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Cloning and Analysis of Transcription of the *mei2* Gene Responsible for Initiation of Meiosis in the Fission Yeast *Schizosaccharomyces pombe*

CHIKASHI SHIMODA,[1]* MASAHIRO UEHIRA,[1] MASAO KISHIDA,[1] HIROSHI FUJIOKA,[1] YUICH IINO,[2] YOSHINORI WATANABE,[2] AND MASAYUKI YAMAMOTO[2]

Department of Biology, Faculty of Science, Osaka City University, Sumiyoshi-ku, Osaka 558,[1] and Laboratory of Molecular Genetics, Institute of Medical Science, University of Tokyo, Tokyo 108,[2] Japan

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We have isolated a hybrid plasmid, pDB(*mei2*)2, containing a 7.4-kilobases (kb) DNA fragment from a *Schizosaccharomyces pombe* genomic library which is able to complement the *mei2* mutation of *S. pombe*. Integration of the cloned DNA sequence at the *mei2* site on chromosome I demonstrated that it contained the *mei2* gene. This gene was localized on a 4.7-kb *Hin*dIII-*Pvu*II fragment in the subclone pFMV402. Transcriptional regulation was studied by Northern blot analysis in which polyadenylated RNA was prepared from a heterozygous (h^{+N}/h^{-S}) diploid strain cultured either in nitrogen-rich growth medium or in nitrogen-free sporulation medium. The size of the major *mei2* mRNA, which always gave a broad band, was estimated to be 4.2 ± 0.2 kb, and a few minor bands (e.g., 3.2 and 1.8 kb) appeared as well. These transcripts appeared more abundantly in sporulating cells than in growing cells. Neither the mating type genes (*mat*) nor the *mei3* gene was essential for transcription of the *mei2* gene, since ample *mei2* mRNA was detected in sporulation-deficient cells transferred to sporulation medium, such as h^{+N}/h^{+N} and h^{-S}/h^{-S} homozygotes, as well as *mei1* and *mei3* mutants.

Nutritional starvation is one of the major signals for sexual reproduction in a wide range of organisms. In the fission yeast *Schizosaccharomyces pombe*, sexual processes (mating in haploids and meiosis in diploids) are induced by the depletion of a nitrogen source from the culture medium (8, 9).

Heterozygosity at the mating type locus (*mat*) is another prerequisite for the initiation of meiosis, implying that both *mat1*-P and *mat1*-M products are required for this process (9). In addition to the *mat* genes, two additional genes, *mei2* and *mei3*, are involved in the initial step of meiosis as positive regulators (5, 16).

Recently, Iino and Yamamoto (11, 12) and Nurse (15) have isolated a conditional mutant, *pat1* or *ran1*, which is able to undergo meiosis at the restrictive temperature even in the presence of a nitrogen source and from a haploid state. The meiotic defect caused by the mutations in *mat* and *mei3* genes is bypassed by *pat1*, whereas the *mei2* mutation suppresses the *pat1* mutation. On the basis of these facts, Iino and Yamamoto (12) proposed a negative control mechanism for meiosis in the fission yeast. The model suggests that *pat1* products repress *mei2* activity to block the initiation of meiosis under nitrogen-rich growth conditions. Thus, derepression of *mei2* activity appears to be a key step for the induction of meiosis.

To elucidate the regulatory network operating during the initiation of meiosis, we cloned the *mei2* gene and studied its transcriptional regulation.

MATERIALS AND METHODS

Strains and culture conditions. The *S. pombe* and *Escherichia coli* strains used in the present study are listed in Table 1. *E. coli* cells were cultured in L broth at 37°C. When necessary, the medium was supplemented with ampicillin (40 μg/ml) or tetracycline (20 μg/ml).

Culture media used for *S. pombe* were YEA or SSL+N for growth medium and either MEA (solid), SSA (solid), or SSL−N (liquid) for sporulation medium. These media are described by Gutz et al. (10) and Egel and Egel-Mitani (9). The incubation temperature was 30°C for vegetative growth and 28°C for meiosis.

Plasmids. The yeast-*E. coli* shuttle vector pDB248′ contains pBR322 and a part of the 2μm DNA and *LEU2* of *Saccharomyces cerevisiae* (1). YIp5 and YIp32 are yeast integration vectors carrying *URA3* or *LEU2* as a selectable marker, respectively (4). The *Saccharomyces cerevisiae LEU2* gene is able to complement *leu1* of *S. pombe* (1) and will be designated *LEU2*(sc) in this article. The *E. coli* plasmid pBR322 was also used.

Transformation. Transformation of *E. coli* was carried out by the method of Davis et al. (6) with minor modifications. *S. pombe* transformation was performed as described by Beach et al. (2). Spheroplasts were produced by treatment with Novozym SP234 (a generous gift from Novo Industry, Japan).

Preparation of DNA and RNA. Total *S. pombe* DNA was prepared from spheroplasts by the method of Davis et al. (7). RNA was prepared from *S. pombe* whole cells by the method of Jensen et al. (13).

Blot hybridization. Blotting and hybridization of DNA and polyadenylated [poly(A)$^+$] RNA have been described by Shimoda and Uehira (17).

RESULTS

Cloning of the DNA sequence complementing the *mei2* mutation. Cloning of *mei2* was achieved by the procedure reported by Shimoda and Uehira (17). Mutant strain C132-14D (h^{90} *mei2*-N3 *leu1*) was transformed with an *S. pombe Hin*dIII gene bank constructed in pDB248′. Leu$^+$

* Corresponding author.

TABLE 1. *E. coli* and *S. pombe* strains used

Strain	Relevant genotype	Source
E. coli		
KH802	*hsdM*$^+$ *hsdR met supE*	
JA221	*hsdM*$^+$ *hsdR leuB trpE lacY recA1*	
S. pombe		
L975	h^{90}	U. Leupold
B102	h^{90} *mei1*-B102 *ade6*-M210	R. Egel
B71	h^{90} *mei3*-B71 *ade6*-M210	R. Egel
SG168	h^{-S} *leu1*	H. Gutz
C213-8D	h^{+N} *leu1 ade2*	C. Shimoda
C132-1A	h^{90} *mei1*-P149 *leu1 ade6*-M216 *ura1*	C. Shimoda
C134-14D	h^{90} *mei2*-N3 *leu1 ade6*-M216 *ura1*	C. Shimoda
C145-3B	h^{90} *mei3*-B71 *leu1 ade6*-M216 *ura1*	C. Shimoda
C133-1D	h^{90} *mei4*-P572 *leu1 ade6*-M216 *ura1*	C. Shimoda
C185-2B	h^{90} *mes1*-B44 *leu1 ade6*-M216 *ura1*	C. Shimoda
INT3	h^{-S} *leu1* [YIp(*mei2*)B2]	This study
JY274 (diploid)	h^{-S} *ade6*-M216 *his2*/h^{+N} *ade6*-M210	Y. Iino
JY275 (diploid)	h^{+N} *ade6*-M216/h^{+N} *ade6*-M210	Y. Iino

transformants were allowed to form colonies on SSA medium. As the host strain is homothallic, mating occurred in these colonies and sporulation was expected to take place if the *mei2* function was supplied. A few meiosis-proficient (Mei$^+$) transformants were indeed found among the Leu$^+$ colonies. Plasmid DNA was extracted from one of the transformants. This plasmid, named pDB(*mei2*)2, could transform C134-14D to Mei$^+$ Leu$^+$. The frequency of asci in the *mei2* mutants harboring pDB(*mei2*)2 was 40 to 60% after 2 days in SSL−N, and the germination rate of spores was greater than 90%. Other meiosis mutants (those carrying mutations *mei1*, *mei3*, *mei4*, and *mes1*) were not complemented by pDB(*mei2*)2.

Restriction mapping and subcloning of pDB(*mei2*)2. Plasmid pDB(*mei2*)2 contained two *Hin*dIII fragments of 6.4 and 1.0 kilobases (kb). The restriction sites on pDB(*mei2*)2 are shown in Fig. 1.

Subcloning experiments were conducted to determine the region necessary for the complementation of *mei2*. Various restricted fragments were cloned into pDB248′, and their ability to complement the *mei2* mutants was examined. The DNA region between the right *Pvu*II site and the left *Hin*dIII site (4.7 kb in length) proved to be essential for restoring Mei$^+$ functions to *mei2* mutants (Fig. 1).

Southern hybridization analysis of total *S. pombe* DNA restricted with *Hin*dIII was carried out with the cloned 6.4-kb *Hin*dIII fragment as a probe. Autoradiograms showed a single intense band corresponding to 6.4 kb (data not shown). Therefore, the cloned DNA insert was derived from the *S. pombe* genome and probably exists as a unique sequence in *S. pombe*.

Identification of the cloned gene. To determine whether the 6.4-kb fragment carries the *mei2* gene or an extragenic suppressor, this fragment was inserted into YIp32 [designated YIp(*mei2*)B2] and then integrated into the *S. pombe* chromosome after transformation of recipient strain SG168, which is a heterothallic haploid strain carrying the *leu1* mutation. One stable Leu$^+$ transformant (INT3) was crossed to C134-14D (h^{90} *mei2 leu1*), and tetrads of the hybrid diploid were dissected. The tetrad data indicate regular segregation for both the Leu$^+$/Leu$^-$ and Mei$^+$/Mei$^-$ phenotypes (2$^+$:2$^-$). As expected, close linkage between *LEU2*(sc) and *mei2* was demonstrated, in that all of 53 dissected asci were of the parental ditype.

The *mei2* gene has been located on chromosome I, 16.6 centimorgans from *ade2* (16). To confirm the linkage between YIp(*mei2*)B2 and *ade2*, INT3 was crossed to C213-8D (h^{+N} *leu1 ade2*). *LEU2*(sc) was shown to be linked to *ade2*, the map distance being 24.2 centimorgans. These tetrad data clearly show that the plasmid carrying the 6.4-kb fragment was integrated at the *mei2* site, verifying that the cloned DNA contained the *mei2* gene itself.

Northern blot analysis. Expression of the *mei2* gene at the transcriptional level was examined by Northern analysis. A 3.2-kb *Cla*I fragment (Fig. 1) was cloned into pBR322. This plasmid, pFMV1, was labeled by nick translation.

A sporogenic diploid strain, JY274 (h^{+N}/h^{-S}), cultured in growth medium (SSL+N) was transferred to sporulation medium (SSL−N) and incubated for 4 h at 28°C. Poly(A)$^+$ RNA was prepared before and after the transfer, fractionated on a 1% denatured agarose gel, and blotted onto nylon membranes (Biodyne A; Nihon Pall Co.). The membranes were then probed with ^{32}P-labeled pFMV1. As shown in Fig. 2, a hybridization band appeared preferentially in the RNA sample from the sporulation culture (lane B), although a trace of hybridization was detectable in the RNA from the growing culture (lane A). It is likely that the difference in hybridization reflects synthesis rather than degradation of *mei2* mRNA, as more degradation of RNA is generally observed in sporulating cells than in growing cells (9). The intensity of the hybridization band for RNA prepared from vegetative cells was extremely low in some experiments, but consistently increased as the culture entered the stationary phase (data not shown). The size of the major *mei2* transcript, which reproducibly gave a broad band, was estimated to be 4.2 ± 0.2 kb, while a few minor bands (e.g., 3.2 and 1.8 kb) were also observed. All these transcripts carried at least a part of the same open reading frame, whose disruption led

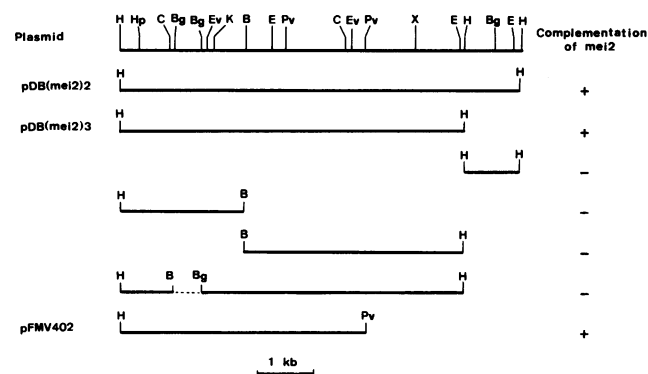

FIG. 1. Restriction map of an *S. pombe* DNA insert in pDB (*mei2*)2 and subcloning of the DNA fragments with and without the ability to complement the *mei2* mutation. A broken line represents a deleted sequence. Complementation of *mei2*: +, complements; −, does not complement. Restriction enzyme cleavage sites: B, *Bam*HI; Bg, *Bgl*II; C, *Cla*I; E, *Eco*RI; Ev, *Eco*RV; Hp, *Hpa*I; K, *Kpn*I; Pv, *Pvu*II; X, *Xho*I; H, *Hin*dIII.

to a Mei2$^-$ phenotype. Furthermore, it is known that the major band itself is a mixture of multiple transcripts with different starting points (Y. Watanabe et al., manuscript in preparation).

The mating type alleles play an indispensable role in the initiation of meiosis. We thus examined whether *mei2* gene expression is under *mat* control. A homozygous diploid (h^{+N}/h^{+N}) was cultured in sporulation medium for 4 h at 28°C. Poly(A)$^+$ RNA was prepared and examined by Northern analysis. Intense hybridization bands appeared even in the homozygous diploid transferred to sporulation medium (Fig. 2, lanes C and D). The same result was obtained with h^{-S}/h^{-S} diploids (data not shown). These results indicate that transcriptional activation of the *mei2* gene is not dependent on the action of the *mat* locus.

Finally, the effect of the *mei1*, *mei2*, and *mei3* mutations, which affect initiation of meiosis, on *mei2* gene transcription was examined. Homothallic haploid strains carrying the *mei* mutations and the wild-type strain (L975 h^{90}) were cultured in SSL−N at 28°C. A number of diploid zygotes were formed within the first 4 h. Poly(A)$^+$ RNA was prepared from each strain after 7 h of incubation. Northern blot analysis (Fig. 3) showed that the *mei2* transcript appeared in all of the preparations. The amount of the transcript in the wild-type strain appeared to be less abundant than in the mutants, perhaps due to degradation of the *mei2* mRNA in the wild type. We observed that the *mei2* transcript level in this h^{90} wild-type strain reached its maximum at 5 to 7 h after transfer to SSL−N and then declined rather rapidly (data not shown). Thus, it was concluded that transcription of the *mei2* gene was not impaired by either the *mei1* (in *mat2*-P) or the *mei3* mutation. Furthermore, functional *mei2* products are not required for expression of the *mei2* gene itself.

DISCUSSION

The *mei2* gene of the fission yeast *S. pombe* is essential for the initiation of meiosis. Since meiosis in diploid cells is triggered by starvation of nitrogen, the *mei2* gene could be expected to be repressed during vegetative growth and to be derepressed only after the nutritional shift to nitrogen-free medium. We indeed observed that the *mei2* gene was preferentially transcribed in the absence of a nitrogen source (Fig. 2). Heterozygosity at the *mat* locus was not necessary for the accumulation of *mei2* mRNA (Fig. 2 and 3), although it is another prerequisite for meiosis in *S. pombe*. It has been found that the *mei3* gene is also transcribed in sporulation medium, despite the homozygosity at the *mat* locus (C. Shimoda, unpublished data). Thus, the *mat1*-P and *mat1*-M products may function coordinately at the posttranscriptional level to express the *mei2* and *mei3* gene function.

Iino and Yamamoto (11, 12) proposed a negative control mechanism for meiosis. According to that model, the product of the wild-type *pat1* gene blocks cells from entering meiosis. Under nitrogen starvation conditions, the *mat1* and *mei3* gene products may cause inactivation of *pat1* products, resulting in derepression of the *mei2* activity or a step which calls for *mei2* activity as an essential positive factor. According to this hypothesis, *pat1* products must be active in *mei3* mutants or diploids homozygous at the *mat* locus even in the absence of nitrogen. In *mei3* mutants and h^{+N}/h^{+N} or h^{-S}/h^{-S} diploid strains, however, *mei2* gene transcription actually occurs. Therefore, if *mei2* activity is under the regulation of *pat1*, its derepression caused by inactivation of *pat1* products must be attained after *mei2* mRNA is produced. Hence, it is probable that expression of the *mei2* gene is regulated at both the transcriptional and posttranscriptional levels. In this respect, it is noteworthy that the *pat1* gene has been suggested to encode a protein kinase (3). Furthermore, it is an open question whether the unusual heterogeneity of *mei2* mRNA has any relevance to such regulation.

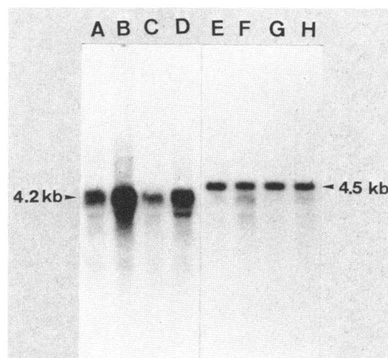

FIG. 2. Northern blot analysis of poly(A)$^+$ RNA prepared from diploid strains JY274 (h^{+N}/h^{-S}) and JY275 (h^{+N}/h^{+N}). Cells were cultured in growth medium (SSL+N) and then incubated in sporulation medium (SSL−N) for 4 h at 28°C. Nick-translated pFMV1 (*mei2* probe) and pFNA2302 (*aro3* probe) were used for hybridization. Each lane received poly(A)$^+$ RNA corresponding to 2.5×10^8 cells in the original culture. Lanes A and E, JY274 in growth medium. Lanes B and F, JY274 in sporulation medium. Lanes C and G, JY275 in growth medium. Lanes D and H, JY275 in sporulation medium. The same filter was probed with pFMV1 (lanes A to D) and with pFNA2302 (lanes E to H), which is known to hybridize with a 4.5-kb *aro3* transcript (14). The amount of *aro3* mRNA was nearly constant in all preparations. Smears in lanes F and H are due to incomplete decay of ^{32}P in lanes B and D.

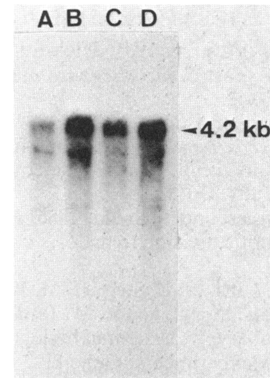

FIG. 3. Northern blot analysis of poly(A)$^+$ RNA prepared from a wild-type strain and three meiosis-deficient mutants. Cells were incubated in SSL−N for 7 h at 28°C. Lanes: A, L975 (h^{90} wild type); B, B102 (h^{90} *mei1*); C, N3 (h^{90} *mei2*); D, B71 (h^{90} *mei3*). Input RNA was 3.2 μg per lane. Size markers used were 25S rRNA (3.36 kb) and 18S rRNA (1.16 kb) and *Saccharomyces cerevisiae URA3* mRNA (0.9 kb). Probe, ^{32}P-labeled pFMV1.

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