Posttranslational Regulation of Ty1 Retrotransposition by Mitogen-Activated Protein Kinase Fus3

DARRYL CONTE, JR.,¹ ELLEN BARBER,¹ MUKTI BANERJEE,¹ DAVID J. GARFINKEL,²
AND M. JOAN CURCIO^{1*}

Molecular Genetics Program, Wadsworth Center & School of Public Health, State University of New York at Albany, Albany, New York 12201-2002,¹ and Gene Regulation and Chromosome Biology Laboratory, National Cancer Institute-Frederick Cancer Research and Development Center, ABL-Basic Research Program, Frederick, Maryland 21702-1201²

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Ty1 retrotransposons in *Saccharomyces cerevisiae* are maintained in a state of transpositional dormancy. We isolated a mutation, *rtt100-1*, that increases the transposition of genomic Ty1 elements 18- to 56-fold but has little effect on the transposition of related Ty2 elements. *rtt100-1* was shown to be a null allele of the *FUS3* gene, which encodes a haploid-specific mitogen-activated protein kinase. In *fus3* mutants, the levels of Ty1 RNA, protein synthesis, and proteolytic processing were not altered relative to those in *FUS3* strains but steady-state levels of TyA, integrase, and reverse transcriptase proteins and Ty1 cDNA were all increased. These findings suggest that Fus3 suppresses Ty1 transposition by destabilizing viruslike particle-associated proteins. The Fus3 kinase is activated through the mating-pheromone response pathway by phosphorylation at basal levels in naive cells and at enhanced levels in pheromone-treated cells. We demonstrate that suppression of Ty1 transposition in naive cells requires basal levels of Fus3 activation. Substitution of conserved amino acids required for activation of Fus3 derepressed Ty1 transposition. Moreover, epistasis analyses revealed that components of the pheromone response pathway that act upstream of Fus3, including Ste4, Ste5, Ste7, and Ste11, are required for the posttranslational suppression of Ty1 transposition by Fus3. The regulation of Ty1 transposition by Fus3 provides a haploid-specific mechanism through which environmental signals can modulate the levels of retrotransposition.

Retroviruses and endogenous retrovirus-like elements are ubiquitous in the eucaryotic kingdom and have been involved in the formation of a significant portion of the typical eucaryotic genome (45). Hence, eucaryotes have evolved many types of regulatory mechanisms to control the replication and mobility of retroelements. However, only a few host genes that control retroviral replication in vertebrates have been identified. A recent example is the mouse *Fv1* gene, whose product is derived from the *gag* domain of an endogenous retroelement and is postulated to inhibit murine leukemia virus replication by interacting with the viral capsid protein (4).

Aside from the infectivity of the retroviral particle, the steps of retrotransposition are analogous to retroviral replication. A well-characterized model system to study host regulation of retrovirus-like elements is the Ty1 retrotransposon in the yeast *Saccharomyces cerevisiae* (8, 50). Ty1 elements have two long terminal repeats (LTRs) surrounding a central region consisting of two overlapping open reading frames: TyA, which encodes a structural capsid protein, and TyB, which encodes protease (PR), integrase (IN), and reverse transcriptase (RT) activities. Replication of Ty1 occurs in the following sequence of events: a chromosomal Ty1 element is transcribed from LTR to LTR by RNA polymerase II into a terminally redundant RNA that is polyadenylated and transported to the cytoplasm. TyA and TyA-TyB fusion protein are synthesized from the full-length transcript, the latter requiring a translational frameshift event that occurs with about 3% efficiency (37).

Subsequently, both Ty1 proteins associate with the Ty1 transcript to form viruslike particles (VLPs). The VLP is the site of proteolytic maturation of TyA protein and processing of TyB into separate PR, IN, and RT proteins. Ty1 protein processing is performed by PR and is essential for transposition (57). Reverse transcription of the Ty1 RNA into a full-length linear duplex cDNA occurs in the VLP, using tRNA-Met(i) as a primer (11, 23). The Ty1 cDNA is then transported into the nucleus of the same cell, where nonhomologous integration is catalyzed by the IN protein. In the absence of IN, Ty1 cDNA is used as a template for gene conversion of preexisting Ty1 elements or LTRs (51).

Most of the 25 to 30 copies of Ty1 in the haploid yeast genome are competent for transposition and highly transcribed; however, individual elements transpose in only 1 of 10⁵ to 10⁷ cells per generation (15, 17). This “transpositional dormancy” is overcome when a Ty1 element is expressed at high levels from the inducible *GAL1* promoter, which increases the efficiency of Ty1 transposition at a posttranslational step, perhaps by overwhelming or evading a host-encoded inhibitor (30, 50). One type of inhibition may occur at the level of maturation of Ty1 proteins in the VLP, which is very inefficient in normal yeast cells and greatly enhanced by induction of *GAL1*-Ty1 expression (16). Since the formation of mature VLPs is an essential step in transposition, inhibition of VLP formation or VLP instability in normal yeast cells could account for the low levels of transposition.

The *FUS3* gene encodes a haploid-specific mitogen-activated protein (MAP) kinase that is activated through a conserved signal transduction cascade in response to mating pheromone. Pheromone secreted by cells of one of the two mating types of haploid yeast, either **a** or **α**, is bound by a serpentine

* Corresponding author. Mailing address: Molecular Genetics Program, Wadsworth Center & School of Public Health, SUNY at Albany, P.O. Box 22002, Albany, NY 12201-2002. Phone: (518) 473-6078. Fax: (518) 474-3181. E-mail: joan.curcio@wadsworth.org.

TABLE 1. Yeast strains used in this study

Strain	Genotype	Source or reference
JC297	<i>MATα ura3-167 his3Δ200 trp1::hisG Ty1his3AI-270</i>	This work
JC358	<i>MATα-ura3-cry1 ura3 his3Δ200 ade2 Ty1his3AI-270</i>	This work
JC801	<i>MATα fus3-187 ura3 his3Δ200 trp1::hisG Ty1his3AI-270</i>	This work
JC935	<i>MATα fus3-187 ura3 his3Δ200 trp1::hisG Ty1his3AI-270</i>	This work
JC953	<i>MATα fus3-187 ura3 his3Δ200 Ty1his3AI-270</i>	This work
JC980	<i>MATα fus3-187, rad52::hisG-URA3-hisG, ura3, his3Δ200, Ty1his3AI-270</i>	This work
JC984	<i>MATα fus3-187 ura3 his3Δ200 trp1::hisG ade2-101 Ty1his3AI-270</i>	This work
JC1065	<i>MATα rad52::hisG-URA3-hisG ura3-167 his3Δ200 trp1::hisG Ty1his3AI-270</i>	This work
JC515	<i>MATα ura3-167 his3Δ200 trp1::hisG ade2Δ::hisG Ty1ade2AI-515</i>	21
JC1038	<i>MATα fus3Δ::HIS3 ura3-167 his3Δ200 trp1::hisG ade2Δ::hisG Ty1ade2AI-515</i>	This work
JC560	<i>MATα ura3-167 his3Δ200 trp1::hisG Ty2his3AI-1</i>	This work
JC1510	<i>MATα fus3Δ::hisG ura3-167 his3Δ200 trp1::hisG Ty2his3AI-1</i>	This work
JC563	<i>MATα ura3-167 his3Δ200 trp1::hisG Ty2his3AI-19</i>	This work
JC1515	<i>MATα fus3Δ::hisG ura3-167 his3Δ200 trp1::hisG Ty2his3AI-19</i>	This work
L1451	<i>MATα ade1 leu2 trp5 lys9 met10 pet9</i>	G. Fink
GRY340	<i>MATα-URA3-cry1 ade2 his3Δ200 ura3-52 trp1Δ1 leu2Δ1, lys2-8Δ1 tyr7-1</i>	20
JC242	<i>MATα ura3-167 his3Δ200 Ty1his3AI-242</i>	17
JC1516	<i>MATα fus3Δ::hisG ura3-167 his3Δ200 Ty1his3AI-242</i>	This work
JC1430	<i>MATα leu2::hisG ura3-167 his3Δ200 Ty1his3AI-242</i>	This work
JC1566	<i>MATα fus3Δ::hisG leu2::hisG ura3-167 his3Δ200 Ty1his3AI-242</i>	This work
JC1634	<i>MATα ste4Δ::LEU2 leu2::hisG ura3-167 his3Δ200 Ty1his3AI-242</i>	This work
JC1635	<i>MATα ste4Δ::LEU2 fus3Δ::hisG leu2::hisG ura3-167 his3Δ200 Ty1his3AI-242</i>	This work
JC1602	<i>MATα ste5Δ102::URA3 leu2::hisG ura3-167 his3Δ200 Ty1his3AI-242</i>	This work
JC1581	<i>MATα ste5Δ102::URA3 fus3Δ::hisG leu2::hisG ura3-167 his3Δ200 Ty1his3AI-242</i>	This work
JC1651	<i>MATα ste7Δ3::URA3 leu2::hisG ura3-167 his3Δ200 Ty1his3AI-242</i>	This work
JC1653	<i>MATα ste7Δ3::URA3 fus3Δ::hisG leu2::hisG ura3-167 his3Δ200 Ty1his3AI-242</i>	This work
JC1600	<i>MATα ste11Δ6::URA3 leu2::hisG ura3-167 his3Δ200 Ty1his3AI-242</i>	This work
JC1579	<i>MATα ste11Δ6::URA3 fus3Δ::hisG ura3-167 his3Δ200 Ty1his3AI-242</i>	This work

receptor on the surface of cells of the opposite mating type. Pheromone binding triggers the activation of a heterotrimeric G-protein consisting of Ste4, Ste18, and Gpa1, which transmits the signal to the Ste20 kinase (39). In turn, Ste20 activates a MAP kinase module consisting of Ste11 (a MAP kinase kinase [MEK kinase]), Ste7 (a MAP kinase kinase [MEK]), and Fus3 (a MAP kinase) (24, 29, 44). The module is held together by interaction with the LIM domain containing protein Ste5 (12). Once activated, Fus3 phosphorylates the cyclin-dependent kinase inhibitor Far1, which mediates G₁ arrest (10, 26, 46). In addition, Fus3 activates the transcription factor Ste12, which binds to pheromone response elements of mating-specific genes and induces their transcription (26, 27). The products of mating-specific genes are required for morphological changes that lead to cell fusion and formation of the α/α diploid cell.

Multiple MAP kinase cascades function in haploid yeast cells, and the components of these pathways can be used in different MAP kinase modules to respond to different extracellular signals. For example, the signalling pathway that triggers invasive growth in haploid cells utilizes Ste20, Ste7, Ste11, and Ste12 (48). Upon activation of the invasive growth pathway, the MAP kinase Kss1 is phosphorylated by Ste7, which switches Kss1 from an inhibitor to an activator of invasive growth (13, 41). Activation of Kss1 leads to the binding of Ste12 in combination with Tec1 to genes containing a Ste12/Tec1 composite binding site, referred to as a filamentous and invasive growth response element (FRE) (3, 40, 43). Subsequent expression of genes that contain FREs is required for invasive growth. Interestingly, the promoter of Ty1 contains an FRE, and Ty1 RNA levels are decreased by mutations in components of the invasive growth pathway (3, 22, 28, 40). Recently, Madhani et al. (41) have shown that in the absence of Fus3, the invasive growth pathway MAP kinase Kss1 is inappropriately activated by the mating signalling pathway, leading to

transcription of both mating-specific genes and invasive growth genes. Hence, Fus3 has a kinase-independent function as a negative regulator of invasive growth.

Here we describe the isolation of a mutation, *rtt100-1*, that results in high levels of retrotransposition of genomic Ty1 elements. We identify the *rtt100-1* mutation as a null allele of the *FUS3* gene and show that Fus3 acts as a posttranslational step in the retrotransposition cycle after processing of Ty1 proteins in the maturing VLP. Our results indicate that the stability of Ty1 proteins in VLPs is increased in *fus3* mutants and that this increase leads to elevated levels of Ty1 cDNA and increased transpositional integration of Ty1 cDNA. Furthermore, we demonstrate that activation of Fus3 by the basal activity of the mating pathway in vegetative cells is required to fully suppress Ty1 transposition. Hence, the modulation of Ty1 transposition by Fus3 links retrotransposition to environmental signals through evolutionarily conserved signal transduction cascades. Since the mating pathway is specific to haploid cells, we propose that this regulatory mechanism is one way in which haploid yeast cells limit insertional mutagenesis by Ty1 transposition.

MATERIALS AND METHODS

Plasmids. Plasmid YIpFUS3 was generated by subcloning the 3.7-kb *Bam*HI-*Hind*III fragment from plasmid pYEE81 (provided by G. Fink) into the yeast integrating vector, pRS406. Plasmid pFUS3 Δ HIS3, containing the *HIS3* gene fused to nucleotide 584 of *FUS3* (25), was created by inserting the *Sma*I-*Eco*RV fragment of pGEM-HIS3 (16) in place of the *Hpa*I fragment in YIpFUS3. Plasmid pFUS3 Δ carries a *fus3 Δ ::hisG-URA3-hisG* allele, which has a deletion of *FUS3* nucleotides 500 to 849. The oligomer AX032 (TAGCTAGCTAGATCT GC) containing a *Bgl*II site was inserted into the *Sac*II site (nucleotide 500) of *FUS3* in YIpFUS3. The resulting plasmid was digested with *Bgl*II, and the 3.8-kb *Bam*HI-*Bgl*II fragment of pNKY51 (1) was inserted. The 6.0-kb *Bam*HI-*Eco*RI fragment of this plasmid, containing *fus3 Δ ::hisG-URA3-hisG*, was subcloned into pBST-KS(+) (Stratagene) to create pFUS3 Δ .

Yeast strains. Yeast strains are described in Table 1. Strain JC297 is a *trp1::hisG* derivative of a strain containing the genomic Ty1his3AI-270 element, which

was isolated following induction of transposition from plasmid pGTy1-H3*his3AI* (17) in strain DG733 (20). The *tp1::hisG* allele was introduced as described by Alani et al. (1). Strain JC358, which has a *URA3* gene integrated between the *MATa* and *cry1* loci, is an ascospore derived by crossing strain JC297 to strain GRY340 and then backcrossing a selected ascospore to strain JC297 twice. Strains JC297 and JC358 are congenic.

The *rtt100-1* strain 18-6A was isolated following ethyl methanesulfonate (EMS) mutagenesis of strain JC358 (see the next section for details). It was crossed to strain JC297 to obtain the *rtt100-1* ascospore JC801. Strain JC801 was backcrossed to JC358 to obtain the *rtt100-1* ascospore JC935, which was backcrossed to JC358 again to obtain *rtt100-1* ascospores JC953 and JC984.

Strains JC560 and JC563, each containing a single Ty2*his3AI* element, were isolated following induction of Ty2 transposition from a *his3AI*-marked derivative of plasmid pGTy2-917 (20) in strain JC299 (*MATa ura3-167 his3Δ200 tp1::hisG*), as described previously (17).

The *fus3Δ::hisG* allele in strains JC1510, JC1515, and JC1516 was introduced by single-step gene disruption with the 6.0-kb *Bam*HI-*Eco*RI fragment of plasmid pFUS3Δ, followed by selection for FOA⁺ derivatives of Ura⁺ transformants. The *fus3Δ::HIS3* allele in strain JC1038 was introduced by gene disruption of strain JC515 with a 1.5-kb fragment containing the 5' end of the *FUS3* open reading frame (nucleotides 1 to 584), the *HIS3* gene, and the last 55 nucleotides of the *FUS3* open reading frame (nucleotides 1008 to 1062). The *fus3Δ::HIS3* fragment was generated by PCR from the template DNA pFUS3ΔHIS3 with oligomers LFUS3INT (ATGCCAAAGAGAATTGTATAC) and R2FUSHIS (CTAACTAAATATTTTCGTTCCAAATGAGTTTCTTGAGGTCTTTTCGT CGTTAGTCTCTTGCCCTCCTCTAGTA). *rad52::hisG-URA3-hisG* strains JC980 and JC1065 were constructed by single-step gene disruption with plasmid pRAD52-GB (15).

Strains JC1430 and JC1566 are *leu2::hisG* derivatives of JC242 and JC1516, respectively, constructed with plasmid pNKY85 (provided by N. Kleckner). The *ste11Δ6::URA3* allele was introduced into JC1430 and JC1566 by using plasmid pNC202, the *ste5Δ102::URA3* allele was introduced by using plasmid pJB221, and the *ste4Δ::LEU2* allele was introduced by using plasmid pDJ154. (All three plasmids were provided by H. Madhani and G. Fink.) The *ste7Δ3::URA3* allele was introduced into strains JC1430 and JC1566 by using plasmid pNC149 (a gift of B. Errede). All transformants were verified by Southern analysis.

Media were prepared as described by Rose et al. (49). Synthetic dropout mixtures were obtained from Bio 101, Inc. Glucose (2%) was used as the carbon source except where stated.

Isolation of the *rtt100-1* mutant. Strain JC358 was mutagenized with EMS (49). EMS-treated cells, diluted and plated onto yeast extract-peptone-dextrose (YPD) medium, were grown for 5 days at 20°C. Subsequently, colonies were replicated to synthetic complete medium lacking histidine (SC-His) plates and incubated at 30°C for 3 days. Colonies with more than three His⁺ papillae (the parental strain yields zero or one papilla per colony) were clonally purified and retested. The *rtt100-1* strain 18-6A had a significant increase in the number of His⁺ papillae when pregrown at 20°C but no significant increase when grown at 30°C.

Strain 18-6A was crossed to the unmutagenized strain JC297. Following tetrad dissection, an α -factor-resistant spore with a hypertransposition phenotype, JC801, was backcrossed to strain JC358 twice. The hypertransposition phenotype segregated 2:2 in 39 of 39 tetrads from the third backcross, as indicated by growing each ascospore as a patch, approximately 2 cm², on YPD plates at 20°C for 3 to 5 days, replicating the patch to SC-His plates, and scoring His⁺ papillae after 3 days of growth at 30°C. Cosegregation of a bilateral mating defect with the Rtt⁺ phenotype was demonstrated by genetic selection for diploid formation. Strain 18-6A and all a ascospores were tested for resistance to α -factor-induced growth inhibition in a halo assay (31).

Because Ty1 and *RTT100* (*FUS3* [see below]) are haploid-specific genes whose expression is down-regulated in α/α diploids, dominance of the *rtt100-1* mutation was tested in α/α diploid strains. The *MATa-URA3* allele in the *rtt100-1* strain 18-6A and the *RTT100* strain JC358 was used to make the *MAT* loci homozygous in diploids following mating to JC297 (*RTT100*). *MATa-URA3*/ α diploids were grown on 5-fluoroorotic acid medium to select derivatives that had lost the *URA3* marker. Derivatives that became α/α at the *MAT* locus were identified by their ability to mate to a *MATa* tester strain. His⁺ prototroph formation in the *rtt100-1/RTT100* diploids was tested and compared to the *RTT100/RTT100* control strain by the patch test described above.

The genetic distance between *rtt100-1* and *pet9* was determined by tetrad analysis of a cross between strains JC801 and L1451 (provided by G. Fink). The *rtt100-1* mutation was scored in tetrads by a failure to form diploids when ascospores were mated to a known *rtt100-1* mutant of the opposite mating type. Genetic distance was calculated from the results of 26 tetrads (19 parental ditypes, 0 nonparental ditypes, and 7 tetratypes) (49). Complementation of the *rtt100-1* mutation by *FUS3* was performed by integrative transformation of strains JC953 and JC984 with plasmid YpFUS3 linearized with *Eco*RI.

DNA sequencing. A 1.2-kb PCR fragment including the *FUS3* transcription unit from positions -114 to +1062 was generated from genomic DNA of strain JC953 and cloned into plasmid pBST-KS(+). Two independent clones were sequenced with Sequenase version 2.0 (U.S. Biochemicals).

Tyhis3AI and Tyade2AI element transposition assays. The rate of transposition in strains harboring a chromosomal Ty1*his3AI*, Ty2*his3AI*, or Ty1*ade2AI*

element was determined by the maximum-likelihood method (38) as described previously (17, 21).

The frequency of Ty1*his3AI*-242 transposition in the *fus3Δ::hisG* strain JC1566 transformed with the *LEU2-CEN* vector pRS415 or with the *LEU2-CEN* plasmid pRS315 carrying *FUS3*, *fus3-T180D*, or *fus3-Y182E* alleles (plasmids pJB236, pJB290 and pJB291 respectively; provided by H. Madhani and G. Fink) was determined as follows. Four independent transformants of each plasmid were grown overnight in SC-Leu medium at 30°C and then diluted 1:100 into YPD medium and grown for 2 days at 20°C. Aliquots of each culture were plated on SC-Leu to determine the frequency of Leu⁺ cells and on SC-Leu-His to determine the frequency of His⁺ prototrophs.

Ty1 integration in the *CAN1* locus. The rate of canavanine resistance in strains JC297 and JC801 was determined by the maximum-likelihood method (38). Strains were grown overnight in YPD medium at 30°C. Approximately 500 cells were used to start 2-ml cultures of YPD, which were grown to saturation at 20°C. The titers of four cultures were determined by plating on YPD medium, and the number of canavanine-resistant (Can^r) cells was determined by plating each culture on SC-Arg+canavanine medium (49).

One Can^r colony was picked from each of 40 SC-Arg+canavanine plates, and genomic DNA was prepared. PCR analysis was performed on 250 ng of genomic DNA. Each reaction mixture also contained 10 mM Tris (pH 8.3), 50 mM KCl, 2.5 mM MgCl₂, 250 μ M each deoxynucleoside triphosphate, 2 U of *Taq* polymerase (Boehringer Mannheim), and 250 ng of each of two DNA oligomers. The reaction conditions were 30 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 1 min in a Perkin-Elmer 9600 thermocycler. *CAN1*-specific oligomers AX016 (GAAATTTTCGAGGAAGACGATAAGG) and AX019 (CAATGCTTCTA CTCCGTCTGC) were used to amplify a 2,265-bp sequence of the *CAN1* gene. DNA samples that failed to amplify the *CAN1* gene in this PCR were subjected to two additional PCR amplifications with the *CAN1*-specific oligomer AX016 or AX019 and a Ty1 LTR-specific oligomer, AX015 (GCCTTTATCAACAATGG AATCCC) to amplify a Ty1:*can1* junction fragment, if present.

Northern analysis. Northern hybridization of approximately 20 μ g of total cellular RNA from each strain was performed as described previously (19). [³²P] RNA probes were synthesized by in vitro transcription with SP6 or T7 polymerase, using the following DNA templates: plasmid pGEM-PYK1 for synthesis of antisense *PYK1* RNA; plasmid pGEM-TyA1 for synthesis of the antisense Ty1 RNA probe; pGEM-HIS3 for synthesis of a sense strand *HIS3* RNA to detect the Ty1*his3AI* transcript (16); and pSPADE2 for synthesis of a sense strand *ADE2* RNA to detect the Ty1*ade2AI* transcript (21). Quantitation of hybridization bands was performed either by scanning densitometry with a Howtek Scanmaster 3+ and Scanalytics software or by phosphorimager analysis with a Molecular Dynamics PhosphorImager and ImageQuant Software.

Immunoprecipitation of ³⁵S-labeled TyA protein. Labeling of cells with [³⁵S] Met, chasing with excess unlabeled Met, and immunoprecipitation of ³⁵S-TyA from denatured cell lysates were performed as described previously (16), except that TyA1 antiserum was used in place of VLP antiserum. TyA1 antiserum to a TrpE-TyA fusion protein, which was synthesized from a pATH-TrpE expression vector carrying nucleotides 1032 to 1317 of Ty1-H3, was raised in rabbits as described by Garfinkel et al. (33).

Western analysis of Ty1 proteins. Strains were grown overnight at 30°C in YPD broth and then diluted 20-fold in fresh YPD broth and grown for 4 h at 20°C. Total-cell proteins were extracted from a 1-ml sample of each culture by trichloroacetic acid precipitation (55). Equal amounts of protein (as determined by Coomassie blue staining) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to a polyvinylidene difluoride membrane (Bio-Rad) with a Semi-dry Horizontal Transfer Cell (Bio-Rad), and probed with TyA1 antibody.

VLP-enriched cell fractions were obtained as follows. Yeast strains were grown in YPD broth overnight at 30°C. The cultures were diluted 50-fold in YPD broth and grown overnight at 20°C and then diluted 5-fold in YPD broth again and grown at 20°C to an optical density at 600 nm of 1.25. VLPs from a 1-liter culture were fractionated on a sucrose step gradient as described by Eichinger and Boeke (23) with the following modifications. Cells were resuspended and disrupted in buffer A (10 mM HEPES-KOH [pH 7.8], 15 mM KCl, 5 mM EDTA) containing 30 mM dithiothreitol, 2 mM phenylmethylsulfonyl fluoride, 10 μ g of aprotinin per ml, and 5 μ g of leupeptin per ml. The lysate was centrifuged, and the resulting supernatant was loaded onto a sucrose step gradient composed of 5 ml of 75% sucrose in buffer A, 3 ml of 45% sucrose in buffer A, 5 ml of 30% sucrose in buffer A, and 12 ml of 20% sucrose in buffer A. The gradients were centrifuged for 3 h at 25,000 rpm in a Beckman SW28 rotor at 4°C, and the 45% layer containing VLPs was removed (~5 ml) and diluted twofold in buffer A. VLP-enriched fractions were concentrated by ultracentrifugation in a Beckman NV190 rotor at 55,000 rpm for 30 min at 4°C. Pellets were resuspended in 500 μ l of VLP storage buffer (20 mM HEPES-KOH [pH 7.6], 140 mM KCl, 1 mM EDTA, 10% glycerol). The protein concentration of VLP-enriched fractions was determined with the Bio-Rad Dc protein assay system. Protein samples (25 μ g) were subject to SDS-PAGE, transferred to a polyvinylidene difluoride membrane, and probed with TyA1, TyB2 (57), and TyB8 (33) antisera. Immune complexes were detected with the enhanced chemiluminescence Western blot detection system (Amersham). Scanning densitometry was performed with a Howtek Scanmaster 3+ and Scanalytics software.

Southern blot analysis of total cellular Ty1 DNA. For each strain analyzed, a single colony grown on YPD medium at 20°C was used to inoculate a 10-ml culture of YPD broth and the cultures were grown to saturation at 20°C. Spheroplasts were prepared from each culture by incubating yeast cells for 30 min at 37°C in 0.9 M sorbitol–0.1 M EDTA containing 1 μ l of β -mercaptoethanol per ml and 0.6 mg of Zymolyase 100T (Seikagru) per ml, and then total cellular DNA was prepared with the G NOME kit (Bio 101). DNA samples were digested with *Pvu*II, subjected to electrophoresis on a 1% SeaKem Gold (FMC) agarose gel, and transferred to a Hybond N+ nylon membrane (Amersham) in 0.4 N NaOH. The filter was probed with a 934-bp *Hind*III–*Bgl*II fragment of Ty1-H3 (nucleotides 4627 to 5561 [6]), radiolabeled with High Prime DNA labeling mix (Boehringer Mannheim) as specified by the manufacturer. Hybridization bands were quantitated by scanning densitometry with a Howtek Scanmaster 3+ and ScanaLytics software.

RESULTS

Isolation of hypertransposition mutants. Transposition of a single genomic Ty1 element under the control of its native promoter can be detected when the element is marked with the retrotransposition indicator gene *his3AI* (17) or *ade2AI* (21). These indicator genes contain an artificial intron (AI) cloned in an antisense orientation into the coding sequences of *HIS3* or *ADE2*, respectively. The marker genes are contained in the 3' untranslated region of a genomic Ty1 element, such that the transcriptional orientations of Ty1 and *his3AI* (or *ade2AI*) are opposed. In this orientation, the antisense strand of the marker gene is transcribed as part of Ty1 RNA and the AI can be spliced from the Ty1 transcript. Use of the spliced Ty1 RNA as a template for cDNA synthesis and genomic integration results in the formation of a Ty1 element containing a functional *HIS3* (or *ADE2*) gene. Hence, the rate of transposition of a Ty1*his3AI* or Ty1*ade2AI* element is proportional to the rate of His⁺ or Ade⁺ prototroph formation, respectively.

To identify genes that are involved in the maintenance of Ty1 transpositional dormancy, we performed a genetic screen for mutations that increased the transposition of the Ty1*his3AI*-270 element in strain JC358. Yeast cells were mutagenized with EMS, and colonies were screened to identify *rtt* (regulator of Ty1 transposition) mutants with an elevated level of His⁺ papillation following nonselective growth on YPD at 20°C, a permissive temperature for retrotransposition. Chromosomal Ty1 element transposition is inhibited at 30°C, but His⁺ prototrophs can arise by homologous recombination of a Ty1*HIS3* cDNA with Ty1 elements in the genome. To eliminate mutants with increased homologous recombination of Ty1*HIS3* cDNA, only those that increased His⁺ papillation at 20°C but not at 30°C were selected.

One mutation identified, *rtt100-1*, segregated 2:2 through three backcrosses to a congenic wild-type strain containing Ty1*his3AI*-270. The *Rtt*[−] phenotype is recessive in α/α diploids that are heterozygous for *rtt100-1*. The transposition rate of Ty1*his3AI*-270 was determined in the wild-type strain JC297 and compared to that in JC953, a congenic *rtt100-1* ascospore from the third backcross of the 18-6A mutant. The *rtt100-1* mutant had a 39-fold increase in the rate of His⁺ prototroph formation per generation (Table 2).

Identification of *RTT100* as *FUS3*. A second phenotype was found to cosegregate with the hypertransposition phenotype in tetrad analysis of *rtt100-1* mutants: failure to form diploids in bilateral mutant crosses. Microscopic analysis of *rtt100-1* $\times$ *rtt100-1* mating mixtures showed defects in conjugation typical of cell fusion mutants, which were not observed in *rtt100-1* $\times$ *RTT100* mixtures. In addition, haploid *MATa* *rtt100-1* strains were found to be resistant to α -factor-induced arrest of growth. The bilateral mating defect and the failure to undergo pheromone-induced arrest in *rtt100-1* mutants were reminiscent of phenotypes observed in *fus3* and *far1* mutants (10, 25). The *rtt100-1* mutation was mapped and shown to be located 13.5

TABLE 2. Effect of *fus3* mutations on Ty1 transposition

Strain	Relevant genotype	Marked Ty element	Transposition rate	Fold effect of <i>fus3</i> mutation
JC297	WT ^c	Ty1 <i>his3AI</i> -270	$(3.1 \pm 1.4) \times 10^{-7a}$	
JC953	<i>rtt100-1</i> (<i>fus3-187</i>)	Ty1 <i>his3AI</i> -270	$(1.2 \pm 0.5) \times 10^{-5a}$	39
JC242	WT	Ty1 <i>his3AI</i> -242	$(2.1 \pm 0.8) \times 10^{-7a}$	
JC1516	<i>fus3Δ::hisG</i>	Ty1 <i>his3AI</i> -242	$(3.7 \pm 1.4) \times 10^{-6a}$	18
JC515	WT	Ty1 <i>ade2AI</i> -515	$(3.2 \pm 1.2) \times 10^{-8b}$	
JC1038	<i>fus3Δ::HIS3</i>	Ty1 <i>ade2AI</i> -515	$(1.8 \pm 0.6) \times 10^{-6b}$	56
JC1065	<i>rad52</i>	Ty1 <i>his3AI</i> -270	$(4.8 \pm 1.7) \times 10^{-6a}$	
JC980	<i>fus3-187 rad52</i>	Ty1 <i>his3AI</i> -270	$(1.7 \pm 0.6) \times 10^{-5a}$	4
JC560	WT	Ty2 <i>his3AI</i> -1	$(7.1 \pm 4.2) \times 10^{-8a}$	
JC1515	<i>fus3Δ::hisG</i>	Ty2 <i>his3AI</i> -1	$(3.7 \pm 2.6) \times 10^{-7a}$	5
JC563	WT	Ty2 <i>his3AI</i> -19	$(3.3 \pm 1.6) \times 10^{-8a}$	
JC1510	<i>fus3Δ::hisG</i>	Ty2 <i>his3AI</i> -19	$(9.2 \pm 6.0) \times 10^{-8a}$	3

^a The rate of His⁺ prototroph formation per cell per generation.

^b The rate of Ade⁺ prototroph formation per cell per generation.

^c WT, wild type.

centimorgans (cM) from *pet9* on chromosome II, which is the same distance reported for *FUS3* (25).

To test whether *FUS3* could complement the *rtt100-1* allele, the *FUS3* gene on a *URA3*-based integrating vector was introduced into *rtt100-1* strains JC953 and JC984. *Ura*⁺ transformants of each strain showed complementation of the bilateral mating defect and reversion of the hypertransposition defect. Therefore, the *fus3* coding region in strain JC953 was sequenced to identify the *rtt100-1* mutation. A transition from G to A was found at position 561, which introduces a nonsense codon in place of the codon for Trp187, resulting in truncation of nearly half of the 353-amino-acid Fus3 protein. Herein, the *rtt100-1* mutation is referred to as *fus3-187*. To confirm that hypertransposition of Ty1 is a null phenotype of *fus3* mutants, a *fus3Δ::HIS3* allele, in which sequences coding for the carboxy-terminal 158 amino acids of Fus3 are deleted, was introduced into a strain that harbors the chromosomal element, Ty1*ade2AI*-515. The rate of Ade⁺ prototroph formation in the *fus3Δ::HIS3* strain JC1038 was 56-fold higher than that in the isogenic *FUS3* strain JC515 (Table 2). We also tested a *fus3Δ::hisG* allele in strain JC242, which contains a different Ty1*his3AI* element from the one in the mutagenesis strains JC297 and JC358. The *fus3Δ::hisG* allele has a deletion of sequences encoding amino acids 167 to 284 of Fus3. The rate of His⁺ prototroph formation was increased 18-fold in the *fus3Δ::hisG* strain JC1516 relative to the isogenic wild-type strain, JC242 (Table 2). Both the *fus3Δ::HIS3* and *fus3Δ::hisG* strains were mating defective in bilateral mutant crosses, and *fus3Δ::hisG* has been shown to be defective in blocking Kss1-dependent mating activity relative to the catalytic-site mutation, *fus3-K42R* (41). In summary, *fus3* null mutants have an 18- to 56-fold increase in the rate of transposition of a single chromosomal Ty1 element.

We further determined whether the frequency of transposition of a Ty1*ade2AI* element expressed from the *GAL1* promoter was affected by a *fus3* null mutation. After galactose induction of plasmid pGTy1*ade2AI* in strain JC418 for 24 h (21), the frequency of Ade⁺ prototrophs was 3.1×10^{-3} , whereas two independent *fus3Δ::HIS3* derivatives had frequencies of 1.4×10^{-3} and 5.2×10^{-3} Ade⁺ prototrophs. Perhaps

TABLE 3. De novo transposition into the *CAN1* locus

Strain	Relevant genotype	No. of Ty1 insertions/no. of Can ^r colonies analyzed	Rate of Can ^r /generation	Rate of Ty1 insertion into <i>CAN1</i> /generation
JC297	<i>FUS3</i>	1/40	$(3.9 \pm 1.1) \times 10^{-7}$	$(9.8 \pm 2.8) \times 10^{-9}$
JC801	<i>fus3-187</i>	8/40	$(2.8 \pm 0.9) \times 10^{-7}$	$(5.6 \pm 2.0) \times 10^{-8}$

these data, which demonstrate that pGTy1 transposition is not increased in *fus3Δ* mutants, indicate that the high levels of Ty1 protein processing and VLP accumulation in cells expressing pGTy1 (16, 32) are able to overcome the negative effect of *Fus3* on transposition.

The effect of *fus3* is specific for the Ty1 retrotransposon. Regulatory mechanisms for one type of retrotransposon may be shared by other classes of transposons. In *Drosophila virilis*, a host-mediated hybrid dysgenesis that causes mobility of the *Ulysses* retrotransposon also mobilizes four other classes of transposons (47). To determine if *FUS3* regulates other retrotransposons in yeast, we examined the effect of a *fus3* mutation on the transposition of Ty2 elements, which are highly related to Ty1 elements. The *fus3Δ::hisG* allele was introduced into two strains containing independent chromosomal Ty2*his3AI* elements, and the rate of His⁺ prototroph formation was determined (Table 2). Compared to isogenic wild-type strains, transposition of the Ty2*his3AI*-1 element was increased approximately fivefold and that of the Ty2*his3AI*-19 element was increased threefold in *fus3Δ* derivatives. Hence, *fus3Δ* causes a small increase in Ty2 retrotransposition, but the effect is significantly lower than the 18- to 56-fold effect of *fus3* null mutations on Ty1 transposition. In contrast, expression of a pGTy1 element causes *trans* activation of both genomic Ty1*his3AI* and Ty2*his3AI* elements to equivalent levels (18). Our findings indicate that expression of a pGTy1 element and expression of the *fus3* mutation overcome transpositional dormancy by different mechanisms, the latter of which is more specific for Ty1 retrotransposition.

Transpositional integration of Ty1 cDNA is increased in *fus3-187* mutants. Ty1 cDNA can enter the genome by two distinct pathways. The transposition pathway, mediated by IN, results in integration of Ty1 at nonhomologous sites and generates a 5-bp target duplication. A second pathway is the *RAD52*-dependent gene conversion of Ty1 elements or LTRs in the genome. Although cDNA-mediated gene conversion is normally rare, it is stimulated by conditions that negatively affect Ty1 IN-mediated integration (51). Either pathway could be stimulated in *fus3* mutants, giving rise to increased His⁺ prototroph formation. To determine if the increase in His⁺ prototroph formation in *fus3* mutants is dependent on *RAD52*, a *rad52* mutation was introduced into the *fus3-187* strain JC953 and the *FUS3* strain JC297. The 39-fold increase in transposition in a *fus3-187* mutant was not abolished by introduction of the *rad52* mutation but instead was slightly enhanced, resulting in a 55-fold increase in transposition relative to the wild-type strain JC297 (Table 2). The data demonstrate that His⁺ prototroph formation in a *fus3-187* strain is *RAD52* independent. The *rad52* mutation by itself increases Ty1*his3AI*-270 transposition about 15-fold, in agreement with previous results (15), and transposition in the *fus3-187 rad52* double mutant is about 4-fold higher than in the *rad52* mutant. The findings clearly argue that the *RAD52*-independent process of transposition is responsible for the increase in His⁺ prototrophs in *fus3* mutants.

To confirm our hypothesis that transposition into de novo targets is increased, we quantitated transposition events that inactivate a selectable target gene, *CAN1*, in the *FUS3* strain, JC297 and a congenic *fus3-187* strain, JC801 (Table 3). Loss-of-function mutations in the *CAN1* gene result in resistance of the strain to growth inhibition in the presence of canavanine. The rate of canavanine resistance was similar in the *FUS3* and *fus3-187* strains, but the fraction of independent Can^r colonies that sustained a Ty1 insertion into the *CAN1* gene was increased eightfold in the *fus3-187* strain relative to the *FUS3* strain (Table 3), yielding an overall increase of sixfold in the rate of Ty1 insertion into *CAN1* in the *fus3-187* mutant. The relatively smaller increase in Ty1 transposition into *CAN1* compared to the increase in Ty1*his3AI* and Ty1*ade2AI* transposition into the whole genome is probably because open reading frames of RNA polymerase II genes are relatively poor targets for Ty1 transposition (36). Taken together, the data show that the *fus3-187* mutation significantly increases de novo integration of Ty1.

The Ty1 RNA level is not increased in *fus3* mutants. To understand how *FUS3* regulates Ty1 transposition in normal cells, we analyzed different steps in the retrotransposition process in *fus3* mutants. Hypertransposition could be caused by an increase in the amount of Ty1 RNA; therefore, RNA prepared from cells in the log phase of growth was analyzed by Northern blotting to quantitate Ty1*his3AI*-270 and Ty1*ade2AI*-515 transcripts from individual elements and from Ty1 elements collectively, relative to a control transcript, *PYK1* RNA (Fig. 1). The level of Ty1*his3AI* RNA in the *fus3-187* strain JC953 was not significantly increased relative to that in the congenic *FUS3* strain JC297. Similarly, the level of Ty1*ade2AI* RNA in the *fus3Δ* strain JC1038 was not increased relative to that in the isogenic *FUS3* strain JC515. Total Ty1 RNA levels were also not significantly altered as a result of either the *fus3-187* or *fus3Δ::HIS3* mutation. Although both minor increases and decreases in the level of RNA from individual Ty1 elements occur in *fus3* mutants relative to isogenic or congenic *FUS3* strains, a consistent increase in the Ty1 RNA level that could explain the 18- to 56-fold increase in Ty1 transposition has not been observed. In addition, Northern analysis of RNA from cells in the stationary phase showed no change in the levels of Ty1, Ty1*his3AI* or Ty1*ade2AI* RNA (normalized to *PYK1* RNA) in *fus3* mutants relative to *FUS3* strains (data not shown). The data indicate that derepression of Ty1 transposition in *fus3* mutants occurs at the posttranscriptional level.

Another possibility to explain the increased His⁺ prototroph

FIG. 1. Northern analysis of total RNA from strains JC297 (*FUS3*) and JC953 (*fus3-187*), containing the Ty1*his3AI*-270 element, and strains JC515 (*FUS3*) and JC1038 (*fus3Δ*), containing the Ty1*ade2AI*-515 element. The riboprobes used to detect Ty1*his3AI* and Ty1*ade2AI* RNA (top), total Ty1 RNA (center), and *PYK1* RNA (bottom) are described in Materials and Methods.

FIG. 2. Posttranslational regulation of Ty1 by *FUS3*. (A) JC297 (*FUS3*), JC953 (*fus3-187*), JC515 (*FUS3*), and JC1038 (*fus3Δ*) cells were labeled with [35 S]Met for 1 h and immunoprecipitated with TyA1 antiserum. The control (c) contains no antiserum. The products were analyzed by SDS-PAGE (10% polyacrylamide). (B) Cells were pulse-labeled with [35 S]Met for 15 min and then chased with excess unlabeled methionine. Samples were harvested 15 min (lanes c, 1, and 5), 1 h (lanes 2 and 6), 8 h (lanes 3 and 7), and 20 h (lanes 4 and 8) after the addition of [35 S]Met, and denatured cell lysates were incubated with preimmune serum (lane c) or TyA1 antiserum (lanes 1 to 8). The products (p190-TyA/TyB, p58-TyA, and p54-TyA) were analyzed by SDS-PAGE (10% polyacrylamide). (C) Total cellular proteins isolated from JC297 (*FUS3*), JC953 (*fus3-187*), and JC801 (*fus3-187*) were separated by SDS-PAGE (10% polyacrylamide) and subjected to Western analysis with TyA1 antiserum.

formation in *fus3* mutants is that the efficiency of splicing of the AI from Ty1*his3AI* RNA is increased. Therefore, relative levels of spliced Ty1*HIS3* RNA and unspliced Ty1*his3AI* were measured in the *fus3-187* strain 18-6A and the unmutagenized strain JC358 by quantitative reverse transcription-PCR with primers in *HIS3* that flank the AI (51). No difference in the ratio of Ty1*HIS3* RNA to Ty1*his3AI* RNA was observed (data not shown). Hence, the increased rate of Ty1*his3AI* transposition in *fus3-187* strains cannot be accounted for by an increase in the efficiency of splicing of the AI.

Posttranslational regulation of Ty1 transposition by *FUS3*. To determine if the efficiency of Ty1 protein synthesis is increased in *fus3* mutants, cells were labeled for 1 h with [35 S]Met and TyA protein was immunoprecipitated from denatured cell lysates (Fig. 2A). The levels of unprocessed p58-TyA and processed p54-TyA were equivalent in the *fus3-187* strain JC953 and the wild-type strain JC297. Similarly, the levels of pulse-labeled TyA proteins in the *fus3Δ* strain JC1038 showed no difference from those in the isogenic *FUS3* strain, JC515. Therefore, the higher levels of retrotransposition in *fus3* mutants cannot be attributed to an increase in the synthesis of TyA protein.

To determine if the rate of Ty1 protein processing is increased in *fus3* mutants, TyA proteins were immunoprecipitated from lysates of cells pulse-labeled with [35 S]Met for 15 min and chased with excess unlabeled Met for up to 20 h (Fig. 2B). Processing of 35 S-labeled p58-TyA to 35 S-labeled p54-TyA was relatively inefficient in both wild-type and mutant strains, which has been shown previously for wild-type strains (16). An increase in TyA processing was not detected in the *fus3-187* strain JC953 relative to the *FUS3* strain JC297. Furthermore, both 35 S-labeled p58-TyA and 35 S-labeled p190-TyA/TyB were detected after a 15-min pulse-labeling step in this analysis (Fig. 2B, lanes 1 and 5), and there was no difference in their level of synthesis in *fus3-187* and *FUS3* strains. In summary, these results indicate that in *fus3* null mutants, the 18- to 56-fold increase in Ty1 transposition is not a result of a detectable in-

crease in the level of Ty1 RNA, Ty1 protein synthesis, or proteolytic processing of TyA.

A small increase in the ratio of p54-TyA to p58-TyA was noticed in *fus3* mutants after 8 h (Fig. 2B, compare lanes 3 and 7), suggesting that the p54-TyA protein might be stabilized. Therefore, Western blot analysis of total-cell extracts was performed with TyA1 antibody (Fig. 2C). Steady-state levels of TyA proteins were increased in *fus3-187* mutants JC953 and JC801 relative to the congenic *FUS3* strain JC297, and this difference was attributable mostly to an increase in the level of the processed form of TyA, p54. The increase in the level of p54 was also observed in the *fus3Δ* mutants JC1038 and JC1516 relative to the isogenic *FUS3* strains (data not shown). Since neither an increase in TyA protein synthesis nor an increase in TyA processing was detected, the data argue that the stability of p54-TyA is increased in *fus3* mutants.

The levels of VLP-associated Ty1 proteins and total Ty1 cDNA are increased in *fus3* mutants. The preferential accumulation of the processed form of TyA in *fus3* mutants suggested that Ty1 proteins were stabilized after the formation of VLPs. Therefore, a cell fractionation procedure developed to isolate VLPs from cells expressing a *GALI*:Ty1 element (transposition-induced cells) was used to obtain a cytoplasmic fraction that was enriched for VLPs from uninduced cells. Although Ty1 proteins are not a major component of this sucrose gradient fraction prepared from uninduced cells as they are when prepared from transposition-induced cells (33), the VLPs were significantly concentrated to allow both TyA and TyB proteins to be detected by Western analysis (Fig. 3). Equal amounts (25 μ g) of protein from VLP-enriched cell fractions were analyzed by immunoblotting with TyA1, TyB2, and TyB8 antibodies against the TyA, IN, and RT domains, respectively (Fig. 3). TyA proteins p58 and p54 were five- to sevenfold

FIG. 3. Ty1-VLP synthesis is increased in *fus3* mutants. Western blot analysis of Ty1-VLPs from *FUS3* (+) and *fus3* (-) strains. VLPs were separated by SDS-PAGE (7.5% polyacrylamide). Western blots were probed with B2 antiserum to detect p90-IN (top panel). Size labels on the left indicate the position of p90-IN, which appears as a doublet (9), p190-TyA/TyB, and processing intermediates (p140 and p160). The band at 110 kDa is attributable to nonspecific proteolysis of TyB (33). The relative levels of p90 doublet in *fus3* strains compared to that in the isogenic or congenic *FUS3* strains, determined by scanning densitometry, are as follows: JC953/JC297, 43; JC1038/JC515, 38; JC1516/JC242, 21. B8 antiserum (center panel) was used to detect p60-RT. The p60 band from JC953 is visible in darker exposures of the Western blot. The relative levels of p60 in *fus3* strains compared to the isogenic or congenic *FUS3* strain are as follows: JC953/JC297, >14; JC1038/JC515, 200; JC1516/JC242, 66. TyA1 antiserum (bottom panel) detected p58 and p54 TyA. Lower-molecular-weight species recognized by this antiserum are probably a result of nonspecific proteolysis. The relative levels of p58 and p54 in *fus3* strains compared to the isogenic or congenic *FUS3* strain are as follows: JC953/JC297, 7; JC1038/JC515, 5; JC1516/JC242, 5.

FIG. 4. The level of Ty1 cDNA is increased in *fus3* mutants. (Top) Structure of the unintegrated linear Ty1 cDNA and location of relevant *PvuII* (P), *HindIII* (H), and *BglII* (B) sites. The *HindIII*-*BglII* Ty1 DNA fragment that was used as a probe in Southern analysis is indicated by the horizontal line marked with asterisks. The 1.9-kb Ty1 cDNA band generated by *PvuII* digestion is represented as the line with crossbars. (Bottom) Total cellular DNA from two independent cultures of *FUS3* (+) and *fus3* (-) strains or an *spt3-101* control strain (lane C) grown at 20°C was digested with *PvuII*, subjected to electrophoresis on a 1% agarose gel, and hybridized to the Ty1 DNA probe described above. Random-primer labeled 1-kb marker DNA (lane M) was included on the agarose gel as a size control. The 1.9-kb Ty1 cDNA band, indicated by the arrow, and three Ty1-genomic DNA junction fragments, indicated by solid circles, were quantitated by scanning densitometry. The ratio of the 1.9-kb Ty1 cDNA band to each of the three Ty1-genomic DNA junction bands was determined, and the three ratios from each of two independent DNA samples were averaged for each strain analyzed. The average ratio of Ty1 cDNA fragment to Ty1-genomic DNA fragment was 0.4 for strain JC297, 2.7 for strain JC953, 0.3 for strain JC515, and 3.1 for strain JC1038.

more abundant in VLP fractions from the *fus3* strains JC953, JC1038, and JC1516 than in those from the *FUS3* control strains JC297, JC515, and JC242, respectively (Fig. 3, bottom panel). A more significant increase in the level of processed TyB proteins from VLP-enriched fractions of *fus3* strains was observed. The level of p90-IN was 21- to 43-fold higher (Fig. 3, top panel) and the level of p60-RT was >14- to 200-fold higher (Fig. 3, center panel) in *fus3* mutants than in *FUS3* strains. In addition, processing intermediates p140, p160, and p190 are detected in strain JC515 by the B2 antibody, and the level of these proteins in VLP fractions is also increased in the isogenic *fus3Δ* strain JC1038 (Fig. 3, top panel). In summary, TyA and TyB proteins that cosediment with VLPs are significantly more abundant in *fus3* mutants. Taken together, the data argue that the stability of VLP-associated proteins is enhanced in *fus3* mutants. Alternatively, the assembly of Ty1 proteins into VLPs

could be increased in *fus3* mutants, which might enhance their stability relative to Ty1 proteins not associated with VLPs.

If the increase in TyA and TyB levels leads to an increase in activity that is responsible for the elevated rates of transposition in *fus3* strains, the amount of Ty1 cDNA might be increased in *fus3* mutants. The Ty1 cDNA present in VLP-enriched fractions was difficult to detect and varied between different preparations of the same strain (data not shown). Therefore, we compared the amount of unintegrated, linear Ty1 cDNA present in total cellular DNA in *FUS3* strains to that in total cellular DNA in *fus3* strains. DNA was prepared from each strain grown at 20°C, digested with *PvuII*, and subjected to Southern hybridization with a probe derived from the 3' end of Ty1 (Fig. 4). The probe detected a 1.9-kb fragment of unintegrated Ty1 cDNA from the *PvuII* site at nucleotide 3944 of Ty1 to the 3' end. In addition, larger *PvuII* fragments representing the junction between the 3' end of integrated Ty1 elements and genomic DNA were detected.

In an *spt3-101* strain in which Ty1 elements are not expressed (54), the 1.9-kb Ty1 cDNA fragment was not detected, even when the autoradiogram was overexposed, indicating that its appearance is dependent on Ty1 expression (Fig. 4). The level of the Ty1 cDNA band was quantitated relative to the levels of three different Ty1-genomic DNA junction fragments in *FUS3* and *fus3* strains (Fig. 4). The *fus3-187* strain JC953 had a 7-fold increase in Ty1 cDNA relative to the congenic *FUS3* strain JC297, and the *fus3Δ* strain JC1038 had a 10-fold increase relative to the isogenic *FUS3* strain JC515. The data demonstrate that higher levels of Ty1 transposition are correlated with an increase in total cellular Ty1 cDNA levels in *fus3* mutants.

Fus3 is activated by the mating-pheromone response pathway in vegetative cells. In response to mating pheromone, Fus3 is rapidly phosphorylated on threonine-180 and tyrosine-182 residues, and this phosphorylation is required for activation of Fus3 to mediate the mating response (34). During vegetative growth, Fus3 kinase is partially activated by the basal activity of the mating-pheromone response pathway (52). We investigated the role of activation of Fus3 in suppression of Ty1 transposition in vegetative cells by examining the ability of phosphorylation site substitution alleles *fus3-T180D* and *fus3-Y182E* to suppress transposition in a *fus3Δ* strain. The *fus3-T180D* and *fus3-Y182E* alleles are completely unable to rescue the mating defect of a *fus3Δ kss1Δ* double mutant (data not shown), indicating that these alleles cannot be activated. The frequency of His⁺ prototroph formation from the genomic Ty1*his3AI-242* element was determined for each allele (Table 4). *FUS3* lowered transposition 74-fold compared to the *LEU2-CEN* vector pRS415 alone, while the *fus3-T180D* and *fus3-Y182E* alleles

TABLE 4. Complementation of Ty1*his3AI-242* hypertransposition in a *fus3Δ* strain

Allele ^a	Transposition frequency ^b	Relative decrease in transposition frequency ^c
Vector only	2.0×10^{-4}	1
<i>FUS3</i>	2.7×10^{-6}	74
<i>fus3-T180D</i>	1.3×10^{-5}	15
<i>fus3-Y182E</i>	2.2×10^{-5}	9

^a Contained on a *LEU2-CEN* vector.

^b Number of His⁺ Leu⁺ prototrophs as a fraction of the total number of Leu⁺ colonies.

^c Relative to the transposition frequency in the strain containing the vector only.

FIG. 5. An intact mating-pheromone response pathway is required for Fus3-mediated repression of Ty1 retrotransposition. (A) Major components of the mating-pheromone response pathway. The receptor Ste2 (or Ste3), the components of the heterotrimeric G protein (Ste4, Ste18, and Gpa1), the scaffolding protein Ste5, and the MAP kinase Fus3 are shown in boldface type to indicate that they are specific to the pheromone response signal cascade. (B) The rate of His⁺ prototroph formation was determined for each strain as described in Materials and Methods. (C) Northern analysis of steady-state RNA from pheromone response pathway mutants. Each lane is labeled below with the relevant genotype of the strain analyzed. Riboprobes to detect Ty1his3AI-242 RNA (top), total Ty1 RNA (center) and PYK1 RNA (bottom) are described in Materials and Methods. The level of Ty1his3AI-242 RNA was normalized to PYK1 RNA. The ratios of Ty1his3AI-242 RNA to PYK1 RNA in each strain, relative to the *FUS3* strain, were as follows: *FUS3*, 1.0; *fus3Δ*, 1.3; *ste4Δ*, 0.5; *ste4Δ fus3Δ*, 0.5; *ste5Δ*, 0.3; *ste5Δ fus3Δ*, 0.3; *ste11Δ*, 0.1; *ste11Δ fus3Δ*, 0.1; *ste7Δ*, 0.1; *ste7Δ fus3Δ*, 0.1. The ratios of total Ty1 RNA to PYK1 RNA relative to the *FUS3* strain were as follows: *FUS3*, 1.0; *fus3Δ*, 0.9; *ste4Δ*, 1.1; *ste4Δ fus3Δ*, 1.0; *ste5Δ*, 0.7; *ste5Δ fus3Δ*, 0.7; *ste11Δ*, 0.5; *ste11Δ fus3Δ*, 0.4; *ste7Δ*, 0.1; *ste7Δ fus3Δ*, 0.1.

decreased transposition 15- and 9-fold, respectively. Hence, the activity of the *fus3-T180D* and *fus3-Y182E* alleles in suppressing Ty1 transposition is significantly lower than that of *FUS3*. These findings argue that basal levels of Fus3 phosphorylation are involved in maintaining normal levels of suppression of Ty1 transposition.

To determine if an intact pheromone response pathway is required for Fus3 to repress Ty1 transposition, we examined the effect of deleting genes that act upstream of Fus3 in the pheromone response pathway (Fig. 5A). *ste11Δ*, *ste7Δ*, *ste4Δ*, and *ste5Δ* mutations were introduced into the *FUS3* strain JC1430 or the isogenic *fus3Δ* strain JC1566 to allow a comparison of *ste fus3Δ* double mutants to single *ste* mutants (Fig. 5B). The rate of Ty1his3AI-242 transposition in a *ste11Δ fus3Δ* mutant was equivalent to that in a *ste11Δ* mutant. Similarly, Ty1his3AI-242 transposition was increased less than threefold in the *ste7Δ fus3Δ* mutant relative to the *ste7Δ* mutant. This small increase may be because Fus3 is present in a complex with Ste7 in naive cells (2) and hence may have increased access to its targets in the absence of Ste7. In addition, transposition was not increased in *ste4Δ fus3Δ* or *ste5Δ fus3Δ* double mutants relative to *ste4Δ* or *ste5Δ* mutants, respectively. The level of total Ty1 RNA or Ty1his3AI-242 RNA was the same in each *ste fus3Δ* mutant relative to the corresponding single *ste* mutant (Fig. 5C), indicating that the absence of an increase in transposition in *ste fus3Δ* double mutants is not a consequence of lower Ty1 RNA levels. Hence, deletion of *FUS3* is insufficient to derepress transposition in the absence of upstream components of the mating pathway.

Because activation of Fus3 is required for suppression of transposition, mutations that prevent Fus3 activation might be expected to cause the same phenotype as *fus3* null mutations do. However, strains with mutations in upstream components of the mating pathway showed decreased rather than increased transposition. Compared to the *fus3Δ* strain, the rate of Ty1his3AI-242 transposition was reduced 513-fold in a *ste11Δ* mutant, 385-fold in a *ste7Δ* mutant, 118-fold in a *ste4Δ* mutant, and 50-fold in a *ste5Δ* mutant (Fig. 5B). One explanation is that the production of Ty1 RNA is dependent on components of the mating pathway. We found a 2-fold reduction in the level of Ty1 RNA in *ste11Δ* mutants and a 10-fold reduction in *ste7Δ* mutants (Fig. 5C, center panel, lanes 7 and 9). However, RNA from the Ty1his3AI-242 element was reduced ten-fold in both *ste11Δ* and *ste7Δ* mutants (Fig. 5C, top panel, lanes 7 and 9). The levels of Ty1 RNA were equivalent to the wild-type level in *ste4Δ* mutants and 70% of the wild-type level in *ste5Δ* mutants (Fig. 5C, center panel, lanes 3 and 5). The *ste4Δ* and *ste5Δ* mutations had a more significant effect on Ty1his3AI-242 RNA, reducing it to 50 and 30% of the level in wild-type strains, respectively (Fig. 5C, top panel, lanes 3 and 5). The pattern of regulation for total Ty1 RNA is consistent with previous results (22, 28) and with the idea that transcription of many Ty1 elements is at least partially under the control of the invasive growth pathway. The invasive growth pathway uses Ste11 and Ste7 proteins but does not use Ste4 and Ste5 proteins, which are unique to the mating pathway. Hence, the finding that Ty1his3AI-242 RNA shows some degree of dependence on all four components of the mating pathway indicates that individ-

ual Ty1 elements may also be regulated as mating-specific genes.

The absence of Ste4 or Ste5 has a small effect on Ty1 RNA levels but significantly reduces Ty1*his3AI*-242 transposition. These findings argue that *fus3Δ* is not epistatic to these *ste* mutations for the regulation of transposition. (Likewise, *fus3Δ* is not epistatic to *ste* mutations in the mating response.) In *ste* mutants, neither Fus3 nor Kss1 can activate mating-specific gene expression but Kss1 can substitute for the activation of mating-specific genes in the absence of Fus3 (24, 41). Therefore, *ste4Δ* and *ste5Δ* mutants may not have the same hypertransposition phenotype as *fus3Δ* mutants because they lose expression of a mating-specific gene whose product activates Ty1 transposition at the posttranscriptional level.

Kss1 is not redundant with Fus3 in regulating Ty1 transposition. To determine if Kss1 is redundant with Fus3 in regulating transposition, we examined the effect of a *kss1Δ* mutation on Ty1 transposition. The rate of transposition of the Ty1*his3AI*-242 element was 2.0×10^{-8} in a *kss1Δ* mutant, which is fourfold lower than in the isogenic wild-type strain (Fig. 5B). Ty1 RNA levels are also reduced about twofold in a *kss1Δ* mutant relative to the isogenic wild-type strain (data not shown). These data demonstrate that Kss1 is not redundant with Fus3 in repressing Ty1 transposition. The rate of transposition in a *kss1Δ fus3Δ* double mutant was 1.0×10^{-9} , which is similar to that in the *ste7Δ* and *ste11Δ* mutants. There is a concomitant decrease in the level of Ty1 RNA in the *kss1Δ fus3Δ* double mutant to a level similar to that in a *ste7Δ* mutant (data not shown). The regulation of transpositional dormancy by Fus3 but not by Kss1 is consistent with two models: (i) Ty1 transposition in *fus3Δ* mutants is increased because the invasive growth response is activated, resulting in increased expression of an invasive growth gene whose product is an activator of Ty1 transposition; and (ii) transposition is increased in *fus3Δ* mutants by the loss of a negative regulatory interaction between the Fus3 kinase and a Ty1-encoded target protein.

DISCUSSION

The yeast MAP kinase Fus3 is a specific negative regulator of Ty1 transposition. Transpositional dormancy of Ty1 elements in yeast results from multiple levels of regulation that affect Ty1 transcription, protein synthesis, VLP formation and maturation, cDNA synthesis, and integration (5, 8, 16, 30, 50, 51). We have used a *his3AI*-based assay for transposition of individual genomic Ty1 elements to screen for host regulators of transpositional dormancy. The hypertransposition mutant *rtt100-1* was isolated in this screen, and *RTT100* was shown to be allelic with *FUS3* (25). Null mutations in *FUS3* increase the transposition rates of individual Ty1*his3AI* and Ty1*ade2AI* elements 18- to 56-fold, depending on the individual chromosomal Ty1 element that is assayed (Table 2). The increase in His⁺ prototroph formation in *fus3* mutants occurs independently of the *RAD52* gene and therefore cannot be attributed to elevated levels of homologous recombination of Ty1*HIS3* cDNA (Table 2). Moreover, Ty1 integration into the *CAN1* gene is elevated in *fus3* mutants (Table 3). These results demonstrate that integration of Ty1 into de novo targets is increased by null mutations in the *FUS3* gene.

In contrast, the *fus3Δ* mutation increases the transposition of Ty2*his3AI* elements only three- to fivefold (Table 2). One interpretation of this data is that Fus3 interacts directly or indirectly with a Ty gene product that is divergent in Ty1 and Ty2 elements. These elements have two regions of sequence divergence, one encompassing *TYA* and the PR domain of *TYB* and the other including the carboxy-terminal domain of IN

encoded within *TYB*. There is 50% identity and 66% similarity in the primary amino acid sequence of the *TYA* product between Ty1 and Ty2 elements, whereas the corresponding *TYB* gene products have 77% identity and 86% similarity in the primary amino acid sequence. Hence, the specificity of Fus3 for the regulation of Ty1 transposition may suggest that TyA1 is the target of the Fus3 kinase.

Null mutations in *FUS3* increase stability of VLP-associated Ty1 proteins. Our analysis of Ty1 RNA levels suggests that hypertransposition in *fus3* mutants does not result from increases in Ty1 RNA synthesis or stability. Steady-state levels of Ty1 RNA were not detectably increased in *fus3-187* or *fus3Δ* strains relative to those in congenic or isogenic control strains (Fig. 1 and 5). Similarly, the RNA level from marked Ty1*his3AI* and Ty1*ade2AI* elements was not consistently increased in *fus3* mutants. The simplest explanation of this data is that Ty1 transposition is regulated at a posttranscriptional step by Fus3. However, we cannot rule out the possibility of small increases in the Ty1 RNA level that were not detected in our Northern analyses and that lead to significant increases in the rate of genomic Ty1 element transposition. However, this possibility seems quite unlikely, given that increasing the copy number of Ty1 does not derepress the transposition of Ty1 elements (7, 17). Moreover, a 25% increase in the total Ty1 RNA level created when a Ty1*ade2AI* element on a high-copy-number plasmid is maintained in the cell actually decreases the transposition of the genomic Ty1*his3AI*-242 element (14).

Immunoprecipitation of pulse-labeled Ty1 proteins demonstrated that p58-TyA and p190-TyA/TyB were synthesized at equivalent levels in *fus3* and wild-type strains (Fig. 2A and B). These results are consistent with the observation that Ty1 RNA levels are not altered and indicate that higher levels of Ty1 retrotransposition in *fus3* mutants are not a result of an increase in the synthesis of Ty1 proteins. The rate of maturation of p58-TyA to p54-TyA, a step in retrotransposition that is very inefficient in normal cells (16), was also not detectably altered in *fus3* strains. However, there was a higher level of processed p54-TyA in *fus3* total-cell extracts (Fig. 2C). Furthermore, Western analysis of cytoplasmic fractions enriched for VLPs revealed that the levels of TyA, p90-IN, and p60-RT proteins were all increased in *fus3* mutants. We were unable to detect the highly unstable p23-PR protein (33), but if its levels are increased in *fus3* mutants, it does not significantly enhance the rate of TyA protein processing (Fig. 2B). Taken together, these findings suggest that transposition is inhibited by Fus3 at a posttranslational step after Ty1 protein processing in VLPs and that Fus3 contributes to the instability of Ty1 proteins. Similar to the regulation of retrotransposition in *fus3* mutants, induction of expression of a pGTy1 element does not change the efficiency of protein synthesis per Ty1 transcript. However, the rate of TyA protein processing is increased in transposition-induced cells, and this gives rise to dramatically higher steady-state levels of TyA and TyB proteins, Ty1 VLPs, and transposition per Ty1 RNA transcript. (16, 32). Our analysis of *fus3* mutants suggests that increasing the stability of Ty1 proteins can also stimulate Ty1 retrotransposition. A posttranslational increase in VLP-associated proteins in *fus3* mutants is likely to be the direct cause of the increase in transposition, because *fus3* mutants also have 7- to 10-fold-higher levels of total cellular Ty1 cDNA. Although we do not know if this cDNA is VLP associated, the simplest explanation of this finding is that higher levels of VLPs result in an increase in the synthesis of the substrate for integration, which in turn leads to higher levels of Ty1 transposition in *fus3* mutants.

The pheromone response pathway activates Fus3 to mediate the suppression of Ty1 transposition. Our results indicate that

basal activation of Fus3 is required to suppress Ty1 transposition at the posttranslational level. Alleles of *FUS3* with amino acid substitutions in the activating residues, *fus3-T180D* and *fus3-Y182E*, were significantly less active than wild-type *FUS3* in complementing Ty1 hypertransposition in a *fus3Δ* strain, suggesting that phosphorylation of Fus3 is involved in mediating the repression of Ty1 transposition in vegetative cells (Table 4). The *fus3-T180D* and *fus3-Y182E* alleles did exhibit a low level of activity in suppressing transposition. This activity could result from these alleles having retained the kinase-independent inhibitory function of Fus3 whereas null *fus3Δ* alleles lack this function (41).

The requirement for activation of Fus3 was confirmed by demonstrating that in the absence of Ste4, Ste5, Ste11, and Ste7, Ty1 transposition is not suppressed by Fus3 (Fig. 5B). Both Ste4 and Ste5 are specific to the mating-pheromone pathway. Hence, the data suggest that for Fus3 to suppress Ty1 transposition, it must be activated by upstream components of the pheromone response pathway. This modulation of Fus3 activity in regulating Ty1 transposition at the posttranslational level is therefore entirely consistent with its modulation in response to mating pheromone. In contrast to the increase in Ty1 transposition in *fus3* mutants, Ty1 transposition is reduced in the four *ste* mutants analyzed and in a *fus3Δ kss1Δ* double mutant. Since *ste4Δ* and *ste5Δ* mutants had nearly wild-type levels of Ty1 RNA, this finding suggests that an activator of Ty1 transposition is encoded by a mating-specific gene, whose expression would be blocked in *ste* and *kss1Δ fus3Δ* mutants but not in a *fus3Δ* mutant. Despite this possibility, the rate of transposition remains sufficiently high in *ste4Δ* and *ste5Δ* mutants (6.5- and 2.7-fold lower than in an isogenic wild-type strain, respectively [Fig. 5B]) that the postulated activator must not be absolutely required for Ty1 transposition and the effect of deleting *FUS3* could be easily detected if it occurred in the absence of activation by upstream components of the pheromone response pathway.

Complex regulation of Ty1 RNA levels by components of the pheromone response and invasive growth pathways. The promoter of Ty1 contains an FRE that is bound by Ste12 and Tec1 and is responsive to conditions that stimulate invasive growth genes (3, 40, 43). Mutations in components of the invasive growth pathway, including Ste11, Ste7, Ste12, and Tec1, abolish the expression of an *FRE(Ty1)::lacZ* reporter. However, components specific to the mating pathway, including Ste4 or Ste5, do not affect the expression of *FRE(Ty1)::lacZ* (40, 43). In general agreement with these findings, we have found that Ty1 RNA levels are reduced in *ste7Δ* and *ste11Δ* mutants, slightly lowered in a *ste5Δ* mutant, and unaltered in a *ste4Δ* mutant (Fig. 5B). However, our findings suggest that Ty1 promoters in their native context may not all be regulated identically to *FRE(Ty1)::lacZ*. For example, *FRE(Ty1)::lacZ* expression was abolished in a *ste11Δ* mutant (40), but we found that the Ty1 RNA level was reduced only twofold in *ste11Δ* mutants. Second, while *FRE(Ty1)::lacZ* expression in a *fus3Δ kss1Δ* double mutant is similar to that in a wild-type strain, Ty1 RNA levels decrease dramatically in a *fus3Δ kss1Δ* double mutant (data not shown), which is more characteristic of mating-specific genes than of invasive growth genes. These differences might be attributable to a posttranscriptional effect on Ty1 RNA levels, since we are measuring steady-state levels of Ty1 RNA rather than promoter activity. Alternatively, there might be heterogeneous regulation of individual Ty1 promoters. The effect of *ste* mutations on RNA from the marked *Ty1his3AI-242* element is consistent with the possibility that at least some Ty1 elements are regulated with characteristics of mating-specific genes. The *Ty1his3AI-242* RNA level was lowered three- and

twofold in *ste5Δ* and *ste4Δ* mutants, respectively, which is a more significant decrease than that of the total Ty1 RNA level in these strains (Fig. 5B). Hence, the expression of individual Ty1 elements may be affected to different extents by the mating and invasive growth pathways.

Direct or indirect interaction of Fus3 with Ty1? TyA is a phosphoprotein (42, 56) whose steady-state levels are elevated in *fus3* mutants (Fig. 2C and 3). Therefore, a simple hypothesis to explain the repression of transposition by Fus3 is that TyA is phosphorylated by the Fus3 kinase and that this modification targets TyA for degradation. Fus3 has multiple targets (26), and TyA has several MAP kinase consensus sites (S/T-P), but the phosphorylation sites in TyA that are used in vivo have not been identified. Xu and Boeke (56) found that when cells were exposed to mating pheromone, thereby activating Fus3, transposition of a pGTy1 element was inhibited. Moreover, TyA was hyperphosphorylated and VLP-associated p90-IN and Ty1 cDNA levels were reduced. Taken together with the results presented here, these findings are consistent with the hypothesis that Fus3 phosphorylates TyA and that this modification decreases the stability of VLPs and reduces transposition levels. An intermediate protein that is phosphorylated by Fus3 and in turn affects the phosphorylation state of TyA and the stability of VLP-associated proteins would also explain these observations.

Another mechanism by which Fus3 might mediate the post-translational regulation of Ty1 is through its role in activating Ste12, which in turn promotes the expression of mating-specific genes. In this scenario, Fus3 would suppress transposition by stimulating the expression of a mating-specific gene that encodes an inhibitor of Ty1 transposition. Several observations argue against this interpretation. First, mating-specific genes are still expressed in a *fus3* mutant, because Kss1 can substitute for Fus3 in the activation of Ste12 (24, 41). Second, the large decrease in *Ty1his3AI-242* transposition in *ste4Δ* and *ste5Δ* mutants relative to that in a *fus3Δ* mutant (Fig. 5B) suggests that there is an activator of transposition encoded by a mating-specific gene, rather than an inhibitor. Third, the fact that Kss1 is not redundant with Fus3 for the suppression of *Ty1his3AI-242* transposition suggests that transpositional dormancy is not regulated via an inhibitor encoded by a mating-specific gene.

A third scenario by which Fus3 might regulate transposition is via its role as a negative regulator of invasive growth and FRE-dependent gene expression (40, 48). Fus3 has a kinase-independent inhibitory activity that prevents the inappropriate activation of invasion by the mating cascade; therefore, Kss1 can be erroneously activated by the mating pathway in *fus3Δ* mutants, resulting in stimulation of both mating-specific and invasive-growth gene expression (41). Hence, Fus3 may inhibit the expression of an invasive growth gene that encodes an activator of Ty1 transposition. Several of our observations are consistent with this hypothesis. Unactivable alleles of Fus3, *fus3-T180D* and *fus3-Y182E*, retain some ability to suppress transposition (Table 4), consistent with the expectation that they retain the kinase-independent function of blocking the invasive-growth pathway. A second relevant observation is that Ty1 transposition is dramatically reduced in *ste11Δ* mutants even though the total Ty1 RNA level is reduced by only twofold (Fig. 5). Similarly, invasive growth and FRE-dependent gene expression are completely blocked in *ste11Δ* mutants (40, 48). In contrast, transposition is only slightly reduced in *kss1Δ* mutants, even though Ty1 RNA levels are comparable to those in *ste11Δ* mutants. This is consistent with the moderate reduction in FRE-dependent gene expression and weak invasion defect in *kss1Δ* mutants that results from loss of both positive and negative regulation of invasion by Kss1 (13, 40, 41). Fur-

ther experiments will be needed to determine whether the Fus3 kinase interacts directly with Ty1 proteins or whether it acts through the invasive growth pathway to mediate the post-translational suppression of Ty1 transposition.

Significance of regulating Ty1 transposition through conserved signal transduction cascades. Because insertional mutations caused by Ty1 transposition can be deleterious in haploids, the haploid-specific Fus3 kinase may have been adapted to specifically repress transposition during the haploid stage of the yeast life cycle. This mechanism of regulation also provides flexibility for the host cell to modulate transposition in response to environmental signals. We have demonstrated that Ty1 transposition is regulated not only by Fus3 but also by Ste4, Ste5, Ste11, Ste7, and Kss1. Therefore, transposition is potentially responsive to environmental signals in both haploid and diploid yeasts. The inhibition of transposition upon exposure of cells to mating pheromone is likely to be a direct effect of activating the Fus3 kinase. The fact that the Ty1 promoter is regulated as a filamentous or invasive growth gene predicts that environmental conditions that promote invasive growth will also activate Ty1 transposition, potentially in both haploids and diploids. Furthermore, the activity of Fus3 can be repressed by activation of other signal transduction cascades, such as the osmoregulatory pathway (35). Therefore, growth in high-osmolarity medium could increase Ty1 transposition in haploids. Although transposition is potentially deleterious, yeast cells might encounter severe conditions in which it is advantageous to activate transposition because of the ability of Ty1 to cause adaptive mutations by altering the regulation of target genes (53). Hence, under conditions of stress (such as conditions that induce invasive growth) where survival is threatened, Ty1 could be activated to induce genetic change, thereby providing a means for the cell to adapt to its environment.

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