Regulation of the pattern of basal bodies within the oral apparatus of *Tetrahymena thermophila*

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SUMMARY

The number and arrangement of basal bodies included in the four compound ciliary organelles making up the mature oral apparatus of *Tetrahymena thermophila* ordinarily vary only slightly. Severe starvation brings about formation of oral structures with a reduced number of basal bodies within these organelles, and sometimes with a complete loss of one of the component organelles. Such reductions are stringently specified in spatial terms, but they do not represent simple and proportional shrinkage of the organelle complex. Instead, certain spatial features remain essentially unaltered, while others undergo major quantitative reductions, resulting in large changes in the internal proportions of the structures. This selective regulation can be explained in terms of the different parallel and sequential processes taking place during the development of this organelle complex. There is also no strict proportionality between the size of the oral apparatus and that of the cell; instead, oral apparatuses become relatively larger as cells become smaller. This is due in part to the inherent temporal discontinuity of oral development, but there is probably also a real change in the oral/body size relation at the time of oral development. The 'French flag' rule fails when applied to the relative sizes and internal proportions of organelle systems in this and in other ciliates.

INTRODUCTION

Ciliated protozoa, like many multicellular organisms, can maintain their integrity over a broad range in body size. This situation allows one to observe the adjustment of pattern to variation in size, and thus to achieve some insight into how the pattern itself is set up. One simple solution would be for the parts to retain the same relative sizes as absolute size varies, as proposed in Wolpert's (1969) 'French flag' rule. Recent detailed analyses of multicellular systems indicate that such strict proportionality often does not hold (Stenhouse & Williams, 1977; Bode & Bode, 1982). In ciliates, the initial location of primordia of cell surface structures relative to established landmarks may be proportional

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Fig. 1. A schematic diagram of the organization of basal bodies in the oral apparatus of *Tetrahymena thermophila* grown in nutrient medium at 28 °C. Basal bodies are indicated by circles. Those bounded by solid lines are always present, those bounded by dashed lines are sometimes present. Filled circles indicate basal bodies that are always ciliated, stippled circles show basal bodies that are sometimes ciliated, and open circles represent basal bodies that are never ciliated. The lines connecting basal bodies are drawn solely for recognition of spatial patterns of basal bodies. UM indicates the undulating membrane; M1, M2, and M3 – the first, second and third membranelle, respectively. S and C refer to the "sculptured" and "columnar" regions of M1 and M2. In the latter regions, the numbers and small letters indicate the columns and rows respectively.

To some measure of overall size (Nanney, 1966; Lynn & Tucker, 1976; Lynn, 1977). However, the few studies done to date indicate that within sets of ciliary organelles the internal organization does not regulate proportionately (Jerka-Dziadosz, 1976; Bakowska & Jerka-Dziadosz, 1980; Bakowska, 1980, 1981).

We here report on an analysis of regulation of the number of ciliary elements in the oral apparatus of *Tetrahymena thermophila*, using starvation to generate variation in size. As expected, the parts of the oral structures can become diminished. The diminution is not, however, a simple proportional shrinkage. We will show that this lack of proportionality is due to a conservation of basic developmental patterns irrespective of nutritional state.
MATERIALS AND METHODS

*Tetrahymena thermophila* B-2079 (II) stock cultures were maintained at 22 ± 1 °C in tubes containing 5 ml of 1 % proteose peptone plus 0·1 % Difco yeast extract, with loop transfer every other week. Flask cultures for oral isolations were grown overnight at 29 ± 1 °C in one of three different nutrient media: (a) 1 % proteose peptone, (PP), (b) 2 % proteose peptone plus 0·5 % yeast extract (PPY) and (c) Thompson's (1967) medium, containing 2 % proteose peptone, 0·2 % yeast extract, 0·5 % glucose, and Fe³⁺ at 9 × 10⁻⁶ M in the form of an Fe-EDTA complex (PPYGFe) made as described by Nelsen, Frankel & Martel (1981). The inoculum, from a fresh tube culture, was adjusted to yield cell densities of about 1 × 10⁶ cells per ml for oral isolation. Cells grown in PPyGFe were in rapid exponential phase of culture growth at the time of oral isolation, cells in PPY were in deceleratory phase, while cells grown in PP were at or close to stationary phase (cf. Nelsen *et al.* (1981), fig. 4).

The starvation protocol of Kaczanowski (1978) was employed. Two flasks of cells were grown overnight at 29 °C in PP to 6 × 10⁴ to 1 × 10⁶ cells per ml; cells from the two flasks were then pooled and resuspended in 80–100 ml of 1 mm-CaCl₂ plus 1 mm-Tris-HCl buffer, pH 7·2, and incubated at 38 ± 0·5 °C for 4 days. Cell density during starvation ranged from 1·5 × 10⁵ to 2·5 × 10⁵ cells per ml. When good silver preparations were desired, 4-day starved cells were diluted into PP, and maintained at a density of 2·5 × 10⁴ cells per ml, at 29 °C, for 1–2 h prior to fixation.

Oral isolation began with preparation of pellicles by the method of Wolfe (1970) as modified by Vaudaux & Williams (1979). The concentration of sucrose in the SEMT (sucrose-EDTA-mercaptoethanol-Tris) medium was raised to 1·67 M (instead of the usual 1 M) for isolations from cells starved in Tris-CaCl₂ medium. Immediately following extraction by Triton X-100, the preparation was pumped through a Logeman homogenizer (Rannested & Williams, 1971), liberating the oral apparatuses. This was then centrifuged at 900 g for 10–15 min, the supernatant decanted, and the slurry containing oral apparatuses fixed for 2–5 min in 1–2 ml of cold 1 % OsO₄. The material was then washed through an ethanol series (50–70–95–100 %) and prepared for scanning electron microscopy by the procedure of Ruffolo (1974), modified as described earlier (Nelsen & DeBault, 1978). The oral structures were observed in a JEOL 35-C scanning electron microscope.

Chatton–Lwoff silver impregnation was carried out using the procedure of Frankel & Heckmann (1968) as modified by Nelsen & DeBault (1978). Cell and oral lengths and maximum widths were measured in suitably oriented cells with a filar micrometer eyepiece (American Optical). Values proportional to cell or oral areas or volumes were obtained by multiplying measured cell length (L) by width (W) (=LW) or by the square of cell width (=LW²), respectively.

Statistical tests were performed as described by Sokal & Rohlf (1969), with
Figs. 2-5. Isolated OAs from cells lysed following growth in PPYGFe (Figs. 2-4) or PP (Fig. 5). Three symbols are used throughout: arrowheads designate basal bodies that lacked cilia prior to lysis, straight solid arrows indicate partially obscured ciliated basal bodies of M3, and wavy arrows show ciliated basal bodies that are dislocated relative to their usual position. All photographs are oriented so that the cell's left side corresponds to the viewer's right. The UM is thus always on the viewer's left, and the membranelles on the viewer's right, with M1 most anterior and M3 most posterior.

Fig. 2. A typical isolated OA. M2 and M3 are seen in exterior view, with the posteriormost basal bodies of M3 (arrows) partly hidden in the deep part of the buccal cavity. In this view, ciliated basal bodies appear as dense rings, while unciliated basal bodies (arrowheads) appear as faint white rings. M1 and the UM are
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the addition of a ‘short-cut’ 2 × 2 factorial ANOVA technique (Harvey, 1960), an analysis of covariance to test homogeneity of slopes (Winer, 1971), and a nonparametric test for goodness of fit of a regression line (MacWilliams, personal communication).

RESULTS

I. Organization of the oral apparatus of cells growing in nutrient medium

We have analysed the pattern of basal bodies within oral apparatuses (OAs) from detergent-extracted *T. thermophila* cells. In these preparations, basal bodies are embedded within a fibrogranular subsurface layer known as epiplasm, the cell membrane being dissolved during extraction. The epiplasmic layer sometimes stands out clearly (e.g. Fig. 10), and sometimes merges with the surrounding mounting surface (e.g. Fig. 4). Although cilia have been removed during preparation, it is possible to see which basal bodies were ciliated prior to lysis and which were not ciliated. Cilia are broken off within the proximal region of the cilium itself (Williams, Vaudaux & Skriffer, 1979), and the remnants appear as prominent, thick-walled rings, while basal bodies that did not bear cilia have distal terminations in the plane of the epiplasm. These appear either as distinctive ‘terminal plates’ (Wolfe, 1970; Williams & Bakowska, 1982), or as thin rings with raised edges (Williams & Bakowska, 1982).

seen partly in external view, and partly in side view, owing to the folding-over of the epiplasmic ridge. In side view the basal bodies appear as columnar structures, and ciliated basal bodies cannot be distinguished from unciliated basal bodies. Row a of M1 terminates at its left (viewer’s right) end one cilium short of the termination of row c and probably also row b. M1-S is largely obscured. × 8600.

Fig. 3. A preparation similar to that in Fig. 2. Note the general similarity in positioning of basal bodies, with differences involving only unciliated basal bodies. There are two unciliated basal bodies at the left end of row b of M1, one at the left end of row c of M1, and one rather than two visible in M2-S (arrowheads). The posterior quadrilateral of M1-S is in clear view, but the anterior quadrilateral is obscured. × 8600.

Fig. 4. A highly flattened and partly disrupted OA preparation. The UM is no longer in its normal spatial relationship to the rest of the OA. The anterior epiplasmic ridge is absent or flattened, so that all of M1 can be seen clearly. The epiplasm between ciliary rows b and c has been ripped. Note the configuration of M1-S, and the characteristic location of the unciliated basal bodies in M1-C. × 10 500.

Fig. 5. A preparation showing an anomaly in M2-S. The ‘sculptured’ portion of M2 was scored as having nine ciliated basal bodies instead of the usual six (the two white masses indicated by the open arrows are not basal bodies, but bits of detritus). Three basal bodies have non-standard positions: the one indicated by the wavy arrow is a basal body of column 1, row c (cf. Fig. 1) that has been displaced posteriorly; the basal body immediately anterior to it is a similarly displaced basal body of column 1, row b, while the posteriormost basal body of the anterior triangle has been displaced somewhat posteriorly and to the cell’s left (viewer’s right), probably in concert with the posterior displacement of the basal bodies of rows b and c in column 1. × 8600.
Table 1. *Number of basal body columns in M1 and M2, and number of ciliated basal bodies in the UM*

<table>
<thead>
<tr>
<th>Medium</th>
<th>M1-C</th>
<th>M2-C</th>
<th>Number of ciliated basal bodies in the UM</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP</td>
<td>1*</td>
<td>2</td>
<td>3 1 — 4 12 2 — 1 5 — 2 1</td>
</tr>
<tr>
<td>PPy</td>
<td>1</td>
<td>3</td>
<td>4 3 — 2 10 5 — 2 1 1 —</td>
</tr>
<tr>
<td>PPyGFe</td>
<td>8</td>
<td>12</td>
<td>3 — 1 14 6 — 1 3 7 8 — —</td>
</tr>
</tbody>
</table>

* The values represent number of OAs in each medium with the indicated number of basal bodies (or columns).

Table 2. *Number of basal bodies in sculptured portions of membranelles*

<table>
<thead>
<tr>
<th>Medium</th>
<th>M1-S</th>
<th>M2-S</th>
<th>M3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ciliated</td>
<td>Ciliated</td>
<td>Unciliated</td>
</tr>
<tr>
<td>PP</td>
<td>—</td>
<td>—</td>
<td>17*</td>
</tr>
<tr>
<td>PPy</td>
<td>—</td>
<td>—</td>
<td>17</td>
</tr>
<tr>
<td>PPyGFe</td>
<td>1</td>
<td>4</td>
<td>28</td>
</tr>
</tbody>
</table>

* The values represent the number of OAs in each medium with the indicated number of basal bodies.
† No OAs were scored in these categories.
‡ Cases in which the sculptured region was expanded due to a displacement of basal bodies belonging to the first column of the columnar region of M2 (see text).

The organization of the oral ciliature is illustrated schematically in Fig. 1, and photographically in Figs. 2–5. The undulating membrane (UM) is located on the posterior-right of the OA, and three membranelles (M1, M2, and M3) on its anterior-left (right and left are designated as seen from inside the cell, and so are opposite to the viewer’s right and left). The UM consists of two staggered rows of basal bodies, the outer one ciliated and the inner one unciliated (Figs. 1, 2, 3). The major portion of M1 and M2 consists of three parallel rows of basal bodies organized into regular columns of three basal bodies each (Fig. 1). In M1, the first row of basal bodies is somewhat shorter than the other two rows, so that one or two left-most columns possess two rather than three basal bodies (Figs. 1–5).*

* In this morphological description, the rows of basal bodies within the membranelles are labelled in an anterior to posterior order, for descriptive convenience only. The order of development is the opposite, posterior-to-anterior (Bakowska, Nelsen & Frankel, 1982).
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Fig. 6. Relationship of the number of basal body columns in M2 to the number of basal body columns in M1 (A) and to the number of basal bodies in the ciliated row of the UM (B). Crosses indicate OAs isolated from cells grown in nutrient media, circles represent OAs isolated from cells starved for 4 days in Tris-CaCl₂. The open circles indicate OAs with three membranelles, and the filled circles represent OAs with two membranelles.

The organization of the right ends of M1 and M2 is substantially modified during oral development (Bakowska, Nelsen & Frankel, 1982). We will here describe these regions in terms of arbitrary but uniform ‘constellations’ of basal bodies (connected by thin lines in Fig. 1). M1 possesses two quadrilaterals with (generally) four ciliated basal bodies each (Figs. 1, 4), while M2 has two triangles, each with a characteristic form (the posterior one approximating a reversed comma), and each invariably possessing three ciliated basal bodies (Figs. 1–4). These modified portions of M1 and M2 will be designated collectively as the ‘sculptured’ (S) regions (M1-S, M2-S), the remainder as the columnar regions (M1-C, M2-C). All of M3 is ‘sculptured’, with 12 ciliated basal bodies, six of them forming an anterior wavy line (‘wave’) (Figs. 1, 2, 3, 5), the remainder a posterior hexagon (Figs. 1, 2).

The disposition of unciliated basal bodies is less regular in membranelles than in the UM. In M1, the farthest-left three to five basal bodies of row (a) are invariably unciliated (Figs. 1, 4, 5), and the single farthest-left basal body in rows (b) and (c) frequently lacks cilia as well (Figs. 1, 3, 4, 5). Unciliated basal bodies are also seen in the sculptured region of M2, and in M3, near the regular
'constellations' described above. The locations of such unciliated basal bodies are shown in Fig. 1; in each OA basal bodies are visible at only some of these locations. Comparison with unpublished electron micrographs, including some kindly lent by N. E. Williams (T. pyriformis) and M. Jerka-Dziadosz (T. thermophila), strongly suggests that unciliated basal bodies are actually present at most or all of these locations, but may not be seen due to their failure to penetrate the epiplasmic layer.

Basal bodies were counted in 19 isolated OAs from cells grown in PP, 17 OAs from PPY, and 35 OAs from PPYGFe. Oral structures from cells grown in these three different media differ only slightly in number of basal bodies in the columnar regions of M1 and M2 and in the UM (Table 1). The sculptured regions of M2 invariably possess six ciliated basal bodies (Table 2), except in two cells in which column 1 is modified sufficiently (Fig. 5) for it to be tallied as part of the sculptured region. Scantier data for M1-S and M3 indicate a similar near-constancy (Table 2). The spatial configurations of the 'constellations' of ciliated basal bodies also vary little. The number of unciliated basal bodies observed in M2-S and M3 is more variable (Table 2), but most of this variation may be due to a failure to detect all of these basal bodies.

The estimated composite average number of basal bodies in the entire OA is 140 in PP, 142 in PPY, and 136 in PPYGFe. If additional undetected unciliated
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basal bodies are taken into account, the true number may be higher by about 5 basal bodies.

Patterns of covariation were analysed for data-sets in which a sufficient number of within-cell comparisons could be made. Within M2, there is no correlation between the number of columns of basal bodies and the number of basal bodies visible in the sculptured region (not shown). The number of columns of basal bodies in M2-C is positively correlated with the number of columns in M1-C (Fig. 6A, ×), but not significantly so with the number of ciliated basal bodies in the UM (Fig. 6B, ×).

OAs isolated from a culture grown in PP at 38 °C were also examined. With only one exception out of 12 specimens, all aspects of basal body pattern were the same as observed at 28 °C.

II. Organization of the oral apparatus of starved cells

Cultures of *T. thermophila* starved in Tris-CaCl₂ medium at 38 °C after previous growth in PP responded in one of two ways: either all of the cells were uniformly tiny after 4 days of starvation, or most cells were tiny while a minority were as large as, or larger than, cells grown in nutrient medium. OAs isolated from populations with both big and tiny cells fell into three classes:

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Figs. 11-14. Isolated OAs from cells lysed following 4 days of starvation in Tris-CaCl₂, with M3 severely diminished or absent. Symbols and orientation are as in Figs. 2-5.

Fig. 11. An OA preparation with a severely reduced M3. The preparation is tilted so that M1 and M2 are seen obliquely, and the UM is largely obscured. M3, however, is clearly visible and is made up of 4 ciliated and 2 unciliated basal bodies, in a totally non-standard configuration. × 13200.

Fig. 12. An OA preparation with a normal M1, nearly normal M2, and no M3. A somewhat unusual feature in M2 is a slight displacement of the posterior end of the posterior triangle of M2-S, bringing it closer to the posterior basal body of column 1 (open arrow). Also, two unciliated basal bodies are located within the otherwise ciliated row of the UM; the position of these in the middle rather than at an end of the row is unique to this cell. × 11200.

Fig. 13. An OA preparation with a (probably) normal M1, truncated M2 and missing M3. There are five basal bodies in row a of M2-C, four in row b, and three in row c. This difference is not observed in cells in which M3 is present. Note also a milder example of the type of displacement of M1-S shown in Fig. 10 (wavy arrow) and the somewhat displaced posterior end of the posterior triangle (open arrow) as in Fig. 12. M2 occupies a position in the buccal cavity resembling that normally characteristic of M3. × 11200.

Fig. 14. An extensively modified M2 in an OA in which M3 is absent. There are only four columns in M2-C, and row c is missing in all but the first column. Lines are drawn between basal bodies to indicate how this highly modified M2 resembles M3. The anterior ‘wave’ is straighter than in M3, while the posterior triangle has been displaced to the cell’s left, to generate a posterior hexagon that resembles that typical of M3. Only three basal bodies are not included in an M3-like pattern. The relationship of the hexagon to the deeply recessed part of the buccal cavity is also similar to that encountered in M3. × 11200.
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Fig. 15. A bar graph indicating the number of basal body columns in M1 (M1-C), and in M2 (M2-C) and the number of basal bodies in the ciliated row of the UM. The shaded bars above the axis indicate OAs from cells grown in PP, while the bars below the axis represent OAs from cells starved for 4 days in Tris-CaCl₂. Shaded bars below the axis represent the subset of large OAs presumed to be derived from normal-sized cells within the starved population, open bars represent small OAs with three membranelles, and stippled portions of bars indicate OAs with two membranelles.

OAs of normal size and organization (Fig. 7), OAs of nearly normal organization but reduced size (Figs. 8–10), and OAs of reduced size, with M3 missing (Figs. 12–14). OAs isolated from populations of uniformly tiny cells were all of reduced size. We therefore presume that the normal-sized OAs isolated from heterogeneous cultures were derived from the large cells, and the small OAs were from the tiny cells.

Normal-sized OAs from starved cultures were normally organized, with only occasional minor deviations from the typical pattern (Fig. 7). Small three-membranellar OAs possessed a typical UM, and membranelles with columnar and sculptured regions (Figs. 8–10). Only a few alterations of the standard pattern were evident: M1-S was frequently somewhat ‘undersculptured’, with its posterior quadrilateral retaining substantial continuity with the adjacent two posterior rows of M1-C (Figs. 8 and 9); also, one or two basal bodies in the anterior quadrilateral (or triangle, as in Fig. 8) were sometimes unciliated. In M2-C, an anterior displacement was sometimes encountered within column 1 (Fig. 10). The organization of M3 was typical in all cells but one, in which M3
Fig. 16. Bar graph indicating the total number of basal bodies in the sculptured part of M1 (M1-S), the number of ciliated and unciliated basal bodies in the sculptured part of M2 (M2-S), and the total number of basal bodies in M3. Graphical conventions are as in Fig. 15, except that for M1-S and M3 the scantiness of the data compelled the use of data from OAs isolated from cells grown in PPY and PPYGFe as well as in PP for the bars above the line.

was deficient in basal bodies and grossly atypical in organization (Fig. 11). A special search uncovered other cases of deficient M3s, which may be transitional to loss of this membranelle.

Cells in which M3 was missing are illustrated in Figs. 12–14. In such cells, the organization of basal bodies in M1 was completely normal. M2, however, commonly underwent certain modifications, mostly in the direction of the M3 pattern. The middle and posterior rows of M2-C were often truncated at their left ends (Figs. 13 and 14). A subtle change in the inclination of M2-S brought its posterior basal body closer to the posterior basal body of the first column (compare Figs. 12 and 13 to Figs. 8, 9 and 10). Finally, in a few cases the posterior triangle of M2 was shifted leftwards to a position almost directly posterior to the posteriormost basal body of the anterior triangle, generating a 'hexagon' similar to that typically observed in M3, though at a somewhat different location within the membranelle (compare Fig. 14 to Figs. 2, 7 and 9). These modifications were associated with a shift in the location of M2 towards a site similar to that expected of M3.
Table 3. Number of unciliated basal bodies located at left ends of membranelles

<table>
<thead>
<tr>
<th>Membranelle</th>
<th>Row</th>
<th>Medium</th>
<th>Nutrient*</th>
<th>Tris-CaCl₂†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>a</td>
<td>3.50 ± 1.00</td>
<td>3.45 ± 1.92</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>0.61 ± 0.58</td>
<td>3.82 ± 1.81</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>0.21 ± 0.41</td>
<td>2.19 ± 0.81</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>a</td>
<td>0.03 ± 0.04</td>
<td>1.07 ± 1.07</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>0</td>
<td>0.32 ± 0.63</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>0</td>
<td>0.40 ± 0.65</td>
<td>25</td>
</tr>
</tbody>
</table>

* Data from all three nutrient media (PP, PPY, and PPYGF).  
† Data from the subpopulation of large OAs (shaded bars in Fig. 15) excluded.

Quantitative comparisons between OAs of starved cells and OAs of cells grown in PP are presented in Figs. 15 and 16. In starved cells, the number of basal body columns in M1 and M2 and the number of basal bodies in the ciliated row of the UM was bimodally distributed (Fig. 15). The minor modes coincided with the modes of controls harvested from PP, while the major modes were at substantially lower values. OAs in which M3 was missing (stippled portion of bars) tended to possess fewer basal bodies in M2 and the UM, but not in M1, than did OAs that retained M3.

There was a strong positive association between the number of basal body columns in M2 and the number of basal bodies in the ciliated row of the UM (Fig. 6B, circles). The association between M1 and M2 was obscured by substantial scatter, mainly due to a disproportionately high M1/M2 ratio in cells that lack M3 (Fig. 6A, closed circles). The association was very clear in the subset of OAs with three membranelles.

Three methods of regression/covariance analysis (see Materials and Methods) of the M1-C vs. M2-C comparison in all OAs with three membranelles gave results consistent with a simple linear regression, with no significant deviation from linearity. The regression line could be extrapolated to a point very near the origin (Fig. 6A), consistent with a coordinate regulation of the length of the columnar regions of M1 and M2. The UM vs. M2 comparison (Fig. 6B) did not fit a simple linear regression, and there was no support for the hypothesis of exact proportionality between the two structures.

The number of basal bodies in the sculptured region of M1 and of M2, as well as in all of M3, remained essentially the same in all classes of OAs from starved cells (Fig. 16). The increased count of unciliated basal bodies in M2-S observed in OAs of starved cells was probably a result of superior detection of
III. Relationship of the size of the oral apparatus to the size of the cell

We assessed the relationship of oral size to cell size by light microscopy of silver-impregnated cells (Figs. 17–19). Results of measurements of cells from two different experiments are presented in Table 4. Experiment 1 utilized a culture fixed during ‘fast exponential’ growth in PP (6 × 10^3 cells/ml), while experiment 2 mimicked the conditions of the oral isolation experiments, in which cells in late-deceleratory phase of culture growth (6 × 10^4 cells/ml) were washed into the Tris-CaCl₂ starvation medium. In expt. 1, cells in different
Table 4. *Dimensions of cells and of OAs*

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Medium</th>
<th>Growth phase</th>
<th>Stage*</th>
<th>n</th>
<th>Cell dimensions†</th>
<th>OA†</th>
<th>Relative dimensions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Area LW (μm²)</td>
<td>Volume LW² (μm³)</td>
<td>Area LW (μm²)</td>
</tr>
<tr>
<td>1</td>
<td>PP</td>
<td>Exponential</td>
<td>d.p.</td>
<td>20</td>
<td>479 ±41</td>
<td>8585 ±1339</td>
<td>56-5±5</td>
</tr>
<tr>
<td>1</td>
<td>PP</td>
<td>Exponential</td>
<td>0</td>
<td>20</td>
<td>714 ±99</td>
<td>12903 ±3002</td>
<td>5-2</td>
</tr>
<tr>
<td>1</td>
<td>PP</td>
<td>Exponential</td>
<td>1-5§</td>
<td>50</td>
<td>845 ±79</td>
<td>16412 ±2753</td>
<td>(n = 40)</td>
</tr>
<tr>
<td>2</td>
<td>PP</td>
<td>Deceleratory</td>
<td>0</td>
<td>20</td>
<td>872 ±174</td>
<td>15899 ±6094</td>
<td>6-1</td>
</tr>
<tr>
<td>2</td>
<td>Tris-CaCl₂</td>
<td>Starved</td>
<td>0</td>
<td>40</td>
<td>246 ±67</td>
<td>2909 ±1134</td>
<td>38-5</td>
</tr>
</tbody>
</table>

* d.p. = incipient division products of cells in terminal phases of cytokinesis. Other stages are the standard stages of oral development (Frankel & Williams, 1973), with stage 0 indicating cells that lack oral primordia.
† Means ± standard deviations.
‡ Combined data from two samples, one of stage-0 cells (n = 20) and the other of stage 4-early 5 cells (n = 20).
§ Combined data from two samples, one of stage-1 cells (n = 30) and the other of stage 4-early 5 cells (n = 20).
∥ Combined data from cells refed for 1 h (n = 20) and cells refed for 2 h (n = 20) prior to fixation. There were no significant differences between these samples.
Table 5. Dimensions of cells and of OAs in a starved population, tabulated according to the number of membranelles present

<table>
<thead>
<tr>
<th>Number of membranelles</th>
<th>Cell dimensions*</th>
<th>OA*</th>
<th>Relative dimensions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Area LW (μm²)</td>
<td>Volume LW² (μm³)</td>
<td>Area LW (μm²)</td>
</tr>
<tr>
<td>3</td>
<td>270 ± 65</td>
<td>3300 ± 1139</td>
<td>41.4 ± 8.0</td>
</tr>
<tr>
<td>2</td>
<td>217 ± 57</td>
<td>2431 ± 952</td>
<td>34.9 ± 8.1</td>
</tr>
</tbody>
</table>

* Means ± standard deviations.

stages of development were measured, while in expt. 2 all measured cells were in stage 0. In expt. 2, cells were uniformly small after starvation.

In the fast-exponential population (expt. 1), estimated cell volume approximately doubled during the cell cycle. Cells in the deceleratory-phase culture (expt. 2) were on the average similar in size to exponential-phase cells that were entering division, but were much more variable. At the end of the four-day starvation period, average estimated cell area was reduced to about three-tenths and cell volume to less than one-fifth of the pre-starvation values. The starved cells were also substantially smaller than the incipient cell division products measured in expt. 1.

The estimated (OA surface)/(cell surface) and (OA surface)/(cell volume) ratios became halved during the cell cycle (expt. 1). This occurred because cells can grow while OAs can not. In deceleratory-phase cultures (expt. 2), the average OA was slightly larger than in fast exponential cultures. After 4 days of starvation, the average estimated OA area became reduced to five-eighths of what it had been previously. However, since cell size was reduced much more, the estimated (OA area)/(cell area) ratio more than doubled, while the estimated (OA area)/(cell volume) ratio more than tripled. Even the most conservative comparison, between starved cells and the incipient division products of expt. 1, indicates that the area of the OA became disproportionately large in the severely starved cells.

Correlation coefficients were computed to assess the relationship between oral size and cell size within each sample for which both OA measurements and cell measurements were available. These coefficients uniformly failed to attain statistical significance in samples harvested directly from nutrient medium, despite the fact that separate computations were made for different developmental stages. On the other hand, in the samples from the starved population modest but highly significant correlation coefficients of 0.62 and 0.59, respec-
respectively, were obtained for the relationship of estimated OA area and cell area, and for the relationship of estimated OA area and cell volume.

Both the average estimated OA areas and the average estimated cell areas and volumes were significantly smaller \((P < 0.01)\) in the two-membranellar than in the three-membranellar starved cells, while mean relative oral/cell dimensions were similar in both (Table 5). OA areas correlated significantly with cell areas within both subpopulations. However, there was no evidence for any precise proportional relationship between oral size and cell size.

We can relate the light microscopical (LM) and scanning electron microscopical (SEM) observations by comparing the degree of diminution of the OA during starvation as estimated by the two techniques. This was done by comparing relative dimensions of the OA (LM) to relative numbers of basal bodies (SEM), in both cases dividing the average values of OAs from starved cells by the average values of OAs from fed cells. For cells with three membranelles after starvation, the relative OA area of starved cells was 0.67 (41.4 divided by 61.5) and the relative number of basal bodies was 0.60 (84 divided by 140). For cells with two membranelles after starvation, the relative OA area was 0.57 (34.9 divided by 61.5), while the relative number of basal bodies was 0.49 (69 divided by 140). In both cases the diminution of SEM counts was greater than that of LM measurements, but the differences were not great, suggesting that the LM measurements, though far cruder than the SEM counts, can none the less provide a reasonably reliable indication of true changes in dimensions of the oral apparatus.

**DISCUSSION**

We have analysed the regulation of the arrangement and number of basal bodies of the oral apparatus of *Tetrahymena thermophila* in two steps: first, by describing and quantitating this pattern in cells grown in various nutrient media, and secondly, by comparing this to the patterns expressed by severely starved cells. The first step showed that the arrangement of basal bodies in oral structures is remarkably constant (cf. Williams & Bakowska, 1982), while the number of basal bodies varies only slightly. The second step revealed that the arrangement is largely preserved in the face of substantial variation in cell and oral size, but that the number changes in a manner that violates the ‘French flag’ rule. More specifically, we can assert the following. (1) Regulation occurs mainly by adjusting the number of basal body pairs in the undulating membrane (UM) and the number of basal body columns in the membranelles, while the length of each membranellar column remains unchanged. The size of the sculptured region of membranelles also does not change. Therefore, both the membranellar width/length and sculptured/columnar ratios change as overall size changes. (2) Within an oral apparatus, adjacent membranelles undergo parallel regulation; at least for the two larger membranelles, reduction in one is proportional to reduction in the other. This limited proportionality does not, however, extend
to the relationship between membranelles and the UM. (3) When size reduction is extreme, the third membranelle may be absent; the second membranelle then develops further posteriorly and sometimes is modified in a manner suggestive of an approach to the organization of the third membranelle. (4) The reduction in size of OAs of starved cells, though substantial, is much less than the reduction in size of the cells themselves; oral apparatuses thus become relatively larger as cells become smaller (Figs. 17-19).

The interpretation of these findings depends critically upon how OAs become smaller as cells starve. Either pre-existing OAs shrink, or new, smaller OAs are formed during starvation. We assume that it is the latter that takes place. The process of oral replacement, involving development of a new OA immediately posterior to an old one that is then resorbed (Frankel & Williams, 1973), is known to take place following transfer of cells to nutrient-free medium (Nelsen, 1978), and probably occurs repeatedly (Kaczanowski, 1978). The alternative, in situ diminution of a pre-existing oral structure, is observed in *Tetrahymena vorax* macrostomes transforming to microstomes, but only during pre-division oral development (Buhse, 1966), when even non-polymorphic tetrahymenas undergo a partial regression and reconstruction of preformed OAs (Buhse, Stamler & Corliss, 1973; Nelsen, 1981; Bakowska et al. 1982). As there is no evidence for reconstruction of old *Tetrahymena* OAs in cells not forming new OAs, we are reasonably confident that the diminished OAs found in non-dividing starved cells are products of development of new, smaller OAs.

If the above is true, then the analysis of oral development provides a key to understanding regulation of the final pattern. Since developing oral primordia are sparse in severely starved cultures, we have to rely on extrapolation from analyses of pre-division oral development in well-fed cells [as far as we know, the details of oral replacement development are similar to those of pre-division oral development, except for the mode of formation of the initial oral field (Frankel, 1969; Kaczanowski, 1976; Nelsen, 1978)]. Three features of normal oral development are of particular importance. (a) Assembly of membranelles: basal body couplets line up to produce ‘promembranelles’ with two ciliary rows each, followed by the addition of a third row through formation of one additional basal body anterior to each of the original couplets (Williams & Frankel, 1973; Jerka-Dziadosz, 1981; Bakowska et al. 1982). This strategy of membranellar development is probably stereotyped (Bakowska et al. 1982), so that cells regulate the size of membranelles primarily by altering the number of couplets that enter the promembranelles. (b) Independent development of the UM: this structure forms initially by a lining up of single basal bodies on the right side of the developing OA, at an oblique angle relative to the promembranelles, and then goes through a complex sequence of developmental manoeuvres quite different from those simultaneously going on in the developing membranelles (Nelsen, 1981; Bakowska et al. 1982). The lack of strict proportionality between lengths of membranelles and UMs is thus not surprising.
Sculpturing of membranelles: this takes place very late during oral development, and appears to involve a movement of certain basal bodies of otherwise fully formed membranelles; this movement is associated with the outgrowth of a set of structures (the ribbed wall microtubules) that are probably involved in the modelling of the buccal cavity (Bakowska et al. 1982). Since the size of the sculptured region depends only upon the number of basal bodies which are moved, a lack of correlation between the size of the sculptured region and the overall length of membranelles is to be expected. The likelihood that the cause of sculpturing is extrinsic to the membranelles also explains the dependence of the shape of a membranelle on its location within the developing OA (as in M2 of OAs that lack a third membranelle).

We can thus appreciate why the internal proportions of the oral apparatus do not regulate according to the ‘French flag’ rule. An intracellular structure as complex as the oral apparatus is not modelled like a piece of clay, all at once by a single sculptor following a single plan. Rather, there is a succession of different developmental steps, each following its own rules, and each flexible in certain circumscribed ways and inflexible in others. The present analysis coupled with a simultaneous study of normal oral development has given us some notion of what these rules might be, and a mutational analysis that is currently under way will test our hypothesis that parallel and sequential processes exist which are subject to separate modification.

The nature of oral development also illuminates the nonproportionality between oral size and cell dimensions. OAs develop discontinuously, while cells change their size continuously. During the normal cell cycle, cells grow while OAs remain static, resulting in a halving of the ratio of oral size to cell size (cf. DeTerra, 1969; Jerka-Dziadosz, 1976).* When starving, cells become smaller, so the oral size/cell size ratio increases until oral replacement takes place. Thus, the relatively oversized OAs of severely starved cells were formed at some earlier time, when cells were presumably larger. We have not ruled out the possibility that OAs, when they develop, always manifest the same size relative to the cell; however, the great disproportion observed in severely starved cells leads us to a strong suspicion that they do not.

Many of the findings reported and discussed here are comparable to observations made earlier on the hypotrich ciliate, *Paraurostyla*. Particularly striking are the consistent violations of the ‘French flag’ rule, also observed in the internal organization of membranelles (Bakowska & Jerka-Dziadosz, 1980) and cirri (Bakowska, 1981) of *Paraurostyla*, in the relationship of sizes of ciliary primordia to the cell as a whole (Bakowska & Jerka-Dziadosz, 1980), and in

* DeTerra (1969) postulated that this change in ‘oral/somatic ratio’ was what triggered new oral development and (if absolute size was sufficient) cell division. A curious coincidence between DeTerra’s data and ours is that the estimated oral/somatic ratios in her study on *Stentor* and ours on *Tetrahymena* are the same: 0·12 at the beginning of the cell cycle and 0·06 at the end. We of course do not know whether this coincidence is meaningful.
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the relation between the sizes of different ciliary systems (Bakowska, 1980). Violations of a quite different order, involving the relative dimensions of cell regions, have been uncovered in yet another ciliate, *Dileptus*, by Golinska & Kink (1977) and Golinska (1979). It is only the relative location of cortical landmarks, such as the contractile vacuole pores of *Tetrahymena* (Nanney, 1966), that may express global proportionality during the establishment of intracellular pattern.

Regulation of form and pattern in at least some multicellular organisms manifests complexities that are remarkably parallel to those encountered in ciliates. In *Hydra*, the diameter of the hypostome and of the body column vary relatively little over very large ranges of body size, but there is a compensating extreme variation in tentacle mass and body length; thus as hydras get smaller they become broader relative to their length, and also become relatively deficient in tentacles (Bode & Bode, 1982). In *Drosophila*, when the size of the second-leg basitarsus is caused to vary by any one of several means, the number of rows of bristles remains constant at 8, but both bristle number per row and the spacing of bristles varies, apparently as a function of cell number (Held, 1979). In both of these multicellular examples, the system as a whole regulates harmoniously but not as a ‘French flag’, the changes in proportions being due to an interplay of different pattern-generating processes that act at different times and places and display varying degrees of regulative capacity. Although the detailed mechanisms are most likely quite different in these diverse systems, the underlying developmental logic may differ much less.

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