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Isolation and Characterization of *pos* Mutants Defective in Correct Positioning of Septum in *Schizosaccharomyces pombe*

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ABSTRACT—We have isolated mutants of fission yeast defective in correct positioning of septum. In visual screening, we obtained 16 clones showing unequal septation at restrictive temperature, which were classified into three complementation groups. At restrictive temperature, all the mutants underwent nuclear division normally. In cytokinesis, however, a contractile ring was formed at the site independent of the mitotic spindle. These results suggest that positional information for cytokinesis are not accurately transmitted to the cell equator. Furthermore, all the mutants frequently displayed incorrect orientation and/or distortion of septum, which suggests that the septum positioning is closely related to correct orientation and organization of septum.

INTRODUCTION

In cytokinesis of animal cells, a contractile ring consisting mainly of actin bisects a cell at an appropriate position. It usually occurs along the equatorial plane of the cell and the position is strictly defined, insuring that chromosomes segregated by the mitotic spindle become equally contained in each daughter cell.

The molecular mechanism of determination of the division plane is one of central questions in cytokinesis. Many experiments have demonstrated that the placement of the division plane is connected with preceding mitosis, and mitotic apparatus specifies the position of the cleavage furrow at a critical period in the cell cycle, reviewed in (Mabuchi, 1986). A signal stimulating cell cortex to form the cleavage furrow is called "cleavage stimulus". It may be transmitted from the cytoplasm to the cortex via the astral microtubules. The nature of the cleavage stimulus is unknown for over 30 years. The study on the positioning mechanism of division plane is thus important for understanding linkage between mitosis and cytokinesis and also various cell motilities based on actin filaments and/or microtubules.

Fission yeast, *Schizosaccharomyces pombe*, is attractive as a model system for investigating cytokinesis. Excellent molecular genetic techniques can be applied, and it is found that fission yeast has similar cytokinetic machinery to animal cells, forming a contractile ring at the cell equator (Kanbe *et al.*, 1989). Moreover, it is also shown that an actin-binding protein, profilin, is involved in cytokinesis in fission yeast (Balasubramanian *et al.*, 1994) like in the organisms undergoing animal cell-type of cytokinesis (Edamatsu *et al.*, 1992; Haugwitz *et al.*, 1994). Several mutants concerning cytokinesis have been isolated, reviewed in (Fankhauser and Simanis, 1994), but no mutant defective in correct positioning of septum has been reported so far.

In this study, we have succeeded in isolating *pos* mutants (called for *pos*itioning of septum) displaying incorrect positioning of septum in fission yeast.

MATERIALS AND METHODS

Schizosaccharomyces pombe strains used were 972h^-, JY6 (h^+ *leu1 his2*), JY333 (h^- *leu1 ade6-M216*) and JY336 (h^+ *leu1 ade6-M210*). In isolation of *pos* mutants, a 972h^- strain was first mutagenized by N-methyl-N'-nitro-N-nitrosoguanidine (Uemura and Yanagida, 1984). After incubated at 25°C for several days for recovery, the cells were transferred to 36°C for 5 to 6 hr to be visually screened by a microscope (Olympus CK) equipped with a micromanipulator (Narishige MN-151). The mutants showing unequal septation with a high frequency were back-crossed to JY6 strain three times prior to analyses.

Standard genetic procedures for linkage analysis, tetrad analysis and analysis of pos^+/pos^- diploids are carried out according to (Alfa *et al.*, 1993). In pairwise crosses of the linkage analysis and construction of pos^+/pos^- diploids, the mutants were first crossed to JY333 (h^- *leu1 ade6-M216*) or JY336 (h^+ *leu1 ade6-M210*) to obtain stable diploids using intragenic complementation between the two *ade6* allele and were then used in the analyses.

To examine the phenotype of the mutants, synchronous cul-

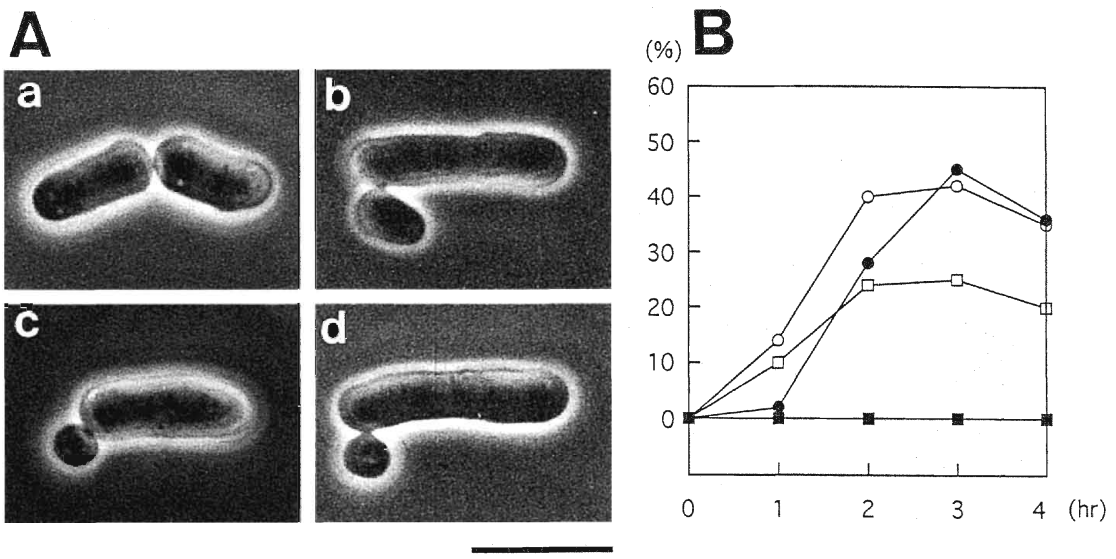

Fig. 1. Unequal septation of *pos* mutants. (A) Phase contrast micrographs of *pos* mutants showing unequal septation, (a) wild-type, (b) *pos1*, (c) *pos2*, (d) *pos3*. Bar, 10 μm. (B) Percentages of cells showing unequal septation in all septated cells. Synchronous cultures were incubated at 36°C. Samples were taken at one-hour intervals, stained with Calcofluor and analyzed by a fluorescence microscopy. Unequal septation is defined as one occurring at less than one third of the cell length. (■) wild-type, (○) *pos1*, (□) *pos2*, (●) *pos3*.

tures were prepared on lactose gradients by the method of Mitchison (Mitchison and Vincent, 1965). The procedure for DAPI (4',6'-diamidino-2-phenylindole, Sigma) and Calcofluor (fluorescent brightener 28, Sigma) staining, and immunofluorescence microscopy were followed as described in (Alfa *et al.*, 1993). For microtubule staining, anti-alpha-tubulin monoclonal antibody (Sigma) and FITC-conjugated anti-mouse IgG polyclonal antibody (Bio Source International) were used, and rhodamine-conjugated phalloidin (Sigma) was used for actin staining.

RESULTS AND DISCUSSION

Mutants defective in correct positioning of septum must produce big and small daughter cells resulting from unequal septation and not be lethal. We therefore carried out the visual screening for isolation of the mutants rather than that based on lethality at restrictive temperature widely used for isolating cell cycle mutants such as *cdc* mutants.

First of all, a haploid 972h^- strain was mutagenized by N-methyl-N'-nitro-N-nitrosoguanidine. About one thouthand of cells displaying unequal septation were visually selected by a micromanipulator. Among the cells, 16 strains frequently displayed unequal septation at restrictive temperature (Fig. 1A). After back-crossed three times, all the strains showed 2:2 segregation of the temperature-sensitive phenotype in tetrad analysis, and thus the phenotype is caused by a single mutation. All pos^+/pos^- diploids were normal phenotype, so the mutations were recessive. Random spore analysis in linkage analysis demonstrated that the mutants were classified into three linkage groups, *pos1-pos3* (Table 1). The result were confirmed by pairwise crosses among the mutants. Although most of the mutations (14/16) were on the

Table 1. Linkage analysis of *pos* mutants

strain	11A7	11B1	11B8	11C1	11C3	16A8	17C4	17C9	18A6	18B4	18B6	18C9	18C10	18F3	18F5	locus
11A6	N	N	N	N	N	N	N	N	N	Y	N	N	N	Y	N	*pos1*
11A7		N	N	N	N	N	N	N	N	Y	N	N	N	Y	N	*pos1*
11B1			N	N	N	N	N	N	N	Y	N	N	N	Y	N	*pos1*
11B8				N	N	N	N	N	N	Y	N	N	N	Y	N	*pos1*
11C1					N	N	N	N	N	Y	N	N	N	Y	N	*pos1*
11C3						N	N	N	N	Y	N	N	N	Y	N	*pos1*
16A8							N	N	N	Y	N	N	N	Y	N	*pos1*
17C4								N	N	Y	N	N	N	Y	N	*pos1*
17C9									N	Y	N	N	N	Y	N	*pos1*
18A6										Y	N	N	N	Y	N	*pos1*
18B4											Y	Y	Y	Y	Y	*pos2*
18B6												N	N	Y	N	*pos1*
18C9													N	Y	N	*pos1*
18C10														Y	N	*pos1*
18F3															Y	*pos3*
18F5																*pos1*

Y, ≥0.05% wild-type recombinants upon random spore analysis; N,<0.05% wild-type recombinants upon random spore analysis

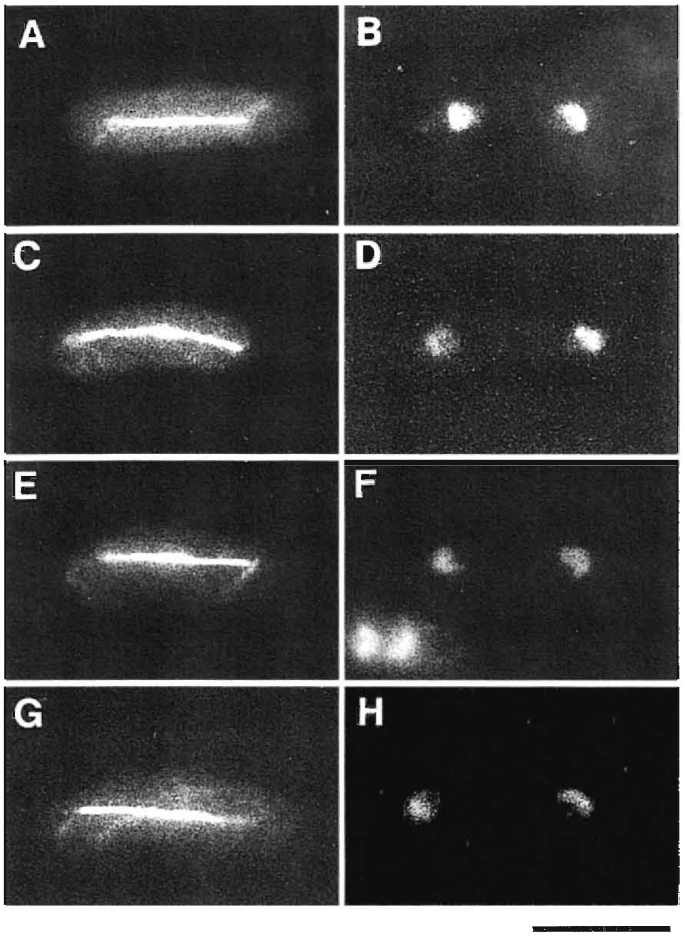

Fig. 2. Mitosis of *pos* mutants at restrictive temperature. Cells exponentially grown at 25°C were incubated at 36°C for 2.5 hr. Cells were stained by anti-alpha-tubulin antibody (A, C, E, G) and DAPI (B, D, F, H). (A, B) wild-type; (C, D) *pos1*; (E, F) *pos2*; (G, H) *pos3*. Bar, 10 μm.

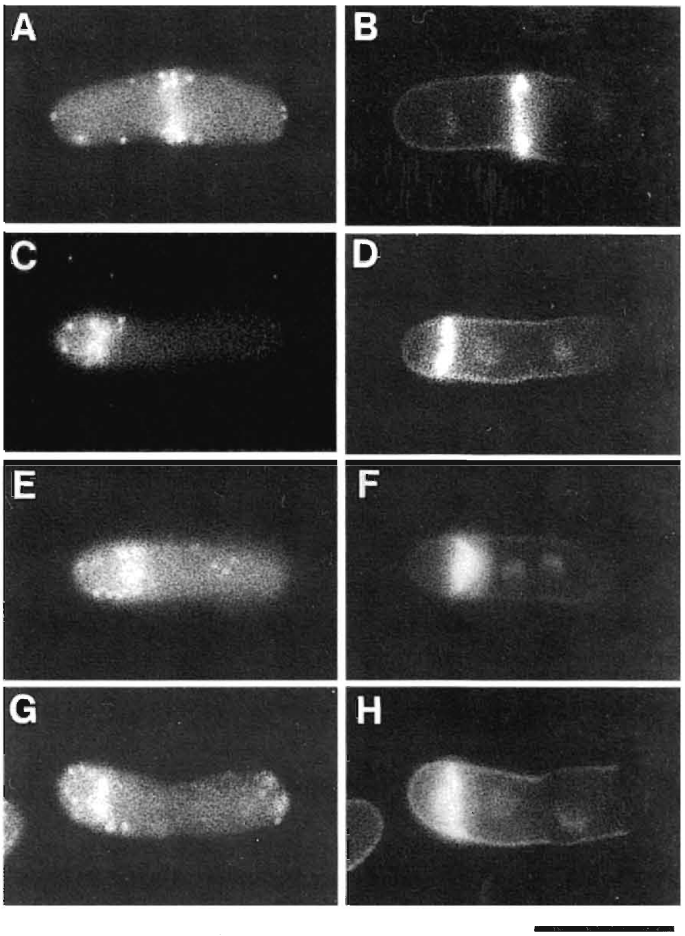

Fig. 3. Cytokinesis of *pos* mutants at restrictive temperature. Cells exponentially grown at 25°C were incubated at 36°C for 2.5 hr. Cells were stained by rhodamine-conjugated phalloidin (A, C, E, G) and DAPI/ Calcofluor (B, D, F, H). (A, B) wild-type; (C, D) *pos1*; (E, F) *pos2*; (G, H) *pos3*. Bar, 10 μm.

same locus, the reason is not known.

In examining the phenotype of *pos* mutants, the septum was distant from the normal equatorial position with a high frequency, 42% (*pos1*), 25% (*pos2*) and 45% (*pos3*) at 3 hr after temperature shift-up (Fig. 1B). The position is quite divergent from the cell equator to the pole.

We then examined mitosis and cytokinesis of *pos* mutants by an immunofluorescence microscopy. At restrictive temperature, *pos1-pos3* were able to form normal mitotic spindles at the cell center like a wild-type cell, which separated chromosomes and elongated to complete nuclear division (Fig. 2). In cytokinesis, however, a contractile ring was located at the site independent of the mitotic spindle, so it was frequently distant from the cell equator (Fig. 3). These observations suggest that the positional information for cytokinesis is not accurately transmitted to cortex of the cell equator in the mutants.

Moreover, it was found that the cells unequally septated often exhibited wrong orientation and/or distortion of septum at restrictive temperature. This irregular septation was also observed in equally septated cells. In the cells of irregular septation, the septum was greatly tilted from the transverse plane (Fig. 4A, c) or even longitudinal to the long axis of the cell (Fig. 4A, b), and/or wavy septum was also observed (Fig. 4A, d). As shown in Fig. 4B, 55-70% of septated cells formed such irregular septum at 3 hr after temperature shift-up. No significant difference is detected among three *pos* mutants. These results suggest the possibility that the septum positioning is intimately related to the correct orientation and organization of septum. In spite of such irregular septation, the mutants were able to form colony at restrictive temperature, although the growth rate was severely reduced.

In this study, we carried out visual screening for isolating *pos* mutants influencing septum positioning, because the mutants are not lethal at restrictive temperature unlike *cdc* mutants. Recently, a similar method was used for isolating the mutants displaying abnormal morphologies, spherical (*orb1-5*), T-shaped (*tea1*), or banana-shaped (*ban1*) (Snell and Nurse, 1994). Thus the visual screening can be widely

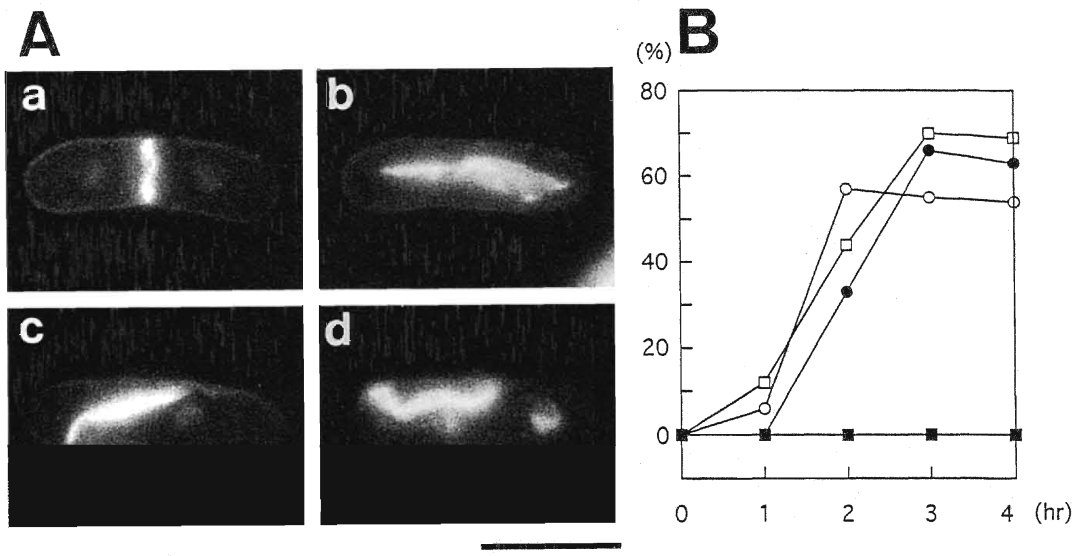

Fig. 4. Incorrect orientation and/or distortion of septum in *pos* mutants. Synchronous cultures were incubated at 36°C. Samples were taken at one-hour intervals, stained with DAPI and Calcofluor, and analyzed by a fluorescence microscopy. (A) Fluorescence micrographs of *pos* mutants with irregular septum, (a) wild-type, (b) *pos1*, (c) *pos2*, (d) *pos3*. Bar, 10 μm. (B) Percentages of cells with incorrect orientation or distortion of septum in all septated cells. Septum of incorrect orientation is defined as one tilted to the cell axis at more than half rectangle. (■) wild-type, (○) *pos1*, (□) *pos2*, (●) *pos3*.

applied for isolating various types of mutants.

The *pos* mutants exhibit quite different phenotypes from the other mutants influencing septum formation isolated so far. Those mutants, mainly isolated as *cdc* mutants, can not form septum (*cdc7, cdc11, cdc14* and *cdc15*) (Nurse *et al.*, 1976), deposit disorganized septum material (*cdc3, cdc4, cdc8* and *cdc12*) (Streiblova *et al.*, 1984), show multiple septum (*cdc16*) (Minet *et al.*, 1979), or can not or can partially separate in the septation (*sep1* and *spl1*) (Spiczki *et al.*, 1993). In those mutants capable of forming septum, the septum positioning is not defective. Therefore, it is unlikely that the gene products are directly implicated in the positioning of septum.

In transition from mitosis to cytokinesis, many events occur concerning initiation of cytokinesis as well as positioning of the division plane. Recently, it is suggested that protein phosphorylation regulates the initiation events, using the inhibitor of myosin light chain kinase, ML-9 (Mabuchi and Takano-Ohmuro, 1990) and that of protein phosphatases, calyculin A (Tosuji *et al.*, 1992). It is also suggested that a small G-protein, rho, is involved in the events (Kishi *et al.*, 1993; Mabuchi *et al.*, 1993). Furthermore, uncoordinated cytokinesis cuts an undivided nucleus in the *cut* mutants, thus *cut* gene products may play a role in the initiation process (Hirano *et al.*, 1986; Samijima *et al.*, 1993). Initiation of furrow formation occurs at the site defined by preceding positioning process, so it is likely to interact between the factors concerning positioning and the initiation processes. The investigation of *pos* gene products, combined with that of the factor for the initiation, will lead to elucidating coordination between mitosis and cytokinesis. We are now attempting to identify *pos* gene products using molecular genetic techniques.

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