; the abnormality may be described in part as a reversal of global asymmetry (Jerka-Dziadosz & Frankel 1979; Frankel 1982). It should be emphasized that this condition bears no resemblance to the parabiotic doublet state (figure 3*c*), in which the duplex organization is produced by lateral cell fusion, there is no genic difference between doublets and singlets, and both sets of oral structures are normal and reliably reproduced except in transient situations when the doublet is regulating back to the singlet state (Nanney 1966*b*; Nanney *et al.* 1975).

† This rule differs from Nanney's procedure of assigning the c.v.p. location according to its actual position, typically about three-quarters of the distance between one ciliary row and the next. Thus, for a c.v.p. immediately to the cell's left of row 5, Nanney assigns a position of 4.75 and we assign a position of 5. The rationale for our procedure is the recent evidence that the fine-positioning of the c.v.p. is controlled by the adjacent ciliary row Ng 1977), and that a new c.v.p. is induced in a rigorously defined position adjacent to a basal body within that row (Ng 1979*a*). In both Nanney's system and ours, relative c.v.p. location is proportional to true circumferential distance *only* if ciliary rows are uniformly spaced, a condition that appears to hold, at least approximately.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Using the definitions developed in the previous section, we can describe the primary effect of the *jan* gene on c.v.p. cytogeometry as a widening of the c.v.p. arc (figure 4). This gives rise to many permutations of c.v.p. arrangements that are never observed in wild-type cells (figure 5). Simultaneously, there is an increase in the frequency of 'skipped' rows within the c.v.p. arc (figure 5). This is probably due in part simply to an increase in the total width of the c.v.p. arc

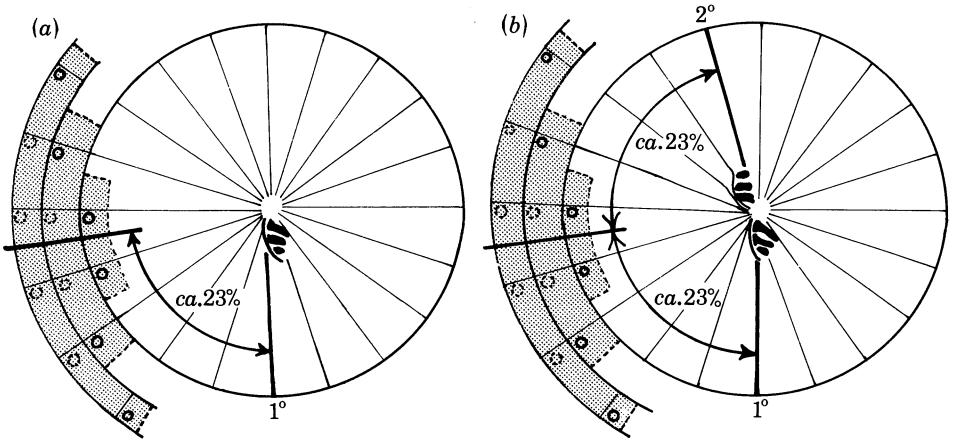


FIGURE 4. Different grades of expression of the c.v.p. arc in *janus* cells with (*a*) one o.a. and (*b*) two o.as. The normal primary (1°) oral axis is shown below, the secondary (2°) oral axis with an abnormal o.a. above. The c.v.p. midpoints are indicated by the heavy line on the left side of each diagram. In each diagram, three c.v.p. arcs of progressively greater widths (shaded) are represented in parallel. Within each of these three arcs, positions at which c.v.ps always appear are indicated by solid circles, those at which they may or may not be present by broken circles. The average distance from each oral axis (right postoral ciliary row) to the c.v.p. midpoint is indicated as a percentage of the cell circumference.

combined with an inherent limit in the number of c.v.p. rows 'permitted' within an arc (never more than five, rarely more than four). But there is an additional effect of *jan* that actively brings about subdivision, since the central row of a three row c.v.p. arc is 'skipped' much more commonly in cells of established *jan* clones than in wild-type cells (figure 5).

We can now briefly summarize the properties of the expanded set of c.v.ps of *jan* cells in the form of a list of general features, with limited supporting documentation. We shall categorize these into 'internal' features that can be ascertained without reference to any cellular landmarks outside the c.v.p. arc, and 'relational' features for which other cellular reference points are essential.

(*a*) *Internal features*

1. No more than five of the ciliary rows within a c.v.p. arc actually bear c.v.ps.

2. All non-c.v.p.-bearing rows are contiguous (e.g. ○○--○○, almost never ○-○-○, using c.v.p. notation as in figure 5).

3. Non-c.v.p.-bearing rows, when present, tend to be centrally located within the c.v.p. arc (59 cases of ○○-○○ patterns were tallied compared with 5 ○○○-○; 19 cases of ○○--○○ compared with 5 of ○○○--○).

4. As the width of the c.v.p. arc increases, *both* the mean number of c.v.ps in the c.v.p. arc and the prevalence and size of the central 'skipped' region increase (figure 5).

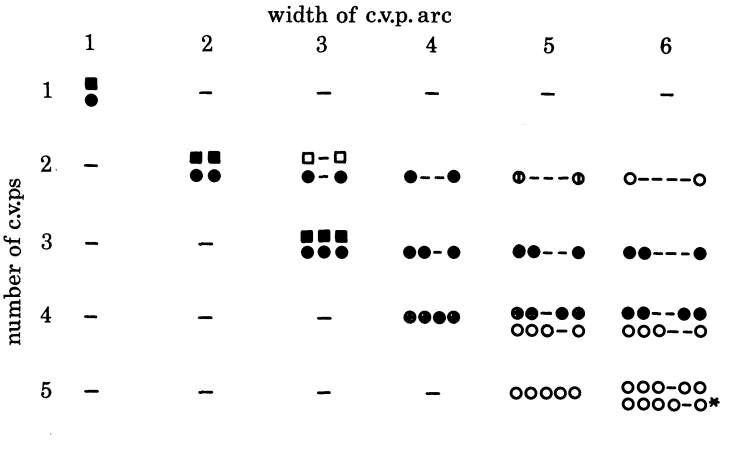
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

(*b*) *Relational properties*

5. There is little or no directional bias in the positioning of c.v.ps within the c.v.p. arc (22 cases of 1°—○○–○—2° against 31 of 1°—○–○○—2°; 29 cases of 1°—○○––○—2° against 22 of 1°—○––○○—2°).

6. The width of the c.v.p. arc tends to increase as the total number of ciliary rows in the cell increases.

7. The average c.v.p. midpoint, measured from the 1° oral axis (as shown in figure 4*a*), is the same in *jan* and wild-type cells.



		1	2	3	4	5	6
mean no. of c.v.p. rows	*jan*+	1	2	2.97	—	—	—
	jan	1	2	2.44	2.82	3.33	3.64
percentage with skipped rows	*jan*+	(0)	(0)	3	—	—	—
	jan	(0)	(0)	44	81	93	100

FIGURE 5. C.v.p. patterns encountered in wild-type and *janus* cells. The configuration of c.v.p. arcs is indicated by squares (wild-type) or circles (*jan*) representing c.v.p. rows with c.v.ps, and short dashes indicating ciliary rows within the c.v.p. arc that lack c.v.ps ('skipped' rows). Where gaps are off-centre, only one of the two possible spatial orientations is shown. The shading within each symbol indicates the commonness of the indicated pattern *relative to others with the same c.v.p. arc.* ■, ●, over 30%; ⊕, 15–30%; ⊙, 8–15%; □, ○, 0–8%; ○*, only one case observed. The data tabulated at the foot of the diagram are arranged in columns that correspond to the width-classes of c.v.p. arc given at the top.

8. The average c.v.p. midpoint is unaffected by variation in the width of the c.v.p. arc.

9. The widths of c.v.p. arcs are mutually uncorrelated, and the c.v.p. midpoints only weakly correlated ($r = 0.2$–0.3), in the two presumptive division products derived from the same parent cell.

10. Both the c.v.p. midpoint and the width of the c.v.p. arc are unaffected by the presence or absence of a 2° o.a.

11. In the subset of *jan* cells in which 2° o.a.s are expressed, the average c.v.p. midpoint is halfway or very near halfway between the two oral axes (figure 6; illustrated schematically in figure 4*b*).†

† This conclusion is sensitive to assumptions concerning the locations of the c.v.ps and the oral axes. We have identified the oral axes with the right-postoral ciliary rows, and the c.v.p. longitudes with the c.v.p. rows. Other assumptions may generate c.v.p. midpoints that may either be somewhat off-centre, or also central, depending on the combination chosen (cf. Jerka-Dziadosz & Frankel 1979).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The last two observations, taken together, suggest that the 2° oral axis of *jan* cells plays an important role in the positioning of c.v.ps even in cells that are not forming o.as along this axis. This was visualized earlier as control of the position of the second of two c.v.p. sets by a field of a handedness opposite to that which controls the position of the first c.v.p. set (Jerka-Dziadosz & Frankel 1979; Frankel 1979, fig. 9). This reversed direction of determination contrasts markedly

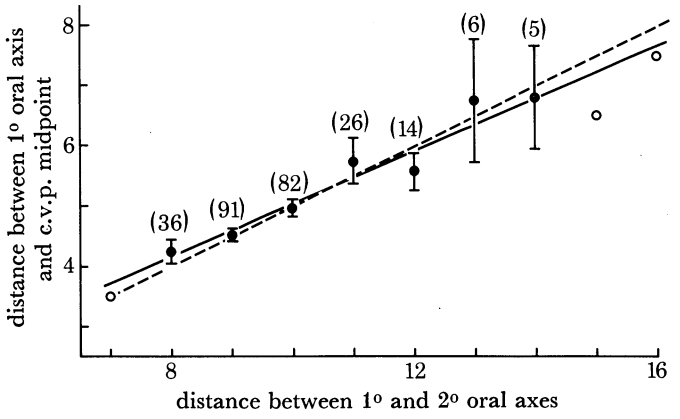


FIGURE 6. Regression of the distance between the 1° oral axis and the c.v.p. midpoint on the distance between the 1° and 2° oral axes. Both sets of distances are expressed in number of ciliary-row intervals. Solid circles indicate averages of more than one observation (numbers given above each), open circles indicate single observations. Vertical bars give 95% confidence limits. The solid line indicates the best-fit linear regression computed from the data, while the dashed line indicates the regression line expected if the average c.v.p. midpoint were always exactly midway between the two oral axes, i.e. a line with a slope of 0.5 and a y intercept of 0.

to the situation in parabiotic doublets generated by side-by-side cell fusion, where one can visualize the coexistence of two separate and similarly oriented gradient systems (figure 3*c*), which can be propagated longitudinally without direct mutual interference and with limited mutual interaction (Nanney *et al.* 1975). However, as soon as one reverses the orientation of one of the two gradients, then the two are likely to overlap. It is hard to imagine how such an overlap could be maintained, especially if we viewed the two gradients as having a similar basis. We shall return to this problem after exploring how the 2° axis is created or altered when *jan* begins to be expressed in cells previously expressing *jan*$^+$.

(*c*) *From wild-type to* janus

By employing certain tricks of *Tetrahymena* genetics, we were able to bring about a synchronous switch from expression of *jan*$^+$ to expression of *jan* in 75–95% of a mass population (Frankel & Nelsen 1982). Secondary o.as first appeared about three fissions after this switch; within ten fissions the proportion of 2° o.a.s was similar to that in comparable established *jan* clones. The very first 2° o.a.s resembled the 2° o.a.s in cells of established *jan* clones, and appeared at nearly the usual *jan* positions in otherwise normal cells that maintained the previous number of ciliary rows. The appearance of these 2° o.a.s was unattended by any microscopically visible structural rearrangement of the cell surface. This mode of 'insertion' of a second o.a. differs fundamentally from the origin of second oral systems in experimentally constructed doublets, where the duplex condition is a consequence of lateral cell fusion or of regulatory adjustments after microsurgically generated rearrangements.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The c.v.p. patterns characteristic of *jan* emerged gradually. The first c.v.p. arrays unique to *jan*, generally of the ○○○○ variety, where observed about two fissions after the switch from *jan*$^+$ to *jan* expression. The characteristic *jan* patterns became more frequent and more extreme thereafter, but with no cell-by-cell correlation between the breath of the c.v.p. arc and the simultaneously emerging appearance of 2° o.a.s. The average c.v.p. midpoint remained the same.

The present descriptive framework, which stresses the breadth of the c.v.p. arc rather than the degree to which it is interrupted, allows us to recognize that the presence of *jan* (or absence of *jan*$^+$) had begun to affect the c.v.p. pattern even when few or no cells expressed the diagnostic c.v.p. arc of four or more ciliary rows. Following the *jan*$^+$ to *jan* phenotypic substitution, the proportion of cells expressing three (instead of the more typical two) c.v.p. rows increased significantly within one fission after the substitution, and was dramatically elevated by two fissions, while in an otherwise parallel cross using the same stocks in which the *jan*$^+$ allele continued to be expressed, no such increase took place (Frankel & Nelsen 1982). Thus, the average width of the c.v.p. arc began to increase at or shortly after the time of the switch from *jan*$^+$ to *jan* expression, and expanded continuously during the next several fissions until the final state characteristic of established *jan* clones was attained, at or before ten fissions.

This increase in breadth of the c.v.p. arc does not, however, provide a full description of the conversion from a wild-type to a *jan* c.v.p. pattern. We noted earlier that the c.v.p. pattern of established *jan* clones was characterized not only by an increase in the average breadth of the c.v.p. arc, but also by an increased proportion of c.v.p. arcs with 'skipped' rows. As *jan* came to expression, the increase in proportion of 'skipped' rows started later than the increase in field breadth. For example, of the cells with a c.v.p. arc of four rows (hence unmistakably expressing *jan*), only 20% manifested a (single) 'skipped' row at two to three fissions after the onset of *jan* expression, compared with 80% that manifested 'skipped' rows later on (a percentage similar to that in established *jan* clones).

Hence the two major aspects of the expression of *jan* c.v.p. patterns are, at least to a limited degree, dissociable. This dissociability is a major reason for describing the effect of *jan* on c.v.p cytogeometry primarily in terms of a broadening of the c.v.p. arc rather than the creation of two c.v.p. sets as was done earlier.

Discussion

We wish to advance the notion that all tetrahymenas, not just those homozygous for *janus*, possess two longitudinally propagated reference axes of mirror-image asymmetry, and use these jointly to specify c.v.p. cytogeometry. To render this speculative idea plausible, we shall argue backwards from the established *jan* state to the wild-type condition from which it emerged after the appropriate allelic substitution. We have seen that in established *jan* clones the c.v.p. midpoint is roughly equidistant from the two oral axes, suggesting that the 2° oral axis might serve as a supplementary reference boundary for c.v.p. positioning, with an effect equal in strength but opposite in direction to that of the 1° oral axis. The independence of c.v.p. parameters from expression of the 2° o.a. in *jan* cells indicates that this referencing function is independent of expression of the 2° o.a.: the *jan* allele affects both in a genuinely pleiotropic manner. One may therefore think of *jan* clones as maintaining two continuously propagated reference borders, one occupied by normal oral structures and the other subject to sporadic appearance of abnormal o.a.s with strong indications of reversed global asymmetry (Jerka-

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Dziadosz & Frankel 1979). Proceeding backwards, when *jan* first comes to expression, 2° o.a.s first appear at (or near) the characteristic location. The c.v.p. arc simultaneously 'spreads' in both directions, the spread beginning even before the 2° o.a.s make their appearance. Hence, if the second reference border is created by the *jan* allele (or by the absence of *jan*$^+$), it must be created within the first two fissions after *jan* comes to expression, and become progressively 'strengthened' thereafter. But the fact that the average c.v.p. midpoint does not change during

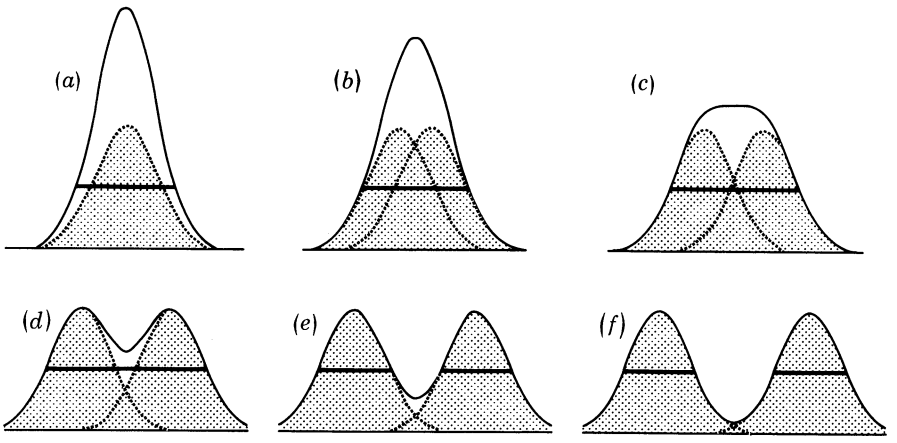


FIGURE 7. A tentative model of determination of c.v.p. locations in *janus* cells. The shaded areas under the broken lines represent pairs of independently established normal frequency distributions of a hypothetical c.v.p. determinant. All of these distributions are identical in size and form, but differ in degree of overlap: (*a*) total overlap; (*b–e*) incomplete overlap, with means separated by (*b*) 1σ; (*c*) 2σ; (*d*) 3σ; (*e*) 4σ; (*f*) 5σ. The solid curves represent the summed ordinates of the two frequency distributions. The heavy horizontal lines represent a threshold for c.v.p. formation, which is arbitrary but unchanging. For further explanation, see the text.

this entire process is consistent with the alternative idea that the second reference boundary was always there, even before the *jan*$^+$ to *jan* phenotypic substitution. The idea that more than one reference boundary can participate in determination of c.v.p. longitudes is not new, as it was proposed by Kaczanowska (1981) for c.v.p. positioning in another ciliate, *Chilodonella*.

Can one translate this intuition into a concrete model of control of c.v.p. cytogeometry? Figure 7 presents an initial attempt, which borrows heavily from Richelle & Ghysen's (1979) model of positioning of bristles in *Drosophila*. We assume that appropriate levels of positional information could bring about c.v.p. formation by generating a frequency distribution of a 'porogen' (cf. Richelle & Ghysen's 'chaetogen'). The positional system depicted in figure 3, whose reference boundary is located at the 1° oral axis and which slopes clockwise, generates one such frequency distribution. We now assume that an analogous second positional system with a reference boundary at the 2° oral axis and a counter-clockwise slope generates a second frequency distribution of 'porogen'. The two oppositely directed positional systems are distinct and independent, and hence can overlap without either summing or interfering with each other. But the 'porogen' concentrations that are determined by these two positional systems do sum, and act additively to promote c.v.p. formation, with a threshold level being necessary to stimulate a posterior basal body within a ciliary row to act as a generating site for a c.v.p. In wild-type cells the two positional systems interpenetrate fully, each wrapping around the entire cell, and the two distributions of the 'porogen' overlap totally (figure 7*a*). *jan* (or absence of

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

jan$^+$) might alter the characteristics of both positional systems, or change the way in which they are locally interpreted, so that the frequency distributions of 'porogen' that were formerly completely overlapping would begin to separate. This would first bring about a broadening of the territory in which c.v.ps can develop (figure 7*b*, *c*) and eventually cause a saddle to form (figure 7*d*). Once the level of the saddle falls beneath the c.v.p. threshold, 'skipped' rows would begin to appear within the centre of the c.v.p. arc (figure 7*e*). More extreme cases of separation (figure 7*f*) would approach two spatially separate frequency distributions of porogen, each specified by its own positional system and each responsible for a separate c.v.p. set. Each set would, on the average, be smaller than the unitary set produced when the two frequency distributions overlap completely (compare figure 7*f* and 7*a*). This condition, however, only represents one extreme of a continuum of states that are variable even in established *jan* clones.

This model can accommodate the major features of c.v.p. expression that were listed earlier, although a probability distribution would probably have to be substituted for the all-or-none c.v.p. threshold to account for the occasional asymmetric location of 'skipped' rows within the c.v.p. arc. The model does not, however, account adequately for the increase in proportion of 'skipped' rows that is superimposed on the broadened c.v.p. field in *jan* clones. The fact that when *jan* first comes to expression the c.v.p. arc widens before the proportion of 'skipped' rows increases suggests that *jan* may have more than one effect on c.v.p. cytogeometry. We nevertheless present this simple model as a suggestion of one way in which apparently overlapping fields might cooperate to generate a coherent pattern.

We might finally point out that the phenomena of large-scale patterning considered in this paper, and the discontinuities and overlaps that they appear to entail, are hard to envision in terms of diffusion gradients, especially in an organism such as a ciliate, which lacks cellular compartments.

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References (Frankel & Nelsen)

Aufderheide, K. J., Frankel, J. & Williams, N. E. 1980 Formation and positioning of surface-related structures in Protozoa. *Microb. Rev.* **44**, 252–302.

Beisson, J. & Sonneborn, T. M. 1965 Cytoplasmic inheritance of the organization of the cell cortex in *Paramecium aurelia*. *Proc. natn. Acad. Sci. U.S.A.* **53**, 275–282.

Child, C. M. 1903 Studies on regulation. I. Fission and regulation in *Stenostoma*. *Wilhelm Roux Arch. EntwMech. Org.* **15**, 187–237.

Elliott, A. M. & Bak, I. J. 1964 The contractile vacuole and related structures in *Tetrahymena pyriformis*. *J. Protozool.* **11**, 250–260.

Frankel, J. 1974 Positional information in unicellular organisms. *J. theor. Biol.* **47**, 439–481.

Frankel, J. 1979 An analysis of cell surface patterning in *Tetrahymena*. In *Determinants of spatial organization* (ed. S. Subtelny & I. R. Konigsberg) (37th Symposium of the Society for Developmental Biology), pp. 215–246. New York: Academic Press.

Frankel, J. 1982 (In preparation.)

Frankel, J. & 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. & Nelsen, E. M. 1982 (In preparation).

Frankel, J., Nelsen, E. M. & Jenkins, L. M. 1977 Mutations affecting cell division in *Tetrahymena pyriformis*, syngen 1. II. Phenotypes of single and double homozygotes. *Devl Biol.* **58**, 255–275.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Frankel, J., Nelsen, E. M. & Martel, E. 1981 Development of the ciliature of *Tetrahymena thermophila*. II. Spatial subdivision prior to cytokinesis. *Devl Biol.* (In the press.)

Jerka-Dziadosz, M. & 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.

Kaczanowska, J. 1981 Polymorphism and specificity of positioning of contractile vacuole pores in a ciliate, *Chilodonella steini*. *J. Embryol. exp. Morph.* (In the press.)

Lawrence, P. A. 1970 Polarity and patterns in the postembryonic development of insects. *Adv. Insect Physiol.* **7**, 197–266.

Lawrence, P. A. & Green, S. M. 1975 The anatomy of a compartment border. The intersegmental boundary in *Oncopeltus*. *J. Cell Biol.* **65**, 373–382.

Naitoh, Y. & Eckert, R. 1969 Ionic mechanisms controlling behavioral responses in *Paramecium* to mechanical stimulation. *Science, N.Y.* **164**, 963–965.

Nanney, D. L. 1966*a* Cortical integration in *Tetrahymena*: an exercise in cytogeometry. *J. exp. Zool.* **161**, 307–317.

Nanney, D. L. 1966*b* Corticotypes in *Tetrahymena pyriformis*. *Am. Nat.* **100**, 303–318.

Nanney, D. L. 1967*a* Cortical slippage in *Tetrahymena*. *J. exp. Zool.* **166**, 163–169.

Nanney, D. L. 1967*b* Comparative corticotype analyses in *Tetrahymena*. *J. Protozool.* **14**, 690–697.

Nanney, D. L. 1975 Patterns of basal body addition in ciliary rows of *Tetrahymena*. *J. Cell Biol.* **65**, 503–512.

Nanney, D. L., Chow, M. & Wozencraft, B. 1975 Considerations of symmetry in the cortical integration of *Tetrahymena* doublets. *J. exp. Zool.* **193**, 1–14.

Nelsen, E. M., Frankel, J. & Martel, E. 1981 Development of the ciliature of *Tetrahymena thermophila*. I. Temporal coordination with oral development. *Devl Biol.* (In the press.)

Ng, S. F. 1977 Analysis of contractile vacuole pore morphogenesis in *Tetrahymena pyriformis* by 180°-rotation of ciliary meridians. *J. Cell Sci.* **25**, 233–246.

Ng, S. F. 1979*a* The precise site of origin of the contractile vacuole pore in *Tetrahymena* and its morphogenetic implications. *Acta protozool.* **18**, 305–312.

Ng, S. F. 1979*b* Interdependence of cell cycle events in *Tetrahymena thermophila*: formation of contractile vacuole pore and fission gap in ciliary meridians. *J. Protozool.* **26**, 583–586.

Ng, S. F. & 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.

Ogura, A. & Machemer, H. 1980 Distribution of mechanoreceptor channels in the *Paramecium* surface membrane. *J. comp. Physiol.* **135**, 233–242.

Richelle, J. & Ghysen, A. 1979 Determination of sensory bristles and pattern formation in *Drosophila*. *Devl Biol.* **70**, 418–437.

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**, 243–251.

Tartar, V. 1967 Cell division after removal of the division line in *Stentor*. *Nature, Lond.* **216**, 695–697.

Tartar, V. 1968 Micrurgical experiments on cytokinesis in *Stentor coeruleus*. *J. exp. Zool.* **167**, 21–36.

Tokunaga, C. & Gerhart, J. C. 1976 The effect of growth and joint formation on bristle pattern in *D. melanogaster*. *J. exp. Zool.* **198**, 79–96.

Uhlig, G. 1959 Polaritätsabhängige Anlagenentwicklung bei *Stentor coeruleus*. *Z. Naturf.* **14b**, 353–354.

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

Whitman, C. O. 1893 The inadequacy of the cell theory of development. *J. Morph.* **8**, 639–658.

Wright, D. & Lawrence, P. E. 1981 Regeneration of the segment boundary in *Oncopeltus*. *Devl Biol.* (In the press.)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES