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Genetics

Negative control for the initiation of meiosis in *Schizosaccharomyces pombe*

(*pat1* mutation/haploid sporulation/premeiotic DNA synthesis/recombination/chromosome segregation)

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ABSTRACT **Temperature-sensitive mutants of the *pat1* gene of *Schizosaccharomyces pombe* are able to produce poorly viable spores from the haploid state without the requirement for nitrogen starvation if exposed to the restrictive temperature. Three lines of evidence strongly suggest that this gene codes for a factor whose physiological role is inhibition of initiation of meiosis. First, *pat1* haploids arrested in G_1 phase undergo apparent premeiotic DNA synthesis at the restrictive temperature. Second, the *pat1* gene was found to exert its function at a specified stage of the meiotic pathway by comparison with other meiotic mutants. Third, meiotic recombination and chromosome segregation take place quite normally in *pat1* diploids at the restrictive temperature, resulting in the production of highly viable spores. A negative control mechanism for meiosis is proposed.**

Meiosis is an essential step of the sexual reproduction of eukaryotes. The molecular mechanisms triggering its initiation are undoubtedly intriguing, but little is known about them. In yeasts, meiosis occurs as the first step of spore formation in diploid cells. Two main prerequisites for meiosis are known in these lower eukaryotes: heterozygosity of the mating-type loci and nutritional starvation, especially that of nitrogen.

The so-called α1–α2 model was proposed for *Saccharomyces cerevisiae* (1), whereby coexistence of active **a** and α mating-type loci in a cell directs specific transcription of these loci, resulting in products essential for the expression of the **a**/α diploid phenotype. This notion was later substantiated (2). Nutritional starvation appears to lead to a reduction in the intracellular level of cAMP, which, in turn, results in the reduction of cAMP-dependent protein kinase activity. Lowering of this enzymatic activity appears essential for the initiation of meiosis (3). However, it remains obscure how a key reaction for the initiation of meiosis ignites under satisfaction of the two requirements or if such a key reaction exists at all.

Nurse (4) and our group (5) have independently isolated mutants of *Schizosaccharomyces pombe* that can sporulate even in the haploid state. At the restrictive temperature, these mutants sporulate, totally disregarding the two prerequisites mentioned above. Further analysis of our mutants (*pat1* mutants) strongly suggests that the *pat1* gene product is involved in the key reaction for the initiation of meiosis. In this article, we demonstrate by three lines of experimentation that the sporulation induced by the *pat1* mutation is indistinguishable from the wild-type process. We propose a negative control mechanism for the initiation of meiosis, in which the *pat1* gene product acts as a repressing factor whose inactivation is essential and sufficient for the initiation of meiosis and subsequent sporulation processes.

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MATERIALS AND METHODS

Strains. *S. pombe* strains used in this study are derivatives of those originally described by Leupold (6). The isolation and basic characterization of *pat1* mutants have been reported (5). *mei2-P192* and *spo5-P2* mutants were isolated and were kindly supplied by C. Shimoda. Other mutants defective in meiosis originate from Bresch *et al.* (7).

Media. Complete medium YPD and synthetic medium SD were described in our previous publication (5) and are prepared essentially according to a standard recipe of the Cold Spring Harbor Laboratory (8). Sporulation media SP and ME are defined in Gutz *et al.* (9). Media SSL+N and SSL−N are described in Egel and Egel-Mitani (10). Medium SPY, which is conveniently used in this study as the medium to induce *pat1*-dependent sporulation, is a 9:1 mixture of media SP and YPD.

Construction of Double Mutants Carrying *pat1* and a Second Meiotic Mutation. HS114-13 (h^{90} *pat1-114 ade6-M216*) was crossed with either of the *mei/mes* strains (h^{90} *mei/mes ade6-M210*) and zygotes were selected by complementation of *ade6* markers. The incubation temperature was 20°C throughout the procedure. Sporulation was induced on ME agar plates and spores were dissected. Asci containing two Pat^+ Mei/Mes^+ (i.e., Ts^+ and sporulation proficient) progeny spores were judged to be nonparental ditype and the two remaining spores were assumed to be *pat1 mei/mes* double mutants. Their retention of these two mutations was confirmed by backcrosses to a wild-type strain.

Construction of Diploids Homozygous for the Mating-Type Locus and *pat1*. The procedure is essentially according to Flores da Cunha (11). HS114-3 (h^+ *pat1-114 ade6-M210 ura1*) was crossed with either HS114-4 (h^- *pat1-114 ade6-M216 lys1*) or HS114-5 (h^- *pat1-114 ade6-M216 lys3*). Ade^+ diploids were selected and they were grown in medium YPD at 20°C. After about 5 days of incubation, the culture was plated on medium YPD for colony formation. White (Ade^+) colonies that were not stained brown by iodine vapor (spore-free) were chosen. Their mating-type (h^+/h^+ or h^-/h^-) was determined by crosses with tester haploid strains.

Other Methods. General genetic methods are according to Gutz *et al.* (9). The diphenylamine method to quantitate DNA is according to Burton (12), additional details of which have been described (5).

RESULTS

DNA Synthesis During Sporulation of Haploids. *S. pombe* haploid cells harboring the *pat1-114* mutation sporulate without mating when subjected to a temperature shift from 20°C to 30°C, resulting in the formation of spores that contain on average only 1/2C DNA (1C: amount of DNA in a haploid spore) (5). These spores showed poor viability, which can be accounted for by the insufficient amount of DNA (see below).

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A kinetic profile of this haploid sporulation is shown in Fig. 1. After temperature shift, one or two doublings were observed prior to sporulation when cells were grown in medium SPY (Fig. 1*A*; see *Discussion*). DNA synthesis in this unusual sporulation process was examined by measuring the DNA content of sporulating cells chronologically by the diphenylamine method (Fig. 1*B*). When exponentially growing *pat1* haploid cells (HS114-12) were subjected to the temperature shift, the amount of DNA per cell (or ascus) remained constant at 2C throughout the process. The starting haploid cells in an exponentially growing phase have almost 2C DNA on average because *S. pombe* has short G_1 and S periods (14).

In the natural sporulation conducted by h^+/h^- diploids, the G_1 period is the exclusive point of entry for meiosis. As shown in Fig. 2, such diploids, initially containing almost 4C equivalent DNA per cell, arrest at G_1 for a certain period before premeiotic DNA synthesis. Therefore, the results with HS114-12 shown in Fig. 1 suggest two possibilities about the *pat1*-driven sporulation. One is that the sporulation embarks from any stage of the cell cycle upon the temperature shift. The alternative is that sporulation can be initiated only from some specific stage(s), but the arrest period is not detectable in the present asynchronous culture. Although it is difficult to differentiate between these two possibilities, the experiments described below show that at least G_1 can serve as an entrance point for meiosis in the *pat1*-driven sporulation.

Haploid *pat1* cells starved for nitrogen at 20°C for 10.5 hr stopped growth and their DNA content decreased to 1C (Fig. 3). The cells thus arrested in G_1 were shifted to 30°C under the same nutritional conditions. As shown in Fig. 3*B*, the amount of DNA per cell doubled before the first nuclear division. This means that the haploid cells at the G_1 stage enter meiosis by carrying out "premeiotic DNA synthesis" initially in this sporulation system. Therefore, the sporulation driven by the *pat1* mutation does not appear as unphysiological as might be expected. This point becomes more evident in the following experiments.

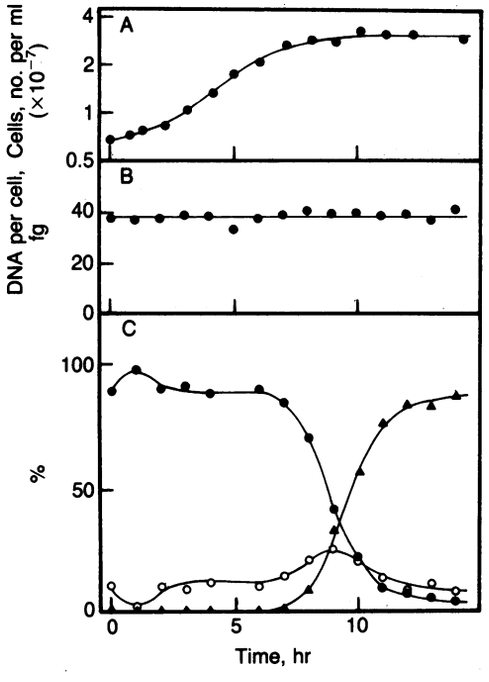

FIG. 1. Kinetics of haploid sporulation. HS114-12 (h^+ *pat1-114*) grown asynchronously in medium SPY at 20°C was shifted to 30°C at time 0. The following characteristics were measured at the times indicated: cell number, determined with a hemocytometer (standard deviation, <3%) (*A*); amount of DNA per cell, determined by the diphenylamine method (12). 1C corresponds to roughly 20 fg (5) (*B*); percentage of cells carrying either one (●), two (○), three or four (▲) nuclei. Nuclei were stained with 4',6-diamidino-2-phenylindole (13) (*C*).

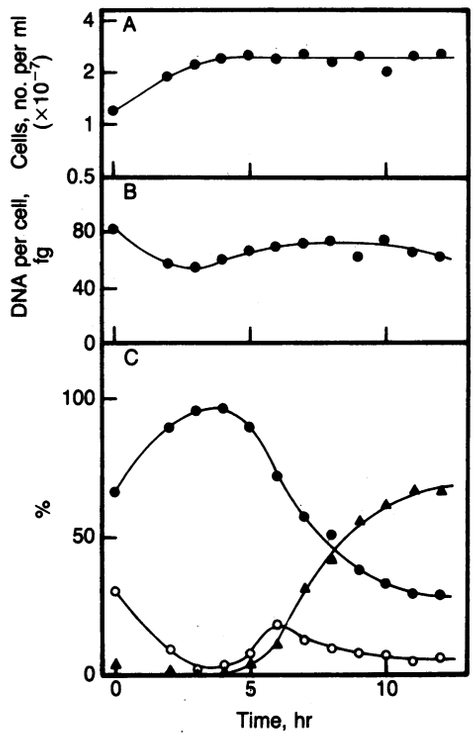

FIG. 2. Kinetics of sporulation displayed by an h^+/h^- diploid. JY274 (h^+/h^- +/*his2 ade6-M216/ade6-M210*) grown in medium SSL+N was shifted to medium SSL−N at time 0. The incubation temperature was 30°C throughout. The characteristics measured and shown in *A–C* are the same as in Fig. 1. The apparent decrease of cellular DNA content after 8 hr probably occurred because the efficiency of extraction was lowered due to maturation of spore walls. Spores produced from haploids are generally more fragile.

Analysis of Double Mutants Carrying *pat1* and a Second Mutation That Interrupts Meiosis. Several mutations that block the progression of meiosis are identified in *S. pombe* and their genes are denoted as *mei* or *mes*. Bresch *et al.* (7) isolated *mei1*, *mei2*, *mei3*, *mei4*, and *mes1*. Subsequently, Shimoda isolated *mei5* (15). The *mei5* mutation used in this study was recently found to be allelic to *mei2*, which was once thought lost, and the allele will be called *mei2-P192* hereafter.

The gene functions of *mei1*, *mei3* (10), and *mei2* (C. Shimoda, A. Hirata, M. Kishida, T. Hashida, and K. Tanaka, personal communication) are required before premeiotic DNA synthesis. *mei1* is now known to be a defect in the *mat2-P* mating-type cassette gene (16). The function of *mei4* is not required for premeiotic DNA synthesis but is necessary for the first nuclear division to take place, and *mes1* is required for the second nuclear division (10). Another mutation used in this study, *spo5*, blocks nuclear migration immediately after the second nuclear division (C. Shimoda, personal communication).

The *pat1-114* mutation was combined with each of these mutations as described in *Materials and Methods*. Resultant double mutants were examined for their ability to sporulate in response to the temperature shift. The results are summarized in Table 1. The *pat1-114* mutation was originally isolated as a suppressor of a *mei1* mutation (*mat2-P-102*), and *pat1-mei1* double mutants could duly sporulate. *pat1-mei3* double mutants also sporulated upon the temperature shift. On the other hand, *mei4*, *mes1*, and *spo5* mutations blocked

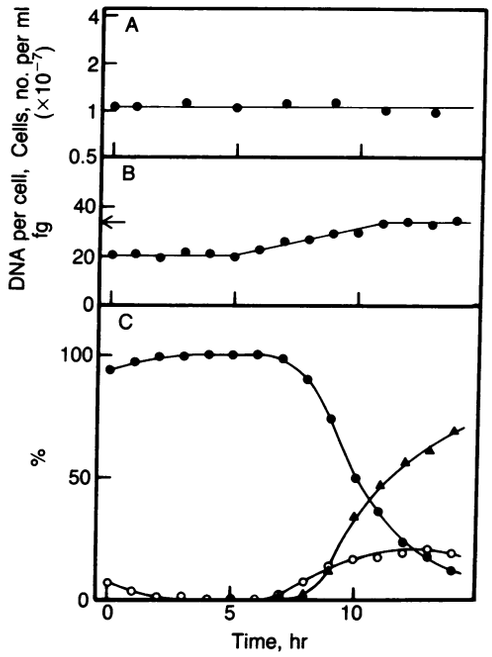

FIG. 3. Kinetics of haploid sporulation starting from a G_1 state. HS114-12, initially grown in medium SD, was starved for nitrogen in medium SD without ammonium sulfate for 10.5 hr at 20°C. The culture was shifted to 30°C at time 0. *A–C* are the same as in Fig. 1. The arrow in B indicates the cellular DNA content before starvation.

the progression of the *pat1*-driven sporulation and the cells eventually lysed at the high temperature. As we reported previously (5), *mei2* mutants act as suppressors of *pat1*—i.e., the double mutants could not sporulate at the high temperature but still had the ability to resume vegetative growth if nutrition was supplied. It is notable that the predominant effect of the *pat1* mutation on the initiation of sporulation is masked by the loss of the *mei2* function.

In summary, the *pat1*-driven sporulation skips early gene functions required for the normal initiation of meiosis (*mat2-P*, *mei3*) but still relies upon those gene functions required after premeiotic DNA synthesis (*mei4*, *mes1*, *spo5*). Furthermore, we can position the functional point of *pat1* action immediately before or at the same stage as *mei2*. Their intimate cooperation in the meiotic process will be discussed later.

Sporulation of Diploids Homozygous for *pat1*. Diploid strains homozygous for *pat1* as well as the mating-type loci (h^+/h^+ or h^-/h^-) were constructed as described in *Materials and Methods*. These strains sporulated after the temperature shift, irrespective of nutritional conditions. Spore viability, however, was found to be much greater than that of spores produced by *pat1* haploids in random spore analysis. This indicates that the poor viability of the latter is not due to the process of sporulation initiated by the *pat1* mutation but due to the insufficient amount of DNA available for distribution. Similarly, dissection of 33 four-spored asci derived from haploids gave no viable progeny at all, whereas similar analysis of asci derived from diploid strains homozygous for the mating-type locus and *pat1* yielded viable colonies at a frequency of about 50% (ranging evenly from 0/4 to 4/0 viable/nonviable). Consistent with these genetic results, the four nuclei in the ascus produced by the diploid appear to have more DNA and a more even distribution of DNA than in the haploid (Fig. 4).

Table 1. Characterization of haploid *pat1-mei/mes* double mutants

Mutations	Production of spores at 30°C	Growth at 36°C
pat1-114	+	−
pat1:mei1-102	+	−
pat1:mei3-B71	+	−
pat1:mei2-P192	−	+
pat1:mei4-P572	−	−
pat1:mes1-B44	−	−
pat1:spo5-P2	−	−
mei/mes/spo	−	+

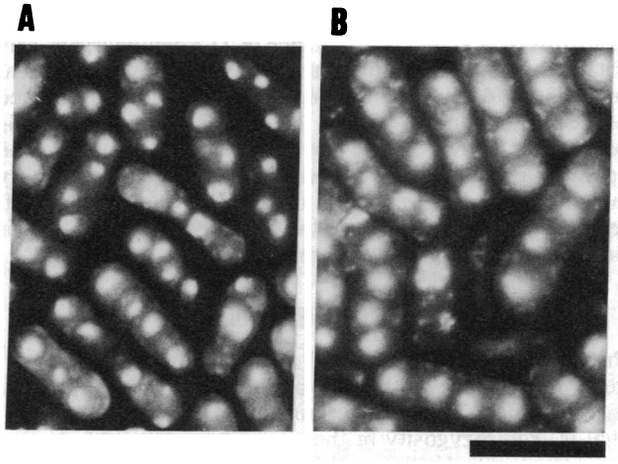

FIG. 4. Comparison of nuclei in haploid and diploid *pat1* strains during the final stage of sporulation. (*A*) HS114 (h^- *pat1-114 ade6-M210 his2*) incubated at 30°C for 9 hr on medium YPD and stained with 4'6-diamidino-2-phenylindole. (*B*) HS114-101 (h^-/h^- *pat1-114/pat1-114 ade6-M210/ade6-M216 his2/his2*) treated as above. (Bar = 10 μm.)

Conventional tetrad analysis was carried out with asci produced by these diploids (HS114-341: h^-/h^- *pat1/pat1 ade6-M210/ade6-M216 ura1/+ +/lys1* and HS114-351: h^+/h^+ *pat1/pat1 ade6-M210/ade6-M216 ura1/+ +/lys3*). Three heterozygous chromosomal markers (*ade6*, *lys1*, and *ura1*) always segregated 2:2 when four spores were viable and 2:1 when three were viable. *lys3* segregated 2:2 in some clones but 4+:0− in others, suggesting gene conversion events and accumulation of convertants during the strain construction (see *Materials and Methods*). No mating-type interconversion had taken place, confirming that the sporulation analyzed here did not depend on mating-type heterozygosity.

Two distant markers on chromosome I (17)—namely, *lys1* and *ura1*—displayed a nonlinkage segregation pattern close to PD:T:NPD = 1:4:1 (where PD = parental ditype; T = tetratype; NPD = nonparental ditype), suggesting frequent crossing-over between them (Table 2). The standard linkage between *lys3* and *ura1* determined by Kohli *et al.* is 21.1 centimorgans (17). The distance between the same genes was calculated to be 7.3 centimorgans in *pat1*-driven meiosis of HS114-351 (Table 2). When we induced meiosis by nitrogen

Table 2. Linkage analysis of chromosomal markers in *pat1*-driven diploid sporulation

Strain	Markers analyzed	Segregation pattern* PD	T	NPD
HS114-341	+/*ura1* and *lys1*/+	3	10	2
HS114-351	+/*ura1* and *lys3*/+	35	6	0

PD, parental ditype; T, tetratype; NPD, nonparental ditype.
*Only tetrads giving three or four viable progeny spores were scored.

starvation at 20°C in a h^+/h^- diploid strain otherwise homologous to HS114-351, a distance of 22.8 centimorgans was obtained. As the sample number is too low, statistical analysis to determine the significance of the difference between the values obtained, 7.3 and 22.8 centimorgans, is not feasible. However, we may conclude that recombination operates in the *pat1*-driven meiosis at a reduced, yet clearly meiotic, level. The reduction may reflect physiological differences during pachytene between the two kinds of meiosis that have been initiated differently.

DISCUSSION

Previously, we have reported a novel mutation in the *pat1* gene of *S. pombe* that enables this yeast to sporulate disregarding the two main prerequisites for natural meiosis—that is, heterozygosity of the mating-type loci and nitrogen starvation (5). In this article, we demonstrated that the meiosis/sporulation events induced in haploids by thermal inactivation of the *pat1* gene product mimic those of the natural process. According to preliminary electron microscopic observations (unpublished results), spindle pole bodies, which organize microtubule assembly in spindles, undergo modification in the second nuclear division of the *pat1*-driven haploid meiosis in the same manner as reported for natural meiosis (18). Furthermore, events induced in diploids by thermal inactivation of the *pat1* gene product are essentially indistinguishable from those that take place in natural meiosis. In addition, analysis of DNA synthesis in *pat1* haploids suggested that the *pat1* gene product exerts its function before premeiotic DNA synthesis and this was confirmed genetically by the analysis of double mutants carrying *pat1* and one of several *mei/mes* mutations.

Judging from the nature of the mutation, the *pat1* gene product must be a negative factor for meiosis. It is striking that the inactivation of this gene product is sufficient to initiate an apparently normal meiotic process leading to the completion of sporulation. We, therefore, propose that the physiological role of this gene product is to repress the initiation of meiosis. We speculate further that the satisfaction of the two natural prerequisites for meiosis results in inactivation of the *pat1* gene product through the function of the *mei3* gene. The necessity for *mat2-P* or *mei1* is equivalent to the necessity for heterozygosity at the mating-type locus and can consistently be skipped by the defect in *pat1*.

A close relationship apparently exists between the function of *pat1* and that of *mei2*. The *mei* and *mes* genes, including *mei2*, should code for a positive factor required for meiosis. Destruction of the function of the *mei2* gene product antagonizes the derepression of meiosis triggered by the inactivation of the *pat1* gene product. The simplest notion is that the *pat1* gene product represses the expression or the function of the *mei2* gene product at either a transcriptional, a translational, or a protein–protein interaction level. If this is the case, expression of the *mei2* function appears to activate premeiotic DNA synthesis and make the process irreversible. Alternatively, the *mei2* gene product may be a positive cofactor for the gene function that is derepressed by inactivation of the *pat1* product. Thus, the molecular identity of the *pat1* and *mei2* gene products and the nature of their interaction are urgent questions to be answered. Our negative control model for the initiation of meiosis is shown schematically in Fig. 5.

Two subsidiary points about the nature of the *pat1*-driven meiosis deserve comment. The average viability of the spores produced by the *pat1* diploids was around 50%. Since we sometimes encounter such a low regeneration frequency even in ordinary crosses, it is difficult to assess whether that value is significantly low or not. Indeed, spore viability was 54% when sporulation was induced in a h^+/h^- *pat1/pat1* diploid strain by nitrogen starvation at 20°C. However, it is difficult to exclude completely the possibility that, when the *pat1* gene product is inactivated, mischievous events may occur, leading to the formation of nonviable spores in a fraction of cells, possibly at a certain stage of the cell cycle. At the same time, we cannot answer decisively the question of whether cells in G_2 can go directly to the first nuclear division upon inactivation of the *pat1* gene product, thereby substituting premeiotic DNA synthesis with mitotic DNA synthesis, although we can argue against it because *pat1* cannot skip the step before premeiotic DNA synthesis, which depends on *mei2*. In any case, the natural meiosis calls initially for G_1 arrest, which may ensure the accurate progression of the meiotic program.

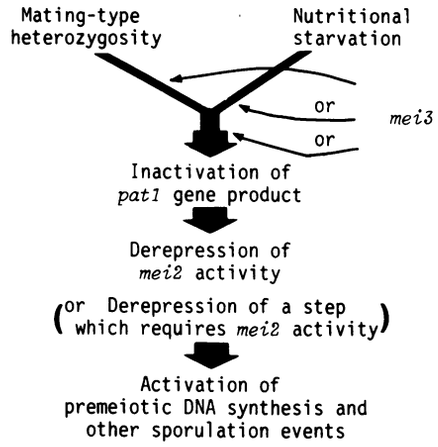

FIG. 5. A proposed negative control model for the initiation of meiosis.

Another point to be noted is the comparison of the *pat1* mutation and the *ran1* mutation isolated and described by Nurse (4). Although allelic, there are some phenotypic differences between them. Nurse reports that the *ran1* mutation, besides evoking sporulation, stimulates mating in a homothallic cell population at the restrictive temperature. The *pat1* mutants appear to form spores only as haploids, even with a homothallic genetic background. According to Nurse, the *ran1* mutants require partial nutritional starvation for the initiation of meiosis at the restrictive temperature. We note some acceleration of the *pat1*-driven meiosis by partial starvation in such medium as YPD or SPY but it is not evident in other media rich in nitrogen. This may mean that certain nutritional starvation affects the structure of the *pat1/ran1* gene product, making it more thermolabile. Finally, suppressors of *ran1* appear to be more common than those of *pat1*. In summary, *pat1* appears less leaky and less pleiotropic than *ran1*. It remains to be seen if the pleiotropy and leakiness displayed by *ran1* reflects a versatile nature of the *pat1/ran1* gene product.

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