Disruption of a Silencer Domain by a Retrotransposon

Molly F. Mastrangelo,[1] Keith G. Weinstock, Brenda K. Shafer, Anne-Marie Hedge, David J. Garfinkel and Jeffrey N. Strathern

Laboratory of Eukaryotic Gene Expression, NCI-Frederick Cancer Research and Development Center, ABL-Basic Research Program, Frederick, Maryland 21702-1201

Manuscript received January 3, 1992
Accepted for publication March 4, 1992

ABSTRACT

A galactose-inducible *Ty* element carrying the *HIS3* gene has been used as an insertional mutagen to generate α-factor resistant mutants. This collection of *Ty*-induced mutations includes insertions into the gene for the α-factor receptor (*STE2*), several nonspecific *STE* genes, and mutations that lead to the expression of the normally silent *HMLα* locus. The *hmlα* "on" mutations fall into two classes, those that disrupt *trans*-acting regulators involved in silencing *HMLα* and a novel class of mutations that activate *HMLα* by insertion at that locus. The *hmlα::Ty* "on" mutations illustrate the unusual ability of these retrotransposons to activate genes by overcoming gene silencing mechanisms. The *hmlα::Ty* "on" mutations include examples of multimeric *Ty* arrays. Single *Ty* and solo δ insertion derivatives of these *Ty* multimers restore the ability of the silencing mechanism to repress *HMLα*.

IN *Saccharomyces cerevisiae*, silent copies of the genes that regulate mating type are present at *HML* and *HMR* (reviewed by HERSKOWITZ 1989). Changes of cell type in homothallic yeast occur by using *HML* and *HMR* as donors in a gene conversion mechanism that transfers copies of these regulatory genes to a locus (*MAT*) where they are expressed. The *HML* and *HMR* loci are transcriptionally repressed due to an active silencing mechanism involving several *trans*-acting factors that recognize sites flanking the repressed genes. Some of the factors involved in the silencing mechanism have been identified as the result of mutations that lead to the expression of *HML* and *HMR* (RINE *et al.* 1979; KLAR, FOGEL and MACLEOD 1979; HABER and GEORGE 1979; RINE and HERSKOWITZ 1987; WHITEWAY *et al.* 1987; MULLEN *et al.* 1989). Additional factors have been identified as proteins that bind to the regulatory sites flanking *HML* and *HMR* (BUCHMAN *et al.* 1988; DIFFLEY and STILLMAN 1988; SHORE and NASMYTH 1987). The DNA sequences involved in the silencing mechanism have been identified by deletion analyses. In this report we describe the isolation of mutations that result in the expression of *HMLα* caused by insertion of the yeast retrotransposon *Ty*.

The insertion of the yeast transposable element *Ty* can generate null alleles by the disruption of the coding region or can alter the regulation of a gene by separating it from its regulatory sequences and bringing the gene under the control of sequences contained within the *Ty* (see review by BOEKE 1989). The usefulness of *Ty* elements as insertional mutagens has been substantially increased by placing them under the control of the galactose inducible *GAL1* promoter (BOEKE *et al.*1985), and including in the element a selectable marker gene (GARFINKEL *et al.* 1988).

In this paper we have exploited a *Ty*-based insertional mutagenesis system to isolate several classes of α-factor resistant mutants. These mutants include *Ty* insertions into genes involved in the α-factor response pathway and genes involved in the silencing of the normally silent copy of *MATα* resident at *HMLα*. One novel class of mutants was obtained that are the result of *Ty* insertion into the *HMLα* locus leading to its expression. Several of the *HMLα::Ty* mutations are the result of the insertion of multimers of the *Ty* element (WEINSTOCK *et al.* 1990). We demonstrate here that the multimeric arrays are necessary for these alleles to release *HMLα* from the silencing mechanism imparted by the *SIR* and *MAR* genes.

MATERIALS AND METHODS

Materials: BioTrace RP, a charge-modified nylon membrane, was purchased from Gelman Sciences. The 1-kb DNA ladder and the high molecular weight DNA markers were purchased from Bethesda Research Laboratories. Synthetic α-factor was purchased from Sigma.

Yeast strains: The yeast strains used in this study are described in Table 1. To facilitate following the *MAT* alleles among the meiotic products of diploid strains that carry mutations causing sterility, the *MATα* allele was marked with the *URA3* gene. The *URA3* gene fragment was derived from the 1.2-kb *Hin*dIII genomic fragment by the addition of *Eco*RI linker oligonucleotides, and was inserted into the *Eco*RI site in the 4.2-kb *MATα Hin*dIII fragment. This *URA3* marked *MATα* fragment was inserted into chromosome *III* at the *MAT* locus by transforming with *Hin*dIII digested DNA and selecting for the Ura$^+$ phenotype. The *mat*Δ

[1] Current address: Department of Biology, Allegany Community College, Cumberland, Maryland 21502.

TABLE 1

List of strains

JSS105-2A	*MATα:URA3 HMLα his3-Δ200 lys2-801 ura3-52 tyr7-1*
JSS104-15B	*MATa HMLα his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ1 ura3-52*
JSS222-7B	*MATα:URA3 HMLα his3-Δ200 leu2-Δ1 lys2-801 trp1 ura3-52 tyr7-1*
JSS56-11B	*MATa HMLα his3-Δ1 leu2-3,112 trp1-289 ura3 can1*
MMA9	*MATa HMLα ste2-609::Ty* (see JSS56-11B)
MMA12	*MATa HMLα ste7-612::TyHIS3* (see JSS56-11B)
MMB12	*MATa hmlα-712::TyHIS3* (see JSS56-11B)
MMB21	*MATa hmlα-721::TyHIS3* (see JSS56-11B)
MFM137-7D	*MATα:URA3 hmlα-721::TyHIS3*
MMC39	*MATa HMLα ste2-839::TyHIS3* (see JSS56-11B)
MFM85-4D	*MATα:URA3 HMLα ste2-839::TyHIS3 his3 tyr7-1 trp1-289 ura3*
MMD25	*MATa hmlα-925::TyHIS3* (see JSS56-11B)
MMD25*matΔ*	*matΔ::URA3 hmlα-925::TyHIS3* (see JSS56-11B)
MMD31	*MATa HMLα ste5-931::TyHIS3* (see JSS56-11B)
MMD37	*MATa hmlα-937::TyHIS3* (see JSS56-11B)
MFM120-8A	*MATα:URA3 hmlα-937::TyHIS3*
MM1-7	*MATa HMLα fus3-107::TyHIS3* (see JSS56-11B)
DG1002	*matΔ:URA3 HMLα* (see JSS56-11B)
DG1031	*matΔ::URA3 hmlα-2078::TyHIS3* (see JSS56-11B)
DG1032	*matΔ::URA3 hmlα-2079::TyHIS3* (see JSS56-11B)
DG1033	*matΔ::URA3 hmlα-2081::TyHIS3* (see JSS56-11B)
DG1034	*matΔ::URA3 hmlα-2084::TyHIS3* (see JSS56-11B)
DG1035	*matΔ::URA3 hmlα-2087::TyHIS3* (see JSS56-11B)
DG1036	*matΔ::URA3 hmlα-2091::TyHIS3* (see JSS56-11B)
DG1037	*matΔ::URA3 hmlα-2098* (see JSS56-11B)
DG1038	*matΔ::URA3 hmlα-2099::TyHIS3* (see JSS56-11B)
DG1039	*matΔ::URA3 hmlα-2103::TyHIS3* (see JSS56-11B)
DG1059	*matΔ::URA3 ard1-2085::Ty* (see JSS56-11B)
DG1067	*matΔ::URA3 nat1-2104::Ty* (see JSS56-11B)
DC14a	*MATa his1*
DC17α	*MATα his1*
RC687	*MATa sst2-4 ade2 ura1 his6 met1 can1 cyh2 rme1* (R. CHAN)
XBH8-2C	*MATα sst2-4 ura1 his1 met1 cry1* (L. BLAIR)
294	*MATα his3 leu2 trp1 ura3* (J. BROACH)

strains were made by making a substitution of *URA3* for *MAT* sequences by the omega transplacement technique (ROTHSTEIN 1983). The *mat* deletion removes all of the *MAT* W, X, Y regions and most of the Z regions (STRATHERN *et al.* 1980; ASTELL *et al.* 1981).

Ty* mutagenesis:** The plasmid pGTy1H3*HIS3*HH4B#84, is a *TRP1* based high copy number plasmid with the *HIS3* gene inserted into the *Ty* element just upstream of the 3′ LTR (long terminal repeat or δ element) (GARFINKEL *et al.* 1988; WEINSTOCK *et al.*1990). Induction of the galactose-regulated *Ty* elements was as described (GARFINKEL *et al.*1988). *MATa** cells carrying the inducible marked *Ty* were grown on minimal medium containing galactose for 5 days at 22°.

Selection of α-factor resistant mutants: Selection for mutants resistant to α-factor was similar to HARTWELL (1980). Cells in which the marked *Ty* element had been induced were plated at 30° on YEPD containing α-factor at 1 μg/ml. Colonies from these plates were retested for α-factor resistance and were tested for ability to mate and sporulate.

Genetic analysis of sterile strains: The mating defects of the mutants in this study were in general not conditional. Genetic analysis required diploid formation by polyethylene glycol mediated fusion (VON SOLINGEN and VAN DER PLATT 1977). Subsequent sporulation and meiotic analysis was performed as described by SHERMAN, FINK and HICKS (1986).

DNA analysis: Yeast DNA was prepared and analyzed as described in WEINSTOCK *et al.* 1990. *HMLα* probes were obtained from the 6.6-kb *Bam*HI fragment of *HMLα* (FELDMAN, HICKS and BROACH 1984). The *ARD1* probe was derived from plasmid M1p1 that was kindly provided by M. WHITEWAY. The *NAT1-SIR2* probe was derived from plasmid pJM201 that was kindly provided by R. STERNGLANZ. The prove for the *FUS3* gene was derived from a 3.7-kb *Hin*dIII fragment from plasmid pYEE93 (ELION, GRISAFI and FINK 1990), which was provided by ELAINE ELION. The *STE2* probe was prepared from a 4.2-kb *Bam*HI fragment from YCpSTE2B1 (NAKAYAMA, MIYAJIMA and ARAI 1985), which was obtained from NAOKI NAKAYAMA. The entire plasmid, 18KR, containing an internal fragment of the *STE5* gene was used as a probe and obtained from JEREMY THORNER. The *STE7* probe contained a 1-kb *Hin*dIII-*Cla*I fragment from plasmid pSTE7.2 (CHALEFF and TATCHELL 1985), which was obtained from KELLY TATCHELL.

Budding pattern, pheromone tests, mating efficiencies: The budding pattern was determined by following the position of bud emergence as described in HICKS, STRATHERN and HERSKOWITZ (1977). In cells with the **a**/α phenotype, the bud on the daughter is away from the mother (polar). In cells with the **a** or α phenotype the bud of the daughter is toward the mother (medial). Sensitivity to α-factor was assayed both at the single cell level by placing cells next to a mass of cells of the α cell type and by ability to grow on a YEPD plate containing 1 mg/liter α-factor. Ability to produce α-factor was monitored with the α-factor halo test

(CHAN and OTTE 1982). Efficiency of mating was performed essentially as described previously (STRATHERN, HICKS and HERSKOWITZ 1981).

Identification of His$^+$ revertants of *hmlα::TyHIS3*: In *MAT***a**/*MATα* diploids that carried the *hmlα::TyHIS3* multimeric alleles, the presence of a *HIS3* marked **a**-specific sterile caused by *hmlα::Tyhis3* could be monitored by checking the ability of a sporulated patch of cells to mate; the patches included spores capable of mating as **a** cells when *HIS3* was not selected, but when *HIS3* was selected, there was no mating. For the His$^+$ revertants of the *hmlα* mutation, the sporulated patches mated as *α* cells even when His$^+$ was selected.

Construction of *MAT*a/*MAT*a diploids: Diploids were made from the related *MAT***a**/*MATα* strains by selecting Ura$^-$ mitotic recombinants as colonies resistant to 5-fluoroorotic acid (FOA) (see BOEKE *et al.* 1987); the *URA3* allele is closely coupled to the *MATα* allele in these strains.

RESULTS

Isolation of α-factor resistant *MAT*a cells: From 10 independent pools of *MAT***a** cells (JSS56-11B) in which marked *Ty* element transpositions were induced, 179 *α*-factor-resistant mutants were isolated. In 49 of these mutants the His$^+$ phenotype was independent of the plasmid, indicating that a *TyHIS3* insertion had occurred somewhere in the genome. The genetic analysis of these sterile mutants required bypassing the mating process by using polyethylene glycol-mediated fusion of spheroplasts or relied on the leaky nature of some of the mutations. Thirty of these mutants were subjected to genetic analysis to determine whether the mutation causing the *α*-factor resistance and the mating defect was the result of an insertion of the *HIS3* marked *Ty1* element. Of those 30, nine mutations causing sterility proved to be tightly linked to the His$^+$ phenotype (Table 2) suggesting that the defects were caused by the insertion of a marked *Ty1* element. In these crosses the *MATα* allele was marked by insertion of the *URA3* gene at an *Eco*RI site just centromere proximal to *MAT*. Because the recombination between the *URA3* insertion and the *MATα* allele was only about 1%, the allele of *MAT* could be inferred from the Ura phenotype: the nonmating segregants could be identified as sterile *MAT***a** (Ura$^-$) or sterile *MATα* (Ura$^+$) cells. The mating characteristics of these mutants are given in Table 3.

Nonspecific sterile mutations: Three of the *Ty-HIS3* marked mutations resulted in a sterile phenotype in cells of both mating types (see mutants MMA12, MMD31 and MM1-7 in Table 2). In each case about half of the meiotic segregants were sterile, reflecting the observation that each tetrad had two sterile segregants. The mutation caused sterility in both the *MAT***a** (Ura$^-$) and *MATα* (Ura$^+$) segregants. The number of His$^+$ segregants was equal to the number of His$^-$ segregants (the His phenotype segregated 2$^+$:2$^-$ in all tetrads), suggesting that there was a single *HIS3* marked *Ty* transposition. Finally, all of the sterile segregants were His$^+$. From these data we conclude that each of these mutants contains a nonspecific sterile mutation that is unlinked to *MAT*. Further, these data show that the mutations are tightly linked to, and probably caused by, a *HIS3* marked *Ty* insertion.

At least six genes that are directly involved in mating by both **a** and *α* cells (nonspecific sterile genes) have been defined by mutants resistant to *α*-factor (MANNEY and WOODS 1976; HARTWELL 1980; ELION, GRISAFI and FINK 1990). Because the *Ty*-induced mutations affected mating ability and were not conditional, a classical complementation analysis with mutants defective in the known *ste* genes was not attempted. Instead, we took advantage of the fact that the insertion of the *Ty* element should cause an alteration of the restriction endonuclease cleavage pattern of the target gene. Restriction digests of DNAs from mutants MMA12, MMD31 and MM1-7 were blotted to nylon membranes and hybridized with DNAs from the *STE5*, *STE7* and *FUS3*, genes (see Figure 1). The mutation in MMA12 proved to be a disruption of the *STE7* gene (designated *ste7-612*), the mutation in MMD31 was a disruption of the *STE5* gene (*ste5-931*), and the mutation in MM1-7 was a disruption of *FUS3* (*fus3-107*). The isolation of a *FUS3* mutation suggests that the parental strain (JSS56-11B) and the tester strain (JSS105-2A) are defective in the functionally redundant gene *KSS1* (ELION, BRILL and FINK 1991), a mutation common to several laboratory strains of yeast.

a-Specific sterile mutations: Mutations that cause *α*-factor resistance and **a**-specific mating defects have been identified in three genes: *STE2*, *ARD1* and *NAT1* (HARTWELL 1980; WHITEWAY *et al.* 1987; MULLEN *et al.* 1989). Two of the nine mutations we characterized were the result of insertions of the marked *Ty* element into the *STE2* gene. One of these, *ste2-217::TyH3HIS3*, has been described previously (GARFINKEL *et al.* 1988). The mutation in strain MMC39 was another example of this class. The cross with MMC39 (MFM85), exhibited segregation characteristic of a *HIS3* marked **a**-specific sterile mutation; all of the Ura$^+$ (*MATα*) segregants were capable of mating as *α*'s, but only half of the Ura$^-$ (*MAT***a**) segregants could mate like **a** cells. All of the sterile segregants were Ura$^-$ and His$^+$, but the excess of His$^+$ to His$^-$ segregants (about 3:1) was consistent with the segregation of an additional *HIS3* marked *Ty* element independent of the *TyHIS3* insertion causing sterility. Analysis of the DNA from this strain confirmed that the *STE2* gene had undergone a rearrangement (designated *ste2-839*) consistent with the insertion of a single *HIS3* marked *Ty* element.

Activation of *HMLα* by *Ty* insertion: Genetic analysis demonstrated that four more of the *TyHIS3*

TABLE 2

Segregation pattern of marked *Ty* steriles

	Ura$^+$ (*MATα*)				Ura$^-$ (*MATa*)			
	α His$^+$	α His$^-$	Ste His$^+$	Ste His$^-$	a His$^+$	a His$^-$	Ste His$^+$	Ste His$^-$
ste7-612								
MFM99 (MMA12 × JSS105-2A) (28 His$^+$:31His$^-$)	0	15	14	0	0	16	14	0
ste5-931								
MFM86 (MMD31 × JSS105-2A) (26 His$^+$:31 His$^-$)	0	14	14	0	0	17	12	0
fus3-107								
MFM3 (MM1-7 × JSS105-2A) (29 His$^+$:24 His$^-$)	0	11	14	0	2	12	13	1
ste2-839								
MFM85 (MMC39 × JSS105-2A) (41 His$^+$:15 His$^-$)	22	7	0	0	5	8	14	1
hmlα-925								
MFM1 (MMD25 × JSS105-2A) (40 His$^+$:34 His$^-$)	23	19	0	0	0	15	17	0
hmlα-712								
MFM20A (MMB12 × JSS105-2A) (53 His$^+$59His$^-$)	23	34	0	0	2	25	30	0
hmlα-721								
MFM144 (MFM137-7D × JSS114-8Ba#3) (55 His$^+$:54 His$^-$)	31	26	0	0	1	28	23	0
hmlα-937								
MFM152 (MFM120-8A × JSS56-11B) (44 His$^+$:42 His$^-$)	18	25	0	0	0	26	17	0

TABLE 3

Phenotypes of the mutants

	Efficiency of mating	a-Factor production	Budding pattern
JSS56-11B	1 (0.3–0.7)	++	Medial
MMA12 (*ste7-612*)	$<10^{-5}$	Weak	Medial
MMB12 (*hmlα-712*)	10^{-3}	−	Polar
MMB21 (*hmlα-721*)	0.03	Weak	Polar
MMC39 (*ste2-839*)	$<10^{-5}$	+	Medial
MMD25 (*hmlα-925*)	0.05	Weak	Polar
MMD31 (*ste5-931*)	$<10^{-5}$	Weak	Medial
MMD37 (*hmlα-937*)	0.006	−	Polar
MM1-7 (*fus3-107*)	0.17	+	Medial
MM2-17 (*ste2-217*)	0.03	+	Medial

marked mutants (Table 2, strains MMB12, MMB21, MMD25 and MMD37) had lesions that resulted in an **a**-specific defect in mating. However, these mutants did not show any disruption of the *STE2*, *ARD1* or *NAT1* genes. These mutants had the polar budding pattern (Table 3) characteristic of **a**/α cells (CRANDALL, EGEL and MACKAY 1976). *HMLα MAT***a** strains that are unable to repress *HMLα* exhibit most features of the **a**/α phenotype including the polar budding pattern. Restriction enzyme analysis of DNA from these four strains using *HMLα* as a probe showed that they all have a disruption of *HML* (WEINSTOCK *et al.* 1990; see also Figure 3). This suggests that insertion of the marked *TyHIS3* element in or near *HMLα* caused the expression of that locus.

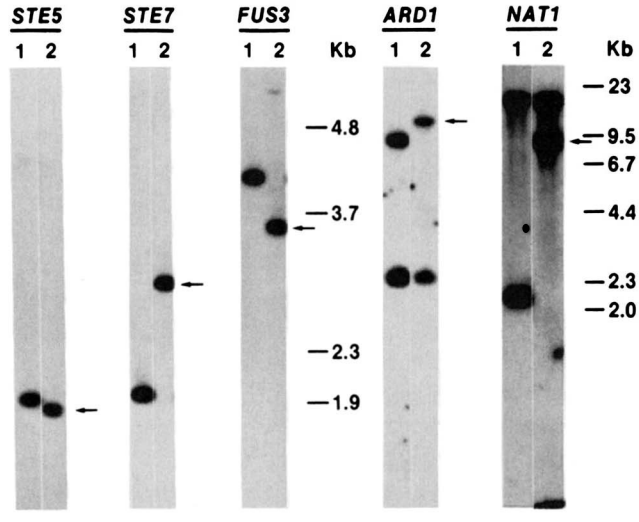

FIGURE 1.—Southern analysis of *Ty*-induced mutations at *STE5*, *STE7*, *FUS3*, *ARD1* and *NAT1*. Each panel consists of the parental DNA and DNA from the mutant identified by corresponding probe: *STE5* lane 1 JSS56-11B, lane 2 MMD31 (*ste5-931*); *STE7* lane 1 JSS56-11B, lane 2 MMA12 (*ste7-612*); *FUS3* lane 1 JSS56-11B, lane 2 MM1-7 (*fus3-107*); *ARD1* lane 1 DG1002, lane 2 DG1059 (*ard1-2085*); *NAT1* lane 1 DG1002, lane 2 DG1067 (*nat1-2104*). DNA was digested with either *Hin*dIII (*STE5*, *STE7* and *FUS3* panels) or *Bam*HI (*ARD1* and *NAT1* panels). The arrows point to *Ty*/target gene junction fragments. Size standards derived from bacteriophage λ appear alongside the blots.

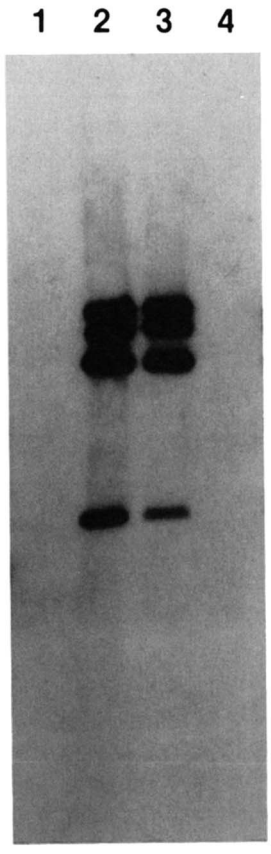

FIGURE 2.—A blot of *Eco*RI-digested DNAs probed with *HIS3*. Tracks 1–4 show a tetrad of MFM144 demonstrating cosegregation of four *HIS3* bands. Tracks 1 and 4 are from spore clones that are *HMLα* (the *his3-Δ200* allele removes all homology to the probe). Tracks 2 and 3 are spore clones with the *hmlα-721* allele.

The genetic analysis of MMD25 was straightforward (Table 2). Histidine prototrophy segregated $2^+:2^-$ in all tetrads, and the **a**-specific sterile was linked to His^+. The mutation in this strain is designated *hmlα-925*. The analysis of the mutation in MMB12 shown in cross MFM20A gave a similar conclusion. The mutation in MMB12 is designated *hmlα-712*.

The genetic analyses of MMD37 and MMB21 were complicated by the presence of several unlinked insertions of *TyHIS3*. However, it was possible to segregate the desired mutation (designated *hmlα-721*) away from the superfluous *TyHIS3* insertions by repeated backcrosses of *MATα:URA3 hmlα-721* segregants to a *MAT***a** *HMLα* strains. Cross MFM144 showed equal number of His^+ and His^- segregants with the **a**-specific sterile cosegregating with the His^+ phenotype. A similar series of backcrosses allowed the segregation of the mutation in MMD37 (designated *hmlα-937*) from unlinked *TyHIS3* insertions (Table 2).

Multiple *TyHIS3* insertions linked to *hmlα* "on" mutations: The simple expectation for cross MFM-144, in which the His phenotype segregates $2^+:2^-$, is that they would have a single novel *HIS3* gene linked to the activated *HML* gene. However, as shown in Figure 2, there are four novel *HIS3* bands linked to the *hmlα-721* mutation from MMB21. Three tetrads showed this pattern. These results indicate that there are multiple *HIS3* marked *Ty* elements inserted at or near this *hmlα* "on" mutation. A similar analysis of the mutations in MMD25, MMD37 and MMB12 showed that they also have multiple *HIS3* bands linked to the activated *hmlα* alleles. A physical analysis of the *hmlα-712*, *hmlα-721*, *hmlα-925* and *hmlα-937* alleles demonstrated that they were caused by insertion of multimeric arrays of *Ty* elements (WEINSTOCK *et al.* 1990; see also Figure 3). These *Ty* arrays were a mixture of *TyHIS3* and unmarked *Ty* elements.

HMLα::Ty* alleles are dominant:** Activation of *HMLα* by *Ty* insertion at that locus would be expected to be dominant. To test this prediction, *MATa**/*MAT***a** diploids carrying one copy of the *hmlα::Ty* mutations were constructed as described in MATERIALS AND METHODS. *MAT***a**/*MAT***a** derivatives of the *hmlα-925*/*HMLα* diploids had the **a**/α phenotype (nonmating and sporulation proficient). As predicted, tetrads from these *MAT***a**/*MAT***a** *hmlα-925*/*HMLα* diploids segregated 2 **a** and 2 sterile spores in each tetrad (29 tetrads). The sterile segregants were all His^+. The absence of α segregants confirmed that the diploids had no *MATα* allele. Therefore, the **a**/α phenotype of the *MAT***a**/*MAT***a** diploids was the result of the dominant expression of the α2 gene at the *hmlα-925* allele. Similar observations demonstrated that the *hmlα-712*, *hmlα-721*, and *hmlα-937 Ty* induced mutations are also dominant.

Reversion of a *Ty* array: The multimers observed at *HML* are in marked contrast to the normal single insertion events seen at other target loci (BOEKE 1989). This suggests either that multimeric *Ty* insertions are required to overcome the SIR-MAR silencing mechanism or that some property of *HML* maintained multimers that would normally be reduced to monomers at other sites. To address the first proposal, we selected for reversion of the sterile phenotype of these *MAT***a** *hmlα::Ty*-multimer mutants by selecting for mating to an α cell. The matings were selected in the presence of histidine and the resulting diploids were screened for mating type and the ability to grow in the absence of histidine.

We analyzed 406 rare matings between MMD25 (*hmlα-925*) and a *MATα his3-Δ200* strain (JSS222-7B). These came from 10 independent pools. As might be expected from the fact that MMD25 has some residual mating ability (Table 3), most (399/406) of these diploids (Table 4) had the same phenotype as similar diploids made by spheroplast fusion. That is, they were His^+, they had the **a**/α phenotype (nonmating and sporulation proficient), and meiotic segregation yielded an **a**-specific mating defect linked to His^+. DNAs from ten independent strains with these phenotypes were cut with *Bam*HI and analyzed

TABLE 4

Diploids made by rare matings

Cross	Phenotype of diploids		Phenotype of His$^+$ spores	
	His$^+$	His$^-$	His$^+$ α and His$^+$ **a**	His$^+$ α but no His$^+$ **a**
*hmlα-925 MAT***a** × *MATα* MMD25 × JSS222-7B	399	7	0	399
*hmlα-937 MAT***a** × *MATα* MFM152-3D × JSS222-7B	354	56	43	311
*hmlα-712 MAT***a** × *MATα* MMB12 × JSS222-7B	84	311	0	84
*hmlα-721 MAT***a** × *MATα* MFM144-2A × JSS222-7B	428	1	4	425

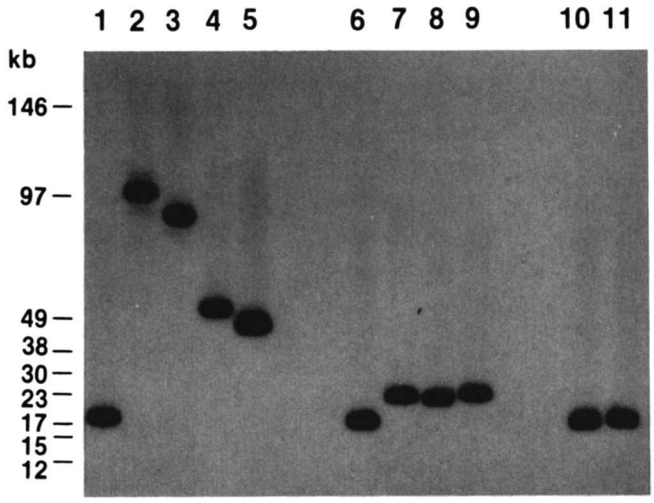

FIGURE 3.—Pulsed field gel electrophoresis of *Stu*I digests of the multimeric *Ty* insertions and revertants. Chromosomal DNA was digested with *Stu*I and separated by CHEF electrophoresis. The DNA was transferred to BioTrace RP and probed with the 1.7-kbp *Hin*dIII-*Bam*HI fragment of *HMLα*. JSS56-11B (starting strain): lanes 1, 6, and 10. Multimeric *Ty* insertions: lane 2, MMB12; lane 3, MMB21; lane 4, MMD25; lane 5, MMD37. Monomeric *Ty* revertants: lane 7, MFM-152D (revertant of MMB12); lane 8, MFM197-3D (revertant of MMD25); lane 9, MFM199-1C (*HIS3* marked revertant of MMD37). Single LTR revertant: lane 11, GRY943 (revertant of MMD25). Size markers (in kilobases) are indicated on the axis.

by filter hybridization. The *hmlα-925* allele in these ten strains appeared unchanged with either *HMLα* or *HIS3* as probes (data not shown).

There were seven His$^-$ diploids (representing five independent events) among the 406 rare matings involving the *hmlα-925* allele. Three of the independent His$^-$ diploid strains contained a novel sized *HMLα* DNA fragment whose size is consistent with the insertion of a single unmarked *Ty* element (Figure 3). Dissection of these diploids confirmed that *MAT***a** *hmlα::Ty* segregants with single *Ty* insertions are mating competent. This observation strongly suggests that the mechanism of disruption of the *SIR-MAR* silencing of *HMLα* caused by the *hmlα-925 Ty* allele is dependent upon its multimeric structure, not the site of its

TABLE 5

Phenotypes caused by *HMLα* expression

	Budding pattern			α-Factor sensitivity		
	Medial	Polar	Unclear	Shmoo	Bud	E.O.M.
*MAT***a** *HMLα* (JSS56-11B)	34	1	2	206	3	1.0
*MAT***a** *hmlα-925 Ty* multimer (MMD25)	1	34	2	3	198	0.005
*MAT***a** *hmlα-925.Z10 Ty* monomer (MFM197-3B)	42	1	3	133	3	1
*MAT***a** *hmlα-925.151* solo delta (GRY943)	45	0	3	142	0	1

E.O.M. = efficiency of mating.

insertion or the presence of a *Ty* enhancer element. One of the His$^-$ strains contained an *HMLα* allele about 300 bases larger than the starting *HMLα* allele from JSS56-11B, consistent with the reduction of the *Ty* array to a solo LTR. The multimeric array in *hmlα-925* contains *Xho*I restriction sites within each of the two outermost LTRs (WEINSTOCK *et al.* 1990). DNA from strains containing the multimeric *Ty* array, a monomeric *Ty*, or a single LTR insertion digested with *Xho*I, yield identical *HMLα* fragments, indicating the presence of the LTR at the same position in each strain. Haploid *MAT***a** segregants with the *HMLα*:solo LTR allele had no mating defect. One His$^-$ event resulted from loss of the *hmlα-925* allele, only the *HMLα* allele from JSS222-7B (which is on a slightly smaller *Bam*HI fragment than the *HMLα* allele in the parent of MMD25, JSS56-11B) was present. A comparison of the effects of *HMLα*, *hmlα-925 Ty* multimer, *hmlα-925.Z10 Ty* monomer, and *hmlα-925.151* solo LTR alleles on budding pattern and α-factor response is given in Table 5.

Similar analyses of about 400 diploids made by rare matings (from 10 independent pools) were performed for strains carrying each of the other *hmlα:Ty* array alleles (*hmlα-712*, *hmlα-721* and *hmlα-937*). Strains in which the *hmlα::Ty* array was altered so that they were His$^-$ and lost the sterile phenotype were isolated from each of these backgrounds (Table 4). These included single *Ty* and solo LTR derivatives of *hmlα-712* and *hmlα-937*, as well as strains in which some or all of the *hmlα::Ty* allele was deleted. These rare matings also identified revertants of *hmlα-937* and *hmlα-721* that had regained the ability to mate like an **a** cell while retaining the His$^+$ phenotype (see MATERIALS AND METHODS). Two such independent strains from *hmlα-721* and six independent strains from *hmlα-937* were shown by Southern blot analysis (Figure 3 and data not shown) and meiotic analysis to be single *TyHIS3* derivatives of the *Ty* arrays at *HMLα* that no longer

resulted in a mating defect in *MAT***a** cells. These observations again suggest that the activation of *HMLα* in the *hmlα:Ty* multimeric alleles is dependent on the multimeric structure.

Expression of the *hmlα::Ty* alleles: The **a**/α-like phenotypes described above for the *MAT***a** *hmlα::Ty*-multimer mutants (α-factor resistance, nonmating, polar budding and the ability to allow sporulation of **a**/**a** diploids) reflect the expression of the α2 protein. The *MATα* locus encodes two regulatory proteins, α1 and α2 (STRATHERN, HICKS and HERSKOWITZ 1981; ASTELL *et al.* 1981). The α1 function is not required for the **a**/α phenotype . The α1 transcript is repressed in **a**/α cells by the combined action of α2 and **a**1 proteins (KLAR *et al.* 1981; NASMYTH *et al.* 1981). As expected, these mutants that have *hmlα* "on" and *MAT***a** did not express α-specific functions. To determine whether the *Ty* insertions would allow the expression of α1 from *hmlα* in the absence of **a**1/α2 protein repression, we deleted the *MAT***a** locus. Haploid cells that have the *MAT* locus deleted and *HML* and *HMR* turned off mate like **a** cells although the zygotes that are formed do not have the **a**1 function required to establish the **a**/α cell type. This is designated the **a**-like-faker or Alf phenotype (STRATHERN, HICKS and HERSKOWITZ 1981). The simple expectation for the *hmlα::Ty* strains is that if both the α1 and α2 proteins can be expressed from the activated *HML* alleles, deletion of *MAT* should result in the α phenotype. If only α2 is expressed, they should have a sterile phenotype similar to a *matα1* mutant. Surprisingly, the *hmlα::Ty matΔ* strains were able to mate well both like **a** and like α cells (bimater, strong α, strong **a**). Microscopic examination of the cells revealed that frequent mating between sibling cells occurred. Thus some of the cells in the population were able to express both the α1 and α2 functions and have the α phenotype while other cells were able to repress *HML* and had the Alf mating phenotype characteristic of *mat* deletion strains.

Phenotypic instability: To monitor the stability of the two phenotypes exhibited by these *HMLα::Ty matΔ* strains, we tested the sensitivity of single cells to the mating pheromone α-factor by pedigree analysis (HICKS and HERSKOWITZ 1976). Single cells were placed next to a patch of α-factor secreting cells (strain 294) and their growth monitored microscopically. For wild-type *HMLα MAT***a** cells (strain JSS56-11B) the cells were arrested by α-factor. Under the conditions of these pedigree analyses, **a** cells do not recover from the arrest. About one-half of the *hmlα-925 matΔ* cells were unable to divide in the presence of α-factor. The remainder divided but they frequently produced α-factor sensitive cells within the first few divisions. Changes in phenotype from α-factor resistant to α-factor sensitive were common in pedigrees of these *hmlα* "on" *matΔ* cells in contrast to the metastable expression or repression of *HMLα* seen in *sir1* mutants (PILLUS and RINE 1989). These observations suggest that *HMLα* is expressed in some cells resulting in α-factor insensitivity, but that repression of *HMLα* can occur in some cells resulting in α-factor sensitivity. Similar phenotypic instability was observed with *hmlα-937*, *hmlα-712* and *hmlα-721* strains carrying *matΔ* alleles.

The use of the *matΔ* approach provided genetic evidence that the *Ty* monomer derivatives of the *hmlα::Ty* arrays were not completely silenced. *matΔ* strains carrying the *hmlα-925.Z10 Ty* monomer allele were made and tested for mating phenotype. Rather than having only the **a**-like-faker phenotype expected if the *HMLα* locus were completely silenced, these strains showed a weak bimating phenotype (weak α, strong **a**).

Isolation of α-factor resistant mutants in a *matΔ* background: We used the bimater phenotype of *hmlα::Ty matΔ* strains as a secondary screen in the α-factor resistance selection to identify additional *Ty*-induced mutations that activate *HMLα* more than *HMR***a**. These could arise by *Ty*-induced activation of *HML* or by mutations in other genes, such as *NAT1* (MULLEN *et al.* 1989) and *ARD1* (WHITEWAY *et al.* 1987), that affect *HML* and *HMR* asymmetrically. *Ty* element transposition was induced using strain DG1002, which contains a complete deletion of *MAT*, and α-factor-resistant mutants were selected. About 3% (34/1084) of these α-factor-resistant mutants had the bimater phenotype and were analyzed further. The strength of the bimating phenotype was variable.

Several mutant classes were identified by Southern filter hybridizations (Figure 1) and phenotypic tests. Four of the mutants were caused by *Ty* element insertion in *ARD1*. Fifteen mutants contained either a *Ty* (13/15) or solo LTR (2/15) element insertion in the *NAT1-SIR2* region (Figure 1). Interestingly, all 15 of these mutants mapped within a 708-bp region that is outside the *NAT1* coding sequence, but covers the *SIR2* 5′-noncoding region and beginning of the coding sequence (data not shown). These mutations will be referred to as *nat1* alleles, but this characterization does not rule out an effect on the expression of *SIR2*. Six mutants remain unidentified.

The *HMLα* locus was disrupted in 9 of the 34 bimater strains obtained in the selection. The general structure and approximate location of these mutations was determined in a manner similar to that used for mapping the initial multimeric *Ty* insertions (WEINSTOCK *et al.* 1990; Figures 4 and 5, data not shown). One of the mutations (*hmlα-Δ2098*) was a deletion that enters *HML* from the telomere proximal end and removes the E site (data not shown). One breakpoint occurred within a 5.4-kb *Eco*R1 fragment that covers

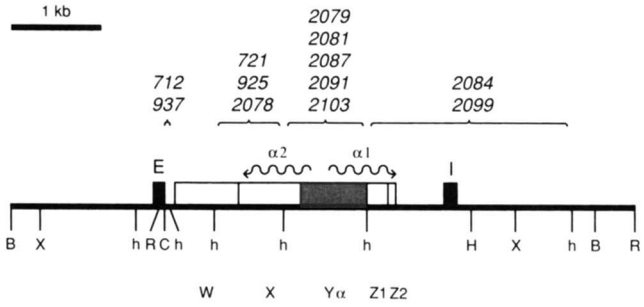

FIGURE 4.—Position of *Ty* insertions. The site of the *Ty* insertion in the various *hmlα::Ty* alleles was determined using subclones of *HMLα* as probes. B = *Bam*HI, R = *Eco*RI, X = *Xho*I, C = *Cla*I, H = *Hin*dIII, h = *Hha*I.

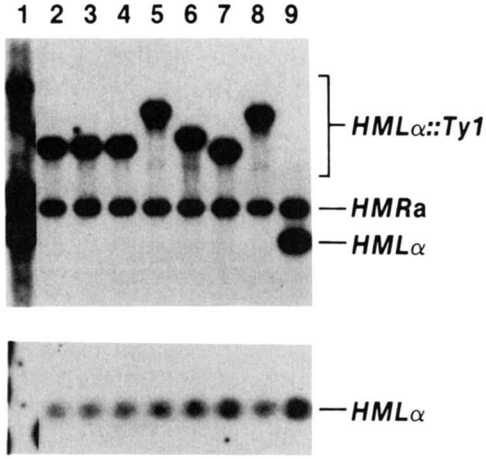

FIGURE 5.—Physical analysis of eight *hmlα::Ty1* mutants isolated in a *matΔ* strain. DNA from strains DG1031 (*hmlα-2078*, lane 1), DG1032 (*hmlα-2079*, lane 2), DG1033 (*hmlα-2081*, lane 3), DG1034 (*hmlα-2084*, lane 4), DG1035 (*hmlα-2087*, lane 5), DG1036 (*hmlα-2091*, lane 6), DG1038 (*hmlα-2099*, lane 7), DG1039 (*hmlα-2103*, lane 8), and the parental strain DG1022 (*HMLα*, lane 9) was digested with *Xba*I, separated on a 0.7% agarose gel, and then blotted to charged nylon membrane. The filter was hybridized with a radiolabeled *Bam*HI-*Hin*dIII fragment that contains the entire *HMLα* locus. The longer exposure for lane 1 is necessary because the *Ty* multimer inserted into a relatively small (1.3 kb) *Xba*I fragment from *HMLα*. This wild-type fragment is shown in the lower segment of the autoradiograph (lanes 2–9). The parts of the autoradiograph that do not contain rearranged *HMLα* fragments have been removed.

the rest of *HML*. The location of the other breakpoint was not determined.

The remaining 8 *HML* mutants are caused by *Ty* insertions (Figures 4 and 5). Southern hybridizations done on DNA digested with *Stu*I (not shown) and *Xba*I (Figure 5), which do not cleave *Ty1* elements, were useful for estimating the number of *Ty* elements inserted at *HML* and whether any of these elements were marked with *HIS3*. Three mutations were caused by multimeric *Ty* insertions that contained both marked and unmarked *Ty* elements. One of these mutations (*hmlα-2078*) is a dominant mutation with a complex *Ty* multimeric structure similar to those discussed above. The *hmlα-2078* allele caused sterility when segregated into *MAT***a** cells. We estimate that *hmlα-2078* contains a *Ty* element array about 30 kb in size. These physical analyses suggested that *hmlα-2087* contains 3 *Ty* elements and *hmlα-2103* contains 2 *Ty* elements. Five of the *hmlα::Ty* mutations were caused by either marked (*hmlα-2091*) or unmarked (*hmlα-2079*, *hmlα-2081*, *hmlα-2084* and *hmlα-2099*) monomeric *Ty* elements. These mutations caused the weak *α*; strong **a**, bimating phenotype similar to that of the *matΔ hmlα-925.Z10 Ty* monomer-bearing strain described above. These alleles would not support the sporulation of *matΔ/MAT***a** diploids. The *matΔ* mutants carrying *hmlα-2079*, *hmlα-2084*, *hmlα-2091*, and *hmlα-2099* were transformed to *MATα* (see MATERIALS AND METHODS). This allowed the *hmlα-2079*, *hmlα-2084*, *hmlα-2091* and *hmlα-2099* alleles to be crossed into *MAT***a** segregants. These *hmlα::Ty* monomer segregants showed no mating defect, and no *α*-factor resistance, consistent with the suggestion that the mutations caused by single *Ty* insertions were more subject to the silencing mechanism than the larger arrays.

DISCUSSION

Several extensive studies have been done with mutants obtained by selecting for insensitivity to the mating pheromone, *α*-factor (MANNEY and WOODS 1976; HARTWELL 1980; WHITEWAY *et al.* 1987; MULLEN *et al.* 1989). These studies have identified 12 genes that can mutate to cause *α*-factor resistance. Thus this selection provided a good demonstration of the usefulness of the *Ty* insertional mutagenesis system and it allowed us to ask whether this novel mutagen might identify additional classes of mutants. Our initial characterization of nine mutations caused by the insertion of a marked *Ty* element has identified two alleles of *ste2::TyHIS3* (the receptor for *α*-factor), one allele each of *ste5::TyHIS3*, *ste7::TyHIS3*, and *fus3::TyHIS3*, and four activated alleles of *hmlα::TyHIS3*. Using an extension of this screen we identified multiple alleles of *ard1::TyHIS3* and *nat1::TyHIS3* and eight additional alleles of *hmlα::TyHIS3*. The *hmlα::TyHIS3* class is particularly interesting because of the novel mechanism of gene activation by a retrotransposon and the phenotypic instability the mutants display. These *hmlα::Ty* mutations include alleles with multiple copies of the *TyHIS3* element.

HMLα is normally not transcribed due to repression by the *SIR/MAR* system. Five genes have been identified that encode products directly or indirectly involved in that repression. Characterization of the *HML* locus has identified two sites involved in *SIR/MAR* repression. These sites are outside of the sequences common to *HML* and *MAT*. The site to the left (centromere distal) of *HML* is called the "E" (essential) site and the site to the right of *HML* is called the "I" (important) site (FELDMAN, HICKS and BROACH

1984). Similar sites were identified at *HMR* (ABRAHAM *et al.* 1983, 1984). The action of the *SIR/MAR* regulators causes a change in the chromatin structure of the *MAT* related sequences between the E and I sites correlated with their inability to be transcribed and their inability to act as substrates for the *HO* endonuclease (KLAR, STRATHERN and HICKS 1981; NASMYTH 1982; STRATHERN *et al.* 1982). The *SIR/MAR* regulation system has been shown to be able to repress (or "silence") other genes (BRAND *et al.* 1985) placed between the E and I sites. Even a tRNA gene placed between E and I sites is subject to *SIR/MAR* regulation (SCHELL and RINE 1986). We have identified 12 *Ty* insertions that disrupt *SIR/MAR* regulation of the *HMLα* locus. GRUENSPAN and EATON (1983) characterized a *cis*-dominant mutation of *HMR***a** that resulted in the expression of that locus. Preliminary characterization of that mutation indicates that it is also the result of a *Ty* element insertion (A. KLAR personal communication).

Three distinct models can be proposed for how the *Ty* insertions cause the expression of *HML*. (1) The insertions could disrupt the E or I sites. (2) The *HML* sequences could be under the control of the *Ty* element. (3) The *Ty* element may have disrupted a domain of chromosome *III* under the control of the *SIR/MAR* regulation. Several observations argue against the first two models. Three of the alleles that yield maximal release from *SIR/MAR* repression (*hmlα-925*, *hmlα-721*, *hmlα-2078*) are clearly outside of the E and I sites. Two *Ty* array insertions (*hmlα-937* and *hmlα-712*) were near the "E" site as defined by FELDMAN, HICKS and BROACH (1984) and MAHONEY and BROACH (1989), but the position of these insertions to the right of the *Cla*I site (Figure 4) indicates that they did not directly disrupt even the expanded "E" site (the "D" region) defined by MAHONEY *et al.* (1991). MAHONEY and BROACH (1989) recently suggested that either the E or I site is sufficient for repression of *HML*. However, we describe five alleles with *hmlα::Ty* insertions between "E" and α2 that give expression of α2 and α1 without altering their position relative to the "I" site. The model that *HML* is activated because of the presence of *Ty* sequences is inconsistent with having obtained the *Ty* insertions in several positions and both orientations. *Ty* enhancer-induced gene activation usually occurs when *Ty* transcription is oriented away from the adjacent gene. The recovery of repression in revertants that retain single *Ty* insertions also indicates that *Ty* sequences are not sufficient to overcome *SIR/MAR* repression. The concept of a domain of *SIR/MAR* repression was suggested by the observation of ABRAHAM *et al.* (1984) that there was a size constraint on the distance between the "E" and "I" sites of *HMR***a** on a plasmid that could be brought under the control of the *SIR/MAR* regulation. The insertion of the *Ty* elements at *HML* may have resulted in the expression of *HML* as a consequence of violating a similar size constraint at *HML*. Physical characterization of seven of the *hml::Ty* mutants isolated in this study indicates that they were the result of the insertion of *Ty* multimers of up to 100 kbp between the E and I sites (Figures 3 and 4; WEINSTOCK *et al.* 1990). The difference between the phenotypes of the multimeric *Ty* insertions and the single *Ty* insertion derivatives indicates that *SIR/MAR* repression can tolerate at least 7-kb (but not 30-kb) insertions in *HMLα*. The concept of a size constraint is easily envisioned if there are sites on both sides of *HML* required for *SIR/MAR* repression. A circular chromosome *III* derivative that results from the fusion of *MAT* to *HMLα* deletes the distal ("E") side of *HMLα* and results in its activation (STRATHERN *et al.* 1979, 1980). Our results imply either that there is a site that provides a different function than the "E" site or that there are additional sites centromere distal to *HML* that can substitute for "E" in the assay of MAHONEY and BROACH (1989).

These *hmlα::Ty* mutations have the unusual property that not all of the cells in a clone have the same phenotype. As judged by α-factor sensitivity, the colonies are made up of some cells in which *HML* is activated and others in which it is silenced. Metastable repression of *HMLα* has been observed in *sir1* mutants (PILLUS and RINE 1989). MAHONEY *et al.* (1991) observed metastable expression of *HMLα* in strains that had the I site deleted and an additional mutation in the E site. For the *hmlα::Ty* mutants characterized here, the high rate of change from α-factor resistant to sensitive means that we cannot determine whether *HMLα* alternates between fully silenced and fully expressed or is expressed at a continuum of levels which the α-factor assay divides into two classes (GIESMAN, BEST and TATCHELL 1991; MAHONEY *et al.* 1991). We observed that some α-factor arrested *hmlα::Ty* cells were able to begin dividing again in the continued presence of α-factor. This ability to become resistant is not observed in *HMLα MAT***a** cells or in the metastable strains described by PILLUS and RINE (1989), or MAHONEY *et al.* (1991).

The results presented here demonstrate that insertion of a *Ty* element into *HML* can overcome the *SIR/MAR* repression mechanism. The activation of *HMLα* by a *Ty* insertion is an example of a more general mechanism by which transposable elements or retroviruses can alter the regulation of genes. The activation of cellular genes by retroviruses may not always reflect the activity of the enhancer elements, but may be caused by the physical separation of the activated gene from a negative regulatory site as appears to be the case for these *hml::Ty* mutations.

We thank AMAR KLAR for comments and PATTI HALL for manuscript preparation. Research was sponsored by the National

Cancer Institute, Department of Health and Human Resources (DHHS), under contract No. NO1-CO-74101 with ABL. The contents of this publication do not necessarily reflect the view or policies of the DHHS, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government. Additional support was obtained from National Science Foundation grant DMB 8812128 and from the Eloise Gerry Fellowship Fund of Sigma Delta Epsilon/Graduate Women in Science.

LITERATURE CITED

ABRAHAM, J., J. FELDMAN, K. A. NASMYTH, J. N. STRATHERN, A. J. S. KLAR, J. R. BROACH and J. B. HICKS, 1983 Sites required for position-effect regulation of mating-type information in yeast. Cold Spring Harbor Symp. Quant. Biol. **47:** 989–998.

ABRAHAM, J., K. A., NASMYTH, J. N. STRATHERN, A. J. S. KLAR and J. B. HICKS, 1984 Regulation of mating-type information in yeast. Negative control requiring sequences both 5′ and 3′ to the regulated region. J. Mol. Biol. **176:** 307–331.

ASTELL, C. R., L. AHLSTROM-JONASSON, M. SMITH, K. TATCHELL, K. A. NASMYTH and B. D. HALL, 1981 The sequence of the DNAs coding for the mating-type loci of *Saccharomyces cerevisiae*. Cell **27:** 15–23.

BOEKE, J. D., 1989 Transposable elements in *Saccharomyces cerevisiae*, in *Mobile DNA*, edited by D. BERG and M. HOWE, American Society for Microbiology. Washington, D. C.

BOEKE, J. D., D. J. GARFINKEL, C. A. STYLES and G. R. FINK, 1985 Ty elements transpose through an RNA intermediate. Cell **40:** 491–500.

BOEKE, J. D., J. TRUEHEART, G. NATSOULIS and G. R. FINK, 1987 5-Fluoroorotic acid as a selective agent in yeast molecular genetics. Methods Enzymol. **154:** 164–175.

BRAND, A. H., L. BREEDEN, J. ABRAHAM, R. STERNGLANZ and K. NASMYTH, 1985 Characterization of a "silencer" in yeast: a DNA sequence with properties opposite to those of a transcriptional enhancer. Cell **41:** 41–48.

BUCHMAN, A. R., W. J. KIMMERLY, J. RINE and R. D. KORNBERG, 1988 Two DNA-binding factors recognize specific sequences at silencers, upstream activating sequences, autonomously replicating sequences, and telomeres in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8:** 210–225.

CHALEFF, D. T., and K. TATCHELL, 1985 Molecular cloning and characterization of the *STE7* and *STE11* genes of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **5:** 1878–1886.

CHAN, R. K., and C. A. OTTE, 1982 Isolation and genetic analysis of *Saccharomyces cerevisiae* mutants supersensitive to G1 arrest by **a** factor and α factor pheromones. Mol. Cell. Biol. **2:** 11–20.

CRANDALL, M., R. EGEL and V. L. MACKAY, 1977 Physiology of mating in three yeasts, in *Advances in Microbial Physiology*, Vol. 15, edited by A. H. ROSE and D. W. TEMPEST. Academic Press, New York.

DIFFLEY, J. F. X., and B. STILLMAN, 1988 Purification of a yeast protein that binds to origins of DNA replication and a transcriptional silencer. Proc. Natl. Acad. Sci. USA **85:** 2120–2124.

ELION, E. A., J. A. BRILL and G. R. FINK, 1991 FUS3 represses CLN1 and CLN2 and in concert with KSS1 promotes signal transduction. Proc. Natl. Acad. Sci. USA **88:** 9392–9396.

ELION, E. A., P. L. GRISAFI and G. R. FINK, 1990 *FUS3* encodes a cdc2$^+$/CDC28-related kinase required for the transition from mitosis into conjugation. Cell **60:** 649–664.

FELDMAN, J. B., J. B. HICKS and J. R. BROACH, 1984 Identification of sites required for repression of a silent mating type locus in yeast. J. Mol. Biol. **178:** 815–834.

GARFINKEL, D. J., M. F. MASTRANGELO, N. J. SANDERS, B. K. SHAFER and J. N. STRATHERN, 1988 Transposon tagging using Ty elements in yeast. Genetics **128:** 95–108.

GIESMAN, D., L. BEST and K. TATCHELL, 1991 The role of RAP1 in the regulation of the *MAT*α locus. Mol. Cell. Biol. **11:** 1069–1079.

GRUENSPAN, H., and N. R. EATON, 1983 A mutation allowing expression of normally silent **a** mating-type information in *Saccharomyces cerevisiae*. Genetics **104:** 219–234.

HABER, J. E., and J. P. GEORGE, 1979 A mutation that permits the expression of normally silent copies of mating-type information in *Saccharomyces cerevisiae*. Genetics **93:** 13–35.

HARTWELL, L. H., 1980 Mutants of *Saccharomyces cerevisiae* unresponsive to cell division control by polypeptide mating omone. J. Cell Biol. **85:** 811–823.

HERSKOWITZ, I., 1989 A regulatory hierarchy for cell specialization in yeast. Nature **342:** 749–757.

HICKS, J. B., and I. HERSKOWITZ, 1976 Interconversion of yeast mating types. I. Direct observations of the action of the homothallism (*HO*) gene. Genetics **83:** 245–258.

HICKS, J. B., J. N. STRATHERN and I. HERSKOWITZ, 1977 Interconversion of yeast mating types. III. Action of the homothallism (*HO*) gene in cells homozygous for the mating type locus. Genetics **85:** 395–405.

KLAR, A. J. S., S. FOGEL and K. MACLEOD, 1979 *MAR1*–a regulator of the *HM***a** and *HM*α loci in *Saccharomyces cerevisiae*. Genetics **93:** 37–50.

KLAR, A. J. S., J. N. STRATHERN and J. B. HICKS, 1981 A position-effect control for gene transposition: state of expression of yeast mating-type genes affects their ability to switch. Cell **25:** 517–524.

KLAR, A. J. S., J. N. STRATHERN, J. R. BROACH and J. B. HICKS, 1981 Regulation of transcription in expressed and unexpressed mating type cassettes of yeast. Nature **289:** 239–244.

MAHONEY, D. J., and J. R. BROACH, 1989 The *HML* mating-type cassette of *Saccharomyces cerevisiae* is regulated by two separate but functionally equivalent silencers. Mol. Cell. Biol. **9:** 4621–4630.

MAHONEY, D. J., R. MARQUARDT, G.-J. SHEI, A. B. ROSE and J. R. BROACH, 1991 Mutations in the *HML* E silencer of *Saccharomyces cerevisiae* yield metastable inheritance of transcriptional repression. Genes Dev. **5:** 605–615.

MANNEY, T. R., and V. WOODS, 1976 Mutants of *Saccharomyces cerevisiae* resistant to the α mating-type factor. Genetics **82:** 639–644.

MULLEN, J. R., P. S. KAYNE, R. P. MOERSCHELL, S. TSUNASAWA, M. GRIBSKOV, M. COLAVITO-SHEPANSKI, M. GRUNSTEIN, F. SHERMAN and R. STERNGLANZ, 1989 Identification and characterization of genes and mutants for an *N*-terminal acetyltransferase from yeast. EMBO J. **8:** 2067–2075.

NAKAYAMA, N., A. MIYAJIMA and K. ARAI, 1985 Nucleotide sequences of STE2 and STE3, cell type-specific sterile genes from *Saccharomyces cerevisiae*. EMBO J. **4:** 2643–2648.

NASMYTH, K. A., 1982 The regulation of yeast mating-type chromatin structure by *SIR*: an action at a distance affecting both transcription and transposition. Cell **30:** 567–578.

NASMYTH, K. A., K. TATCHELL, B. D. HALL, C. ASTELL and M. SMITH, 1981 A position effect in the control of transcription at yeast mating type loci. Nature **289:** 244–250.

PILLUS, L AND J. RINE, 1989 Epigenetic inheritance of transcriptional states in *S. cerevisiae*. Cell **59:** 637–647.

RINE, J., and I. HERSKOWITZ, 1987 Four genes responsible for a position effect on expression from *HML* and *HMR* in *Saccharomyces cerevisiae*. Genetics **116:** 9–22.

RINE, J., J. N. STRATHERN, J. B. HICKS and I. HERSKOWITZ, 1979 A suppressor of mating-type locus mutations in *Saccharomyces cerevisiae*: evidence for and identification of cryptic mating-type loci. Genetics **93:** 877–901.

ROTHSTEIN, R. J., 1983 One-step gene disruption in yeast. Methods Enzymol. **101:** 202–211.

SCHELL, R., and J. RINE, 1986 A position effect on the expression

of a tRNA gene mediated by the *SIR* genes in *Saccaromyces cerevisiae*. Mol. Cell. Biol. **6:** 494–501.

SHERMAN, F., G. R. FINK and J. B. HICKS, 1986 *Laboratory Course Manual for Methods in Yeast Genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

SHORE, D., and K. NASMYTH, 1987 Purification and cloning of a DNA binding protein from yeast that binds to both silencer and activator elements. Cell **51:** 721–732.

STRATHERN, J. N., J. HICKS and I. HERSKOWITZ, 1981 Control of cell type in yeast by the mating type locus. The α1-α2 hypothesis. J. Mol. Biol. **147:** 357–372.

STRATHERN, J. N., C. S. NEWLON, I. HERSKOWITZ and J. B. HICKS, 1979 Isolation of a circular derivative of yeast chromosome *III*: implications for the mechanism of mating type interconversion. Cell **18:** 309–319.

STRATHERN, J. N., E. SPATOLA, C. MCGILL and J. B. HICKS, 1980 Structure and organization of transposable mating type cassettes in *Saccharomyces* yeasts. Proc. Natl. Acad. Sci. USA **77:** 2839–2843.

STRATHERN, J. N., A. J. S. KLAR, J. B. HICKS, J. A. ABRAHAM, J. M. IVY, K. A. NASMYTH and C. MCGILL, 1982 Homothallic switching of yeast mating type cassettes is initiated by a double-stranded cut in the *MAT* locus. Cell **31:** 183–192.

VON SOLINGEN, P., and J. B. VAN DER PLATT, 1977 Fusion of yeast spheroplasts. J. Bacteriol. **130:** 946–947.

WEINSTOCK, K. G., M. F. MASTRANGELO, T. J. BURKETT, D. J. GARFINKEL and J. N. STRATHERN, 1990 Multimeric arrays of the yeast retrotransposon Ty. Mol. Cell. Biol. **10:** 2882–2892.

WHITEWAY, M., R. FREEDMAN, S. VAN ARSDELL, J. W. SZOSTAK and J. THORNER, 1987 The yeast *ARD1* gene product is required for repression of cryptic mating-type information at the *HML* locus. Mol. Cell. Biol. **7:** 3713–3722.

Communicating editor: E. W. JONES