Positive and Negative Regulatory Elements Control Expression of the Yeast Retrotransposon Ty3

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ABSTRACT

We report the results of an analysis of Ty3 transcription and identification of Ty3 regions that mediate pheromone and mating-type regulation to coordinate its expression with the yeast life cycle. A set of strains was constructed which was isogenic except for the number of Ty3 elements, which varied from zero to three. Analysis of Ty3 expression in these strains showed that each of the three elements was transcribed and that each element was regulated. Dissection of the long terminal repeat regulatory region by Northern blot analysis of deletion mutants and reporter gene analysis showed that the upstream junction of Ty3 with flanking chromosomal sequences contained a negative control region. A 19-bp fragment (positions 56–74) containing one consensus copy and one 7 of 8-bp match to the pheromone response element (PRE) consensus was sufficient to mediate pheromone induction in either haploid cell type. Deletion of this region, however, did not abolish expression, indicating that other sequences also activate transcription. A 24-bp block immediately downstream of the PRE region contained a sequence similar to the **a**1-α2 consensus that conferred mating-type control. A single base pair mutation in the region separating the PRE and **a**1-α2 sequences blocked pheromone induction, but not mating-type control. Thus, the long terminal repeat of Ty3 is a compact, highly regulated, mobile promoter which is responsive to cell type and mating.

EXPRESSION of integrated retroviruses and retrovirus-like elements is governed by some of the most intricate regulation known in prokaryotic and eukaryotic cells. This reflects the balanced demands of host genome fitness and extra-genomic element proliferation. Examples of highly regulated expression of viruses include the tissue-specific, glucocorticoid-responsive transcription of mouse mammary tumor virus and the *trans*-activation of complex lentiviruses (MAJORS 1990). Among transposable elements, regulation of expression can be similarly stringent as evidenced by the antisense regulation of Tn*10* (SIMONS and KLECKNER 1983) and the germline-specific splicing of *P* element RNA (MULLINS, RIO and RUBIN 1989). Initial studies have suggested that expression of the *Saccharomyces cerevisiae* retrotransposon, Ty3, is also highly regulated. Its expression is coordinated with the yeast life cycle and is representative of the haploid-specific, pheromone-inducible class of genes.

Wild-type *S. cerevisiae* is a diploid organism which can grow vegetatively, or under conditions of nutrient deprivation, undergo meiosis and sporulation. Haploids are of two mating types, **a** and α, which are restricted to mitotic growth, but which are capable of conjugation to produce the **a**/α diploid cells. Cell identity, **a**, α or **a**/α, is determined by the regulated expression of genes which determine cell-type functions. These genes can be classified with regard to the cell type in which they are expressed, such as **a**- or α-, or haploid-cell specific. The differential expression of all these genes is controlled at the level of transcription by interaction of positive and negative transcription factors with promoter elements and is ultimately specified by the allele(s) **a** or α, represented at the mating-type (*MAT*) locus (reviewed by MARSH, NEINMAN and HERSKOWITZ 1991). The protein products of the *MAT***a** and the *MAT*α loci, **a**1, α1 and α2, mediate this control. In haploid α cells, α1 binds with the *MCM1* product (a serum response factor-like protein) (JARVIS, CLARK and SPRAGUE 1989) to *PQ* control elements to promote transcription of α-specific genes (BENDER and SPRAGUE 1987; JARVIS, HAGEN and SPRAGUE 1988; TAN, AMMERER and RICHMOND 1988). In haploid **a** cells, the *MCM1*-encoded protein binds to the promoter sequence, *P*, and contributes to activation of transcription of **a**-specific genes (BENDER and SPRAGUE 1987; TAN, AMMERER and RICHMOND 1988; KRONSTAD, HOLLY and MACKAY 1987). The *MCM1*-encoded product in combination with α2, the *SSN6*-encoded tetratricopeptide repeat protein (TPR) (SIKORSKI *et al.* 1990) and the β-transducin-like, *TUP1*-encoded protein (WILLIAMS and TRUMBLY 1990), represses trancription of **a**-specific genes in α cells by interaction at promoter sequences recognized by α2 (MILLER, MACKAY and NASMYTH 1985; JOHNSON and HERSKOWITZ 1985; KELEHER, GOUTTE and JOHNSON 1988; MUKAI, HARASHIMA and OSHIMA 1991; KELEHER *et al.* 1992; FUJITA *et al.*

1992). In diploid cells, the transcription of genes restricted to haploid cell types is repressed directly by binding of **a**1 and α2 homeodomain proteins (SHEPHERD *et al.* 1984; LAUGHON and SCOTT 1984; SCOTT, TAMKUN and HARTZELL 1989) in conjunction with the *TUP1*- and *SSN6*-encoded proteins (GOUTTE and JOHNSON 1988; DRANGINIS 1990; MUKAI, HARASHIMA and OSHIMA 1991; KELEHER *et al.* 1992; FUJITA *et al.* 1992) to **a**1-α2 operator sites (MILLER, MACKAY and NASMYTH 1985; SILICIANO and TATCHELL 1986) or indirectly because of reduced levels of *STE12*-encoded protein (FIELDS and HERSKOWITZ 1987).

A subset of genes encoding products required for conjugation of **a** and α cells, are regulated by exposure to pheromone [reviewed by SPRAGUE (1991)]. Interaction of the pheromone, **a**- or α-factor, with the respective receptor stimulates a signal transduction pathway. Genes regulated by α-factor induction contain copies of a 7- or 8-bp pheromone response element [PRE; (A)TGAAACA] (KRONSTAD, HOLLY and MACKAY 1987; VAN ARSDELL and THORNER 1987). The *STE12*-encoded protein has been shown to interact with this sequence and also to mediate induction (COMPANY, ADLER and ERREDE 1988; DOLAN, KIRKMAN and FIELDS 1989; ERREDE and AMMERER 1989; HWANG-SHUM *et al.* 1991). Although the PRE-dependent α-factor response in **a** cells has been more intensively studied, the PRE sequence has also been shown to mediate **a**-factor induction of the haploid-specific gene *FUS1* in α cells by **a**-factor (HAGEN, MCCAFFREY and SPRAGUE 1991). In the absence of a consensus PRE element in the promoter region of the α-cell specific gene *STE3*, however, it was argued that a *PQ* element mediated the **a**-factor response (SENGUPTA and COCHRAN 1990).

The retrotransposon, Ty*3*, is 5.4 kbp in length and consists of an internal domain flanked by 340-bp long terminal repeats (LTRs) or σ elements. It occurs in one to four copies in the typical haploid genome (CLARK *et al.* 1988). The 5.2-kb Ty*3* transcript is initiated in the upstream LTR and terminated in the downstream LTR. It contains two open reading frames, *GAG3* and *POL3*, which encode structural and catalytic proteins required for transposition (HANSEN, CHALKER and SANDMEYER 1988) and it is analogous to the retroviral genome. Haploid yeast genomes also contain about 30 copies of solo Ty*3* LTRs. Both isolated LTRs and Ty*3* elements are found within 16–19 bp of mature tRNA coding sequences [referenced in SANDMEYER *et al.* (1988)] and *de novo* integration has been found to be specific for the region of polymerase III transcription initiation (CHALKER and SANDMEYER 1992).

The results from previous studies suggested that Ty*3* expression is regulated by α-factor in *MAT***a** cells and by mating type in *MAT***a**/α cells. Northern analysis using a Ty*3* probe indicated that the 5.2-kb Ty*3* genomic RNA was among the transcripts induced by α-factor treatment of **a** cells. Ty*3* RNA was detected in haploid cells of both mating types (CLARK *et al.* 1988), but not in **a**/α cells (VAN ARSDELL, STETLER and THORNER 1987; CLARK *et al.* 1988). Because levels of some transcripts derived from solo sigma elements are responsive to these forms of regulation, the LTR was predicted to contain sequences sufficient for α-factor induction and mating-type repression (VAN ARSDELL, STETLER and THORNER 1987; CLARK *et al.* 1988). Inspection of the Ty*3* LTR revealed four sequences with similarity to the 8-bp PRE sequence, but only one was an exact match to the consensus and one was a six of eight match (VAN ARSDELL and THORNER 1987). Two regions that showed some similarity to the **a**1-α2 operator sequence which mediates mating-type control were also detected within the LTR. The functional significance of these sequences was not known. Further, whether Ty*3* has UAS elements in addition to PRE was not known.

The Ty*3* promoter by virtue of its mobility and position specificity is potentially subject to regulation in addition to mating-type and pheromone regulation. The tRNA gene initiation factor, TFIIIB, binds over the region up to approximately 40 bp upstream of the tRNA gene (KASSAVETIS *et al.* 1989). Where the Ty*3* element is in the common, transcriptionally divergent orientation with respect to the tRNA gene (SANDMEYER *et al.* 1988), this transcription factor could be bound over the upstream end of the LTR. This portion of the LTR contains potential regulatory regions for α-factor and mating-type control of Ty*3* expression, and the formation of the tRNA transcription complex might occlude binding of transcription factors to Ty*3*. Furthermore, Ty*3* expression could also be subject to the types of genomic context effects or autoregulation which is documented for retroviruses (reviewed by VARMUS and BROWN 1989; MAJORS 1990).

MATERIALS AND METHODS

General methods: Plasmid amplification and recombinant DNA manipulations were carried out in *Escherichia coli* strain HB101 using standard methods (AUSUBEL *et al.* 1992). Yeast culture conditions were as described by SHERMAN, FINK and HICKS (1986). Yeast were transformed by a modification of the LiAc method (ITO *et al.* 1983). Gene transplacement was based on the procedure described by ROTHSTEIN (1983). The mating type of several yeast strains was changed by activity of the *HO* gene product introduced on plasmid pHO-c12 (HERSKOWITZ and JENSEN 1991).

Yeast strains: The *S. cerevisiae* strains used in this work are listed in Table 1. To facilitate the analysis of transcription of Ty*3*, an isogenic set of strains containing three, two, one and zero Ty*3* elements was constructed from strain VB109. VB109 contains three Ty*3* elements designated I, II and III. Ty*3* elements in VB109 were transplaced with a 6-kbp *Xho*I Ty*3* fragment containing the 1.1-kbp *URA3*

TABLE I

***S. cerevisiae* strains used in this study**

Strain	Genotype	Source
VB109	*MAT***a** *ade2-101 his3Δ200 leu1-12 lys2-1 trp1 Δ901 ura3-52 gal3 can1-100* [Ty3I Ty3II Ty3III]	This work
VB129	*MATα* (as VB109)	This work
VB111	*MAT***a**/*α* (as VB109)	This work
VB116	*MAT***a** *ste2* (as VB109)	This work
VB117	*matax50*/*α* (as VB111)	This work
J12-8C	*MAT***a** *ade2-1 leu1-12 lys2-1 met4-1 trp5-2 ura1-1 can1-100*	KURJAN and HALL (1982)
XP635-10C	*MAT***a** *leu2-3,12 gal2 bar1*	HARTIG *et al.* (1986)
RC634	*MAT***a** *ade2 his6 met1 rme1 sst1-3 ura1*	JULIUS *et al.* (1983)
VB155	*MAT***a** [Ty3I::*URA3*] (as VB109)	This work
VB147	*MAT***a** [Ty3II::*URA3*] (as VB109)	This work
VB144	*MAT***a** [Ty3III::*URA3*] (as VB109)	This work
VB141	*MAT***a** [Ty3IΔ] (as VB109)	This work
VB159	*MAT***a** [Ty3IIΔ] (as VB109)	This work
VB160	*MAT***a** [Ty3IIIΔ] (as VB109)	This work
VB164c	*MATα* (as VB159)	This work
VB165b	*MATα* (as VB160)	This work
VB170a	*MAT***a** [Ty3I Ty3IIΔ Ty3IIIΔ] (as VB109)	This work
VB171b	*MAT***a** [Ty3IΔ Ty3II Ty3IIIΔ] (as VB109)	This work
VB173d	*MATα* (as VB171b)	This work
VB174d	*MAT***a** [Ty3IΔ Ty3IIΔ Ty3III] (as VB109)	This work
VB175a	*MATα* [(as VB174d)	This work
VB110	*MAT***a** [Ty3-null] (as VB109)	This work
VB194c	*MAT***a** [Ty3-null] (as VB109)	This work
VB198d	*MATα* [Ty3-null] (as VB109)	This work
VB114	*MATα* [Ty3-null] (as VB109)	This work
VB115	*MAT***a**/*α* [Ty3-null] (as VB109)	This work

fragment inserted at the *Sal*I site. Transformants were selected in uracil-minus medium. Southern analysis of genomic DNA using *URA3* and Ty3 probes identified strains in which the marked Ty3 had transplaced Ty3I, Ty3II or Ty3III (VB155, VB147 and VB144, respectively). Strains in which the internal domain of the Ty3::*URA3* element was deleted by recombination between the LTRs, were selected by growth on medium containing 5-fluoro-orotic acid (5-FOA) (BOEKE, LACROUTE and FINK 1984). These strains were VB141 (Ty3IΔ), VB159 (Ty3IIΔ) and VB160 (Ty3IIIΔ). The Ty3 complement of strains was determined by Southern blot analysis of genomic DNA using Ty3 and LTR probes. To derive strains containing either Ty3I, Ty3II or Ty3III only, the mating type of strains VB159 (Ty3IIΔ) and VB160 (Ty3IIIΔ) was changed from *MAT***a** to *MATα* described in General Methods) to yield strains VB164c and VB165b. The strains containing deletions of individual Ty3 elements were mated [VB141 (*MAT***a**Ty3IΔ) × VB165b (*MATα*Ty3IIIΔ); VB141 (*MAT***a**Ty3IΔ) × VB164c (*MATα*Ty3IIΔ); VB160 (*MAT***a**Ty3IIIΔ) × VB164c (*MATα*Ty3IIΔ)], and diploids were isolated, and sporulated. Colonies from individual spores were tested by Southern analysis, and strains containing a single Ty3 element (VB170a, Ty3I only; VB171b and VB173d, Ty3II only; and VB174d and VB175a, Ty3III only) were identified. The strains containing a single Ty3 element were mated (cross A: VB170a × VB173d; cross B: VB170a × 175a; cross C: VB171b × 175a), sporulated, and the Ty3-null strains were identified. These strains were VB110 and VB114 (from cross A), VB194c and VB198d (from cross C). Ty3-null strains were derived from cross B, but were not used in this work. Strains VB110 (*MAT***a**) and VB198d (*MATα*) were mated to derive the Ty3-null strain VB115 (*MAT***a**/*α*).

Plasmids: The plasmids used in this work are listed in Table 2. Two sets of plasmids were used to study Ty3 expression. In the first set, the effects on Ty3 expression of specific base pair changes in the Ty3 LTR were tested. The changes in plasmids pPK199, pVB275, pVB276, pVB186 and pVB278 were made by oligonucleotide-directed mutagenesis (KUNKEL 1985) of single-stranded DNA derived from pVB192. Plasmid pVB192 has the *Hin*dIII-*Sal*I fragment of Ty3-1, which contains the 5′ LTR of Ty3, inserted into pIBI20 (International Biotechnologies, Inc.). All changes were confirmed by DNA sequence analysis. Ty3 DNA containing the changes replaced wild-type Ty3-1 sequence in pVB191. To facilitate this cloning, plasmid vector pVB190 was constructed by digestion of YCp50 DNA with *Sal*I and *Bam*HI, incubation with the Klenow fragment of DNA polymerase and nucleotides, and religation. The resulting plasmid was deleted for the sequences between the *Sal*I and *Bam*HI restriction endonuclease sites, and both restriction sites were destroyed. The deletions in plasmids pPK199, pVB198, pVB194, pVB482, pVB485 and pVB185 were constructed by digestion of mutagenized Ty3-1 and religation of appropriate DNAs.

In the second set of experiments, Ty3 DNA fragments were inserted into the *lacZ* reporter plasmid, pLGΔ-312 (GUARENTE and MASON 1983; GUARENTE *et al.* 1984). The plasmid pLGΔ-312 consists of the *CYC1* promoter region, including UAS1 and UAS2 (centered at positions −275 and −225, respectively, relative to the transcription initiation site), fused to the gene for bacterial β-galactosidase (GUARENTE *et al.* 1984; GUARENTE and MASON 1983; LOWRY *et al.* 1983). The abilities of Ty3 LTR fragments inserted into an *Xho*I site at position −178 to influence expression of the reporter gene were determined. The pLGΔ-312-based plasmids were constructed in two ways. In plasmids pVB481

TABLE 2

***S. cerevisiae* plasmids used in this study**

Plasmid	Insert	Base plasmid	Source
pHO-c12	*HO*	YCp50	RUSSELL *et al.* (1986)
pZV63	*ste2*	pUC12	HARTIG *et al.* (1986)
196	*matax50*	YRp7	TATCHELL *et al.* (1981)
pTy3-1	Ty*3*-1	pIBI20	CLARK *et al.* (1988)
pSBS6	[sigma]	pSP64	CLARK *et al.* (1988)
YIp5			STRUHL *et al.* (1979)
pVB190	Δ(376/654)	YCp50	This work
pVB191	Ty*3*-1	pVB190	This work
pVB192	Ty*3*-1 (*Hin*dIII-*Sal* I)	pIBI20	This work
pPK199	Ty*3*Δ(−88/−11)	pVB190	This work
pVB275	Ty*3*(−13/−8)	pVB190	This work
pVB276	Ty*3*(18/23)	pVB190	This work
pVB198	Ty*3*Δ(−11/18)	pVB190	This work
pVB186	Ty*3*(76)	pVB190	This work
pVB278	Ty*3*(91/95)	pVB190	This work
pVB194	Ty*3*Δ(79/91)	pVB190	This work
pVB482	Ty*3*Δ(−11/91)	pVB190	This work
pVB485	Ty*3*Δ(19/91)	pVB190	This work
pVB185	Ty*3*Δ(76) [Δ-600/76]	pVB190	This work
pLGΔ-312	*CYC1-lac Z*		GUARENTE and MASON (1983)
pVB509	Ty*3*(−26/105)	pLGΔ-312	This work
pVB542	Ty*3*(31/105)	pLGΔ-312	This work
pVB514	Ty*3*(105/31)	pLGΔ-312	This work
pJC631	Ty*3*(M31/105)	pLGΔ-312	This work
pJC632	Ty*3*(M105/31)	pLGΔ-312	This work
pVB481	Ty*3*(56/97)	pLGΔ-312	This work
pVB480	Ty*3*(97/56)	pLGΔ-312	This work
pJC633	Ty*3*(−26/31)	pLGΔ-312	This work
pVB512	Ty*3*(31/−26)	pLGΔ-312	This work
pJC634	Ty*3*(31/56)	pLGΔ-312	This work

and pVB480, complementary oligonucleotides corresponding to the Ty*3* sequence 56/97 bp, and flanked by sequences to form *Xho*I cohesive ends, were synthesized (oligonucleotide 131, 5′-tcgaGATAAAACACATATGAAACAA-CCTTATAACAAAACGAACAACAc-3′ and oligonucleotide 132, 5′-tcgaGTGTTGTTCGTTTTGTTATAAGGTTGTTTCATATGTGTTTTATc-3′). The oligonucleotides were annealed and cloned into the *Xho*I site in pLGΔ-312. For the remainder of the pLGΔ-312-based plasmids, *Xho*I-flanked Ty*3* fragments were derived by polymerase chain reaction (PCR) (SAIKI *et al.* 1988). The oligonucleotide primer sets contained *Xho*I recognition sites near their 5′-ends [5′-GCTCCTCGAG-(22/23 nucleotides of Ty*3* sequence) 3′, *Xho*I site underlined] and Ty*3*-1 DNA was used as the template. The PCR-derived fragments were digested with *Xho*I and cloned into the *Xho*I site of pLGΔ-312. The sequence and orientation of the inserted Ty*3* DNA in pLGΔ-312 was determined by DNA sequence analysis.

DNA and RNA analysis: Yeast genomic DNA was prepared as described (BOEKE *et al.* 1985). For Southern blot analysis, DNA was digested with restriction enzymes, fractionated by electrophoresis in 1% agarose gels and transferred to nitrocellulose. Hybridization conditions were as previously described (CLARK *et al.* 1988). DNA size markers were *Hin*dIII-digested lambda DNA fragments which were 5′-end-labeled with [γ-^{32}P]ATP in a reaction with T4 polynucleotide kinase. Total cellular RNA from yeast was isolated according to the method of ELDER, LOH and DAVIS (1983). For Northern analysis, RNA was glyoxylated (MCMASTER and CARMICHAEL 1977), fractionated by electrophoresis in 1.1% agarose gels and transferred to nitrocellulose (THOMAS 1980). Hybridization was as described (CLARK *et al.* 1988). Relative levels of transcripts were determined by scanning densitometry in cases where intensities were in a linear range. RNA size markers were made by labeling an RNA size ladder purchased from Bethesda Research Laboratories with [5′-^{32}P]cytidine 3′,5′-bisphosphate using T4 RNA ligase (PIRTLE *et al.* 1980). Probes for Southern and Northern analysis were prepared by labeling gel-purified DNA fragments with [α-^{32}P]ATP by the random-primer method (FEINBERG and VOGELSTEIN 1983, 1984). The probes were: σ (LTR), 339-bp *Xho*I-*Taq*I fragment from pSBS6; Ty*3*, 1.6-kbp *Acc*I or 2.9-kbp *Bgl*II fragment from pTy*3*-1; and *URA3*, 1.1-kbp *Hin*dIII fragment from YIP5.

α-Factor and a-factor treatment: Yeast cultures were routinely grown to densities of $OD_{600} \cong 0.5$ and treated with 3.5 μM α-factor (Sigma Chemical Co.) for 20 min. For the α-factor time course experiment, cultures were treated with 3.5 μM α-factor for 0, 5, 10, 15, 30, 60, 120 and 180 min. Conditioned YPD (medium containing **a**-factor) was prepared for the **a**-factor experiments by growing RC634 cells to $OD_{600} \cong 1.1$ and removing of the cells by filtration through a 0.2-μm pore membrane. For **a**-factor treatment of VB129 (*MAT*α), cultures were grown in YPD medium to $OD_{600} \cong 0.4$–0.5. Cells were pelleted, resuspended in fresh, prewarmed, conditioned medium and grown for 60 min. Aliquots of cells were fixed and the percentage of unbudded cells was determined. For Northern blot experiments, RNA was prepared from pheromone-treated cultures at the times indicated.

MAT*a*1* disruption:** In order to test for mating-type regulation, *MATa***1* in VB111 **a**/α cells was replaced by a nonfunctional *mat***a***1* allele, *matax50*, which contains an *Xho*I linker insertion, from plasmid 196 (TATCHELL *et al.* 1981). VB111 was cotransformed with the 4.2-kbp *Hin*dIII fragment from plasmid 196 and a YCp50 derivative containing *URA3*. Transformants were selected on uracil-minus medium and screened for their ability to mate to an **a** tester strain, J12-8C. The disruption was confirmed by Southern blot analysis of genomic DNA using a *MAT***a***1* probe from plasmid 196 (data not shown). The strain containing the disrupted *MAT***a***1* allele is VB117.

β-Galactosidase assays: Assays were carried out on newly transformed VB110 (*MAT***a**), VB114 (*MAT*α), VB115 (*MAT***a**/α) and VB117 (*MAT***a**$^-$/α). Cells were grown overnight in selective medium, diluted to fresh selective medium and grown to log phase ($OD_{600} \cong 0.45$–0.6). In the α-factor experiment, cultures were treated with 3.5 μM α-factor for 60 min prior to assay. In the **a**-factor experiment, the **a**-factor was administered in the form of conditioned medium containing **a**-factor described above. Yeast transformants were grown overnight in selective medium, diluted into fresh selective medium, and grown to log phase ($OD_{600} \cong 0.45$–0.6). Cultures were diluted into prewarmed YPD to $OD_{600} \cong 0.2$ and allowed to grow to $OD_{600} \cong 0.4$. Cells were pelleted by centrifugation, resuspended at the same density in prewarmed **a**-factor-conditioned YPD medium and grown for 60 min prior to assay. β-Galactosidase assays were performed using *o*-nitrophenyl-β-galactopyranoside (ONPG) as the substrate (ROSE, CASADABAN and BOTSTEIN 1981). Assays were carried out in triplicate on two to four transformants and the results were averaged. The range of values was within 30% of the average.

RESULTS

Effect of pheromones on Ty3 RNA levels correlated with cell cycle arrest: Initial studies (VAN ARSDELL, STETLER and THORNER 1987; CLARK *et al.* 1988) showed that the levels of Ty*3* transcripts and some sigma transcripts were increased in **a** cells by α-factor treatment. Pheromones also mediate a block at G_1 in the cell cycle (MARSH, NEINMAN and HERSKOWITZ 1991). We first determined the time course of Ty*3* induction by α-factor and compared it to the time course of G_1 cell cycle arrest. In order to achieve the greatest possible sensitivity, this was carried out in a strain with a defect in the gene encoding the protease which degrades α-factor (*bar1*), as well as in a strain with the wild-type allele (*BAR1*). Logarithmically growing **a** cells (VB109) were treated with 3.5 μM α-factor and total RNA was prepared at times from 0 to 180 min. The results of Northern analysis using a Ty*3* probe are shown in Figure 1. The intensity of the band representing hybridization to the 5.2-kb Ty*3* RNA was quantitated using a scanning densitometer on an autoradiogram exposed in the linear range, and the values were normalized to the intensity of the band of *URA3*-hybridizing RNA on the same blot. The relative intensity at different experimental time points is shown in the graph in Figure 2A. The level of the 5.2-kb RNA in VB109 cells increased markedly, by 5 min and continued to increase rapidly up to 15 min after α-factor treatment was initiated (Figure 1A, lane 6; Figure 2A). After 15 min, the level of the 5.2-kb Ty*3* transcript increased more slowly and reached a maximum of 15-fold that in the untreated cells at 60 min. The level declined after this point. No significant changes occurred in the level of the 5.2-kb RNA in untreated cultures over the time course (Figure 1A, lanes 1–4; Figure 2A).

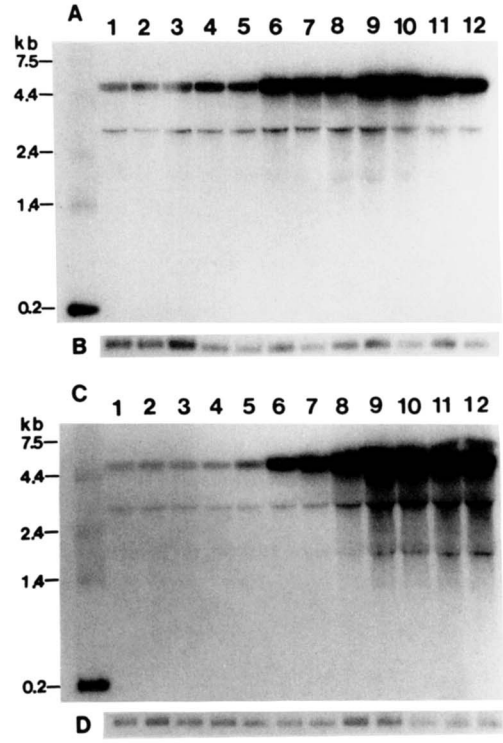

FIGURE 1.—Ty*3* RNA levels following α-factor treatment. Total RNA (20 μg/lane) from untreated or α-factor-treated cultures was subjected to Northern analysis with a radiolabeled Ty*3* probe (A) or with a radiolabeled *URA3* probe (B). Lanes 1–4, RNA from control cultures isolated at 0, 60, 120 and 180 min; lanes 5–12, RNA from α-factor treated cultures isolated at 0, 5, 10, 15, 30, 60, 120 and 180 min after α-factor addition. (A) VB109 (*BAR1*) RNA hybridized to the Ty*3* probe. (B) Hybridization of the samples used in A to the *URA3* probe. (C) XP635–10c (*bar1*) RNA hybridized to the Ty*3* probe. (D) Hybridization of the samples used in C to the *URA3* probe. Sizes of RNAs were extrapolated from RNA markers end-labeled with ^{32}P and shown in the left lane.

The α-factor is known to be degraded in cultures of **a** cells by the *BAR1*-encoded protease (HICKS and HERSKOWITZ 1976; MACKAY *et al.* 1988). To determine the kinetics of induction in the absence of α-factor turnover, the α-factor time course experiment was repeated using strain XP635-10c, which contains a mutation in *BAR1*. The results of this experiment are shown in Figure 1C and Figure 2A. The basal level of Ty*3* transcripts in this strain is comparable to that in *BAR1*-containing (VB109) cells (Figure 1, A and C, lane 1; Figure 2A). Treatment with α-factor caused a rapid increase in the level of the 5.2-kb Ty*3*-hybridizing RNA which was similar to that observed in the *BAR1*-containing strain up to 15 min. The level

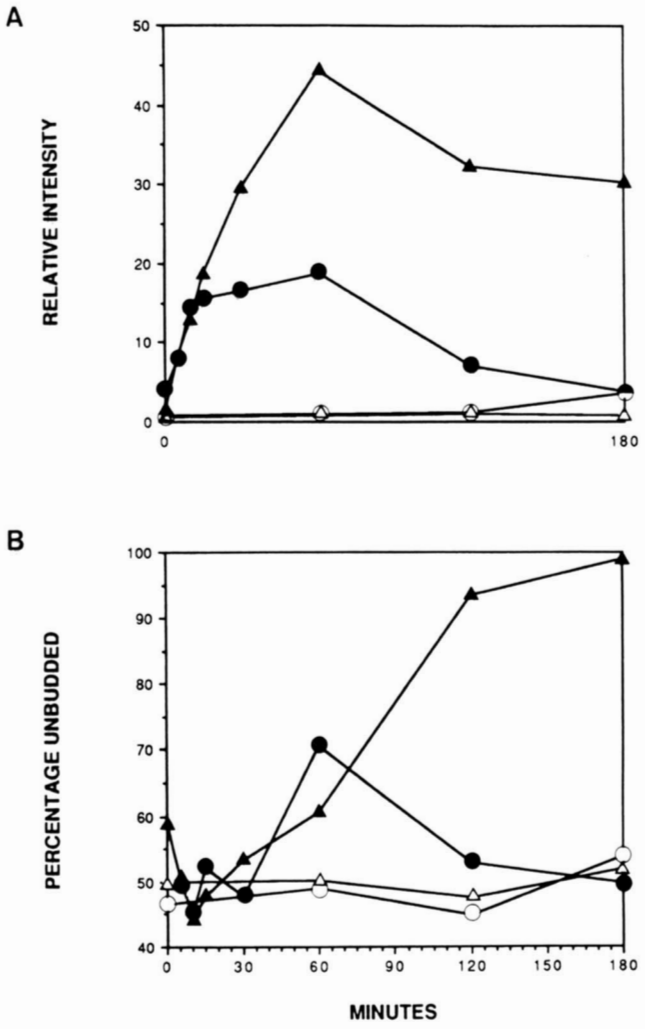

FIGURE 2.—Comparison of changes in levels of the 5.2-kb Ty*3* RNA and percentage of G_1-arrested cells over a time course following α-factor treatment. The strains were VB109 (*BAR1*), untreated (open circles) or α-factor-treated (solid circles); and XP635-10c (*bar1*), untreated (open triangles) or α-factor treated (solid triangles). (A) Levels of the 5.2-kb Ty*3* RNA from *BAR1*- and *bar1*-containing strains quantitated from Northern analysis by scanning densitometry and normalized to *URA3* RNA levels for 0–180-min time points. The autoradiographs scanned were lighter exposures than the ones shown in Figure 1, and were within a linear range. (B) Percentage unbudded cells for *BAR1*- and *bar1*-containing strains from cultures analyzed in A.

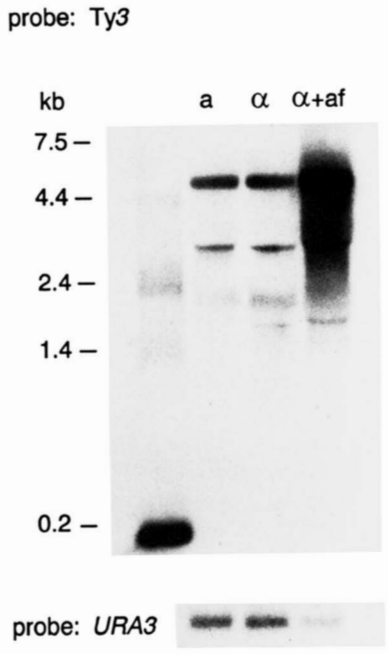

FIGURE 3.—Ty*3* RNA levels following **a**-factor treatment. Total RNA (20 μg/lane) from untreated or **a**-factor treated cultures was subjected to Northern analysis with a radiolabeled Ty*3* (top) or with a radiolabeled *URA3* probe (bottom). RNA was isolated from untreated VB109 (*MAT***a**) and VB129 (*MAT*α) cultures and **a**-factor-treated VB129 cultures. VB109 and VB129 are isogenic except for different *MAT* alleles. End-labeled RNA size markers are shown in the left lane.

in this strain, however, continued to increase to approximately 40-fold basal levels by 60 min. In contrast to the results with the *BAR1*-containing strain, the relative intensity was still approximately 30-fold basal levels at 180 min after α-factor addition. These results indicated that the decline in Ty*3* transcription in *BAR1*-containing cells can be explained by degradation of α-factor during the experiment.

The different response of *BAR1*- and *bar1*-containing strains to α-factor over the time course was also measured by monitoring cell-cycle arrest in G_1. Determination of the percentage of cells arrested in G_1 (percentage unbudded) during the α-factor treatment time course was carried out in parallel with the RNA experiments. In contrast with the RNA results, no change in the percentage of unbudded cells was detected for approximately 30 min after initiation of α-factor treatment. The percentage of unbudded *BAR1*-containing cells rose from 50% at 0–30 min to about 70% at 60 min after α-factor treatment, which correlated with maximum Ty*3* RNA levels (Figure 2). The eventual decrease in the percentage of unbudded cells parallels the decrease in 5.2-kb Ty*3* RNA. This is consistent with degradation of α-factor in the culture medium and recovery of cells from G_1 arrest. The percentage of unbudded cells in the *bar1*-containing strain increased initially in a similar manner to the *BAR1*-containing strain, but continued to increase to 98% at 180 min. This result is expected if the concentrations of α-factor are constant in the culture of the *bar1*-containing strain. The results showed that the effect of α-factor on Ty*3* RNA levels was rapid and was consistent with a continuous requirement for α-factor to maintain elevated expression of Ty*3*.

To determine if Ty*3* expression is regulated by **a**-factor as it is by α-factor, the effect of **a**-factor on Ty*3* RNA levels was measured in α cells (VB129). Northern analysis of total RNA isolated from **a**, α and α cells treated with **a**-factor-conditioned medium showed that the levels of the Ty*3* RNAs were the same in both **a** and α cells. The level of the 5.2-kb

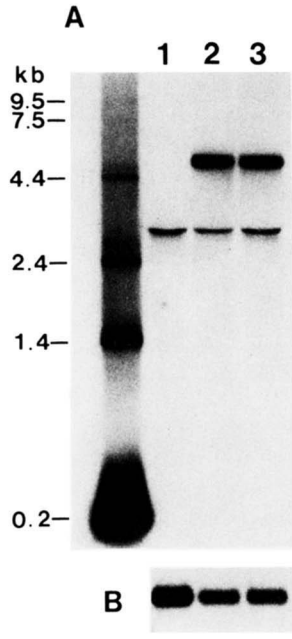

FIGURE 4.—Effect of *MAT***a***1* disruption on Ty*3* RNA levels. Northern analysis of total RNA (20 μg/lane) from **a**/α, **a**$^-$/α, and **a** cell cultures using Ty*3* and *URA3* radiolabeled probes. (A) RNA hybridized to the Ty*3* probe is from: lane 1, VB111 (*MAT***a**/α); lane 2, VB117 (*MAT***a**$^-$/α); and lane 3, VB109 (*MAT***a**). (B) RNA in blot from panel A hybridized to a *URA3* probe.

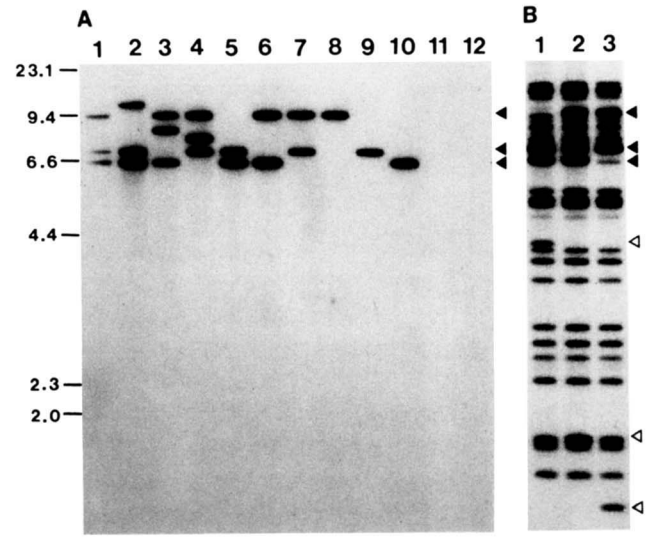

FIGURE 5.—Ty*3*-null strain construction. Southern analysis of *Eco*RI-digested genomic DNA from isogenic yeast strains used in derivation of Ty*3*-null strains. DNA was hybridized with radiolabeled Ty*3* (A) and σ (B) probes. (A) DNA is from: lane 1, VB109 (parental strain); lane 2, VB155; lane 3, VB147; lane 4, VB144; lane 5, VB141; lane 6, VB159; lane 7, VB160; lane 8, VB170a; lane 9, VB171b; lane 10, VB174d; lane 11, VB110; lane 12, VB194c. (B) DNA is from: lane 1, VB141; lane 2, VB159; lane 3, VB160. Solid arrowheads indicate positions of Ty*3* elements I, II and III. Open arrowheads indicate positions of new σ-hybridizing *Eco*RI fragments resulting from deletion of Ty*3* internal regions.

RNA was elevated in **a**-factor-treated α cells (Figure 3).

Effect of *MAT*a*1* disruption on Ty*3* RNA levels: The level of some σ-hybridizing and Ty*3*-hybridizing RNAs was decreased in **a**/α diploid cells in comparison to haploid levels (CLARK *et al.* 1988). The combined activities of the regulatory proteins **a**1 and α2, are responsible for repression of transcription in **a**/α diploids, and mutation of either the *MAT***a***1* or the *MATα2* locus results in loss of mating-type control. To determine whether Ty*3* transcripts are regulated by mating type, the effect of *mat***a***1* disruption on transcription of Ty*3* in diploid cells (**a**$^-$/α) was investigated by Northern blot analysis (Figure 4). In RNA from **a** cells, the Ty*3* probe detected major transcripts of 5.2 and 3.1 kb. Only the 5.2-kb transcript level was decreased in RNA from **a**/α cells. The levels of the 5.2 and 3.1 kb transcripts in RNA from **a**$^-$/α cells were indistinguishable from Ty*3* hybridizing transcripts in **a** cells, as expected if the 5.2-kb Ty*3* RNA is under mating-type control.

Analysis of strains containing zero to three Ty*3* elements: An isogenic set of strains containing three, two, one and zero Ty*3* elements was constructed to investigate transcription of individual Ty*3* elements, and to determine whether expression of Ty*3* was regulated by copy number. Southern blot analysis of *Eco*RI-digested VB109 DNA with a Ty*3* probe showed that VB109 contained three Ty*3* elements. These were designated Ty*3*I, Ty*3*II and Ty*3*III and corresponded to fragment sizes of 9.4, 7.4 and 6.5 kbp, respectively (Figure 5A, lane 1). Integrative transformation with a Ty*3* fragment containing *URA3* resulted in three strains which each had a *URA3* insertion in a different Ty*3* element as demonstrated by an increase in size of the Ty*3*-containing fragment (Figure 5A, lanes 2–4) and by hybridization of the fragment to a *URA3* probe (data not shown). Cells in which the internal domains of Ty*3*::*URA3* elements were deleted were selected by growth of cells on 5-FOA-containing medium. Deletion of the internal domain was presumed to occur through recombination between the LTRs consistent with the appearance of new σ-hybridizing fragments in Southern blots (Figure 5B, lanes 1–3). Strains containing a single Ty*3* element (Figure 5A, lanes 8–10) were isolated from among the progeny of Ty*3*-deletion strains. Single element strains were mated to derive the Ty*3*-null strains, which contained no Ty*3*-hybridizing sequences (Figure 5A, lanes 11–12). No obvious differences in growth rate, based on the size of colonies from germinating spores, were observed among strains containing different numbers of Ty*3* elements and Ty*3*-null strains.

The expression of integrated retroviruses can be affected by sequence variation in the promoter region, genomic location, and by copy number. Northern blot analysis with a σ probe showed that not all σ-hybridizing RNAs were regulated in the same way (CLARK *et al.* 1988). To investigate the expression of individual genomic Ty*3* elements, Northern analysis was

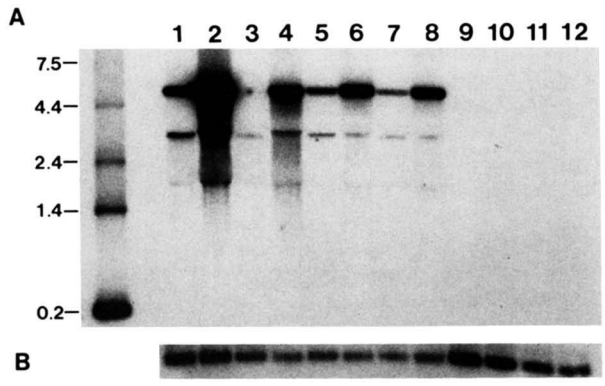

FIGURE 6.—Analysis of Ty*3* transcripts in strains containing Ty*3* elements I, II or III, and Ty*3*-null strains. Northern analysis of total RNA (20 μg/lane) using radiolabeled Ty*3* and *URA3* probes. (A) RNA is from: lane 1, VB109, untreated; lane 2, VB109, α-factor treated; lane 3, VB170a, untreated; lane 4, VB170a, α-factor treated; lane 5, VB171b, untreated; lane 6, VB171b, α-factor treated; lane 7, VB174d, untreated; lane 8, VB174d, α-factor treated; lane 9, VB110, untreated; lane 10, VB110, α-factor treated; lane 11, VB194c, untreated; lane 12, VB194c, α-factor treated. End-labeled RNA size markers are shown in the left lane. (B) RNA in the same blot as A was hybridized with a *URA3* probe.

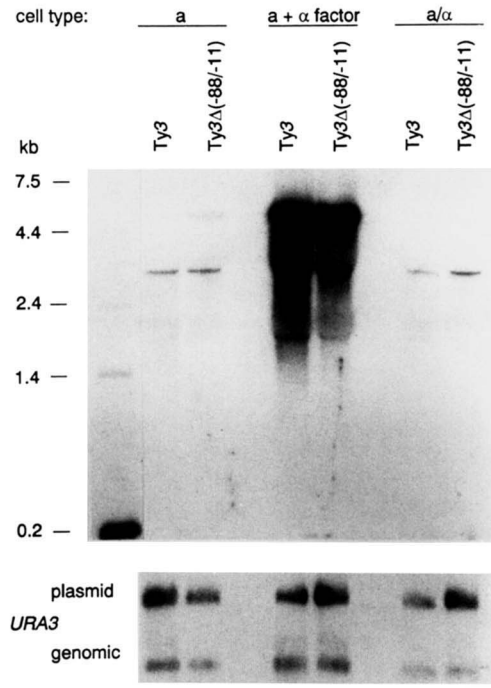

FIGURE 7.—Effect of deletion of tRNA^{Cys} adjacent to Ty*3*-1 on Ty*3* expression. Northern analysis of total RNA from **a** (VB110), α-factor-treated **a** cells and **a**/α (VB115) cells containing either plasmid pVB192 (Ty*3*-1) or pPK199 (Ty*3*Δ−88/−11). RNA was hybridized with a radiolabeled Ty*3* probe. The same blot was hybridized with a *URA3* probe. End-labeled RNA size markers are shown on the left.

performed on RNA prepared from α-factor-treated and untreated cultures of single-element strains. Ty*3* RNAs of 5.2, 3.1 and 1.8 kb were detected in the starting strain, VB109 (Figure 6, lane 1). The same RNAs were detected in each of the strains containing only one Ty*3* element (Figure 6, lanes 3, 5 and 7). Treatment with α-factor increased levels of the 5.2-kb RNA in the starting strain, as well as in the single-Ty*3*-element strains. The level of RNA in the single-element strains was reduced compared with that of the original (Figure 6, lanes 2, 4, 6 and 8). As expected, no Ty*3*-hybridizing RNAs were detected in total RNA prepared from untreated or α-factor-treated Ty*3*-null strain cultures (Figure 6, lanes 9–12). The results showed that each Ty*3* element originally present in starting strain VB109 was expressed, and was inducible by α-factor. Thus, neither position nor copy number appeared to affect expression of these Ty*3* elements.

Effect of tRNA^{Cys} on the transcription of Ty*3*-1: In the genome, Ty*3* elements are located 16–19 bp upstream of the coding sequence of mature tRNAs and therefore overlap the region occupied by TFIIIB when bound to the tRNA gene (KASSAVETIS *et al.* 1989). The proximity of these RNA polymerase III and RNA polymerase II promoters suggested that interactions between transcription factors or regulatory proteins might occur. KINSEY and SANDMEYER (1991) showed that a σ element or a Ty*3* element inserted upstream of the tRNA^{Tyr} gene, *SUP2*, resulted in a modest increase in expression of the gene. Transcription from a σ element (LTR) was also affected by the adjacent tRNA gene. In the case where transcription from σ and the tRNA was in the same orientation, expression of the tRNA gene interfered with expression from the σ promoter. The effect of the tRNA gene in the divergent, more common, orientation on Ty*3* expression was not previously tested.

To examine the effect of the divergent tRNA^{Cys} gene on Ty*3*-1 expression, the Ty*3*-null strains (VB110, *MAT***a**) and (VB115, *MAT***a**/α) were transformed with plasmid pVB191, containing the divergent Ty*3*-1 element and tRNA^{Cys} gene. RNA was prepared from untreated and α-factor treated cultures of transformed cells and subjected to Northern blot analysis (Figure 7). The Ty*3* probe detected RNAs of 5.2 and 3.1 kb in **a** cells. The regulation of plasmid-borne Ty*3* was the same as that observed with genomic Ty*3* elements. No changes were detected in regulation of the 5.2-kb Ty*3* RNA as the result of deletion of the tRNA^{Cys}. A point mutation in the *box* B promoter element of the tRNA gene, which renders the gene transcriptionally inactive, did not have a significant effect on the level of Ty*3* transcripts in untreated **a** cells (data not shown).

DNA sequence analysis of Ty*3* region −11/110: DNA sequence of the region implicated in regulation of Ty*3* transcription by α-factor and mating type is shown in Figure 8. This DNA region was analyzed for sequences similar to the PRE consensus found in the promoters of genes responsive to α-factor (KRON-

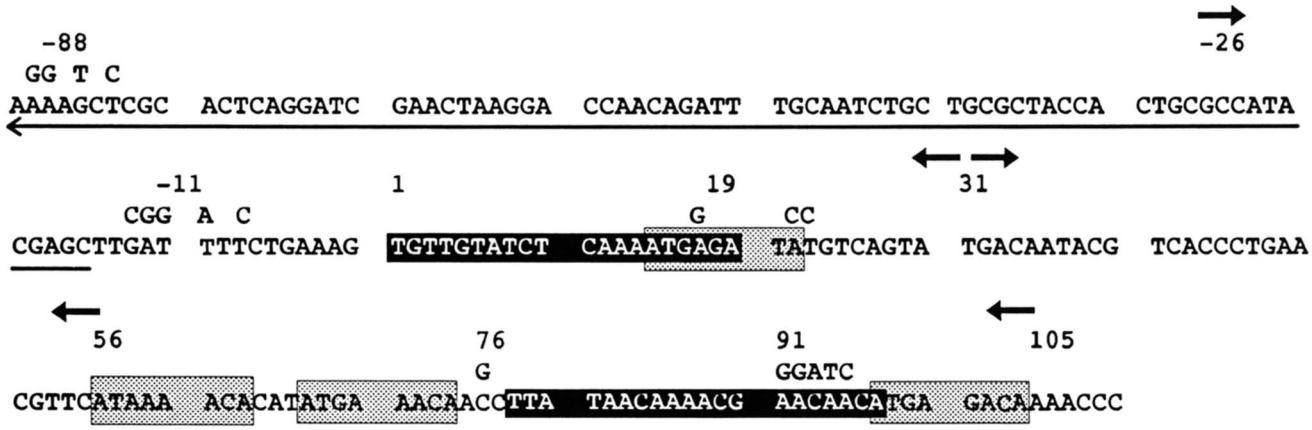

FIGURE 8.—DNA sequence of the first 110 bp of the Ty3-1 LTR and including the $tRNA^{Cys}$ located upstream of Ty3-1. The first base pair of the Ty3 LTR is designated position 1. The direction of Ty3 transcription is left to right. The mature $tRNA^{Cys}$ coding sequence is indicated by an arrow below the sequence, showing direction of transcription. Letters above the sequence show specific changes made by oligonucleotide-directed mutagenesis. The numbers above the nucleotide changes indicate the positions of restriction endonuclease cut sites. Numbers accompanied by arrows indicate the positions of the 5′ termini and orientation of oligonucleotides used to prepare Ty3 fragments by PCR for cloning into plasmid pLGΔ-312. The black boxes denote sequences that have similarity to the **a**1-α2 consensus sequence of MILLER, MACKAY and NASMYTH (1985). Gray boxes indicate sequences that are identical to, or have similarity to PRE sequences (KRONSTAD, HOLLY and MACKAY 1987; VAN ARSDELL and THORNER 1987).

STAD, HOLLY and MACKAY 1987; VAN ARSDELL and THORNER 1987). Four sequence blocks with similarity to the PRE occur in the first 100 bp of Ty3, beginning at 15, 56, 67 and 97 bp (Figure 8). These sequences were previously noted (VAN ARSDELL and THORNER 1987). The Ty3 sequence was analyzed for sequences with similarity to the **a**1-α2 mating-type control consensus of MILLER, MACKAY and NASMYTH (1985). Two regions which have 80%, and 70% similarity to the Miller consensus sequence are located between bp 1 and 20 and between bp 78 and 97, respectively (Figure 8). In the course of DNA sequence analysis we determined that the Ty3 LTR also contains one near-perfect match to the heat shock element consensus sequence, CNNGAANNTTCNNG, at position 45–58 (PELHAM and BIENZ 1982). Heat shock elements are located in the promoters of genes whose transcription is activated by exposure to high temperature. Analysis of RNA from cultures that were grown at 23° and shifted to 39 for 30 min showed that levels of Ty3 RNAs were greatly reduced following heat shock treatment (our unpublished results).

Effects of mutations and deletions within the Ty3 LTR on α-factor and mating-type regulation of Ty3: In order to identify those DNA sequences actively involved in regulation of Ty3 transcription, a set of YCp50-based plasmids was made which contained site-directed mutations and restriction fragment deletions in the Ty3 promoter. The plasmids were transformed into **a** and **a**/α Ty3-null strains for analysis. The level of the 5.2-kb Ty3 RNA from untreated, and α-factor-treated, cultures was determined by Northern blot analysis with a Ty3 probe and a compilation of the results is presented in Figure 9A. Ty3 sequences deleted or altered in these constructs are shown in Figure 8.

The level of Ty3 RNA in untreated **a** cells transformed with mutant elements Ty3Δ(−11/18), Ty3Δ(−11/91) and Ty3Δ(19/91) was elevated compared to the level in cells transformed with the Ty3-1 control plasmid (Figure 9A). The simplest interpretation is that sequences in the regions −11/18 and 19/91 negatively affect basal transcription levels. The apparent modest increase in Ty3 RNA levels in **a** cells containing Ty3(91/95) is eliminated when samples are adjusted for loading (Figure 9A, lower panel).

The level of Ty3 5.2-kb RNA in **a** cell cultures treated with α-factor was compared with the level in untreated cultures. The results show that most mutations or deletions had no effect on the increase in Ty3 RNA following α-factor treatment. In plasmid Ty3Δ(−11/91), however, the level of Ty3 RNA was the same as in untreated cultures. These results indicated that sequences within the region −11/91 were responsible for α-factor induction of Ty3. The regulation of Ty3Δ(−11/91) was restored by the inclusion of sequences −11/18 in plasmid Ty3Δ(19/91). The single C to G change in Ty3(76) also completely eliminated the effect of α-factor on Ty3 RNA levels (Figure 9A).

The same set of plasmids were also analyzed in VB115 (*MAT***a**/α) cells in order to ascertain the effect of mutations and deletions on the repression of Ty3 transcription observed in diploid cells (Figure 9A). The levels of Ty3 RNA from construct Ty3Δ(−11/91) were equivalent to those in **a** cells. Therefore, sequences conferring mating-type control were within this region. Deletion Ty3Δ(19/91) did not affect mat-

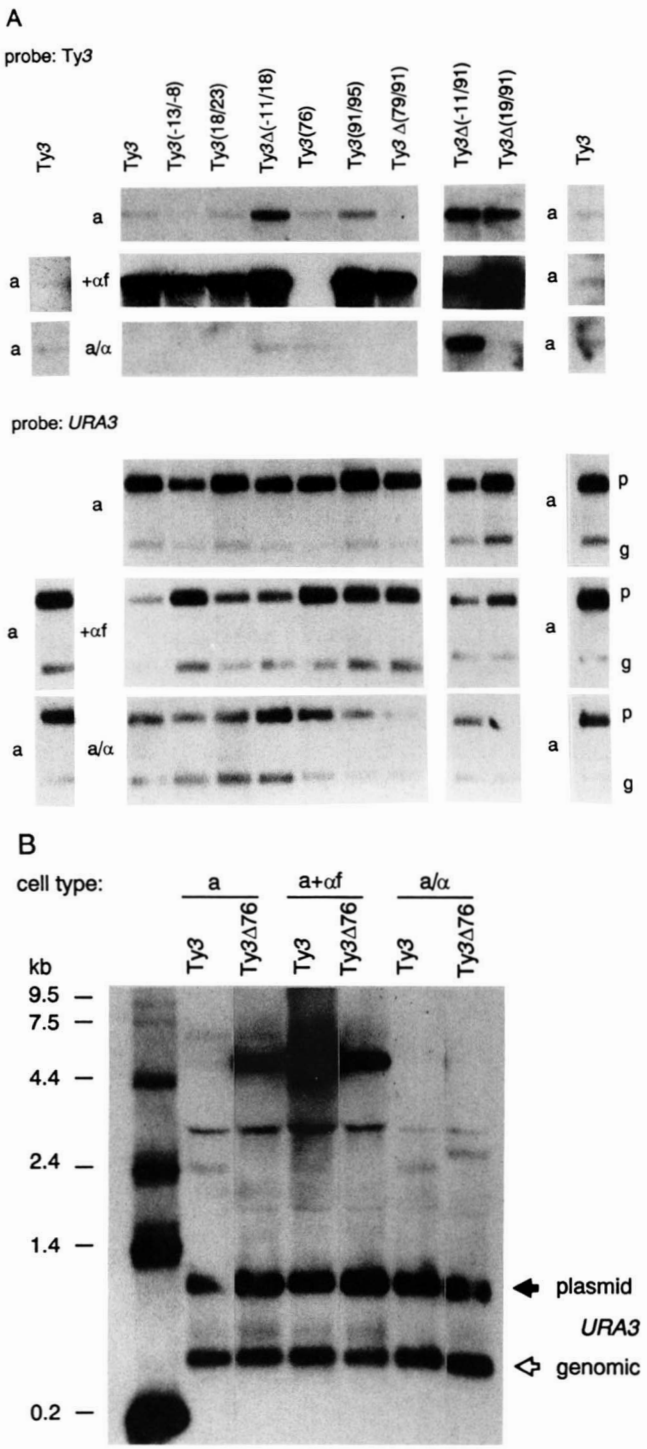

FIGURE 9.—Effect of point mutations and deletions on Ty*3* RNA levels in **a**, α-factor-treated **a** cells and **a**/α cells. Northern analysis of total RNA (20 μg/lanc) was done using a radiolabeled Ty*3* probe. RNA was from untreated and α-factor-treated **a** cells (VB110), and **a**/α (VB115) cells that were transformed with Ty*3*-containing plasmids as indicated. The same blots were hybridized with a *URA3* probe as a control. The probe detected RNA derived from the plasmid (p) and a smaller RNA from the genomic *ura3-52* locus (g). (A) To facilitate comparison of the samples, only the portion of the blot containing hybridization to the 5.2-b Ty*3* RNA or *URA3* RNAs is presented. The experiments were done in two sets as grouped. Controls from the first set are shown on the left, and controls from the second set are shown on the right. A defect in transfer of the plasmid-derived *URA3* RNA occurred for the Ty*3*Δ(19/91) sample and the intensity of hybridization in this portion of the blot does not reflect a difference in loading. (B) Autoradiogram of RNA isolated from untreated and α-factor treated **a** cells and **a**/α cells containing Ty*3* and Ty*3*Δ76 and hybridized with a mixture of Ty*3*- and *URA3*-specific probes.

ing-type control, but the mutation, Ty*3*(76), which is located within this region appeared to relieve repression. In cells containing plasmid Ty*3*(−11/18), Ty*3* RNA was also detected, but the level was lower than in **a** cells. It can not be determined from this analysis whether the level of transcription in the Ty*3*(−11/18) construct is a partial relief of mating-type repression, or the result of a general increase in transcription.

Northern analysis showed that sequence required for α-factor induction and mating-type control was contained in the region −11/91 (Figure 9A). The DNA sequence analysis predicts that deletion of the sequences upstream of bp 76, which contains three of the four PRE sequences and is within the defined region −11/91, would result in loss of α-factor inducibility, but not mating-type control. To test this prediction, plasmid pVB185 (Ty*3*Δ76) was constructed. This plasmid is deleted for all sequences 5′ to bp 76, including the tRNA gene. The plasmid was introduced into **a** (VB110) and **a**/α (VB115) cells. RNA from untreated and α-factor-treated cultures was analyzed on Northern blots (Figure 9B). In **a** cells, the level of Ty*3* RNA detected by the Ty*3* probe from Ty*3*Δ76-containing cells was elevated. The level of Ty*3* RNA from α-factor-treated cultures was similar to that of the untreated cultures. Therefore, the sequence required for α-factor response was located within the −11/76-bp region. In **a**/α cells, the level of Ty*3* RNA was less than in **a** cell cultures (Figure 9B). Taken together with previous results (Figure 9A), these data indicated that a mating-type control region occurs within 76/91.

Ty*3* LTR fragments confer regulation on a reporter gene: Deletion analysis indicated that at least two blocks of sequence were involved in α-factor induction, implicated two blocks of sequence in mating-type control, and demonstrated a negative control element in the region −11/18. To identify regions sufficient for these effects, and to determine whether the same regions mediated both α-factor and **a**-factor induction of Ty*3* expression, a series of plasmids was constructed which contained portions of Ty*3* sequences cloned into the promoter region of the *lacZ* reporter gene plasmid pLGΔ-312 (MATERIALS AND METHODS). Each plasmid was transformed into **a** (VB110), α (VB114) **a**/α (VB115) and **a**$^-$/α (VB117) cells. The β-galactosidase activity was measured in cellular extracts from pheromone-treated and un-

TABLE 3

Ty3 LTR fragments confer regulation on a reporter gene

Plasmid	ORI	a	a+αf	a+αf/a	α	α+af	α+af/α	a/α	a⁻/α	a⁻/α/a/α
pLGΔ-312		5.37	2.60	0.48	14.91	11.43	0.77	2.28	2.53	1.11
Ty3(−26/105)	+	2.37	73.00	30.80	3.94	55.73	14.14	0.25	12.58	50.32
Ty3(31/105)	+	1.94	22.14	11.41	1.61	30.01	18.64	0.17	4.42	26.00
Ty3(105/31)	−	2.42	25.43	10.51	2.16	27.34	12.66	0.10	3.60	36.00
Ty3(M31/105)	+	5.14	3.55	0.69	13.63	25.11	1.84	0.18	13.67	75.94
Ty3(M105/31)	−	6.28	76.42	12.17	13.38	150.26	11.23	0.20	15.96	79.80
Ty3(56/97)	+	18.78	247.50	13.18	11.29	102.30	9.06	0.31	56.68	182.84
Ty3(97/56)	−	25.55	307.78	12.05	16.42	129.36	7.87	0.24	39.36	164.00
Ty3(−26/31)	+	0.26	0.18	0.69	0.60	0.45	0.75	0.10	0.11	1.10
Ty3(31/−26)	−	0.34	0.21	0.62	0.49	0.46	0.94	0.13	0.08	0.65
Ty3(31/56)	+	2.61	1.26	0.48	4.70	2.21	0.47	1.34	1.06	0.79

Strains were transformed with the indicated plasmid and grown as described in MATERIALS AND METHODS. β-Galactosidase activities were determined in permeabilized cells and are reported in Miller units. Values are the means of triplicate determinations performed on two to four transformants.

treated haploid cell cultures and diploid cell cultures. The results are presented in Table 3.

The results from untreated **a** and α cells (Table 3) showed that Ty3 fragments −26/105, 31/105 and 31/56 had no effect on promoter activity. The introduction of a mutation at position 76 in the LTR sequence, Ty3(M31/105) resulted in increased expression in both **a** and α cells compared to the wild-type fragment. The effect was orientation insensitive. Ty3 fragment −26/31 caused a 15–20-fold reduction in β-galactosidase activity when inserted in either orientation. The Ty3 sequence 56/97 enhanced β-galactosidase activity approximately five fold in either orientation in the pLGΔ-312 plasmid, and also in the reporter plasmid, SX178, which lacks all *CYC1* UAS sequences (data not shown). This analysis demonstrated that the sequence −26/31 was sufficient to repress the activity of a heterologous promoter and that the sequence 56/97 contained UAS activity.

The ability of Ty3 LTR fragments to confer pheromone inducibility on the reporter gene was determined by comparison of β-galactosidase activity in treated and untreated cells. The results in **a** cells treated with α-factor (Table 3) showed that α-factor had no effect on β-galactosidase expression from pLGΔ-312 alone. The set of Ty3 sequences −26/105, 31/105 and 56/97 each caused a 10–30-fold induction of β-galactosidase activity when inserted in either orientation in the test plasmid. These regions contained PRE sequences (Figure 8). The C-G mutation at bp 76 in the complete Ty3 eliminated α-factor induction (Figure 9A). When this mutation was included in the 31/105 Ty3 sequence (M31/105), it eliminated inducibility in the natural (+) but not in the opposite orientation. Ty3 sequences −26/31, 31/−26 and 31/56 had no effect on β-galactosidase activity in α-factor-treated cultures when inserted into the promoter, although induction by −26/105 was more than that of 31/105. The effect of Ty3 fragments on β-galactosidase activity in **a**-factor-treated cultures of α cells was similar to those in α-factor-treated cultures.

The ability of Ty3 LTR fragments to confer mating-type control was tested by comparing the β-galactosidase activity in **a**/α cells and **a**⁻/α cells. The β-galactosidase activity in **a**⁻/α cells was greater than the activity in **a**/α cells for all plasmids containing the region 56/97. This effect was independent of fragment orientation. The 56/97 region, therefore, contains sequences that confer mating-type control. The base-pair change in the Ty3(M31/105) construct had no effect on mating-type control regulation. In keeping with the results in haploid cells, the activity of the construct was higher in **a**⁻/α cells than Ty3(31/105) sequences. The activity from plasmids containing Ty3 sequences −26/31 and 31/56 was insensitive to **a**/α control. This showed that these regions were not able to confer mating-type control.

The fragment −26/31 caused a reduction (16–32-fold) in β-galactosidase activity in all cell types when inserted in either orientation in the test plasmid. Thus, this negative effect was not cell type-specific. The 31/56 region, which was also negatively acting in all cell types, had a more modest effect.

DISCUSSION

Regulation of Ty3 expression by pheromones: Deletion analysis of the upstream LTR demonstrated that the sequences between positions −11 and 74 were responsible for α-factor induction of Ty3 expression. Subsequent analysis with a reporter gene construct showed that the 56/97 fragment was sufficient for pheromone induction. This region contains two copies of the PRE sequence separated by 4 bp (KRONSTAD, HOLLY and MACKAY 1987; VAN ARSDELL and THORNER 1987). The downstream copy is an exact match to the consensus sequence ATGAAACA, and the

upstream copy, ATAAAACA, is a 7 of 8-bp match. The same fragment mediated responsiveness to both **a**- and α-factor. This has also been observed for other genes, such as *FUS1*, expressed in both haploid cell types (McCaffrey *et al.* 1987; Trueheart, Boeke and Fink 1987; Hagen, McCaffrey and Sprague 1991). The Ty*3* fragment did not contain any PQ sequences which were reported to mediate **a**-factor responsiveness of *STE3* in α cells (Sengupta and Cochran 1990). Our results indicated that for Ty*3*, PREs were responsible for pheromone induction in both haploid cell types. The other two PRE-like sequences in the LTR were not able to function independently as PRE elements. The fragment −26/31, which contained a 6 of 8-bp match to the consensus, beginning at position 15, did not confer inducibility to the reporter gene in either orientation. This result could reflect the weak activity of single PRE elements (Hagen, McCaffrey and Sprague 1991; Sengupta and Cochran 1990), but could also reflect masking of activity by negatively acting sequences on the same fragment. The PRE-like sequence ATGAGACA downstream of the **a**1-α2 box at position 97 did not mediate induction of the Ty*3*Δ(76) deletion mutant.

Ty*3* contains multiple UAS regions: The fragment 56/97, which contained the PREs, exhibited UAS activity. Inclusion of this fragment in the promoter region of the reporter gene conferred inducibility in both haploid cell types. This fragment also acted as a UAS in a reporter plasmid containing no other UAS sequences (data not shown). In *FUS1*, the PRE provided the only UAS activity found in the promoter (Hagen, McCaffrey and Sprague 1991). However, two lines of evidence indicated that Ty*3* contained pheromone-independent UAS activity. Deletion of sequences between positions −11 and 91 of the LTR, which eliminated pheromone responsiveness, did not eliminate transcription. In addition, disruption of the α-factor receptor, *STE2*, in **a** cells did not affect basal levels of Ty*3* RNA (our unpublished results).

Regulation of Ty*3* expression by mating type: The Northern analysis of deletion mutants implicated two blocks of sequence −11/18 and 74/91 in mating-type control. In the reporter gene assay, only region 56/97 independently caused repression of reporter gene activity in **a**/α cells. The sequence TTATAACAAAACGAACAACA, 78/97, which has 70% similarity to the **a**1-α2 consensus sequence (Miller, MacKay and Nasmyth 1985; Siliciano and Tatchell 1986) occurs within this fragment. This repression was probably not the result of an indirect effect due to a reduction in *STE12*-encoded protein levels, because *STE12* expression is reduced 5–10-fold in diploids and reporter gene expression was 162–182-fold lower in **a**/α diploids than in $\mathbf{a}^-$/α diploids. In addition, fragments mediating pheromone induction could be separated from sequences which conferred mating type regulation. The mutation at position 76, which is adjacent to the PRE sequences and blocked PRE activity in the natural orientation, did not affect mating-type control in either orientation, and the deletion up to position 76, which eliminated three of the four PREs and inducibility, did not affect mating-type control.

Negative and positive regulatory regions are clustered: PRE sequences are clustered with other control elements in the upstream regions of some pheromone responsive genes. For example, in the *STE2* and *BAR1* genes, PREs are located adjacent to P boxes which enhance transcription (Errede and Ammerer 1989; Hwang-Shum *et al* 1991; Keleher, Passmore and Johnson 1989). In Ty*3*, the PRE sequences are adjacent to the **a**1-α2 sequence. This may reflect a general pattern since in the haploid-specific gene, *FUS3*, PRE-like sequences also flank a potential **a**1-α2 sequence. Two observations from our work suggest that the association of these control elements is important and that interactions occur between proteins complexed at these adjacent control elements. First, a mutation at bp 76 in the region separating an upstream PRE and the **a**1-α2 box blocked pheromone responsiveness. Second, deletion analysis showed that joining the Ty*3* regions upstream of position 18 to the regions downstream of position 91 produced a construct that was pheromone responsive and mating-type repressed. It is possible that the joining of these sequences created a new control region by juxtaposing an **a**1-α2-like sequence in −11/18 with a PRE-like sequence, ATGAGACA at position 97. This junction also created a tandem repeat of two PRE-like sequences, in which a 5 of 8-bp match to the PRE consensus sequence from the upstream region is directly adjacent to the PRE-like sequence from the downstream region. These two PRE-like sequences may function in pheromone induction in this construct. An alternative explanation for these results is suggested by the results of *FUS1* promoter analysis (Hagen, McCaffrey and Sprague 1991). A region was identified which did not confer pheromone responsiveness, but was capable of affecting the magnitude of the response from an adjacent control element. The Ty*3* region −11/18 could function similarly to modulate expression, although sequence comparison of the Ty*3* and *FUS1* regions did not reveal obvious similarities.

The 5′ region of the Ty*3* LTR contains a URS: Deletion of the region −11/18 resulted in an elevation of the basal level of Ty*3* transcription in **a** and **a**/α cells, and inclusion of the region −26/31 in the reporter plasmid decreased expression in all cell types and in either orientation. In its natural location in the Ty*3* LTR, this region functioned upstream of other

Ty3 regulatory elements, and it also functioned downstream of a UAS in the reporter plasmid context. These properties indicated that the Ty3 region −26/31, contained URS activity. The existence of a negative regulatory element near the upstream junction of LTR and flanking chromosomal sequences is not unprecedented. For example, in human immunodeficiency virus a negative control region is located near the 5′-end of the LTR and in murine leukemia virus, a regulatory element, in a similar location, binds a negative transcription factor (ROSEN, SODROSKI and HASELTINE 1985; FLANAGAN *et al.* 1989, 1992). Sequence comparisons of the Ty3 −26/31 region with LTR regions of Ty1 and Ty2 (δ), and Ty4 (τ) revealed that both δ and τ contained a region which matched the Ty3 sequence, TCAAAATGA, at 7 of 9 bp. The Ty3 sequence is located beginning at position 10 and the sequences in δ and τ were located in a similar position, beginning at positions 9 and 18 with respect to the 5′-end of the LTR. Although deletions of bp positions 78–151 of the 5′ δ from Ty1 and Ty2 elements showed little effect on transcription (LIAO, CLARE and FARABAUGH 1987; FARABAUGH *et al.* 1989; FULTON *et al.* 1988), it is possible that these deletions had multiple effects, some of which masked the presence of a URS.

The organization of Ty3 transcriptional control regions is different from Ty1 and Ty2: Previous analysis of transcription in the yeast retrotransposons, Ty1 and Ty2, showed that in contrast to the major regulatory regions of Ty3, regions that promote Ty1 and Ty2 transcription are located downstream of the transcription start site and within the coding region (LIAO, CLARE and FARABAUGH 1987; FARABAUGH *et al.* 1989; YU and ELDER 1989; FULTON *et al.* 1988). Solo δ sequences had little or no ability to promote transcription (LIAO, CLARE and FARABAUGH 1987; FULTON *et al.* 1988). Ty917, a Ty2 element, contained an upstream region which increased transcription, but it only functioned in combination with downstream sequences (LIAO, CLARE and FARABAUGH 1987). Repression of Ty1 and Ty2 transcription in **a**/α cells is also mediated by sequences within the internal domain (ELDER *et al.* 1981; ROEDER, ROSE and PEARLMAN 1985; FULTON *et al.* 1988).

Ty1 and Ty2 do not show the level of pheromone responsiveness displayed by Ty3. In one genetic background, pheromone treatment had no effect on the level of Ty1 transcripts, although the level of Ty3 transcripts increased dramatically (data not shown). In another genetic background, levels of transcripts from the *CYC7* promoter under regulation of an adjacent Ty1 element increased by twofold upon pheromone treatment of cells (C. ADLER and B. ERREDE, personal communication). Although *STE12*-encoded protein interacts at a Ty1 control element within the internal domain (COMPANY and ERREDE 1988; ERREDE and AMMERER 1989), this isolated region mediated less than a twofold induction of a reporter fusion (M. BAUR and B. ERREDE, personal communication). Ty2 transcription is not induced by pheromone treatment (P. FARABAUGH, personal communication).

Our analysis of Ty3 indicated that positive control elements for pheromone induction, negative control elements for mating-type control, and regions with UAS function were located upstream of the transcription start site in Ty3. In contrast to the LTR (δ) of Ty1 and Ty2 then, the LTR of Ty3 (σ) controls expression of the Ty3 genomic RNA and has organization similar to other yeast promoters.

Effects of genomic context on Ty3 regulation: We constructed an isogenic set of strains differing in their complement of Ty3 elements. These strains allowed us to examine gene expression of different Ty3 elements in various genomic contexts. Because Ty3 is most often inserted adjacent to tRNA genes in the divergent orientation (SANDMEYER *et al.* 1988), the effect of deletion of such a tRNA gene on Ty3 expression was examined. Analysis showed that the presence of the $tRNA^{Cys}$ gene had no effect on regulation by α-factor or mating type. It is possible that the location of Ty3 elements next to tRNA genes shelters the regulatory elements in Ty3 from genomic context effects. Analysis of Ty3 expression in strains which vary only in their Ty3 complement showed only modest variation in the level of expression of any of the three elements examined, and the level of Ty3 transcripts under all conditions of expression showed no evidence of feedback regulation.

In this work we present the results of our initial analysis of genomic influences on Ty3 transcription and an analysis of control regions in Ty3 which coordinate Ty3 expression with the yeast life cycle. In **a**/α cells, the common state of wild-type yeasts, transcription is repressed and transposition is restricted. Expression is low in both haploid cell types, and *de novo* transposition is not readily detectable under conditions of mitotic growth. Studies showing that Ty3 is naturally regulated by pheromones suggest that transient induction of transcription during conjugation may be sufficient to initiate Ty3 transposition in a mating pair. The effects of pheromones and cell cycle arrest on Ty3 transposition would then differ from that on Ty1, in which transposition was decreased in pheromone arrested cells (XU and BOEKE 1991). These findings suggest that Ty3 expression and transposition are naturally limited to the stage in the yeast life cycle when an increase in ploidy could potentially buffer cells from the damage incurred from genomic rearrangement. They argue that highly regulated transcription, in addition to position-specific transpo-

sition, facilitates retention of Ty*3* in the yeast genome.

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