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The *S.pombe mei2* gene encoding a crucial molecule for commitment to meiosis is under the regulation of cAMP

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The complete nucleotide sequence of the *mei2* gene of *Schizosaccharomyces pombe*, which is essential for initiation of meiosis, is presented and four transcriptional start sites assigned. Transcription of *mei2* and other genes involved in life cycle control of *S.pombe*, which is inducible by nitrogen starvation, is inhibited by addition of cAMP, suggesting that cAMP can mediate the signal of nitrogen supply in *S.pombe*. *mei2* is the furthest downstream among target genes regulated by cAMP and genetic or physiological factors so far shown to block uncontrolled meiosis in *S.pombe*, which is provoked by inactivation of the *pat1* gene product, are either mutations at the *mei2* locus or inhibitors of its expression. Cooperation of two regulatory pathways, one leading to the inactivation of *pat1* activity and the other to the supply of the *mei2* product, appears to commit cells to meiosis in *S.pombe*.

Key words: fission yeast/nitrogen source/cAMP/transcriptional inhibition/meiotic control

Introduction

The analysis of meiotic mutants and relevant gene clones of *Schizosaccharomyces pombe* has established a sequential pathway of gene functions that regulates the initiation of meiosis in this fission yeast. Mating type heterozygosity, i.e. a joint function of *mat1-P* and *mat1-M*, and nitrogen starvation are the two major prerequisites for meiosis (Leupold, 1970; Egel, 1971). The *mei3* gene is expressed only when these two prerequisites are satisfied and its artificial expression results in uncontrolled sporulation (McLeod *et al.*, 1987). The same phenotype was shown to be caused by loss of the *pat1 (ran1)* gene activity (Iino and Yamamoto, 1985a; Nurse, 1985). The meiotic process induced by inactivation of the *pat1* product disregards the two prerequisites yet reflects natural meiosis. For instance, pre-meiotic DNA synthesis and meiotic recombination take place normally and it was proposed that the *pat1* product is a repressor of the initiation reaction for meiosis which is inactivated when the natural requirements for meiosis are satisfied (Iino and Yamamoto, 1985b). The *pat1* mutation is effective in the absence of *mei3* activity (Iino and Yamamoto, 1985b; Beach *et al.*, 1985), indicating the *pat1* is functionally downstream to *mei3*. Thus, it is reasonable to assume that *mei3* activity is necessary to inactivate the pat1 product, directly or indirectly, in meiotic control.

Inactivation of the *pat1* product is not the event which commits cells to meiosis. Defects in the *mei2* gene, whose function is required prior to pre-meiotic DNA synthesis in natural meiosis (Bresch *et al.*, 1968; Shimoda *et al.*, 1985), completely neutralize the effect of the *pat1* mutation (Iino and Yamamoto, 1985a; Beach *et al.*, 1985). The *mei2* activity is thus critical in switching from the mitotic cell cycle into the meiotic pathway and is most likely to be a key determinant of the commitment to meiosis in *S.pombe*. A characteristic of this gene is that nitrogen starvation strongly induces its transcription, but this induction can be seen in cells of any mating type (Shimoda *et al.*, 1987). Thus it is obvious that expression of *mei2* is not sufficient by itself for the commitment.

To understand better the process of commitment to meiosis, we have determined the complete nucleotide sequence of the *mei2* gene and precisely assigned its four start sites for transcription. Furthermore, three conditions which suppress the *pat1* mutation and are simultaneously inhibitory for natural meiosis have been analyzed and the reason for such suppression is shown to be that *mei2*, as well as some other genes specific to the sexual life cycle, becomes uninducible by nitrogen starvation under these conditions. The conditions analyzed include elevation of intracellular levels of cAMP. It is also shown that *mei2* is the furthest downstream among genes whose expression is essential for meiosis and is negatively controlled by cAMP.

This article presents the basic characteristics of the *mei2* gene and demonstrates the critical nature of a pathway leading from nitrogen starvation to expression of this gene during commitment to meiosis in *S.pombe*. A possible involvement of a cAMP cascade in control of this pathway is also revealed. This is particularly interesting as the physiological role of cAMP in *S.pombe* has so far been unclear.

Results

Structure of the mei2 gene

The original *mei2* clone used in this study was described previously (Shimoda *et al.*, 1987). A 5.5 kb *Xho*I–*Hin*dIII DNA fragment has been sequenced and Figure 1 summarizes the relevant nucleotide sequence determined. The sequence from −1051 to 2674 (*Pvu*I–*Cla*I) in Figure 1 is the shortest region demonstrated to complement *mei2* defects *in vivo* when carried on a multicopy plasmid and to possess a promoter activity by itself. This region contains a coding capacity of 750 amino acids, whose sequence reveals no strong homology with any known proteins. The deduced amino acid sequence of the *mei2* product is shown in Figure 2. Preliminary experiments have shown that a protein of this size can be precipitated from *mei2*$^+$ cells but not from

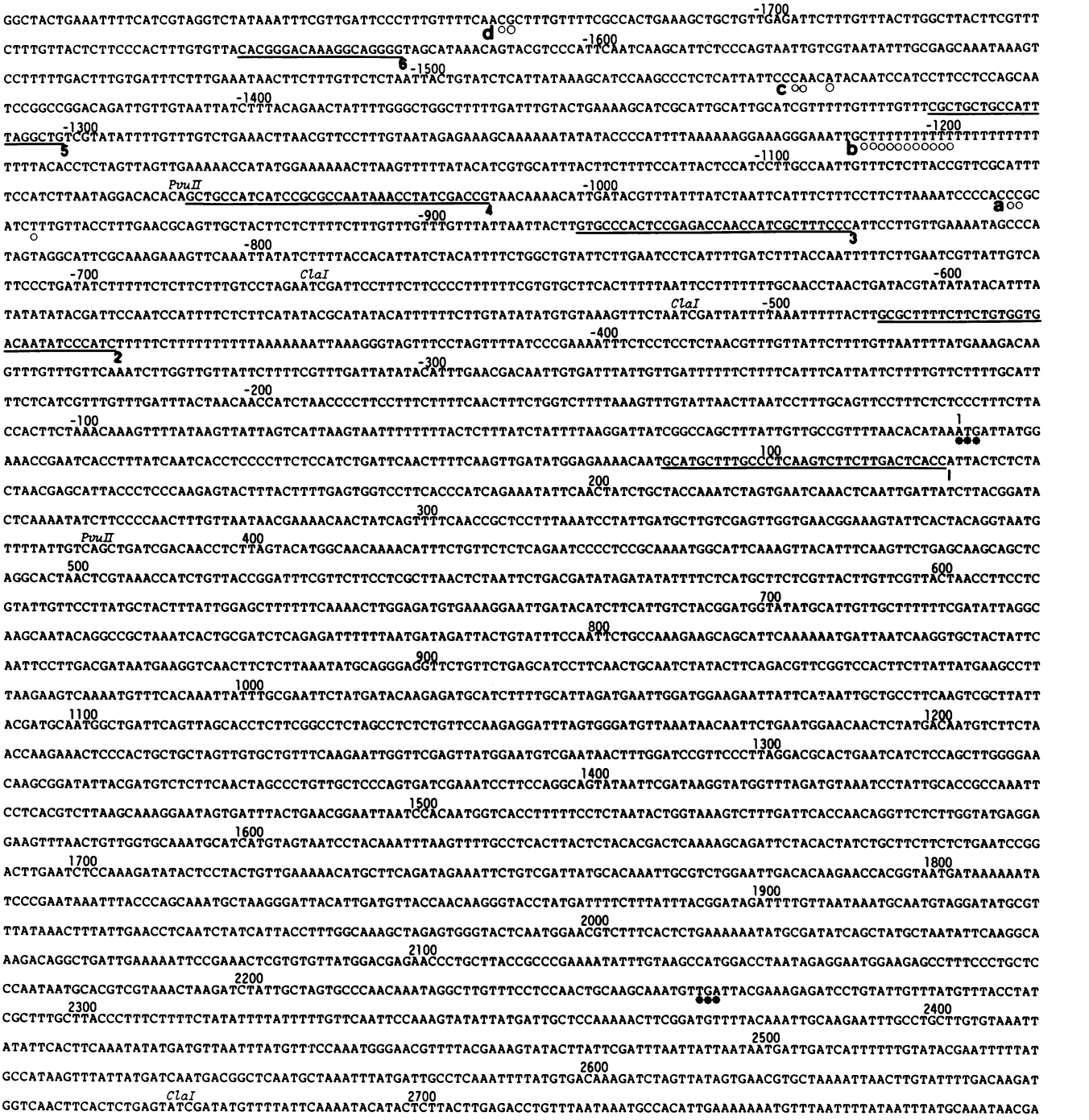

Fig. 1. Nucleotide sequence of the *mei2* region. The deduced initiation and termination codons are marked with asterisks. Numbering starts at the initiation codon. No intron has been detected in the deduced *mei2* coding region. Four major transcription start sites are shown by letters a, b, c and d. Open circles indicate nucleotides at which transcription initiates in each site. The larger circle indicates a nucleotide showing the strongest initiation activity in that site. Although not indicated, possible TATA boxes characteristic of eukaryotic promoters (Gannon *et al.*, 1979) can be assigned 30−40 bp upstream of each start site. Areas underlined, 1−6, indicate the sequence to which each primer used in the primer-extension analysis hybridizes. The sequence AATAAA at position 2717 is a possible poly(A) addition signal (Proudfoot and Brownlee, 1976), which matches the length of the transcripts, although no information has been obtained of the 3′-termini of the transcripts. Recognition sites of the restriction endonucleases *Cla*I and *Pvu*II are indicated.

mei2-disrupted cells by antibodies raised against a fused protein (Watanabe and Yamamoto, unpublished results), suggesting that the coding capacity identified here indeed corresponds to the *mei2* product. The deduced product is rich in serine (14%) and appears to be a hydrophilic protein.

Transcription start sites for mei2

We have previously noted that transcripts of *mei2* are always heterogeneous, giving a broad major band of ~4.2 ± 0.2 kb in length on Northern analysis (Shimoda *et al.*, 1987). To analyze this heterogeneity, primer extension experiments

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MIMETESPLSITSPSPSDSTFQVDMEKTMHALPSSLLDSPLLSTNEHYPP
KSTLLLSGPSPIRNIQLSATKSSESNSIDYLTDTQNIFPNFVNNENNYQF
STAPLNPIDACRVGERKVFTTGNVLLSADRQPLSTWQQNISVLSESPPQN
GIQSYISSSEQAAQALTRKPSVTGFRSSSLNSNSDDIDIFSHASRYLFVT
NLPRIVPYATLLELFSKLGDVKGIDTSSLSTDGICIVAFFDIRQAIQAAK
SLRSQRFFNDRLLYFQFCQRSSIQKMINQGATIQFLDDNEGQLLLNMQGG
SVLSILQLQSILQTFGPLLIMKPLRSQNVSQIICEFYDTRDASFALDELD
GRIIHNCCLQVAYYDAMADSVSTSSASSLSVPRGFSGMLNNNSEWNNSMT
MSSNQETPTAASCAVSRIGSSYGMSNNFGSVPLGRTESSPAWGTSGYYDV
SSTSPVAPSDRNPSRQYNSIRYGLDVNPIAPPNSSRLKQRNSDLLNGINP
QWSPFSSNTGKVFDSPTGSLGMRRSLTVGANASCSNPTNLSFASLTLHDS
KADSTLSASSLNPDLNLQRYTPTVEKHASDRNSVDYAQIASGIDTRTTVM
IKNIPNKFTQQMLRDYIDVTNKGTYDFLYLRIDFVNKCNVGYAFINFIEP
QSIITFGKARVGTQWNVFHSEKICDISYANIQGKDRLIEKFRNSCVMDEN
PAYRPKIFVSHGPNRGMEEPFPAPNNARRKLRSIASAQQIGLFPPTASKC
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Fig. 2. Deduced amino acid sequence of the *mei2* protein. A total of 750 amino acids, including the first methionine, is shown. The mol. wt of the putative *mei2* protein is ~83 000. Serine is the most abundant amino acid (106 residues).

were carried out and start sites for transcription determined. Two primers were prepared, one of which (Primer 1) hybridizes inside the coding region and the other (Primer 2) hybridizes to the putative 5′ untranslated region of *mei2* mRNA (Figure 1). Following extension using either primer, four major stop sites and some minor sites were detected (Figure 3). Four new primers (Primers 3–6), which hybridize to the region ~70–160 bases downstream of each major stop site, were then prepared and used in more detailed analysis. As shown in Figure 3, each site revealed further stop site microheterogeneity. It is concluded that transcription of *mei2* starts from four major sites (a, b, c and d in Figure 1), separated from each other by ~250 bases. This appears to explain the observed heterogeneity of the mRNA. However, *mei2* mRNA species are apparently transcribed coordinately under various conditions (Shimoda *et al.*, 1987), and the physiological significance of the multiple start sites for *mei2* transcription remains unclear. When the results of the extension analysis are combined, it is evident that no extensive splicing event is involved in the production of *mei2* mRNA. It is also concluded that the transcriptional start site furthest downstream (a in Figure 1) alone can provide sufficient *mei2* expression when carried on a plasmid.

Effects of cAMP on meiosis

Preliminary evidence suggesting that cAMP has a role in meiosis in *S.pombe* was provided by the finding that a high concentration of cAMP partially inhibits sexual events including conjugation and sporulation (Calleja *et al.*, 1980), although the *S.pombe* strain employed in this study is different from the Leupold strain currently used in genetic studies. Furthermore, cAMP at a concentration of 50 mM was shown to suppress the *pat1* mutation (Beach *et al.*, 1985). We examined the effect of exogenous cAMP on natural sporulation of the Leupold strain. If 2 mM of cAMP is added to malt extract agar, a convenient medium for mating and sporulation of *S.pombe*, the conjugation efficiency of haploids was reduced to 5.2% and the sporulation efficiency of diploids to 3.3%. Higher concentrations of cAMP were not much more effective. Despite the effects on sexual events, addition of 10mM cAMP did not affect the growth rate of vegetative *S.pombe* cells.

Nitrogen starvation induces transcription of *mei2* (Shimoda *et al.*, 1987), indicating that expression of this gene is

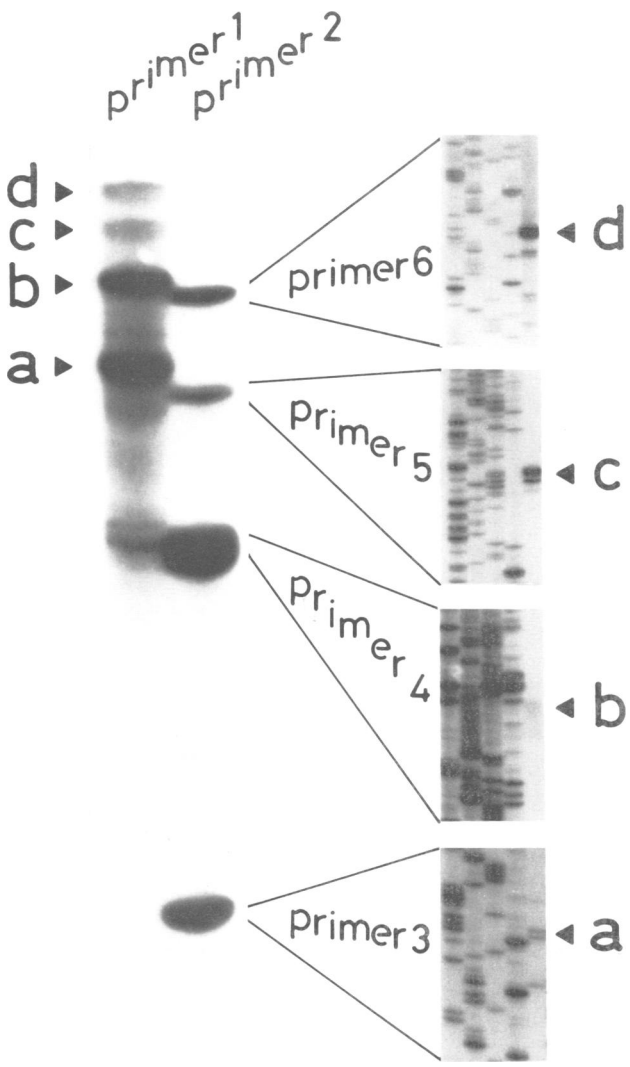

Fig. 3. Primer-extension analysis of the transcription start sites in the *mei2* gene. Four major start sites were detected by using Primers 1 and 2 (large panel). Each start site was more precisely analyzed by using Primers 3–6, respectively, and the results are shown in the lane furthest right on each small panel. Sizes of detected bands were estimated from a DNA sequencing ladder run side-by-side (the four other lanes in each small panel). Nucleotides corresponding to the start sites are marked in Figure 1.

regulated by nutritional conditions. We therefore examined the effect of cAMP addition on *mei2* expression. As shown in Figure 4A, 10 mM cAMP added together with 5 mM caffeine, a phosphodiesterase inhibitor which boosts the effect of cAMP, was found to inhibit transcription of *mei2* dramatically even under conditions of nitrogen starvation. This must account, at least partially, for the inhibitory effect of cAMP on sporulation via meiosis.

Suppression of pat1 by cAMP

An exceptionally high concentration of cAMP (50 mM) or a combination of cAMP (2 mM) and caffeine (1 mM) given exogenously restores growth of cells deficient in *pat1* (Beach *et al.*, 1985). The most efficient suppressor of *pat1-114* is a mutation in *mei2* (Iino and Yamamoto, 1985a). Hence, loss of *mei2* transcription in the presence of cAMP, as was demonstrated above, explains suppression of *pat1* by cAMP

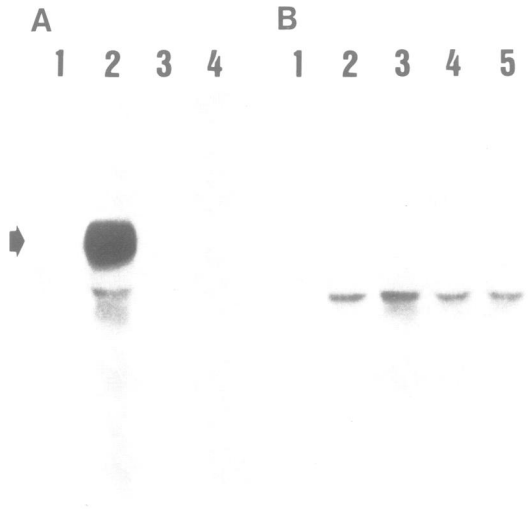

Fig. 4. (**A**) Inhibition of transcription of the *mei2* gene by cAMP. *S.pombe* JY362 was grown at 30°C in PM with or without the addition of 10 mM cAMP and 5 mM caffeine. Rapidly growing cells were sampled at a cell density of 5×10^6/ml and the remaining cells were starved for nitrogen in PM-N with or without cAMP and caffeine for 4 h. Total RNA was prepared from each sample, run on agarose gels after denaturation with glyoxal, blotted onto a nitrocellulose filter and probed with ^{32}P-labeled *mei2* DNA. **Lane 1**, cells rapidly growing in PM; **lane 2**, cells starved for nitrogen in PM-N; **lane 3**, cells rapidly growing in PM with cAMP and caffeine; and **lane 4**, cells starved for nitrogen in PM-N with cAMP and caffeine. Each lane has ~25 μg RNA. The arrow indicates the position of *mei2* mRNA, with an average size of 4.2 kb. Bands seen in the lower part of lane 2 have been judged to be degradation products of *mei2* mRNA pushed down by ribosomal RNAs in the gel. (**B**) Transcription of the *mei2* region carried on plasmid pDB(mei2)9. The host strain JY776 has most of the chromosomal *mei2* sequence deleted and carries the *ura4* gene instead. It can carry out meiosis if transformed with pDB(mei2)9, as described in the text. Transcription of the *mei2* region in the transformant was measured under the same conditions as in **A**. **Lane 1**, JY776 cells carrying the vector pDB248, starved for nitrogen in PM-N. No intrinsic transcription of *mei2* is seen. **Lanes 2–5**, cells carrying pDB(mei2)9: **2**, cells rapidly growing in PM; **3**, cells starved for nitrogen in PM-N; **4**, cells rapidly growing in PM with cAMP and caffeine; and **5**, cells starved for nitrogen in PM-N with cAMP and caffeine.

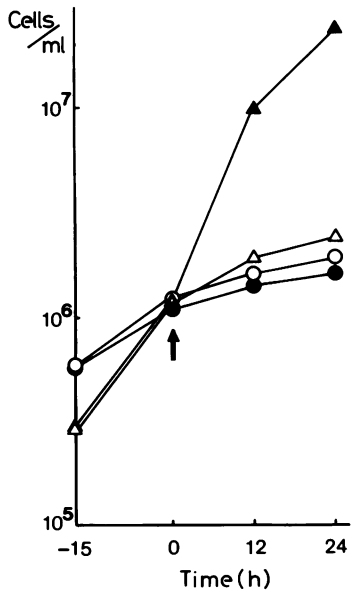

Fig. 5. Neutralization of the effects of cAMP by constitutive expression of *mei2* in suppression of the *pat1* mutation. A *pat1* strain JY712 was transformed with either pDB(mei2)9 or the vector pDB248. They were grown at 24°C in PM with or without 10 mM cAMP and 5 mM caffeine. At a cell density of ~10^6/ml (Time 0, indicated by the arrow), they were shifted to 34°C. Increase in cell number was followed for the next 24 h: cells carying pDB(mei2)9, in the presence (●) and absence (○) of cAMP, and cells carrying pDB248, in the presence (▲) and absence (△) of cAMP.

Table I. Sporulation efficiency of JY712 transformants

Transformed with	cAMP	Sporulation efficiency (%)
pDB(mei2)9	−	33
pDB(mei2)9	+	18
pDB248	−	38
pDB248	+	<2

The experimental conditions are the same as in Figure 5. The number of cells which formed haploid asci were counted 24 h after temperature shift-up and the sporulation efficiency calculated.

quite well. However, after finding that the *pat1* product is a putative protein kinase, McLeod and Beach (1986) proposed that loss of *pat1* kinase activity might be compensated by hyperactivation of cAMP-dependent kinase in phosphorylation of hypothetical common substrates. We thus decided to test if loss of *mei2* transcription is the primary reason for the suppression of *pat1* by cAMP and the following experiments were designed.

Among a series of plasmids carrying the *mei2* gene, pDB(mei2)9 was of particular interest. This plasmid has a 3.2 kb *Cla*I fragment (−513 to 2674 in Figure 1), which encompasses the *mei2* coding region, inserted at the *Bam*HI site of an *S.pombe* multicopy vector pDB248 (Beach and Nurse, 1981). The *Bam*HI site is located in the *tet* gene derived from pBR322 and the *mei2* coding frame is set in the opposite direction to that of *tet*. Although the *Cla*I fragment bears no authentic promoter of *mei2*, pDB(mei2)9 could fully restore a sporulation ability to *mei2* mutants (data not shown). However, such a complementation activity was lost if the direction of the insert was reversed. This indicates the expression of the *mei2* coding region on pDB(mei2)9 depends on read-through from the vector. It has been indeed demonstrated that pBR322 exhibits strong counterclockwise transcription traversing the *tet* gene in *S.pombe* (Losson and Lacroute, 1983). Northern analysis of a pDB(mei2)9 transformant showed that the *mei2* region on this plasmid is constitutively transcribed into RNA of about 3.6 kb in length, which is larger than the full length of the insert by 0.4 kb (Figure 4B). Importantly, expression of this chimeric transcript does not appear to be influenced by nitrogen starvation or cAMP addition (Figure 4B).

A haploid *pat1-114* mutant, JY712, was transformed with this plasmid. The transformants grew slowly, suggesting that constitutive expression of *mei2* is deleterious to cell growth (Figure 5). When the transformants and a control strain carrying only the vector were subjected to a temperature shift in the absence of cAMP, both halted growth and sporulated. However, if cAMP is present at the temperature shift, the control strain continued to grow, while the transformants sporulated (Figure 5 and Table I). Thus cAMP is no longer effective in suppression of *pat1* once transcripts of *mei2* are constitutively supplied, demonstrating that inhibition of *mei2* transcription by cAMP is the primary reason for suppression of *pat1* by this nucleotide.

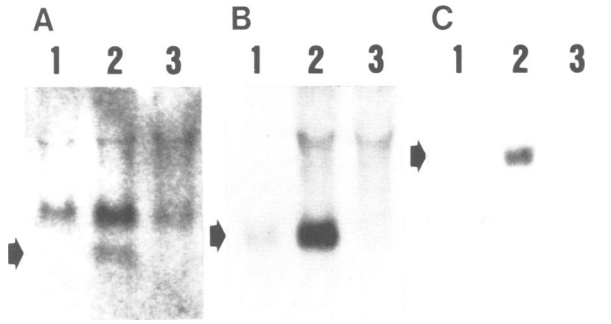

Fig. 6. Inhibition of transcription of life cycle control genes by cAMP. The sporogenic diploid JY362 was starved for nitrogen for 4 h in the presence or absence of cAMP and caffeine, as in Figure 4. Probes used for analysis of RNA are described in Materials and methods. Genes analyzed are *mat1-P* (**A**), *mat2-M* (**B**) and *mei3* (**C**). In each panel: **lane 1**, cells rapidly growing in PM; **lane 2**, cells starved for nitrogen in PM-N; and **lane 3**, cells starved for nitrogen in PM-N supplemented with cAMP and caffeine. The arrow indicates a band which is induced by nitrogen starvation and suppressed by cAMP. Approximate sizes of these bands are 0.6 kb (*mat1-P*), 0.7 kb (*mat1-M*) and 1.3 kb (*mei3*). The constitutive transcript of *mat1-P* (0.8 kb) is seen in **A** but that of *mat1-M* is not detected in **B**. Each lane has 50 μg of RNA. The data shown in **B** are those obtained with JY776, which are essentially the same as those for JY362. The top band seen in most lanes is incompletely digested DNA.

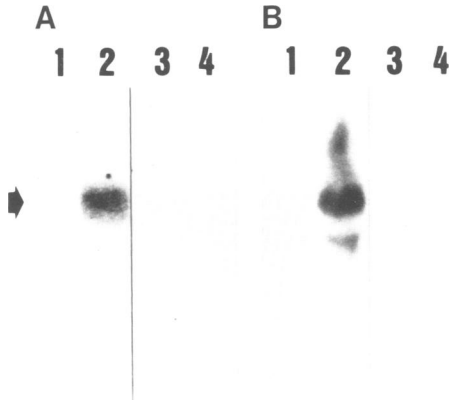

Fig. 7. Analysis of *mei2* transcription in a *steX* strain (**A**) and a transformant carrying pKF1 (**B**). Cells were grown in PM and then starved for nitrogen in PM-N, as in Figure 4. (**A**) **Lane 1**, a *steX*$^+$ control JY3, growing rapidly; **lane 2**, JY3 under nitrogen starvation; **lane 3**, a *steX* strain JY722, growing rapidly; and **lane 4**, JY722 under nitrogen starvation. (**B**) **Lane 1**, JY450 transformed with the vector pDB248, growing rapidly; **lane 2**, the same as 1, but under nitrogen starvation; **lane 3**, JY450 transformed with pKF1, growing rapidly; **lane 4**, the same as 3, but under nitrogen starvation. Each lane in **A** has 25 μg of RNA and each in **B**, 20 μg.

It is logical that interaction of *pat1* with *mei2*, if any, should be post-transcriptional (Yamamoto, 1986), and indeed, inactivation of *pat1* does not affect *mei2* transcription at all (data not shown). As cAMP controls *mei2* transcription, the notion that cAMP-dependent kinase and *pat1* kinase share a common critical substrate cannot be sustained.

Effect of cAMP on other life cycle control genes

Although transformation with pDB(mei2)9 could overcome the inhibitory effect of cAMP on *pat1*-driven meiosis, it failed to restore meiosis in nitrogen-starved h^+/h^- diploid cells under suppression of cAMP (data not shown). It was therefore suspected that cAMP might have inhibitory effects on components other than *mei2*, which are required for natural meiosis but not for *pat1*-driven meiosis. The *mei3* transcript is inducible by nitrogen starvation, as well as one of the two transcripts of mating type locus *mat1*, whether it is *mat1-P* or *mat1-M* (McLeod *et al.*, 1987; Figure 6). Induction of these transcriptions was indeed suppressed by cAMP addition (Figure 6). It thus appears that cAMP generally counteracts nitrogen starvation in induction of life cycle control genes and the results in the previous section consequently indicate that *mei2* is the furthest downstream among such genes.

Genes which cause effects similar to the addition of cAMP

We have so far identified two more genetic conditions that can suppress *pat1*, in addition to *mei2* deficiency. One is a mutation in a gene we tentatively call *steX*. Cells carrying this mutation are sterile and sporulation deficient. The other condition is a high dosage of the gene named *pac1* (*pat1*-compensation), whose structure will be published elsewhere. Introduction of the multicopy plasmid pKF1 carrying this gene converts the host to sterility and sporulation deficiency. Transcription of *mei2* was examined in a *steX* mutant strain as well as a transformant harboring pKF1. As seen in Figure 7, no transcription of *mei2* was induced by nitrogen starvation in these two kinds of cells. These genetic changes apparently mimic the effect of cAMP addition.

Discussion

Commitment to meiosis is conceptually defined as a point of no return to the mitotic cell cycle. Although this terminology has been conveniently used, virtually nothing is known about the molecular mechanism that commits cells to meiosis. Furthermore, once molecular mechanisms are concerned, the terminology appears to reveal inevitable ambiguity. In the well-studied yeast *Saccharomyces cerevisiae*, the commitment to meiosis was inferred to occur during the pre-meiotic S phase and not to be a single event (Sherman and Roman, 1963; Simchen *et al.*, 1972; Esposito and Esposito, 1974; Esposito and Klapholz, 1981). This conclusion was drawn mainly from physiological shift-back experiments and strong support for it came from the observation that diploids can return to the vegetative cell cycle even after meiotic recombination has occurred. Similar studies in *S.pombe* suggested that the same arguments do not hold true for this yeast (Beach *et al.*, 1985). As clearly shown in this study, analysis of gene functions has lead us to the conclusion that a definition of the commitment to meiosis in *S.pombe* could be 'inactivation of *pat1* activity in the presence of *mei2* product, which takes place prior to pre-meiotic DNA synthesis'. Neither *pat1* inactivation nor *mei2* induction can evoke meiosis by itself, but once these two are combined there appears to be no genetic or physiological means to prevent the cells from entering meiosis. It is quite unclear at present if this kind of genetic definition of the commitment to meiosis can be reconciled with the physiological one inferred in *S.cerevisiae*. Alternatively, each organism may have its own way of commitment to meiosis.

Both *pat1* inactivation and *mei2* induction are triggered by nitrogen starvation in natural meiosis, the former being dependent on the heterozygosity of the mating type loci while

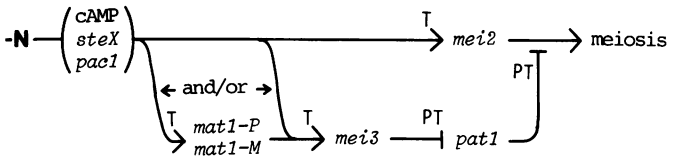

Fig. 8. Schematic illustration of the flow of gene functions leading to initiation of meiosis in *S.pombe* which can be deduced from previous and present studies (Iino and Yamamoto, 1985a,b; Nurse, 1985; Beach *et al.*, 1985; McLeod *et al.*, 1987). The arrow indicates positive or activating regulation and the rectangular line indicates negative or repressive regulation. cAMP and the genes in parentheses are likely to mediate the signal of nitrogen starvation. T stands for transcriptional regulation and PT stands for post-transcriptional regulation.

Table II. *S.pombe* strains used

Strain	Genotype
JY3	h^{90} prototroph
JY362	h^+ *ade6-M210 leu1*
	h^- *ade6-M216 leu1*
JY450	h^{90} *ade6-M216 leu1*
JY712	h^+ *pat1-114 leu1*
JY722	h^{90} *steX-029 ade6-M210 leu1*
JY776	h^+ *mei2::ura4$^+$ ura4-D18 ade6-M210 leu1*
	h^- *mei2::ura4$^+$ ura4-D18 ade6-M216 leu1*

the latter are not. Mating type loci themselves are under control of nitrogen availability. This study has shown that cAMP antagonizes the effect of nitrogen starvation in induction of all these life cycle control genes. Moreover, we have shown that two genes, *steX* and *pac1*, must have an intimate connection with the cAMP effect. It is thus conceivable that cAMP mediates the signal of nitrogen availability in the control of meiosis in *S.pombe*. We made preliminary measurements of cAMP levels in sporulating diploid cells when we characterized *S.pombe ras1*$^-$ strains (Fukui *et al.*, 1986), where intracellular cAMP was found to be reduced by ~35% under nitrogen starvation. It is currently under investigation if this reduction observed *in vivo* is significant in the control of meiosis. It is also noteworthy whether the long promoter region of the *mei2* gene and/or the unusually long untranslated leaders in its mRNA (0.95–1.73 kb) have any relevance to the transcriptional control by cAMP.

Summarizing previous and present data, the genetic control of initiation of meiosis in *S.pombe* is outlined in Figure 8. In the original reports on analysis of *pat1* mutants (Iino and Yamamoto, 1985a,b), we noted that they can sporulate on media rich in nitrogen regardless of their mating type identity, and we proposed a single pathway regulating meiosis. The present study has shown that a scheme which has dual pathways as in Figure 8 is more appropriate. The current interpretation of the previous observation is that a low level of *mei2* mRNA transcribed in such media could support a commitment to meiosis. Obviously small amounts of the *mei2* product are enough for initiation of meiosis if inactivation of *pat1* is complete. Cells carrying *ran1-4*, a weaker allele of *pat1-114* isolated by Nurse (1985), require partial nitrogen starvation to exhibit their sporulating phenotype, probably because more *mei2* product is necessary if *pat1* is not completely inactivated. Thus, enhancement of *mei2* transcription under nitrogen starvation must have practical importance in the decision of timing for initiation of natural meiosis.

Although there is substantial evidence supporting the idea that the *mei2* protein is the key molecule for commitment to meiosis, the deduced amino acid sequence of the *mei2* protein unfortunately does not allow us to predict the biochemical nature of the reaction in which it is involved. Two major questions remain. One is whether the *mei2* product is a substrate for the putative *pat1* protein kinase (McLeod and Beach, 1986), i.e. whether its activity is regulated by phosphorylation. The other, which is more essential, is what exactly is its activity? To investigate these points further, characterization of the *mei2* protein is in progress. Elements involved in the regulation of *mei2* transcription by nitrogen and cAMP are also under investigation.

Materials and methods

Strains, plasmids and media

S.pombe strains used in this study are derivatives of those originally described by Leupold (1950). Their genotypes are summarized in Table II. The shuttle vector pDB248 replicates both in *Escherichia coli* and *S.pombe* and can complement *leu1* in *S.pombe* (Beach and Nurse, 1981). Clones carrying *mei2* (Shimoda *et al.*, 1987) or *mei3* (Shimoda and Uehira, 1985) were described previously. Plasmids carrying either the *matP* cassette or the *matM* cassette were kindly supplied by Drs O.Nielsen and R.Egel. *Pombe* minimal (PM) and *pombe* minimal minus nitrogen (PM–N) are the media used for nitrogen starvation experiments. PM contains per litre 20 g glucose, 5 g NH_4Cl, 1.8 g Na_2HPO_4 and 3 g potassium biphthalate at pH 5.6 (Nurse, 1975; Beach *et al.*, 1985) in addition to vitamins, minerals and salts described in Mitchison (1970). NH_4Cl is ommitted in PM-N. Conventional media for *S.pombe* genetics are according to Gutz *et al.* (1974).

DNA sequencing

The *mei2* region was sequenced essentially by the method developed by Sanger *et al.* (1977). The serial deletion method described by Henikoff (1984), which is advantageous in sequencing long DNA fragments, was employed. Any part shown in Figure 1 has been sequenced in both directions.

Northern blotting and primer-extension analysis

Total RNA was prepared from *S.pombe* cells as described for *S.cerevisiae* (Elder *et al.*, 1983). DNase (RNase free) was sometimes applied to the preparation to decrease backgrounds in the following analysis. Northern blotting was done essentially according to Thomas (1980). The denaturant used was either glyoxal (1 M) or formaldehyde (2 M). The latter was preferentially used for detection of smaller RNAs and the same concentration of formaldehyde was added to the agarose gel in this case. The DNA fragments used as probes are as follows: for detection of *mei2*, a 3.3 kb *Pvu*II–*Hin*dIII fragment which covers the 3′-half of the gene; for *mei3*, a 0.6 kb *Hin*cII fragment which detects the coding region (Shimoda and Uehira, 1985; McLeod *et al.*, 1987); and for *mat1-P* and *mat1-M*, the *matP* and *matM* specific cassette DNA respectively, which resides between the H1 and H2 blocks defined by Beach (1983). Primer-extension analysis was done according to the protocol described in Domdey *et al.* (1984). Oligonucleotides used as primers were synthesized by the automated phosphoramidite method (Matteucci and Caruthers, 1981).

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