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Epigenetic Inheritance of Transcriptional Silencing and Switching Competence in Fission Yeast

Geneviève Thon and Tove Friis

Department of Genetics, Institute of Molecular Biology, University of Copenhagen, Denmark

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ABSTRACT

Epigenetic events allow the inheritance of phenotypic changes that are not caused by an alteration in DNA sequence. Here we characterize an epigenetic phenomenon occuring in the mating-type region of fission yeast. Cells of fission yeast switch between the *P* and *M* mating-type by interconverting their expressed mating-type cassette between two allelic forms, *mat1-P* and *mat1-M*. The switch results from gene conversions of *mat1* by two silent cassettes, *mat2-P* and *mat3-M*, which are linked to each other and to *mat1*. GREWAL and KLAR observed that the ability to both switch *mat1* and repress transcription near *mat2-P* and *mat3-M* was maintained epigenetically in a strain with an 8-kb deletion between *mat2* and *mat3*. Using a strain very similar to theirs, we determined that interconversions between the switching- and silencing-proficient state and the switching and silencing-deficient state occurred less frequently than once per 1000 cell divisions. Although transcriptional silencing was alleviated by the 8-kb deletion, it was not abolished. We performed a mutant search and obtained a class of *trans*-acting mutations that displayed a strong cumulative effect with the 8-kb deletion. These mutations allow to assess the extent to which silencing is affected by the deletion and provide new insights on the redundancy of the silencing mechanism.

EPIGENETIC events contribute to the phenotype of both uni- and multi-cellular organisms. They allow discrete subsets of genetic information to be expressed while others are not, in such a fashion that the various patterns of expression are transmitted during cell division. Several model systems have contributed greatly to the understanding of how specific expression states can be memorized and inherited. Among them are studies of the Polycomb group of repressors (reviewed by PIRROTA 1995), studies of position effect variegation (reviewed by WILSON *et al.* 1990; HENIKOFF 1995), *X*-chromosome inactivation (reviewed by RIGGS and PFEIFER 1992; RASTAN 1994) and imprinting (reviewed by PETERSON and SAPIENZA 1993; BARLOW 1995). These studies performed in higher eukaryotes have led to the view that heritable repression of transcription might often be mediated by self-templating repressive chromatin structures. Parallel studies in unicellular eukaryotes have suggested analogous models. In the yeast *Saccharomyces cerevisiae* transcriptional silencing of the mating-type cassettes (PILLUS and RINE 1989; MAHONEY *et al.* 1991; SUSSEL *et al.* 1993; HOLMES and BROACH 1996; for review, see LAURENSON and RINE 1992) and silencing of genes placed at the vicinity of telomeres (GOTTSCHLING *et al.* 1990) are maintained epigenetically and are accompanied by altered chromatin properties (BRAUSTEIN *et al.* 1996 and references herein; GOTTSCHLING 1992). Similarly, in the fission yeast *Schizosaccharomyces pombe*, epigenetically transmitted repression of transcription operates near telomeres (NIMMO *et al.* 1994) and centromeres (ALLSHIRE *et al.* 1994), where atypical nucleosome patterns are observed (POLIZZI and CLARKE 1991; TAKAHASHI *et al.* 1992; ALLSHIRE *et al.* 1994). Although most known epigenetic effects are explained in terms of regulation of transcription, processes other than transcription can also be regulated epigenetically. Along these lines, the particular properties of transcription at the vicinity of centromeres and telomeres might reflect the presence and properties of structures required for chromosome function. In fission yeast, the existence of an epigenetic component in centromere function was directly demonstrated by STEINER and CLARKE (1994). These authors described *in vivo* conversions from an inactive centromeric state to a functional state which they proposed results from the folding of the centromere into a higher order chromatin structure.

Mating-type switching in *S. pombe* involves three loci: *mat1*, *mat2-P* and *mat3-M*, which are closely linked in the right arm of chromosome *II* (for review, see KLAR 1992). The *mat1* locus is expressed and determines the mating type of the cell, whereas *mat2-P* and *mat3-M* are transcriptionally silent. *mat2-P* and *mat3-M* donate genetic information to *mat1*, allowing it to switch between *mat1-P* and *mat1-M*. At least 16 *trans*-acting functions are important for the process: *swi1–swi10*, *rik1* and *rad22* influence switching (EGEL *et al.* 1984, 1989; GUTZ and SCHMIDT 1985; SCHMIDT *et al.* 1989), and *clr1–clr4* repress transcription in the *mat2-mat3* region

Corresponding author: G. Thon, Department of Genetics, Institute of Molecular Biology, University of Copenhagen, Øster Farimagsgade 2A, DK-1353 Copenhagen K, Denmark. E-mail: gen@biobase.dk

(Thon and Klar 1992; Ekwall and Ruusala 1994; Thon *et al.* 1994). The distinction between switching and silencing factors is not clear cut. The products of *swi6, rik1* and *clr4,* in particular, are important for both mating-type switching (Egel *et al.* 1984, 1989; Gutz and Schmidt 1985; Thon and Klar 1993; G. Thon, unpublished observation) and silencing (Lorentz *et al.* 1992; Ekwall and Ruusala 1994; Thon *et al.* 1994). In addition to their effect on switching and silencing, *rik1, swi6, clr1, clr2, clr3* and *clr4* repress meiotic recombination in the mating-type region (Egel *et al.* 1989; Klar and Bonaduce 1991; Lorentz *et al.* 1992; Thon *et al.* 1994) and alleviate transcription near centromeres and telomeres, indicating they play a role in chromosome function (Allshire *et al.* 1995). Indeed, at least three of these factors: *rik1, swi6* and *clr4,* are important for chromosome segregation (Allshire *et al.* 1995). A proposed interpretation for their multiple roles is that Rik1, Swi6, and Clr1–Clr4 allow the formation or maintenance of particular chromatin structures important for switching and silencing as well as for other chromosomal functions. Consistent with this model, the Swi6 protein contains a chromodomain (Lorentz *et al.* 1994; Aasland and Stewart 1995 and references herein) and colocalizes with centromeres, telomeres and the mating-type region (Ekwall *et al.* 1995).

No silencing function other than *rik1, swi6,* and *clr1–clr4* was identified in two independent searches for silencing-deficient mutants (Ekwall and Ruusala 1994; Thon *et al.* 1994). However, several observations indicate that silencing in the mating-type region is redundant and that additional factors remain to be identified (Thon *et al.* 1994). The arguments in support of the existence of more than one silencing pathway are as follows. First, none of the *trans*-acting mutations reported to date fully derepress transcription of the mating-type genes, nor do pairwise combinations of these mutations, nor does deletion of *swi6, rik1* or *clr1.* Second, deletion of a 1.5-kb DNA fragment flanking *mat2-P* does not noticeably derepress the *mat2-P* genes, but the same deletion in combination with a mutation in any of the six characterized *trans*-acting loci fully derepresses *mat2-P* (Thon *et al.* 1994). Hence the proposition that two pathways repress transcription in the *mat2-mat3* region. One silencing pathway would be mediated by *swi6, rik1,* and *clr1–clr4* acting *via* an as yet unidentified DNA element whereas a second pathway would involve unidentified *trans*-acting factors acting *via* a DNA element centromere-proximal to *mat2-P.*

The three mating-type loci, *mat1, mat2-P* and *mat3-M,* are distributed at ~15-kb intervals, the region between *mat2-P* and *mat3-M* being referred to as the K region (Figure 1). We constructed a strain with a large deletion of ~8 kb between *mat2-P* and *mat3-M.* We designate the mating-type region with this deletion $h^{\Delta K1}$ (for *ho*mothallic $\Delta K1$) by analogy with the designation for the wild-type h^{90}. $h^{\Delta K1}$ cells adopted one of two phenotypes: either they switched mating-type and repressed transcription at the vicinity of *mat2-P* and *mat3-M* in a fashion similar to the wild type, or they were deficient for both processes. Hence, the $h^{\Delta K1}$ strain had properties similar to a strain independently constructed by Grewal and Klar in which the same DNA fragment was deleted from the chromosome and replaced by the *S. pombe ura4* gene (Grewal and Klar 1996). Although the 8-kb deletion derepressed transcription in the *mat2-mat3* region, the derepression was only partial and when combined with a *swi6⁻* allele, the deletion did not cause further derepression than the *swi6⁻* allele alone. These observations prompted us to combine the deletion with a class of *trans*-acting factors that have a cumulative effect with a mutant *swi6* allele. We describe the strategy by which the new class of mutants were obtained, show how the mutation that we tested affected transcription of *mat3-M* in the $h^{\Delta K1}$ mating-type region and discuss the implications of our observations for the current models of mating-type switching and silencing in *S. pombe.*

MATERIALS AND METHODS

Media: *S. pombe* strains were propagated and tested using the following media: YEA (5 g yeast extract, 100 mg adenine, 30 g glucose per liter); YES (5 g yeast extract, 2 g casamino acids, 100 mg adenine, 100 mg uracil, 200 mg L-leucine, 30 g glucose per liter); MSA (Egel *et al.* 1994) supplemented with 100 mg adenine, 100 mg uracil, 200 mg L-leucine per liter as indicated; AA-ura (drop-out medium where uracil has been omitted, Rose *et al.* 1990); FOA (same as AA-ura but supplemented with 1 mg 5-fluoroorotic acid (5-FOA) and 50 mg uracil per liter). *Escherichia coli* was propagated in 2 × YT (Miller 1972). Ampicillin was used at 200 μg/ml. Amino acids, nucleotides and ampicillin were purchased from Sigma; Yeast extract, casamino acids, yeast nitrogen base and tryptone were purchased from Difco laboratories. Salts were purchased from Merck. 5-FOA was purchased from United States Biological.

Strains: The *S. pombe* strains used in this study are listed in Table 1 with their genotype and origin. Strains originating from crosses were obtained by tetrad dissection except PG1306 which was obtained from a random spore preparation. *E. coli* strain DH5α (Hanahan 1983) was used for cloning plasmids.

Plasmid constructions: The *mat3-Mint::ura4* construct (pGT77, Thon and Klar 1992) consists of a 4.2-kb *Hin*dIII *S. pombe* genomic fragment containing *mat3-M* with a 1.8-kb insertion of the *S. pombe ura4* gene at the *Eco*RV site centromere distal to the cassette. The 6-kb *Hin*dIII insert of pGT77 was cloned into the *Hin*dIII site of Bluescript SKII(–) (Stratagene) with the *Pst*I site of the polylinker on the centromere-proximal side of *mat3-M* to create pGT107. The 6.3-kb *Hin*dIII *S. pombe* genomic fragment containing *mat2-P* (Beach 1983) was cloned into PUC19 (Yanish-Perron *et al.* 1985) with the *Pst*I site of the polylinker on the centromere-distal side of *mat2-P* to create pGT81. The 5-kb *Sac*I-*Pst*I fragment of pGT81 was then ligated in between the *Sac*I and *Pst*I sites of pGT107 to create pGT110. pGT110 contains therefore 5 kb of *S. pombe* genomic sequence from the *mat2-P* centromere-proximal *Sac*I site to the *mat2-P* centromere-distal *Hin*dIII site, the sequence AGCTTGCATGCCTGCA from PUC19 polylinker, the sequence GGAATTCGATATCA from Bluescript SKII(–) polylinker and 6 kb of *S. pombe* genomic sequence from the *mat3-Mint::ura4* centromere-proximal *Hin*dIII site to the *mat3-Mint::ura4* centromere-distal *Hin*dIII site. The insert of pGT110 can be released as one 11-kb *Sac*I-*Sal*I fragment.

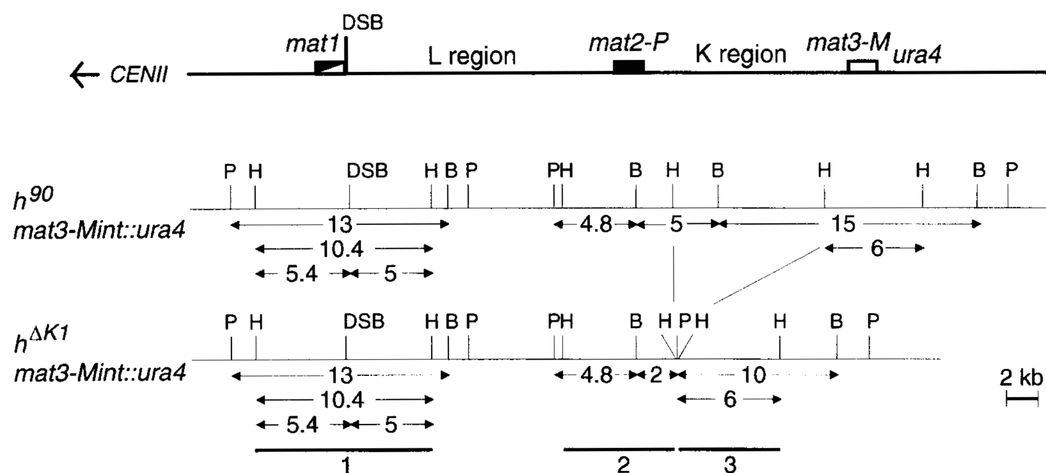

FIGURE 1.—Mating-type region of h^{90} and $h^{\Delta K1}$ strains. The mating-type region is represented at the top of the figure and restriction maps of the wild-type (h^{90}) and deleted ($h^{\Delta K1}$) regions are shown underneath to help interpret the blots in Figure 3. Both regions are depicted with a *mat3-Mint::ura4* allele. Approximate sizes of the relevant restriction fragments are given in kilobases. *CENII,* centromere II; DSB, double-strand break; P, *Pst*I site; H, *Hin*dIII site; B, *Bam*HI site. The thick lines numbered 1, 2 and 3 represent the probes used for the Southern blots in Figure 3.

Construction of $h^{\Delta K1}$ mating-type region: The 11-kb *Sac*I-*Sal*I fragment of pGT110 containing the engineered mating-type region with the $\Delta K1$ deletion was gel purified and used to transform the h^{90} *swi6-115 S. pombe* strain PG1. The *swi6-115* allele in PG1 facilitates insertion and expression of auxotrophic markers in the mating-type region (THON and KLAR 1993). DNA-mediated transformation was achieved by the lithium acetate procedure described by MORENO *et al.* (1991). Ura^+ transformants were isolated and proper integration of the construct was tested by Southern blot. One correct transformant, PG1212, was used for this study.

Iodine staining: Sporulation phenotypes of *S. pombe* colonies can be visualized by iodine staining since the spores, but not the vegetative cells, are stained black by iodine vapors (BRESH *et al.* 1968). Staining patterns indicate how much mating-type switching has occurred in a colony. Dark homogenous iodine staining, such as in colony "TYPE 1" in Figure 2, is observed when mating-type switching has occurred efficiently. Streaks and sectors, such as in colony "TYPE 2," are caused by inefficient mating-type switching. Staining can also be produced by haploid sporulation, a phenomenon observed in specific mutant backgrounds where haploid cells undergo aberrant meiosis and form spores without mating. However, asci originating from haploid meiosis are easily distinguished from zygotic asci by microscopic examination.

Fluctuation tests: PG1247 cells were diluted to a concentration of ~3 cells/ml in YES medium. Aliquots (180 × 100 μl) of type-1 and 180 × 100 μl aliquots of type-2 cells were allowed to grow at 30° for 22 hr in microtiter dishes and plated onto YES medium. After 3 days, the YES plates with colonies were replicated onto MSA sporulation plates supplemented with adenine, uracil and leucine and onto selective MSA plates supplemented with adenine and leucine but lacking uracil. The appearance of variant phenotypes was scored after a 4-day incubation at 30° by examining the sporulation phenotypes of the colonies and their ability to grow in the absence of uracil. The rate of appearance of the variant phenotypes (μ) was inferred from the frequency of cultures that contained no variant (P_0) using the formula $\mu = -(\ln P_0)/N$, where N is the average number of colony forming units per culture. Cultures ($n = 45$) of type-1 cells and 39 cultures of type-2 cells gave rise to colonies, whose composition is presented in Table 2.

Isolation of *esp* mutants: SP1126 contains the unswitchable *mat1-Msmt-0* allele and the mutant *swi6-115* allele. *mat2-P* is only partially derepressed in that strain: <1% of the cells undergo haploid meiosis when starved for nitrogen and consequently SP1126 colonies are not stained by iodine vapors (THON *et al.* 1994). Ten independent cultures of SP1126 were mutagenized with EMS to 75% survival by following the procedure of MORENO *et al.* (1991). The mutagenized cells were plated onto MSA sporulation plates supplemented with adenine and uracil, and allowed to grow at 25° for 6 days. Approximately 40,000 colonies were screened by iodine staining. Thirty black-staining mutants in which high levels of haploid meiosis were observed by microscopic examination were isolated and the levels of expression of *mat2-P* in these mutants were assayed by Northern blot analysis. Seven mutants with increased level of *mat2-P* transcripts were obtained. Three of the mutations: *esp1-1, esp2-1* and *esp3-1* are presented here.

Analysis of linkage between the *esp* loci: Placing the seven *esp* mutations into linkage groups was not straightforward because the mutations had no easily identifiable phenotypes in $swi6^+$ background and they considerably reduced mating in $swi6^-$ backgrounds. One of the methods that we used was to cross h^{90} *swi6Δ::ura4* esp^- *ura4-D18 leu1-32* strains (PG1098 and PG1114) pairwise with h^{90} esp^- *ura4-D18* strains. Leu^+, Ura^+ recombinant spores were selected. Appearance of lightly staining h^{90} *swi6Δ::ura4* esp^+ colonies among the darkly staining h^{90} *swi6Δ::ura4* esp^- colonies indicated the two *esp* mutations in the cross were not linked. Presence of only darkly staining h^{90} *swi6Δ::ura4* esp^- colonies indicated the mutations were linked. By that assay, we determined that three of the mutations were linked to the mutation in PG1098 (*esp3-1*), one was linked to the mutation in PG1114 (*esp1-1*) and one was linked to neither (*esp2-1*).

Analysis of linkage between the *esp* and *clr* loci: *mat1-PΔ17::LEU2* esp^- strains, esp^- representing, respectively, *esp1-1* (PG1134), *esp2-1* (PG1127) and *esp3-1* (PG1147), were crossed pairwise with *mat1-Msmt-0* clr^- strains, clr^- representing, respectively, *clr1-5* (SP1167), *clr2-760* (PG1046), *clr3-735* (PG1029) and *clr4-681* (PG1031) and 12–18 tetrads were dissected for each cross. The four genetic combinations esp^- clr^+; esp^- clr^-; esp^+ clr^+ and esp^+ clr^- were found in the progeny of each cross in ~1:1:1:1 ratios, indicating the *esp* and *clr* genes were unlinked. The *clr* genotypes could be easily assigned because clr^- alleles derepress *ura4* in the mating-type region, which could be assayed in all progeny. The *esp* genotypes were assigned by test crosses. esp^- clr^+ and esp^+ clr^+ were not distinguishable, but esp^- clr^- and esp^+ clr^- had distinct pheno-

TABLE 1

List of strains and their genotypes

Strain	Genotype			Source
	Mating-type region[a]	Silencing loci	Auxotrophic markers	
AL91	h^{90}	*swi6Δ::ura4*	*ura4-D18 ade6-M210 leu1-32*	LORENTZ *et al.* (1994)
SP1126	*mat1-Msmt-0 mat2-Pint::ura4*	*swi6-115*	*ura4-D18 ade6-M210*	THON *et al.* (1994)
SP1167	*mat1-Msmt-0 mat2-Pint::ura4*	*clr1-5*	*ura4-D18 ade6-M216*	THON *et al.* (1994)
PG1	h^{90}	*swi6-115*	*ura4-D18 ade6-M216 leu1-32*	SP837 × SP107[b]
PG9	h^{90} *mat3-Mint::ura4*		*ura4-D18 ade6-M216 leu1-32*	THON and KLAR (1992)
PG447	*mat1-PΔ17::LEU2 mat3-Mint::ura4*		*ura4-D18 ade6-M216 leu1-32*	THON and KLAR (1992)
PG1029	*mat1-Msmt-0 mat2-Pint::ura4*	*clr3-735*	*ura4-D18 ade6-M216*	THON *et al.* (1994)
PG1031	*mat1-Msmt-0 mat2-Pint::ura4*	*clr4-681*	*ura4-D18 ade6-M216*	THON *et al.* (1994)
PG1046	*mat1-Msmt-0 mat2-Pint::ura4*	*clr2-760*	*ura4-D18 ade6-M216*	THON *et al.* (1994)
PG1058	*mat1-Msmt-0 mat2-Pint::ura4*	*swi6-115 esp2-1*	*ura4-D18 ade6-M210*	Mutagenesis of SP1126
PG1059	*mat1-Msmt-0 mat2-Pint::ura4*	*swi6-115 esp1-1*	*ura4-D18 ade6-M210*	Mutagenesis of SP1126
PG1063	*mat1-Msmt-0 mat2-Pint::ura4*	*swi6-115 esp3-1*	*ura4-D18 ade6-M210*	Mutagenesis of SP1126
PG1093	h^{90}	*swi6Δ::ura4 esp2-1*	*ura4-D18 ade6-M210*	AL91 × PG1058
PG1098	h^{90}	*swi6Δ::ura4 esp3-1*	*ura4-D18 ade6-M210 leu1-32*	AL91 × PG1063
PG1114	h^{90}	*swi6Δ::ura4 esp1-1*	*ura4-D18 ade6-M210 leu1-32*	AL91 × PG1059
PG1127	*mat1-PΔ17::LEU2 mat3-Mint::ura4*	*esp2-1*	*ura4-D18 ade6-M210*	PG447 × PG1093
PG1129	*mat1-PΔ17::LEU2 mat3-Mint::ura4*	*swi6Δ::ura4 esp2-1*	*ura4-D18 ade6-M216 leu1-32*	PG447 × PG1093
PG1134	*mat1-PΔ17::LEU2 mat3-Mint::ura4*	*esp1-1*	*ura4-D18 ade6-M210 leu1-32*	PG447 × PG1114
PG1135	*mat1-PΔ17::LEU2 mat3-Mint::ura4*	*swi6Δ::ura4 esp1-1*	*ura4-D18 ade6-M216 leu1-32*	PG447 × PG1114
PG1144	*mat1-PΔ17::LEU2 mat3-Mint::ura4*	*swi6Δ::ura4 esp3-1*	*ura4-D18 ade6-M210 leu1-32*	PG447 × PG1098
PG1146	*mat1-PΔ17::LEU2 mat3-Mint::ura4*	*esp3-1*	*ura4-D18 ade6-M216 leu1-32*	PG447 × PG1098
PG1147	*mat1-PΔ17::LEU2 mat3-Mint::ura4*	*esp3-1*	*ura4-D18 ade6-M210*	PG447 × PG1098
PG1177	*mat1-PΔ17::LEU2 mat3-Mint::ura4*	*swi6Δ::ura4*	*ura4-D18 ade6-M216 leu1-32*	AL91 × PG447
PG1212	$h^{\Delta K1}$ *mat3-Mint::ura4*	*swi6-115*	*ura4-D18 ade6-M216 leu1-32*	Transformation of PG1
PG1217	h^{90}		*ura4-D18 ade6-M210 his2*	SP982 × SP1126[c]
PG1247	$h^{\Delta K1}$ *mat3-Mint::ura4*		*ura4-D18 ade6-M216 leu1-32*	PG1212 × PG1217
PG1306	*mat1-PΔ17::LEU2 ΔK1 mat3-Mint::ura4*		*ura4-D18 ade6-M216 leu1-32*	PG1247 × PG81
PG1310	*mat1-PΔ17::LEU2 ΔK1 mat3-Mint::ura4*	*esp3-1*	*ura4-D18 ade6-M216 leu1-32*	PG1306 × PG1098

[a] The *mat1-Msmt-0* allele was obtained by ENGELKE *et al.* (1987) and sequenced by STYRKARSDOTTIR *et al.* (1993). The *mat1-PΔ17::LEU2* was constructed by ARCANGIOLI and KLAR (1991).

[b] SP107 was described by KLAR and BONADUCE (1991) and SP837 by THON *et al.* (1994).

[c] SP982 was described by THON and KLAR (1992).

types, the *esp⁻ clr⁻* combinations leading to increased haploid meiosis. Crosses with *rik1⁻* strains were not performed, but allelism of the *esp* genes with *rik1* was ruled out because none of the *esp* mutations was linked to *ade6* whereas *rik1* is (EGEL *et al.* 1989).

Southern blot analysis: *S. pombe* DNA was prepared as in MORENO *et al.* (1991) and digested with restriction enzymes according to instructions from the supplier (New England Biolabs). Gel electrophoresis was performed as described in SAMBROOK *et al.* (1989), and the gels were blotted onto Hybond-N nylon membrane as directed by the manufacturer (Amersham). Hybridizations were performed at 42° for 16–20 hr using DNA probes prepared by random priming with a kit from Stratagene and a hybridization solution composed of 0.25 M $NaHPO_4$ pH 7.2, 0.25 M NaCl, 7% SDS, 1 mM EDTA, 50% formamide, 10% PEG(4000), 5× Denhardt's solution, and 200 μg/ml yeast RNA. The blots were washed 10 min in 2 × SSC, 60 min in 2× SSC, 1% SDS and 30 min in 0.1× SSC, 1% SDS at 65° and autoradiographed on Agfa Curix x-ray films.

Northern blot analysis: Nitrogen starvation experiments, RNA preparation and Northern blot analysis were conducted as described by NIELSEN and EGEL (1990) except that we used Hybond-N membrane (Amersham) instead of Genescreen (NEN). An antisense RNA transcribed from the *mat1-M* 1016-bp *BclI-TaqI* DNA fragment (KELLY *et al.* 1988; NIELSEN and EGEL 1990) was used to detect the Mc transcript. An antisense RNA transcribed from the 665-bp *XbaI-HindIII* fragment of the *cdc2* gene (HINDLEY and PHEAR 1984; NIELSEN *et al.* 1992) was used to detect the cdc2 transcript.

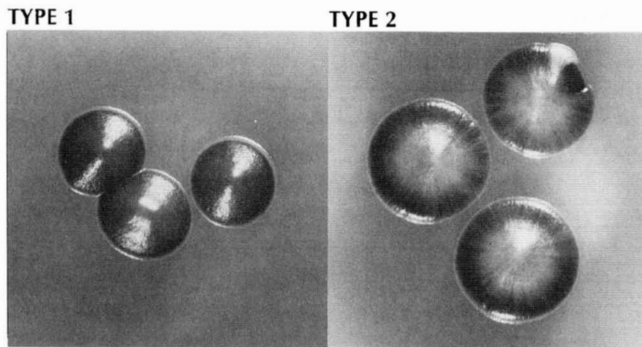

FIGURE 2.—Variegation of switching-competence in $h^{\Delta K1}$ strain. "TYPE 1": colonies of efficiently switching $h^{\Delta K1}$ cells; "TYPE 2": colonies of poorly switching $h^{\Delta K1}$ cells. One colony contains a sector of type-1 and a sector of h^{+N} cells.

RESULTS

Construction of a strain with a deletion in the K region: The mating-type region of *S. pombe* occupies ~30 kb in the right arm of chromosome *II* (Figure 1). The mating-type cassettes were cloned by BEACH (1983). We utilized a construct derived from BEACH's clones to introduce a deletion of ~8-kb between *mat2-P* and *mat3-M*. We refer to this deletion as $\Delta K1$ and to the mating-type region bearing the deletion as $h^{\Delta K1}$. One of our goals when constructing $h^{\Delta K1}$ was to test a possible involvement of the K region in the transcriptional silencing of *mat2-P* and *mat3-M* in various mutant backgrounds. Therefore, in addition to introducing the 8-kb deletion, we inserted an *S. pombe ura4* gene on the centromere-distal side of the *mat3-M* cassette (*mat3-Mint::ura4* allele; THON and KLAR 1992). For convenience, the deletion was first introduced in a *swi6-115* background, and the *swi6-115* allele was subsequently outcrossed. We noticed at this stage that the *swi6-115* mutation was epistatic to the deletion: all $h^{\Delta K1}$ *swi6-115* colonies displayed a phenotype similar to h^{90} *swi6-115* colonies where cells switch mating-type inefficiently, express the transplaced *ura4* gene and partially derepress the normally silent mating-type cassettes.

The $\Delta K1$ deletion affects mating-type switching: A major effect of the $\Delta K1$ deletion in a *swi6*$^+$ background proved to be on mating-type switching. However, individual colonies displayed different phenotypes. Two predominant phenotypes were observed (Figure 2). The first type of colonies (type 1) was very similar to wild-type homothallic colonies: most cells mated and sporulated when starved for nitrogen, indicating that mating-type switching had been efficient during the growth of this type of colony. They differed from the wild type in that they often contained a sector of poorly switching cells. The second type of colonies (type 2) contained *P* and *M* cells that interconverted inefficiently. Wild-type sectors arose from these poorly switching colonies. When restreaked, cells from the poorly switching sectors gave rise to type-2 colonies whereas cells from the efficiently switching sectors gave rise to type-1 colonies. We tested whether either of these phenotypes correlated with DNA rearrangements in the mating-type region (Figure 3). No rearrangements able to account for the difference in phenotypes was detected.

Mitotic stability of the two phenotypes caused by $\Delta K1$: We conducted pedigree analysis and fluctuation tests to determine the stability of the two phenotypes adopted by the $h^{\Delta K1}$ cells. In the pedigree analysis, 200 dividing cells of each type were separated for two consecutive divisions by micromanipulation. The cells were then allowed to form colonies whose phenotype was examined. Only one change of phenotype was observed in the colonies originating from the cousin cells. This was a change from type 2 (poorly switching) to type 1 (efficiently switching). This low incidence of variation is in agreement with the results of our fluctuation tests (Table 2) from which we calculated that conversion from type 1 to type 2 occurred at a rate of approximately three events per 10^4 cell divisions whereas conversion from type 2 to type 1 occurred at a rate of eight events per 10^4 cell divisions.

Meiotic stability of the two phenotypes caused by $\Delta K1$: Tetrad dissections were performed with zygotic asci from type 1 and type 2 colonies (Table 3). We found that both phenotypes were extremely stable in meiosis. In addition, type 1 and type 2 $h^{\Delta K1}$ cells were allowed to mate with wild-type h^{90} cells and zygotic asci were dissected (Table 4). Both phenotypes were also stable in such crosses and segregated with the $h^{\Delta K1}$ mating-type region. Hence, the epigenetic events responsible for the maintenance of each phenotype occurred at the mating-type region itself and not at an unlinked locus.

TABLE 2

Stability of the $h^{\Delta K1}$ phenotypes in mitosis

Observed variation	No. of cultures in experiment[a] (A)	Average number of cfu[b] per culture (N)	No. of cultures without variation (B)	No. of variation per cell division -[ln(B/A)]/N
Type 1 to Type 2	45	493	38	3.4×10^{-4}
Type 1 to h^{+N}	45	493	38	3.4×10^{-4}
Type 2 to Type 1	39	688	23	7.7×10^{-4}
Type 2 to h^{+N}	39	688	22	8.3×10^{-4}

[a] The strain used in this experiment was PG1247.
[b] cfu, colony-forming unit.

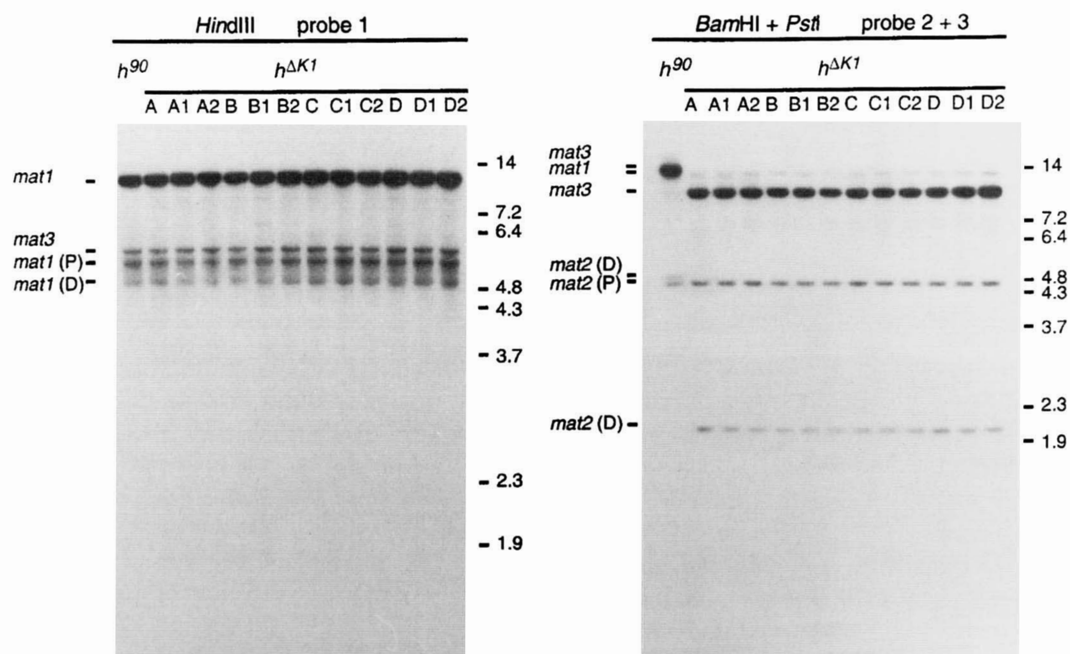

FIGURE 3.—Genomic organization of type-1 and type-2 $h^{\Delta K1}$ cells. The h^{90} (PG9) and $h^{\Delta K1}$ (PG1247) mating-type regions were examined by Southern blot. Both strains have the *mat3-Mint::ura4* allele. A, B, C1, C2, D1, and D2 were cultures of efficiently switching $h^{\Delta K1}$ cells (type 1) and A1, A2, B1, B2, C, and D were cultures of poorly switching $h^{\Delta K1}$ cells (type 2). A1 and A2 were derived from A by isolating type 2 variants; B1 and B2 were derived from B; C1 and C2 from C; D1 and D2 from D. The *Hind*III digests were hybridized to a *mat1-M* probe (10.4-kb *Hind*III fragment; probe 1 in Figure 1). The *Pst*I + *Bam*HI digests were hybridized to a mixture of *mat2-P* (6.3-kb *Hind*III fragment; probe 2 in Figure 1) and *mat3-Mint::ura4* (6-kb *Hind*III fragment; probe 3 in Figure 1). The migration of size markers (λ *Bst*EII) is reported on the left of the blots. D, centromere-distal fragment; P, centromere-proximal fragment. *mat1* (P) and *mat1* (D) originate from the double-strand break at *mat1* (BEACH 1983).

Increased rearrangement rates in the $h^{\Delta K1}$ mating-type region: Two additional phenotypes were occasionally observed with the $h^{\Delta K1}$ strain: sectors and colonies that were Spo$^-$ (type 3), as evidenced by iodine staining and microscopic examination, and others that were largely Spo$^-$ but with dark-staining streaks of zygotic asci (type 4). Both types of colonies contained cassette duplications equivalent to the previously described rearrangement h^{+N}, which has the following structure: *mat1:2 K mat3:1 L mat2 K mat3*, where *mat1:2* represents a fusion between the *mat1* and *mat2* cassettes, flanked by the *mat1* centromere-proximal sequence and the *mat2* centromere-distal sequence whereas *mat3:1* represents a fusion between the *mat3* and *mat1* cassettes, flanked by the *mat3* centromere-proximal sequence and the *mat1* centromere-distal sequence (BEACH and KLAR 1984; Figure 4). The h^{+N} rearrangement might be caused by a resolution error at *mat3-M* during mating-type switching and occurs in cells with a wild-type region once per 10^5 cell divisions (EGEL *et al.* 1980; BEACH and KLAR 1984 and references herein). We determined in our fluctuation tests that the rate of appearance of h^{+N} rearrangements in $h^{\Delta K1}$ cultures was >30-fold higher than it is in the wild type (Table 2). Consistently, these rearrangements were often detected as minor bands in Southern blots of $h^{\Delta K1}$ DNA (Figure 1). The increased occurrence of rearrangements indicates switching in $h^{\Delta K1}$ cells is less accurate than in h^{90} cells. We found it intriguing that the same rearrangement could generate two phenotypes and made two further observations. First, the Spo$^-$ colonies with streaks (type 4) proved to be unstable: when restreaked, they gave rise not only to type 4 colonies but also to colonies that had lost the cassette duplication, their mating-type region having reverted to $h^{\Delta K1}$ (Figure 4). Second, type 3 and type 4 could interconvert. We did not measure the rates of interconversion between the two phenotypes, but their frequency in the populations we examined suggested that

TABLE 3

Stability of the $h^{\Delta K1}$ phenotypes in meiosis

Source of zygotic asci[a]	No. of tetrads examined	Class of tetrads			
		4 Type 1	4 Type 2	2 Type 1:2 Type 2	2 Type 2:2 h^{+N}
Type 1 colony	40	38	0	2	0
Type 2 colony	40	0	36	2	2

[a] The strain used in this experiment was PG1247.

TABLE 4

Stability of the $h^{\Delta K1}$ phenotypes in crosses with h^{90}

Source of zygotic asci	No. of tetrads examined[a]	Class of tetrads	
		2 Type 1:2 h^{90}	2 Type 2:2 h^{90}
Type 1 × h^{90}	23	21	2
Type 2 × h^{90}	18	2	16

[a] A total of 80 asci were dissected for each cross, but only tetrads involving both parents are reported. The mating-type region of each parent could be distinguished because the $h^{\Delta k1}$ parent, PG1247, had a *mat3-Mint::ura4* allele whereas the h^{90} parent, SP837, did not. Colonies that were wild-type for sporulation and Ura^- were scored as h^{90}; colonies that were wild type for sporulation and grew poorly in the absence of uracil were scored as type 1; and colonies that sporulated poorly and grew well in the absence of uracil were scored as type 2.

the conversions might be due to the same epigenetic effect that causes conversions between type 1 and type 2 colonies.

The Δ*K1* deletion alleviates transcriptional silencing: Expression of the *mat2-P* and *mat3-M* cassettes within haploid cells causes the cells to undergo meiosis without prior mating, a phenomenon referred to as haploid meiosis. Haploid meioses were not observed in cells with the $h^{\Delta K1}$ mating-type region, indicating that the donor cassettes were repressed. This result is consistent with the observation of HEIM (1990) that a *mat2:3-P* fusion cassette is not expressed. However, it has been noticed before that silencing is redundant and that impairment of a single pathway can yield weak or undetectable expression of the mating-type genes from *mat2-P* or *mat3-M* (THON *et al.* 1994). Therefore, we also assayed expression of a more sensitive marker, the *ura4* gene. The pattern of expression of the *ura4* gene placed near *mat3-M* showed that silencing was not fully operating in some of the colonies (Figure 5). The *ura4* gene was repressed in the colonies that mated and sporulated like wild type (type 1 colonies). In contrast, the *ura4* gene was well expressed in the slowly switching colonies (type 2 colonies). We concluded that the Δ*K1* deletion affected one level of transcriptional repression maintained in the same manner as the switching competence.

When cultures of h^{90} *mat3-Mint::ura4* cells are plated on medium lacking uracil, colonies appear at approximately one thousandth the frequency they appear in the presence of uracil (Figures 5 and 6). We investigated the stability of the Ura^+ and Ura^- phenotypes by replating h^{90} *mat3-Mint::ura4* cells that had grown on plates either lacking uracil or containing 5-FOA (Figure 6). The same growth patterns were observed independently of the origin of the cells: nearly all cells gave rise to colonies on medium containing 5-FOA indicating the *ura4* gene was repressed and very few, in the order of 1 in 10^3 cells, gave rise to small colonies on medium lacking uracil. Hence, the *ura4* gene had reverted to a repressed state in most cells of the Ura^+ colonies. This

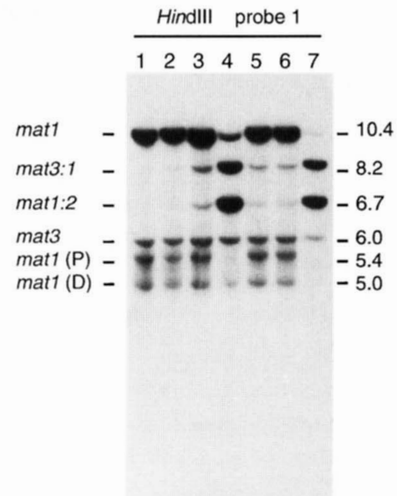

FIGURE 4.—Rearrangements occurring in $h^{\Delta K1}$ strain. DNA prepared from cultures inoculated with h^{90} cells (1, PG9); $h^{\Delta K1}$ cells (2, PG1247); unstable (3–6) and stable (7) Spo^- derivatives of PG1247 was digested with *Hin*dIII and hybridized to the 10.4-kb *Hin*dIII *mat1-M* fragment. The cultures of unstable Spo^- cells have composite hybridization patterns: a pattern characteristic of the h^{+N} rearrangement (8.2-kb *mat3:1* fragment and 6.7-kb *mat1:2* fragment; BEACH and KLAR, 1984) combined with various amounts of nonrearranged mating-type region (10.4-kb *mat1* fragment). The culture of stable Spo^- cells is predominantly h^{+N}.

experiment showed that the *ura4* gene placed near *mat3-M* was sporadically derepressed in the h^{90} mating-type region, but the repression was much more efficiently restored than in the $h^{\Delta K1}$ mating-type region (Figure 5). We investigated further the role of the K region in silencing by constructing double mutants as explained below.

Isolation of mutations that have a cumulative effect with a mutant *swi6* allele: the *esp* (*e*nhancer of *s*wi *p*henotype) mutations: Mutations in *swi6* or in the other known silencing functions only partially derepress transcription in the mating-type region. To search for potential *trans*-acting factors involved in a second pathway, we mutagenized an unswitchable *mat1-Msmt-0* strain containing the deficient *swi6-115* allele (SP1126) and sought mutants with a more strongly derepressed phenotype. In the first part of the screen, we isolated 30 mutants with haploid meiosis levels increased >10-fold in conditions of nitrogen starvation. These mutants were then screened by Northern blot analysis for increased levels of *mat2-P* transcripts. Seven *trans*-acting mutations that increased expression of *mat2-P* were obtained. The other mutations increased the level of haploid meiosis without increasing the amount of *mat2-P* transcripts. These mutations might be in genes that control meiosis such as *pat1* (for review, see EGEL 1994) and they were discarded for this study.

The seven mutations that increased the amount of *mat2-P* transcripts were crossed into an h^{90}, *swi6*Δ::*ura4* background, where they increased haploid meiosis. Mutant *mat1-Msmt-0 swi6*Δ::*ura4* colonies were also ob-

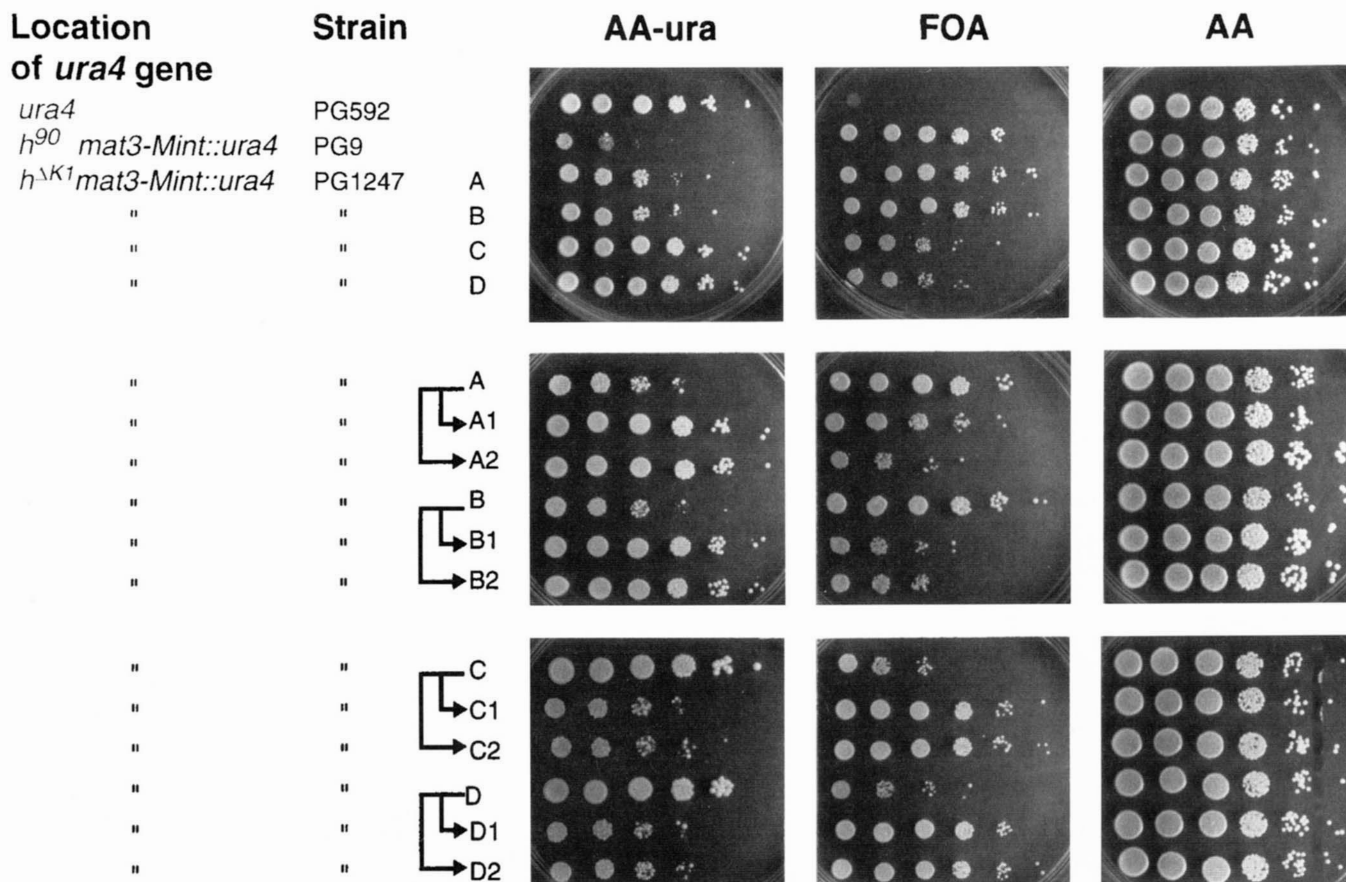

FIGURE 5.—Variegation of transcriptional states in $h^{\Delta K1}$ cells. The level of *ura4* expression in the two types of $h^{\Delta K1}$ populations was estimated by spotting serial 10-fold dilutions of cell suspensions onto complete medium (AA), plates lacking uracil (AA-ura) and plates containing the toxigenic substrate of the Ura4 protein, 5-FOA (FOA). Nomenclature as in Figure 3.

tained in these crosses. They had the same phenotypes as the originally isolated *mat1-Msmt-0 swi6-115* mutants. Consistently, each mutation conferred similar phenotypes to h^{90} *swi6*Δ::*ura4* and h^{90} *swi6-115* cells. Hence, the mutations did not act in combination with a specific *swi6* allele but they had the same effect when *swi6* was mutant or deleted. Crosses were performed between the strains to determine which mutations were linked (MATERIALS AND METHODS). The linkage analysis defined three loci named *esp1*, *esp2* and *esp3*. We determined that the *esp* loci were distinct from the previously characterized *clr1*, *clr2*, *clr3*, *clr4* and *rik1* loci (MATERIALS AND METHODS).

We tested whether mutations representative of each *esp* locus also affected expression of *mat3-M* by constructing unswitchable *mat1-P*Δ*17*::*LEU2 swi6⁻* mutant strains. The sporulation phenotypes of these stable *P* strains were examined, as well as production of *mat3-M* transcripts. High levels of haploid meiosis and *mat3-M* transcripts were observed (Figure 7A). Hence, although the *esp* mutations had been isolated on the basis of their effect on the expression of *mat2-P*, they also allowed increased expression of *mat3-M* in a $swi6^-$ background.

We were especially interested in determining whether the *esp* mutations had a cumulative effect with a defective *swi6* allele or whether they were epistatic to *swi6*. We moved the *esp* mutations to $swi6^+$ backgrounds and found that they had little or no effect on their own, as shown for the mutation that had the strongest effect, the *esp3-1* allele, in Figure 7B. This phenotype is expected for mutations affecting only part of a redundant pathway. We realized that mutations increasing mRNA stability might also increase the amount of mRNA originating from *mat2-P* and *mat3-M* in $swi6^-$ cells and have no effect in $swi6^+$ cells. Thus, such mutations would behave as our *esp* mutations. They could be used as tools to detect small levels of derepression of *mat2-P* or *mat3-M*, but they would not be informative *per se* with regard to silencing. We addressed this issue by examining the amount of ura4 transcript originating from *mat3-Mint*::*ura4* in *swi6-115* cells. We found that *esp1-1*, *esp2-1* and *esp3-1* increased the amount of ura4 transcript originating from *mat3-Mint*::*ura4* whereas they had no effect on the ura4 transcript originating from the *ura4* locus itself (data not shown). Hence, the *esp* loci do not merely affect transcript stability; they have a role in silencing and a cumulative effect with *swi6*.

Cumulative effect of Δ*K1* and one of the *esp* mutations, *esp3-1*: We combined the mutant *esp3-1* allele with Δ*K1*. Crosses were performed both with partially derepressed isolates of an unswitchable Δ*K1* strain, that is, isolates expressing the *ura4* gene placed near *mat3*,

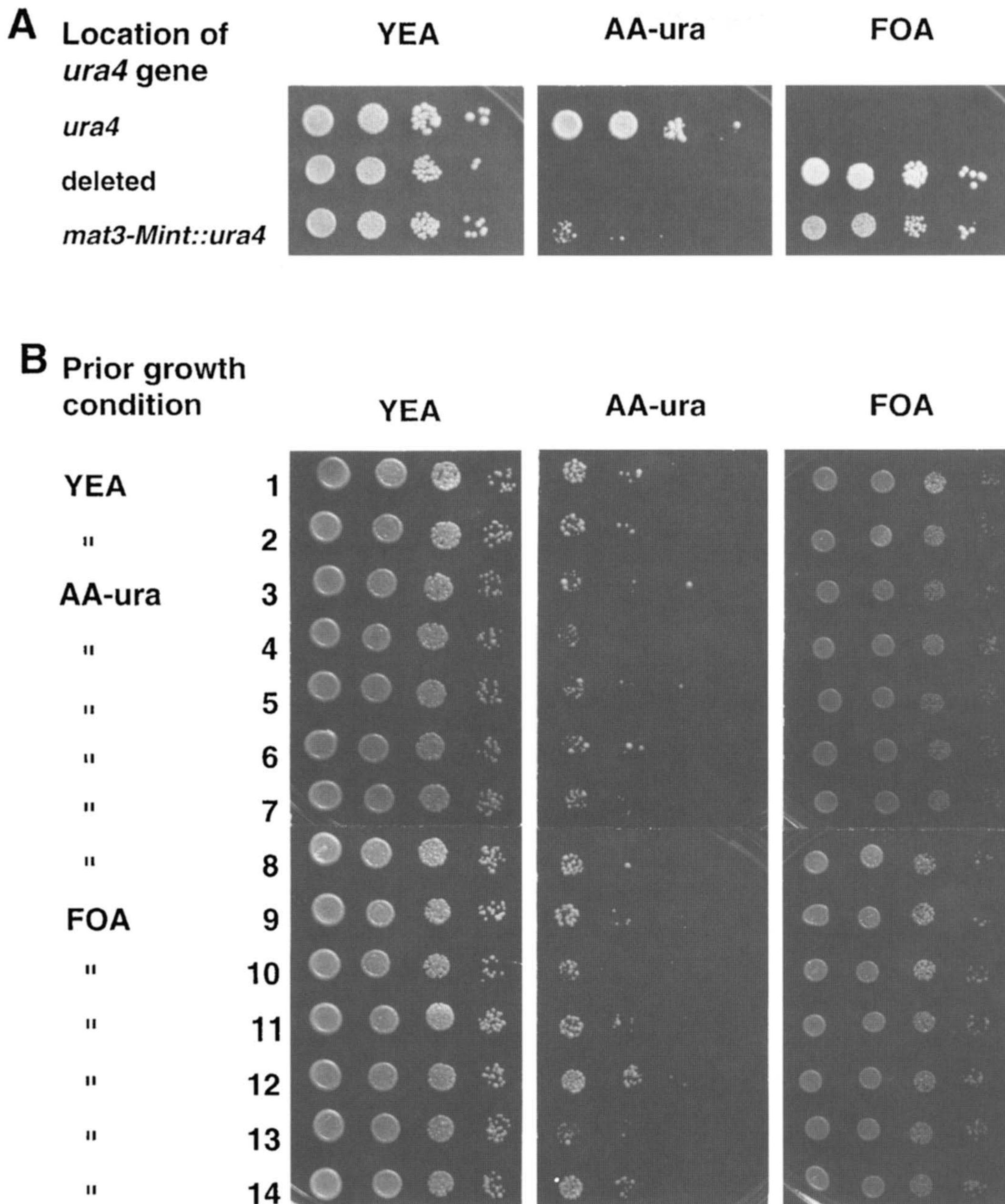

FIGURE 6.—Transient derepression of *mat3-Mint::ura4* in the h^{90} mating-type region. (A) The *ura4* gene is sporadically expressed in h^{90}, *mat3-Mint::ura4* cells. Serial 10-fold dilutions of PG592 (*ura4*), PG1217 (deleted) and PG9 (*mat3-Mint::ura4*) were plated onto the indicated media. A small proportion of PG9 cells were able to form colonies on medium lacking uracil, indicating the *ura4* gene was derepressed in these cells. (B) Ura^+ h^{90}, *mat3-Mint::ura4* cells revert efficiently to Ura^-. Individual colonies of PG9 cells propagated on rich or selective medium were retested for expression of *ura4*. All populations displayed very similar growth patterns characteristic of the repressed state, indicating the occasional expression of the *ura4* gene was very unstable.

and with repressed isolates where *ura4* was silenced. When crossed into partially derepressed $\Delta K1$ cells, the *esp3-1* allele showed a cumulative effect with the $\Delta K1$ deletion. Increased levels of haploid meiosis were observed in the $\Delta K1$ *esp3-1* double mutant, as well as elevated transcription of the *mat3-M* cassette (Figure 7B). When crossed into repressed $\Delta K1$ cells, the *esp3-1* allele did not display a cumulative effect with the $\Delta K1$ deletion: it did not increase expression of *ura4* transplaced near *mat3-M,* nor of the mating-type genes from *mat3-M.* However, following transfer onto sporulation medium lacking uracil, the *esp3-1* $\Delta K1$ progeny with a silenced mating-type region produced Ura^+ papilli displaying high levels of haploid meiosis. Thus, the *esp3-1* allele had a cumulative effect with the derepressed configuration of $\Delta K1$ but not with the repressed configuration of $\Delta K1$, a finding in agreement with *esp3-1* having no phenotype in wild-type cells. The cumulative effect of $\Delta K1$ and *esp3-1* corroborates our previous conclusion that silencing is impaired in the $h^{\Delta K1}$ mating-type region and strengthens models proposing that redundant pathways repress transcription in the mating-type region.

DISCUSSION

An epigenetic effect influences mating-type switching and transcriptional silencing in *S. pombe.* The effect occurs in the mating-type region and causes cells bearing a large deletion between *mat2-P* and *mat3-M* to adopt one of two semistable phenotypes: switching- and silencing-deficient or switching- and silencing-competent. The existence of such an epigenetic effect indicates that both switching and silencing involve an establishment

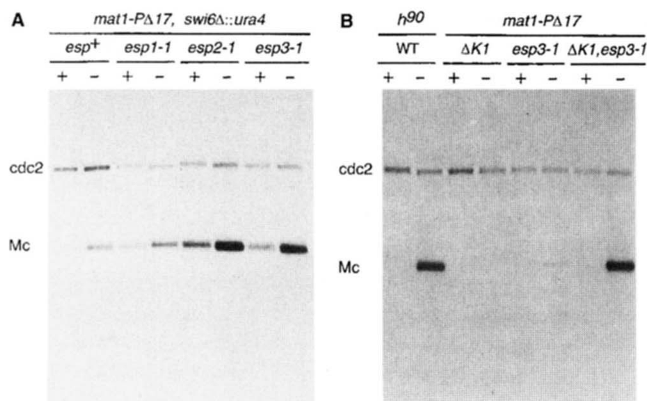

FIGURE 7.—Transcription of *mat3-M* in various mutant backgrounds. (A) Effect of three *esp* mutations in a *swi6⁻* background. Transcription of *mat3-M* can be assayed by Northern blot analysis in unswitchable *mat1-P* cells. *mat3-M* is poorly transcribed in *swi6Δ::ura4* cells (*esp⁺*, PG1177). Larger amounts of Mc transcript are present in *swi6Δ::ura4 esp⁻* cells (*esp1-1*, PG1135; *esp2-1*, PG1129; *esp3-1*, PG1144). (B) Cumulative effect of $\Delta K1$ and a mutant *esp3* locus. Transcription of *mat3-M* is tightly repressed in a *swi6⁺* background (THON and KLAR 1992). Transcription of *mat3-M* was also repressed in $\Delta K1$ cells ($\Delta K1$, PG1306), slightly derepressed by the *esp3-1* mutation (*esp3-1*, PG1146), and further derepressed by the combination of $\Delta K1$ and *esp3-1* ($\Delta K1$ *esp3-1*, PG1310). Transcription of the *mat1-M* Mc gene in h^{90} cells is shown for comparison (WT, PG9). The cells were grown in abundant (+) or limiting (−) nitrogen as indicated, limiting nitrogen being an inducer of transcription of the mating-type genes (KELLY *et al.* 1988). Hybridization to a *cdc2* probe was used to estimate the amount of RNA loaded.

step facilitated by some element present between *mat2* and *mat3*, followed by a maintenance step independent of that DNA element. Our current understanding of mating-type switching and silencing in *S. pombe* suggests simple models for the nature of the establishment and maintenance steps. Here, we will summarize the input of previous studies and the input of the experiments reported here and discuss which aspects of switching and silencing are particularly affected by the epigenetic phenomenon. We will present a model for how the two phenotypes might arise and interconvert and compare our observations with other epigenetic effects occurring in *S. pombe*.

The process of mating-type switching involves several steps revealed by the examination of switching-defective mutants. A double-strand break is formed as *mat1* and persists during all stages of the cell cycle (BEACH 1983). The *trans*-acting functions *swi1*, *swi3* and *swi7* are required for forming the break (EGEL *et al.* 1984) as well as *cis*-acting elements adjacent to *mat1* (reviewed by KLAR 1992). Nine *trans*-acting factors are known to act subsequently in the gene conversion of *mat1* and resolution of the gene conversion. Distinctive phenotypes allow to separate these factors into different classes. Functions important for utilization of the break or for directionality of switching were originally distinguished from functions important for resolution (EGEL *et al.* 1984; GUTZ and SCHMIDT 1985). Further associations can now be proposed that take into account additional roles of the switching factors such as participation in silencing or chromosome segregation. These criteria point to similarities shared by *swi6*, *rik1* and *clr4:* each affects the directionality of mating-type switching (THON and KLAR 1993; G. THON, unpublished observations), recombination in the mating-type region (EGEL *et al.* 1989; KLAR and BONADUCE 1991; LORENTZ *et al.* 1992; THON *et al.* 1994), transcriptional silencing near *mat2* and *mat3* (THON and KLAR 1992; EKWALL and RUUSALA 1994; THON *et al.* 1994), transcriptional silencing near centromeres and telomeres (ALLSHIRE *et al.* 1995) and the efficiency of chromosome segregation (ALLSHIRE *et al.* 1995). Most reports concerned with the roles of Swi6, Rik1 and Clr4 have proposed that these proteins act by modifying the chromatin structure of the mating-type region, telomeres and centromeres or by stabilizing specific structures at these locations. The slowly switching phenotype of the $h^{\Delta K1}$ strain was strikingly similar to the phenotypes conferred by mutations in *swi6*, *rik1* or *clr4:* first, switching was inefficient despite a normal level of double-strand break; second, both switching and silencing were affected at the same time; third, the effect on switching and silencing was only partial; fourth, cassette duplications of the h^{+N} type were unstable, indicating the deletion allowed increased recombination in the mating-type region. These phenotypic similarities lead to propose that the deletion can prevent *swi6*, *rik1* and/or *clr4* from accomplishing their function. Consistently, a mutation in *swi6* was epistatic to the deletion, as expected if the deleted DNA fragment facilitates the function of *swi6*.

Derepression of transcription in the $h^{\Delta K1}$ mating-type region was only partial: expression of a *ura4* gene introduced near *mat3-M* could be detected, but not expression of the mating-type genes themselves. This derepression was somewhat less pronounced than when *swi6*, *rik1* or *clr4* are mutated or deleted but not dramatically so. Impairing *swi6*, *rik1* or *clr4* allows strains whose *ura4* gene is within the mating-type region to grow in the absence of uracil but it does not fully derepress transcription of the *ura4* gene (ALLSHIRE *et al.* 1995). Impairing these functions does not allow much expression of the mating-type genes from the normally silent loci either and only leads to low levels of haploid meiosis. Hence, the transcriptional repression exerted by Swi6, Rik1 and Clr4 and the repression exerted by the DNA fragment that we deleted are within the same range. We were able to refine the comparison between *swi6* and the *K* region by obtaining mutations in a novel class of *trans*-acting loci, the *esp* loci. Mutations at these loci caused increased expression of the *mat2-P* and *mat3-M* cassettes when combined with a mutation in *swi6* (*swi6-115*) or with a deletion of *swi6*. They had a very similar effect when combined with the $\Delta K1$ deletion. The cumulative effect of a mutation in *esp3* with the $\Delta K1$ deletion revealed that the deletion does affect transcription of the mating-type genes although this effect is masked

in an esp^{+} background. Furthermore, the phenotypes of the various double-mutants we constructed support the notion that both *swi6* and an element within the K region act within one of two silencing pathways, the *esp* functions acting in the second pathway.

In addition to its effects on switching and silencing, the $\Delta K1$ deletion increased the rate of cassette duplication leading to h^{+N} rearrangements, indicating the deletion affects resolution. Rates higher than those reported for the wild-type (EGEL *et al.* 1980; BEACH and KLAR 1984 and references herein) were observed both in populations of poorly and efficiently switching cells (Table 2). Hence, this effect might be mediated differently from the epigenetically transmitted effects. Resolution might be affected in $h^{\Delta K1}$ because a DNA element important for resolution was deleted or because *mat2-P* and *mat3-M* were placed close to each other: at ~3.5 kb instead of 12 kb in the wild type. GREWAL and KLAR reported recently the phenotype of a strain with a deletion in the mating-type region (GREWAL and KLAR 1996). In that strain, the fragment deleted from the mating-type region was the same as in $h^{\Delta K1}$, but it was replaced with a 2-kb fragment containing the *ura4* gene ($K\Delta$ :: *ura4* region). Interconversions between two epigenetic states similar or identical to the states we described were observed in the $K\Delta$ :: *ura4* region, but h^{+N} rearrangements were not, indicating the spacing between the two silent cassettes might be important for resolution of the *mat1* gene conversions.

We have pointed out similarities between the slowly switching phenotype of $h^{\Delta K1}$ cells and the phenotypes of mutants in *swi6, rik1* or *clr4*. However, mutations in *swi6, rik1* or *clr4* do not confer an epigenetically maintained phenotype, whereas the $\Delta K1$ deletion does. To accommodate the two observations, we propose that a DNA element within the K region acts as a nucleation site and facilitates the association of factors such as Swi6, Rik1 and Clr4, that are required for the formation of fully functional switching and silencing complexes. In our model, when this element is deleted in the $h^{\Delta K1}$ strain, all factors required for switching and silencing including Swi6, Rik1 and Clr4 can still assemble in the mating-type region, but the assembly is an unlikely event. Once formed, however, the complex can be duplicated and efficiently transmitted to the progeny, both in mitosis and in meiosis, giving rise to colonies similar to the wild type. The persistance of a macromolecular complex or DNA modification associated with the mating-type region during meiosis is expected. It would account for the inhibition of meiotic recombination observed in the mating-type region (EGEL 1984), which is dependent upon the presence of the switching and silencing factors *rik1, swi6,* and *clr1–clr4* (EGEL *et al.* 1989; KLAR and BONADUCE 1991; LORENTZ *et al.* 1992; THON and KLAR 1992; THON *et al.* 1994).

We would like to draw a parallel between the effects reported here and the epigenetic effects at *S. pombe* centromeres. The three *S. pombe* centromeres occupy ~38, 65 and 97 kb, respectively, and consist of a nonrepetitive central core flanked by large inverted repeats (reviewed by ALLSHIRE 1995). Cen3-based minichromosomes lacking part of the centromeric repeats proceed inefficiently through an establishment step when introduced into *S. pombe* by transformation, after which they are more stably maintained, not only in mitosis, but also in meiosis (STEINER and CLARKE 1994). Hence, the epigenetic maintenance of silencing and switching competence in the mating-type region and the epigenetic maintenance of centromere function both belong to a poorly documented class of epigenetic effects that are stable in meiosis and not simply explained in terms of epigenetic regulation of transcription. Another study (ALLSHIRE *et al.* 1995) has shown that when *swi6, rik1* or *clr4* are mutated, centromeres function inefficiently, resulting in high loss rates. By analogy with our model for the epigenetic maintenance of a switching and silencing complex in the mating-type region, one might suggest that the centromeric repeats missing in the epigenetically stabilized minichromosomes contain a nucleation site for the Swi6, Rik1 or Clr4 proteins.

We thank HENNING SCHMIDT for providing a strain containing the *swi6*Δ::*ura4* allele (AL91), PERNILLA BJERLING, STANLEY BROWN, RICHARD EGEL and OLAF NIELSEN for editorial suggestions and P. B., S. B., R. E., and O. N. and members of the genetics department for comments on the experiments. This work was supported by a long-term fellowship from the Human Frontier Science Program Organization to G.T., by the Danish National Science Foundation and by the Novo Nordisk Foundation.

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