Effects of cystment on cells of *Oxytricha fallax* possessing supernumerary dorsal bristle rows

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**SUMMARY**

Cells of *Oxytricha fallax* possessing cytotactically inherited supernumerary dorsal bristle rows can redevelop those dorsal supernumerary rows after cystment, even though supernumerary ventral cortical structures are permanently lost through cystment. Previous work has demonstrated: (1) that cystment involves a complete dedifferentiation of all ciliary structures—all cilia, basal bodies, microtubules and fibres; and (2) that all ventral ciliary structures arise from a single ciliary primordium during excystment.

These observations suggest the following conclusions. (1) The information for the redevelopment of supernumerary dorsal bristle rows during excystment is associated with some ultrastructurally unidentifiable molecular structure of the cyst cortex. (2) Cytotactic information for the development of cortical patterns is retained in at least two locations in the resting cyst; one location specifies the site of development of the ventral ciliature whereas the other specifies the location and pattern of the dorsal ciliature.

The patterning and organization of new structure in eukaryotic cells can, in certain cases, be controlled by existing structure. Sonneborn illustrated this in *Paramecium* two ways. (1) Cells with a doublet phenotype (individual cells with almost two complete sets of cortical structures) develop true to type and are inherited, sexually and asexually, independent of changes in nuclear genotype or fluid cytoplasm (Sonneborn, 1963). (2) Inverted rows of cilia and basal bodies maintain their inverted polarity during morphogenesis and cell division, again independent of changes in nuclear genotype or fluid cytoplasm (Beiss & Sonneborn, 1965). These observations led to formulation of the concept of 'cytotaxis'—the organization of new structure under the influence of pre-existing structure (i.e. the molecular architecture of the cell which includes visible structure) (Sonneborn, 1964, 1975). Cytotaxis thus represents an information system for pattern development and regulation which is not under direct nuclear control. Several examples of cytotaxis have been demonstrated.

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The hypotrichous ciliate *Oxytricha fallax* has proven to be especially useful in investigations of cytotaxis and pattern development because of its ability to undergo cystment processes. Encystment results in complete dedifferentiation of all cortical organelles and microtubules, yet upon excystment all cortical organelles and microtubules can redevelop within two hours (Grimes, 1973a, b). Thus, cystment allows a distinction between the role of visible organellar structure in development and patterning from other controlling elements.

Previous work also has shown that certain types of cytotactic information are retained in the cyst in the absence of visible cortical structure (Grimes, 1973b, c; Hammersmith, 1976b). In these cases the cyst apparently contains information determining the number of complete sets of ventral ciliature formed upon excystment. Thus homopolar doublets, heteropolar doublets and monster cells all encyst and excyst true to type (Grimes, 1973c; Hammersmith, 1976b). Although it is unclear exactly how the information for determining the number of ventral surfaces is stored in the cysts, presumably the information is associated with some localized molecular structure of the cyst cortex (Grimes, 1973c; Hammersmith, 1976b). These data led to the postulation of a determinative region associated with the ventral cortex which serves as the single initiation region of a ciliary primordium which in turn gives rise to a single complete set of the ciliature of a ventral surface (Grimes, 1973c).

This determinative region has boundaries (identifiable by microsurgery) and also is subdivisible; any part is capable of giving rise to a complete set of ventral ciliature (Grimes, 1973c, 1976; Grimes & Adler, 1978). Thus the determinative region possesses many of the properties of a classical embryonic field (Aufderheide, Frankel & Williams, 1980), except that it is cytotactically inherited, because if once lost it cannot be regained (Grimes, 1976).

Not all cytotactically inherited structure, however, is retained through cystment. Recent work has also shown that certain supernumerary cortical structures (marginal cirral rows) are propagated cytotactically during vegetative reproduction but are permanently lost during cystment (Grimes & Hammersmith, 1980). These observations suggest that two levels of cytotactic control exist in *Oxytricha fallax*: one is dependent on visible ciliature whereas the other is dependent on the ultrastructurally unidentifiable molecular architecture of the determinative region.

The question remains, 'Are any cortical components cytotactically inherited through cystment independent of other ciliary organelles and the hypothetical determinative region?'

To answer this question, we analyse the redevelopment of dorsal bristle rows after cystment.
MATERIALS AND METHODS

The stocks of cells used in this investigation were isolated from a lake near Bloomington, Indiana and tentatively have been identified as a subspecies of *Oxytricha fallax*. Several stocks with different mating types have been isolated; two of the stocks proved to be unique in that they formed abortive conjugants when mixed with mating reactive normal stocks of complementary mating type. Abortive conjugants fuse to form what initially appears to be a typical singlet cell without the intervention of any meiotic processes. These cells rapidly return to an asexual state if supplied with appropriate food, or can encyst if deprived of food.

Stocks of complementary mating type were grown in phosphate-buffered Cerophyl pH 7-4, allowed to deplete the food source, and mixed together. Abortive conjugant pairs usually formed within 2–4 h. Several hundred abortive pairs were selected by micropipette and pooled. Pairs were randomly divided into two groups; one for encystment, the other immediately fixed either for protargol staining or for SEM.

Encystment was induced in the first group by washing the cells through two changes of depleted Cerophyl and leaving them in a third change for encystment. Resting cysts were usually formed within 3–4 h, although considerable individual variation in encystment time was observed. Resting cysts were stored for approximately one week in depleted Cerophyl, then transferred to fresh medium inoculated 24 h earlier with *Enterobacter aerogenes*. Excystment occurred within approximately 3 h. Excysted cells were then fixed for either protargol or SEM, usually within 1–2 h of excystment.

Procedures for fixation and preparation of cells for SEM have been described previously (Hammersmith, 1976a, b; Grimes, 1976). Briefly, cells of the desired stage were fixed in glutaraldehyde–osmium mixture, dehydrated, and critical-point dried with CO₂. After drying, cells were mounted on stubs, coated with palladium–gold and observed in a Hitachi HHS-2R scanning electron microscope operated at 20 KV. Photographs were made on Kodak type 4127 film. Protargol silver-protein impregnation preparations were made by a modified Tuffrau technique (Tuffrau, 1969) to be described elsewhere.

RESULTS

Cortical morphology of vegetative cells

The cortical morphology of *Oxytricha fallax* has been described previously (Grimes, 1972); however, a brief review is necessary to understand data presented below. This subspecies of *Oxytricha fallax* is approximately 90–100 μm long and 30–40 μm wide, and the ventral surface is concave whereas the dorsal surface is convex.

The ventral ciliature consists of three major groups of ciliary structures:
Fig. 1. An excysted cell with typical ventral ciliature. AZM: adoral zone of membranelles; UM: undulating membranes; C: cirri of ventral surface; MC: marginal cirral rows. SEM: ×2300.

Fig. 2. Dorsal view of a typical singlet cell possessing six dorsal bristle rows (numbers and arrows). SEM: ×2100.

(1) the adoral zone of membranelles (AZM) and undulating membranes (UM) which comprise the feeding structures; (2) a series of cirri (C) positioned in three groups on the ventral surface; and (3) the marginal cirral rows (MC) which extend from anterior to posterior on both the left and right margins of the ventral surface (Fig. 1). All of these structures are compound ciliary organelles composed of basal bodies, cilia, microtubules and associated fibres.
**Supernumerary dorsal bristle rows in Oxytricha**

On the dorsal surface are six longitudinally arranged dorsal bristle rows (ciliary rows) (Fig. 2). Each individual dorsal bristle in a row consists of a pair of basal bodies (a short cilium arising from the anterior basal body; a ciliary stub from the posterior one) and associated microtubular and fibrillar components (Grimes & Adler, 1976).

During morphogenesis, dorsal bristle ciliary primordia are initiated in existing dorsal bristle rows 1, 2 and 3 (if those rows are within the proliferative zones; Grimes & Adler, 1976). New dorsal bristle row 4 arises from a splitting of developing row 3, and rows 5 and 6 are derived from the right marginal cirral primordia (MCP). The basal body pairs within each primordium spread out longitudinally to the anatomical right of existing rows and form the new dorsal bristle rows. All old dorsal bristles which are not incorporated into primordia are later resorbed, and this results in a dorsal surface of newly formed rows. Thus, direct structural continuity exists for the morphogenetically competent dorsal bristle rows 1–3.

**Origin of cells with supernumerary dorsal bristle rows**

Cells containing supernumerary dorsal bristle rows (Fig. 3) arise as a result of abortive conjugation. Initially, reactive cells of complementary mating type make contact in dissimilar locations and eventually form a typical conjugation fusion area on the anterior left side of one number, and the anterior right side of the other, with both members' ventral surface facing the substratum (Hammersmith, 1976a). In abortive conjugation the fusion proceeds rapidly posteriorly, and eventually the two cells completely fuse. Meiosis is not induced and cells return to their asexual status. As a result of fusion, almost two complete ventral and dorsal surfaces are incorporated on to the resultant cell. The ventral surface of the fused cell rapidly regulates (one to three divisions) to that of a typical singlet cell. (The ventral ciliature of the anterior division products of the fused cell usually regulates to that of a typical singlet cell in one cell division, whereas the posterior product usually requires two to three cell divisions to obtain the typical singlet structure.)

The number of supernumerary dorsal bristle rows present on the dorsal surface of fused cells ranges from one to five supernumerary rows, the exact number apparently a function of the exact position and degree of fusion. Occasionally a marginal cirral row also will be displaced and retained on the dorsal surface (Grimes & Hammersmith, 1980). Table 1 (a, b) provides the number of dorsal rows found on fused cells prior to encystment and their relative frequency.

If these cells are fed and allowed to divide instead of encyst, these supernumerary dorsal bristle rows initiate primordia in a precise pattern during morphogenesis if they are in the proliferative zone(s), and consequently redevelop in subsequent generations (Hammersmith, unpublished).
Fig. 3. Dorsal view of a pre-cystment fused cell possessing ten dorsal bristle rows.
SEM: × 2400.

Fig. 4. Dorsal view of an excysted cell possessing supernumerary dorsal bristle rows.
SEM: × 1800.

Supernumerary dorsal bristle rows have undergone development and been retained for at least ten generations after formation of the fused cells (Hammersmith, unpublished). Thus these supernumerary rows exhibit cytotoxic properties during divisional morphogenesis.

**Encystment and excystment of cells with supernumerary dorsal bristle rows**

When cells containing supernumerary dorsal bristle rows were allowed to encyst and then excyst, up to 74% (43 of 58 and 51 of 75 of cells observed) of
Supernumerary dorsal bristle rows in Oxytricha

Table 1a. Frequency of supernumerary dorsal bristle rows before encystment (protargol)

<table>
<thead>
<tr>
<th>Number of dorsal bristle rows</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency (no. of cells in a sample of 50)</td>
<td>0</td>
<td>5</td>
<td>7</td>
<td>20</td>
<td>17</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

100% had extra dorsal bristle rows. Mean no. of rows 9.04 ± 0.989. Typical singlet cells have six dorsal bristle rows.

Table 1b. Frequency of supernumerary dorsal bristle rows before encystment (SEM)

<table>
<thead>
<tr>
<th>Number of dorsal bristle rows</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency (no. of cells in a sample of 50)</td>
<td>2</td>
<td>8</td>
<td>12</td>
<td>13</td>
<td>12</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

96% had extra dorsal bristle rows. Mean no. of rows 8.68 ± 1.28. Typical singlet cells have six dorsal bristle rows.

the excysted cells possessed supernumerary dorsal bristle rows (Table 2a, b). In all cases, typical singlet ventral ciliature was observed in these excysted cells (75/75 protargol; 21/21 SEM) (Fig. 1). Thus supernumerary dorsal bristle rows can and do redevelop after encystment (Figs. 4, 5).

The mean number of dorsal bristle rows observed after encystment was reduced when compared to the pre-cystment controls (Table 1 vs. Table 2). The significance of this decline in mean number is not known; however, the maximum number of possible rows (10 dorsal bristle rows) for excysted cells was obtained in some cases. [Ten dorsal bristle rows would be the maximum number of rows expected after encystment, because in singlets dorsal bristle rows 5 and 6 are derived from marginal cirral primordia (MCP) and are not themselves morphogenetically competent. Thus, fused cells possess nearly two sets of dorsal bristle rows, but the left set does not have a competent MC row on its right edge and therefore apparently cannot give rise to two short dorsal bristle rows equivalent to dorsal bristle rows 5 and 6. The right set of the fused cell does possess a competent MC row on its right and therefore does give rise to two short dorsal bristle rows. The ten rows in fused cells would result from two sets of four morphogenetically competent rows plus two rows derived from the single right MCP during encystment.]

One of the earliest signs of excystment in typical singlets is the appearance of one contractile vacuole pore (CVP) on the left dorsal surface. During the excystment of fused cells, two contractile vacuole pores frequently were
Table 2a. Frequency of supernumerary dorsal bristle rows after excystment (protargol)

<table>
<thead>
<tr>
<th>Number of dorsal bristle rows</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency (no. of cells in a sample of 75)</td>
<td>24</td>
<td>12</td>
<td>24</td>
<td>10</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

68% had extra dorsal bristle rows. Mean no. of rows 7.46 ± 1.25. Typical singlet cells have six dorsal bristle rows. (75 out of 75 ventral surfaces possessed the typical singlet structure.)

Table 2b. Frequency of supernumerary dorsal bristle rows after excystment (SEM)

<table>
<thead>
<tr>
<th>Number of dorsal bristle rows</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency (no. of cells in a sample of 58)</td>
<td>1</td>
<td>14</td>
<td>13</td>
<td>22</td>
<td>7</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

74.1% had extra dorsal bristle rows. Mean no. of rows 7.396 ± 1.09. Typical singlet cells have six dorsal bristle rows. (21 out of 21 ventral surfaces possessed the typical singlet structure.)

observed approximately 45 degrees apart (as viewed from the pole). Furthermore, several recently excysted cells were observed to have two CVPs positioned between dorsal bristle rows (approximately 45 degrees apart) (Fig. 5). This suggests that other cortical features (e.g. CVPs) of the dorsal surface redevelop upon excystment. (Contractile vacuole pores are not ultrastructurally visible in the resting cyst.)

**DISCUSSION**

Cystment previously has been shown to result in complete dedifferentiation of visible ciliary components – all cilia, basal bodies and microtubules (Grimes, 1973a, b; Hammersmith, 1976b). Thus, information for redevelopment of a new ciliation must be retained within the ultrastructurally unidentifiable molecular architecture of the cyst cortex. The exact type of information retained in the cyst is unknown; however, previous results suggest that cysts of *Oxytricha fallax* possess information [determinative factor(s)] *only* for determining the number of complete sets of ciliation formed during excystment (Grimes, 1973c; Hammersmith, 1976b). Furthermore, Grimes demonstrated that the determinative factor(s) for the number of sets of ciliation could be subdivided by microsurgical procedures (Grimes, 1973c; Grimes & Adler, 1978; Grimes & L’Hernault, 1979). These determinative factors are similar in concept to those of classical embryonic fields (Aufderheide et al., 1980). These observations lead
Supernumerary dorsal bristle rows in Oxytricha

Fig. 5. Dorsal view of an excysted cell possessing two contractile vacuole pores (CVP). SEM: × 1600.

to a conceptualization of a subdivisible determinative region which gives rise to at least the ventral ciliature by serving as the single initiation region for a ventral ciliary primordium, this primordium, giving rise to a complete set of ciliature via intracortical communication among primordia (Grimes & Adler, 1978).

The question remains: are the dorsal bristle rows inherited through cystment independent of other cortical components and the hypothesized determinative region (originally proposed based upon data obtained from analysis of the ventral cortex only)? The answer to this question is a definitive ‘yes’ in Oxytricha. When cells possessing supernumerary dorsal bristle rows are encysted, then excysted, supernumerary dorsal bristle rows redevelop. This occurs even though extra ciliary components of the ventral surface located on the dorsal surface are permanently lost through cystment (Grimes & Hammersmith, 1980). These results with dorsal bristles are thus in contrast to those obtained for
supernumerary dorsally positioned marginal cirral rows (typically ventral ciliary structures) which do not redevelop after cystment even though they can be cytotactically inherited in asexual growth (Grimes & Hammersmith, 1980).

Based upon these results we suggest the existence of independent determinative factor(s) for the ventral and for the dorsal ciliary components: the determinative region associated with the ventral surface determines the region of initiation for one complete set of ventral ciliary structures; the determinative factor(s) for the dorsal surface (with the possible exception of dorsal bristle rows 5 and 6) determines the location and pattern of (supernumerary) dorsal bristle rows (six rows in typical singlets; more than six rows in fused cells). At least two possible alternatives exist for the mechanism determining the number of dorsal bristle rows which redevelop upon excystment. (1) Some ultrastructurally unidentifiable component associated with each morphogenetically competent dorsal bristle row present prior to encystment acts as a determinative factor(s) in initiating one new dorsal bristle row upon excystment. A prediction of this alternative is that no excysting cell would contain more dorsal bristle rows than it possessed prior to encystment. (2) A separate ‘determinative region’ for the dorsal surface exists which serves as a single initiation region for a complete set of dorsal bristle rows. If this alternative were true the fused cells with supernumerary dorsal bristle rows would be incomplete doublets which possess two dorsal determinative regions. Each of these regions would have the capacity to determine a complete set of dorsal bristle rows (excluding those rows which typically arise from marginal cirral primordia). A prediction of this alternative would be that occasionally an excysted cell would possess a greater number of rows than it possessed prior to encystment. Experiments to distinguish between these alternatives are being initiated.

(The observation that many of the excysting cells with supernumerary dorsal bristle rows possessed two contractile vacuole pores approximately 45 degrees apart suggests that the second alternative may be correct. However, CVPs might be determined completely independently from the dorsal bristle rows.)

Whatever the determinative factors are for the dorsal surface, they do not exhibit the totipotency of the ventral surface. A single ventral primordium is sufficient for development of a complete set of ventral ciliature, but a single dorsal primordium apparently is insufficient for development of a complete set of dorsal ciliature. The evidence for this is twofold. (1) The initiation and development of dorsal bristles has never been observed to occur in the absence of ventral surface morphogenesis. (2) If one or more dorsal bristle rows are eliminated by microsurgical procedures, the ventral ciliature contributes primordia to form new dorsal bristle rows (Grimes & Adler, 1978); however, the reverse apparently is never true. Dorsal primordia have never been observed to contribute to ventral ciliary structures. These observations indicate that the development of dorsal bristle rows is at least temporally dependent on the
morphogenetic activity of the ventral surface, and on whatever factor(s) is responsible for development of dorsal bristle rows upon excystment. Those factors, however, are unable to direct the formation of dorsal bristle rows de novo during vegetative or reorganizational morphogenesis.

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