ANALYSIS OF CONTRACTILE VACUOLE PORE MORPHOGENESIS IN TETRAHYMENA PYRIFORMIS BY 180° ROTATION OF CILIARY MERIDIANS

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SUMMARY
The contractile vacuole pores (CVPs) in Tetrahymena pyriformis are usually found to the left of ciliary meridians (CVP meridians). When the CVP meridians are experimentally rotated 180° ('inverted'), the CVPs are now found to the right of the rotated CVP meridians. In other words, the CVP is almost invariably found on the same side with respect to the CVP meridian. Thus, the 2 sides of the CVP meridian have different morphogenetic properties and such differences are determinative in the asymmetrical fine-positioning of the CVP.

The number of CVPs per animal and their general placement on the animal (i.e. with which ciliary meridians the CVPs are associated) are known to be a function of the total number of ciliary meridians possessed by the animal. Rotation of the CVP meridians affects both the number of CVPs per animal and their general placement. The specificity of such effects appears to depend on whether both, or one, or which one, of the CVP meridians is rotated. Rotation of ciliary meridians may be used as a tool in analysing the mechanism determining the number of CVPs per animal and their general placement.

INTRODUCTION
In the study of organelle morphogenesis in ciliated protozoans, some questions frequently asked are: (1) What regulates the number of a particular organelle in an animal? (2) What controls the general placement of the organelle in relation to the whole animal; and (3) What determines the fine-positioning of the organelle in question in relation to other organelles in its neighbourhood? A favourable system for the pursuit of such questions is found in the contractile vacuole pores (CVPs) – the opening of the contractile vacuoles – of Tetrahymena pyriformis.

In T. pyriformis, Protargol staining reveals that each animal usually possesses one to three CVPs placed near the posterior end of the animal in a quadrant to the right of the mid-ventral surface (Fig. 1). Typically, each CVP is closely associated with a ciliary meridian (row of basal bodies, some ciliated) and is positioned to the immediate left of the meridian.† Thus a CVP is typically seen to be closer to the ciliary meridian

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† Throughout this report, 'right' and 'left' are defined in terms of the observer's right and left respectively, assuming that he stands inside the animal, lines up antero-posteriorly with the animal and faces the cortical region which is being examined.
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on the right of the CVP (hence, CVP meridian) than to the ciliary meridian on the left of the CVP. About 10% of the CVPs, however, do not exhibit such asymmetry of positioning in association with the CVP meridian: some are found centrally on the CVP meridian and do not show a distinct right/left positional difference in relation to the CVP meridian (Fig. 2); others are found midway between 2 ciliary meridians and cannot therefore be assigned readily to either of the ciliary meridians on the 2 sides (Fig. 3). Only rarely is the CVP found closer to the right side of a ciliary meridian (Fig. 4).

The first 2 questions, namely, the number of CVPs per animal and their general placement in relation to the whole animal, have been dealt with by Nanney (1968, 1972). The present investigation adopts a quasi-surgical approach to address directly the third question concerning the asymmetrical fine-positioning of CVPs near the CVP meridian and also has significant bearings on the first 2 questions. It is well known that ciliary meridians can be rotated 180° (hence, 'inverted') and that the inversions thus created can be perpetuated in *Paramecium* (Beisson & Sonneborn, 1965). Inverted meridians have also been obtained and perpetuated in *Tetrahymena pyriformis* (Ng & Frankel, 1977); our findings agree with the earlier conclusion reached in studies on *Paramecium* (Beisson & Sonneborn, 1965; Sonneborn, 1970) that the micro-environment around the basal bodies plays an often decisive role in the fine positioning of structures associated with the ciliary meridian (such as basal bodies, parasomal sacs, kinetodesmal fibres and microtubular bands). It is therefore possible that the right and left sides of the CVP meridians may possess different capacities for CVP morphogenesis and that such a difference is determinative in the fine-positioning of its associated CVP.

The CVP meridian is therefore experimentally rotated 180° and the question asked is simply whether the associated CVP is still found to the left of the inverted CVP meridian, or, now to the right. If the former were true, i.e. the fine-position of

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Fig. 1. For abbreviations used on micrographs, see p. 237. CVPs normally found to the left of normal ciliary meridians.

Fig. 2. One CVP found centrally on a normal ciliary meridian.

Fig. 3. CVP found midway between 2 normal ciliary meridians.

Fig. 4. Exceptional case of CVP being closer to the right side of a normal ciliary meridian.

Fig. 5. Two CVPs found to the left of the same normal ciliary meridian.

Fig. 6. Anterior part of normal animal showing asymmetrical arrangement of major cortical microtubular bands (longitudinal, *lm*; transverse, *tm*; the postciliary, *pm*) around basal bodies (*bb*) on ciliary meridians.

Fig. 7. Anterior part of animal bearing one inverted ciliary meridian.

Fig. 8. An animal from line 56 showing CVPs to the right of inverted CVP meridians no. 5 and 6.

Fig. 9. An animal from line 67 showing 2 CVPs to the right of inverted CVP meridian no. 6 and one CVP to the left of normal CVP meridian no. 5.

Fig. 10. An animal from line 5 showing one CVP to the right of inverted CVP meridian no. 5 and one CVP to the left of normal CVP meridian no. 6.
the CVP were unchanged even though the CVP meridian is inverted, then obviously the ciliary meridian would exhibit no right-left difference in CVP-morphogenetic capacity; some other factors outside the ciliary meridian would henceforth have to be sought to account for the typically asymmetrical positioning of the CVP near the CVP meridian. On the other hand, if the CVP were found to the right of the inverted CVP meridian, then there would be no escape from the conclusion that the right and left sides of the ciliary meridian are different in CVP-morphogenetic capacity and that such a difference is determinative in CVP fine-positioning. As shown below, the latter is found to be true.

MATERIALS AND METHODS

Stocks

All experimental animals are of a derivative of strain B of *Tetrahymena pyriformis* that is homozygous for the recessive gene *mol* (Frankel, Jenkins, Doerder & Nelsen, 1976). Such animals frequently form heteropolar duplexes at 39 °C as a result of failure in separation of the 2 daughters during fission. Such heteropolar duplexes are removed from 39 °C and used in the generation of animals bearing inverted ciliary meridians (see Ng & Frankel, 1977, for details). Heteropolar duplexes when left at 39 °C form monsters later on and die unless returned to a lower temperature. Animals of corticotype 18 (i.e. possessing 18 ciliary meridians) have been chosen for study. These typically have their CVPs associated with ciliary meridians 5 and 6, and sometimes with 4 or 7. (The numbering of ciliary meridians starts with the ventral ciliary meridian associated with the formation of the oral primordium (no. 1) and goes clockwise toward the right of the animal.) Characteristically, only one CVP is associated with a ciliary meridian; sometimes, two CVPs are associated with a ciliary meridian (Fig. 5).

Line 67 (line = progeny from a single animal) is the founder line in which ciliary meridians no. 6 and 7 are inverted. Lines 67a and 67b are sublines originated from two different single-cell isolates from line 67 selected 2 and 4 weeks respectively after its initiation and also have ciliary meridians no. 6 and 7 inverted.

Line 6 (with ciliary meridian no. 6 inverted) was isolated from line 67 about 2 weeks after its initiation.

Line 56a (with ciliary meridians no. 5 and 6 inverted) was derived from a spontaneous monster in line 67b that was isolated about one week after its initiation. Line 56b (with ciliary meridians no. 5 and 6 inverted) was derived from a monster selected after exposure of line 67b to 39 °C at about 14 weeks after its initiation.

Line 5 (with ciliary meridian no. 5 inverted) was derived from a spontaneous monster isolated from line 67b about one week after its initiation.

Control lines without inverted ciliary meridians include line C1, isolated from line 67 about 3 weeks after its initiation, and C3 and C4, which are derived from animals found in cultures of lines 56a and 5 respectively, 3 days after their initiation.

Culture method

The animals were cultured in 1 % proteose peptone–0.1 % yeast extract medium at about 25 °C. Animals were isolated into depression slides and the isolates were allowed to grow for 1-5 days before being transferred (drop-transfer) to culture tubes each containing 5 ml of medium. Thereafter the animals were transferred daily or one every 1-5 days (loop-transfer) into fresh tubes. The animals were maintained in log phase of growth at about 7 fissions/day throughout the course of investigation.
Staining of cortical features and identification of inverted ciliary meridians

An improved version of the Protargol technique (Ng & Nelsen, 1977) reveals unambiguously the contractile vacuole pore as a ring-like structure and also, in favourable preparations, the associated water-collecting channels which confer a stellate appearance to the structure.

Recognition of the inverted ciliary meridian is possible due to the asymmetrical arrangement of major microtubular bands around the basal bodies (Fig. 6): normally, the longitudinal band runs along the entire length of the animal on the right side of each row of basal bodies; the transverse band originates anterior to each mature basal body, extends leftward across the inter-meridian space and stops short of the next ciliary meridian; the short post-ciliary band starts near the right-posterior corner of the basal body, extends right-posteriorly and stops near the longitudinal band. When these microtubular bands are seen to be 180° from the above described positions, then the ciliary meridian clearly has been inverted 180° (Fig. 7).

Statistics

G-test is used throughout for class-distribution comparisons (Sokal & Rohlf, 1969). The comparisons are conservative: Yate's correction is used when \( n < 200 \); significance level is set at \( P > 0.01 \).

The control lines (C1, C2 and C3) show no significant variations in all the parameters studied; they are therefore grouped together. No difference is found among lines 67, 67a and 67b; they are also grouped together. Lines 56a and 56b are not different from each other and are likewise grouped together. Comparisons of lines having inverted ciliary meridians with control lines are based on grouped data.

Abbreviations

\( i \) = inverted ciliary meridian; \( n \) = normal ciliary meridian; \( oa \) = position of oral apparatus (in some cases not in focus) to denote orientation of the animals; arrows indicate contractile vacuole pores (CVPs) of interest. All figures are of the same magnification (\( \times 1400 \)) except Fig. 6 (\( \times 2000 \)) and 7 (\( \times 1800 \)). In all figures, the anterior end of the animal points towards the top of the page.

RESULTS

Fine-positioning of CVP

From the control lines without inverted ciliary meridians (C1, C2 and C3), a total of 347 animals have been studied. These possess 581 CVPs that are asymmetrically positioned next to CVP meridians, and are all on the left side of the CVP meridians. From all the lines having inverted ciliary meridians (see Materials and methods for the position of the inversion in different lines), 758 animals were tallied. These yield: (i) 484 CVPs that are to the left of normal ciliary meridians; (ii) 557 CVPs that are to the right of inverted ciliary meridians (Figs. 8–11) and 5 CVPs to the left of inverted ciliary meridians (Fig. 12). Apart from the 5 exceptional CVPs to the left of inverted ciliary meridians, the present result clearly shows that, as the CVP meridian is inverted, the CVP is now positioned on the right side of the inverted meridian. The rule that asymmetrically positioned CVPs are close to the left of normal CVP meridians, but to the right of inverted CVP meridians, holds even when both situations occur in a single animal. Since the right side of the inverted CVP meridian corresponds structurally to the left side of the normal CVP meridian, it is therefore inferred that the left side of the normal CVP meridian is unique in CVP-morphogenetic capacity.
and, as such, is determinative in CVP fine-positioning. The 5 exceptional cases do not come as a surprise since exceptional cases (CVP to the right of normal CVP meridian, see Fig. 4) are also occasionally encountered in other observations on normal animals.

Another aspect of fine positioning of the CVP is shown in Table 1 (see also Table 4 for results of G-tests). In all of the lines having inverted CVP meridians, the frequency of a CVP occurring centrally on ciliary meridian 6 (Fig. 13) is much higher than that in the control group (11-33% v. < 1%). On the other hand, the frequency of a

Fig. 11. An animal from line 56 undergoing transverse cell fission (f-f = fission zone). Two newly-formed CVPs are found anterior to the fission zone: one to the right of inverted CVP meridian no. 6, the other to the left of normal CVP meridian no. 5. Posterior to the fission zone but remote from the posterior part of the animal is a CVP to the right of inverted CVP meridian no. 7.

Fig. 12. Exceptional case of CVP to the left of inverted CVP meridian no. 6.

Fig. 13. CVP found centrally on inverted CVP meridian no. 6, and remote from the posterior end of the animal.

CVP occurring centrally on ciliary meridian 5 is the same (about 10%) for all of the lines studied. This phenomenon of 'central-shifting' of CVP position on the CVP meridian apparently is not an effect of the inversion of the CVP meridian in question per se, at least in the case of lines 56a, 56b and 5: (i) in these lines, CVP meridian 5 is inverted but no increase in the frequency of central-shifting of the CVP is observed on CVP meridian 5; (ii) in line 5, CVP meridian 5, but not 6, is inverted; yet there is an increase in the frequency of central-shifting of the CVP on meridian 6.

The number of CVPs per animal and the general placement of CVP

Inverting ciliary meridians in the area where CVPs are normally placed not only introduces variations in the fine-positioning of the CVP on the CVP meridians as shown above, but also affects the number of CVPs per animal and the general place-
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ment of CVPs on different ciliary meridians. Tables 2, 3a and 3b summarize the data on these two aspects and Table 4 furnishes the results of statistical comparisons between the control group and other lines with inverted ciliary meridians.

Lines 67, 67a, 67b and 6. Most (82%) animals in the control group have 2 CVPs (Table 2) which are found most frequently on ciliary meridians 5 and 6 (Table 3a). However, when ciliary meridian 6, but not 5, is inverted, as lines 67, 67a, 67b and 6, the frequency of CVPs associating with meridian 6 is lowered (Table 3a). This is readily seen in the subpopulation of animals possessing only one CVP (Table 3b): in none of these is the CVP found in association with meridian 6 (v. 8% in the control). The percentage of animals possessing only one CVP also is double that of the control group (Table 2). It appears therefore that when meridian 6 is inverted, it becomes less favourable as a site for CVP formation. The effect appears to be restricted to meridian 6: the incidence of CVPs associated with meridian 5 is not affected (see Table 3a: number of CVPs associated with meridian 5/total number of animals = 353/347, 279/269, 160/148 for control, lines 67 + 67a + 67b, and 6 respectively).

Table 1. Fine-positioning of CVP on ciliary meridians number 5 and 6

<table>
<thead>
<tr>
<th>Line</th>
<th>Ciliary meridian no. 5</th>
<th>Ciliary meridian no. 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M*</td>
<td>L/R†</td>
</tr>
<tr>
<td></td>
<td>no. of CVPs</td>
<td>no. (%)</td>
</tr>
<tr>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>C1 + C2 + C3</td>
<td>36</td>
<td>1 (0.1)</td>
</tr>
<tr>
<td>67, 67a, 67b</td>
<td>33</td>
<td>43 (0.3)</td>
</tr>
<tr>
<td>6</td>
<td>13</td>
<td>26 (0.3)</td>
</tr>
<tr>
<td>56a, 56b</td>
<td>18</td>
<td>21 (0.15)</td>
</tr>
<tr>
<td>5</td>
<td>17</td>
<td>7 (0.11)</td>
</tr>
</tbody>
</table>

*M = CVP placed centrally on CVP meridian.
L/R† = CVP on left or right of CVP meridian.

Table 2. Distribution of animals possessing 1–4 CVPs

<table>
<thead>
<tr>
<th>Line</th>
<th>No. of animals</th>
<th>No. (%)</th>
<th>No. (%)</th>
<th>No. (%)</th>
<th>Total (animals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1 + C2 + C3</td>
<td>7 (2)</td>
<td>286 (82)</td>
<td>54 (16)</td>
<td>347</td>
<td></td>
</tr>
<tr>
<td>67, 67a, 67b</td>
<td>4 (1)</td>
<td>169 (63)</td>
<td>95 (35)</td>
<td>269</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>4 (3)</td>
<td>99 (67)</td>
<td>45 (30)</td>
<td>148</td>
<td></td>
</tr>
<tr>
<td>56a, 56b</td>
<td>16 (7)</td>
<td>151 (70)</td>
<td>48 (22)</td>
<td>215</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>14 (11)</td>
<td>81 (64)</td>
<td>31 (25)</td>
<td>128</td>
<td></td>
</tr>
</tbody>
</table>

readily seen in the subpopulation of animals possessing only one CVP (Table 3b): in none of these is the CVP found in association with meridian 6 (v. 8% in the control).
Table 3a. General placement of CVPs on ciliary meridians*

<table>
<thead>
<tr>
<th>Meridian number</th>
<th>No. of CVPs (%)</th>
<th>Line</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>C₁ + C₂ + C₃</td>
<td>15 (2)</td>
<td>353 (57)</td>
</tr>
<tr>
<td>67, 67a, 67b</td>
<td>1 (&lt;1)</td>
<td>279 (66)</td>
</tr>
<tr>
<td>6</td>
<td>6 (2)</td>
<td>160 (66)</td>
</tr>
<tr>
<td>56a, 56b</td>
<td>24 (7)</td>
<td>187 (53)</td>
</tr>
<tr>
<td>5</td>
<td>10 (5)</td>
<td>139 (65)</td>
</tr>
</tbody>
</table>

* CVP occurring midway in between 2 ciliary meridians not included (28 in C₁ + C₂ + C₃, 24 in 67, 67a, 67b, 11 in 6, 46 in 56a, 56b and 20 in 5).

Table 3b. General placement of CVPs on ciliary meridians; data from Table 3a divided into 3 subpopulations according to the number of CVPs found in an animal*

<table>
<thead>
<tr>
<th>Line</th>
<th>Position of inverted ciliary meridian(s) (number)</th>
<th>1 CVP/animal</th>
<th>2 CVPs/animal</th>
<th>3 CVPs/animal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Meridian number</td>
<td>No. of CVPs (%)</td>
<td>No. (%)</td>
<td>No. of CVPs (%)</td>
</tr>
<tr>
<td>C₁ + C₂ + C₃</td>
<td>—</td>
<td>46 (8)</td>
<td>14 (3)</td>
<td>256 (54)</td>
</tr>
<tr>
<td>67, 67a, 67b</td>
<td>6, 7</td>
<td>85 (0)</td>
<td>1 (&lt;1)</td>
<td>187 (57)</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>41 (0)</td>
<td>6 (3)</td>
<td>112 (59)</td>
</tr>
<tr>
<td>56a, 56b</td>
<td>5, 6</td>
<td>25 (46)</td>
<td>22 (8)</td>
<td>144 (52)</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>20 (20)</td>
<td>10 (7)</td>
<td>94 (63)</td>
</tr>
</tbody>
</table>

* See note on Table 3a.
Tetrahymena inverted meridians CVP position

Furthermore, line 6 is not different from lines 67, 67a and 67b in these aspects; therefore, inverting meridian 7 in addition to meridian 6 (lines 67, 67a and 67b) does not produce any further change than does simply inverting meridian 6 (line 6).

Line 5. Although only one line with meridian 5 inverted (line 5) has thus far been obtained, it provides an interesting counterpart to line 6 which has meridian 6 inverted. The previous paragraph shows that the frequency of CVPs associating with the inverted meridian 6 in line 6 (as well as in lines 67, 67a and 67b) is lowered. Now, if meridian 5 is inverted, would one find a similar lower frequency of CVPs associating with meridian 5? The answer in this case is, surprisingly, no. Instead,

<table>
<thead>
<tr>
<th>Line 5 control</th>
<th>Table 1</th>
<th>Table 2</th>
<th>Table 3a</th>
<th>Table 3b</th>
</tr>
</thead>
<tbody>
<tr>
<td>(C1 + C2 + C3)</td>
<td>Meridian</td>
<td>Meridian</td>
<td>1 CVP/animal</td>
<td>2 CVPs/animal</td>
</tr>
<tr>
<td>67, 67a, 67b, 6</td>
<td>n</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>56a, 56b</td>
<td>n</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>n</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

\[ n, 1 > P > 0.1; 2, 0.005 > P > 0.001; 3, 0.001 > P. \]

* The parameter in Table 1 compared is the fine position of CVP on ciliary meridians. The parameter in Table 2 compared is the distribution of animals possessing 1-4 CVPs. The parameter in Tables 3a and 3b compared is the distribution of CVPs on different ciliary meridians.
† Lines 67, 67a, 67b, 6, 56a, 56b and 5 as a group compared with control.

the frequency of CVPs associating with meridian 6 which is normal is lowered (Table 3a). Examination of subpopulations in the sample (Table 3b) indicates that animals having 2 CVPs exhibit a tendency (compared to control) to disfavour placing CVPs next to meridian 6. This shows that (i) the frequency of CVPs associating with an inverted meridian is not always lowered, and appears to depend on the particular CVP meridian in question and (ii) the frequency of CVPs associating with a normal ciliary meridian (in this case, no. 6) can be lowered if the CVP meridian on its left (no. 5) is inverted, i.e. the effect of inverting CVP meridian 5 is manifested through CVPs associated with CVP meridian 6. Furthermore, in animals having only one CVP, there is no indication of favouring placement of CVP with meridian 6 instead of with 5 (Table 3b), although by proportion more animals now develop only one CVP than in the control group (Table 2).

There is also an increase in the percentage of animals in line 5 developing 3 CVPs, compared with control (Table 2).

Lines 56a and 56b. Lines 56a and 56b furnish us with an example of the effect having 2 major CVP meridians (5 and 6) inverted. In these lines, there is a slight increase of the proportion of animals having 1 and 3 CVPs (Table 2).
Interestingly, when conditions permit formation of only one CVP per animal, meridians 5 and 6, both inverted, are equally favourable for CVP formation, unlike the situation in controls (Table 3b). Thus in normal animals, whatever determines meridian 5 to be the preferred site of CVP formation when conditions allow only one CVP per animal, is rendered inoperative when both meridians 5 and 6 are inverted. When conditions allow formation of 2 CVPs per animal, more CVPs are now found in association with meridian 4 (and probably also with meridian 7) than in controls (Table 3b). This suggests a relaxation of the restriction of CVPs mostly to ciliary meridians 5 and 6 when both of these meridians are inverted.

**CVP midway between two ciliary meridians**

This class of CVPs has not been included in the above analysis (except in Table 2). This is because many, though not all, of them are found between the posterior part of 2 adjacent ciliary meridians which come close to each other. Such apparently happens when, in the process of cell fission, the constriction between the anterior and posterior daughter animals brings the posterior parts of the ciliary meridians in the anterior daughter close to each other. Therefore, this class of CVPs probably reflects a technical difficulty in assigning a CVP to either of the 2 adjacent meridians based on proximity rather than providing any meaningful parameter for comparison.

**Antero-posterior distribution of CVPs**

By and large, CVPs associated with inverted ciliary meridians, like those with normal ciliary meridians, are restricted to the posterior region of the animal. However, even without involving measurement and statistical analysis, it is apparent that there is a higher incidence of CVPs associated with inverted meridians being found less posteriorly (Figs. 11, 13) than those associated with normal meridians. An explanation may be sought based on the observation that basal bodies along inverted meridians are sometimes, and at various latitudes of the animal, more distant from each other than normal; such ‘gaps’ of inverted CVP meridians are sometimes associated with CVPs. A parallel to this situation may be found in another mutant (also from strain B) homozygous for the mutant gene mol* (Frankel et al. 1976). In this mutant, CVPs are occasionally seen to be associated with gaps in normal ciliary meridians, even when such gaps develop on the anterior of the animal (Frankel, personal communication). However, analysis of the antero-posterior distribution of CVPs in the lines presently reported is complicated by the fact that all of the lines are homozygous for the mol* gene. Animals of this genotype, even in the absence of inverted ciliary meridians, are not entirely normal with regard to the antero-posterior distribution of CVPs: sometimes CVPs are posterior to the fission zone in dividers. Therefore mol* animals are not favourable materials for a simple analysis of the effect of inverted CVP meridians on the antero-posterior distribution of CVPs.
DISCUSSION

The problem of asymmetrical fine-positioning of CVPs on CVP meridians in *Tetrahymena* in a sense presents a challenge to the idea that pre-existing structures impose a restraint on the development and positioning of new structures (Beisson & Sonneborn, 1965; Sonneborn, 1970). The most rigorous and clearcut demonstration of this idea in *Paramecium* (Beisson & Sonneborn, 1965) and in *Tetrahymena* (Ng & Frankel, 1977) comes from observations on the positioning/orientation of new basal bodies, kinetodesmal fibres, microtubular bands and parasomal sacs in 180°-inverted ciliary meridians. Such new structures are all parts of a ciliary meridian. The question is therefore raised whether the ciliary meridian also imposes a restraint on the development and positioning of structurally different and functionally independent organelles in its immediate neighbourhood. The CVP, being unique in structure and function, in this regard serves as a good test. It is not associated with every ciliary meridian in an animal, although it can be potentially associated with any ciliary meridian (Nanney, 1967).

The present results leave little doubt that the CVP meridian does impose a restraint on the asymmetrical fine-positioning of CVPs next to it. Apparently, the microenvironment on the side opposite to the longitudinal microtubular band of the ciliary meridian provides an almost exclusive site for the formation of CVP. This is true even when the CVP meridian is 180° rotated. Hence, CVPs are almost exclusively found to the left of normal CVP meridians, but to the right of inverted CVP meridians.

The reason for the other aspect of CVP fine positioning, namely, the higher incidence of shifting of the CVPs to a more central position along the ciliary meridian as a result of introduction of an inversion, remains to be investigated. It is interesting
to note that (i) inverted CVP meridians do not necessarily lead to such a behaviour of their associated CVPs (e.g. CVP meridian 5 of lines 5, 56a and 56b) and (ii) CVPs associated with normal CVP meridians (no. 6) may exhibit such behaviour when a neighbouring CVP meridian (no. 5) is inverted (line 5).

The present investigation also bears upon questions of the regulation of the number of CVPs per animal and their general placement. Such parameters are known to vary according to the total number of ciliary meridians an animal possesses. To account for such variations, Nanney (1968, 1972) has put forth a formal explanation which basically consists of 2 elements (Figs. 14–16): (i) An 'inductive angle' between ciliary meridian 1 and the area in which CVPs are found. This angle, about 90°, stays constant within a syngen irrespective of the number of ciliary meridians an animal may have. (ii) A 'field angle' of about 36° which defines a field (zone) on the surface of the animal within which spots (presumably close to ciliary meridians) may be competent in yielding CVPs. Thus in the case of an animal possessing a greater number of ciliary meridians (Figs. 14, 16), more ciliary meridians would be found within the surface area subtending the same inductive angle which remains constant. The CVPs are thus placed adjacent to ciliary meridians that assume higher numbers, since numbering of ciliary meridians is consecutive, starting from ciliary meridian 1. Also, more ciliary meridians would fall within the confines of the surface as defined by the field angle, resulting in a greater number of CVPs found in the animal.

The present results clearly show that both the number of CVPs per animal and the general placement of the CVPs are affected as a result of inverting either or both of the 2 major CVP meridians (5 and 6 in animals having 18 ciliary meridians). As a preliminary probe in this area, the present investigation yields the following information: (i) Inverting ciliary meridians 6 and 7 (lines 67, 67a and 67b) apparently produces no changes additional to those produced by inverting ciliary meridian 6 alone (line 6). (ii) Inverting CVP meridian 6 (line 6) results in a lowering of frequency of CVPs associating with it, but has no effect on CVP morphogenesis on meridian 5. (iii) Inverting CVP meridian 5 (line 5) does not result in a lowering of the frequency of CVPs associating with it, but (iv) this is correlated with a lowering of the frequency of CVPs associating with CVP meridian 6. (v) Inverting both ciliary meridians 5 and 6 results in a slight increase in the frequency of CVP associating with ciliary meridian 4 (and probably with meridian 7) and (vi) this also yields an equal chance of CVPs associating either with ciliary meridian 5 or 6 when conditions permit only one CVP per animal. All of these observations are based on animals having 18 ciliary meridians in total. Such animals having their CVP meridians inverted differ from the controls in the number of CVPs per animal and their general placement; such differences, in addition, depend on the number and the location of the inverted CVP meridian. This shows that a straightforward application of Nanney's explanations to the present observations is not possible.

How can these observations be understood? To begin with, it appears that as far as formation of CVPs is concerned there is a hierarchy of susceptibility to disturbance exhibited by the CVP meridian: CVP meridian 6 is easily disturbed (observation (ii)), whereas CVP meridian 5 is not (observation (iii)). This is corroborated by the
observation that in normal animals, when conditions permit only one CVP per animal, the CVP is more frequently found in association with meridian 5. When both CVP meridians 5 and 6 are inverted, apparently the disturbance is so great that the hierarchy partially breaks down (observation (vii)).

While the consistency of observations (iii) and (iv) remains to be tested, since they are presently exemplified by only one case (line 5), these have nevertheless stimulated the following conjectures, which may be a key to understanding the control of CVP number and general placement.

Observation (ii) implies that an inversion (meridian 6) does not affect CVP formation on the normal meridian (no. 5) towards its left. This is corroborated by observation (i) that the inverted meridian 7 does not affect CVP formation on meridians to its left (6 and 5). Observation (iv) may be taken to imply that the inversion (meridian 5) does affect CVP formation on the normal meridian (no. 6) to its right. Thus hypothetically there appears to be a directionality which governs the transmission of disturbance: the disturbance (as a result of an inverted meridian) is transmitted toward the right, but not toward the left. The slight increase in the frequency of CVPs associated with meridians 4, and probably 7, when both meridians 5 and 6 are inverted (observation (v)) may render a modification of the above hypothesis necessary if the observation is shown to be consistent in future studies. The directionality of transmission of disturbance may be understood in terms of a signal regulating CVP morphogenesis coming from the left, e.g. from ciliary meridian 1 (because CVP placement is closely related to ciliary meridian 1, see Nanney, 1967). This signal may be propagated in the form of conformational changes from one unit to the next in the cortical components of the animal (see ‘Gradion hypothesis’, Roth, Pihlaja & Shigenaka, 1970; Sonneborn, 1974). The effect of disturbance at any point may then be manifested at that particular point or at places on the right of the disturbance but not on its left. This hypothesis can be tested by imposing ciliary inversions in between ciliary meridian 1 and the area where CVPs are normally found. The hypothesis would predict under such situations a disturbance in CVP morphogenesis even though the CVP meridians are not inverted.

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