Establishment of Septum Orientation in a Morphologically Altered Fission Yeast, \textit{Schizosaccharomyces pombe}

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Among the spheroidal fission-yeast \textit{(Schizosaccharomyces pombe)} cells resulting from aculeacin A treatment were found cells whose putative growth axis and polarity differed from those of their progenitor, that is they were changed in the orientation of their septum. The ratio of cell length (measured perpendicular to the septum plane) to septum diameter of these cells equalled or exceeded unity without exception, whether the septum orientation changed or not and whether the cellular shape was spherical, spheroidal or cylindrical. From these results we conclude that the septum is always oriented perpendicular to the plane including the longest axis of the cell even if the morphology is irregular or the new septum has become perpendicular to the previous septum. There is no cellular region forbidden to new septa.

\textbf{INTRODUCTION}

The septum at division not only divides a cell into two cells but also indicates an axis of the cell. The position and orientation of the septum, therefore, are of fundamental morphogenetic importance for subsequent cellular growth, especially for differentiation and morphogenesis in plants. Cells of the sausage-shaped fission yeast \textit{Schizosaccharomyces pombe} usually grow cylindrically and form their transverse septa near the middle of the major axis. However, questions of how the septum is oriented or whether some rules exist related to axial orientation of the cell remain unanswered, especially if the cellular morphology is altered.

Fission-yeast cells grow spherically in the presence of low doses of aculeacin A (Acu), an antifungal antibiotic (Miyata \textit{et al.}, 1985). When the temperature-sensitive mutant \textit{cdc} 10-121 \textit{h} was cultured at the restrictive temperature in the presence of Acu, the cellular morphology changed to ‘round-bottomed flask (RBF) like’ (see Fig. 2h). When these RBF-like cells were transferred to fresh medium without Acu and cultured at the permissive temperature, the cells began to divide after recovery growth for about 3 h – the ‘recovery system’. The first division of an RBF-like cell showed the remarkable feature that its septum was formed without exception at the boundary plane between the spheroidal and the cylindrical region: the RBF-like cell divided into a spheroidal and a cylindrical sib. The second division divided the spheroidal and the cylindrical progeny into two spheroidal and two cylindrical sibs, respectively. The alterations of morphology caused by Acu were simple and reversible; the wall components were changed, resulting in the alteration of cellular morphology, but the growth of the protoplasm of the RBF-like cells seemed to equal that of the controls (Miyata \textit{et al.}, 1985, 1986). RBF-like cells in the recovery system are therefore convenient for investigating the relationship between septation and cellular morphology. Using the recovery system, we found a correlation between the

\textbf{Abbreviations:} Acu, aculeacin A; RBF, round-bottomed flask; CL, cell length; SD, septum diameter.
aberrant morphology of the cell and the asymmetry of septum placement, i.e. the greater the deviation from the cylindrical form, the greater the degree of asymmetry (Miyata et al., 1986).

Here another new system, the ‘non-recovery system’ is introduced, in which the RBF-like cells are recultured with a low dose of Acu at the permissive temperature. This non-recovery culture of RBF-shaped fission-yeast cells seems an elegant system for examining septum and axial orientation. We report here observations on the orientation of the septum and the change of major axis in morphologically altered fission-yeast cells, and discuss the relationship between cell morphology and the subsequent establishment of the septum.

METHODS

Schizosaccharomyces pombe cdc 10-121 h-1, a temperature-sensitive mutant of wild-type strain 972h+ (Nurse et al., 1976), was cultured in EMM2 (a minimal salts medium; Mitchison, 1970) as before (Miyata et al., 1986). The RBF-like cells (Fig. 2h) were obtained by treating cells (cultured for 3 h at 35 °C) with Acu (1 μg ml⁻¹) for 3 h at 35 °C as described in detail previously (Miyata et al., 1985). Control cells were cultured under the same conditions without Acu. The uni-, bi- and tri-septation indices are the percentages of ‘cells’ with one, two and three visible septa, respectively. Methods of measurement of lengths were given by Miyata et al. (1986). Acu (1 mg) was dissolved in 2 ml methanol and stored at -25 °C (Mizoguchi et al., 1977; Miyata et al., 1980).

RESULTS

When RBF-like cells (Fig. 2h) were transferred to fresh medium without Acu at a cell population density of 3·0 × 10⁶ cells ml⁻¹ and cultured by shaking at 25 °C, the cells continued to grow at their spherical and/or cylindrical end(s) and then divided synchronously (three synchronous divisions in 10 h; Fig. 1a; Miyata et al., 1986). Thus this ‘recovery system’ is a kind of induction synchrony. Using it, we were able to quantify symmetry and asymmetry of division of a variety of septate cells (Miyata et al., 1986). The first septum was formed at the boundary plane between the spheroidal and the cylindrical region as described previously. At the second cycle, some cells formed their second septum before completing fission of the first septum. This is also inferred from the fact the minima of the uni-septation index curve in RBF-like cells are higher than those of the control (Fig. 1a). Nevertheless, the frequency of bi-septate cells or tri-septate cells was not higher than 5% (see Fig. 1a). Among such cells we occasionally found spheroidal cells with their septum orientation changed by as much as 90° from the original one at the second cell cycle (Fig. 2i–I).

In an attempt to determine the rules governing the cell’s behaviour in establishing a new axis at cell division, we examined a different system in which we could quantitatively observe dividing cells (a) with altered morphology (spherical, quite different from cylindrical), and/or (b) with changing axis (noted because sibs were inseparable through failed fission during at least two cell cycles). This is a ‘non-recovery system’, in which the RBF-like cells were transferred to fresh medium containing Acu (1 μg ml⁻¹), instead of the medium without Acu in the recovery system, and cultured at 25 °C (Fig. 1b). Owing to the presence of Acu, the RBF-like cells in the non-recovery system continued to grow spherically again at the spherical and/or cylindrical end(s) (see Fig. 2m). After 8 h, their cellular morphologies were RBF, dumbbell, spheroidal and cylindrical shapes; the uni-, bi- and tri-septation indices became constant, i.e. 33, 17 and 6%, respectively [compare Fig. 1b with Fig. 1a (the absence of Acu); Fig. 2i–m]. After 20 h, the typical survivors – about 30% of cells had lysed – were spheroidal (Fig. 2a–e); 37% of the cells had a septum or septa (532/1453); the uni-, bi- and tri-septation indices were 27, 7 and 3%, respectively; half of the multi-septate cells had changed the orientation of their septa. These septation indices were much higher than the 5% found after 20 h in the recovery system. The comparison shows that the cells in the non-recovery system were delayed in both septation and fission. However, there were no cells with more than three septa.

The spheroidal cells present after 20 h of culture – about 25% in the recovery system and about 98% in the non-recovery system – can recover cylindrical morphology after repeated transfer to the Acu-free medium.

In a morphometric analysis of septum orientation, we calculated the ratio of cell length (CL), defined as being perpendicular to the septum plane, versus septum diameter (SD) in cells from
Fig. 1. Septum formation and fission inferred from the uni-septate (○, control cylindrical cells; ●, RBF-like cells), bi-septate (△) and tri-septate (▲) indices. (a) The recovery system; the control cells were only uni-septate; note the synchronous division as an induction synchrony and that the minima of the curve of the progeny of RBF-like cells are higher than those of the control. (b) The non-recovery system; note that 45% of the cells are uni-septate, 15% are bi-septate, 15% are tri-septate and 25% non-septate after 6.5 h.

Fig. 2. Photographs of cells in various types of axial orientations. (a) A spherical cell after 20 h in the non-recovery system; (b–e) spheroidal, axis-changed cells after 20 h in the non-recovery system; (f) a spheroidal (pear- or lemon-shaped) cell after 6 h in the recovery system; (g) a control cell after 6 h in the recovery system; (h) an RBF-like cell at zero time (same in both the systems); (i–r) axis-changed progenies of RBF-like cells after 6 h in the recovery system; (m) a dumbbell-shaped cell after 6.5 h in the non-recovery system.
Fig. 3. Distribution of the ratio of cell length (CL), of an axis defined as being at right angles to septal plane, versus septum diameter (SD). (a) Spherical cells (like the cell shown in Fig. 2a) after 6 h in the recovery system or after 20 h in the non-recovery system; 110 cells were measured and calculated. (b) Spheroidal axis-changed cells (like the cells shown in Fig. 2i–f) after 6 h in the recovery or non-recovery system; 110 cells were measured and calculated. (c) Spheroidal axis-changed cells (like the cells shown in Fig. 2b–e) after 20 h in the non-recovery system; 140 cells were measured and calculated. (d) Spheroidal (pear- or lemon-shaped) cells (like the cell shown in Fig. 2f) after 6 h in the recovery system; 110 cells were measured and calculated. (e) Cylindrical cells (like the cells shown in Fig. 2g) grown for 16 h (mid-exponential phase) in the normal culture (EMM2, 25 °C); 110 cells were measured and calculated.

both the recovery and the non-recovery system (Fig. 3). The CL/SD ratios in various morphological classes from spherical to cylindrical and in various axis-types were examined. The first class comprised spherical uni-septate cells that resulted from the recovery (after 6 h) or non-recovery (after 20 h) system (Fig. 2a). The low value of the ratios (1.00 to 1.40 in Fig. 3a) indicates that the cells were spherical or only slightly ellipsoidal. The narrow distribution indicates that these cells form a single recognizable class regardless of whether they were younger (from the recovery system) or older (from the non-recovery system). The second class comprised spheroidal cells with changed septum orientation which remained in the RBF-like form (Fig. 2i–f). Their CL/SD ratios ranged from 1.00 to 1.80 (Fig. 3b), regardless of the extent of change of septum orientation. The third class comprised the spheroidal, multi-septate cells with changed septum orientation, after 20 h in the non-recovery system (Fig. 2b–e). Their CL/SD ratios ranged from 1.00 to 2.40 (Fig. 3c). These results indicate that the second and third classes of cells identified their longer axis in spite of remaining attached to their sister cell. The fourth class comprised the pear- or lemon-shaped, uni-septate cells found after 6 h in the recovery system (Fig. 2f). Their CL/SD ratios ranged from 1.00 to 4.20 (Fig. 3d), a broad range
indicating much variation of the basic shape. However, most of the ratios were under 2.80, less than those of the majority of the cylindrical cells, whose ratios ranged from 2.40 to 5.80 (Fig. 3e). All of the above classes stand in strong morphometric contrast to the cylindrical cells (Fig. 2g).

The ratios of all classes equalled or exceeded unity without exception (a combined total of 580 cells was measured). Thus, the septum was always oriented perpendicular to the long axis, the longer 'diameter' if spheroidal, of a cell, even if the morphology was irregular and even if the new septum became perpendicular to the previous septum.

**DISCUSSION**

Septa of fission yeasts are rarely laid down near or across old division scars. Johnson et al. (1982) suggested that there exists a cell-wall region near old scars that is forbidden to new septa. They did illustrate a scar crossed by the septum of a succeeding division, but because this seemed exceptional, having been observed only for germinating spores (and only in four cases), they inclined to the conclusion that the freshly germinated spore was a special case of lowered constraints.

In contrast, septa crossing pre-existing septa are common among the dividing spheroidal products of RBF-like cells. The dividing spheroidal cell also draws attention to a rule that might have gone unnoticed because of its commonplace nature: the septum is laid down transaxially, perpendicular to the major axis of the fission-yeast cell. This rule applies even if that new septum must bisect an old scar or a septum not yet subjected to fission. From these observations we conclude that there is no cellular region forbidden to new septa, but only a simple rule that the septum is formed perpendicular to the major axis, the longer 'diameter' if spheroidal, of the cell.

Having recorded perpendicular septa in 250/470 spheroidal cells, we are satisfied that they are a completely normal response dictated by an unusual morphology. If so, they might be seen in other circumstances. Branching, multi-septate cells (Johnson & McDonald, 1983) seemed a possibility, and in fact the first published illustration of *Schizosaccharomyces pombe* cells (Lindner, 1893) shows a branching 'cell' with crossed, perpendicular septa.

Similar observations have been noted for the growth and cell division of the polypodious fern protonema. Protonemal growth is by elongation and cell division, both of which usually take place only in the apical cells. The formation of the new cell plate usually occurs at a constant distance from the tip where the nucleus of growing apical cells has been localized (Ito, 1969). When elongation growth of the apical cell is repressed under blue light just after cell division, its growth axis changes so that the orientation of the new cell plate becomes perpendicular to the previous cell plate. Hence, the apical cell of the protonema monitors the relationship between the long axis and the short axis in cell plate formation (personal communication to H. M. from Dr Michio Ito).

How do the fern protonema apical cell and the fission-yeast cell perceive their long axis and then place their septa at right angles to it? We speculate that the crossed septum is formed perpendicular to the longest spindle microtubules of the mitotic nucleus; thus septum formation is closely related to mitosis. Many *cdc* mutants are unable to initiate septation when mitosis is blocked (Nurse & Fantes, 1981; Nasmyth & Nurse, 1981), and mutants *cdc* 13.117 and *cdc* 16.116 form their septa abnormally between the dividing or divided nuclei (Nurse & Fantes, 1981; Minet et al., 1979). Mitosis in *Schizosaccharomyces pombe* (McCully & Robinow, 1971; Tanaka & Kanbe, 1986) is essentially identical with that in *Saccharomyces cerevisiae* (Peterson & Ris, 1976): the double SPBs (spindle pole bodies), which have two classes of microtubules (long and short), move apart from each other on the nuclear periphery, while elongating their microtubules, and become almost opposed to each other. We infer that the longest spindle microtubules in our spheroidal cells also become oriented to a position within the cell that allows them their greatest length (along a new major axis), possibly by the extension force of the mitotic spindle (Tanaka & Kanbe, 1986) or even involving guidance by cytoplasmic microtubules. The extended microtubules thereby guide the formation of the septum, but we recognize that the new axis might already have been established before mitosis, possibly by the cytoplasmic microtubules.
In conclusion, dividing fission-yeast cells with unusual shape identify their major (longer) axis and place their septum transverse to that long axis near its midpoint, even though those septa were sited asymmetrically by a length or a volume parameter (Miyata et al., 1986). It is reasonable to believe that fission-yeast cells with the usual, cylindrical shape behave at division in precisely the same way.

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REFERENCES


