Autoregulated Expression of *Schizosaccharomyces pombe* Meiosis-Specific Transcription Factor Mei4 and a Genome-Wide Search for Its Target Genes

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Manuscript received October 1, 1999
Accepted for publication December 20, 1999

ABSTRACT

The *Schizosaccharomyces pombe* mei4+ gene encoding a forkhead transcription factor is necessary for the progression of meiosis and sporulation. We searched for novel meiotic genes, the expression of which is dependent on Mei4p, since only the spo6+ gene has been assigned to its targets. Six known genes responsible for meiotic recombination were examined by Northern blotting, but none were Mei4 dependent for transcription. We determined the important cis-acting element, designated FLEX, to which Mei4p can bind. The S. pombe genome sequence database (The Sanger Centre, UK) was scanned for the central core heptamer and its flanking 3' sequence of FLEX composed of 17 nucleotides, and 10 candidate targets of Mei4 were selected. These contained a FLEX-like sequence in the 5' upstream nontranslatable region within 1 kb of the initiation codon. Northern blotting confirmed that 9 of them, named mde1+ to mde9+, were transcriptionally induced during meiosis and were dependent on mei4+. Most mde genes have not been genetically defined yet, except for mde9+, which is identical to spo5+, which encodes one of the septin family of proteins. mde9+ and a related gene pit1+ encode proteins related to Saccharomyces cerevisiae Ime2. The double disruptant frequently produced ascii having an abnormal number and size of spores, although it completed meiosis. We also found that the forkhead DNA-binding domain of Mei4p binds to the FLEX-like element in the putative promoter region of mde4 and that the maximum induction level of mde4 mRNA required functional mei4 activity. Furthermore, expression of a reporter gene driven by the authentic mei4 promoter was induced in vegetative cells by ectopic overproduction of Mei4p. These results suggest that mei4 transcription is positively autoregulated.

In multicellular organisms, gametes differentiate into morphologically and functionally specialized cells. Sporulation in single-celled eukaryotes such as yeasts is a morphogenetic process equivalent to gametogenesis, because an ascospore is a highly specialized cell and its formation is preceded by meiotic nuclear division. Programmed gene expression guarantees an accurate progression of ordered events during cellular morphogenesis. Accordingly, a number of specific transcription factors might be involved in gametogenesis of higher eukaryotes and sporulation of yeasts.

Transcriptional control in the course of sporulation has been studied extensively in the budding yeast *Saccharomyces cerevisiae* (Kupiec et al. 1997). To understand genome-wide alterations in the expression level of individual genes during sporulation, DNA microarray technology has been applied (Chu et al. 1998). Among ~6200 protein-encoding genes in the *S. cerevisiae* genome, ~500 are transcriptionally upregulated during sporulation. Such a shift in gene expression might be attained by transcriptional cascades. Ndt80 is supposed to be one of the most important sporulation-specific transcription factors, because it can affect the expression of >200 genes (Chu et al. 1998). Ndt80 is also a key mediator of the meiotic recombination checkpoint, which represses the activity of Ndt80, resulting in a meiosis blockade at the pachytene stage (Chu and Herskowitz 1998; Hepworth et al. 1998).

To date, only Mei4 has been found as the meiosis-specific transcription factor in the fission yeast *Schizosaccharomyces pombe* (Horie et al. 1998). Mei4p is required primarily for progression through prophase-I, because mei4 mutants arrest in this stage. The only defined target of Mei4p is spo6+ (Horie et al. 1998). The arrest phenotype of mei4 mutants (Bresch et al. 1968; Olson et al. 1978; Shimoda et al. 1985) cannot be explained by the failure of spo6 expression, because spo6+ is required only for meiosis-I and sporulation (T. Nakamura, personal communication). Therefore, more meiosis-specific genes are probably expressed under Mei4 control, and some of them must be indispensable for prophase-I. Mei4 protein contains a forkhead DNA-binding domain at its N-terminal region (Horie et al. 1998). The domain is composed of ~120 amino acids and is evolutionarily conserved (Weigel and Jackle 1990). The cis-acting element located in the 5' upstream region of spo6 was determined by gel mobility shift assay (Horie et al. 1998). The Mei4 forkhead domain binds 27-bp oligonucleotides containing the heptamer core, GTAAACA,
which shares an identical binding motif for the human forkhead protein, FREAC (Pierré et al. 1994). This nucleotide sequence was designated FLEX (FREAC-like element of spo6). Mutational analysis revealed that the core heptamer is essential and that its 3' flanking sequence of 10 nucleotides is important for recognition by Mei4p (Horie et al. 1998).

The environmental cue for meiosis is nutrient depletion, especially nitrogen starvation. Most of the S. pombe genes responsible for sexual reproduction are transcriptionally induced by a nitrogen starvation signal mediated by the HMG family transcription factor, Ste11 (Sugimoto et al. 1991). Since me4 is not transcribed in haploid cells starved for a nitrogen source, Ste11 is not sufficient to induce me4 transcription. The Mei2 RNA-binding protein is a crucial inducer of meiosis in S. pombe (Wat anabe and Yamamoto 1994). The transcription of me4 requires me2 function and proceeds rapidly in diploid cells shortly after commitment to meiosis (Horie et al. 1998). Little is known about the transcriptional regulation of the me4 gene, in spite of its importance. The aims of this study were to identify novel genes, the expression of which is governed by Mei4, and to confirm positive autoregulation of me4 gene expression.

**MATERIALS AND METHODS**

**Strains, media, and genetic techniques:** The S. pombe strains listed in Table 1 were cultured in media as described (Egel and Egel-Mitani 1974; Gut z et al. 1974; Moreno et al. 1990). Cells were grown on YEA complete or SD minimal media at 30 °C. Mating and sporulation were induced at 28 °C on the malt-extract agar medium (MEA). For liquid sporulation culture, cells were incubated in PM, shifted to PM-N, a nitrogen-free version of PM, and incubated at 28 °C with shaking.

Standard procedures for S. pombe genetics followed those of Gutz et al. (1974). S. pombe was transformed using lithium acetate (Okazaki et al. 1990).

**Synchronous meiosis in pat1 temperature-sensitive mutants:** The temperature-sensitive pat1-114 mutant (Lino and Yamamoto 1985; Nurse 1985) was cultured on YE minimal medium at 24 °C for 2 days. The cells were transferred to PM-N at a density of OD530 = 0.65 and were shaken at 24 °C for 15 hr to arrest the cell cycle at the G1 phase. Shifting the incubation temperature to 34 °C induced meiosis, which proceeded in a synchronous fashion (see Figure 1).

**Northern blotting:** Total RNA was prepared from S. pombe cultures according to the method of Jensen et al. (1983). DNA probes were labeled with 32P by random priming. The templates for random priming of all the me4 genes, as well as rec6, rec12, and rec14, were gel-purified polymerase chain reaction (PCR) products. The synthetic oligonucleotides used in the PCR reaction are listed in Table 2. Other templates consisted of the following fragments: me4, a 2.3-kb HindIII-Hpal fragment (Horie et al. 1998); rec7, a 1.1-kb BglII fragment; rec8, a 0.6-kb BamHII-Hsal fragment (Lin et al. 1992); rec10, a 2.2-kb SalI fragment (Lin and Smit h 1995); and dmc1, a 0.3-kb EcoRI fragment (A. Shinohara, unpublished data). Northern hybridization proceeded under standard conditions (Thomas 1980). Ribosomal RNA stained by ethidium bromide was used as the loading control. Hybridization with the S. pombe rec14 probe was the internal reference (Nakanishi and Yamamoto 1984; Lino et al. 1995). The intensity of the hybridization bands was quantified with a Fuji BAS1000 Bio-Imaging analyzer.

**Gene disruption of mde3 and pit1:** A 2970-bp DNA fragment containing the mde3 open reading frame (ORF) was amplified by PCR, with the forward primer GGGACGCTTGGTATAC and reverse primer CTTTACTCATGGCG. The HindIII fragment of the amplified fragment was cloned into pBluescript II SK' (Stratagene, La Jolla, CA). The mde3::ura4+ null allele was produced by a one-step gene disruption method (Rothstein 1983). A BglII fragment of 965 bp was replaced by a 1.8-kb us4+ cassette (Grimm et al. 1988). A diploid strain (C525) was transformed with the HindIII fragment having this disrupted mde3 allele, and stable Ura+ transformants were isolated. Disruption was confirmed by the size of the PCR products using several different pairs of primers and also by tetrad dissection of the diploid disruptants.

### TABLE 1

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**TABLE 2**

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<td>rec14</td>
<td>0.6-kb BamHII-Hsal fragment</td>
<td>Lin et al. 1992</td>
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<td>dmc1</td>
<td>0.3-kb EcoRI fragment</td>
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A 2720-bp DNA fragment containing the pit1 ORF was amplified by PCR, with the forward primer CCCCTCGAG(Xho I)CACGGTGG CTTACAATTCGAA and reverse primer CCCGCAGGC(Gm2)AAAGCAACAAAATTCGGC. The PCR product was digested with XhoI and NotI and cloned into pBluescript-II SK (Stratagene). A 0.3-kb SalI/BamHI fragment was replaced by a 2.2-kb LEU2 cassette containing the S. cerevisiae LEU2 gene. A diploid strain (C525) was transformed with the XhoI/NotI fragment having the disrupted pit1 allele, and stable Leu+ transformants were isolated. Disruption was confirmed by the methods mentioned above.

**Construction of a mei4-lacZ fusion plasmid:** The 3.3-kb BamHI fragment containing the Escherichia coli lacZ gene cut from pMC1871 (Cassadan et al. 1983) was fused to the mei4 ORF on pAU(me4)KHL, yielding pAU(me4)NL. This plasmid thus contained the authentic me4 promoter, part of the 5’-terminal Mei4p-coding region (1–275 amino acids) and LacZ (8–1021 amino acids; see Figure 7A).

**β-Galactosidase assay:** The heterothallic haploid strain JY741 was transformed with pAU(me4)NL and cultured in PM liquid medium at 30°C to the early stationary phase. β-Galactosidase activity was assayed according to Horie et al. (1998).

**Preparation of GST-Mei4 fusion protein:** Plasmid pGEX(me4) contains a forhead DNA-binding domain of Mei4p fused to glutathione-S-transferase (GST; Horie et al. 1998). The E. coli strain BL-21 (Studier and Moffatt 1986) was transformed with pGEX(me4) and the GST-Mei4 fusion protein was expressed by adding isopropyl-β-d-thiogalactopyranoside in Luria-Bertani medium. Cells were homogenized by sonication (INSONATOR 201M, Kubota Manufacturing Co., Ltd.) in a buffer containing 30 mM Tris-HCl (pH 7.5) and 30 mM NaCl at 4°C. The supernatant fraction obtained by centrifugation at 10,000 × g for 30 min at 0°C was used as a crude preparation of the GST-Mei4 fusion protein.

**Gel mobility shift assay:** Three sets of complementary oligonucleotides, Fmei4-D, Fmei4-U, and FLEX-D, were synthesized and annealed to generate double-stranded DNA fragments with the following nucleotide sequences (only one strand is presented): Fmei4-D, 5'-ATACCGGTTAATGTTAAATACGAGCAAGA-3'; Fmei4-U, 5'-TATAAATTGAAATACGAGCAAGA-3'; FLEX-D, 5'-AAATATGGTGAAATACGAGCAAGA-3'. These fragments were labeled with [γ-32P]dATP using polynucleotide kinase (Takara Shuzo Co.). A standard reaction mixture (20 μl) contained 24 ng of radiolabeled double-stranded oligonucleotide probes, and an E. coli crude extract containing 9 ng of protein, 2 μg of poly(dI-dC), and 8.4 μg of salmon sperm DNA in binding buffer (100 mM Tris-HCl [pH 8.0], 10 mM MgCl2, 60 mM KCl, 1 mM spermidine, 0.1% Nonidet P-40, 7 mM β-mercaptoethanol, and 10% glycerol). The reaction mixture was placed on ice for 60 min and then loaded onto 4% native polyacrylamide gels in TGE buffer. Electrophoresis proceeded at 15 mA in TGE buffer at 4°C until free probes reached the bottom of the gel. Resolved bands were fixed with 7% acetic acid and then exposed to X-ray film (Fuji NIF-RX film) for 12–18 hr at –80°C.

**RESULTS**

**Mei4-independent transcription of early meiotic genes:** To date, only spo6+ has been recognized as a target gene for the Mei4 transcription factor (Horie et al. 1998). Although me4Δ cells arrest in meiotic prophase-I (Bresch et al. 1968; Olson et al. 1978; Shimoda et al. 1985; Horie et al. 1998), spo6+ is required for meiois-II and sporulation (T. Nakamura, unpublished data). We surmised that Mei4p governs the transcription
of some of the genes that function during the early stages of meiosis. Other meiosis-deficient S. pombe mutants with a phenotype similar to that of mei4Δ have not yet been identified. In the search for novel target genes of Mei4p, the dependence on mei4+ activity of several typical early meiotic genes, such as rec6+, rec7+, rec8+, rec10+, rec12+ (Lin et al. 1992; Lin and Smith 1994, 1995), and dmc1+ (A. Shinohara, personal communication), has been examined. Some of these genes are transcribed exclusively during the early meiotic process (Lin et al. 1992; Lin and Smith 1994, 1995). In addition, spo5+ was tested as a representative late meiotic gene (H. Asakawa, personal communication). In the following experiments, pat1-driven meiosis was adopted to achieve fairly good synchrony in meiotic divisions (see materials and methods; Iino et al. 1995). The level of synchrony attained is shown in Figure 1. mei4Δ cells were arrested at the mononucleate stage in pat1-driven meiosis (Figure 1). Portions of the synchronous culture were removed, and RNA was purified and Northern blotted (Figure 2). Hybridization of mei4+ cultures with specific probes did not reveal signals for the rec/dmc genes at 0 hr. Signals were detected at 2 hr, the intensity of which peaked 4 hr after the temperature shift, and then rapidly declined. These genes were also transcribed in mei4Δ cultures (Figure 2), indicating that these early genes do not rely on Mei4p for transcription.

In mei4Δ cells, the elevated transcript level persisted, in contrast to wild-type cells in which such elevation was only transient (Figure 2). This finding raises the notion that the transcripts of meiosis-specific genes are stable in mei4Δ cells. To test this, the turnover rates of specific RNA molecules were determined according to Surosky and Esposito (1992). The turnover rates of the spo5 and dmc1 mRNA molecules were not significantly different between mei4+ and mei4Δ cells (data not shown). Therefore, it seems less likely that the persistence of these mRNA molecules in mei4Δ cells is due to their increased stability. These results indicate that Mei4p is required for turning off the transcription of some meiosis-specific genes. Persistent transcription might be the secondary effect of arrest at prophase-I in mei4Δ cultures.

A genome-wide screen of target genes with Mei4p-dependent transcription: Since Mei4-dependent target genes were not identified among known early meiotic genes, we screened for novel genes that are dependent on Mei4p for transcription. A large volume of data is available in the S. pombe genome sequence database at The Sanger Centre (UK). In addition, our mutational analysis of the FLEX sequence of spo6 revealed that the central core heptamer (GTAACA) and its 3' flanking sequence (AACAAAATCA) are very important for Mei4 binding (Horie et al. 1998). Using this 17-nucleotide sequence and its complementary sequence as a query, we conducted a computer-aided search of the S. pombe genome sequence database. Since no complete match was found except for spo6, up to four mismatch bases were allowed in the 3' flanking sequence. Position of the FLEX-like sequence in the respective genes was also examined. We found 10 genes whose FLEX-related sequence resides in the 5' upstream nontranslatable region within range of 1 kb from the initiation codon.

The transcription of these potential Mei4-dependent genes was examined by Northern blotting. The gene symbol mde was adopted to indicate Mei4-dependent expression. We compared the expression of 10 putative mde genes in JZ670 (mei4+) and AB4 (mei4Δ) strains. Nine genes, designated mde1+ to mde9+, were transcribed when meiosis was induced in wild type, but not in mei4Δ under the same conditions (Figure 3A). The transcript level of one gene, SPAC19A8.10, was too low to determine whether or not the expression is Mei4p dependent (data not shown).

As mentioned above, mei4+ was not transcribed in vegetative cells. If Mei4p is involved directly in the activation of transcription, the ectopic expression of mei4+ might cause the transcription of these genes. To test this theory, me4 was expressed by the thiamine-repressible nmt1 promoter in mitotic cells. C525C-1A transformed with pREP(mei4+) was incubated in PM medium with or without thiamine.
Figure 2.—Transcription profile of meiosis-specific genes in wild-type and mei4Δ cells. RNA was prepared from diploid strains JZ670 (mei4+) and AB4 (mei4Δ) and was analyzed by Northern blot hybridization as described in materials and methods. Meiosis was synchronously induced as described in the legend to Figure 1. The approximate equality of RNA was checked by staining gels with ethidium bromide, which reveals the rRNA.

Seventeen hours after transfer to thiamine-free PM medium, mei4 was induced in the cells. Figure 3B shows that the overexpression of mei4 stimulated the transcription of these putative candidate mde genes even in growth medium. As mde1 was expressed in the medium under repressed conditions (containing thiamine), this gene might be transcribed in response to the very low level of Mei4p present in the presence of thiamine. The mde4 transcript was also detected when cells harboring pREP1 vector plasmid were incubated for 17-19 hr in PM with or without thiamine (data not shown), indicating that transcription of mde4 was not due to overproduction of Mei4p. The transcript level of mde4 was very low after incubation in nitrogen-free medium for 15 hr and was enhanced after pat1-driven meiosis dependent on mei4+ (Figure 3A). We concluded that these nine genes, mde1+ to mde9+, are likely targets of Mei4p.

The position of the likely FLEX sequence in the mde1+ to mde9+ genes relative to the initiation codon and alignment of the FLEX sequence is shown in Table 3. As suggested in our previous study (Horie et al. 1998), the 3’ flanking sequence seemed important and in fact 5 of the 10 nucleotides were conserved in all of the 9 mde+ genes. We concluded from these results that the consensus cis-element is GTAAACAAACA-A.

Mde3p is homologous to S. cerevisiae Ime2 kinase and is necessary for normal sporulation: Sequence data of these mde genes indicated that they are mostly novel genes with unknown biological functions (Table 3). The exception was mde9+, which is identical to spn5+ and encodes a putative septin protein (Longtine et al. 1996). In addition, Mde3p attracted our attention because it has sequence similarity to the S. cerevisiae meiosis-specific protein kinase, Ime2 (Kominami et al. 1993; Foiani et al. 1996). We studied the function of mde3+ in meiosis and sporulation.

The S. pombe genome sequence project has revealed that another gene, SPAC3C7.06c, also encodes an Ime2-
related protein. Hereafter, this gene is designated pit1 (S. pombe Ime1 homolog). Figure 4A shows the sequence similarity, especially in the kinase subdomain I-X, among Ime2p, Mde3p, and Pit1p. In contrast to mde3Δ, 3.3-kb pit1Δ mRNA was present in vegetative cells and its abundance was not enhanced after shift to nitrogen-free sporulation medium (data not shown).

To know the role of mde3Δ and pit1Δ, both genes were disrupted (Figure 4B). These null mutants showed no growth defects at incubation temperatures ranging from 20° to 37°. The S. cerevisiae IME2 gene plays an indispensable role in controlling timing of premeiotic DNA replication and meiosis (Folani et al. 1996; Dirick et al. 1998). Thus, we studied whether the mde3Δ and pit1Δ mutants were defective in meiosis and sporulation. The homothallic haploid strain harboring mde3Δ could mate and could undergo meiosis and sporulation. The sporulating culture, however, contained nonsporulating zygotes and aberrant asci with less than four mature spores (Figure 5B). Apparently, small immature spores were also produced (Figure 5B). The frequency of these aberrant asci with abnormal size and number of spores is significantly higher than wild-type strain (Figure 5A). These defects were observed also with pit1Δ and were more remarkable in the mde3Δ pit1Δ double disruptant strain. Next, kinetics of meiotic nuclear division was monitored by DAPI staining. No differences in the progression of meiosis between wild-type and the mutant strains were observed (data not shown). These results indicate that mde3Δ and pit1Δ play an important role in meiosis and sporulation.

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**Figure 3.** Northern blots indicating Mei4-dependent transcription of the mdeΔ genes. (A) Effect of mei4 disruption on transcription of mdeΔ genes in pat1-driven synchronous meiosis. Meiosis of the diploid strains harboring homozygous pat1-114, JZ670 (mei4Δ), and AB4 (mei4Δ) was synchronized and at intervals total RNA was prepared and analyzed by Northern blot hybridization as described in materials and methods. (B) Effect of ectopic expression of mei4Δ on mdeΔ transcription. C525C1A transformed with pREP(mei4Δ) was incubated in PM with (+) or without (−) thiamine (20 μm). After 17 and 19 hr of incubation, samples from both cultures were examined by Northern blotting. The approximate equality of RNA was checked by staining gels with ethidium bromide.
in spore formation, but no indispensable role in the progression of meiosis.

**Transcription of mei4 is positively autoregulated:** We found that the transcript level of me4 is reduced in the me4-P572 mutant (Horie et al. 1998). To confirm this observation, we tested the me4 mRNA level in three independently isolated me4 mutants. Figure 6 shows that me4 mRNA abundance in these mutants was one-half to one-third relative to the wild-type level. Therefore, full expression of me4 requires me4+ function. We next examined whether or not me4 transcription is activated by ectopically overproduced Mei4p. The plasmid pAU(me4)NL (Figure 7A), containing the me4 gene with its promoter fused to the E. coli lacZ gene, was introduced into the h+ haploid strain JY741. To ectopically overexpress me4+, the plasmid pREP(me4+) (Figure 7A) was introduced as well. β-Galactosidase activity due to me4-lacZ was remarkably increased when Mei4p was overproduced by incubating cells in thiamine-free medium (Figure 7B). This activity was low in transformants bearing the empty vector pREP1. These results support the view that me4 transcription is positively upregulated by its own product. This positive autoregulation of me4 transcription might explain the rapid induction of me4 and may play a role in the transcription of downstream genes in response to environmental cues for the induction of meiosis.

We have previously reported that me4+ has no FLEX-like sequences (Horie et al. 1998). We could not find any potential FLEX sequences in the genome-wide search described above. As Mei4p can stimulate the transcription of me4 itself, we reexamined the nucleotide sequence carefully and found the FLEX-like sequence of reverse orientation in the 5' upstream region of this gene. These potential FLEX-like Mei4p-binding sites, designated Fmei4U and Fmei4-D (Figure 8, A and B), are highly diverged from the consensus query sequence. The downstream element (Fmei4-D) contains two tandem repeats of the core heptamer, GTAAAYA. Using bacterially produced recombinant GST-Mei4 protein (Horie et al. 1998), we tested binding to these potential FLEX sequences as probes with a gel mobility shift assay. Figure 8C shows shifted bands with the original spo6-FLEX-D and Fmei4-D, but not with Fmei4-U. The GST protein without the forhead region of Mei4p gave no positive signals. The intensity of the shifted band of Fmei4-D was reproducibly weaker than that of spo6-FLEX-D. Such inefficient binding might be due to the divergence of the Fmei4-D sequence from the consensus. These gel shift assays indicated that the Mei4 fusion protein binds to the likely cis-element of me4. These facts further support the notion that me4 transcription is positively autoregulated.

**DISCUSSION**

**Identification of novel Mei4p targets:** Mei4p is a forkhead family transcriptional regulator that is required for the progression of meiosis and sporulation in S. pombe (Horie et al. 1998). We selected novel target genes of the Mei4 transcriptional regulator by means of a genome-wide screen for its recognition sequence. This strategy necessitates at least two conditions: the identification of DNA-binding motifs and the availability of genome nucleotide sequences. Our previous study revealed that the forhead DNA-binding domain of Mei4p recognizes a cis-element composed of 27 nucleotide pairs (Horie et al. 1998). The S. pombe genome sequence project is ~80% complete.

To find novel Mei4 target genes in the genome, we used a 17-bp stretch of the FLEX element containing the central core and its 3' flanking region as a query.

### Table 3

**List of mde genes**

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<td>TGGAGTGCAATGAAAGCAACATTT</td>
<td>—</td>
</tr>
</tbody>
</table>

1. The name of ORF assigned by the genome sequence project is given in parentheses.
2. Numbered with the translational start point at +1.
3. Arrows indicate the direction of FLEX on the coding strand of the respective ORF.
4. Completely conserved nucleotides are shown in italic type. Dots represent a core heptamer.
This screen revealed nine genes, designated mde1 to mde9, the expression of which was strongly dependent on wild-type mde4 function. The FLEX-like nucleotide sequences of these novel mde genes are aligned in Table 3. Whereas mismatches of 1-4 nucleotides were allowed on screening, 12 nucleotides are completely conserved among these genes. The revised consensus FLEX sequence based on these data is GTAAACAAACA(A/T).
Figure 6.—Northern blots indicating that the level of \textit{mei4} transcript was reduced in \textit{mei4} mutants. (A) Autoradiogram. The level of \textit{mei4} mRNA in three \textit{mei4} mutants during meiosis. Haploid strains harboring \textit{pat1-114} allele were used: AB1-2C (\textit{mei4}1), AB11-15C (\textit{mei4-KA42}), AB12-11B (\textit{mei4-KA5}), and AB13-1D (\textit{mei4-KE76}). Meiosis was induced by shifting the incubation temperature from 24° to 34°. Cultures were sampled after 9 hr at 34°. RNA preparations were subjected to Northern analysis with \textit{mei4}- and \textit{aro3}-specific hybridization probes. (B) Relative intensity of the \textit{mei4} transcripts to \textit{aro3}. The autoradiograms shown in A were quantified using a Fuji bio-imaging analyzer (BAS1000).

A(A/C). This approach to identifying targets of a particular transcription factor might be applicable to other systems if the two prerequisites described above are fulfilled.

Sequence data predict that the \textit{mde} gene products include the \textit{S. cerevisiae} \textit{Ime2} homolog, two septin-like proteins, an \textit{a}-amylase precursor, and a putative RNA-binding protein (Table 3). Four of the identified \textit{mde} genes have neither significant homology with known proteins nor functional motifs. The \text{spn5} mutation allelic to \textit{md9} impaired spore formation in \textit{S. pombe} (J. Bael er, personal communication). The essential role of septin proteins in sporulation was also reported in budding yeasts (Far es et al. 1996; Vir gilio et al. 1996).

The \textit{S. pombe} \textit{mde1}+ gene, which is essential for the meiotic second division (Bre sch et al. 1968; Shimo da et al. 1985), contained one short intron (Kishida et al. 1994). Splicing of the \textit{mde1} mRNA requires \textit{mei4}+ function probably in an indirect manner (Horie et al. 1998). In this context, interestingly, \textit{mde7}+ encodes a putative RNA-binding motif. An attractive hypothesis
that Mei4p regulates the me1 splicing through the mde7+ gene product is worth examining.

Ime2p of the budding yeast is a serine/threonine protein kinase essential for the normal timing of premeiotic DNA replication and meiotic division and the completion of sporulation (Fojani et al. 1996; Dirick et al. 1998). The mde3 mutant frequently produced aberrant asci, which had only zero to three spores and immature spores. However, we could not observe any delay of meiosis, unlike ime2 mutants (Fojani et al. 1996; Dirick et al. 1998). Since S. pombe has another gene, named pit1+ encoding an Ime2-like protein, the weak meiotic phenotype of mde3 cells is possibly due to pit1+, which is expressed constitutively. The mde3Δ pit1Δ double disruptant, however, displayed the normal progression of meiotic nuclear division. Our observation implies that S. pombe Ime2-like proteins regulate sporulation in a substantially different way than S. cerevisiae Ime2p. Of course, we could not exclude the possibility that S. pombe has a third Ime2-related protein that has not yet been identified.

Currently, we are performing gene knockout experiments with the other me genes. Elucidation of the cellular function of these meiosis-specific genes expressed downstream of Mei4p could shed light on meiosis and sporulation in the fission yeast.

Positive autoregulation of mei4 transcription: mei4+ itself is also regulated primarily at the transcriptional level. The following facts suggest a positive autoregulation of mei4 transcription. First, the me4 transcript level is greatly reduced in me4 mutant cells (Figure 6). Sec-
ond, the ectopic expression of Mei4p in vegetative cells induces a reporter gene that is transcribed under the control of the me4 promoter (Figure 7B). Finally, recombinant Mei4p binds to the FLEX-like cis-element of the me4 promoter (Figure 8). These observations imply that the low level of Mei4p that is initially produced enhances further transcription of me4.

This type of positive autoregulation has been found in other yeast genes. For example, the S. cerevisiae PDR3 gene encoding the zinc finger transcription factor implicated in drug resistance is positively autoregulated (Delahodde et al. 1995). Another example is found in the copper detoxification phenomenon in Candida glabrata. The Amt1 transcription factor regulates the expression of a family of metallothioneins and the transcription of AMT1 by copper-induced gene expression by the mating-type locus. Proc. Natl. Acad. Sci. USA 80: 3035-3039.


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Communicating editor: G. R. Smith