Processes of Reversion from Homopolar Doublets to Singlets in *Paramecium bursaria*

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**ABSTRACT**—Morphological changes in the reversion process from the double-form cell (doublet) to the single-form cell (singlet) in an unicellular organism *Paramecium bursaria* was investigated. Though, in the life of doublets, there was a “meta-stable” period in which they produced doublet-type daughter cells, they soon failed to retain their doublet-state and eventually produced singlet daughter cells. In the course of the reversion process, two kinds of aberrant shaped doublets were found: one was the N-cell with a notch at their anterior tip, and another was the P-cell which appeared to be pinched at the anterior portion. Isolation culture experiments of successively dividing cells showed that the P-cell gave rise to the N-cell, and that the N-cell produced a pair of singlets within subsequent several cell divisions. The notch was retained and deepened only in the daughter cell derived from the anterior part of the parental cell in each cell division. When the notch deepened more than 1/2 of the cell length, a pair of singlets were produced in the next division. Two oral apparatuses kept approximately 180° apart symmetric location throughout the reversion process. Thus the process of reversion from doublet to singlet in *P. bursaria* is different from that of *P. tetraurelia*.

**INTRODUCTION**

In an unicellular organism *Paramecium*, the structural basis of the highly ordered cortical pattern resides in thousands of repeating cortical units organized into longitudinally-oriented rows on the cell surface (Fig. 1). Each of the unit is composed of one or two basal bodies associated with cilium and kinetodesmal fiber, parasomal sac and a system of peribasal ridge which delimit the unit [1–4]. During cell division, two types of daughter cells are produced: a *proter*, derived from the anterior part, and an *opisthe*, derived from the posterior part of the parent cell. The process of reproduction of the cortical pattern in these cells before and after cell division has been extensively studied [5–8]. During cell division, not only increase in the number of cortical units but also duplication of the oral apparatus (OA; see Fig. 1B) and of the contractile vacuole pores (CVPs; see Fig. 1C) take place. The proter inherits the anterior part of the parental cell including the old OA, and forms its posterior part anew. On the contrary, the opisthe inherits the posterior part with the new OA, which is formed posteriorly to the old one prior to cell division of the parental cell [9–10], and makes its anterior part anew. The cortical pattern of *Paramecium* is maintained through cell divisions according to the preexisting cortical structures [1, 11]. The preexisting pattern prescribes the correct positioning and orientation of newly produced basal bodies and their accessories along existing ciliary rows. This rule has been confirmed in paramecia which have one or more 180°-rotated ciliary rows [12]. Thus paramecia maintain their cortical pattern in all members of the descendant.

The case is different for the double-form cell (doublet). Doublets have been reported in a number of genera of ciliates including *Paramecium* [13, 14]. They can be obtained by failed in the separation of conjugants during mating [1, 15]. They are characterized by two sets of cortical...
domains, including two OAs and two pairs of CVPs. A doublet produces by cell division two daughter doublets. However, the doublet-form is considered to be a “meta-stable” state, because doublets eventually produce normal single-form cells (singlets) after repeated cell divisions [11, 15-18]. In other words, the doublet cortical pattern cannot be maintained throughout the entire clonal life.

The process of reversion from doublets to singlets has been extensively studied in *Paramecium tetraurelia* [1, 16, 19]. These works suggest that the proximity of two OAs (asymmetric locations of two OAs in a doublet) resulted from regression of a part of cortical domain might be essential for the reversion. In the present work, we report the process of reversion from doublets to singlets in *P. bursaria*. During reversion, neither apparent reduction of cortical domain nor disturbance of symmetric location of the OAs are observed, but a notch is formed at the anterior tip of the doublet.

**MATERIALS AND METHODS**

**Cells and culture methods**

Singlet cells (stocks F36 and F29) and doublet cells (stocks Bd4, Bd6, Bd8 and IB3) of *Paramecium bursaria*, syngen 1, were grown at 25°C in lettuce juice medium infected with *Klebsiella pneumoniae* one day before use [19]. Under these conditions, both types of cells divide about twice a day.

**Induction of doublets**

In order to obtain doublets, conjugating pairs of single cells of complementary mating types were used. At 7-8 hr after the mixing of two complementary mating types, conjugating pairs were treated with 3% (w/v) mannitol in Dryl's solution [20] for 1 hr at 25°C, and then transferred into Dryl's solution. The pairs were then incubated in culture medium for several dyns. Some pairs were connected by cytoplasmic bridge at the postoral region. When these pairs divided, two singlets from the anterior part and one doublet from the posterior part were produced. Doublets obtained in this way gradually became stable, producing two daughter doublets during cell division. Several doublet clones were obtained from different conjugants and used in the present experiments.

**Light microscopy**

Cortical patterns of cells were visualized using Chatton-Lwoff's silver impregnation technique as modified by Frankel and Heckmann [21]. In the case of staining a small number of samples, cells were fixed, impregnated with silver nitrate and exposed to sunlight before embedding in gelatin. Afterward, they were mounted on slides. For detailed observation of living cells, we held cells down with the agar-cover slip sandwich method as described by Yanagi & Hiwatashi [22].

**Scanning electron microscopy**

Preparation of cells for scanning electron microscopy was described previously [23]. Samples were fixed in Parducz's solution [24], dehydrated with ethanol and isoamyl acetate and dried in a Hitachi HCP-1 critical point dryer. The cells were then coated with gold and observed with a JSM-840 scanning electron microscope.

**RESULTS**

**Cortical pattern of singlets and doublets in *P. bursaria***

In normal single-form cells of *P. bursaria* (Fig. 1), the most conspicuous structures on the ventral surface are the oral apparatus (OA) and the suture which is extended both anteriorly and posteriorly from the edge of the oral opening. These structures are on one meridian, i.e. the oral meridian, which was considered to be the midventral line [1]. On the other hand, the most conspicuous features of the dorsal surface are contractile vacuole pores (CVPs). Usually, a single-form cell has one anterior and one posterior CVP and they are located in a same meridian or at most one or two meridians-apart [1]. The CVP-meridian of single-form cells is about 180° from the oral-meridian.

All doublet strains derived from different conjugants produced double-form daughter cells by cell division for a certain period after establishment of the original doublet cell. In Figure 2,
whether N-cells typical doublets exhibit notches were of structures. Because of partition apparent of doublets have doublets shaped at doublet. The 15. (notched cells; Two kinds a after appearance of their depressed shape, doublets tended to exhibit their ventral side on slides (Fig. 2). No apparent partition was seen between the two sets of structures.

**Two kinds of aberrant-shaped doublets, N-cells and P-cells**

After a number of cell divisions, doublet cells began to produce singlets. The time of singlet production varied among different doublet stocks: some produced singlets within a month, others did so more one year after obtaining the original doublet. At this stage, two kinds of aberrant-shaped doublets were also observed. One of them had a notch at the anterior tip, named N-cells (notched cells; Fig. 3). Various depths of the notches were observed amongst N-cells. To know whether N-cells represent an intermediate form during reversion from doublets to singlets, we isolated N-cells from cultures of the three stocks (Table 1). When each isolated cell underwent the first cell division, the proter (the anterior daughter cell) and the opisthe (the posterior daughter cell) were isolated separately and cultured until the next cell division. During these two division cycles, the proter of some N-cells produced singlets in all three stocks, whereas control doublets with no notch never produce singlets (Table 1). Even if some N-cells did not produce any singlets within these two cell divisions, they eventually produced singlets in subsequent divisions. The isolation experiment proves that only the proter inherits the notch in successive cell divisions.

Another type of aberrant-shaped doublet had a
flattened anterior part which appeared as if the cells were pinched at the anterior tip, and hence the cells were designated as P-cells (pinched cell). We next tested whether the P-cell was a precursor of the N-cell in the reversion process. The P-cells were isolated and cultivated for four division cycles. During this period, 22 of 32 P-cells isolated produced N-cells, whereas only one amongst 16 typical doublets produced N-cells (Table 2). This suggests that P-cells are precursors of N-cells in the

TABLE 1. The singlet cells are produced from the doublet with the notch

<table>
<thead>
<tr>
<th>Doublet clone</th>
<th>Notch</th>
<th>Number of doublets observed</th>
<th>Number of doublets producing singlets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bd4</td>
<td>-</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Bd6</td>
<td>-</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Bd8</td>
<td>-</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>24</td>
<td>17</td>
</tr>
</tbody>
</table>

* + and − mean presence and absence of observable notch at the anterior tip, respectively.
Doublet in *Paramecium* 35

**Table 2.** The notched cells are derived from the P-cell

<table>
<thead>
<tr>
<th>Cell type</th>
<th>No. of P-cells examined</th>
<th>No. of P-cells producing N-cells*</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-cells</td>
<td>32</td>
<td>22</td>
</tr>
<tr>
<td>ordinary doublets</td>
<td>16</td>
<td>1</td>
</tr>
</tbody>
</table>

* The P-cells producing N-cells within four cell divisions.

reversion process.

Morphological changes in N-cells

To know whether the notch divides the anterior portion of a doublet at random or at a defined position, the position of the notch relative to the anterior suture in N-cells was examined. It turned out that, in all N-cells investigated, the notch was found exclusively on the right side of the anterior suture: the notch always divided the anterior portion passing the two right-sides of a doublet (Table 3, and Figs. 3, 4). Silver-stained preparations clearly show that the notches are located on the right side of the anterior suture (Fig. 4). Thus, the notch is formed at a defined position of the doublet cell.

**Table 3.** Position of the notch relative to the anterior suture

<table>
<thead>
<tr>
<th>Doublet clones</th>
<th>Position of the notch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right side</td>
</tr>
<tr>
<td>IB3</td>
<td>4</td>
</tr>
<tr>
<td>Bd8</td>
<td>14</td>
</tr>
</tbody>
</table>

* Sometimes shallow notches are located on the anterior suture.

In the reversion process, N-cells with notches of varying depths were observed (Figs. 3, 4). Since the notch seems to deepen in every cell division, a relationship between the depth of the notch and the number of divisions necessary for singlet production was examined. N-cells were classified according to the ratio of notch depth to cell length, and cultivated separately. Then the number of divisions were counted until a pair of singlets were produced. As shown in Table 4, N-cells whose notch depth is more than 1/2 of the cell length produce singlets in the first division, while N-cells whose notch is less than 1/5 of the cell length take more than three divisions to produce singlets. These results suggest that the notch become deepened gradually through cell divisions and that, if a notch deepens over 1/2 of the cell length, division furrow is formed anterior to the bottom of the notch, resulting a pair of singlets as provers and one doublet as the opisthe.

Observations on the N-cells revealed that their two anterior tips were becoming wider apart as the

**Table 4.** Relationship between depth of the notch and the number of cell divisions necessary for the singlet production

<table>
<thead>
<tr>
<th>Relative depth of the notch</th>
<th>Number of cells observed</th>
<th>Number of cell divisions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/2≤a/1*</td>
<td>46</td>
<td>1.0±0.10**</td>
</tr>
<tr>
<td>1/3≤a/1&lt;1/2</td>
<td>19</td>
<td>1.5±0.25</td>
</tr>
<tr>
<td>1/4≤a/1&lt;1/3</td>
<td>22</td>
<td>2.2±0.36</td>
</tr>
<tr>
<td>1/5≤a/1&lt;1/4</td>
<td>23</td>
<td>3.2±0.52</td>
</tr>
<tr>
<td>1/6≤a/1&lt;1/5</td>
<td>14</td>
<td>3.2±0.40</td>
</tr>
</tbody>
</table>

* a/1=depth of the notch/total cell length.
** Mean number of cell divisions with 95% confidence limit necessary for the singlet production.

In the reversion process, N-cells with notches of varying depths were observed (Figs. 3, 4). Since the notch seems to deepen in every cell division, a relationship between the depth of the notch and the number of divisions necessary for singlet production was examined. N-cells were classified according to the ratio of notch depth to cell length, and cultivated separately. Then the number of divisions were counted until a pair of singlets were produced. As shown in Table 4, N-cells whose notch depth is more than 1/2 of the cell length produce singlets in the first division, while N-cells whose notch is less than 1/5 of the cell length take more than three divisions to produce singlets. These results suggest that the notch become deepened gradually through cell divisions and that, if a notch deepens over 1/2 of the cell length, division furrow is formed anterior to the bottom of the notch, resulting a pair of singlets as provers and one doublet as the opisthe.

Observations on the N-cells revealed that their two anterior tips were becoming wider apart as the

**Fig. 5.** Disordered rows of cortical units near the bottom of the notch in a N-cell. An upper focal level (A) and a lower focal level (B) of the same cell showing a blank space at the rearranging sites of the longitudinal rows of cortical units (arrow head). Directions are indicated by arrows (a: anterior, p: posterior). ×1,000.
notch deepened (Figs. 3, 4). During this time, dislocation of the longitudinal rows of the cortical units and bald areas devoid of basal bodies in the two ventral surfaces were observed (Fig. 5).

DISCUSSION

As mentioned above, the cortical pattern of *Paramecium* is retained through cell divisions by a mechanism that pre-existing cortical structures determine the following pattern [1, 11, 25]. This rule is applicable to the doublets at least in the early stage of its clonal life span. However, doublets also has a tendency to return to singlets. So, the process of reversion from doublets to singlets contains a mechanism of deviation from the rule of the cortical morphogenesis of *Paramecium*.

The most typical way to return from doublets to singlets in *P. tetraurelia* has been known as an asymmetric reduction in numbers of the cortical unit-rows and in distance on one side between two OAs [1, 11, 15, 18, 26]. Another type of reversion is also reported in *P. tetraurelia* [27] but it is not main route of the reversion. In *P. bursaria*, the notch formation may be the only way to reverse from the doublet to the singlet. Two OAs are located approximately 180° throughout the reversion process. Cell surface of *Paramecium* is composed of thousands of cortical units which contain one or two ciliary basal bodies and their accessorys. Longitudinal increase of cell surface is accomplished by proliferation of the cortical units [1, 8, 28]. Cortical units proliferate most frequently at the middle portion of the cell-body just prior to cell division, but very rarely at near the anterior and the posterior tips [8, 28, 29, 30]. We saw a P-cell was transformed to a N-cell by the fourth cell division (Table 2). During this period, it’s most unlikely that the low rate of addition of the cortical units to the anterior surface contributes to form or deepen the notch in the line of the successive proters of the P- and N-cells. Because a rearrangement of the cortical units takes place during the reversion, we must consider some possible mechanical forces, which may be generated by elongation of cortical cytoskeletal components [8, 31] and/or by a torsion of cellular axes observed in N-cells (Figs. 4, 5), to explain the cause of notch formation.

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REFERENCES

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