Analysis of the effects of encystment and excystment on incomplete doublets of *Oxytricha fallax*

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**SUMMARY**

Singlet cells of *Oxytricha fallax* possessing supernumerary rows of dorsally located marginal cirri were encysted, excysted, and observed for retention of the supernumerary rows. Without exception, cells lost the supernumerary rows as a result of cystment processes. (Encystment previously has been shown to result in the total resorption of ciliature.) In contrast, supernumerary dorsally located marginal cirral rows develop true-to-type during prefission morphogenesis and thus are inherited from cell to cell. These (and other) observations suggest that at least two levels of cytotactic control of cell patterning are operative on the ciliate cortex; one is dependent upon visible ciliature whereas the other is dependent upon an as yet ultrastructurally unidentifiable molecular architecture.

**INTRODUCTION**

The role of pre-existing structure in the organization and patterning of new structure in ciliated protozoa was first documented thoroughly by Sonneborn (1963), who showed that the doublet phenotype of *Paramecium* was cortically inherited independent of changes in nuclear genotype or fluid cytoplasm. Later, Beisson & Sonneborn (1965) demonstrated that inverted rows of cilia on the *Paramecium* cortex reproduced true-to-type during cell division, again independent of changes in nuclear genotype or fluid cytoplasm. These observations led to the conceptualization of a unique inherited informational system operative within eucaryotic cells, a system based upon the pre-existing molecular architecture (structure) of the cell (i.e. cytotaxis; Sonneborn, 1964).

Since the original postulation of such an information system, similar results on inverted rows have been obtained on a closely related species, *Tetrahymena*, and have led to similar conclusions (Ng & Frankel, 1977). Additionally, cytotactic phenomena have been described in the hypotrich ciliates (Grimes, 1973\(a\), 1976; Hammersmith, 1976; Grimes & L'Hernault, 1979). Initially, the

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faithful retention of the number of sets of ciliary structures (in doublets) during sexual and asexual reproduction was described, as well as the retention of those sets (in doublets and monsters) during encystment and excystment processes. The latter observation is particularly important because encystment involves the complete resorption of ciliature (Grimes, 1973; Hammersmith, 1976). Thus, information for counting complete sets of ciliature must be retained not only in vegetative cells, but also in the resting cysts even in the absence of ciliary organelles.

Rows of compound ciliary organelles (marginal cirral rows) also have been shown to display several cytotactic characteristics. In one case it was shown that the rows of marginal cirri, when inverted on the opposite margin of the cell, underwent morphogenesis in an inverted orientation (Grimes & L’Hernault, 1979), an observation homologous to those on inverted rows of Paramecium (Beisson & Sonneborn, 1965) and Tetrahymena (Ng & Frankel, 1977).

Another demonstration of the cytotactic properties of marginal cirral rows involved the laser microbeam production of incomplete doublets (Oxytricha) which were essentially typical singlet cells possessing supernumerary rows of marginal cirri on the mid-dorsal surface (Grimes, 1976). (Typically, marginal cirral rows are located only on the left and right margins of the ventral surface.) These rows developed on the dorsal surface as marginal cirral rows, thus demonstrating the independence of positioning and morphogenetic fate of the marginal cirral rows.

The exact amount of information retained in the cyst remains unknown. Clearly, the cyst retains information at least for determination of complete sets of ventral ciliature (Grimes, 1973; Hammersmith, 1976). Yet the question remains, ‘Is each ciliary field inherited and determined independently in the absence of those organelles”? We address this question by analyzing the fate of dorsally located supernumerary cirral rows after encystment and excystment processes.

MATERIALS AND METHODS

All relevant techniques for the culturing, processing for SEM, and laser microbeam induction of incomplete doublets of Oxytricha fallax have been published previously (Grimes, 1973a, b, c, 1976; Grimes & Adler, 1978).

RESULTS

Incomplete doublets of Oxytricha fallax have been described in detail elsewhere (Grimes, 1976), but a brief description of their origin and development is important for understanding results and interpretations presented below. As illustrated diagrammatically in Fig. 1, incomplete doublets were induced experimentally by removing the primordia for the oral apparatus and a portion of the ventral ciliature on one half of a doublet cell. The resultant posterior fission product was an essentially typical singlet which possessed an additional two rows
of marginal cirri located mid-dorsally. These rows conferred to the cell a characteristic dorsal ‘hump’, which aided in the selection of live cells during asexual propagation (Fig. 3).

The supernumerary rows of marginal cirri underwent development during cell division as marginal cirri, and were passed faithfully to all posterior fission products (opisthes, Fig. 1). However, the anterior fission products (proters) usually reverted to typical singlet cells within a few fissions (Figs. 1, 2). Occasionally, the proter retained the ‘humped’ phenotype and this ‘humped’ cell behaved as all other ‘humped’ cells (Fig. 1). Therefore, by careful selection it was possible in some circumstances to obtain a large number of cells within a clone which possessed the supernumerary dorsally located marginal cirral rows.

Effect of encystment and excystment on the ‘humped’ phenotype
(a) Laser-microbeam-induced ‘humped’ cells

Six ‘humped’ cells were induced independently by the laser microbeam as illustrated in Fig. 1, and each appeared to be identical to all other ‘humped’
cells. However, the presence of supernumerary cirri on the dorsal surface could not be verified directly. Nevertheless, when each of these six ‘humped’ cells was encysted and excysted, each lost the ‘humped’ phenotype and all progeny cells were apparently typical singlets.

(b) Spontaneous ‘humped’ cells from excysted doublets

In order to obtain a larger sample of ‘humped’ cells within a clone so that the presence of supernumerary cirral rows within the clone could be verified directly, a ‘humped’ cell was isolated from a freshly excysted culture of doublets. [Even though doublets pass through cystment as doublets, occasionally ‘humped’ cells appear spontaneously in cultures of recently excysted doublets. The origin of these ‘humped’ cells is unknown, but they are identical to experimentally induced ‘humped’ cells (Grimes, 1976).] Unlike the cells above, the proters in this clone frequently retained the ‘humped’ phenotype and thus, after selection during several weeks, approximately 100 ‘humped’ cells within this clone were obtained. At this point, the clone was subdivided and portions were either encysted or fixed and prepared for SEM. SEM analysis revealed that individuals of the population were indeed ‘humped’ cells possessing supernumerary marginal cirral rows (Figs. 3–5). Additionally, the variability in the phenotype (i.e. length and number of rows) was similar to that described previously (Grimes, 1976).

The approximately 50 encysted ‘humped’ cells were later excysted. Without exception, all excysted cells possessed the standard singlet phenotype (Fig. 2); i.e. all cells lost the supernumerary dorsally located marginal cirral rows as a result of the encystment–excystment processes.

(c) Spontaneous ‘humped’ cells from abortive conjugation

‘Humped’ cells have also been obtained in another stock of Oxytricha fallax. This stock is characterized by frequent abortive conjugation which results in the fusion of the two cells (Hammersmith, unpublished observations). Within a few cell cycles, the ventral ciliature regulates to a standard singlet phenotype, but these cells frequently possess the ‘humped’ phenotype. Protargol staining and

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**Figures 2-5**

- Fig. 2. An excysted cell that was ‘humped’ before encystment showing the typical singlet dorsal ciliature. × 1000.
- Fig. 3. Lateral view of cell having supernumerary rows of dorsally located marginal cirri (arrow). × 1000.
- Fig. 4. Dorsal view of ‘humped’ cell having two supernumerary marginal cirral rows (arrows). × 1000.
- Fig. 5. Dorsal view of ‘humped’ cell having a single supernumerary row of marginal cirri (arrow). × 1000.
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SEM verify the presence of supernumerary dorsally located marginal cirral rows in these cells. These rows apparently originate by ‘slippage’ of cirral rows onto the dorsal surface during the abortive conjugation and fusion of the abortive conjugants and the exact details of the origin of these ‘humped’ cells currently is being investigated (Hammersmith, unpublished). ‘Humped’ cells of many independently derived clones have been encysted, and without exception, all cells lost the ‘humped’ phenotype as a result of these processes.

DISCUSSION

The results presented herein address specifically the question, ‘Is each cortical component (in this case, marginal cirral rows) inherited independently during encystment and excystment?’ The answer in the case of marginal cirri is unequivocally no; cells possessing dorsally located marginal cirral rows before encystment do not possess them after excystment. At other stages of the life-cycle, the cytotactic nature of marginal cirral rows previously has been demonstrated in two ways. (1) Dorsally located marginal cirral rows on ‘humped’ cells reproduce true-to-type during prefission morphogenesis, indicating that marginal cirral rows can be inherited cytotactically, as demonstrated by the fact that marginal cirral rows exhibit their typical morphogenetic manifestations even when in an abnormal position (Grimes, 1976). (2) If marginal cirral rows are inverted in a morphogenetically competent region, they undergo morphogenesis corresponding to the inherent polarity of the row (Grimes & L’Hernault, 1979), an observation homologous to that on Paramecium (and later Tetrahymena; Ng & Frankel, 1976) which serves as the definitive evidence for cytotaxis (Sonneborn, 1964; Beisson & Sonneborn, 1965).

However, marginal cirri undergo development only when they are present in morphogenetically competent latitudinal zones (‘proliferative zones’; Grimes & Adler, 1978). If marginal cirri (in Stylonychia) are absent in these proliferative zones, then the primordia for the marginal rows are developed from other ventral primordia (Grimes & Adler, 1978). Additionally, it should be re-emphasized that all ciliature is resorbed during encystment (Grimes, 1973b, c; Hammersmith, 1976) and that this process leads to the permanent loss of supernumerary rows of marginal cirri. Thus it appears that marginal cirral rows, when present, are cytotactic elements of the ciliature, but with the loss of the visible ciliature during encystment, this cytotactic information is also lost.

If information for determination of individual ciliary components of the ventral ciliature is not present in the cyst, then what information is retained in the cyst? Previous results indicate that Oxytricha fallax cysts possess information only for determining the number of complete sets of ventral ciliature to be formed during excystment morphogenesis. Several lines of evidence support this conclusion. (1) Complete doublets pass through encystment and excystment true-to-type (Grimes, 1973a). (2) Heteropolar doublets pass through encystment and
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excystment true-to-type (Hammersmith, 1976). (3) ‘Monster’ Oxytricha pass through encystment and excystment as ‘monsters’ (Hammersmith, 1976). (4) Equatorial transection of encysting doublet Oxytricha results in two structurally non-equivalent fragments, both of which excyst as complete doublets, a result indicating the subdivisibility of some determinative factor (Grimes, 1973a; Grimes & L'Hernault, 1979). (5) Longitudinal transection between the two ventral surfaces of encysting doublets results in two structurally equivalent fragments (each of which contains a complete set of ventral ciliature), both of which excyst as normal singlets indicating the separability of these determinative factors (Grimes, 1973a). These data indicate that the information present in the cyst is independent of the number and type of individual ciliary organelles on the encysting cell but rather is a function of some component of the subvisible molecular architecture present on a ventral surface. The data presented above led previously to the hypothesis of a ‘determinative region’ associated with each ventral set of ciliature (Grimes, 1973a; Hammersmith, 1976), the function of which was proposed to be to serve as the initiation region for a single complete set of ventral ciliary organelles. Subsequent development proceeding by intracortical communication among primordia (Grimes & Adler, 1978) would result in the final organization of the ciliary set.

Results presented herein are consistent with the existence and function of such a hypothesized determinative region; extra marginal cirral rows are propagated cytotactically during asexual cellular reproduction, but are not replaced during excystment because the structural continuity of the rows is lost during encystment. These observations illustrate that at least two distinct cytotactically propagated levels of control of cell patterning are operative on the cortex of Oxytricha fallax. The first of these levels is dependent upon the visible ciliature (e.g. marginal cirri; cytotaxis, sensu-stricto) whereas the second depends upon a currently ultrastructurally unidentifiable molecular architecture. Whether these two levels of cytotactic patterning control are governed by the same or different molecular mechanisms remains unknown.

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REFERENCES


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