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Cytoskeletal discontinuities in the cell body cortex initiate basal body assembly and oral development in the ciliate *Stentor*

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SUMMARY

My previous work has shown that disconnecting the oral apparatus of *Stentor* into two parts induces mass assembly of basal bodies on the ventral cell surface and thus initiates oral development. This operation severs the extensive microtubule tracts joining the oral membranelles at their bases. To determine whether basal body assembly and oral development are also induced by permanently disconnecting the longitudinal microtubule fibre tracts (mt fibre tracts) of the cell body cortex, I interposed a ring of inverted (heteropolar) cortex between the anterior and posterior halves of interphase stentors. When successful, this operation made it impossible for these fibre tracts to rejoin at the heteropolar boundaries and always induced basal body assembly and oral development in the graft complex. By contrast, tripartite homopolar graft complexes rarely initiated oral development; when they did, it was apparently in response to the presence of disproportionately small oral structures, which is the normal stimulus for oral development in *Stentor*. The mt fibre tracts of tripartite homopolar grafts also eventually became continuous.

These results support the hypothesis that permanent, extensive discontinuities anywhere within the cortical cytoskeleton can trigger basal body assembly and oral development. Since the onset of these processes is known to initiate cell division in *Stentor*, the results also suggest that development of discontinuities within the cortical cytoskeleton during interphase growth may be the endogenous stimulus initiating cell division in *Stentor*.

INTRODUCTION

In *Stentor*, cell division is initiated by a developmental process in which many thousands of basal bodies are assembled at a specific site on the ventral cell surface to form a primordium which develops into the oral apparatus of the posterior daughter cell; the anterior cell retains the original oral structures (Fig. 1). Understanding the mechanism that initiates oral development is therefore crucial for understanding how the cell cycle is timed in this ciliate.

Previous work has shown that the oral apparatus specifically inhibits oral development (Tartar, 1958*a*) and cell division (de Terra, 1977) during interphase unless it is disproportionately small in relation to cell body size. One obvious way in which the oral apparatus could do this is by producing a diffusible substance that inhibits basal body assembly on the ventral cell surface. To test this hypothesis, I used a glass needle to disconnect the oral apparatus into two parts which remained

Key words: cytoskeleton, basal body, oral development, cell division, ciliate, *Stentor*

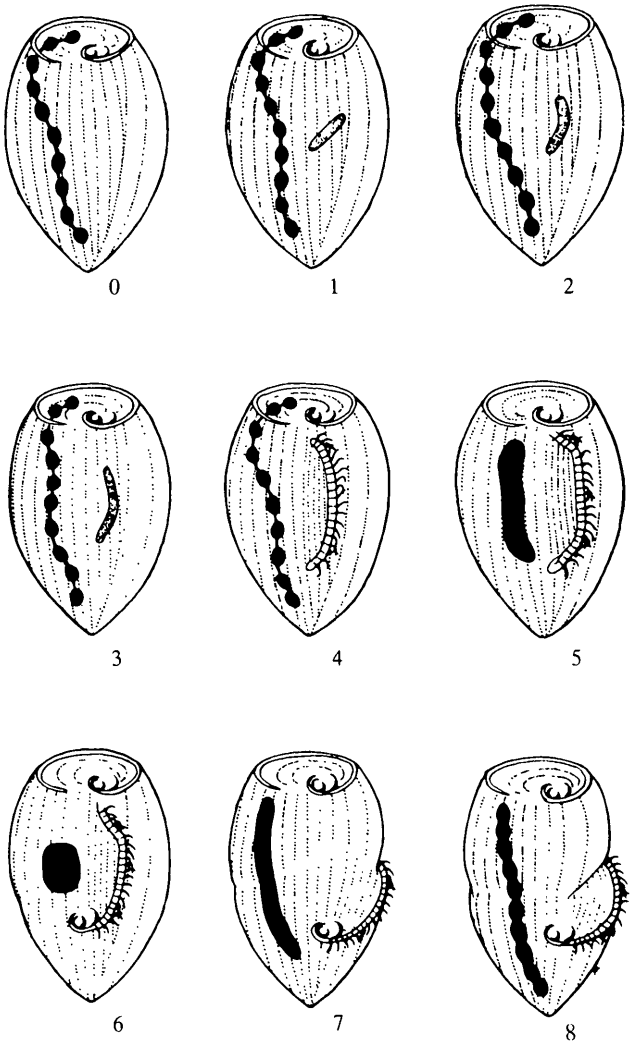

Figure. 1. Cell division in *Stentor*. Stages 1, 2, 3, oral primordium shown as stippled structure located posterior to gullet; stage 4, membranelles have formed; stage 5, macronucleus coalesces; stage 6, gullet forms; stage 7, macronucleus elongates; stage 8, macronucleus nodulates (from de Terra, 1978).

separate at the anterior end of the cell (see the accompanying paper, de Terra, 1985) and found that the operation initiated basal body assembly and oral development. This result is not consistent with the diffusible inhibitor hypothesis but rather suggests that the initiating factor is disconnection of the mt fibre tracts linking adjacent membranelles in the oral apparatus.

The experiments described here were designed to find out whether this phenomenon is a general one; i.e., whether permanently disconnecting the longitudinal mt fibre tracts of the cell body cortex also initiates oral development. I did this by interposing an inverted (heteropolar) ring of cortex between anterior

and posterior halves of interphase stentors. The operation, which prevents healing of the mt fibre tracts, indeed initiates oral development; when a homopolar ring of cortex is inserted instead, the tracts become continuous or mostly so within 24 h and oral development does not usually occur. These results suggest that the mechanism normally initiating cell division in *Stentor* might involve detachment of structural connections within the cytoskeleton as the cell grows.

MATERIALS AND METHODS

The organism

Stentor has already been described to some extent in the accompanying paper (de Terra, 1985). The cytoskeleton of the cell body cortex occupies a layer 3–5 μl deep just beneath the cell membrane in the longitudinal clear stripes. The most prominent structures are the contractile microfilament bundles comprising the myonemes (M-bands) and the mt fibre tracts of the ciliary rows. The microfilaments of the M-bands do not bind H-meromyosin (Kristensen, Engdahl-

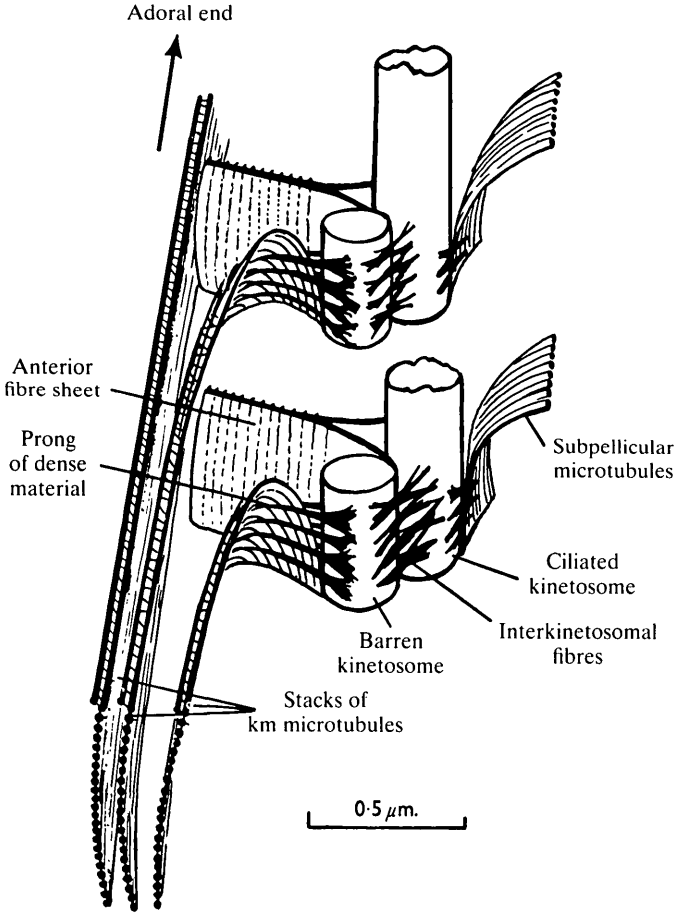

Figure. 2. Diagram showing part of a somatic kinety (from de Terra, 1970; courtesy of L. H. Bannister).

Nielsen & Rostgaard, 1974) and therefore do not seem to be actin but are more likely to resemble the microfilaments in the spasmonemes of vorticellid ciliates (Routledge, Amos, Yew & Weis-Fogh, 1976). Each ciliary row is composed of paired basal bodies, one of which gives rise to a cilium, the other to a ribbon of 20–22 microtubules (Bannister & Tatchell, 1968; Huang & Pitelka, 1973; Fig. 2). The microtubule ribbons arising from adjacent basal bodies in a ciliary row overlap to form a fibre tract; they are linked by cross bridges and can slide freely on each other.

Methods

Stentor was grown and microsurgery done as described previously (de Terra, 1985). Graft complexes were fixed in Champy's solution for 30 min and stained for 15 min with 0·1 % toluidine blue. They were then dissected in half, squashed in water on microscope slides and examined with

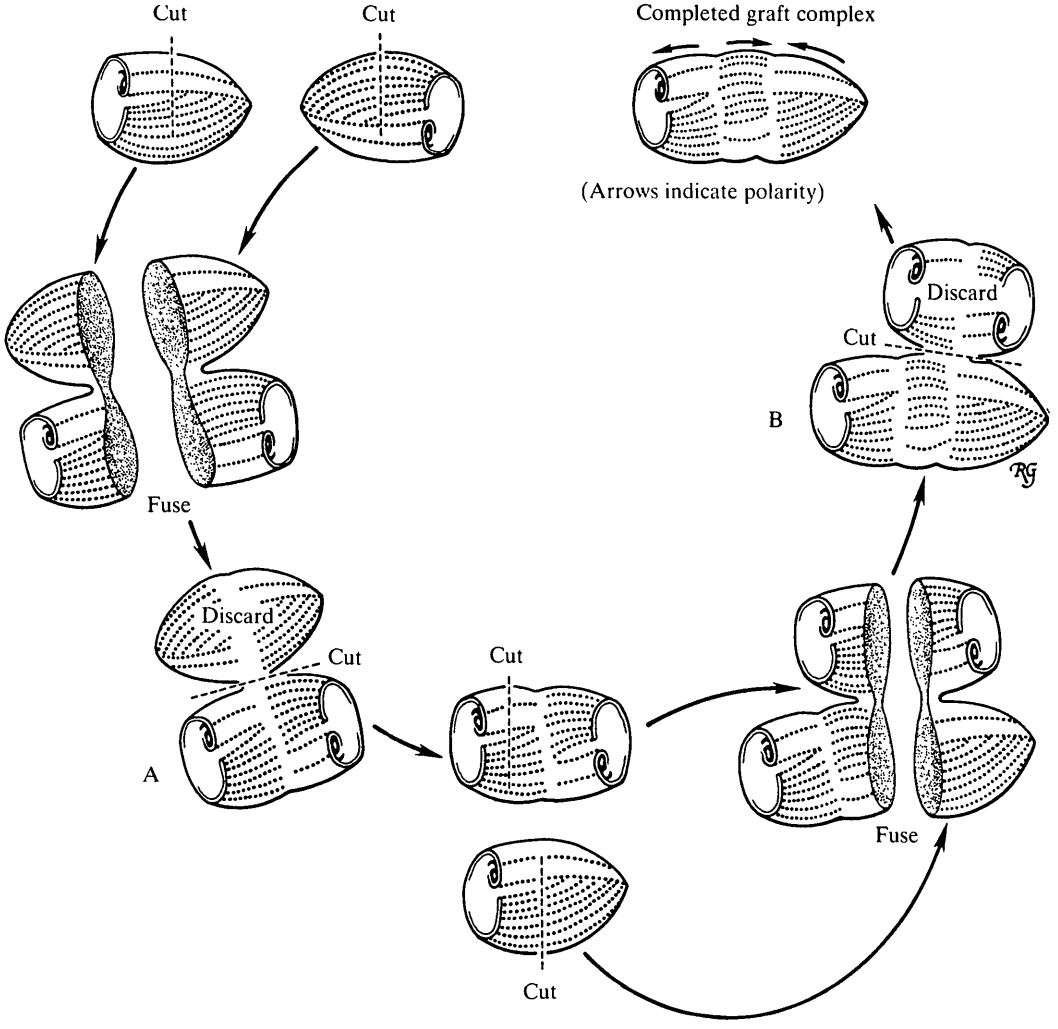

Figure 3. Procedure used in making graft complexes containing a middle ring of heteropolar cortex. The anterior half of a stentor is grafted to a second cell in heteropolar orientation (A). A cut is then made in the second stentor just below the line of heal and the posterior half of a third cell is added in homopolar orientation with respect to the first one (B). Most of the second stentor is removed during the operation so that it persists only as a disc separating the two main graft components.

Nomarski optics. The mt fibre tracts and basal bodies were stained with protargol using a technique slightly modified from that of Pelvat & de Haller (1979). The main differences consisted in post-fixing the bleached cells with 2·5 % glutaraldehyde in phosphate buffer (pH 7·3) for 15 min to keep them from disintegrating during passage through the alcohol series and in using six applications of 5 % collodion (instead of dipping once in 1 % collodion) to keep the cells from falling off the slides during staining and later processing. Because the degree of staining varied widely from cell to cell on a single slide, not all experimental cells could be examined.

RESULTS

Preparation of graft complexes

The operation used to produce graft complexes with heteropolar middle rings is shown in Fig. 3. Two stentors are grafted head-to-head creating a heteropolar doublet which is transferred to Peters' medium. Within 30 min, the posterior half of a third stentor is added by making a tail-to-head graft. This operation generates two heteropolar boundaries, one between the anterior and middle graft components and a second between the middle and posterior ones. Most of the middle graft component was removed during the operation so that it persisted only as a thin ring of cortex. Controls were prepared by making two consecutive head-to-tail grafts using the general technique shown in Fig. 3 so that the resulting graft complexes had only homopolar boundaries. Since the endoplasm of *Stentor* mixes freely after grafting while the cortical cytoskeleton stays in place (de Terra, 1973), these grafts were in effect cortical.

Examination of heteropolar tripartite graft complexes

Fifty graft complexes containing a middle ring of heteropolar cortex were made, placed at 25–26 °C, and examined for primordia 6–8 h after the operation. Those which had not formed primordia were left overnight at 8 °C to prevent them from doing so, a procedure which does not affect the cells, returned to 25–26 °C the next morning at 9 a.m. and observed at intervals during the day. All graft complexes were fixed, bisected, stained with toluidine blue and squashed on slides for examination of their stripe patterns, either at the time when they had formed primordia or at 6 p.m. of the second day of the experiment if they had not done so. It was not difficult to determine which graft complexes had tripartite striping as apposed regions of heteropolar striping appear discontinuous (Figs 5 & 7), while in apposed regions of homopolar striping the clear stripes at the boundaries join or interpenetrate (Figs 4 & 6).

Of 39 graft complexes which formed primordia on days 1 and 2, 29 were found to be completely tripartite and 10 were largely so ($\frac{1}{2}$ or more). The 11 graft complexes which did not form primordia also had no heteropolar boundaries, presumably because the middle ring was incomplete and the two homopolar sections were able to rejoin. The behavior of km fibres at heteropolar boundaries as shown by protargol staining (Fig. 7) reflected the discontinuous appearance of the stripe pattern (Fig. 5). Primordia appeared at all loci of stripe contrast (1sc), confirming

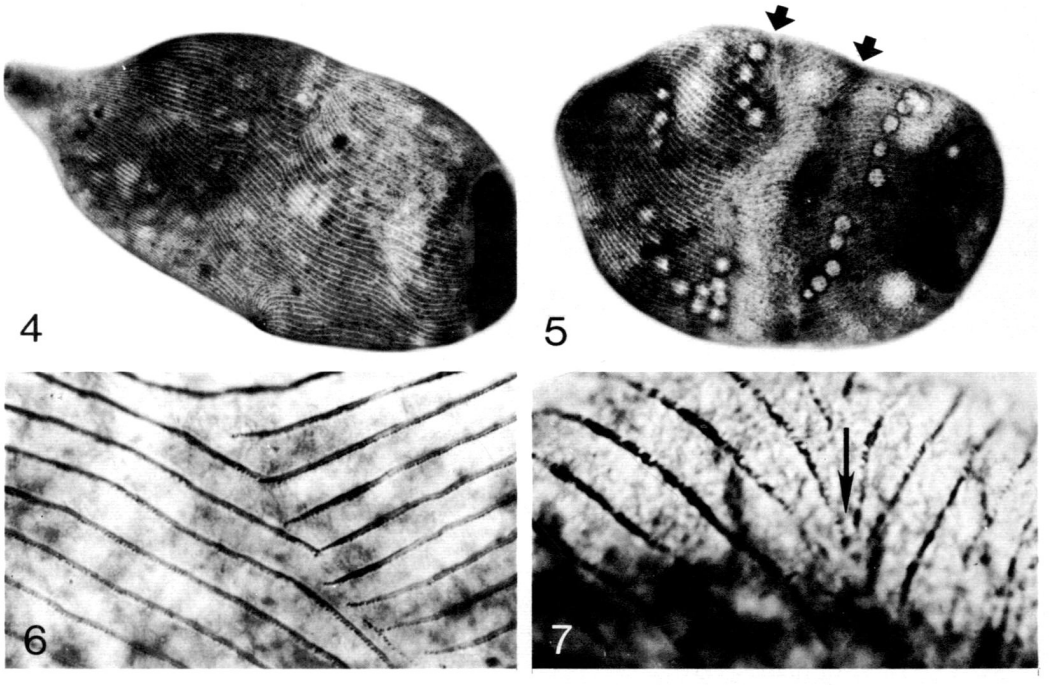

Figure 4. Tripartite homopolar graft complex 4 h after operation. Cortical striping is becoming continuous (best seen in lower centre). Living cell in microcompression chamber. × 230.

Figure 5. Central ring of heteropolar cortex with discontinuous stripe pattern 4 h after operation to produce a tripartite graft complex. Oblique striping can be seen at top centre between arrows demarcating boundaries of central ring. Living cell in microcompression chamber. × 230.

Figure 6. Homopolar boundary 4 h after operation with ciliary rows joining and interpenetrating. Protargol stain. × 2900.

Figure 7. Heteropolar boundary 4 h after operation. Arrow points to site where two heteropolar ciliary rows approach each other but do not meet. Protargol stain. × 3700.

previous work by Tartar (1961) and Uhlig (1960) on the role of such loci in determining the positions at which primordia will develop. Examination of cells bearing early primordia indicated that a primordium first appeared at the lower 1sc but that a second soon appeared at the upper one and the two became synchronized by stage 4 as would be expected from the work of Tartar (1961) on induced primordia. Since some of the cells examined were in late stages of oral development, it cannot be conclusively stated that all cells behaved in this way.

Examination of homopolar tripartite graft complexes

Only 7 of 74 homopolar tripartite graft complexes observed for 2 days formed

primordia. Of the 74, 69 had continuous or mostly continuous clear striping. The other 5 had a more chaotic stripe pattern with clear stripes joining and interpenetrating in blocks. Microscopic observation and protargol staining revealed that the mt fibre tracts gradually became continuous with time by joining and interpenetration (Figs 4 and 6). The 7 graft complexes bearing primordia all had an oral apparatus that was disproportionately small in relation to cell body size; this appears to be the normal trigger for primordium formation (de Terra, 1969; 1977).

DISCUSSION

These experimental results show that graft complexes with heteropolar middle segments undergo oral development and that their mt fibre tracts do not rejoin at the graft boundaries. The ciliary rows at heteropolar boundaries are asymmetric because the mt fibre tracts always lie to the (cell's) right of the basal bodies. Observations on two other ciliates, *Paramecium* (Beisson & Sonneborn, 1965) and *Tetrahymena* (J. Frankel, personal communication) have shown that km fibres in heteropolar orientation do not rejoin. It is therefore not surprising that the same result was found in *Stentor*. By contrast, graft complexes composed of three homopolar segments rarely initiated oral development and their mt fiber tracts eventually became continuous. Tartar (1958*b*) found that cell sectors from regenerating stentors resorb their primordia when grafted to morphostatic cells; the middle ring of the graft complex is therefore not likely to have induced oral development in the two larger morphostatic components on either side of it. The results described in this paper are therefore consistent with the hypothesis that extensive discontinuities in the mt fibre tracts trigger mass assembly of basal bodies and oral development at the primordium site. There are two other experiments supporting this hypothesis. The first showed that basal body assembly and oral development occur when the oral apparatus is cut into two parts which cannot rejoin (see accompanying paper; de Terra, 1985). This operation severs the thick

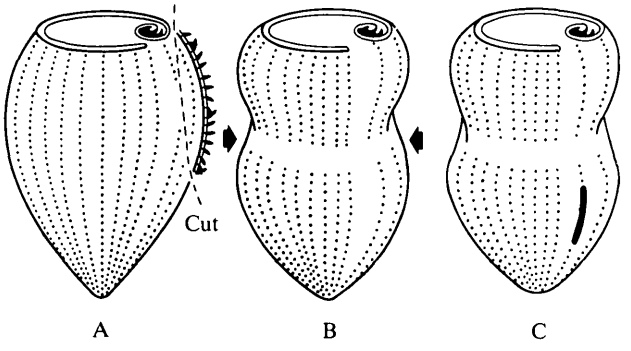

Figure 8. (A) Oral primordium excised from stentor in middle stage 3 of division (membranelles half grown out); (B) Cleavage fails but microtubule fibre tracts disconnect at furrow line; (C) 4–6 h later an oral primordium appears at the lower locus of stripe contrast (from de Terra, 1975).

tracts of microtubules (basal fibre) connecting the bases of the membranelles (Randall & Jackson, 1958). In the second experiment, I excised the oral primordium from cells in stage 3 of division without removing a significant amount of cytoplasm. Cleavage sometimes failed in these cells because of damage to the incipient furrow line but the mt fibre tracts of the cell body disconnected at this line as they normally do during division (de Terra, 1969; Fig. 8). Since the size, surface area and volume of the cell were unchanged, one might have expected the cell to attempt division again. However, these cells did not form an oral primordium immediately at the upper locus of stripe contrast (primordium site), suggesting that disconnection of the mt fibre tracts had removed the stimulus to division from the anterior part of the cell; instead, as in the present work, a primordium first appeared at the posterior locus of stripe contrast, as if disconnection of the mt fibre tracts at the furrow line had triggered oral development, presumably because the posterior half-cell was no longer attached to the oral apparatus by km fibres.

The fact that extensive discontinuities in the mt fibre tracts of the cell body seem to trigger basal body assembly at the primordium site and oral development no matter where they are produced raises the interesting possibility that the stimulus normally initiating these events in *Stentor* also involves changes caused by detachment or deformation of structural connections in the cortical cytoskeleton caused by interphase growth. Possibly, expansion of the cell membrane during interphase eventually moves the basal bodies which are connected to it far enough apart to disconnect or deform links between the individual microtubule ribbons of the mt fibre tracts. Alternatively, torsions and strains arising during interphase growth could deform or detach the microfilament bundles connecting the oral apparatus and the perforated sheet of microfilaments to which the basal bodies of the ciliary rows are attached (L.H. Bannister & E.C. Tatchell, unpublished observations).

However, even if cytoskeletal discontinuities developing as a result of interphase growth do initiate division, the control mechanism must be more complex than this. When two stentors are joined together tail-to-tail, oral development can be induced in one graft component by excising the oral apparatus of the other (Tartar, 1961) although the mt fibre tracts here are discontinuous at the graft boundary and therefore cannot transmit the initiating stimulus. Yet the work of Tartar (1961) and de Terra (1971; 1973; 1975) has indicated that induction of oral development in graft complexes is transmitted through the cortex. Work on cultured mammalian cells (for reviews see Burger, 1978; Pardee, 1975) and lymphocytes (for review see Edelman, 1974) has suggested that cell membrane changes initiate cell division in these cell types and the search for such changes in *Stentor* would therefore be a promising area for future work.

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