Genet. Res., Camb. (1976), **27**, *pp.* 213–226
With 4 plates and 3 text-figures
Printed in Great Britain

Laser microbeam induction of incomplete doublets of *Oxytricha fallax*

BY GARY W. GRIMES
Department of Zoology, Indiana University, Bloomington, Indiana, U.S.A. and Department of Biology, Hofstra University, Hempstead, New York

(*Received* 10 *October* 1975)

SUMMARY

Destruction by laser microbeam of oral and ventral cirral primordia for the future opisthe of 1/2 of a pre-fission doublet results in the permanent loss of ability to form oral and ventral cirral primordia on the irradiated half. This observation provides further evidence for the postulated 'determinative region' present on each ventral surface of these organisms. The opisthe of the irradiated doublet retains both original rows of marginal cirri, which are located mid-dorsally with respect to the remaining ventral surface. These two rows of marginal cirri are reproduced faithfully in all future opisthes, but are lost in most proter lines. The pattern of loss in proters involves migration of the extra rows of marginal cirri to the cell's right margin followed by aberrant morphogenesis and subsequent resorption. These results show that the reproduction of marginal cirri is at least partially dependent upon the presence of marginal cirri and their inherent structural characteristics as well as upon their position on the cell surface.

1. INTRODUCTION

The cortex of several ciliated protozoa has been subjected to a variety of descriptive and experimental analyses that have led investigators to rather diverse conclusions regarding the mechanisms of cortical pattern control. Sonneborn (1963, 1970) and Beisson & Sonneborn (1965) demonstrated the determinative role of pre-existing cortical organization on the orientation of new cortical structures, a phenomenon defined as cytotaxis. Implied in these accounts is an information system based upon an extensive molecular organization of the cortex and translated by the cortical organelles (see Sonneborn, 1970, 1974). However, Frankel prefers to emphasize the role of 'positional information', broadly defined in terms of gradients, without stipulation as to whether or not these gradients are diffusible or (more generally) represent a 'graded distribution of a property', and without precisely defining the reference points for these gradients (see Frankel, 1975).

Previous work on *Oxytricha fallax* has led to the postulation of a 'determinative region', inherited independently of the ciliature, and responsible for initiating the development of a complete set of cortical structures (Grimes, 1973*b*). Such an area is consistent with Sonneborn's cytotaxis but not with Frankel's positional

https://doi.org/10.1017/S0016672300016414 Published online by Cambridge University Press

information described in terms of diffusible gradients. The results presented here on experimentally induced incomplete doublets of *O. fallax* indicate that the apparent difference in interpretation is a matter of definition, and that the interpretations may be related. The mature ciliary structures and developing primordia may provide the reference points for gradients; i.e. one structure (or primordium) determines the site of another, and its morphogenetic fate.

2. MATERIALS AND METHODS

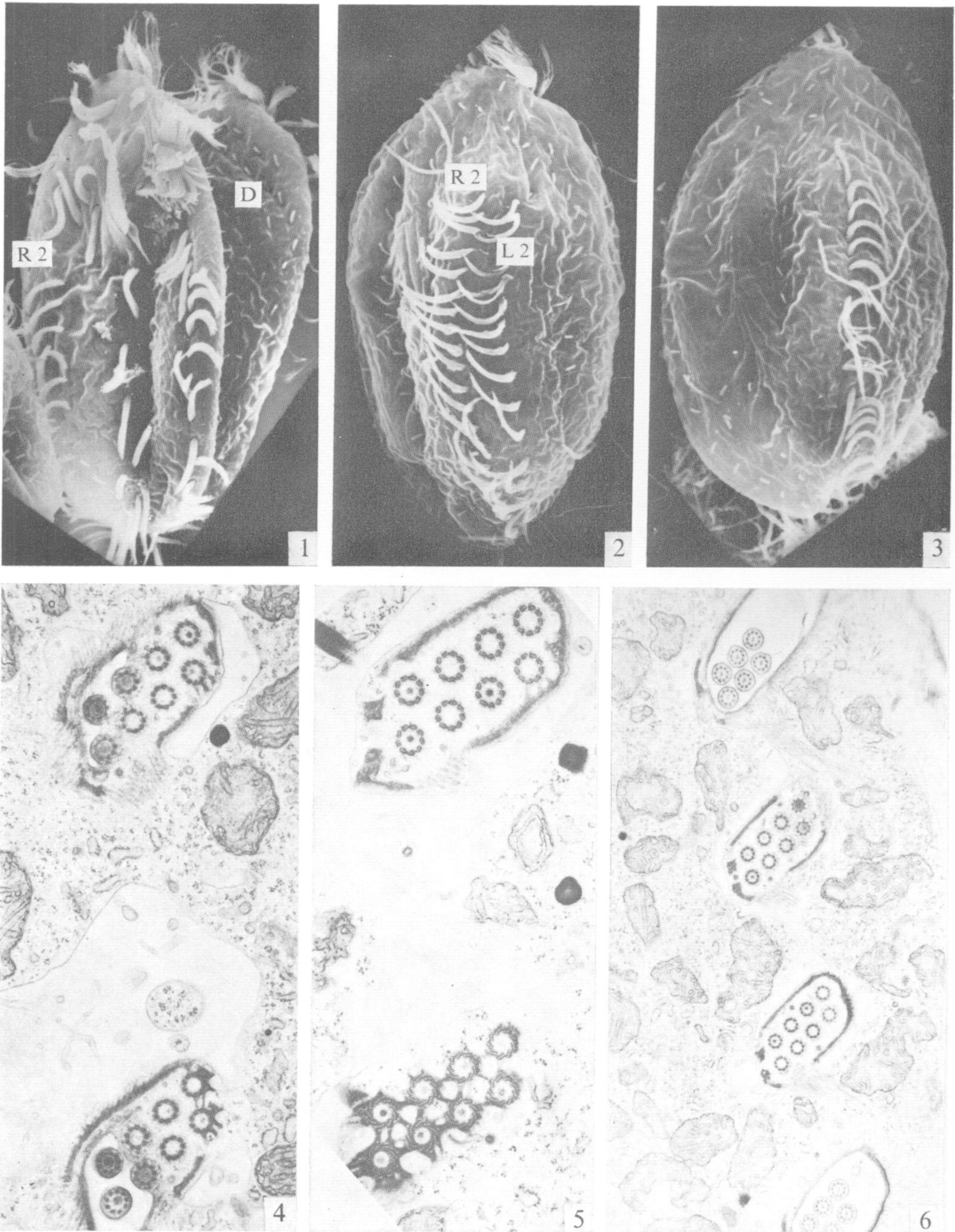
The methods have been described for culture, protargol staining, transmission electron microscopy (TEM) (Grimes, 1972, 1973*b*) and scanning electron microscopy (SEM) (Grimes & Adler, 1976). The laser microbeam technique will be published in detail elsewhere, but the principles are as follows. Log-phase cells were transferred to a solution of fresh 0·005% (w/v) Bismark Brown-Y in Cerophyl for a minimum of 15 min for selective vital staining. Individual cells were transferred to a rotocompression chamber and compressed until immobilized. The chamber was transferred to the stage of a phase-contrast microscope, and the cells observed under 100 × oil-immersion phase-contrast optics. Position and stage of cortical primordia and nuclei can be identified precisely by this method. Irradiation of selected areas was achieved by reflecting an argon-ion laser beam (TRW-B, Quantrad Corp., El Segundo, Calif.) down the optical path of the microscope, thus de-magnifying the entering beam to create a focused spot (at the specimen) approximately 2 μm in diameter. Several successive pulses, each of 40 μsec duration, were usually necessary to inflict the desired damage. Desired damage corresponds to the description of damage by Jerka-Dziadosz (1972, fig. 2C), using a UV microbeam apparatus; i.e. a 'blebbing' off of a small amount of cytoplasm and cortex, thus removing selected ciliary organelles. The total removal of ciliary structures and their attached kinetosomes has been verified by protargol staining and transmission electron microscopy (G. W. Grimes, unpublished). An individual cell may be irradiated as many successive times as is necessary to remove the desired number of cortical organelles.

After irradiation, the cells were removed from the compression chamber and returned to fresh cerophyl medium for growth. Pedigree analyses were performed, and cells were maintained until selected lines were fixed for either protargol staining or electron microscopy.

3. RESULTS

The reader is referred to previously published accounts of ventral, dorsal and doublet morphogenesis (Grimes, 1972, 1973*a*, *b*; Grimes & Adler, 1976) for details of these processes. All doublets used in this study were Type I (Grimes, 1973*a*, fig. 1), although results with Type II doublets would not be anticipated to be different since the only major difference between Type I and Type II doublets is in the arrangement of the *anterior* ciliary organelles.

https://doi.org/10.1017/S0016672300016414 Published online by Cambridge University Press



G. W. GRIMES

(*Facing p.* 215)

https://doi.org/10.1017/S0016672300016414 Published online by Cambridge University Press

(i) *Microbeam irradiation of pre-division doublets*

Five doublets were irradiated in the cirral streak stage, and all cells yielded the same results; the experimental scheme and subsequent cell lineage are depicted in Figs. 7 and 8. The entire oral primordium (OP) and ventral cirral primordia (VCP) of 1/2 of the future doublet opisthe were removed; no apparent lag in cellular division resulted from this treatment. (Comparable irradiation of pre-division singlets appears to inhibit cellular division, until the OP is reinitiated in its characteristic position; G. W. Grimes, unpublished.) The proter of each original irradiated doublet possessed the normal doublet phenotype, and gave rise to a normal doublet line upon subsequent divisions.

The results obtained from the opisthes of each original irradiated doublet were quite different. The immediate product of fission was a singlet cell which was abnormal in that it possessed a distinctive dorsal hump (Fig. 8; Plate 2, fig. 13). Subsequent analysis showed that the humped phenotype was due to the presence of two (or one, see below) rows of marginal cirri located mid-dorsally (Plate 1, figs. 2, 3; Fig. 8). Protargol staining, SEM and TEM analysis have demonstrated that the abnormally placed cirri have the same structure as ventrally located marginal cirri (Plate 1, figs. 4–6). These cirri are presumably the remaining portions of the cortex of the irradiated half of the original doublet opisthe; removal of all structures except the marginal cirral rows is equivalent to transplanting marginal cirral rows onto the dorsal surface of an otherwise normal singlet. These abnormally placed cirral rows will hereafter be termed dorsal marginal cirral rows. The means by which the two dorsal surfaces of the doublet regulate to a single (though abnormal) dorsal surface is unknown.

(ii) *Fate and reproductive pattern of incomplete doublets*

A thorough description of the morphogenetic events associated with the humped phenotype is most easily undertaken by separate consideration of primordia for future proters and opisthes.

PLATE 1

For detailed descriptions of all figures, see text.

Fig. 1. Scanning electron microscopy (SEM) view of a Type I doublet. R 2 and L 2 represent right and left rows of marginal cirri (see also Figs. 8 and 9). D, Lateral dorsal surface of doublet. ×675.

Fig. 2. SEM view of a humped cell containing two dorsal marginal cirral rows (R 2 and L 2 as above). ×700.

Fig. 3. SEM view of a humped cell containing only one dorsal marginal cirral row. ×700.

Figs. 4–6. Transmission electron microscopy (TEM) views through three different rows of marginal cirri on the same humped cell, showing structural homology.

Fig. 4. Right ventral marginal cirri. ×15000.

Fig. 5. Left ventral marginal cirri. ×20000.

Fig. 6. Dorsal marginal cirri. ×10000.

https://doi.org/10.1017/S0016672300016414 Published online by Cambridge University Press

All figures are presented as viewed from outside the cell; views are presented of both the dorsal and ventral ciliatures. The left and right margins of a cell are defined in terms of the ventrally located marginal cirral rows; therefore, the cell's left corresponds to the reader's left on dorsal views, whereas the reader's left corresponds to the cell's right when viewing the ventral surface of the organism.

Fig. 7. Diagrammatic representation of the experiment and results. The stippled area on the pre ÷ doublet represents the laser-damaged area (oral primordium and ventral cirral primordia removed). Marginal cirri are represented by bold dots, whereas dorsal bristle kineties are represented by dashed lines.

When structures are located on the dorsal surface, their left and right as defined by (and consistent with) the Rule of Desmodexy is opposite to the cell's anatomical right and left. Since most of the detailed descriptions presented below discuss the positions of structures on the cell surface and *not* details of ultrastructure, all references to left or right are based upon the *cell's* asymmetry.

(ii *a*) *Fate of dorsal marginal rows in opisthes of humped cells during division*

As indicated in Fig. 7 the opisthe of a humped cell receives, without exception, the dorsal marginal cirral rows and, therefore, the humped phenotype. Pedigree analysis of each experimentally induced deficient doublet (humped cell), one such pedigree carried for over 40 fissions, show that all possessed this common feature. In no case was reversion to the doublet cortical status observed, and cellular viability and fission rates appeared normal.

https://doi.org/10.1017/S0016672300016414 Published online by Cambridge University Press

Study of pre-fission morphogenesis in humped cells reveals that the dorsal marginal cirral rows (R 2, L 2) undergo development at the same time and latitudinal position as the ventral marginal cirri (Figs. 10–14). The dorsal marginal cirral primordia possess their characteristic asymmetry in ciliary length, which is exactly opposite to the asymmetry of the dorsal bristle primordia (Plate 2, fig. 12). Short ciliary buds (immature cilia) are present on the right side of the dorsal marginal cirral primordia, whereas the ciliary buds of dorsal bristle primordia are on the left side. The abnormal reproductive pattern of the dorsal bristle rows leads to the faithful transmission to opisthes of the dorsal marginal cirri.

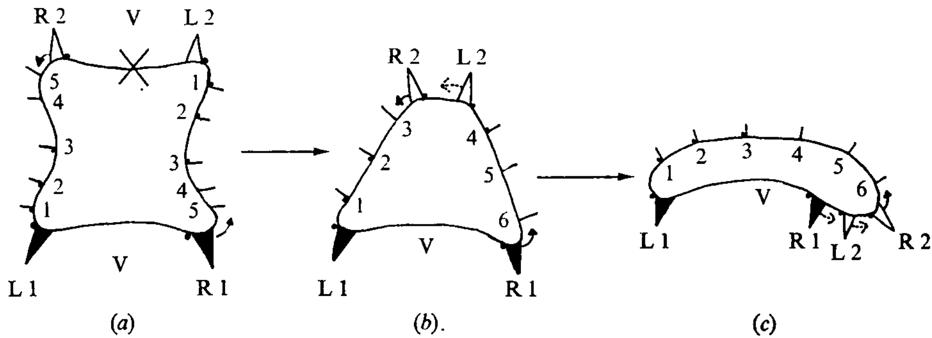


Fig. 8. Diagrammatic representation of major experimental results and structural relationships. View is from the posterior pole of the cell, thus ventral (V) is down (in (*b*) and (*c*)), and the viewer's right corresponds to the cell's right. Dorsal bristle kineties are illustrated as single lines and numbered from left to right (1–6), whereas marginal cirral rows are indicated as points and retain the same designations as in the pre-irradiated cell (R 1, L 1, R 2, or L 2). The dots at the base of structures indicate which rows form primordia, and the location (left or right) of the ciliary buds within primordia. Arrows on marginal cirri indicate which rows produce dorsal bristles, the direction of migration of the dorsal bristles, and whether the bristles are resorbed (dashed arrow) or retained (solid arrows). (*a*) Doublet cell prior to irradiation (the X indicates the laser-damaged ventral surface). (*b*) The humped cell created as a result of irradiation (opisthe of irradiated doublet). (*c*) Singlet cell (proter of a humped cell) possessing multiple rows of marginal cirri on the cell's right ventral margin.

The normal dorsal bristle morphogenetic pattern is illustrated in Fig. 9(i) (see Grimes & Adler (1976) for further details). Each of the three left dorsal bristle rows develops primordia at two A–P locations. Each of the primordia within the first two dorsal bristle rows gives rise to a single row of bristles in subsequent development, whereas each primordium within the third row splits, and gives rise to two new dorsal bristle rows (rows 3 and 4). New bristle rows 5 and 6 are derived from kinetosomes of the anterior-most portions of the two right ventral marginal cirral primordia (one set for the future proter, one set for the future opisthe) which subsequently migrate dorsally; all old bristles not contained within primordia are later resorbed. The observations on normal dorsal morphogenesis have led to the postulation of 'proliferative zones' (Grimes & Adler, 1976); i.e. latitudinal bands of kinetosomal proliferation.

https://doi.org/10.1017/S0016672300016414 Published online by Cambridge University Press

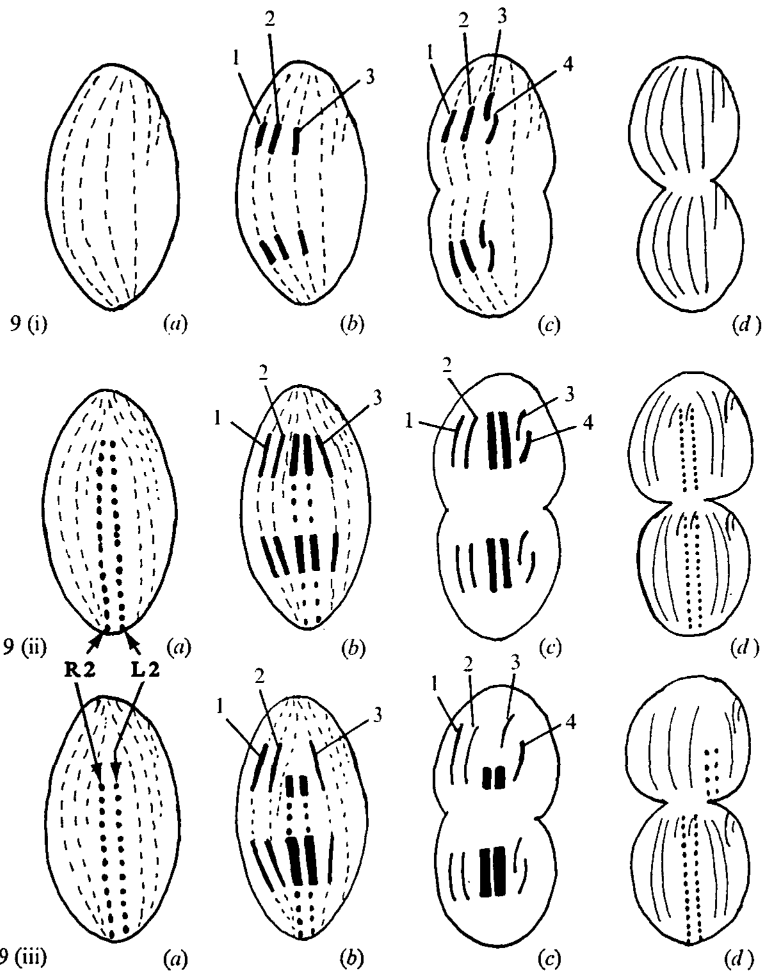


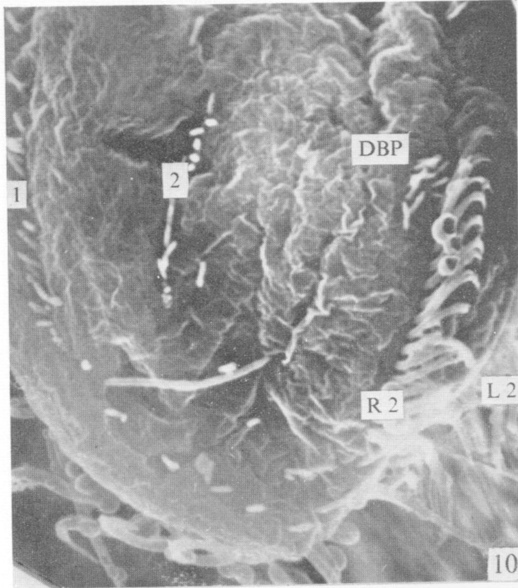
Fig. 9. Diagrammatic representation of prefission dorsal morphogenetic patterns in normal singlets (9 (i)) and humped cells (9 (ii), (iii)). Conventions as for Figs. 7 and 8.

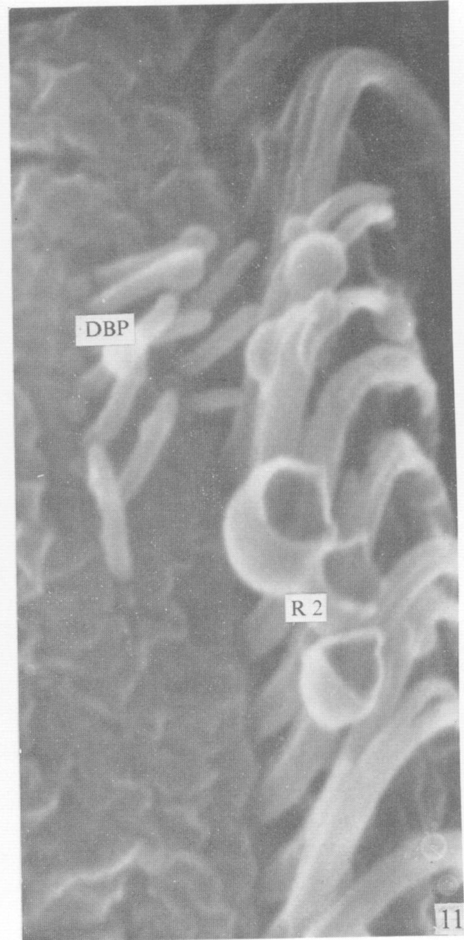
(i) Normal prefission dorsal morphogenesis. (*a*) Morphostatic cell containing six rows of bristles. (*b*, *c*) Development of four new rows (for proter and opisthe) within the three left dorsal bristle rows. (*d*) Late constriction stages showing the six new rows (two short rows on right side for both proter and opisthe develop from the right ventral marginal primordia).

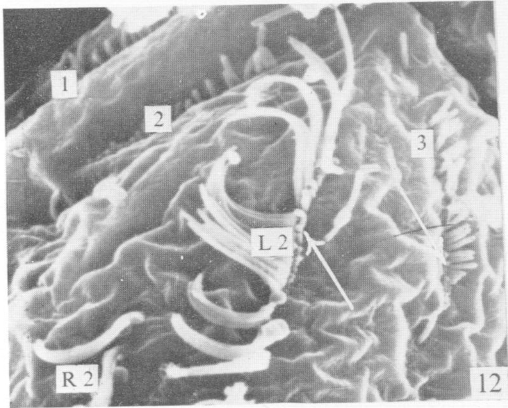
(ii) Morphogenesis leading to humped phenotype in both proter and opisthe. (*a*) The morphostatic humped cell. (*b*, *c*) The two left dorsal bristle rows (1 and 2) form to the left of the marginal cirri in both proter and opisthe, whereas the third dorsal bristle primordium forms to the right. (*d*) Short rows are contributed to the left side by row R 2, and to the right side by the right ventral marginal row (R 1).

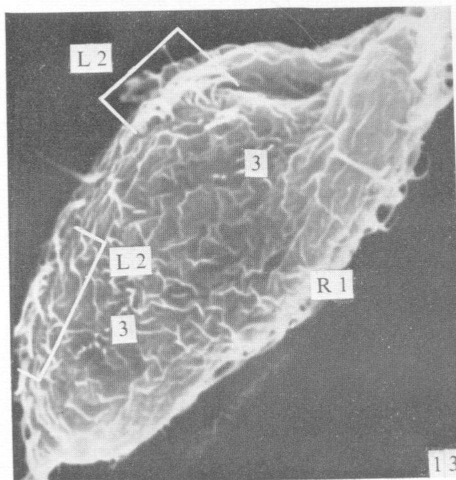
(iii) Morphogenesis leading to a displacement of the dorsal marginal cirral rows in the proter. The opisthe retains the humped phenotype. Neither dorsal marginal cirral row develops dorsal bristles for the proter (*d*), and slippage to the left of dorsal primordia occurs (row 3 in (*b*) and (*c*)). Dorsal marginal cirri are thus displaced in the proter, but maintained in the same position in the opisthe (*d*).

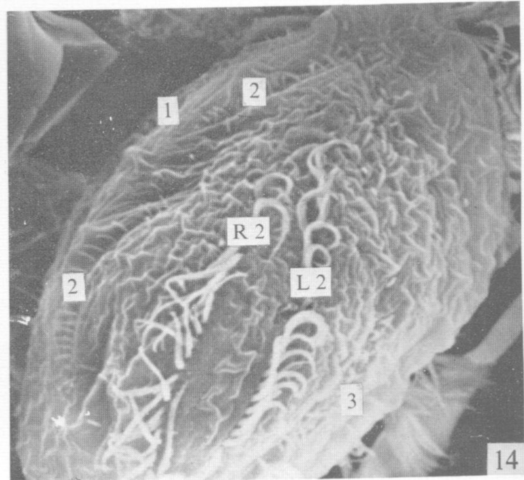
https://doi.org/10.1017/S0016672300016414 Published online by Cambridge University Press











G. W. GRIMES

(*Facing p.* 219)

https://doi.org/10.1017/S0016672300016414 Published online by Cambridge University Press

The morphogenetic pattern of dorsal bristles in humped cells is essentially the same as described above but with the following significant modifications.

(1) The two left-most dorsal bristle rows develop primordia as in the normal pattern (Fig. 9(ii); Plate 2, figs. 10, 12, 14), but the third bristle row (which normally forms primordia) does not undergo development, even though it is located to the left of the dorsal marginal cirri. Instead, the dorsal bristle row immediately to the *right* of the dorsal marginal cirral rows (which normally does not form primordia) develops a primordium (Fig. 9(ii); Plate 2, figs. 12, 13, 14) and splits into two rows at a later stage of morphogenesis.

(2) Dorsal bristle primordia for the future opisthe develop from *both* dorsal marginal cirral primordia (R 2, L 2) and the right ventral marginal cirral primordium (R 1) (Fig. 9(ii); Plate 2, figs. 10, 12). The dorsal bristle primordia from the *left dorsal* primordium (R 2) move to the left, thus reproducing short dorsal bristle rows to the immediate left of the dorsal marginal cirral rows. The dorsal bristle primordia of the *right* dorsal marginal cirral primordium (L 2) are resorbed, as are all old bristles not contained within primordia. The dorsal bristle primordia from the right ventral marginal cirral primordium migrate dorsally, thus reproducing short dorsal bristle rows at the extreme *right* of the dorsal surface. The result of this complex morphogenesis is that the dorsal marginal cirral rows (R 2, L 2) are maintained in the same mid-dorsal position in all successive opisthes.

(ii*b*) *Fate of dorsal marginal rows in proter lines*

Two possibilities exist for the fate of dorsal marginal cirri in proters of humped cells. The first of these is the transmission of the trait in the same position as in the opisthe. If the dorsal marginal cirral rows extend sufficiently far anteriorly to develop dorsal bristle primordia (see below), all dorsal bristle primordia and marginal cirral primordia (R 2, L 2, R 1) develop as described above for opisthes (Fig. 9(ii)). Both proter and opisthe then possess the humped phenotype, and each behaves independently as though it were an individually derived humped cell.

PLATE 2

Figs. 10, 11. Left (cell's) posterior dorsal primordia of prefission humped cell. Dorsal bristle primordia are located in two kineties, 1 and 2, and are differentiating from both marginal primordia, R 2 and L 2 (L 2 dorsal primordia are not shown). Fig. 10, × 1800; Fig. 11, × 9000; SEM.

Fig. 12. Anterior dorsal primordia of humped cell containing marginal cirral rows of unequal length; L 2 is longer and undergoing morphogenesis, R 2 is morphostatic. The three rows of dorsal bristle primordia are evident (1, 2 and 3) one (3) to the right and two (1 and 2) to the left of L 2. The arrows point to the ciliary buds in the primordia. × 2000; SEM.

Figs. 13, 14. Illustrations of morphogenetic stages of humped cells with anteriorly shortened dorsal marginal cirral rows.

Fig. 13. View of right dorso-lateral surface showing lengths of dorsal marginal cirral primordia (brackets) and position of dorsal bristle primordium (row 3). × 600.

Fig. 14. Dorsal view of a cell similar to that in Fig. 13. × 750.

https://doi.org/10.1017/S0016672300016414 Published online by Cambridge University Press

However, in the majority of cases, proters of humped cells eventually give rise to normal singlet lines, although several fissions are required for all cells within the line to assume the normal singlet phenotype. Morphogenetic processes leading to normal singlet proter lines are illustrated diagrammatically in Fig. 9 (iii); the dorsal marginal cirral rows are of insufficient length (anteriorly shortened) to reproduce the pattern faithfully in the proter. Evidence for this is indirect, since the humped cells must be killed at specific times in morphogenesis and the total number of humped cells available for analysis was limited, thus a complete morphogenetic sequence was not obtained. Several features substantiating the diagrammatic representation of Fig. 9 (iii) are illustrated in Fig. 12. Two dorsal marginal cirral rows are present, but the two are of unequal length; a primordium for the future proter is present in only one row (L 2, which extends anteriorly into the proliferative zone). This is a consistent feature of dorsal marginal primordia; marginal primordia form only if the cirri are located within the proliferative zone. Presumably, the resultant proter of the cell illustrated in Plate 2, fig. 12 would possess only one dorsal marginal cirral row, corresponding to the phenotype illustrated in Plate 1, fig. 3. If only a few dorsal marginal cirri are present within the proliferative zone, primordia form only as far anteriorly as the row extends, thus creating anteriorly shortened dorsal marginal cirral primordia (Fig. 9; Plate 2, figs. 13, 14). Additionally, dorsal marginal cirral primordia do not differentiate dorsal bristle primordia from their anterior ends unless the dorsal marginal cirral primordia extend anteriorly to the level where differentiation of dorsal bristles from marginal cirri normally occurs. In this instance, anteriorly shortened dorsal marginal cirral primordia (R 2, L 2) do not differentiate dorsal bristle primordia. These observations are incorporated into Fig. 9 (iii), but it is unknown if the third dorsal bristle primordial row indeed 'slips over', or is initiated to the left of the dorsal marginal cirri. The result, nevertheless, is that the proter possesses the two (or in some cases, one) dorsal marginal cirral rows displaced toward the cell's right ventral margin, as is the hump (Fig. 8; Plate 3, figs. 15–18). Proters of this type possess three *right* ventral marginal cirral rows (R 2, L 2, R 1) as a result of the displacement on the dorsal surface.

The migration of these marginal rows (R 2, L 2) on the dorsal surface toward

---

Plate 3

Figs. 15–18. SEM views of the same cell (reoriented by hand after initial photography) containing the supernumerary rows of marginal cirri displaced toward the cell's right margin.

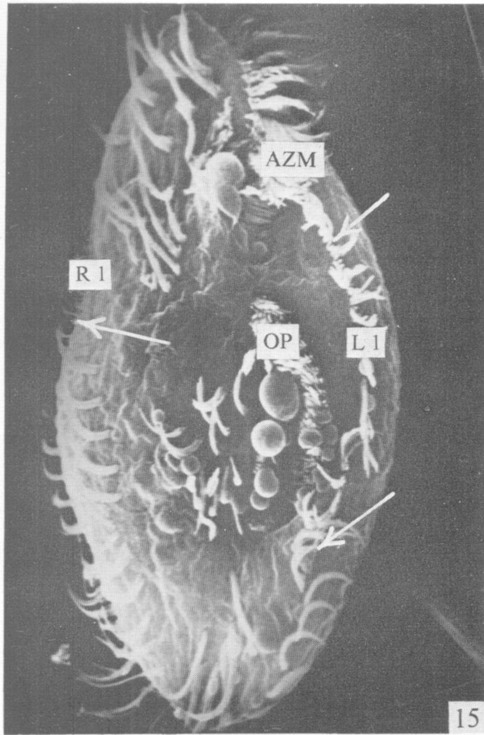
Fig. 15. Ventral view. Normal morphogenesis except for right posterior marginal cirri. × 750.

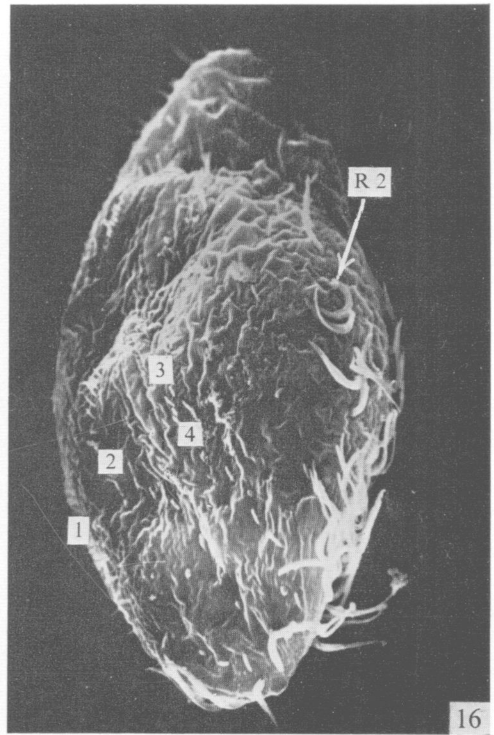
Fig. 16. Dorsal view showing displacement of hump toward the cell's right margin and the normal arrangement of dorsal bristle primordia. × 700.

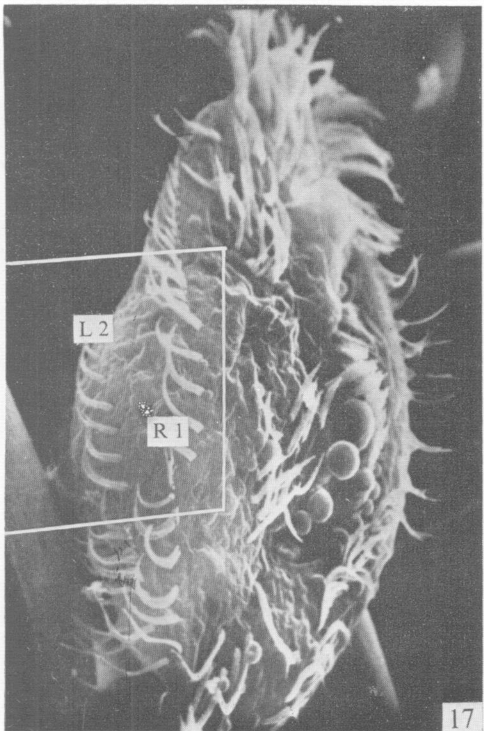
Fig. 17. View of right lateral margin indicating aberrant primordium formation in the marginal cirral rows (R 1 and L 2). × 750.

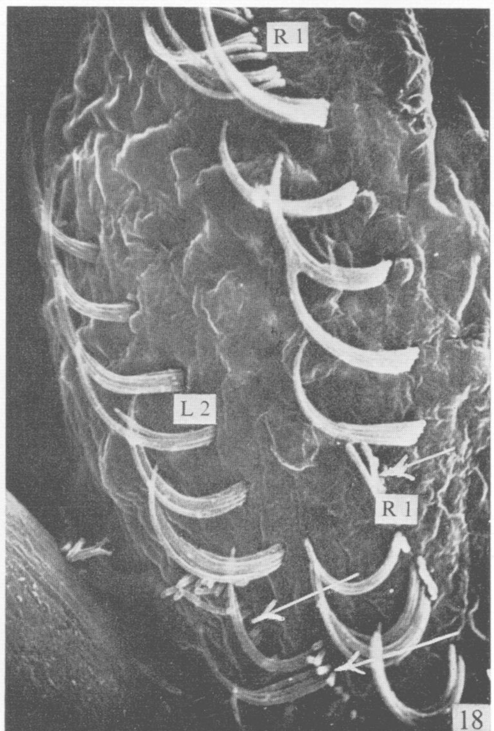
Fig. 18. Higher magnification (area within box on Fig. 17) illustrating irregularities of the posterior primordia (arrows). × 2400.

https://doi.org/10.1017/S0016672300016414 Published online by Cambridge University Press



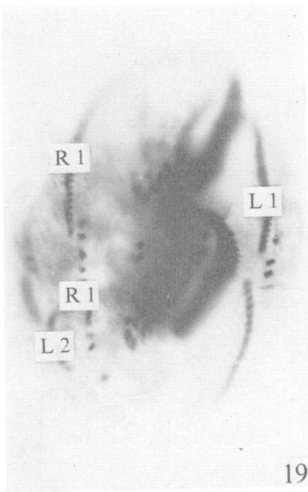


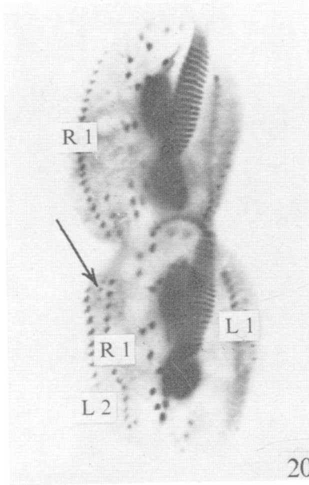


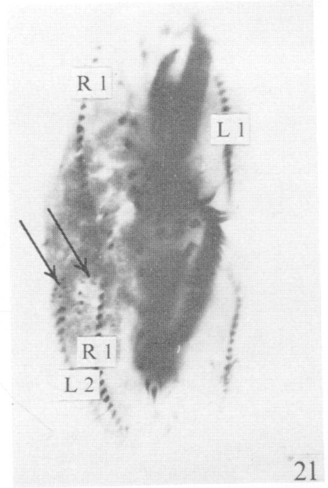


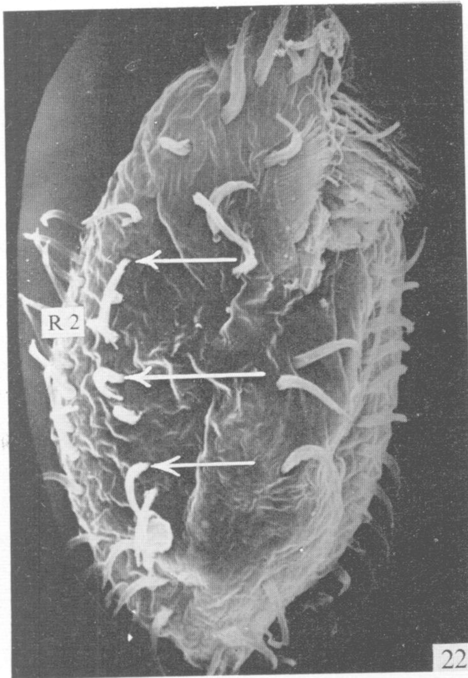
G. W. GRIMES (*Facing p.* 220)

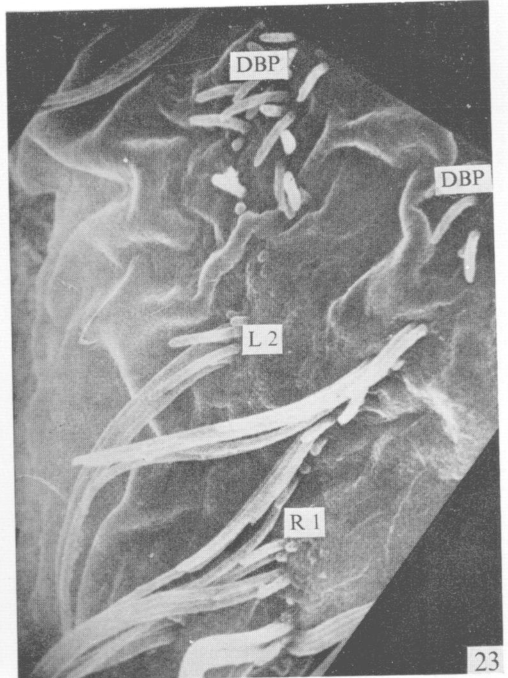
https://doi.org/10.1017/S0016672300016414 Published online by Cambridge University Press











G. W. GRIMES

https://doi.org/10.1017/S0016672300016414 Published online by Cambridge University Press

the cell's right margin is the first step which leads eventually to the normal singlet phenotype in proter lines. *All* cells derived from proters of this type will lose supernumerary marginal rows and possess only one right ventral row of marginal cirri. The remaining right marginal row is most often descended directly from the *left* dorsal marginal cirral row (the original *right* marginal row of the irradiated doublet half, R 2), thus replacing the two most mid-ventrally located rows (L 2, R 1) of marginal cirri (Fig. 8). The means by which the supernumerary rows located on the cell's right margin are lost is illustrated in Plate 3, figs. 15–23. Loss of cirral rows requires prefission morphogenesis; the two midventral-most rows of marginal cirri (L 2, R 1) form abnormal and lagging (in time) primordia (Plate 3, figs. 17, 18; Plate 4, fig. 19). The primordia of these two rows for the future opisthe are sparse and irregularly spaced, even though the spacing of mature marginal cirri of these rows within the proliferative zone appears to be normal. This abnormal formation of primordia within the midventral-most cirral rows is correlated with the presence of other marginal cirral rows toward the right lateral margin. The anterior portion of marginal cirral primordium R 1 is normal, and the extra rows (R 2, L 2) are absent (Plate 3, fig. 17; Plate 4, fig. 19). The posterior portion of the same row (R 1) is abnormal; extra rows R 2 and L 2 are located laterally at this position (Figs. 17, 19). Thus, there is no determination for morphogenetic regularity within a row, but rather, laterally oriented interactions among marginal cirral rows appear responsible for the induction of abnormal developmental patterns, perhaps simply by displacing rows L 2 and R 1 midventrally.

Such abnormal morphogenesis is not always observed. For example, Plate 4, figs. 20 and 21 illustrate the results of apparently normal morphogenesis within all rows. These fission products will possess intact supernumerary marginal cirral rows. Such normal morphogenesis results in cells with visibly normal extra marginal rows, whereas abnormal development yields cells with sparse and displaced marginal cirri, as illustrated in Plate 4, fig. 22. These displaced marginal cirri are resorbed during subsequent cortical morphogenesis.

The pattern of dorsal bristle development in cells with extra rows of right ventral marginal cirri is essentially the same as for normal singlets (Fig. 9(i),

---

Plate 4

Figs. 19–21. Protargol-stained preparations. In all cases, row R 2 is out of the plane of focus.

Fig. 19. Irregular morphogenesis of the right posterior R 1 and L 2 cirral rows. × 400.

Fig. 20. Late constriction stages showing irregular arrangement of cirri in row R 1, and dorsal bristle primordia derived from R 1 (at arrow). × 400.

Fig. 21. Mid-constriction stage showing visibly normal rows R 1 and L 2, both of which have differentiated dorsal bristles (at arrows). × 375.

Fig. 22. SEM of an interfission cell possessing a ventrally displaced sparce row of marginal cirri (examples of cirri at arrows). × 900.

Fig. 23. The production of dorsal bristle primordia (DBP) from the aberrant primordia of marginal cirral rows L 2 and R 1. × 3200.

https://doi.org/10.1017/S0016672300016414 Published online by Cambridge University Press

Plate 3, fig. 16, and description above). New dorsal bristle rows 1–4 develop on the dorsal surface as usual. The shorter 5th and 6th rows are contributed to the dorsal surface by the right-most marginal cirral primordium (Fig. 8). Due to the usual shorter length of the supernumerary rows (R 2, L 2), different marginal rows contribute the dorsal bristle primordia to the proter and opisthe (R 1 to the proter, R 2 to the opisthe). However, if any of the right marginal primordia (including supernumerary) are at the appropriate A-P level (within the proliferative zone), each marginal primordium differentiates dorsal bristles. This is true whether the marginal primordia are 'normal' (Plate 4, figs. 20, 21) or 'abnormal' as described above (Fig. 23). The fate of extra bristles is unclear, although they are presumably resorbed at a later stage of morphogenesis.

(iii) *Spontaneous occurrence of humped cells*

Careful observation of both log-phase and freshly excysted doublet cultures revealed the low frequency (higher among freshly excysted doublets) spontaneous occurrence of the humped phenotype. Pedigree and microscopic analyses of these cells show that they represent exact phenocopies of the experimentally induced humped cells, but their origin is unclear. Injury may account for their formation; for example, when doublets 'decay' into two singlets, pulling and tugging frequently lead to unequal cytoplasmic breakage, thus resulting in extra cortical structures on one decay product. The high propensity of freshly excysted doublets to decay therefore could account for the higher spontaneous frequency of humped cells in those cultures.

4. DISCUSSION

Irradiation of prefission doublets originally was attempted in order to test further the hypothesis of a 'determinative region' located on each ventral cortex of *Oxytricha fallax*; the results from these experiments are consistent with the presence of such a proposed cortical localization. Implicit in the hypothesis is that the region must duplicate at each division and once removed, it cannot be replaced spontaneously. It should therefore be possible to remove one such duplicated region in doublets, thus yielding one doublet and one singlet fission product; this was the experimental result. Furthermore, the irradiated region corresponded to the position of the primordial ciliary fields; thus removal of the primordia permanently (at least for the duration of these analyses) induced the loss of the capacity to form an oral primordium and ventral cirral primordia. The apparent correlation of positions between the hypothesized regions and primordia does not prove causal relationships, but, nevertheless, the correlation is noteworthy. To date, this has been the only irradiation performed on doublets of *O. fallax*, but more extensive analyses (e.g. lasing at different stages and removing different structures) are planned in order to analyse further the determinative region.

The observation that marginal cirri located on the dorsal surface of the organism reproduce as marginal cirri shows that the mere presence of a structure on the

https://doi.org/10.1017/S0016672300016414 Published online by Cambridge University Press

dorsal surface does not lead to the production of dorsal bristles as a result of morphogenesis. The abnormally placed marginal cirri presumably undergo reproduction in response to the normal pre-fission morphogenesis of the remaining ventral ciliature, but always give rise to new marginal cirri. These results are inconsistent with the gradient theories recently proposed by Jerka-Dziadosz (1974) and Frankel (1974, 1975), since these theories would lead to the prediction that any structure on the dorsal surface should produce dorsal ciliature as a result of morphogenesis (i.e. position determines developmental competence).

The reproductive pattern of the dorsal ciliature in humped cells is altered but in a predictable fashion (Fig. 9). The third left dorsal bristle row *does not* develop primordia when extra marginal cirri are located mid-dorsally. Rather, when the dorsal marginal cirral rows are present, the third dorsal bristle primordium develops to the *right* of these rows in a position which is normally morphogenetically inactive. In Frankel's view, it would appear that the underlying gradients of the dorsal cortex have been interrupted due to the presence of marginal cirri, thus implying, in essence, that these marginal cirri, *per se*, modify the cortical gradients.

The existence of two latitudinal proliferative zones suggests the presence of a generalized primordium-induction field, and any marginal cirri within these zones form primordia, regardless of their relative lateral positions to dorsal and ventral ciliature. However, the important points are that the marginal cirri must be within these zones to produce primordia, and that they produce marginal cirral primordia. Significantly, this is true for all marginal cirri as they migrate from their mid-dorsal to right-ventral position. The region through which these marginals move represents the area in which old dorsal bristles normally are resorbed during morphogenesis, yet the marginal cirri are not resorbed. Thus, either the marginal cirri upset the gradients determining areas of resorption, or resorption is area *and* organelle-type specific. It may be that a specific dorsal area is 'determined' to resorb only dorsal bristles within that area; i.e. other ciliary structures (e.g. marginal cirri) located within that cortical region will not be resorbed. Resolution of these alternatives will require further experimental results.

The pattern of dorsal bristle production from marginal cirral primordia is also consistent with a specific zonal localization of developmental potential, although no visible gradient of developmental potential appears to exist between the mid-dorsal and right ventral cortical areas. Any marginal primordium within this region differentiates dorsal bristle primordia as long as its anterior limit is at the appropriate A-P level. The *right* mid-dorsal row of marginal cirri (L 2, corresponding to the original *left* row of the irradiated doublet half) produces dorsal bristle primordia. This indicates that the row is not inherently determined *not* to produce dorsal bristle primordia, but rather that the left marginal cirral primordia of normal cells (i.e. with complete ventral cortical structures) fail to differentiate dorsal structures because of their relationship to other ventral structures. Implied in this specific statement is the more generalized idea that the existing ventral structures 'determine' primordial sites and developmental potential; i.e. the ciliary structures serve as reference points (Frankel, 1974) for the gradients (if they exist).

https://doi.org/10.1017/S0016672300016414 Published online by Cambridge University Press

The experiments presented herein on *O. fallax* doublets are analogous to those performed by Hanson (1962) on *Paramecium* doublets using a UV microbeam for gullet irradiation. More recent results on *Paramecium* (Hanson & Ungerleider, 1974) show that gullet-forming capacity can be maintained through a limited number of cell cycles even in the physical absence of the gullet. These results further extend the analogy to the hypothesized determinative region discussed for *O. fallax* since here, such a region is inherited through the cyst which contains no identifiable remnants of the ciliature. The proposed function of this region (supported by data presented here) is to determine the site of primordium initiation for a full set of cortical structures. Successive interactions and inductions among primordia (perhaps through gradients) then would lead to the mature intact arrangement of ciliary organelles. Such an initiation and organization function is also consistent with the 'cortical picking' experiment previously reported by Sonneborn (1963), and the 'organization area' proposed by Jerka-Dziadosz (1964).

Cytotaxis was originally defined by Sonneborn (1964) as the 'organizing and arranging of new cell structures under the influence of the pre-existing cell structures'. His discussions have assumed a broad definition of cell structure, both organellar and molecular, accompanied by an obvious recognition that cytotactic phenomena ultimately must be due to a subvisible molecular architecture (structure) (Sonneborn, 1970, 1974); the ciliary organelles thus would be visible manifestations of this underlying molecular structure. The redefinition of cytotaxis as 'structural guidance' by Frankel (1974) represents a severe narrowing of the concept as originally discussed. This was apparently done to yield an easier distinction between kinety-type reproductive patterns (production of new ciliary units within an existing row, Frankel's structural guidance) and developmental fields (Frankel's positional information). The implication from these definitions is that two totally distinct and mutually exclusive mechanisms exist for patterning the ciliate cortex. The data presented above indicate that this sharp line delimiting two mechanisms controlling cortical patterns in ciliates is misleading. They suggest, alternatively that a more generalized cytotactic mechanism may exist; i.e. the organization of unorganized structural subunits under the direction of the existing molecular structure of the cortex.

Various individuals have postulated regional biochemical localizations in the cortex (e.g. membrane, Beisson, 1972; Frankel, 1975) as the feature responsible for cytotactic phenomena and morphogenetic fields. The presence of such cortical differentiations is consistent with Frankel's gradients, *if* one accepts gradient as referring broadly to 'a graded distribution of a property' (Frankel, 1975, p. 41) instead of referring only to a *diffusion-based* gradient of that property. According to this broad definition of gradient, highly localized stable cortical differentiations can be viewed as steeply sloped gradients in molecular organization. Such gradients must not be completely rigid, but rather semi-fluid, since the entire cortical organization (gradients and reference points) must change (duplicate) at each cell division. A remaining significant question related to this view is 'What determines

https://doi.org/10.1017/S0016672300016414 Published online by Cambridge University Press

the detailed molecular organization of the cortex?', i.e. 'What are the reference points for the gradients?'

The data from the present study indicate that at least two self-perpetuating reference point systems are present within the cortex of *O. fallax*. The first of these is dependent upon the presence of ciliary organelles. Marginal cirri within the proliferative zone produce marginal cirri as a result of morphogenesis; i.e. these ciliary organelles affect the molecular organization of the cortex such that marginal cirri result at morphogenesis. The second of these, the determinative region, is a different reference point system, and its presence is *not* dependent upon the ciliature. These two systems should not be viewed as totally isolated and independent; through a series of morphogenetic interactions, the determinative region apparently possesses the capacity to organize the entire cortical architecture upon excystment. The mis-reproduction of supernumerary marginal cirri also indicates the capacity of these systems to interact and undergo regulation. One can envision morphostatic cortical structure as the end-result of the reciprocal interactions and induced changes in the molecular structure of the cortex occurring during morphogenesis. This view represents a close approximation to the 'nearest neighbour' hypothesis recently proposed by Sonneborn (1974); i.e. the final pattern is a result of successive multiple 'nearest neighbour' interactions.

This research was supported by grants PHS R01 GM 15410-05 to T. M. Sonneborn, PHS Genetics Training Grant no. GM 82 from Indiana University and NSF BMS74-21599 to G. W. Grimes, and funds from the Department of Biology, Hofstra University. The author gratefully acknowledges the technical assistance of Mr Joseph Adler, and critical readings of the manuscript at various stages by Dr Joseph Frankel, Dr Ruth V. Dippell, Mr Robert Hammersmith, and Mr Joseph Adler.

REFERENCES

Beisson, J. (1972). Déterminants nucléaires et cytoplasmiques dans la biogenèse des structures chez les protozoaires. *Année Biologique* **11**, 401–411.

Beisson, J. & Sonneborn, T. M. (1965). Cytoplasmic inheritance of the organization of the cell cortex in *Paramecium aurelia*. *Proceedings of the National Academy of Sciences, U.S.A.* **53**, 275–282.

Frankel, J. (1974). Positional information in unicellular organisms. *Journal of Theoretical Biology* **47**, 439–481.

Frankel, J. (1975). Pattern formation in ciliary organelle systems of ciliated protozoa. In *Cell Patterning. Ciba Foundation Symposium* **29**, 25–49.

Grimes, G. W. (1972). Cortical structure in non-dividing and cortical morphogenesis in dividing *Oxytricha fallax*. *Journal of Protozoology* **19**, 428–445.

Grimes, G. W. (1973*a*). Differentiation during encystment and excystment in *Oxytricha fallax*. *Journal of Protozoology* **20**, 92–104.

Grimes, G. W. (1973*b*). An analysis of the determinative difference between singlets and doublets of *Oxytricha fallax*. *Genetical Research* **21**, 57–66.

Grimes, G. W. & Adler, J. A. (1976). The structure and development of the dorsal bristle complex of *Oxytricha fallax* and *Stylonychia pustulata*. *Journal of Protozoology* (in the Press).

Hanson, E. D. (1962). Morphogenesis and regeneration of oral structures in *Paramecium aurelia*. An analysis of intracellular development. *Journal of Experimental Zoology* **150**, 45–68.

Hanson, E. D. & Ungerleider, R. M. (1974). The formation of the feeding organelle in *Paramecium aurelia*. *Journal of Experimental Zoology*, **185**, 175–187.

https://doi.org/10.1017/S0016672300016414 Published online by Cambridge University Press

JERKA-DZIADOZ, M. (1964). Localisation of the organisation area in course of regeneration of *Urostyla grandis* Ehrbg. *Acta Protozoologica* **2**, 129–136.

JERKA-DZIADOSZ, M. (1972). An analysis of the formation of ciliary primordia in the hypotrichous ciliate *Urostyla weissei*. II. Results from ultraviolet microbeam irradiation. *Journal of Experimental Zoology* **179**, 81–88.

JERKA-DZIADOSZ, M. (1974). Cortical development in *Urostyla*. II. The role of positional information and preformed structures in formation of cortical pattern. *Acta Protozoologica* **12**, 239–274.

SONNEBORN, T. M. (1963). Does preformed structure play an essential role in cell heredity? In *The Nature of Biological Diversity* (ed. J. M. Allen), pp. 165–221. McGraw-Hill.

SONNEBORN, T. M. (1964). The differentiation of cells. *Proceedings of the National Academy of Sciences, U.S.A.* **51**, 915–929.

SONNEBORN, T. M. (1970). Gene action in development. *Proceedings of the Royal Society of London* B **176**, 347–366.

SONNEBORN, T. M. (1974). Ciliate morphogenesis and its bearing on general cellular morphogenesis. *Actualités Protozoologiques* **1**, 337–355.

https://doi.org/10.1017/S0016672300016414 Published online by Cambridge University Press