The *clr1* Locus Regulates the Expression of the Cryptic Mating-Type Loci of Fission Yeast

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ABSTRACT

The *mat2-P* and *mat3-M* loci of fission yeast contain respectively the *p*lus (*P*) and *m*inus (*M*) mating-type information in a transcriptionally silent state. That information is transposed from the *mat2* or *mat3* donor locus via recombination into the expressed mating-type locus (*mat1*) resulting in switching of the cellular mating type. We have identified a gene, named *clr1* (for *c*ryptic *l*oci *r*egulator), whose mutations allow expression of the *mat2* and *mat3* loci. *clr1* mutants undergo aberrant haploid meiosis, indicative of transcription of the silent genes. Production of mRNA from *mat3* is detectable in *clr1* mutants. Furthermore, the *ura4* gene inserted near *mat3*, weakly expressed in wild-type cells, is derepressed in *clr1* mutants. The *clr1* mutations also permit meiotic recombination in the 15-kb *mat2-mat3* interval, where recombination is normally inhibited. The *clr1* locus is in the right arm of chromosome *II*. We suggest that *clr1* regulates silencing of the *mat2* and *mat3* loci, and participates in establishing the "cold spot" for recombination by organizing the chromatin structure of the mating-type region.

EXPRESSION of a gene can be influenced by long-range position-effect controls. These controls may be mediated by positive or negative regulatory elements, such as enhancers and silencers, or by less-defined chromatin context effects (for reviews, see JONES, RIGBY and ZIFF 1988; FELSENFELD 1992). This study addresses how the expression of the mating-type genes is regulated by their position in the genome of the fission yeast *Schizosaccharomyces pombe*.

Haploid cells of the fission yeast exist in two mating types, called *p*lus (*P*) and *m*inus (*M*) (LEUPOLD 1958). When starved for nitrogen, *P* and *M* cells mate and the resulting zygotic cells proceed to meiosis and sporulation without further vegetative growth. The *P* and *M* cell types are determined respectively by the *mat1-P* and *mat1-M* alleles of the mating-type locus (*mat1*) that resides in linkage group *II* (Figure 1). The *mat1-P* and *mat1-M* alleles each code for two divergently transcribed messages, transcription of which is induced by nitrogen starvation (KELLY *et al.* 1988). The mating-type region consists of three components, *mat1*, *mat2-P* and *mat3-M* (EGEL and GUTZ 1981; BEACH 1983; EGEL 1984; BEACH and KLAR 1984; KELLY *et al.* 1988). The *mat2* locus is located about 15 kilobases (kb) centromere-distal to *mat1*, while *mat3* is located about 15 kb centromere-distal to *mat2* (BEACH and KLAR 1984). The *mat1* locus is transcriptionally active while the same genetic information (including the divergent promoter elements) resident at the *mat2* and *mat3* loci is unexpressed (KELLY *et al.* 1988).

Wild-type cells are homothallic, designated h^{90}, because they switch the cell type by interconverting the *mat1* allele (for reviews, see EGEL 1989; KLAR 1989; GUTZ and SCHMIDT 1990). Cells follow a strict pattern of switching in a cell lineage, such that only one in four granddaughters of a cell switches to the opposite mating type in 80–90% of cell divisions. In particular, *P* cells primarily use the farther *mat3-M* cassette as a donor for the *mat1* conversion, while *M* cells choose the nearby located *mat2-P* as a donor (EGEL 1977; MIYATA and MIYATA 1981; EGEL and EIE 1987; KLAR 1987, 1990; KLAR and BONADUCE 1991). Another unusual feature of the mating-type region is that meiotic recombination in the *mat2-mat3* interval is not observed at a resolution of 0.001 centimorgan (cM) (EGEL 1984). Based on the overall genetic map length of *S. pombe*, the 15-kb intervening sequence should place the *mat2* and *mat3* loci about 3 cM apart. Hence, the region is called a "cold spot" for meiotic recombination.

We have found six mutations that allow expression of the normally silent donor loci. These mutations define one locus, which we have designated *clr1*. These mutations also remove the block to recombination in the *mat2-mat3* region. We suggest that position-effect control is mediated by establishing chromatin structure in the mating-type region and that the *clr1* product plays a key role in this process.

MATERIALS AND METHODS

Strains: *S. pombe* strains and their genotype are presented in Table 1. All strains were constructed in this laboratory.

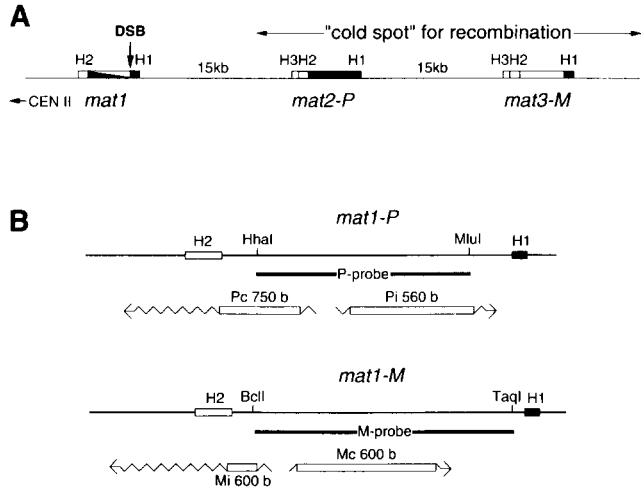


FIGURE 1.—Mating-type region of *S. pombe* and *mat1* transcripts. **A**, The mating-type region is comprised of one expressed cassette, *mat1*, and two silent cassettes, *mat2* and *mat3*, which are closely linked on chromosome *II*. The *mat2* cassette contains the *P* sequence (black box), *mat3* contains the *M* sequence (open box) and *mat1* contains alternatively *P* or *M* sequence. The size of each cassette is approximately 1.1 kb. The distance between two adjacent cassettes is approximately 15 kb. H1 (59 bp), H2 (135 bp) and H3 (57 bp) are short regions of homology shared between two (H3) or three (H1 and H2) cassettes. DSB indicates the location of the double-stranded break at *mat1* that initiates mating-type switching. CENII denotes the centromere of chromosome *II*. **B**, *mat1* transcripts (from KELLY *et al.* 1988). Pc (constitutive) and Pi (inducible) are *P*-specific transcripts, and Mc (constitutive) and Mi (inducible) are *M*-specific transcripts. The approximate size of each transcript is indicated as number of bases (b). Open boxes denote open reading frames. P-probe and M-probe indicate which DNA fragments were used as probes for the Northern blots shown in Figure 5. Restriction sites *Hha*I, *Mlu*I, *Bcl*I, *Taq*I are indicated.

Culture conditions: We used standard conditions for culture, mutagenesis with ethyl methanesulfonate, sporulation, tetrad and random spore analyses, iodine staining, transformation, and construction of diploids from haploid cells (MORENO, KLAR and NURSE 1991). The six *clr1* mutants were obtained from about 2.5×10^4 mutagenized PG19 cells (with about 60% viability).

Construction of the *mat3-M::ura4* locus: The *S. pombe* 1.8-kb *Hin*dIII-*Hin*dIII *ura4* fragment was filled-in with the Klenow fragment of DNA polymerase 1 and was inserted into the *mat3-M* distal *Eco*RV site by blunt-end ligation. This *Eco*RV site is located 150 bp to the right of *mat3* relative to the centromere (BEACH 1983). The resulting *mat3-M::ura4* construct was recombined into the chromosome, and its position was confirmed by Southern analysis (data not shown). The insertion of *ura4* near *mat3* did not affect expression or utilization of the *M* information for switching. Standard procedures for manipulating DNA *in vitro* were used (MANIATIS, FRITSCH and SAMBROOK 1982).

Quantitation of *ura4* expression in *mat3-M::ura4* locus: Cells grown in rich medium were suspended in water and serial dilutions in a volume of 3 μl were spotted on plates containing one of the following media: complete, ura$^-$ drop-out, or synthetic complete containing 5-fluoroorotic acid (FOA, 875 mg/liter). The extent of growth of serial dilutions on ura$^-$ medium indicated the level of *ura4* expression. Conversely, inhibition of growth on FOA-containing medium indicated efficient expression of *ura4* because of the toxicity of FOA (BOEKE *et al.* 1987).

RNA preparation and Northern blot analysis was according to KELLY *et al.* (1988).

RESULTS

Isolation of mutants of *S. pombe* that undergo haploid meiosis: PG19 cells contain *M* information at *mat2* and *P* information at *mat3*. This configuration of the mating-type region is designated as h^{09}, which is the reverse of standard h^{90} (*mat2-P, mat3-M*) strains. A significant difference between h^{90} and h^{09} colonies is that the latter contain fewer zygotic asci, as h^{09} cells switch to the opposite mating type inefficiently (G. THON and A. J. S. KLAR, in preparation). A rough estimate of the efficiency of switching can be obtained by the iodine staining procedure (BRESCH, MULLER and EGEL 1968). The switching-proficient, and therefore sporulation-proficient, h^{90} colonies stain black after exposure to iodine vapors because they accumulate a glycogen-like compound during sporulation. By contrast, h^{09} colonies stain sparingly (Figure 2).

We have employed the iodine staining procedure to screen for darker-staining mutants of an h^{09} strain (PG19). In addition to mutants with increased switching to the opposite mating type (to be published elsewhere), we found six mutants with increased staining due to haploid meiosis. Haploid meiosis is defined as aberrant events producing immature azygotic asci in haploid cells (KELLY *et al.* 1988). In fact, the frequency of zygotic asci was noticeably reduced in all six mutants. Mutant cells displayed the haploid meiosis phenotype only when starved for nitrogen, a condition also essential for normal meiosis and sporulation of diploid cells (EGEL 1989). Through genetic crosses with the strain PG19, we established that each mutant phenotype was conferred by a single mutation. These crosses also established the haploid nature of the mutant strains.

To test the phenotype of the six mutations in the standard h^{90} genetic background, we crossed each h^{09} mutant with an h^{90} strain (PG247). Interestingly, each of the six mutations reduced the frequency of zygotic asci and iodine staining of h^{90} cells (Figure 2) and caused them to undergo haploid meiosis when placed under sporulation conditions (Figure 3). In summary, each of the six mutations causes increased iodine staining of h^{09} cells but decreased staining of h^{90} cells. In both h^{09} and h^{90} mutant colonies the frequency of zygotic asci is reduced and haploid cells undergo aberrant meiosis.

The six mutations that allow haploid meiosis define a unique locus: We crossed the six originally isolated h^{09}, *his2*$^+$ mutants with an h^{90}, *his2*$^-$ strain containing one of the mutations. The resulting diploid strains were subjected to random meiotic spore analysis. Over 300 colonies grown from these spores were examined for each cross. As judged by the iodine

TABLE 1

***S. pombe* strains**

Strain No.	*mat* region	*clr1*	Auxotrophic markers
SP982	h^{90}	+	*ade6-M216, his2*
SP1001	*mat1-Msmt-o*	+	*ade6-M216, his2*
SP1005	*mat1-PΔ17*	+	*leu1-32, ura4-D18, ade6-M216*
SP1064	h^{90}	+	*leu1-32 ade6-M216 ade1-25*
PG9	*mat3-M::ura4*	+	*leu1-32, ura4-D18, ade6-M216*
PG11	*mat3-P::ura4*	+	*leu1-32, ura4-D18, ade6-M216*
PG19	h^{09}	+	*leu1-32, ura4-D18, ade6-M210*
PG122	h^{09}	*clr1-1*	*leu1-32, ura4-D18, ade6-M210*
PG125	h^{09}	*clr1-2*	*leu1-32, ura4-D18, ade6-M210*
PG126	h^{09}	*clr1-3*	*leu1-32, ura4-D18, ade6-M210*
PG147	h^{09}	*clr1-4*	*leu1-32, ura4-D18, ade6-M210*
PG165	h^{09}	*clr1-5*	*leu1-32, ura4-D18, ade6-M210*
PG166	h^{09}	*clr1-6*	*leu1-32, ura4-D18, ade6-M210*
PG247	h^{90}	+	*ura4-D18, ade6-M216, his2*
PG327	*mat3-P::ura4*	*clr1-5*	*leu1-32, ura4-D18, ade6-M216*
PG335	h^{90}	*clr1-5*	*leu1-32, ura4-D18, ade6-M216, his2*
PG377	*mat1-Msmt-o*	*clr1-5*	*leu1-32, ura4-D18, ade6-M210, his2*
PG383	*mat1-PΔ17*	*clr1-5*	*leu1-32, ura4-D18, ade6-M210*
PG391	*mat1-Msmt-0*	*clr1-4*	*ura4-D18, ade6-M216, his2*
PG408	*mat3-P::ura4*	*clr1-4*	*leu1-32, ura4-D18, ade6-M216*
PG410	*mat3-P::ura4*	*clr1-3*	*leu1-32, ura4-D18, ade6-M210*
PG432	*mat3-M::ura4*	*clr1-1*	*leu1-32, ura4-D18, ade6-M216*
PG434	*mat3-M::ura4*	*clr1-2*	*leu1-32, ura4-D18, ade6-M216*
PG436	*mat3-M::ura4*	*clr1-4*	*leu1-32, ura4-D18, ade6-M216*
PG438	*mat3-M::ura4*	*clr1-5*	*leu1-32, ura4-D18, ade6-M216*
PG440	*mat3-M::ura4*	*clr1-6*	*leu1-32, ura4-D18, ade6-M210*
PG442	*mat1-M-smt-o*	+	*ura4-D18, ade6-M210, his2*
PG447	*mat1-PΔ17::LEU2*	+	*leu1-32, ura4-D18, ade6-M216*
PG496	*mat3M::ura4*	*clr1-5*	*leu1-32, ura4-D18, ade6-M210 top1Δ::LEU2*
PG498	*mat1-PΔ17::LEU2*	*clr1-5*	*leu1-32, ura4-D18, ade6-M216*
PG502	*mat1-PΔ17::LEU2*	*clr1-4*	*leu1-32, ura4-D18, ade6-M210*

The *mat* regions containing *mat1, mat2-P* and *mat3-M* are designated h^{90} while the *mat* regions containing *mat1, mat2-M, mat3-P* are designated h^{09}. Unless otherwise indicated, strains contain the *mat2-P, mat3-M* constitution. The unswitchable *mat1-PΔ17::LEU2* and *mat1-Msmt-o* mutant loci are defined in the text.

staining procedure, all segregants contained the mutation. Therefore, the six mutations define a single locus, which we designate *clr1*. A small number of spo$^-$ colonies were found, which could have been heterothallic derivatives of h^{90} or h^{09} (BEACH and KLAR 1984), or products of recombination between *mat2* and *mat3* (see below).

***clr1* is located on chromosome *II*:** Mitotically dividing diploid cells of *S. pombe* frequently become homozygous for all the genetic markers located distal to *mat1* on the right arm of chromosome *II* (EGEL 1981), an event presumably promoted by the double-stranded break found at *mat1* (KLAR and MIGLIO 1986). We noted that a few subclones of a diploid strain that was originally heterozygous for one of the *clr1* mutations (*clr1*$^-$/*clr1*$^+$) became homozygous for the mutation (*clr1*$^-$/*clr1*$^-$) and that a few others became homozygous for the wild-type allele (*clr1*$^+$/*clr1*$^+$). This result suggested that the *clr1* mutations mapped to the right arm of chromosome *II*. We confirmed that this was the case by crossing the *clr1* mutants with strains carrying auxotrophic markers on chromosome *II* and localized the mutated locus between *ade1* and *top1* (Table 2; Figure 4).

***clr1* mutations allow transcription of the cryptic loci:** Mating and meiosis is prohibited in *S. pombe* cells growing in rich medium by a complex pathway, central to which is the *pat1* (also known as *ran1*) protein kinase (EGEL, NIELSEN and WEILGUNY 1990 and references therein). Mutations in *pat1* (NURSE 1985; IINO and YAMAMOTO 1985) or overexpression of *mei3* (MCLEOD and BEACH 1988) cause cells to undergo haploid meiosis, a phenotype similar to the one caused by the mutations in *clr1*. Both *pat1* and *mei3* map to the left arm of chromosome *II*, whereas *clr1* maps to the right arm of chromosome *II*–a result ruling out their identity with *clr1*.

Since expression of both *P* and *M* functions is required for meiosis of wild-type cells (KELLY *et al.* 1988), one class of mutations that would permit haploid cells to sporulate would be those allowing the expression of either or both silent loci. We therefore tested whether *mat2* and *mat3* are expressed in *clr1* mutants.

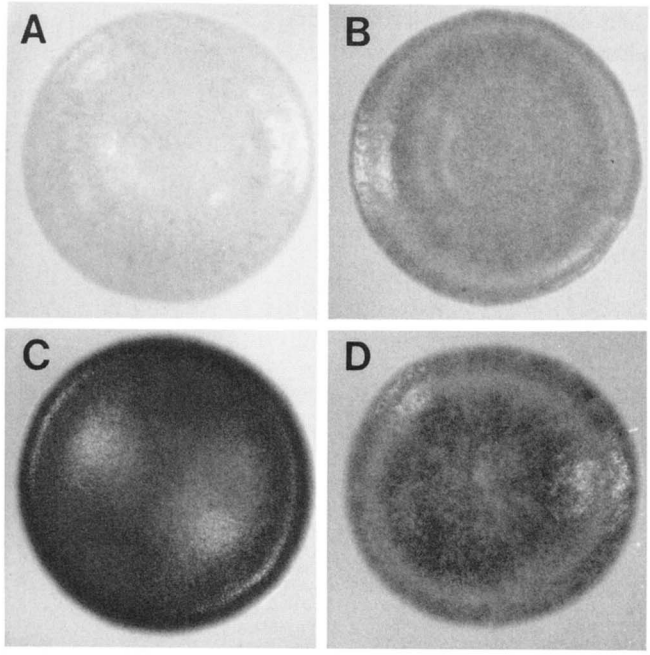


FIGURE 2.—Iodine staining phenotype of *S. pombe* colonies. Photographs of individual colonies sporulated on nitrogen-free medium (see MATERIALS AND METHODS) and stained by iodine vapors are shown. These colonies were from the following strains: **A**, PG19 (h^{09}, *clr1*$^+$); **B**, PG165 (h^{09}, *clr1-5*); **C**, PG247 (h^{90}, *clr1*$^+$); and **D**, PG335 (h^{90}, *clr1-5*).

First, we determined whether the phenotype of the *clr1* mutations depended upon the presence of *P* and *M* sequences. We found that strains containing only *P* cassettes (*mat1-P*, *mat2-P*, *mat3-P*) or only *M* cassettes (*mat1-M*, *mat2-M*, *mat3-M*) did not exhibit the mutant phenotype (data not shown). On the other hand, a stable *P* strain with a *mat3-M* cassette (*mat1-PΔ17*, *mat2-P*, *mat3-M*) exhibited the haploid meiosis phenotype, as did, although to a lesser extent, a strain with a stable *M* mating type (*mat1-Msmt-o*, *mat2-P*, *mat3-M*). Therefore, the haploid meiosis phenotype of the *clr1* mutants is dependent on the genetic content of the donor loci, suggesting that *clr1* normally silences *mat2* and *mat3*. The *mat1-Msmt-o* and *mat1-PΔ17* alleles have small deletions outside the coding region at *mat1* (ENGELKE *et al.* 1987; ARCANGIOLI and KLAR 1991; O. NIELSEN, personal communication). These small deletions prevent formation of the double-stranded break that promotes mating-type switching (BEACH 1983; BEACH and KLAR 1984; NIELSEN and EGEL 1989).

Second, we directly tested whether *mat2-P* and *mat3-M* were expressed in nonswitching *mat1-Msmt-o* and *mat1-PΔ17 clr1*$^+$ and *clr1*$^-$ strains. Transcription of *mat3-M* was detected in *mat1-PΔ17*, *mat2-P*, *mat3-M*, *clr1*$^-$ cells by Northern blot analysis but not that of *mat2-P* in a *mat1-Msmt-o*, *mat2-P*, *mat3-M*, *clr1*$^-$ strain (Figure 5). However, the phenotype of the *mat1-Msmt-o clr1*$^-$ cells indicated that *mat2-P* was also transcribed. In the presence of the *clr1* mutations, morphology of the *mat1-PΔ17* cells changed when the cells were starved for nitrogen. Many cells were enlarged, and a significant number (~10%) contained aberrant spores. Most *mat1-Msmt-o clr1*$^-$ cells did not undergo morphological changes, but a few underwent haploid meiosis (~1%). The assay for sporulation, we imagine, is more sensitive than the Northern blot analysis for detecting *mat2* expression. Therefore, we

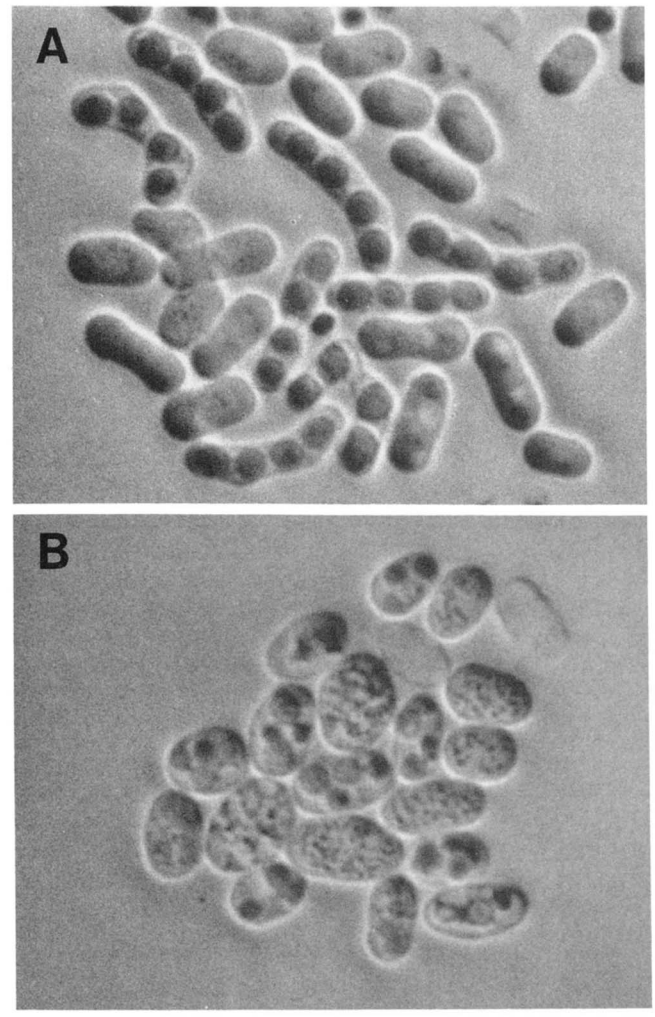


FIGURE 3.—Phase contrast micrographs of sporulated wild-type and *clr1* mutant cells. **A**, PG247 (h^{90}, *clr1*$^+$) shows normal zygotic asci and haploid cells do not sporulate. **B**, PG335 (h^{90}, *clr1-5*) cells instead contain immature spores in haploid cells.

TABLE 2

Tetrad analysis showing linkage of *clr1* with *ade1* and *top1* (SP1064 × PG496)

Gene pair	PD[a]	NPD[a]	T[a]	Map distance[b]
ade1-clr1	108	2	31	15.3
ade1-top1	98	2	41	18.8
clr1-top1	125	0	16	5.6

[a] PD, parental ditype; NPD, nonparental ditype; T, tetra type asci.

[b] Map distance in cM was calculated with the equation cM = 100 [(T + 6NPD)/2(PD + NPD + T)] according to PERKINS (1949).

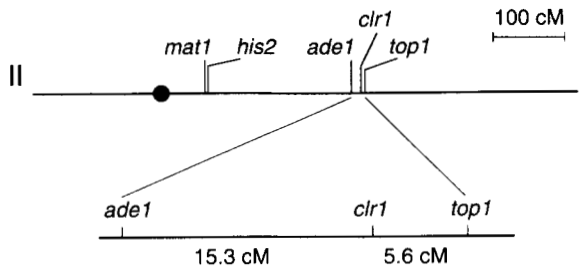


FIGURE 4.—Location of the *clr1* locus on chromosome *II*. The positions of only the loci that were used to map *clr1* are indicated. The closed circle represents the centromere.

interpret these results as showing that both silent loci are expressed in the presence of the *clr1* mutations, with *mat3-M* being transcribed more than *mat2-P*.

The phenotype of the *clr1* mutations is not exhibited when cells are grown on rich medium. Correspondingly, transcription of *mat3* is detectable only in starved cells (Figure 5), showing that it is also under the control of nitrogen starvation. *mat1* is likewise nutritionally regulated in wild-type strains (KELLY *et al.* 1988). As there is no significant sequence homology between *mat1* and *mat3* outside of the cassettes, we suggest that the nitrogen starvation-responsive element(s) are entirely contained within the cassettes.

Mutations in *clr1* derepress a *ura4* gene placed adjacent to *mat3*: We placed the *ura4* gene 150 bp distal to *mat3-M* (designated *mat3-M::ura4*) in the genome to ask whether the expression of *ura4* was inhibited like the expression of *mat2* and *mat3* in wild-type strains, and to determine whether the *clr1* mutations would affect *ura4* expression. These experiments were conducted with strains whose endogenous *ura4* gene was deleted (*ura4-D18* allele). *ura4* expression was measured in two ways by growth of cells on ura$^-$ medium and by resistance to 5-FOA (see MATERIALS AND METHODS). FOA is converted into a toxic metabolite by the *ura4* gene product. Cells expressing normal levels of the *ura4* gene product are inviable on media containing FOA, whereas *ura4*$^-$ cells are resistant to FOA (BOEKE *et al.* 1987).

The *ura4* gene is poorly expressed in *clr1*$^+$ strains, as they grow slowly on ura$^-$ medium and do not die on FOA-containing medium (Figure 6). In the presence of any of the *clr1* mutations, such strains acquire the ability to grow well on medium lacking uracil, with a growth rate similar to that of the wild-type ura$^+$ strains. In addition, the *mat3-M::ura4 clr1*$^-$ mutants die on medium containing FOA as do wild-type ura$^+$ strains. Therefore, *clr1*-mediated repression of transcription extends outside of the *mat3* cassette.

The *clr1* mutations are semidominant: We examined the expression of the *ura4* gene in *mat3-M::ura4*$^+$*/mat3-M* heterozygous diploids carrying a *clr1* mutation on one or the other chromosome. Such an experiment bears the complication that markers located distal to *mat1* frequently become homozygous (EGEL 1981). To prevent homozygosis, we used strains with unswitchable *mat1-PΔ17* and *mat1-Msmt-o* alleles. The *clr1* mutations acted as semidominant since the *clr1*$^-$*/clr1*$^+$ heterozygotes grew better than *clr1*$^+$*/clr1*$^+$ but less than *clr1*$^-$*/clr1*$^-$ strains on ura$^-$ medium (Figure 7). The converse level of growth on FOA-containing medium confirmed this conclusion. Also, the *ura4* gene was equally expressed when the mutations were present in *cis* or in *trans* with the *mat3-M::ura4*$^+$ locus indicating that *clr1* can act in *trans*.

The *clr1* gene functions to inhibit recombination in the *mat2-mat3* cold spot: As stated in the Introduction, the *mat2-mat3* interval is unusual in that both these loci are unexpressed and there is no recombination in this region (EGEL 1984; KELLY *et al.* 1988). It is possible that the specific chromatin organization of the *mat2-mat3* region regulates both transcription and recombination (EGEL 1984; KLAR and MIGLIO 1986). It was, therefore, of interest to check whether recombination in this region is also affected in *clr1* mutants.

Pairwise crosses of mutants were performed. In each cross, one strain contained a *mat1-P, mat2-P, mat3-P::ura4* mating-type region, while the other partner was a *mat1, mat2-M, mat3-P* (h^{09}) strain (Figure 8). A recombination event within the *mat2-mat3* interval should produce h^{09} ura$^+$ segregants that should be capable of sporulation. Analysis of only the ura$^+$ segregants circumvented the aforementioned complication of homozygosis of markers located distal to *mat1*. Segregants obtained by random spore analysis were stained with iodine. As shown in Table 3, we observed that nearly all crosses generated h^{09} ura$^+$ recombinants roughly at a frequency of 1%. The control cross with *clr1*$^+$ strains (PG11 and PG19) did not generate such recombinants in about 2,000 ura$^+$ segregants.

DISCUSSION

The work described in this article attempts to shed light by genetical means on the mechanism of silencing of the *S. pombe mat2* and *mat3* loci and on the "cold spot" for recombination located between them. We find that the transcriptional repression extends outside of the *mat3* cassette to a *ura4* gene introduced in the vicinity of *mat3*. We have identified a locus, *clr1*, whose mutations cause expression of *mat2* and *mat3* and increased expression of the *ura4* gene near *mat3*. In addition, mutations of the *clr1* locus allow meiotic recombination in the *mat2-mat3* interval. The transcriptional repression and cold spot for recombination being affected by the same mutations indicates that the two phenomena proceed, at least in part, from the same mechanism.

Expression of *mat2* and *mat3* is affected by the *clr1*

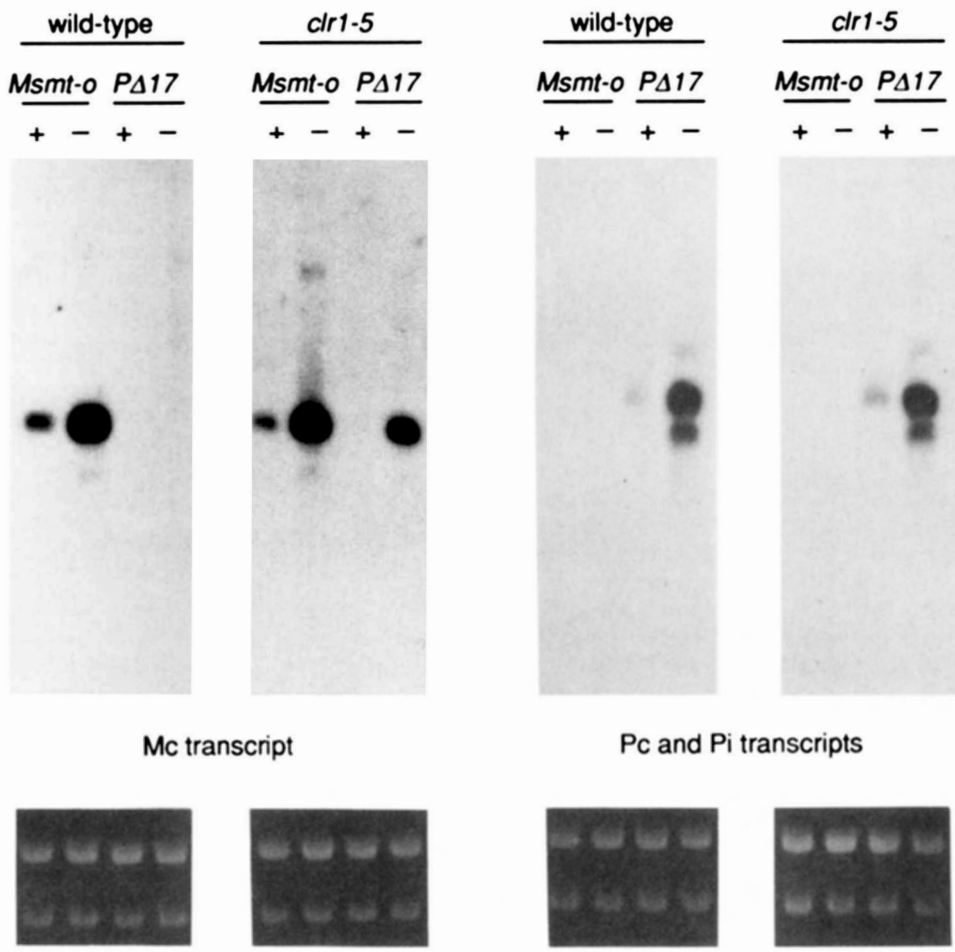
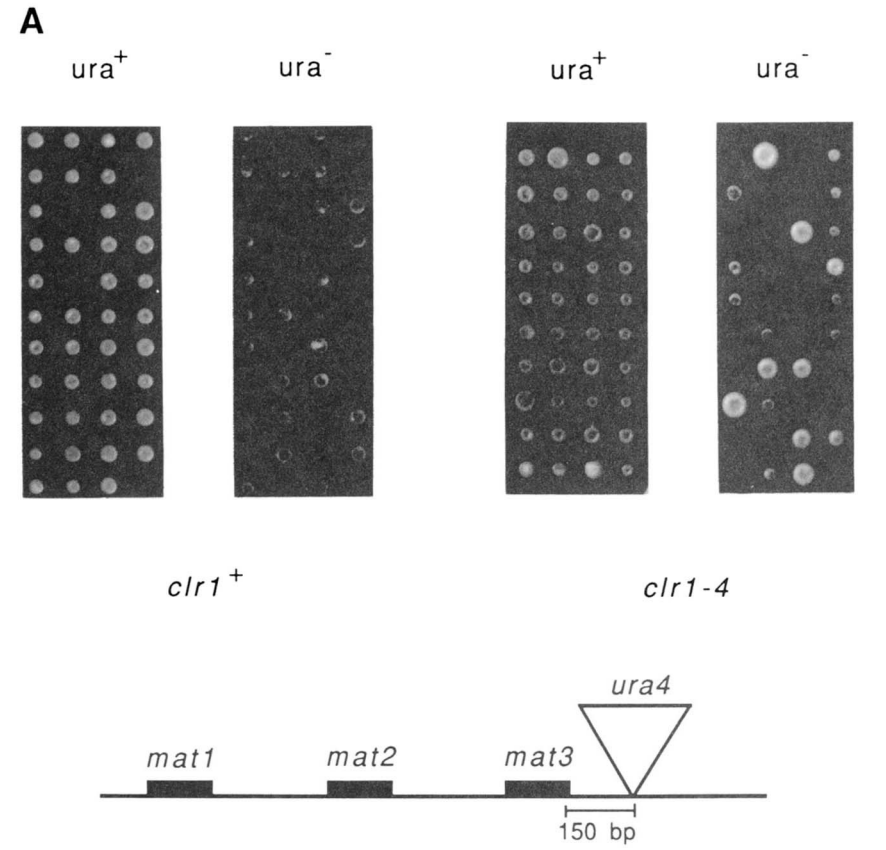


FIGURE 5.—Detection of *mat1*, *mat2* and *mat3* transcripts in unswitchable *clr1*⁻ cells by Northern blot analysis. RNA from unswitchable *M* (*Msmt-o*) or *P* (*PΔ17*) cells, both containing *mat2-P* and *mat3-M*, was hybridized to a probe specific for the *M* (left panels, Mc transcript) or the *P* (right panels, Pc and Pi transcripts) sequence. The probes are defined in Figure 1. The "wild-type" *Msmt-o* (SP1001) and *PΔ17* (SP1005) strains are *clr1*⁺; "*clr1-5*" represents *Msmt-o* (PG377) and *PΔ17* (PG383) mutant strains. Ethidium bromide staining of ribosomal RNA species in the gel prior to transfer is shown in the bottom panels, indicating that roughly equivalent amounts of total RNA were loaded in each lane. The "+" symbol indicates RNA isolated from cells growing in rich medium while the "−" symbol indicates samples from nitrogen-starved cells.

mutations to different extents. The six *clr1* mutations that we isolated allow a sufficient level of expression of the *mat3* Mc transcript to be detected by Northern blot analysis. We were unable to detect the *mat2-P* transcript, but phenotypic assays suggested that *mat2* was also expressed in *clr1* mutants. First, a low level of haploid meiosis was produced by *mat1-Msmt-o*, *mat2-P*, *mat3-M* cells containing a mutated *clr1*. Second, the capacity of these strains to mate as *M* cells was significantly reduced, consistent with the expression of *mat2-P*. The different extent of expression of the two donor loci may be due to their location in the chromosome, or to differences in their genetic contents. We note, however, that *ura4* placed next to *mat3-M* or *mat3-P* is equally enhanced in its expression in *clr1* mutants, partially arguing against the second possibility.

Originally, the *mat2* and *mat3* loci genetically segregated as a single locus since recombination between them was not found (EGEL 1984). These loci were shown to be distinct by physical analysis (BEACH 1983; BEACH and KLAR 1984). Mutations in two loci have been recently shown to allow recombination in the *mat2-mat3* interval. A pleiotropic mutation called *rik1* (for recombination in the *K* region) allowed the separation of *mat2* and *mat3* by about 4–5 cM (EGEL, WILLER and NIELSEN 1989). The *clr1* locus is distinct from *rik1* as *rik1* maps to chromosome *III*, while *clr1* maps to the linkage group *II*. The *swi6* (switching defective) gene function was initially identified as required for switching *mat1* (EGEL, BEACH and KLAR 1984). Mutants of *swi6* contain the double-stranded break at *mat1* but are thought to be defective in a step for utilizing the break for recombination. Recently, it was found that *swi6* mutations also allowed recombination in the *mat2-mat3* interval (KLAR and BONADUCE 1991; LORENTZ, HEIM and SCHMIDT 1992). LORENTZ, HEIM and SCHMIDT (1992) also noted a low level of haploid meiosis in *swi6*⁻ mutant cells. The *swi6* gene is also unlinked to



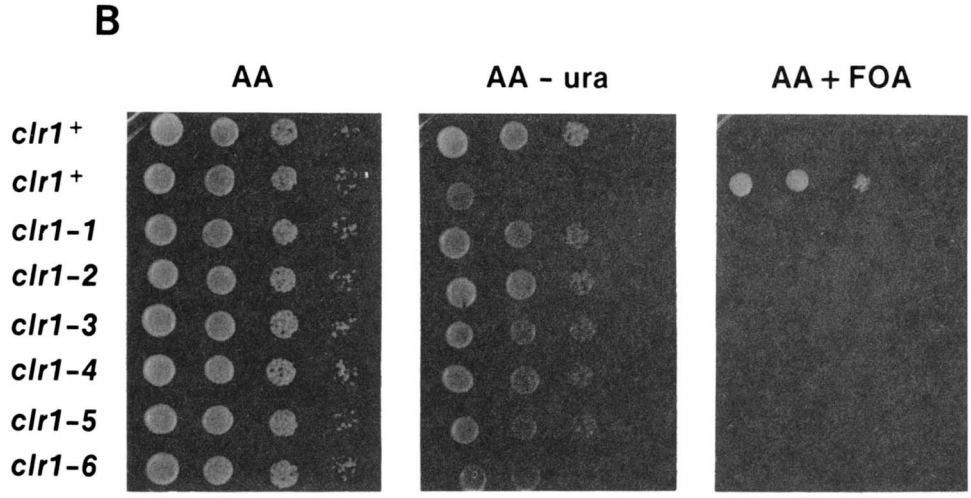


FIGURE 6.—The *clr1* mutations derepress *ura4* placed near *mat3* (*mat3-M::ura4*) in haploid cells. **A**, Tetrad analysis of a sporulated diploid obtained from a cross between PG9 (h^{90}, *mat3-M::ura4, clr1*$^+$) and PG147 (h^{09}, *clr1-4*), showing segregation of *clr1-4* and *mat3-M::ura4* (right two panels). The segregants that grew well in AA-ura medium contained the *clr1-4* mutation. The mutant segregants were identified by their haploid meiosis phenotype. The four spore segregants of each ascus were placed on rich media in a horizontal row by micromanipulation. After growth for 4 days, they were replicated onto plates containing the indicated media. The pictures were taken after 2 days of growth. The left two panels are of a cross involving parental *clr1*$^+$ [PG9 (h^{90} *mat3-M::ura4*$^+$, *clr1*$^+$) × PG19 (h^{09}, *clr1*$^+$)] strains presented as a control for comparison, demonstrating slow growth of ura$^+$ segregants. The second tetrad from the top, we presume, resulted from homozygosis of the *mat3-M::ura4*$^+$ allele. **B**, Growth of wild-type and *clr1* mutant strains containing *mat3-M::ura4* on complete medium (AA), medium lacking uracil (AA-ura) and medium containing FOA (AA + FOA). Cells grown in rich medium were suspended in water and 10-fold serial dilutions of strains SP982 (*clr1*$^+$, *ura4*$^+$ control strain), PG9 (*clr1*$^+$), PG432 (*clr1-1*), PG434 (*clr1-2*), PG410 (*clr1-3*), PG436 (*clr1-4*), PG438 (*clr1-5*) and PG440 (*clr1-6*) were spotted on each plate (see MATERIALS AND METHODS). Approximately the same number of cells were spotted for each strain. Pictures were taken after 3 days of growth.

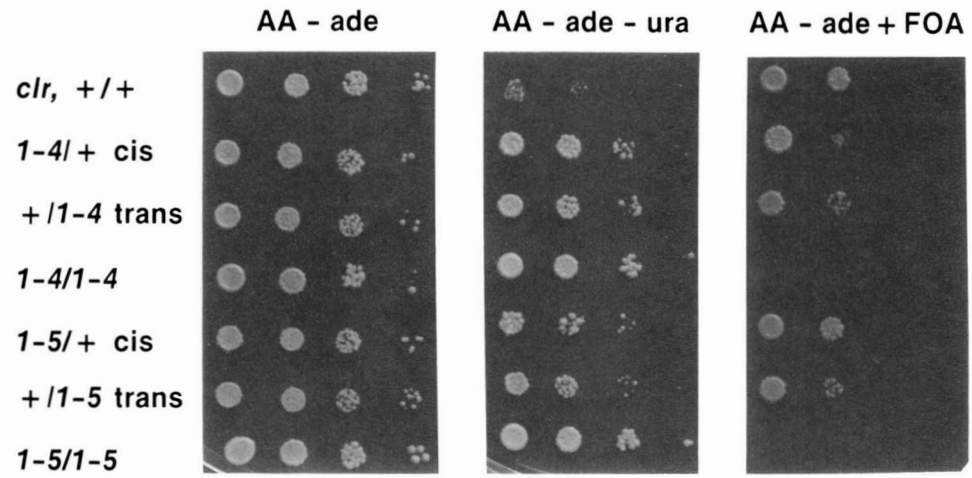


FIGURE 7.—Semidominance of *clr1* mutations for derepression of *ura4* in *mat3-M::ura4* locus. Serial tenfold dilutions of diploid cells were spotted on plates containing synthetic medium deficient in adenine (AA-ade), lacking adenine and uracil (AA-ade-ura) or lacking adenine but containing FOA (AA-ade-FOA). The diploids were heterozygous for *mat3-M::ura4* and either homozygous wild-type for *clr1* (+/+, mating product of PG447 and PG442), heterozygous for a *clr1* mutation (*clr1-4* +/− *cis*, PG502 and PG442; *clr1-4* +/− *trans*, PG447 and PG391; *clr1-5* +/− *cis*, PG498 and PG442; *clr1-5* +/− *trans*, PG447 and PG377), or homozygous for a *clr1* mutation (*clr1-4* −/−, PG502 and PG391 or *clr1-5*, PG498 and PG377). Adenine-deficient media were used to ensure growth of only diploid strains because the *ade6-M210/ade6-M216* cells are ade$^+$ as the mutations complement (MORENO, KLAR and NURSE 1991).

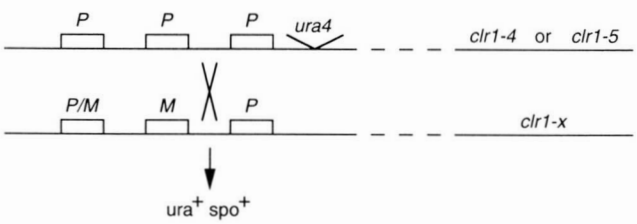


FIGURE 8.—The *clr1* mutations remove the inhibition to recombination in the *mat2-mat3* cold-spot interval. The mating-type region constitution of two strains that were crossed to study recombination is diagrammed. The *mat1*, *mat2* and *mat3* cassettes are presented from left to right as conventionally drawn. Both strains were otherwise ura$^-$ auxotrophs, because they contained the *ura4-D18*-deletion allele.

TABLE 3

Effect of *clr1* mutations on meiotic recombination in the *mat* region

	ura$^+$ spo$^+$/total ura$^+$	
clr1-x	*clr1-4* (PG408)	*clr1-5* (PG327)
clr1-1 (PG122)	6/408	0/100
clr1-2 (PG125)	4/520	6/530
clr1-3 (PG126)	5/328	3/307
clr1-4 (PG147)	7/550	3/130
clr1-5 (PG165)	7/450	4/500
clr1-6 (PG166)	5/275	7/607

Diploids obtained from matings between the strains indicated in parentheses were subjected to random spore analysis. Ratios indicate the numbers of ura$^+$ spo$^+$ recombinants obtained from a total number of ura$^+$ segregants analyzed.

clr1 (data not shown). Studies of *rik1* and *swi6* mutations had led to the suggestion that the mating-type region may be organized in a heterochromatin-like structure to prohibit donor locus expression and may be sequestered to prohibit interactions with the homolog (EGEL, WILLER and NIELSEN 1989; KLAR and BONADUCE 1991). It was suggested that such an arrangement may also be essential for making the donor loci accessible for recombination during *mat1* switching, perhaps by intrachromosomally folding the donor loci onto *mat1* (KLAR and BONADUCE 1991). *clr1* appears to be an additional component that directly or indirectly contributes to establishing the chromosomal structure of the mating-type region.

In the budding yeast *Saccharomyces cerevisiae* chromatin structure has been implicated in silencing the *HML* and *HMR* loci (for a review, see KLAR 1989). These loci, analogously to *mat2* and *mat3*, act as storage of mating- type information, and are required to switch the constitutively active *MAT* locus. Mutations in the three *MAR/SIR* loci allow full expression of the silent genes (IVY, KLAR and HICKS 1986; RINE and HERSKOWITZ 1987). Point mutations in the N terminus of histone H4 and N-terminal acetyl-transferase also relieve repression of *HML* and *HMR* (for a review, see GRUNSTEIN 1990). Additional evidence for the involvement of chromatin in silencing is suggested by the *in vivo* inaccessibility of *HML* and *HMR* to the HO endonuclease (STRATHERN *et al.* 1982) and to the *Escherichia coli dam*$^+$ methylase activity expressed in yeast (SINGH and KLAR 1992). In *mar/sir*, mutants, the *in vivo* accessibility to the HO endonuclease (KLAR, STRATHERN and HICKS 1981; KLAR, STRATHERN and ABRAHAM 1984) and *dam*$^+$ methylase (SINGH and KLAR 1992) is gained or enhanced. Furthermore, the expression of heterologous genes placed in or around the *HM* loci is repressed (MAHONEY and BROACH 1989 and reference therein). Finally, *in vitro* nuclease sen-

sitivity analysis of *HML* and *HMR* suggested that the chromatin structure of *HM* loci is changed in *mar/sir* mutants (NASMYTH 1982). The *clr1* product may be similar to any one of the functions required to silence the *HM* loci.

It was shown earlier that plasmid-borne donor loci are also repressed in *S. pombe* (KELLY *et al.* 1988). A recent study has identified small *cis*-acting elements close to *mat2-P*, two that map on the left and two on the right, that are required to repress the plasmid-borne gene (EKWALL, NIELSEN and RUUSALA 1991). The *clr1* product may act directly or indirectly through these sequences. Since the *clr1* mutations affect recombination in the cold spot and expression of the *ura4* gene inserted 150 bp distal to *mat3*, we propose that *clr1* functions over the entire *mat* region. In this context, it is interesting to note that both of the mutations we tested were semidominant with the wild-type allele; perhaps the mutant protein forms a complex with the normal protein conferring an intermediate phenotype or alternatively, there are multiple target sites for the *clr1* product.

Our results also bear on another position-effect control. That is, the same sequence is cleaved at *mat1* but not in the *mat2* and *mat3* loci (KELLY *et al.* 1988; NIELSEN and EGEL 1989). By analogy with the *S. cerevisiae* system, it may be imagined that the same mechanism that keeps the *mat2, mat3* loci silent, may limit their accessibility to functions required to cleave the *mat* cassettes. It was found that *mat1* was normally cleaved and *mat2* and *mat3* were not cleaved in *clr1* mutants (data not shown). Therefore, the state of expression of the donor loci does not influence the generation of DSB in the *S. pombe* cassettes. We believe that this is because in *S. pombe*, unlike in *S. cerevisiae*, all the sequences required for making the DSB are not present at the silent loci. In particular, the sequences present to the right of *mat1*, outside of the cassette, were previously shown to be essential for cleavage and those sequences do not exist at the *mat2* and *mat3* loci (KLAR, BONADUCE and CAFFERKEY 1991; ARCANGIOLI and KLAR 1991).

One of the purposes of mapping a locus in the chromosome is to determine whether the gene at this locus was previously identified with different phenotypic properties. The *clr1* locus maps close to *swi3* and *top1* (KOHLI 1987; Figure 4). The *swi3* gene is required for *mat1* switching by promoting the formation of the double-stranded break at *mat1* (EGEL, BEACH and KLAR 1984). The *clr1* mutants are not affected in the level of the double-stranded break at *mat1* (data now shown). Secondly, the *swi3* mutants do not undergo haploid meiosis (EGEL, BEACH and KLAR 1984). Thus, these genes are judged to be different. The *top1* gene is also different, as we have genetically separated them during our mapping experiments. Future studies of the *clr1* gene and its product are essential to further define their function in molecular terms.

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