Does the oral apparatus of the ciliate *Stentor* inhibit oral development by release of a diffusible substance?

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SUMMARY

In the ciliate *Stentor*, many thousands of basal bodies assemble on the ventral cell surface to form a new oral apparatus during cell division, regeneration and reorganization (oral replacement during interphase). During interphase, oral development is normally inhibited by the presence of the anteriorly placed oral apparatus. A glass needle was used to cut the oral apparatus of interphase stentors in two so that the parts remained intact but separate at the anterior end of the cell. These cells initiated basal body assembly and oral development, usually within 8 h. Basal body assembly can therefore result from disconnection of fibrous structures within the oral apparatus but is unlikely to be regulated by an inhibitor diffusing from it.

INTRODUCTION

Very little is known about the mechanisms initiating basal body assembly. The ciliate *Stentor* (Fig. 1) is an excellent organism in which to study this problem because it undergoes three morphogenetic processes in which many thousands of basal bodies are assembled at a specific site on the ventral cell surface. The event that initiates cell division in *Stentor* is the mass assembly of basal bodies on the ventral cell surface to form a visible structure, the oral primordium, which develops into a new oral apparatus for the posterior daughter cell; the anterior daughter retains the original oral structures. *Stentor* can also develop an oral primordium to regenerate missing oral structures when the oral apparatus is removed, most simply by cutting it off with a glass needle (Fig. 2). The stages of oral development are essentially the same as during cell division except that the cell and the nucleus do not divide. Finally, *Stentor* can form an oral primordium which replaces the existing set of oral structures with a new, larger set during interphase; this process is called reorganization (Fig. 3). The stages of oral development are the same as during regeneration and cell division except that the original gullet, together with an adjacent section of membranellar band, is resorbed as the new oral structures move upward.

Several important things are known about why *Stentor* initiates basal body assembly, oral development and cell division. Tartar (1961) found that removing most of the oral apparatus except for the gullet induced oral development and that the largest cells usually divided while smaller ones regenerated instead. This showed that

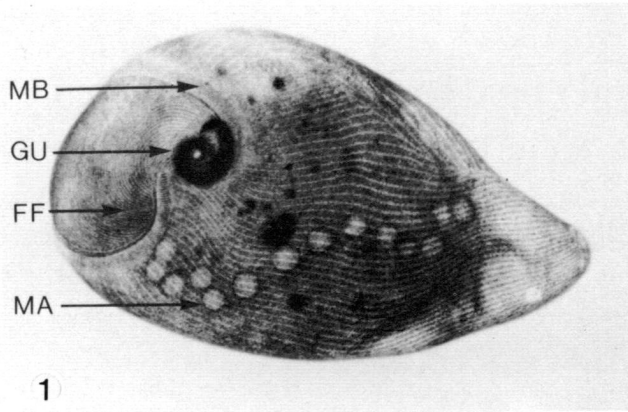*Key words*: cytoskeleton, basal body, oral development, ciliate, *Stentor*.

Fig. 1. *Stentor* flattened in microcompression chamber. MA, chain macronucleus; MB, membranellar band; GU, gullet; FF, frontal field (from de Terra, 1973). × 140.

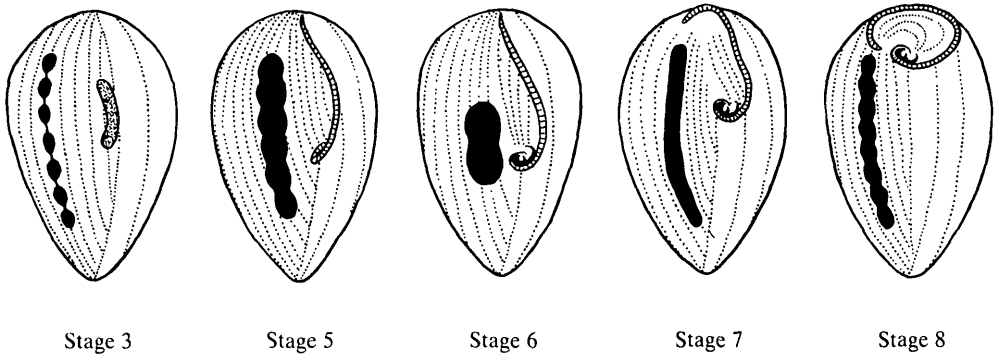Stage 3   Stage 5   Stage 6   Stage 7   Stage 8

Fig. 2. Some stages of oral regeneration in *Stentor*. Stage 3, showing curved oral primordium (stippled); stage 5, nuclear coalescence; stage 6, gullet formation; stage 7, nuclear elongation; stage 8, nuclear nodulation (from de Terra, 1975).

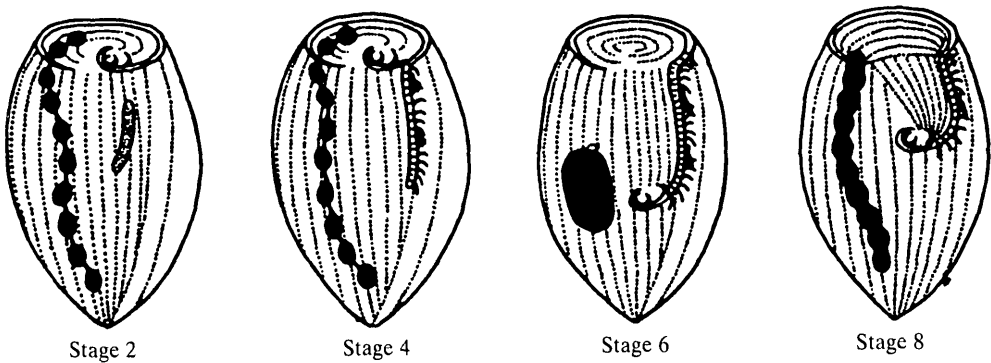Stage 2   Stage 4   Stage 6   Stage 8

Fig. 3. Some stages of reorganization in *Stentor* (see text for details; from de Terra, 1978).

oral development occurs when the oral apparatus is incomplete or dispropor-
tionately small and that whatever endogenous stimulus normally initiates basal
body assembly and oral development in mature cells is also the event which triggers
cell division. Tartar (1958) also demonstrated that the presence of an intact oral
apparatus at the anterior end of the cell normally inhibits oral development during
interphase, as one would expect from the fact that removing this structure initiates
primordium formation. He excised the oral apparatus from morphostatic cells,
waited until early primordia were visible and then grafted the oral apparatus from
another stentor onto the experimental cells which then resorbed their primordia
and returned to interphase. The oral apparatus can apparently no longer inhibit
basal body assembly when the cell divides because the cell body grows during
interphase and the oral apparatus does not, with the result that oral apparatus size
becomes disproportionately small in relation to cell body size and is therefore no
longer able to inhibit basal body assembly and oral development (de Terra, 1969).
I later showed this directly by reciprocal transfers of the oral apparatus between
cells of a large (L) and a small (S) strain of *Stentor*, the S-strain having an oral
apparatus about half the size of the L-strain. Very large L-strain stentors divided
prematurely when their oral apparatus was replaced with one from an S-strain cell.
Conversely, S-strain stentors in early stages of division resorbed their primordia
and returned to interphase when given the oral apparatus of an L-strain cell (de
Terra, 1977).

   The question of how the oral apparatus normally inhibits basal body assembly
and oral development is crucial for understanding what mechanisms control the
timing of basal body assembly, oral development and cell division. The most ob-
vious hypothesis is that the oral apparatus releases a diffusible inhibitor which can
no longer prevent basal body assembly when the cell reaches a certain critical size.
Here I report the results of an experiment which has made this hypothesis seem
unlikely by showing that when the entire oral apparatus is present at the anterior
end of the cell but is disconnected into two parts, the morphostatic cell initiates oral
development. This experiment has also suggested that the time of basal body assem-
bly in *Stentor* may normally be regulated by a mechanism involving detachment of
cytoskeletal structures.

MATERIALS AND METHODS

The organism

   Stentor coeruleus is a ciliate commonly used in microsurgical experiments because of its large
size (up to 1 mm when fully extended) and remarkable powers of wound healing. The anteriorly
placed oral apparatus consists of a gullet, an oral pouch and a band of membranelles enclosing
a circle of cortex called the frontal field. The membranelles are triangular plates of fused cilia.
Each membranelle originates from three rows of underlying basal bodies; these in turn give rise
to microtubules which run downward and finally converge into a thick continuous fibre tract
connecting adjacent membranelles (Randall & Jackson, 1958; Fig. 4). Membranelles are also
joined by continuous bands of M-fibre (myoneme) material (L. H. Bannister & E. C. Tatchell,
personal communication). The cell body cortex consists of alternating clear and dark stripes. The

dark stripes contain rows of blue–green pigment granules and each clear stripe contains a ciliary row consisting of paired basal bodies and the structures originating from them. One of each pair is ciliated; the other gives rise to a ribbon of 20–22 linked microtubules (Bannister & Tatchell, 1968; Huang & Pitelka, 1973) which overlap to form a microtubule fibre tract (mt fibre tract) visible by light microscopy. The pigmented stripes are graded in width around the cell and the oral primordium appears at the site where the widest and narrowest stripes meet on the ventral surface. Here, many thousands of basal bodies appear, sprout cilia and align themselves in rows to form the membranelles; a gullet eventually forms at the posterior end of the primordium and migrates upward to its final position, together with the newly formed membranellar band. The entire process of oral development takes about 8 h at 20 °C.

The Stella strain of *S. coeruleus* was grown as previously described (de Terra, 1977) on the small flagellate *Chilomonas paramecium*. Operations were done freehand with a glass needle. For photography, most of the cell was dissected away with a glass needle, leaving the split oral apparatus and the oral primordium intact. The cells were then fixed in Schaudinn's fluid for 20 min and passed through a graded alcohol series into cedarwood oil.
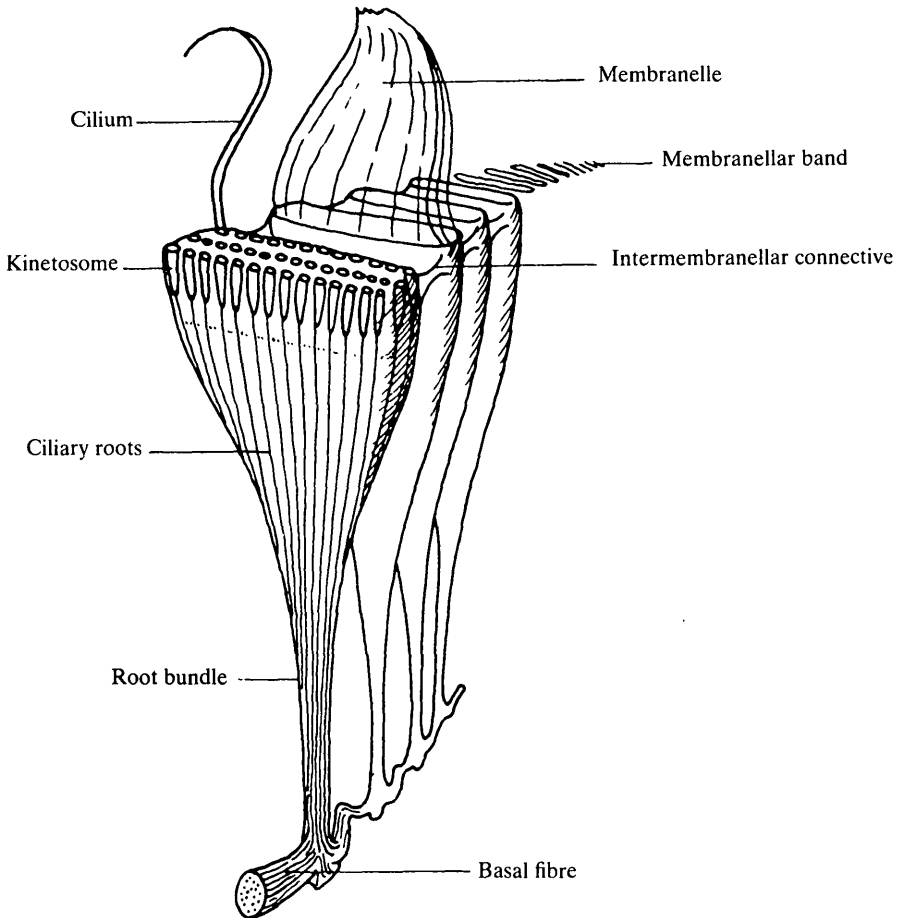

Fig. 4. Diagram showing the structure of the oral membranelles and their underlying basal bodies. These give rise to microtubules which run downward and finally converge into a continuous fibre tract (basal fibre) connecting adjacent membranelles (from Tartar, 1961 after Randall & Jackson, 1958).

RESULTS

In preliminary experiments, I found that isolated parts of the membranellar band tend to curl in on themselves to form partially or entirely closed circles. The operation I used to produce discontinuities in the oral apparatus (Fig. 5) consisted of cutting through the cell longitudinally with a glass needle in such a way that the oral apparatus was split in half; the needle was then used to push the half of the cell containing the gullet posterior to the other half and hold it down for about 1 min so that it would not migrate back immediately to its original position. The needle was also used to twist the part of the cell containing only half of the membranellar band so that a continuous part of the band would face the half of the oral apparatus containing the gullet when this half migrated upward. This helped to keep the two parts of the oral apparatus from healing together. In spite of this, the two parts
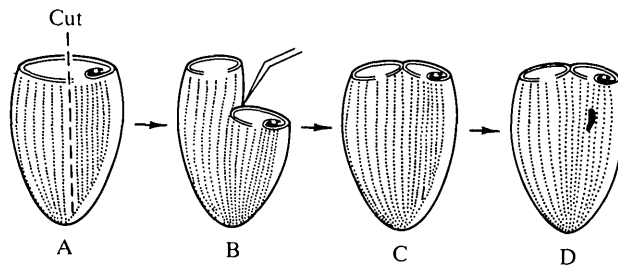

Fig. 5. (A) Longitudinal cut is made through most of cell, separating oral structures into two parts; (B) Half of oral apparatus with gullet is held down with needles; (C) Two parts of oral apparatus are now separate; (D) Primordium forms.
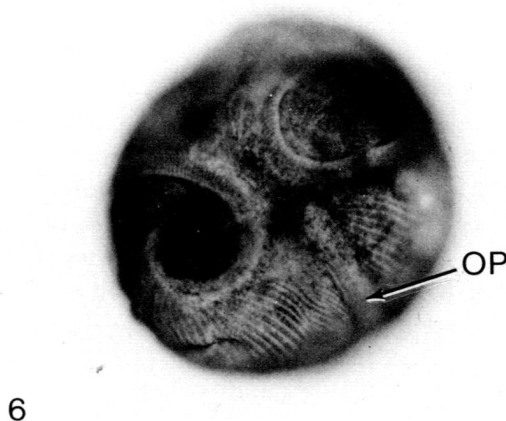

Fig. 6. Cell with oral primordium induced by disconnecting the oral apparatus into two parts. OP = oral primordium. × 515. Normal structure of oral apparatus is shown in Fig. 1.

usually rejoined but sometimes they remained separated at the anterior end of the cell. Both halves always looked healthy and their membranelles continued to beat. Of 64 such operated cells kept at 20–25 °C, 52 formed primordia within 8 h; the majority of cells, which healed the oral apparatus into one continuous structure, did not form primordia. A typical result (cell in which the oral apparatus remains separated into two parts) is shown in Fig. 6. Figure 1 shows the normal structure of the oral apparatus. The primordium almost always appeared in association with the half of the membranellar band containing the gullet, which was resorbed along with an adjacent section of membranellar band while the newly formed gullet and membranellar band migrated upward as in reorganization (Fig. 3). The half of the membranellar band which did not contain the gullet was incorporated into the newly formed oral apparatus, either during oral development or after this process was complete. Cells in which the two halves of the oral apparatus had not healed or formed primordia within 8 h after the operation were examined the next day; all had a normal oral apparatus, indicating that they had either healed or reorganized during the night. The results of these experiments indicate that separating the oral apparatus into two parts provides a strong stimulus for initiation of basal body assembly and oral development.

DISCUSSION

The results obtained here are not consistent with the hypothesis that an inhibitor diffusing from the oral apparatus controls the time of basal body assembly and oral development in *Stentor*. They do suggest that it is simply the presence of an intact oral apparatus of normal size at the anterior end of the cell that inhibits these events, perhaps by causing structural changes in the cortex which affect the properties of basal bodies at the primordium site. These results further suggest that detachment of cytoskeletal structures somewhere in the cell may constitute the endogenous stimulus initiating basal body assembly and oral development in *Stentor*. This hypothesis will be discussed more fully in the accompanying paper (de Terra, 1985) which shows that discontinuities in the microtubule fibre tracts of the cell body cortex also induce oral development in *Stentor*.

The principle of 'change induced by attachment' (or, in this case, detachment) which apparently controls assembly of basal bodies at the primordium site in *Stentor* is well known, both at the molecular level in allosteric enzymes and at the level of supramolecular assemblies such as viruses (Casjens & King, 1975; Wood, 1979), ribosomes (Nomura, 1973) and the flagella of *Salmonella* (Asakura, Eguchi & Iino, 1966) and *Chlamydomonas* (Luck, Piperno, Ramanis & Huang, 1977). It is therefore not surprising to find that such a mechanism may operate at the intracellular level to control the time of basal body assembly and oral development in *Stentor*.

So little is known about the factor or factors initiating assembly of basal bodies and centrioles in metazoan cells that it is difficult to evaluate the significance of the

results obtained with *Stentor* for other cell types. Since the work on *Stentor* has now provided abundant evidence indicating that basal body assembly is initiated by changes in cytoskeletal organization, the possibility that similar mechanisms exist in other cell types should at least be kept in mind.

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