Schizosaccharomyces pombe ste11 encodes a transcription factor with an HMG motif that is a critical regulator of sexual development

Asako Sugimoto, Yuichi Iino, Tatsuya Maeda, Yoshinori Watanabe, and Masayuki Yamamoto

Department of Biophysics and Biochemistry, Faculty of Science, University of Tokyo, Tokyo 113, Japan

Schizosaccharomyces pombe ste11 encodes a member of the family of HMG-box proteins. Its transcript is induced in response to nitrogen starvation and a concomitant decrease of the intracellular cAMP level. Expression of ste11 is essential for induction of sexual development, and its ectopic expression stimulates uncontrolled mating and sporulation. Ste11 protein regulates positively transcription of the following genes required for sexual development: the mating type genes, matP and matM, and the mei2 gene, which is essential for commitment to meiosis. Ste11 protein synthesized in vitro binds specifically to a DNA fragment carrying a 10-base motif TTCTTTGTTY that is an essential cis-acting element for the induction of mei2 and is commonly seen in the upstream regions of the genes inducible by nitrogen starvation. These observations strongly suggest that Ste11 serves as a key transcription factor for sexual development.

[Key Words: Schizosaccharomyces pombe; nitrogen starvation; sexual differentiation; transcription factor; high-mobility group protein box; DNA-binding activity]

Received June 20, 1991; revised version accepted August 19, 1991.

Nutritional starvation, particularly that for nitrogen, induces sexual development in Schizosaccharomyces pombe (Egel 1973; Egel and Egel-Mitani 1974). Haploid cells mate and diploid cells initiate meiosis under nitrogen starvation, and ascospores are subsequently formed. Many genes have to be regulated in an organized manner during these differentiation processes. This study focuses on how nitrogen starvation induces concerted gene expression that commits S. pombe cells to sexual development.

Genes that are activated transcriptionally in response to nitrogen starvation have been reported. They include the four transcription units of the mating-type genes (matPc, matPi, matMc, and matMi) (Kelly et al. 1988), and the mei2 gene (Shimoda et al. 1987), which encodes an essential factor for the initiation of meiosis (Bresch et al. 1968). Function of these genes is required in the initial stages of sexual development. Three lines of evidence indicate that cAMP is involved in the control of the expression of these genes. [1] Addition of cAMP to the medium inhibits induction of these genes (Watanabe et al. 1988); [2] S. pombe cells that lack the gene for adenyl cyclase and do not have a measurable amount of cAMP are highly derepressed for sexual development; [Maeda et al. 1990]; and [3] nitrogen starvation lowers the level of intracellular cAMP by ~50% in S. pombe (Fukui et al. 1986; Maeda et al. 1990; N. Mochizuki and M. Yamamoto, in prep.). Thus, it appears that nitrogen starvation results in a decrease in the intracellular cAMP level which, in turn, functions as a signal for the induction of nitrogen starvation-responsive genes in S. pombe.

We demonstrate in this discussion that the ste11 gene is also inducible by nitrogen starvation and repressible by cAMP and, furthermore, that the function of ste11 is essential for the induction of all the other known nitrogen-responsive genes. These findings indicate a pivotal role for ste11 in the regulation of sexual development in response to nitrogen starvation. Analysis of the ste11 gene product strongly suggests that this protein binds to DNA and functions as a transcription factor.

Results

ste11 mutation

Originally we identified the ste11 mutation as a suppressor of the uncontrolled meiosis driven by the patl (also called ran1) mutation. S. pombe strains carrying the temperature-sensitive patl-110 or patl-114 allele initiate meiosis and hence halt growth at the restrictive temperature (Iino and Yamamoto 1985a; Nurse 1985). More than 150 spontaneous revertants able to grow at 37°C
were isolated from parental pat1 mutants. All of these revertants had acquired suppressor mutations; 90% of the mutations mapped in mei2, whose deficiency had been shown to be a potent suppressor of pat1 (Beach et al. 1985; Lino and Yamamoto 1985a,b). The remainder defined a new gene, ste11, which we referred to as steX in a previous publication (Watanabe et al. 1988), and is now known to be allelic both to the ste11 gene described recently (Kitamura et al. 1990) and to aff1 isolated independently as a pat1 suppressor (Sipiczki 1988). The ste11 mutations separated from the pat1 background conferred complete sterility and meiotic deficiency. Although there are other S. pombe sterility genes that are essential for both mating and meiosis (Girgsdies 1982), ste11 is distinct from them in that none of the others can arrest the uncontrolled meiosis driven by pat1.

Cloning of the ste11 gene

Cloning of the ste11 gene was achieved by complementation of the ste11-29 allele. An S. pombe genomic library was prepared in the vector YIp468, which was expected either to integrate into S. pombe chromosomes or replicate autonomously but poorly. Spo + transformants of Y736 (hsteel2-29) were isolated and plasmids were recovered from them. Among plasmids recovered successfully in Escherichia coli, only three had unarranged sequences, two of which [named pSX1] were identical and able to retransform Y736 to Spo +. The insert in pSX1 was a 9.5-kb BglII fragment. This fragment was recloned in pUC118, and the resultant plasmid was named pSX1-U1.

Structure of the ste11 gene

Deletion analysis of pSX1 suggested that ste11 is carried on a 3.7-kb BamHI–EcoRI fragment (Fig. 1). Sequence analysis of this region revealed an open reading frame [ORF] that potentially encodes 468 amino acids (Fig. 2). To confirm that this ORF corresponds to ste11, gene disruption was performed. A 2.0-kb BamHI–PstI fragment, which covers the amino-terminal one-fourth of the ORF, was excised from pSX1-U1 and replaced by a ura4 + cassette [Fig. 1]. The wild-type allele in a homothallic haploid strain YJ878 was substituted with this construct. Southern analysis of two sterile Ura + transformants indicated that the disrupted allele had integrated by homologous recombination [data not shown]. These transformants were fused with an original ste11 isolate YJ736 by protoplast fusion. All diploid fusion products were Spo +. Because both the original and the disrupted sterile mutations are recessive, these results strongly suggest that the cloned gene is indeed ste11.

The ste11 product carries a conserved motif found in the nuclear high-mobility group proteins

Homology of the deduced ste11 gene product to known proteins was pursued with the aid of Dr. Mark Goebel [Indiana University School of Medicine, Indianapolis]. The Ste11 protein was found to have the highest similarity to the product of S. pombe matMc, which is a transcription unit in the M-mating type locus (Kelly et al. 1988). The matMc gene product has been shown to have homology with SRY [Sinclair et al. 1990], a human gene that is likely to be the testis-determining factor [TDF] [Berta et al. 1990; Jäger et al. 1990; Koopman et al. 1990]. Ste11 and this putative TDF also exhibited considerable homology (Fig. 3). The homologous region in these cases matches the HMG box (Jantzzen et al. 1990; Kolodrubetz 1990), which corresponds to the conserved domains in nuclear high-mobility group protein HMG1 (Tsuda et al. 1988; Wen et al. 1989) and has been demonstrated to be a DNA-binding domain in some of the family members [Wright and Dixon 1988; Jantzzen et al. 1990]. These suggest that Ste11 also could be a DNA-binding protein.

ste11 is inducible by nitrogen starvation and repressible by cAMP

The effect of nitrogen starvation on expression of ste11 was examined. Expression of this gene was higher under nitrogen starvation [Fig. 4, lanes 1,2], irrespective of the mating type of the cell [data not shown]. However, a low level of ste11 expression was consistently observed when cells were growing in rich medium at the maximum growth rate, which contrasts to much lower expression of mei2 under the same conditions [data not shown].

---

**Figure 1.** A restriction map of the ste11 locus and complementation analysis of subclones. The insert in the original clone pSX1 is illustrated at the top. The extent and direction of the ste11 ORF, which encodes 468 amino acids, is shown by a solid arrow. Open arrows indicate the orientation of readthrough transcription from a cryptic promoter on the vector pDB248'. The fragment inserted in pSX-D1 and pSX-D2 apparently carries the authentic ste11 promoter, whereas that in pSX-D3 and pSX-D4 does not. Complementation was assayed by mating and sporulation proficiency of the recipient ste11 + cells. The construct used in disruption of ste11 is shown schematically at bottom. The presumed promoter region and amino-terminal one-fourth of the ste11 ORF was replaced by a ura4 + cassette (Grimm et al. 1988). Restriction sites shown are BamHI [B], BglII [Bg], CiaI [C], EcoRI [E], HindIII [H], MluI [M], PstI [P], PvuII [Pvu], SpI [S], and XhoI [X].
shown). Thus, the regulation of ste11 expression by nutritional conditions appears to be less rigorous.

Because induction of mei2 in response to nitrogen starvation has been shown to be blocked by cAMP [Watanabe et al. 1988], the effect of cAMP on ste11 expression was examined. The addition of 10 mM cAMP, together with 5 mM caffeine, a phosphodiesterase inhibitor, severely suppressed expression of ste11 both in the presence and absence of a nitrogen source [Fig. 4, lanes 3,4]. Conversely, ste11 was highly expressed in cells defective in cyr1, which have no detectable level of adenylyl cyclase and cAMP [Maeda et al. 1990], even in the presence of a nitrogen source [Fig. 4, lanes 5,6]. These results establish that ste11 expression is responsive to changes in the level of cAMP.

ste11 is essential for expression of genes required for sexual development and cells expressing ste11 are sexually derepressed

Induction of mei2 by nitrogen starvation did not occur in a ste11 mutant [Watanabe et al. 1988; also see Fig. 5]. Similarly, the four transcripts of the mating-type genes, which are normally inducible by nitrogen starvation [Kelly et al. 1988], were not induced in ste11− cells (Fig. 5). Apparently, ste11 regulates these genes positively.

---

**Figure 2.** DNA sequence and deduced amino acid sequence of the ste11 gene. The DNA sequence of the 3.67-kb BamHI-EcoRI fragment, carried on pSX1-D1 and pSX1-D2 [Fig. 1], is shown. The deduced amino acid sequence is shown below the DNA sequence in the single-letter code, starting with the first methionine codon. These sequence data have been submitted to the EMBL/GenBank data libraries.
To examine the consequences of unregulated expression of *stel1* in *S. pombe* cells, three plasmids, based on the vector pDB248' (Beach et al. 1982), were constructed that express *stel1* under the control of different promoters. pSX1-D4 carries a 3.2-kb HindIII-BglII fragment, which covers the entire *stel1* ORF but lacks its authentic promoter. The *stel1* ORF on this plasmid, however, could be transcribed in *S. pombe* cells from a cryptic promoter on the vector. pSX1-V2 carries the same fragment connected to the SV40 promoter. pSX1-A1 carries the same fragment connected to the *S. pombe adh* promoter. A homothallic *stel1*-disrupted strain, JZ396, was transformed with these three plasmids. Cells that became Leu+ as a result of the *S. cerevisiae LEU2* marker on the vectors were selected. Apparently heterogeneous transformants could be obtained with pSX1-D4 and pSX1-V2, whereas pSX1-A1 gave <10% as many transformants as the other two plasmids and these transformants showed heterogeneous aberrant phenotypes in growth and sexual development. This is probably because the *S. pombe adh* promoter is too strong; therefore, overproduced Ste11 protein causes deleterious effects on cell growth (see below). The pSX1-D4 and pSX1-V2 transformants could grow, but unlike wild-type cells, they readily produced zygotes and asci on nitrogen-rich SD medium (Fig. 6). Cells (10.1%) of the pSX1-V2 transformant were found to have undergone mating on SD. These observations strongly suggest that artificial expression of *stel1* can induce sexual development irrespective of the nutritional conditions, as was seen in *cyri1* mutants (Maeda et al. 1990). A large portion of these transformants continued to proliferate, although the truncated gene on pSX1-V2 was efficiently transcribed (see Fig. 5). This may mean that the truncated mRNA, lacking most of the 5'-untranslated region, is unable to direct production of a sufficient amount of Ste11 protein. Alternatively, Ste11 protein may not be fully active in the presence of a nitrogen source (see Discussion). As expected from its phenotype, the mating-type genes and *mei2* were expressed in the presence of nitrogen in the pSX1-V2 transformant (see Fig. 5).

*Stel1* protein synthesized in vitro binds to DNA

The results shown above suggest that the Ste11 protein could be a transcription factor that positively regulates
Figure 4. Effect of nitrogen starvation and cAMP on the expression of ste11. Total RNA was extracted from cells either rapidly growing or starved for nitrogen for 4 hr, and analyzed by Northern blotting. The probe used was the HindIII-BglII fragment covering the entire ste11 ORF, whereas a shorter fragment was used to detect ste11 mRNA in other experiments. (Lane 1) JY333 [cyt1 \(^{+}\)] in the presence of nitrogen; (lane 2) JY333 in the absence of nitrogen; (lane 3) the same as lane 1 but 10 mM cAMP and 5 mM caffeine were added; (lane 4) the same as lane 2 but cAMP and caffeine were added; (lane 5) JZ300 [cyt1 \(^{-}\)] in the presence of nitrogen; (lane 6) JZ300 in the absence of nitrogen. Equal loadings of RNA in these lanes were confirmed by ethidium bromide staining of rRNAs [not shown].

at least matP, matM, and mei2. Inspection of these genes revealed that they carry nearly identical 10-base motifs [TTCTTTGTGY] in their 5’-upstream regions (Fig. 7). Hereafter, we call them TR (T-rich) boxes. Five TR boxes are oriented in the same direction [regular orientation] in mei2. Two TR boxes were found in both matP and matM, and they locate in the central region of the two diverging transcription units in each case (Kelly et al. 1988). In the matP locus, they are regularly oriented toward matPi and hence in reverse order toward matPc, whereas they are regularly oriented toward matMc in the matM locus. Gel-shift experiments using oligonucleotides with this motif as a probe showed that S. pombe extracts exhibit a Ste11-dependent activity that binds specifically to the probes [data not shown]. To demonstrate that Ste11 protein itself binds to DNA, we performed reverse gel-shift experiments with Ste11 protein produced in wheat germ cell extract (Fig. 8). The results showed that the Ste11 protein could bind to double-stranded probes with TR motifs [Fig. 8C, lanes 3,5] but not to a mutated TR box in which the invariant G was replaced by T [Fig. 8C, lane 4] or to single-stranded probes [data not shown]. Furthermore, a truncated Ste11 protein that lacks the HMG domain lost the ability to bind to the probe [Fig. 8C, lane 7], suggesting that this domain is necessary for DNA binding.

The TR box is an essential cis-acting element for gene induction under nitrogen starvation

We wanted to determine whether the TR box is required in vivo for gene induction in response to nitrogen starvation. A plasmid carrying part of the mei2 gene was conveniently used for this purpose. This plasmid, pDB[mei2]\(\Delta\)12, carries two of the four transcription initiation sites of mei2 identified previously (Watanabe et al. 1988), two of the five TR boxes found in their vicinity (c and d in Fig. 7), and most of the mei2 ORF [Fig. 9A]. A mei2-disrupted strain, YJ776, was transformed with pDB[mei2]\(\Delta\)12 and its derivatives and analyzed by Northern blotting. As shown in Figure 9B, transcription of mei2 from pDB[mei2]\(\Delta\)12 was considerably inducible by nitrogen starvation [lanes 1,2]. However, this induction was reduced when the conserved G in TR box d was altered to T [pDB[mei2]\(\Delta\)12M1] [Fig. 9B, lanes 3,4]. Induction of the shorter transcript appeared to be inhibited more severely. If the same mutation was also introduced into the remaining TR box c, the inducibility was almost completely abolished [pDB[mei2]\(\Delta\)12M2] [Fig. 9B, lanes 5,6]. These observations establish the requirement of the TR box as a controlling cis-acting element for induction of the mei2 gene. Together with the fact that Ste11 protein binds to a TR box in vitro [Fig. 8], this result strongly suggests that induction of nitrogen starvation-responsive genes is due to binding of the Ste11 transcription factor to TR boxes upstream of these genes.

Figure 5. Summary of Northern blot analysis of genes required for sexual development. Total RNA was prepared from ste11\(^{+}\) and ste11\(^{-}\) cells, either growing exponentially or starved for nitrogen. RNA from cells expressing ste11 in the presence of nitrogen was also analyzed. Genes analyzed are listed at left; the probes to detect each transcript are described in Materials and methods. [Lanes 1,2] YJ450 [ste11\(^{+}\)]; [lanes 3,4] JZ396 [ste11\(^{-}\)] carrying the vector pDB248\(^{+}\); [lane 5] JZ396 carrying pSX1-V2. Cells were either grown in the presence of nitrogen [lanes 1,3,5], or starved for nitrogen for 4 hr [lanes 2,4]. The ste11 transcript in lane 5 (2.4 kb), expressed from the truncated gene on pSX1-V2, is shorter than the authentic ones seen in the other lanes (3.6 kb). The matMc and matM\(i\) transcripts, which are both 0.6 kb in length [Kelly et al. 1988], were measured collectively in this analysis. The sizes of other transcripts are as follows: mei2, 4.2 kb [Watanabe et al. 1988]; matPc, 0.75 kb [Kelly et al. 1988]; matPi, 0.56 kb [Kelly et al. 1988]. [Bottom] The ethidium bromide-stained patterns of rRNA, which verifies approximately equal loadings of total RNA in the five lanes.
indicate that the Stel 1 protein has intrinsic sequence specificity for DNA binding. However, we note that apparently only a small fraction of Stel 1 protein migrated to sexual development in response to nitrogen starvation (Egel 1973; Egel and Egel-Mitani 1974). We have proposed that depletion of nitrogen results in a reduction in the level of intracellular cAMP which, in turn, serves as a signal for the subsequent gene expression. The activity of adenyl cyclase, which catalyzes production of cAMP, appears to be regulated at the enzyme level in *S. pombe* cells (Maeda et al. 1990), but the mechanism that connects nitrogen depletion to reduction in this activity remains unknown. This study, however, has shed light on the subsequent steps of sexual development. It appears that *ste11* is initially induced in response to reduction in the intracellular cAMP level and that other genes required for the commitment to sexual development are subsequently activated by the Stel 1 protein. Because constitutive expression of *ste11* can induce sexual development under conditions otherwise favorable for vegetative growth, expression of *ste11* appears to be the principal target of the regulation by cAMP during sexual development. Consistent with this idea, we recently found that expression of *S. pombe ste6*, which encodes a putative activator of the Ras1 protein (Hughes et al. 1990), is also regulated by Stel 1 [D. Hughes and M. Yamamoto, unpubl.]. Because ras1 function is essential for mating (Fukui et al. 1986; Nadin-Davis et al. 1986), this observation reinforces the view that Stel1 plays a pivotal role in sexual development in *S. pombe*.

The deduced Stel1 protein has an HMG box, which has been shown to bind to DNA in some cases (Wright and Dixon 1988; Jantzen et al. 1990), although it may not direct DNA sequence specificity by itself (Bell et al. 1990). The results of the reverse gel-shift assay [Fig. 8] indicate that the Stel1 protein has intrinsic sequence specificity for DNA binding. However, we note that apparently only a small fraction of Stel1 protein migrated in association with DNA in the reverse gel-shift assay, and it remains an open question whether Stel1 can perform its function in vivo by itself or whether it collaborates with other elements.

Among the HMG-box family of proteins, the function of Ste11, MatMc, and TDF may be related most closely, as they share conserved amino acid residues in the carboxy-terminal region of the HMG box [Fig. 3A, asterisks], although it is not yet clear whether the HMG-box proteins can be classified into subfamilies. Another interesting feature is that Stel1 regulates matMc, which encodes an HMG-box protein. Thus, *S. pombe* uses HMG-box proteins twice, in a cascading manner, to regulate sexual development.

Expression of the *ste11* gene is induced when the level of intracellular cAMP is reduced [see Fig. 4]. How does the cAMP pathway control *ste11* expression? One possibility is that the Stel1 protein is a transcriptional activator of the *ste11* gene itself, which is inactive while phosphorylated by protein kinase A. This hypothesis assumes that nitrogen starvation lowers the activity of protein kinase A and leads to accumulation of dephosphorylated Stel1 protein, which results in transcriptional activation of *ste11* in an autoregulatory fashion. Consistent with this idea, we found a tentative TR box in the 5'-upstream region of *ste11* [Fig. 7]. It remains an interesting question whether such an autoregulatory mechanism operates as a key switch for *S. pombe* sexual development.

A motif similar to the TR box, YYYYATTGTTCTC, has been proposed as a consensus operator sequence of *Saccharomyces cerevisiae* hypoxic regulons and is probably recognized by the ROX1 gene product [Lowry et al. 1990]. Interestingly, Rox1 protein also has an HMG box [C. Lowry and S. Zitomer, pers. comm.]. Although Rox1 regulates gene expression negatively, whereas Stel1 does not allow wild-type cells to mate and sporulate (<1 x 10⁻²). (A) JZ396 [ste11⁺] carrying the vector pDB248. (B) JZ396 carrying pSX1-V2 and expressing ste11 constitutively. White arrowheads point to asci and a possible zygote. Larger cells seen here but not in A are likely to represent diploids.

![Figure 6: Unconditional mating and sporulation displayed by ste11-expressing cells.](image)

**Figure 6.** Unconditional mating and sporulation displayed by ste11-expressing cells. Cells were cultured on SD medium, which does not allow wild-type cells [ste11⁺] to mate and sporulate (<1 x 10⁻²). (A) JZ396 [ste11⁺] carrying the vector pDB248. (B) JZ396 carrying pSX1-V2 and expressing ste11 constitutively. White arrowheads point to asci and a possible zygote. Larger cells seen here but not in A are likely to represent diploids.

**Discussion**

*S. pombe* cells initiate sexual development in response to nitrogen starvation (Egel 1973; Egel and Egel-Mitani 1974). Consensus elements, named TR boxes, of the genes induced at the initial stage of sexual development. These sequences are found in the 5'-upstream region of each ORF. The conserved G in box d of mei2 is essential for binding to Stel1 protein [see text]. The sequences shown here correspond to the following nucleotides defined in the original publication. *mei2*: a, -1896 to -1879; b, -1874 to -1857; c, -1702 to -1685; d, -1522 to -1505; e, -917 to -900 [Watanabe et al. 1988]. *matP*: 528–545 and 543–560 [Kelly et al. 1988]. *matM*: 298–315 and 314–331 [Kelly et al. 1988]. *ste11*: -1381 to -1364 [this study].

Transcription factor for sexual development

**Figure 7.** Conserved elements, named TR boxes, of the genes induced at the initial stage of sexual development. These sequences are found in the 5'-upstream region of each ORF. The conserved G in box d of mei2 is essential for binding to Stel1 protein [see text].
stel1 cells may simply reflect the lack of gene expression necessary to produce or secrete mating factors. This explanation may also be the case for matMi, although there is no direct evidence on this point. The matPc, matMc, and mei2 genes are inducible in heterothallic cells by nitrogen starvation and, hence, are likely to be regulated directly by stel1. Some other genes, like ste6, appear to be regulated in a similar manner.

so positively, the two regulatory systems, both of which respond to changes in environmental conditions, may have conserved cis- and trans-acting elements.

Failure of ste11 mutants to transcribe matPi may result from a defect in synthesis of mating factors. The matPi gene is highly induced by nitrogen starvation in homothallic h^o^o^ cells, which contain both P and M cells, but not in heterothallic h^-^- cells [Nielsen and Egel 1990]. Thus, nitrogen starvation alone is not sufficient for induction of matPi transcription; the M-mating factor is essential for its expression. Because ste11 mutants do not secrete mating factors (Y. Iino and M. Yamamoto, unpubl.), the absence of matPi mRNA in homothallic

Figure 8. Reverse gel-shift assay of the DNA-binding activity of Ste11 protein. (A) Nucleotide sequences of three oligonucleotide probes used. ME1 carries TR box d in the mei2 upstream region (see Figs. 7 and 9); ME2 has the same sequence as ME1 except that the invariant G-C pair in the TR box is substituted by T-A. MP1 corresponds to the upstream region of matP and carries two TR boxes. (B) Ste11 protein produced in wheat germ extract (for details see Materials and methods). (Lane 1) No RNA was added to the wheat germ extract; (lane 2) RNA transcribed from pET(stell)-2 was added; (lane 3) RNA transcribed from pET(stel 1)-1 was added. A full-length Ste11 protein [M, 51,000] and a truncated Ste11 protein that lacks the HMG domain [M, 43,000] are apparently produced in lanes 2 and 3, respectively. Samples were run in a 10% SDS-polyacrylamide gel and fluorographed. Lines at left represent markers for a molecular mass: from top to bottom, 92, 66, 42, 34, and 26 kD. (C) Mobility-shift assay of radioactive Ste11 bands. Each lane received the following combination of protein and probe DNA. (Lane 1) No Ste11, probe ME1; (lane 2) full-length Ste11, no probe; (lane 3) full-length Ste11, ME1; (lane 4) full-length Ste11, MP1; (lane 5) truncated Ste11, no probe; (lane 7) truncated Ste11, ME1. The majority of radioactive Ste11 protein was stacked at the top of the gel, and new shifted bands were seen only in lanes 3 and 5.

Figure 9. Changes in inducibility of mei2 expression by mutations in TR boxes. (A) The structure of the 5'-upstream region of mei2 is illustrated at top. (A1) Five TR boxes shown in Fig. 7. Arrows indicate four transcription initiation sites identified previously [Watanabe et al. 1988]. Some restriction sites are also indicated. The oligonucleotide ME1 used for the in vitro-binding assay [Fig. 8] and three plasmids used in the following analysis are shown schematically below. pDB(meii2)A12 has intact TR boxes c and d. pDB(meii2)A12M1 has intact box c, but box d has a G → T change. The two TR boxes in pDB(meii2)A12M2 are both mutated. Only intact TR boxes are shown as A. (B) JY776 transformed with either of the above three plasmids was analyzed by Northern blotting for expression of the plasmid-borne mei2 gene in the presence or absence of a nitrogen source. Experimental conditions were the same as those in Fig. 5. (Lane 1) pDB(meii2)A12 transformant growing exponentially; (lane 2) the same transformant starved for nitrogen; (lane 3) pDB(meii2)A12M1 transformant growing exponentially; (lane 4) the same transformant starved for nitrogen; (lane 5) pDB(meii2)A12M2 transformant growing exponentially; (lane 6) the same transformant starved for nitrogen. Equal loadings of RNA in these lanes were confirmed by ethidium bromide staining of rRNAs [not shown].
be regulated both by nutritional conditions and by mating factors [D. Hughes, N. Yabana, M. Yamamoto, unpub.]. Investigation of the interaction between the nutrient-sensing and the mating factor response pathways should help us to understand further how different signals are integrated to drive cell differentiation.

Materials and methods

Strains, media, and genetic methods

Table 1 summarizes *S. pombe* strains used in this study. Complete medium YPD and minimal medium SD [Sherman et al. 1986] were used for routine culture of *S. pombe* strains. Malt extract agar medium [MEA] (Gutz et al. 1974), which contains a limited amount of nitrogen, was used to induce mating and sporulation after permitting some cell growth. Minimal medium PM and its nitrogen-free derivative PM-N [Beach et al. 1985; Watanabe et al. 1988] were used for nitrogen-starvation experiments. General genetic methods were as described by Gutz et al. [1974]. Transformation [Beach et al. 1982; Okazaki et al. 1990] and gene disruption [Rothstein 1983; Fukui et al. 1986] were done as described.

**Plasmids**

YIp468, in which the ste11 gene was cloned originally, is a chimeric plasmid that consists of YIp33 developed for *S. cerevisiae* [Botstein et al. 1979] and a 10.4-kb HindIII fragment that carries one repeating unit of the *S. pombe* rRNA gene [Schaak et al. 1982]. Because centromere-bearing plasmids, like *S. cerevisiae* YCp plasmids [Hsiao and Carbon 1981], are not available in *S. pombe*, YIp468 was developed as a possible low-copy vector, although its copy number has not been measured accurately. In other experiments, pDB248' [Beach et al. 1982] was used as the vector to transform *S. pombe*. Three plasmids based on pDB248' were used to express ste11 constitutively. pSX1-D4 has the ste11 ORF inserted at the HindIII site of pDB248' and uses a cryptic promoter on the vector to express ste11. pSX1-V2 has the same structure as pSX-D4 except that a 342-bp HindIII-PvuII fragment carrying the SV40 early promoter was inserted upstream of the ste11 ORF. This promoter has been shown to function in *S. pombe* [Käfer et al. 1985]. pSX1-A1 has a 0.7-kb Sp61–EcoRI fragment carrying *S. pombe* adh promoter [Russell and Hall 1983] inserted at the same site. pDB(meii2)A12, used in the experiments shown in Figure 9, was a derivative of the mei2 clone pDB(meii2)2 [Shimoda et al. 1987]. Mutagenesis of pDB(meii2)A12 to pDB(meii2)A12M1 or pDB(meii2)A12M2 was performed by using oligonucleotides as described previously [Kunkel 1985].

**Table 1. S. pombe strains used**

| Strain | Genotype |
|--------|----------|
| JY333  | h^+ ade6-M216 leu1 |
| JY450  | h^{90} ade6-M216 leu1 |
| JY685  | h^{90} ade6-M216 pat1-110 ste11-29 |
| JY736  | h^{90} ade6-M210 leu1 ste11-29 |
| JY776  | h^+ ade6-M210 leu1 ura4-D18 mei2::ura4^+ |
| JY878  | h^{90} ade6-M216 leu1 ura4-D18 |
| JY990  | h^{90} ade6-M216 pat1-110 |
| JZ300  | h^+ ade6-M216 leu1 ura4-D18 cyr1::ura4^+ |
| JZ396  | h^{90} ade6-M216 leu1 ura4-D18 ste11::ura4^+ |

**Northern blotting analysis**

*S. pombe* cells growing in PM were sampled at a cell density of 5 × 10^6/ml, and the remaining cells were starved for nitrogen in PM-N for 4 hr. Total RNA was prepared from these cells, and RNA-blotting analysis was performed essentially according to Thomas [1980] with modifications as described previously [Watanabe et al. 1988]. DNA fragments used as the probe to detect each transcript are as follows: ste11, 1.3-kb PvuII–PvuII [this study]; mei2, 3.3-kb PvuII–HindIII [Watanabe et al. 1988]; matPc and matPi, 0.9-kb HindIII–MluII [Kelly et al. 1988]; matMc and matMi, 0.37-kb BclI–EcoRI [Kelly et al. 1988].

In vitro production of Ste11 protein

Two plasmids were prepared based on the vector pET-3a, which carries a bacteriophage T7 promoter [Rosenberg et al. 1987]. pET[ste11]-1 carries a 2.3-kb NdeI–BglII fragment that covers amino acid residues 73–468 of Ste11 protein. pET[ste11]-2 was designed to express the complete Ste11 protein. To do so, the NdeI site at histidine 72–methionine 73 of the cloned ste11 gene was mutagenized to CACATG, and a new NdeI site was created at the initiation codon [Kunkel 1985]. The new 2.5-kb NdeI–BglII fragment was cloned in pET-3a. These two plasmids were linearized with MluI and the ste11 constructs were transcribed in vitro by using T7 RNA polymerase [Tabor and Richardson 1985]. After phenol–butanol extraction, RNA was transcribed in wheat germ extract (Promega) containing 60 mm potassium acetate and [35S]methionine (Amersham) in 25 μl of reaction mixture. Incubation was at 25°C for 60 min.

Reverse gel-shift assay of protein–DNA binding

The DNA-binding activity of Ste11 was assayed essentially according to Hope and Struhl [1985]. Each assay sample contained in a total volume of 10 μl, 3 μl of Ste11 solution (made in vitro), labeled with 35S of poly[d(I-C)]–poly[d(I-C)] (Pharmacia), 0.5 μg of pUC118 DNA, 6 mm MgCl₂, 10 mm Tris–HCl (pH 8.0), 0.1 mm EDTA, 10% glycerol, and 0.1 μg of probe DNA to be examined. The assay mixture was incubated at 25°C for 30 min and analyzed by nondenaturing 5% polyacrylamide gel electrophoresis. The gel was fixed and fluorographed.

Acknowledgments

We thank K. Furuhashi-Hirabayashi for her earnest technical assistance, Dr. M. Nishizawa for introduction to the wheat germ extract system, and Dr. D.A. Hughes for critical reading of the manuscript. This work was supported by grants-in-aid to M.Y. from the Ministry of Education, Science, and Culture of Japan and from the Mitsubishi Foundation.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 USC section 1734 solely to indicate this fact.
Beach, D., M. Piper, and P. Nurse. 1982. Construction of a Schizosaccharomyces pombe gene bank in a yeast bacterial shuttle vector and its use to isolate genes by complementation. Mol. Gen. Genet. 187: 326–329.

Beach, D., L. Rodgers, and J. Gould. 1985. RAN1* controls the transition from mitotic division to meiosis in fission yeast. Curr. Genet. 10: 297–311.

Bell, S.P., H.-M. Lantzen, and R. Tjian. 1990. Assembly of alternative multiprotein complexes directs rRNA promoter selectivity. Genes & Dev. 4: 943–954.

Berta, P., I.R. Hawkins, A.H. Sinclair, A. Taylor, B.L. Griffiths, P.N. Goodfellow, and M. Fellous. 1990. Genetic equation proving SRY and the testis-determining factor. Nature 348: 448–450.

Botstein, D., S.C. Falco, S.E. Stewart, M. Brennann, S. Scherer, D.T. Stinchcomb, K. Struhl, and R.W. Davis. 1979. Sterile host yeasts (SHY): A eukaryotic system of biological containment for recombinant DNA experiments. Gene 8: 17–24.

Bresch, C., G. Müller, and R. Egel. 1968. Genes involved in meiosis and sporulation of a yeast. Mol. Gen. Genet. 102: 301–306.

Devereux, J., F. Haebelri, and O. Smithies. 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. 12: 387–395.

Egel, R. 1973. Commitment to meiosis in fission yeast. Mol. Gen. Genet. 121: 277–284.

Egel, R. and M. Egel-Mitani. 1974. Premeiotic DNA synthesis in fission yeast. Exp. Cell Res. 88: 127–134.

Fukui, Y., T. Kozasa, Y. Kaziro, T. Takeda, and M. Yamamoto. 1986. Role of a ras homolog in the life cycle of Schizosaccharomyces pombe. Cell 44: 329–336.

Girgadies, O. 1982. Sterile mutants of Schizosaccharomyces pombe: Analysis by somatic hybridization. Curr. Genet. 6: 223–227.

Grimm, C., J. Kohli, J. Murray, and K. Maundrell. 1988. Genetic engineering of Schizosaccharomyces pombe: A system for gene disruption and replacement using the ura4 gene as a selectable marker. Mol. Gen. Genet. 215: 81–86.

Gutz, H., H. Heslot, U. Leupold, and N. Loprieno. 1974. Schizosaccharomyces pombe. In Handbook of genetics (ed. R.D. King), vol. 1, pp. 395-446. Plenum, New York.

Henikoff, S. 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene 28: 351–359.

Hope, I.A. and K. Struhl. 1985. GCN4 protein, synthesized in vitro, binds HIS3 regulatory sequences: Implications for general control of amino acid biosynthetic genes in yeast. Cell 43: 177–188.

Hsiao, C. and J. Carbon. 1981. Characterization of a yeast replication origin [ARS2] and construction of stable minichromosomes containing cloned yeast centromere DNA (CEN3). Gene 15: 157–166.

Hughes, D.A., Y. Fukui, and M. Yamamoto. 1990. Homologous activators of ras in fission and budding yeast. Nature 344: 355–357.

Iino, Y. and M. Yamamoto. 1985a. Mutants of Schizosaccharomyces pombe which sporulate in the haploid state. Mol. Gen. Genet. 198: 416–421.

Iino, Y. and M. Yamamoto. 1985b. Negative control for the initiation of meiosis in Schizosaccharomyces pombe. Proc. Natl. Acad. Sci. 82: 2447–2451.

Jantzen, H.-M., A. Admon, S.P. Bell, and R. Tjian. 1990. Nucleolar transcription factor hUBF contains a DNA-binding motif with homology to HMG proteins. Nature 344: 830–836.

Jäger, R.J., M. Anvret, K. Hall, and G. Scherer. 1990. A human XY female with a frame shift mutation in the candidate testis-determining gene SRY. Nature 348: 452–454.

Käfer, N.F., V. Simanis, and P. Nurse. 1985. Fission yeast Schizosaccharomyces pombe correctly excises a mammalian RNA transcript intervening sequence. Nature 318: 78–80.

Kelly, M., J. Burke, M. Smith, A. Klar, and D. Beach. 1988. Four mating-type genes control sexual differentiation in the fission yeast. EMBO J. 7: 1537–1547.

Kitamura, K., T. Nakagawa, and C. Shimoda. 1990. Novel sterile mutants of the fission yeast Schizosaccharomyces pombe which are defective in their response to starvation. Curr. Genet. 15: 315–321.

Kolodrubetz, D. 1990. Consensus sequence for HMG1-like DNA binding domains. Nucleic Acids Res. 18: 5565.

Kolodrubetz, D. and A. Burgum. 1990. Duplicated NHP6 genes of Saccharomyces cerevisiae encode proteins homologous to bovine high mobility group protein 1. J. Biol. Chem. 265: 3234–3239.

Koopman, P.A., A. Münsterberg, B. Capel, N. Vivian, and R. Lovell-Badge. 1990. Expression of a candidate sex-determining gene during mouse testis differentiation. Nature 348: 450–452.

Kunkel, T.A. 1985. Rapid and efficient site-specific mutagenesis without phenotypic selection. Proc. Natl. Acad. Sci. 82: 488–492.

Lowry, C.V., M.E. Cerdán, and R.S. Zitomer. 1990. A hypoxic consensus operator and a constitutive activation region regulate the ANB1 gene of Saccharomyces cerevisiae. Mol. Cell. Biol. 10: 5921–5926.

Maeda, T., N. Mochizuki, and M. Yamamoto. 1990. Adenyllyl cyclase is dispensable for vegetative cell growth in the fission yeast Schizosaccharomyces pombe. Proc. Natl. Acad. Sci. 87: 7814–7818.

Nadin-Davis, S.A., A. Nasim, and D. Beach. 1986. Involvement of ras in sexual differentiation but not in growth control in fission yeast. EMBO J. 5: 2963–2971.

Nielsen, O. and R. Egel. 1990. The pat1 protein kinase controls transcription of the mating-type genes. EMBO J. 9: 1401–1406.

Nurse, P. 1985. Mutants of the fission yeast Schizosaccharomyces pombe which alter the shift between cell proliferation and sporulation. Mol. Gen. Genet. 198: 497–502.

Okazaki, K., N. Okazaki, K. Kume, S. Jinno, K. Tanaka, and H. Okayama. 1990. High-frequency transformation method and library transducing vectors for cloning mamalian cDNAs by trans-complementation of Schizosaccharomyces pombe. Nucleic Acids Res. 18: 6485–6489.

Rosenberg, A.H., B.N. Lade, D. Chui, S. Lin, J.J. Dunn, and F.W. Studier. 1987. Vectors for selective expression of cloned DNAs by T7 RNA polymerase. Gene 56: 125–135.

Rothstein, R. 1983. One step gene disruption in yeast. Methods Enzymol. 101: 202–211.

Russell, P.R. and B.D. Hall. 1983. The primary structure of the alcohol dehydrogenase gene from the fission yeast Schizosaccharomyces pombe. J. Biol. Chem. 258: 143–149.

Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. 74: 5463–5467.

Schach, J., J.-I. Mao, and D. Soll. 1982. The 5.8S RNA gene sequence and the ribosomal repeat of Schizosaccharomyces pombe. Nucleic Acids Res. 10: 2581–2584.

Sherman, F., G. Fink, and J. Hicks. 1986. Methods in yeast genetics: Laboratory course manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
Shimoda, C., M. Uehira, M. Kishida, H. Fujioka, Y. Iino, Y. Watanabe, and M. Yamamoto. 1987. Cloning and analysis of transcription of the mei2 gene responsible for initiation of meiosis in the fission yeast Schizosaccharomyces pombe. J. Bacteriol. 169: 93–96.

Sinclair, A.H., P. Berta, M.S. Palmer, J.R. Hawkins, B.L. Griffiths, M.J. Smith, J.W. Foster, A.-M. Frischauf, R. Lovell-Badge, and P.N. Goodfellow. 1990. A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. Nature 346: 240–244.

Sipiczki, M. 1988. The role of sterility genes [ste and aft] in the initiation of sexual development in Schizosaccharomyces pombe. Mol. Gen. Genet. 213: 529–534.

Tabor, S. and C.C. Richardson. 1985. A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes. Proc. Natl. Acad. Sci. 82: 1074–1078.

Thomas, P. 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. 77: 5201–5205.

Tsuda, K.-I., M. Kikuchi, K. Mori, S. Waga, and M. Yoshida. 1988. Primary structure of non-histone protein HMG1 revealed by the nucleotide sequence. Biochemistry 27: 6159–6163.

Watanabe, Y., Y. Iino, K. Furuhata, C. Shimoda, and M. Yamamoto 1988. The S. pombe mei2 gene encoding a crucial molecule for commitment to meiosis is under the regulation of cAMP. EMBO J. 7: 761–767.

Wen, L., J.-K. Huang, B.H. Johnson, and G.R. Reeck. 1989. A human placental cDNA clone that encodes nonhistone chromosomal protein HMG-1. Nucleic Acids Res. 17: 1197–1214.

Wright, J.M. and G.H. Dixon. 1988. Induction by torsional stress of an altered DNA conformation 5′ upstream of the gene for a high mobility group protein from trout and specific binding to flanking sequences by the gene product HMG-T. Biochemistry 27: 576–581.
Schizosaccharomyces pombe ste11+ encodes a transcription factor with an HMG motif that is a critical regulator of sexual development.

A Sugimoto, Y Iino, T Maeda, et al.

Genes Dev. 1991, 5: 1990
Access the most recent version at doi:10.1101/gad.5.11.1990

References
This article cites 47 articles, 11 of which can be accessed free at:
http://genesdev.cshlp.org/content/5/11/1990.full.html#ref-list-1

License

Email Alerting Service
Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or click here.