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Mycelial and syncytial growth in *Schizosaccharomyces pombe* induced by novel septation mutations

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SUMMARY

Mutation in the gene *sep1*$^+$ of the unicellular fission yeast *Schizosaccharomyces pombe* impairs cell separation after cytokinesis and confers a branching mycelial morphology. The mutant is not defective in cell wall β-glucanase activity but shows increased sensitivity to Ca^{2+} and Mg^{2+}, and increased resistance to the microtubule inhibitor benomyl. The mycelial growth of *sep1-1* provides a convenient method for the examination of the polar growth pattern and for pedigree analysis as demonstrated by the segregation of mating types in the homothallic microhyphae. *sep1* is closely linked to *ade1* (0.94 cM) on the right arm of chromosome II. The ts mutation *spl1-1* confers a bent cell shape and causes aberrant septum formation at the restrictive temperature. *sep1*$^+$ and *spl1*$^+$ perform closely related functions as their mutant alleles interact with each other and with another septation mutant *cdc4-8*. These functions may overlap with certain cytoskeletal processes and with the determination of cell polarity because the triple mutant forms huge multinucleate syncytia with promiscuous branching and rare septum formation.

Key words: cytokinesis, septation, cell growth, polarity, mating-type switching, cytoskeleton, fission yeast

INTRODUCTION

The cylindrical cells of the unicellular yeast *Schizosaccharomyces pombe* grow exclusively, but not equally, at the poles, without detectable change in the cell radius. This is thought to be the consequence of the polarity of the cytoskeleton and of intracellular traffic that direct enzymes and wall precursors to the cell tips (Marks et al., 1986). At the beginning of the cell cycle, growth is confined almost exclusively to the "old end", the end that also existed in the previous cycle. At a point in G_2 termed NETO, growth at the "new end" from the septum, is accelerated (Mitchison and Nurse, 1985). Cell extension is maintained until 0.75 of the cell cycle is completed, at which point growth ceases and the sequence of mitosis, cytokinesis and cell separation begins (for a review, see Mitchison, 1989).

Strains containing lethal ts$^-$ mutations in certain *cdc* genes are unable to form septa, but their nuclear cycles continue, resulting in multinucleate cells (Nurse et al., 1976). Those that do not stop growing but increase in cell length are thought to be blocked before the transition point from the growth phase to the constant volume phase (early septation mutants: *cdc7, 11, 14* and *15*). In their cells no septal material can be detected (Nurse et al., 1976; Mateos and Dominguez, 1991). Mutants defective in four other genes (late septation mutants: *cdc3, -4, -8* and *-12*) increase in width rather than in length when shifted to the restrictive temperature and form disorganized depositions of septal material (Nurse et al., 1976; Streiblova et al., 1984; Mateos and Dominguez, 1991). *cdc16* represents a particular sort of septation mutant; its cells undergo uncontrolled multiple septation at the non-permissive temperature (Minet et al., 1979). In a recent paper, Marks et al. (1992) demonstrated that the products of the *cdc7, cdc11, cdc14* and *cdc16* genes interact and may regulate the activity of the *cdc15* gene product.

A series of mitotic mutants described recently are also unable to carry out cytokinesis (Yanagida, 1989). In *nuc* mutants a septum is formed but cell separation is blocked, whereas inactivation of the *nda* and *dis* genes prevents both septation and cell separation. Interestingly, the inability to complete mitosis does not necessarily abolish septum formation. Strains defective in *top* or *cut* genes are not inhibited in septation. This diversity of the mitotic mutants indicates that cytokinesis in not dependent on the completion of mitosis but requires certain mitotic functions. Consistent with this, the cells treated with the microtubular drug benomyl or with leptomycin B, an antifungal antibiotic, were blocked in nuclear division but elongated and had one or several cell plates (Hamamoto et al., 1985; Walker, 1992). Interestingly, the disruption of *kin1*$^+$ (a putative protein kinase gene) also results in poor cell separation at low temperatures (Levin and Bishop, 1990).

Accomplishment of the division process seems to be dependent on physiological factors that can be influenced by culturing conditions. Under certain chemostat conditions multiseptate, ephemeral and sometimes branched hyphae appeared (Johnson et al., 1982). Adding aniline blue fluorochrome, a dye having a strong affinity for β-(1→3)-glucans, generated bi-septate and tri-septate cells (Pancaldi et

al., 1989). These phenomena are reversible, the multiseptate growth pattern ceasing as soon as the culturing conditions were changed.

Here we show that a stable hyphal form (mycelium) can be induced by a single nuclear mutation (*sep1-1*). As cell separation is impaired in the mutant, it forms branching hyphae composed of up to 15 cells. Branching is due to the bipolar growth pattern of the cells, which is not affected by the mutation; neither nuclear division nor sexual processes are affected. Another mutation (*spl1-1*), which is temperature sensitive, confers a bent cell shape and blocks the fission process. Both mutations interact with the late septation mutation *cdc4-8*, so that the triple mutant is almost completely deficient in septum formation and forms multinucleate syncytia with disturbed cell polarity even at the permissive temperature.

MATERIALS AND METHODS

Strains and media

All strains used are derived from three wild-type strains of *S. pombe*/synonym of *S. pombe* var. *pombe* (Sipiczki et al., 1982)/, *L972* h^-, *L975* h^+ and *L968* h^{90}. The *cdc*$^-$ mutants were kindly provided by Peter Fantes. The auxotrophic strains used for crosses and mapping were received from the Bern collection. Yeast-extract agar (YEA), yeast-extract liquid (YEL), minimal agar (MMA), and malt-extract agar (MEA) were described previously (Sipiczki and Ferenczy, 1977). SPA is a synthetic sporulation medium (Gutz et al., 1974). YEPG is YEL supplemented with 1% peptone.

Sensitivity to cations and drugs was tested at 25˚C, either in YEL or on YEA supplemented with various concentrations of the salts, caffeine, benomyl (methyl-1-(butylcarbamoyl)-benzimidazole-2-yl-carbamate) or *p*-fluoro-phenylalanine. For determination of mitotic segregation, heterozygous non-sporulating diploids were constructed by protoplast fusion, incubated on YEA, YEA + 0.6 mg/ml *p*-fluoro-phenylalanine and YEA + 8 μg/ml benomyl at 25˚C. After four days samples were taken and spread on YEA. The colonies growing at 25˚C were replica-plated on MMA, and the percentage of auxotrophic segregants was determined.

Genetic techniques

Diploids were constructed by protoplast fusion (Sipiczki et al., 1985). All other genetic methods, including tetrad analysis and mapping, were essentially the same as those described by Gutz et al. (1974).

Microscopic techniques

Growth of individual cells was followed by time-lapse photomicrography. Cells from stationary phase cultures were plated onto a microscopic slide with a thin film of YEA. The preparation was then covered with a coverglass, placed in a Petri dish and examined by phase-contrast microscopy at 25˚C. The cells were photographed every 15-20 minutes. Sporulation of homothallic microhyphae was also examined *in situ* on thin agar surface, but the medium was SPA.

Nuclei were visualized with DAPI (4′,6-diamidino-2-phenylindole) as described by Moreno et al. (1991). Calcofluor staining was carried out according to Mitchison and Nurse (1985).

Glucanase assay

The β-(1-3)-glucanase activity of the cell wall was determined as described by Fleet and Phaff (1974). Cells were cultivated in YEPG at 30˚C. For glucanase assays, samples were taken after 7 h, 12 h and 24 h of incubation.

RESULTS

Recognition of a mycelial mutant

In a programme aimed at isolating spontaneous *S. pombe* mutants impaired in sexual development, we isolated colonies from a homothallic *ura1* strain that stained grey (Sipiczki et al., 1984) instead of black when treated with iodine vapour. One of these was remarkably large and had a rough surface. As revealed by microscopic observation, it contained long multiseptate cells and branching mycelium-like forms (Figs 1 to 3). In the wild-type *S. pombe*, the cells separate after septation, thus becoming independent, and a new round of cell division takes place only after the completion of cell separation. This regulation is apparently broken down in the mutant. As the defect is manifested as incomplete separation, the mutant will be referred to as *sep1-1* throughout this paper.

sep1-1 is a recessive mutation and defines a new gene on chromosome II

The homothallic mutant *sep1-1 ura1* was hybridized by protoplast fusion with heterothallic strains carrying various complementing auxotrophic markers. None of the prototrophic fusants showed the hyphal morphology, demonstrating that the *sep1-1* mutation was recessive.

The fusants sporulated when cultivated on MEA and the *sep*$^-$ phenotype segregated 2:2, as the product of a single mutation. During these crosses, genetic linkage was noted between the *sep1-1* mutation and markers of the middle part of the right arm of chromosome II. Its location was estimated by tetrad analysis: it was found to be 36.1 cM from *his4* (32PD:26T:3NPD), 23.3 cM from *leu3* (21PD:8T:1NPD) and 0.97 cM from *ade1* (101PD:2T:0NPD). Since no other mutation conferring a related phenotype has been mapped into this part of the genome, the *sep1-1* mutation defines a hitherto unknown gene.

Incomplete cell separation in *sep1-1* inhibits tip growth but does not affect the polar extension patterns

In wild-type cells growth begins only when the cytokinesis of the previous cell cycle has been completed and both ends are physically free. In the hyphae of *sep1-1*, however, cell tips remain covered by the uncleaved septa for multiple generation. Exceptions are found only at the old ends of the two “outer” cells. If, as suggested by previous studies (Johnson et al., 1982), cell growth at the new ends can begin only when these ends have differentiated to maturity, one would expect that in the “outer” cells no NETO would take place and that the “inner” cells could not grow at all.

This, however, turned out not to be the case. *sep1-1* cells inoculated into fresh media first formed linear microhyphae composed of four cells, but then the two “inner” cells also started growing at the subapical regions of their old ends. As this happened at lateral positions, the extension resulted in bent cells and concomitant torsions of the microhyphae. In agitated liquid cultures they frequently broke at this stage. Three characteristic stages of microhyphal development in liquid cultures can be seen in Fig. 1C to E.

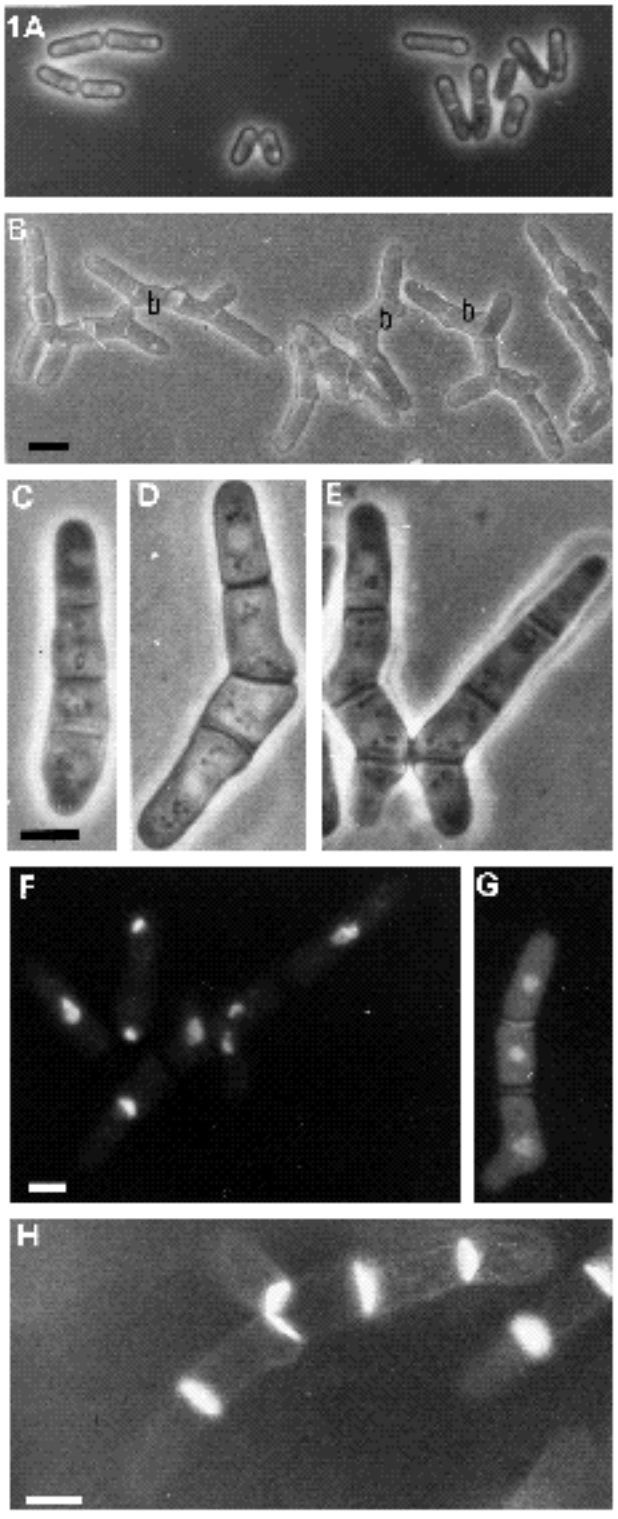

Fig. 1. Morphology of *sep1-1*. Cells of the wild-type L972 (A) and the mutant *sep1-1* (B) grown in YEL. (C), (D) and (E) show three characteristic stages of microhypha development in YEL. Nuclear morphology in DAPI-stained logarithmic phase (F) and stationary phase (G) *sep1-1* microhyphae. (H) Septal morphology in Calcofluor-stained microhyphae. b, cells growing bipolarly. Bars, 5 μm.

We were able to study the growth pattern of the mutant better when cells were grown on the surface of solid media. In Fig. 2 a set of time-lapse photomicrographs is shown. Interestingly, most "outer" cells grew in a monopolar fashion, whereas the "inner" cells frequently extended bipolarly (marked with b; see also marked cells in Fig. 1, and cells 1/11 and 1/21 in Fig. 3C). The bipolar growth followed two patterns: either the old end predominated or both ends grew at equal rates. Three additional pedigrees are illustrated by single photographs taken at more advanced stages of microhyphal development (Fig. 3). In microhyphae A and C, old-end growth dominated the extension of the cells. The third microhypha (B) contains a cell (1/11) that violated this rule, i.e. it started growing at its new end.

Conjugation and sporulation in the microhyphae of *sep1-1*

Wild-type *S. pombe* responds to pheromone signals by extension at the cell end and by bending towards the signal source (Leupold, 1987). The somewhat reduced iodine reaction of the homothallic *sep1-1* colonies (see above) could be an indication of impaired communication ability of the cells caused by the delay in cell tip maturation. To test this possibility, *sep1-1* h^{90} cells were spread on the sporulation meduim SPA and examined microscopically. As shown in Fig. 4, cells conjugated after a few divisions, regardless of their positions in the microhyphae. The "inner cells" developed their conjugation tubes from lateral (usually subapical) positions, and because of the filamentous mode of growth, many of them had to form unusually long tubes to be able to match a partner. Interestingly, the sporulation rate did not seem significantly reduced, so the "grey" iodine reaction cannot be attributed to impaired fertility.

Homothallic cells are known to switch between two alternative mating-types (− and +) in the course of vegetative propagation (for a review of the mechanism, see Klar, 1992). This can be directly demonstrated in *sep1-1*. Fig. 4C shows the conjugation of a homothallic *sep1-1* microhypha with heterothallic wild-type cells: three cells of the microhypha formed zygotes. In most four-celled examples one (− − + −), two (− − + +) or three (− + + +) (but never four) cells conjugated (data not shown). If conjugation took place within the microhyphae, either one ascus (− − + − and + + − +) or two asci (− − + +) were formed. Both patterns can be interpreted in terms of the strand-segregation model (Klar, 1987), which describes the pattern of mating-type switching.

Impaired separation is not due to low β-glucanase activity

The septum of the fission yeast is composed of a primary septum, and a secondary septum on each side of the primary septum. Cell separation occurs by the progressive dissolution (fission) of the primary septum, presumably through the action of β-glucanase (Horisberger and Rouvet-Vauthey, 1985). To learn if the *sep1-1* mutation is defective in this enzyme we assayed β-(1→3)-glucanase activity in cell wall preparations of *sep1-1* h^-. Table 1 shows that the cell wall-associated glucanase activity of *sep1-1* was not significantly different from that of the wild-type. These

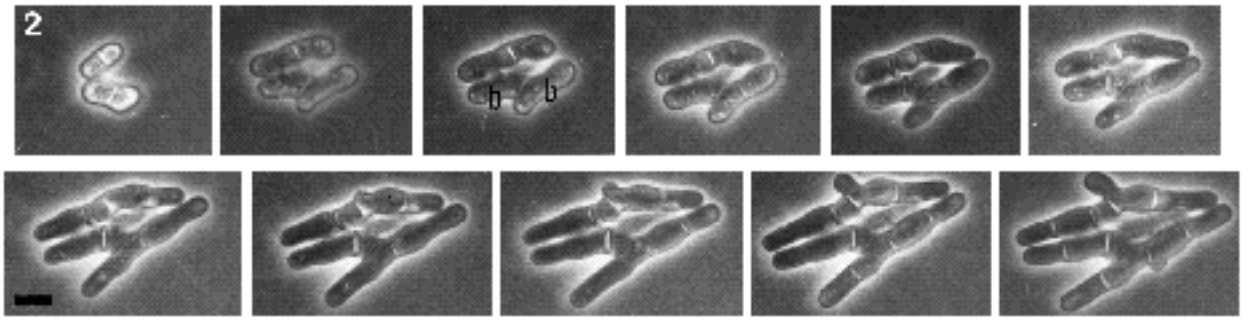

Fig. 2. Time-lapse photomicrographs of *sep1-1* grown on the surface of YEA film. The upper cell line follows the standard growth pattern (old end growth dominates cell extension), whereas the lower cell line shows a bipolar growth pattern in the second round of division (marked with b). Cells were photographed every 45 min. Bar, 5 μm.

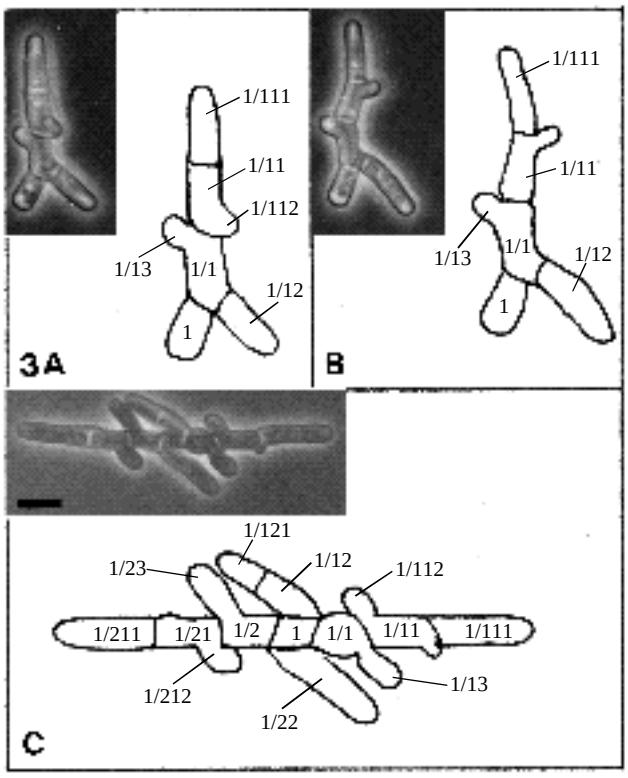

Fig. 3. Growth pattern in microhyphae of *sep1-1*. (A) and (C) Cell lines that grew predominantly at their old ends. (B) A mixed line containing a cell (1/11) that violated the bipolar growth rule, because it started growing at its new end. The tracings show the chronological order of the cell divisions. Number 1 marks the mother cell of the hypha. The sibs are numbered in the order of their birth. For example: 1/1 and 1/2 cells are the first and second daughters of the initial cell, respectively; 1/11, 1/12 and 1/13 are the first, the second and the third sibs of the cell 1/1; etc. Bar, 5 μm.

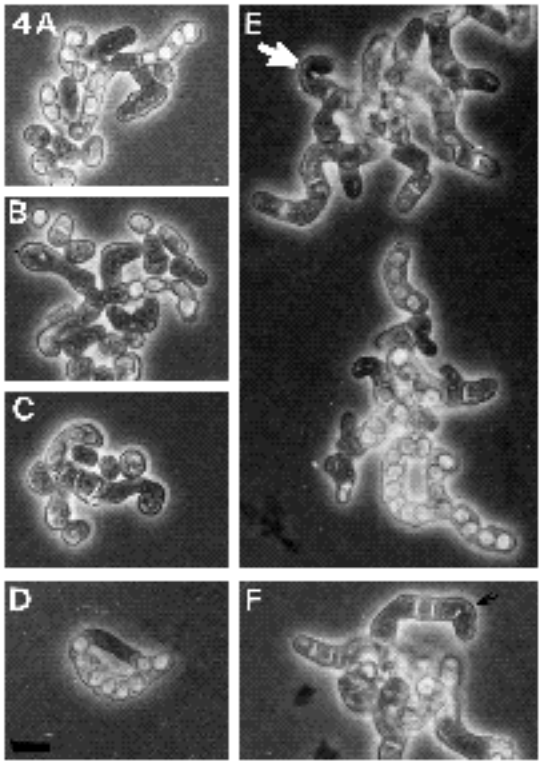

Fig. 4. Conjugation and sporulation of *sep1-1*. Conjugation of a *sep1-1* h^- microhypha with sep^+ h^+ cells (A). Conjugation of a *sep1-1* h^+ microhypha with sep^+ h^- cells (B). Conjugation of a *sep1-1* h^{90} microhypha with sep^+ h^- cells (C) Conjugation and sporulation within *sep1-1* h^{90} microhyphae (D, E and F) Arrows in E and F show conjugation of sister cells. Bar, 5 μm.

results suggest that the separation delay was not due to a β-glucanase defect. The same conclusion could be drawn from treatment of cells with enzymes specific for various cell wall polysaccharides. Neither lyticase (β-glucanase) nor Novozyme (α-glucanase) (Levin and Bishop, 1990) could lyse the septa.

sep1-1 does not affect septum initiation

To examine whether $sep1^+$ functionally interacts with other genes involved in cytokinesis and cell separation, tetrads were isolated from the fusion hybrids constructed between *sep1-1 arg4-8* h^+ and *cdc7-24* h^-, *cdc11-123* h^-, *cdc15-140* h^- (early septation mutants), *cdc3-6* h^-, *cdc4-8* h^- and

Table 1. Comparison of the cell wall β-(1→3)-glucanase activities in *sep1-1 h⁻* and *L972 h⁻*

Strain	Incubation time (h)	β-(1→3)-Glucanase activity (unit/g (dry wt) walls)*
L972 h⁻	7	1.75
	12	2.01
	24	0.96
sep1-1 h⁻	7	1.65
	12	1.85
	24	0.98

*1 unit = 1 μM reducing sugar per minute.

cdc16-116 h⁻ (late septation mutants), *nda3-KM311 leu1 h⁻* and *nda4-108 h⁻* (nuclear division mutants). The *sep1-1 cdc* and *sep1-1 nda* recombinants formed microhyphae and did not grow at the restrictive temperatures (37˚C for *cdc* and 18˚C for *nda*). At temperatures semirestrictive for the *cdc* mutations (30-32˚C) and for the nuclear division mutations (22˚C), they grew slowly and formed microhyphae usually with irregularly positioned septa. However, these irregularities did not differ from those characteristic of the *cdc* and *nda* mutations, suggesting either that none of the genes carrying these mutations overlaps functionally with *sep1⁺* or that they simply all act upstream from it in the regulation of the septation process.

The recombinant *sep1-1 cdc16-116* was studied further as it offered a means of examining whether the *sep1-1* mutation had any effect on the timing of septum initiation. *cdc16-L116* is a mutation that causes a postmitotic cell cycle block but allows several rounds of successive septation after the shift to the restrictive temperature (Minet et al., 1979). If *sep1-1* delayed the initiation of septum formation, the "extra septa" would have to appear with a delay in the double mutant *sep1-1 cdc16-116*. However, this was not the case. The first cells containing two or three centrally located septa appeared in both the double mutant and the control *cdc16-116* cultures between 45 and 60 minutes after shift to 35˚C. Their number then grew progressively, and after 135 minutes 95% of the cells were multiseptate in both cultures.

A novel mutation interacting with *sep1-1* and *cdc4-8*: production of multinucleate syncytia

One of the hybrids from the fusion *sep1-1 arg4-55 h⁺* × *cdc4-8 h⁻* frequently gave incomplete tetrads (18 out of 45) or tetrads with one or two spores forming very small colonies after prolonged incubation at 25˚C (14 out of 45). The small colonies produced a limited number only of viable cells when streaked out, and the surviving cells grew very slowly. As deduced from the genotypes of the viable spores, the inviable ones must have been *sep1-1 cdc4-8* recombinants; so were the small colonies. However, recombinants of the same genotype also occurred among the normally growing spore clones, suggesting that the low viability could not be attributed merely to an interaction between *sep1-1* and *cdc4-8*.

Crosses of the *sep1-1 cdc4-8* segregants with the wild-type L972 revealed the existence of a third mutation in the slowly growing clones. In a cross, 28 tetrads were isolated,

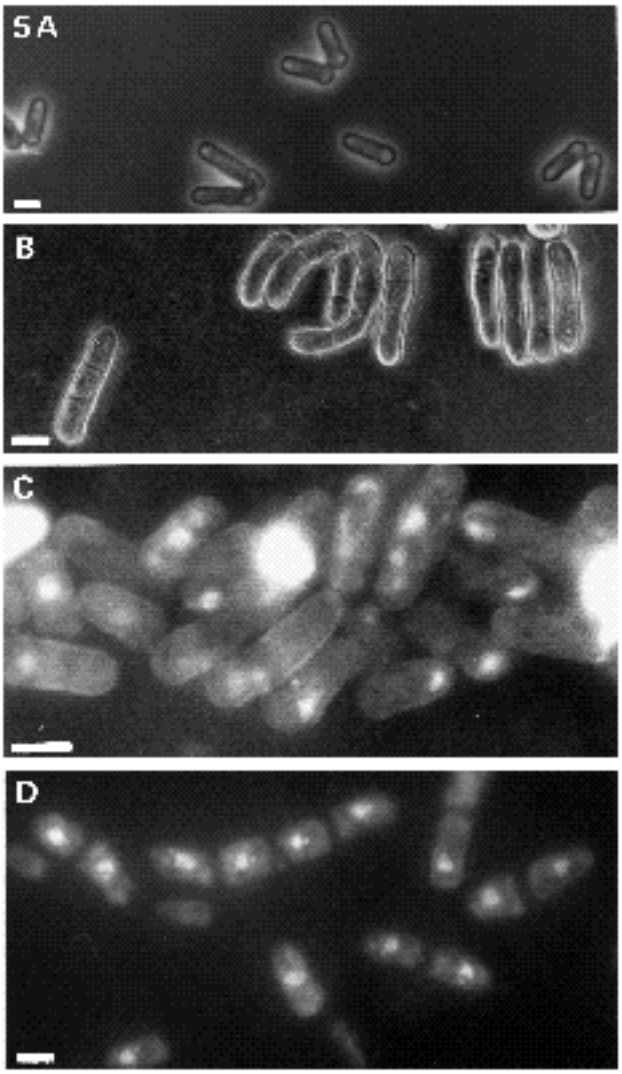

Fig. 5. Microscopic phenotype of *spl1-1*. Logarithmic phase culture at 25˚C (A). Cells after incubation at 35˚C for 24 h (B). Calcofluor-stained (C) and DAPI-stained (D) images of cells after incubation at 35˚C for 24 h. Bars: 5 μm (A and B); 3 μm (C and D).

among them were ten incomplete ones. Eleven produced one or two slowly growing colonies with highly aberrant cell morphology. Unexpectedly, in 12 out of the 18 complete tetrads, the temperature-sensitive phenotype segregated either as $3ts^-:1ts^+$ or $4ts^-:0ts^+$ in proportion to the wild-type. This pattern indicated that the segregant crossed with L972 contained two unlinked ts^- mutations: *cdc4-8* and a novel one. The latter probably arose in the fusion hybrid as a spontaneous mutation, because the other hybrids analysed showed the $2ts^-:2ts^+$ segregation.

At 37˚C, one or two ts^- clones of the 3:1 and 4:0 tetrads showed a defective cell morphology unambiguously different from that of *cdc4-8*. Their cells were somewhat bent, considerably elongated, uninuclear, and usually with one to five uncleaved or partially cleaved septa or irregular depositions of septal material (Fig. 5). Because the latter feature resembles the *sep1-1* phenotype but with the genetic lesion

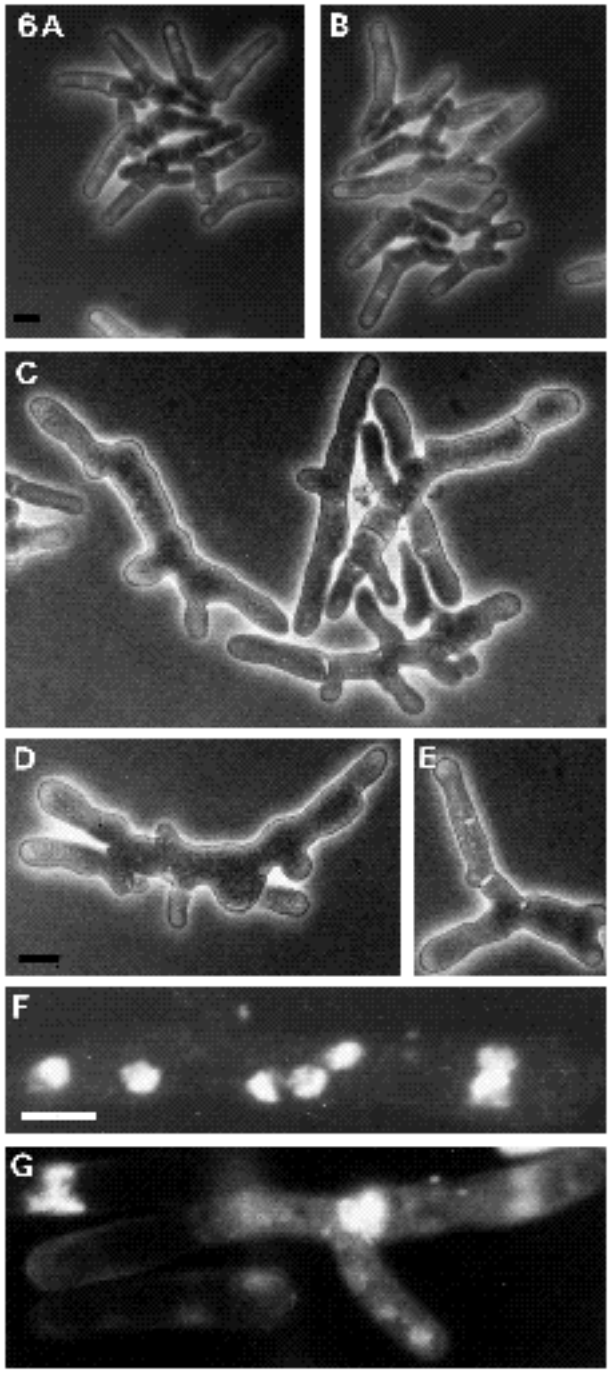

Fig. 6. Morphology of double and triple mutants at 25˚C. Cells of *sep1-1 spl1-1 h*$^-$ (A), *sep1-1 cdc4-8 h*$^-$ (B), and *sep1-1 spl1-1 cdc4-8 h*$^-$ (C-D). DAPI-stained (F) and Calcofluor-stained cells of *sep1-1 spl1-1 cdc4-8 h*$^-$. Black bars, 5 μm; white bar, 3 μm.

that accounts for it being located in a locus unlinked to *sep1* (free recombination in the tetrads), this novel mutation will be referred to as *spl1-1* (s*ep-l*ike). A series of tetrad analyses using 10 strains with auxotrophic markers revealed that *spl1-1* is 9.8 cM apart from *ura3* in the left arm of chromosome I (45PD:11T:0NPD). As no mutant of similar phenotype has been described in this region, *spl1-1* probably defines a novel septation gene.

The double mutants *spl1-1 cdc4-8* and *spl1-1 sep1-1* showed aberrations of both mutations simultaneously: the growth block at the restrictive temperature and, in the latter case, mycelial morphology at the permissive temperature. However, the triple mutant (Fig. 6) grew very slowly and formed huge elongated multinucleate branching syncytia even at 25˚C. Most cells contained septa or irregular depositions of septal material. Division was completed only rarely and brought about multinucleate syncytial siblings. The inability of certain spores to form a colony in the tetrad analysis was probably a consequence of this low division efficiency.

sep1-1 alters susceptibility to the inhibitory effect of certain ions and drugs

Morphological aberrations are frequently consequences of, or associated with, deficiencies of the cytoskeleton. To examine whether *sep1-1* alters the response of the cell to drugs and agents that had been reported to interfere with cytoskeletal functions (Gutz, 1966; Schliwa, 1976; Walker, 1982; Hepler and Bonsignore, 1990) the mutant *sep1-1 h*$^-$ was tested for sensitivity to certain difunctional ions, benomyl, *p*-fluoro-phenylalanine and caffeine. Table 2 shows that *sep1-1 h*$^-$ was slightly more sensitive to Ca^{2+} and Mg^{2+} than the wild-type. Surprisingly, Ca^{2+} proved to be much more toxic when it was applied in liquid medium. The inhi-

Table 2. Effect of ions and drugs on growth at 25˚C after 48 h

Agent			*L972 h*$^-$	*sep1-1 h*$^-$
Control		(YEA)	+++	+++
		(YEL)	+++	+++
$CaCl_2$:	5 mM	(YEL)	+++	++
	7.5 mM	(YEL)	+++	++
	20 mM	(YEL)	++	(+)
	30 mM	(YEL)	+	−
	50 mM	(YEA)	+++	+++
	200 mM	(YEA)	+++	++
	250 mM	(YEA)	+++	+
	300 mM	(YEA)	++	−
	450 mM	(YEA)	+	−
$MgCl_2$:	250 mM		++	(+)
	325 mM		++	−
	500 mM		+	−
$ZnSO_4$:	4 mM		(+)	(+)
	5 mM		−	−
$MnSO_4$:	20 mM		+++	+++
	50 mM		+++	+++
	400 mM		(+)	(+)
p-Fluoro-phenylalanine:				
	0.5 mg/ml		+++	++
	0.7 mg/ml		++	+
	0.9 mg/ml		(+)	(+)
Benomyl:	8 μg/ml		+	++
	10 μg/ml		−	+
	14 μg/ml		−	(+)
	16 μg/ml		−	−
	8 μg/ml + 0.6 mg/ml *p*-Fluoro-phenylalanine		−	−
Caffeine:	3.2 mg/ml		+	+
	4.0 mg/ml		−	−

−, No growth. +++, Normal growth.

Table 3. Mitotic segregation in diploids

Hybrid					Experiment	Benomyl (μg/ml)	*p*-Fluoro-phenylalanine (mg/ml)	Segregation (% auxotrophs)
ura1	h^-	+	*arg1*		1		0	4.90
+	h^-	*ade6*	+				0.6	15.67
					2	0		1.23
						8		11.06
					3	0		0.8
						8		4.12
					4	0		0.47
						8		79.64
					5	0		0.53
						8		60.19
sep1	h^-	+	*leu1*	+	1		0	17.16
sep1	h^-	*ura5*	+	*ade6*			0.6	48.83
					2	0		1.81
						8		4.89
					3	0		2.73
						8		2.44
					4	0		14.23
						8		42.05
					5	0		2.94
						8		19.56

bition observed was not attributable to the increased osmotic pressure in the medium, as none of the supplements, KCl, NaCl sorbitol or mannitol, was more inhibitory to the mutant cells (data not shown).

In contrast to the difunctional ions, benomyl was less toxic to the mutant *sep1-1* than to the wild type (Table 2). Supplementation of the benomyl medium with 600 μg/ml *p*-fluoro-phenylalanine significantly increased the inhibitory effect in both the wildtype and the mutant. Both drugs impaired mitotic stability and increased chromosome nondisjunction in diploids (Table 3). Consistent with its effect on the haploids, benomyl induced less haploidization in sep^-/sep^- than in sep^+/sep^+ diploids. Caffeine sensitivity was not affected by the mutation.

DISCUSSION

Cytokinesis is a seemingly straightforward process of a cell dividing to form two new cells. In cells surrounded by a rigid cell wall, the division is in essence the production and cleavage of a septum. In *S. pombe*, temperature-sensitive mutants have been isolated that form long aseptate multinucleate cells at the restrictive temperatures (Nurse et al., 1976), suggesting that multiple rounds of the cell cycle can occur when there is no cell division.

The mutant *sep1-1* described in this paper is defective in the cleavage of the septum, the concluding event of cell division. As the septum otherwise fulfils its function and severs the cell after karyokinesis into two uninuclear entities, the mutant is as viable as the wildtype. Because of the deficiency in cell separation, the strain forms branching threads composed of up to 15 cells. Using the definition given by van der Walt and Yarrow (1984), these forms are true mycelia because their cells reproduce by fission (in contrast to the budding yeasts, which form pseudomycelia when cell separation is incomplete).

In the budding yeast *Saccharomyces cerevisiae*, the lack of chitinase activity encoded by *CTS1* results in the inability of the cells to separate normally (Kuranda and Robbins, 1991). This enzyme is an endochitinase, which hydrolyses the chitin-rich primary septum between the mother cell and the new daughter cell (the former bud) (Elango et al., 1982). The delayed separation in *sep1-1* could also be due to a deficiency in the degradation process of the primary septum. The *S. pombe* primary septum, whose enzymatic lysis concludes cell separation, is composed of β-(1→3)-glucan (Duffus et al., 1984; Horisberger and Rouvet-Vauthey, 1985). However, the mutant cells showed the same β-glucanase activity as the wildtype, indicating that the gene $sep1^+$ is probably not involved in the production and regulation of β-glucanases

In principle, a late initiation of septum formation could account for the delayed separation, because it might prolong the cleavage step far into the next cell cycle, after the point where cell growth is accelerated. The rearrangement of the cytoskeleton associated with the growth acceleration (Marks et al., 1986) might then interrupt septal maturation before cleavage. However, the experiments with *cdc16* and *cdc16 sep1* did not show any delay in the appearance of the septa in the double mutant, suggesting that the initiation of septation in *sep1-1* is timed normally.

Separation delay could also be caused by a deficiency or a delay in the maturation process of the septum. For example, the *S. cerevisiae* cells harbouring the *rsf1-1* mutation accumulate in a budded state with delayed conversion of the chitin-rich annulus into the primary septum (Veinot-Drebot et al., 1991). In *S. pombe*, a very similar phenotype has been described in the nuclear division mutants *nuc2* (Hirano et al., 1988), and the tubulin mutants *nda2*, *nda3* and *nda4* at semirestrictive temperatures (Toda et al., 1983; Hiraoka et al., 1984; Yanagida, 1989). These mutants are blocked in spindle functions, and in *nuc2* even the cytoplasmic microtubules are absent, which suggests that the maturation and fission of the septum require cytoskeletal functions. However, the defect in *sep1-1* is not related directly to that of *nda2*, *nda3* or *nda4*, as the $sep1^- nda^-$ double mutants did not show any interaction between the

mutations. Nevertheless, the finding that benomyl inhibited the growth of the mutant cells less severely than that of the wildtype and induced less mitotic segregation (non-disjunction of chromosomes) in the *sep1-1*/*sep1-1* diploids than in the *sep1*$^+$/*sep1*$^+$ diploids argues for the notion that the *sep1-1* mutation is associated with a cytoskeletal deficiency. The high spontaneous instability of the *sep1-1*/*sep1-1* diploid can also be attributed to the same alteration of the intracellular structure. It is pertinent to note here that actin duplication increases the proportion of cells undergoing septation and causes lethality in the benomyl-resistant *ben4.D3* mutant (J.S. Hymans, personal communication).

Consistent with the supposed involvement of the cytoskeleton in septation, the *sep1-1* cells showed an increased sensitivity to Ca^{2+} and Mg^{2+}. The role of these divalent cations in the regulation of microtubules, vesicle fusion and cell plate formation is well established in a broad spectrum of eukaryotic species (e.g. see Schliwa, 1976; Wolniak et al., 1980; Keith et al., 1983; Prescott et al., 1988; Cyr, 1991). In fungi, including *S. pombe*, it is assumed that the cytoplasmic vesicles contribute to cell wall synthesis, and their movement to the site of the synthesis is directed by cytoplasmic microfilaments (Kanbe et al., 1989; Heath, 1990). The *sep1-1* mutation might impair this system, thus making the whole process more vulnerable to Ca^{2+} and to Mg^{2+}. The latter has been reported to substitute for Ca^{2+} in certain cytoskeletal functions (Schliwa, 1976). When interpreting the observed altered sensitivities in *sep1-1* to drugs and ions, it cannot be ruled out that they were only secondarily caused by altered cell wall permeability, although it is hardly conceivable that a single change in the wall structure accounted for the altered uptake of the diverse agents.

The polarized transport of wall vesicles to the hyphal tip occurs parallel to actin filaments (Heath, 1990). In *S. pombe* actin is involved in the determination of growth polarity and septal positioning (Marks and Hyams, 1985). In addition to actin, the fission yeast contains a system of cytoplasmic microtubules extending from one end of the cell to the other, which is also thought to be involved in the establishment and maintenance of cell morphology (Hagan and Hyams, 1988). As the cells of *S. pombe* grow at their poles, it is tempting to surmise that the cytoplasmic actin and microtubules are organized in a bipolar system that allows growth and wall extension only at the cell ends.

The finding that cells within the mycelium of *sep1-1* grow subapically suggests that the establishment of cell polarity is not an exclusive function of the cytoplasmic skeleton but rather the result of a sort of communication between the cytoplasm and the cell wall. The cell probably probes its apical region for amenability to growth and, if it finds the tips immature (covered by an unsplit septum), launches an extension subapically at the nearest possible site. This then results in torsion of the cell axis and the linearity of the cytoskeleton, owing to which both the migration of the nuclei and the placement of the septum are adjusted to the abnormal cell shape. However, the cell retains the biopolar growth pattern (Mitchison and Nurse, 1985) and retains the growth direction of the hyphal tip cells, as demonstrated by the arrangements of the branches in the microhyphae (Figs 2 and 3). The mutant *spl1-1* also shows a deficiency in linearity of growth at the restrictive temperature, but in a different way. Although its cells grow apically, their ends bend frequently (Fig. 5). The deficiency caused by *sep1-1* has a deleterious effect on the cell when combined with *cdc4-8* and *spl1-1*. *cdc4-8* is a late septation mutation that impairs septum formation and causes heterologous morphological aberrations at the restrictive temperature (Mateos and Dominguez, 1991). The cells of *spl1-1* cease growing at the restrictive temperature after a few incomplete cell divisions as short multiseptate threads. They do not seem to interact with *sep1-1* in the double mutants, but severely affect growth and cell morphology in the triple mutant, even at permissive temperatures. The interaction involves frequent abortion of septum formation and the collapse of the bipolar growth pattern. In contrast to the septation mutants that grow only at their ends even after being shifted to the restrictive temperature (Mateos and Dominguez, 1991), cells of the triple mutant developed into multinucleate syncytia growing promiscuously at any site of the cell surface. Apparently, the interaction of the mutants does not affect growth and the nuclear cycle but the mechanisms that ensure polarity and cytokinesis.

A possible conclusion that can be drawn from these results and considerations is that both *sep1*$^+$ and *spl1*$^+$ are late septation genes that functionally interact with *cdc4*$^+$ and indirectly affect cell polarity.

One of the mutants, *sep1-1*, described in this paper provides a suitable opportunity to do a pedigree analysis and to investigate certain biological phenomena in situ. As shown in Figs 2 and 3, the bipolar growth pattern (Mitchison and Nurse, 1985) can be demonstrated directly, and any case of violation of it can be spotted easily. The same holds true of the strand-segregation model (Klar, 1987), which describes the pattern of mating-type switching. We believe, that the mutant will provide a means for direct demonstration of any changes in both patterns. Culturing the cells of *sep1-1* under starvation conditions further showed that neither the conjugation nor the preceding pheromonal communication require free cell tips.

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