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Chromatin-Associated Genes Protect the Yeast Genome From Ty1 Insertional Mutagenesis

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ABSTRACT

Chromosomal genes modulate Ty retrotransposon movement in the genome of *Saccharomyces cerevisiae*. We have screened a collection of 4739 deletion mutants to identify those that increase Ty1 mobility (Ty1 restriction genes). Among the 91 identified mutants, 80% encode products involved in nuclear processes such as chromatin structure and function, DNA repair and recombination, and transcription. However, bioinformatic analyses encompassing additional Ty1 and Ty3 screens indicate that 264 unique genes involved in a variety of biological processes affect Ty mobility in yeast. Further characterization of 33 of the mutants identified here show that Ty1 RNA levels increase in 5 mutants and the rest affect mobility post-transcriptionally. RNA and cDNA levels remain unchanged in mutants defective in transcription elongation, including *ckb2*Δ and *elf1*Δ, suggesting that Ty1 integration may be more efficient in these strains. Insertion-site preference at the *CAN1* locus requires Ty1 restriction genes involved in histone H2B ubiquitination by Paf complex subunit genes, as well as *BRE1* and *RAD6*, histone H3 acetylation by *RTT109* and *ASF1*, and transcription elongation by *SPT5*. Our results indicate that multiple pathways restrict Ty1 mobility and histone modifications may protect coding regions from insertional mutagenesis.

THE Ty1, -2, and -5 and Ty3 retrotransposons of Saccharomyces belong to the Ty1/Copia and the Ty3/Gypsy superfamilies, respectively, which are present in every eukaryotic genome examined to date (Boeke and Devine 1998; Eickbush and Malik 2002; Sandmeyer *et al.* 2002; Voytas and Boeke 2002; Lesage and Todeschini 2005). Ty-element structure, expression strategy, and the process of retrotransposition resemble those of retroviruses, except Ty elements lack an envelope gene and retrotransposition is not infectious. Ty elements are flanked by long terminal repeats (LTRs) and transcribed from end to end, forming a transcript that is a template for translation and reverse transcription. Translation results in the synthesis of Ty Gag, a retroviral-like capsid protein, and a Gag-Pol fusion protein containing protease (PR), integrase (IN), and reverse-transcriptase (RT) domains. Synthesis of Gag-Pol results from a specific frameshifting event within a small region of overlapping coding sequence in *GAG* and *POL*. Gag and Gag-Pol proteins form cytoplasmic virus-like particles (VLPs) within which protein maturation and reverse transcription occur, and assembly of functional Ty3 VLPs takes place in association with P-bodies (Beliakova-Bethell *et al.* 2006; Larsen *et al.* 2007). *Cis*-acting signals for Ty translational frameshifting, RNA packaging and dimer formation, and the initiation and strand-transfer events necessary for reverse transcription are present on Ty mRNA. Like retroviruses, a Ty preintegration complex (PIC) minimally containing Ty cDNA and IN may be released from cytoplasmic VLPs. The Ty PIC traverses the nuclear membrane in a manner similar to human immunodeficiency virus (HIV) infection of quiescent cells, via a nuclear localization signal present on IN. Most Ty1- and all Ty3-element insertions occur near genes transcribed by RNA polymerase III (Chalker and Sandmeyer 1990; Kirchner *et al.* 1995; Devine and Boeke 1996; Yieh *et al.* 2000; Bachman *et al.* 2005), while Ty5 inserts into subtelomeric regions and adjacent to the silent-mating cassettes (Zou *et al.* 1996; Zou and Voytas 1997; Xie *et al.* 2001). Ty1 elements are capable of insertional mutagenesis, where they preferentially insert into promoter regions; however, insertion into coding sequences also occurs (Natsoulis *et al.* 1989). In addition, Ty cDNA undergoes recombination with chromosomal elements, especially when transpositional integration is blocked.

The Ty life cycle's nuclear and cytoplasmic phases set the stage for interactions with a variety of cellular genes and processes. The first nuclear phase involves Ty transcription, RNA processing, and export, while the second involves nuclear import of the PIC followed by transpositional integration. Distinct cytoplasmic phases de-

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rive from the fact that Ty RNA is used for translation and is also encapsidated into VLPs for reverse transcription and formation of the PIC. Identifying cellular genes that have been co-opted by Ty1 and Ty3 elements has not only resulted in a deeper understanding of retroelement replication and control, but has also helped elucidate the normal function of these genes in the cell. Certain Ty–host cell interactions are conserved with retroviruses and, therefore, may help identify new antiviral targets for HIV (see the recent review by Maxwell and Curcio 2007). Highlighted among these are mammalian genes required for DNA repair, genome maintenance, and protein trafficking that also modulate retroviral replication and virion budding (Yoder and Bushman 2000; Li *et al.* 2001; Kilzer *et al.* 2003; Aye *et al.* 2004; Lau *et al.* 2004; Irwin *et al.* 2005; Beliakova-Bethell *et al.* 2006; Lloyd *et al.* 2006; Smith and Daniel 2006; Yoder *et al.* 2006; Larsen *et al.* 2007).

Minimizing Ty1 retrotransposition is important for maintaining the integrity of the yeast genome since these elements can mutate genes and are involved in genome rearrangements (Voytas and Boeke 2002; Lesage and Todeschini 2005). A variety of genetic screens have identified host genes modulating Ty1 RNA level (Winston 1992; Yamaguchi *et al.* 2001; Timmers and Tora 2005), translational frameshifting (Xu and Boeke 1990; Kawakami *et al.* 1993; Farabaugh 1995), protein processing and VLP maturation (Conte *et al.* 1998), RT activity (Bolton *et al.* 2002; Yarrington *et al.* 2007), cDNA level (Lee *et al.* 1998; Rattray *et al.* 2000; Scholes *et al.* 2001; Griffith *et al.* 2003) and stability (Lee *et al.* 2000), target-site preference (Liebman and Newnam 1993; Bachman *et al.* 2005; Gelbart *et al.* 2005; Mou *et al.* 2006), or the overall level of retrotransposition (Scholes *et al.* 2001; Griffith *et al.* 2003). Analyzing deletion and transposon libraries has also revealed functions that modulate Ty3 transposition involving chromatin dynamics, RNA metabolism and translation, tRNA processing, vesicular trafficking, nuclear transport, and genome integrity (Aye *et al.* 2004; Irwin *et al.* 2005; Beliakova-Bethell *et al.* 2006; Larsen *et al.* 2007).

An important cellular modulator of Ty retrotransposition, *RAD6*, encodes an E2 ubiquitin-conjugating enzyme involved in DNA repair and transcription elongation (Broomfield *et al.* 2001; Osley 2004). *RAD6* inhibits Ty1 and Ty3 retrotransposition and minimizes Ty1 integration in coding sequences and transcription units (Picologlou *et al.* 1990; Kang *et al.* 1992; Liebman and Newnam 1993; Irwin *et al.* 2005). Rad6p acts together with the E3 ubiquitin ligases Rad18p and Bre1p to ubiquitinate Pol30p (PCNA) (Hoege *et al.* 2002) and histone H2B (Robzyk *et al.* 2000; Hwang *et al.* 2003; Wood *et al.* 2003a), respectively. The RNA polymerase II-associated Paf complex is also required for Rad6p-mediated H2B ubiquitination (Ng *et al.* 2003; Wood *et al.* 2003b; Xiao *et al.* 2005).

Here, we report the results of a systematic screen for Ty1 restriction genes, which complements and expands the number and functional classes of retrotransposition inhibitory genes obtained from previous studies. Bioinformatic analyses have been utilized to illustrate the various biological processes that help or restrict Ty1- and Ty3-element movement in the genome. We have also identified additional genes involved in histone transactions and transcription that minimize Ty1 insertional mutagenesis in coding sequences, as well as at their preferred targets upstream of genes transcribed by RNA polymerase III.

MATERIALS AND METHODS

Genetic techniques, media, and strains: Yeast genetic techniques and media were used as described previously (Sherman *et al.* 1986; Guthrie and Fink 1991). The haploid *MAT*α deletion collection (Giaever *et al.* 2002) was obtained from Invitrogen (Carlsbad, CA). A total of 4739 deletion mutants derived from BY4742 (*MAT*α *his3-Δ1 leu2-Δ0 lys2-Δ0 ura3-Δ0*) (Brachmann *et al.* 1998) were transformed with pBJC573, a *URA3*-based integrating plasmid carrying a complete Ty1 element tagged with a modified indicator gene *his3-AId1* (designated *his3-AI*) (Curcio and Garfinkel 1991), which cannot undergo recombination with the internal deletion of *HIS3* (*his3-Δ1*) present in BY4742. Briefly, the *his3-AId1* allele was constructed by cotransforming pBDG208, which contains the *HIS3* gene oriented such that transcription of the *GAL1*-promoted Ty1 (pGTy1) and the *HIS3* gene is in opposite directions, and a PCR-amplified disruption fragment containing the artificial intron (AI) flanked by 40 bp of *HIS3* coding sequences into the Ty-less strain DG1768 (Garfinkel *et al.* 2003). The PCR primers used were ΔAIT2 (5′-GGATCATCT CGCAAGAGAGATCTCCTACTTTCTCCCTTTGGTATGTTA ATATGGAC-3′) and ΔAIB2 (5′-TCTTTCGAACAGGCCGTAC GCAGTTGTCGAACTTGGTTTGCTGTTATAAATAATACC-3′) and the AI template was pUC/intron 4 (Yoshimatsu and Nagawa 1989). The PCR primers flanked a region 400–480 bp from the start of the *HIS3* coding sequence. To identify pGTy1*his3-AId1* recombinants, His^- Ura^+ transformants identified by replica plating were spread on synthetic complete medium lacking uracil (SC −Ura) + galactose, incubated for 4 days at 20°, and then replicated to SC −His −Ura + glucose medium. Candidate pGTy1*his3-AId1* plasmids were recovered from transformants that remained His^- when propagated on glucose but produced many His^+ papillae when induced with galactose. The presence of the AI in the expected position within *HIS3* was confirmed by DNA sequencing. To construct pBJC573, the *his3-AId1* gene was subcloned into a *URA3*-based Ty1-integrating plasmid at the *Bgl*II site in *TYB1* adjacent to the 3′ LTR. Further details on pBJC573 can be found elsewhere (Bryk *et al.* 2001; Scholes *et al.* 2001). Integrants of pBJC573 upstream of the *HIS4* gene were enriched for by linearizing the plasmid with *Pac*I, which cleaves once in the *HIS4* sequences adjacent to the Ty1*his3-AI* element. Strain DG2122 was constructed by introducing pBJC573 into the parental strain BY4742. Strain JC3787 was derived from BY4742 after transposition induction of cells harboring pBDG945 (pGTy1-H3*his3-AId1*) and contains a genomic Ty1*his3-AId1* element (Mou *et al.* 2006). Strain DG3027 [*MAT***a** *his3-Δ1 leu2-Δ0 met15-Δ0 ura3-Δ0 can1-Ty1(26)* (pBJC573)] was generated by crossing BY4743 (*MAT***a** *his3-Δ1 leu2-Δ0 met15-Δ0 ura3-Δ0*) (Brachmann *et al.* 1998) and DG3016 [DG2122; *can1-Ty1(26)*] followed by tetrad analysis. One-step gene disruptions were performed using *KanMX4*-targeting fragments (Wach *et al.* 1994), amplified from the deletion mutants using the gene-

specific flanking primers A and D (http://www-sequence.stanford.edu/group/yeast_deletion_project/Deletion_primers_PCR_sizes.txt). Mutant identity was verified by PCR using A and D primers, phenotypic analyses, and complementation tests.

Ty1 *his3-AI* mobility assay used for systematic screening: To detect Ty1 mobility events, four independent transformants containing pBJC573/Ty1*his3-AI* from each deletion mutant along with the wild-type strain DG2122 were streaked for single colonies on SC −Ura and incubated at 20° for 5 days, which is optimal for Ty1 retrotransposition (Paquin and Williamson 1984). Ura^+ cells were replica plated to SC −His plates and incubated at 30° for 3–4 days. The levels of His^+ papillation from the deletion mutant and DG2122 were compared. Candidate mutants containing pBJC573 that displayed a higher level of Ty1*his3-AI*-mediated His^+ papillation in at least three of the four transformants were retested and used for further analyses.

Gene ontology enrichment analysis: Gene ontology biological processes (GOBP) enrichment analysis was performed using Whole Pathway Scope (WPS) software (Yi *et al.* 2006). Yeast gene ontology annotations were obtained from the Saccharomyces Genome Database (http://www.yeastgenome.org/). The GOBP enrichment was performed using overrepresentation analysis. Fisher's exact test was performed on 2×2 contingency tables, to determine whether a gene is in a given list or not *vs.* whether this gene is associated with a GOBP term or not. A one-sided Fisher's exact test was used to determine which biological processes and pathways had a statistically significant enrichment within the Ty1 restriction gene list or other gene lists. The associated GOBP terms were ranked into a term enrichment list on the basis of their Fisher's exact test *P*-values with the most enriched terms at the top. Comparison of gene lists of Ty modulators from different sources at the GOBP level was performed using an extended version of WPS (M. Yi and R. M. Stephens, unpublished results). Briefly, enrichment levels of each GOBP term were computed in a batch mode for each of the lists and the results were merged into a Stanford format file with a matrix of enrichment scores [−Log(*P*-value) of Fisher's exact test *P*-values], which were filtered using the criteria of list hits >1 (list hit: genes from the intended list that are associated with the corresponding GOBP term) and *P*-values <0.05. The results were displayed in color-coded "heat maps" to reveal the patterns of significantly altered biological processes from the multiple-gene lists. The color coding of the heat maps is related to the enrichment of genes in a GOBP term [−Log(*P*-value) based]. The gradient of red color in the heat map indicated the enrichment levels with the maximal red color (enrichment score ≥3; *P*-value ≤0.001) and black denoting no enrichment (enrichment score 0; *P*-value >0.05). Underrepresented GOBP terms were not included in this analysis as a separate feature. Hierarchical clustering was obtained using the average linkage and Euclidean distance of the GOBP terms in the rows. All rows were organized into a binary tree as a dendrogram. The lower height of a subtree indicated a greater similarity in enrichment levels of GOBP terms across all the gene lists in the heat map. The vertical lines across the hierarchical lines determined the number of clusters at a certain depth.

Frequency of Ty1*his3-AI* mobility: Each strain was streaked for single colonies on SC −Ura at 20°. A single colony was suspended in 10 ml of SC −Ura and ~10^3 cells were inoculated into four individual 1-ml SC −Ura liquid cultures and grown at 20° until saturation. Aliquots of the cultures were spread on SC −Ura and SC −His −Ura plates, followed by incubation at 30° for 5 days. The frequency of Ty1*his3-AI* mobility was calculated by dividing the average number of His^+ Ura^+ cells per milliliter by the average number of Ura^+ cells per milliliter.

Monitoring Ty1 insertions at *SUF16* by PCR: The deletion mutants and the wild-type strain DG2122 were grown on SC −Ura plates at 20° for 4 days. Three single colonies per strain were inoculated into individual tubes containing 10 ml of SC −Ura liquid media and grown at 20° until saturation. Total genomic DNA was isolated by glass-bead/phenol lysis (Hoffman and Winston 1987) and used as a template in a PCR reaction with oligonucleotide primers specific to Ty1 (TyB OUT: 5′-GAACATTGCTGATGTGATGACA-3′) and *SNR33* (SNR33 OUT: 5′-TTTTAGAGTGACACCATCGTAC-3′), which is adjacent to the 3′ end of *SUF16*, as described previously (Sundararajan *et al.* 2003). An aliquot of the reaction was analyzed by electrophoresis on a 1% agarose gel in Tris–Borate–EDTA running buffer. The ethidium bromide-stained gel was scanned on a Typhoon Trio phosphorimager (GE Healthcare, Piscataway, NJ), and the intensity of the Ty1 insertion pattern was compared to that obtained with the wild-type strain DG2122. To ensure that the DNA samples were PCR competent, control reactions were performed with primers containing sequence from the *CPR7* locus (CPR7A: 5′-GTTT GTGATTTATCTCTGGACTGCT-3′ and CPR7D: 5′-AGTTCGT CTCTCCTTCATATTCTCA-3′).

Northern blot analysis of Ty1 RNA: A single colony from each strain was inoculated into 10 ml of SC −Ura liquid medium and grown until late log phase at 20°. Total RNA was isolated using the MasterPure yeast RNA purification kit (Epicentre, Madison, WI). RNA samples were separated on a 1.2% agarose–formaldehyde gel and transferred to Hybond-N (GE Healthcare). An RT-domain probe was obtained by purifying a 1.6-kb *Pvu*II–*Cla*I fragment from pGTy1Cla (Garfinkel *et al.* 1988), using a QIAquick gel extraction kit (QIAGEN, Valencia, CA). The RT-domain fragment was labeled by randomly primed DNA synthesis using the Megaprime DNA labeling system and [α-^{32}P]dCTP (GE Healthcare). The levels of Ty1 and Ty1*his3-AI* transcripts were normalized to the 18S and 28S rRNA bands visualized by staining with ethidium bromide. Gel electrophoresis and Northern hybridizations were performed as described previously (Lee *et al.* 1998). The hybridization and ethidium bromide fluorescence signals were quantified using a Typhoon Trio phosphorimager and ImageQuant TL software (GE Healthcare). Lane background was subtracted using the rolling-ball method, which calculates the background as if a disc with a radius setting of 200 (default) were rolling underneath each lane profile. We also verified the level of Ty1 RNAs for 16 (*asc1*Δ, *asf1*Δ, *bre1*Δ, *bud27*Δ, *cdc40*Δ, *cdc73*Δ, *ckb2*Δ, *ipk1*Δ, *leo1*Δ, *paf1*Δ, *pbs2*Δ, *rpd3*Δ, *rtf1*Δ, *spt5*Δ#, *ssk2*Δ, and *ypl183c*Δ*/rtt10*) of the 33 Ty1 restriction mutants.

Southern blot analysis of Ty1 cDNA: The deletion strains and the wild-type strain DG2122 were grown on SC −Ura plates at 20° for 4 days. An individual colony from each strain was inoculated into 10-ml SC −Ura liquid cultures and grown until mid- to late-log phase at 20°. Total genomic DNA was isolated as described above, digested with *Pvu*II, separated on a 0.8% agarose gel at 4°, and transferred to Hybond-N (GE Healthcare). A ^{32}P-labeled DNA probe was derived from the Ty1 RT region as described above. Gel electrophoresis and Southern hybridizations were performed as described previously (Lee *et al.* 1998). The intensity of the 2-kb cDNA band was determined by phosphorimage analysis and normalized to three conserved Ty1-chromosomal junction fragments.

Monitoring Ty1 insertions at *CAN1*: Spontaneous canavanine-resistant (Can^R) mutants from selected deletion strains as well as DG2122 were obtained by streaking cells for single colonies on SC plates. After incubating for 4–5 days at 20°, cells were replicated to SC −Arg +Can plates and incubated at 30° until Can^R papillae appeared (Rinckel and Garfinkel 1996). Independent Can^R mutants were clonally purified on SC −Arg +Can plates prior to isolation of DNA. The frequency of Can^R

was determined by inoculating ~10^3 cells from an independent colony of each mutant into four individual 1-ml SC liquid cultures followed by incubation at 20° until the cultures saturated. Dilutions of each culture were spread onto SC plates and SC −Arg +Can plates and incubated at 30° for 5 days. The frequency of Can^R was calculated by dividing the average number of Can^R cells per milliliter by the average number of total cells per milliliter. PCR was used to detect and map the positions of Ty1 insertions that disrupted *CAN1*. Total genomic yeast DNA was initially analyzed using primers CAN1(−317) (5′-GTCTCTATCAATGAAAATTTCGAGG-3′) and CAN1(+1966) (5′-GTTTCAAATGCTTCTACTCCGTCTGC-3′) that bracket *CAN1* and include 317 bp of 5′-noncoding sequence, 1773 bp of coding sequence, and 193 bp of 3′-noncoding sequence. Can^R mutants lacking the 2283-bp *CAN1* amplification product were analyzed using Ty1-specific primers midLTR OUT (5′-ATTCATTGATCCTATTACATTATC-3′) and midLTR IN (5′-GATAATGTAATAGGATCAATGAAT-3′) and primers adjacent to the *CAN1* start codon CAN1 IN (5′-ATGACAAATT CAAAAGAAGACG-3′) and CAN1 OUT (5′-CGTCTTCTTTT GAATTTGTCAT-3′). Chi-square analysis was performed to determine if the incidence of 5′-noncoding insertions *vs.* coding sequence insertions was significantly altered in the deletion mutants. Since the fraction of spontaneous Ty1-induced *can1* mutations in wild-type cells is too low to be recapitulated here, we compiled the data from hundreds of Ty1 transposition events at *CAN1* obtained in wild-type backgrounds from previous studies showing that ~50% of Ty1 insertions occur in the *CAN1* promoter, defined as a 317-bp region upstream of the initiation codon, and the rest were in the 1773-bp *CAN1* coding sequence (Wilke *et al.* 1989; Picologlou *et al.* 1990; Liebman and Newnam 1993; Rinckel and Garfinkel 1996). The number of trials used for the wild-type sample was adjusted to the number of trials for a given restriction mutant in the chi-square analysis. For example, one promoter region and 19 coding sequence insertions of Ty1 were obtained at *CAN1* in a *paf1*Δ mutant. Therefore, we assumed that 10 promoter and 10 coding sequence insertions occurred in the wild type to estimate the *P*-value. DNA sequencing of Ty1 or solo-LTR insertions at *CAN1* was performed using Ty1 primer LTR (+89) (5′-CATTTGCGTCATCTTCTA ACACCG-3′) or the midLTR primers, respectively.

RESULTS AND DISCUSSION

Identifying genes that restrict Ty1 mobility: We introduced a functional Ty1 element under the control of its native promoter and carried on a *URA3*-integrating plasmid, pBJC573 (Bryk *et al.* 2001; Scholes *et al.* 2001), into 4739 haploid *MAT*α deletion mutants (Figure 1). The Ty1 element contained the *his3-AI* (artificial intron) retroelement indicator gene, which allows Ty1 movement to be monitored by the formation of His$^+$ colonies, following Ty1*his3-AI* RNA splicing and reverse transcription (Curcio and Garfinkel 1991). His$^+$ cells usually arise by *de novo* retrotransposition of Ty1*HIS3* to a new chromosomal location in wild-type cells, but homologous recombination between Ty1*HIS3* cDNA and a resident element also occurs (Sharon *et al.* 1994). Therefore, the term "Ty1 mobility" is used to describe the Ty1*HIS3* events that arise from Ty1 retrotransposition and cDNA recombination. Integrative recombination of pJBC573 containing Ty1*his3-AI* was targeted to the 5′-noncoding region of *HIS4* by digestion with *Pac*I.

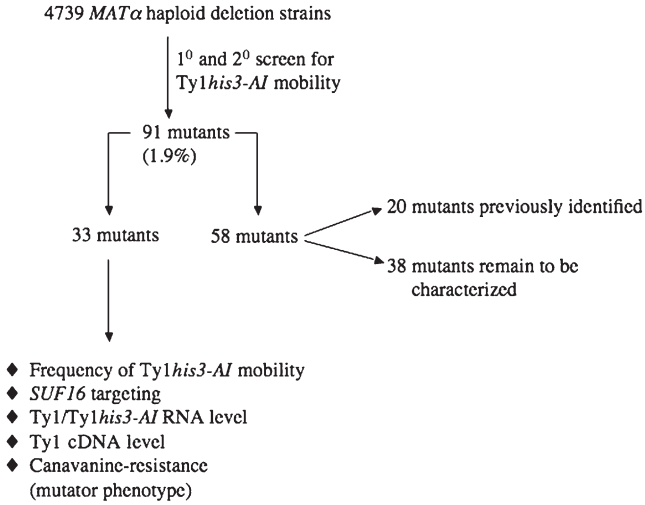

Figure 1.—Characterization of Ty1 restriction mutants. Ninety-one mutants (1.9%) were identified from 4639 *MAT*α deletion strains, on the basis of an increased level of His$^+$ papillation mediated by a chromosomal Ty1 element containing the retrotransposon mobility marker, *his3-AI*. Thirty-three mutants were chosen for further analyses after considering the function of the deleted gene and the level of Ty1*his3-AI* mobility, 20 mutants were identified in other screens for Ty1 mobility, and 38 mutants remain to be characterized.

Four independent Ura$^+$ transformants of each deletion mutant were tested along with a wild-type strain containing pJBC573 (DG2122) for an increase in His$^+$ papillation to identify Ty1 restriction genes. An increase of threefold in Ty1*his3-AI* mobility could be reproducibly detected using this qualitative assay and at least three of the four independent transformants from a given mutant were required to show increased Ty1*his3-AI* mobility to be saved for further analyses. We identified 91 mutants with a higher level of Ty1*his3-AI* mobility when compared with DG2122 (Figure 1, supplemental Table S1 at http://www.genetics.org/supplemental/). The Ty1 restriction mutants were placed in the following categories (Figure 1): Thirty-three novel mutants were chosen for further analysis on the basis of the function of the deleted gene or their level of Ty1 mobility, 20 mutants were identified in previous screens, and 38 mutants remain to be characterized.

Bioinformatic analyses reveal functional relationships between Ty modulators: Computational approaches were used to determine if the 91 Ty1 restriction genes were functionally related (Table 1, supplemental Table S1) and the degree of overlap with previous genetic screens for Ty1 restriction genes (Scholes *et al.* 2001), Ty1 helper genes (Griffith *et al.* 2003), and Ty3 restriction and helper genes (Irwin *et al.* 2005) (Figures 2 and 3, supplemental Figure S1 at http://www.genetics.org/supplemental/). Eighty-five of the 91 Ty1 restriction genes identified here were associated with at least one GOBP (Table 1, list total), while six ORFs remained uncharacterized (*YBR239C, YGR110W, YJR142W, YLR282C,*

TABLE 1

Overrepresentation analysis of Ty1 restriction mutants

Gene ontology biological process (GOBP)	List hits	List total	Population hits	Population total	*P*-value ($\times 10^{-10}$)
Double-strand break repair	12	85	39	6455	0.000358
Nucleic acid metabolism	51	85	1490	6455	0.00223
Nonrecombinational repair	10	85	27	6455	0.00641
Response to DNA damage stimulus	19	85	194	6455	0.0374
Double-strand repair via homologous recombination	8	85	17	6455	0.143
Recombinational repair	8	85	17	6455	0.143
Chromosome organization and biogenesis (*sensu* Eukaryota)	20	85	237	6455	0.15
Negative regulation of DNA transposition	6	85	8	6455	1.2
Nuclear organization and biogenesis	20	85	297	6455	8.91
DNA recombination	16	85	187	6455	17.7
Transcription/DNA dependent	23	85	441	6455	58.2
Meiotic recombination	8	85	39	6455	286
Cell cycle	23	85	530	6455	1830
Establishment and maintenance of chromatin architecture	14	85	202	6455	2950
Telomerase-independent telomere maintenance	5	85	13	6455	4170
Cell proliferation	24	85	601	6455	4360
Chromatin modification	13	85	178	6455	4490

List hits, number of Ty1 restriction genes associated with the designated GOBP term; list total, number of Ty1 restriction genes associated with at least one GOBP term; population hits, the number of unique genes annotated for the designated GOBP term; population total, the number of unique genes annotated for a GOBP term; *P*-value, one-sided Fisher's exact test.

YML009W-B, and *YPL183C*). Most of the genes described here contain multiple GOBP terms, ranging from a specific term such as telomerase-independent telomere maintenance to a general term such as nucleic acid metabolism. The number of Ty1 restriction genes (list hits) associated with at least one GOBP term ranged from 5 (telomerase-independent telomere maintenance) to 51 (nucleic acid metabolism), while the number of unique yeast genes (population hits) annotated to a GOBP ranged from 8 (negative regulation of transposition) to 1490 (nucleic acid metabolism). When the list hits of Ty1 restriction genes and population hits of unique yeast genes were compared, the top-scoring GOBP terms were enriched for DNA repair and recombination, regulation of transposition, transcription, the cell cycle, cell proliferation, and chromatin transactions, with *P*-values ranging from 3.58×10^{-14} to 4.9×10^{-7}. Furthermore, 80% of the 85 annotated genes identified here are involved in nuclear processes. Together, these results suggest that a limited number of cellular functions inhibit Ty1 movement in the yeast genome.

To compare the enrichment levels and patterns of GOBP terms associated between the gene list obtained in our screen for Ty1 restriction genes and the terms obtained in previous screens for Ty1 and Ty3 cellular modulators, GOBP heat maps (supplemental Figure S1 and Figure 2) were generated using the data from our systematic screen (A-R), a subgenomic screen for Ty1 restriction genes using transposon mutagenesis (B-R) (Scholes *et al.* 2001), a systematic screen for Ty1 helper genes (C-H) (Griffith *et al.* 2003), and a systematic screen for Ty3 restriction (D-R) and helper genes (D-H) (Irwin *et al.* 2005). Briefly, enrichment levels of each GOBP term were computed for gene lists from each Ty modulator screen, and the results were combined into a data matrix used for the comparison. The matrix was displayed in color-coded heat maps to reveal the patterns of related biological processes identified by comparing different Ty modulator gene lists. The color coding of the heat maps is related to the enrichment of genes with specific GOBP terms. Increasing shades of red indicate higher enrichment and black indicates no enrichment. Two heat maps are included. The first (supplemental Figure S1) compares all GOBP terms associated with the 85 Ty1 restriction genes identified here (supplemental Table S1) and the second (Figure 2) compares more detailed GOBP terms from these restriction genes. Also note that novel genes and GOBP terms obtained in the other screens are not represented in the heat maps because comparisons were made with the GOBP terms identified in our screen for Ty1 restriction genes.

The systematic screen for Ty1 restriction genes presented here verified and expanded the number of GOBP terms identified by Scholes *et al.* (2001) (compare A-R with B-R) (supplemental Figure S1 and Figure 2). There was significant overlap between a subset of GOBP terms, because we recovered 12/21 mutants (Figure 3) previously identified as nonmarginal inhibitors of Ty1 mobility. These included general nuclear processes such as DNA replication, repair, and transcription, as well as the regulation of transposition (supplemental Figure S1 and Figure 2). We identified genes with novel GOBP terms for stress responses and signaling, transcriptional regulation

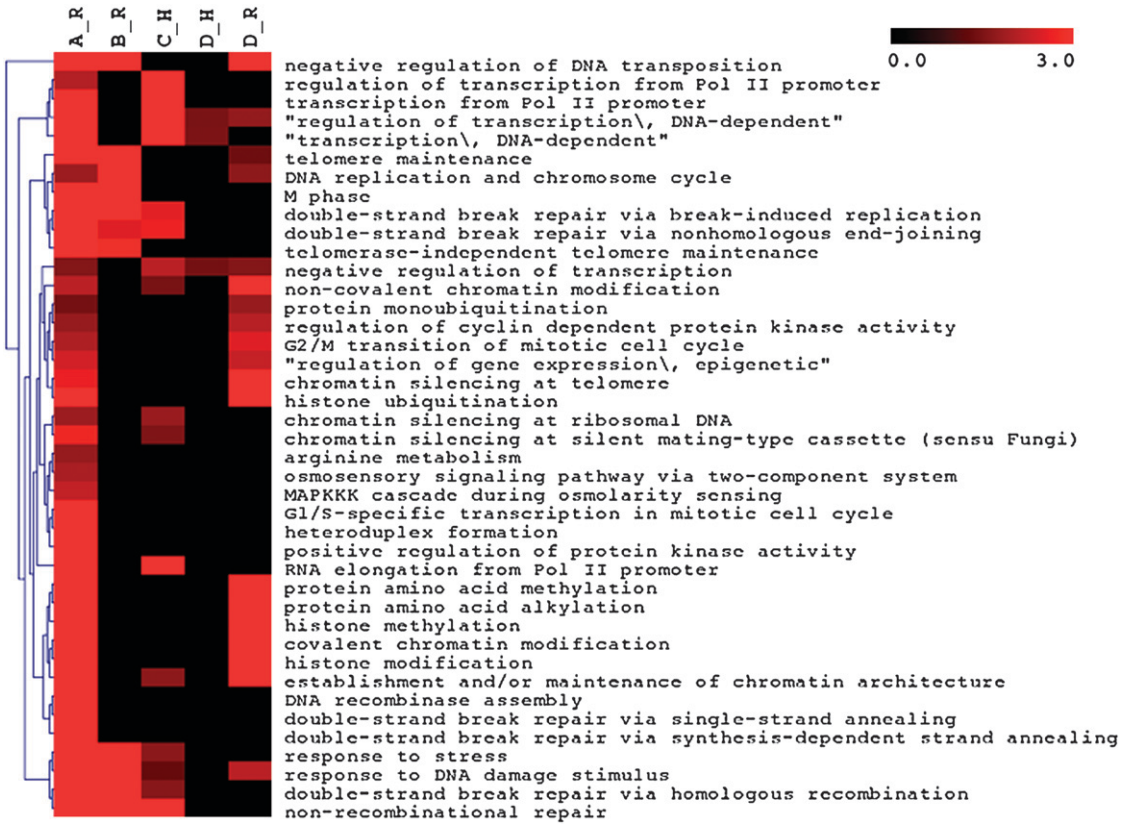

Figure 2.—Heat map of enrichment scores of GO biological process terms (GOBPs) for gene lists derived from independent screens for Ty1 (Scholes *et al.* 2001; Griffith *et al.* 2003) and Ty3 modulators (Irwin *et al.* 2005). The gradient of red color indicates the enrichment levels with black representing no enrichment [maximum enrichment ≥3 (*P*-value ≤0.001), no enrichment = 0 (*P*-value >0.05)]. Rows of the heat map are GOBPs and columns are the genetic screens for Ty modulators. A-R: more detailed GOBPs associated with Ty1 restriction genes identified in this work (supplemental Table S1). A heat map containing all GOBPs associated with Ty1 restriction genes identified here is shown in supplemental Figure S1. B-R: GOBPs associated with Ty1 restriction genes identified by transposon mutagenesis (Scholes *et al.* 2001). C-H: GOBPs associated with Ty1 helper genes identified by systematic screening of a diploid deletion library (Griffith *et al.* ;2003). D-R: GOBPs associated with Ty3 restriction genes identified by systematic screening of a haploid deletion library (Irwin *et al.* 2005). D-H: GOBPs associated with Ty3 helper genes identified by systematic screening of a haploid deletion library (Irwin *et al.* 2005). Hierarchical clustering of the GOBPs for Ty modulators is shown on the left.

and elongation, and several aspects of chromatin structure and function.

Several common GOBP clusters were also shared between the screen reported here and the systematic screen for Ty1 helper genes performed by Griffith *et al.* (2003) (supplemental Figure S1 and Figure 2; compare A-R and C-H). The GOBP terms tended to be more general (refer to the extreme top and bottom clusters of the heat map, supplemental Figure S1), although more specific processes such as negative regulation of transcription, RNA elongation, and DNA double-strand break repair were identified in both screens (supplemental Figure S1 and Figure 2). However, an apparent conflict was created by common GOBP terms associated with several genes identified in both screens, because the Griffith *et al.* (2003) systematic screen identified Ty1 helper genes, whereas our screen identified Ty1 restriction genes (see below, Figure 3). Genes involved in RNA processing and turnover, translation, and protein folding and trafficking are required for Ty1 mobility (Griffith *et al.* 2003), but were not highly represented in our screen for restriction genes. Together, these results suggest that common as well as distinct processes help or restrict Ty1 mobility, a property that may reflect the interactive capacity of regulatory networks in yeast (Harrison *et al.* 2007).

Although Ty1 and Ty3 are both LTR retrotransposons inhabiting the Saccharomyces genome, they are distantly related (Eickbush and Malik 2002) and, therefore, may have unique interactions with their host cells. Consistent with this idea, the heat map analyses (supplemental Figure S1 and Figure 2) show several processes that restrict Ty1 but not Ty3 mobility (compare A-R and D-R), such as stress responses and DNA double-strand break repair. However, several closely related processes restricted Ty1 and Ty3 mobility, including chromatin transactions and chromatin-based gene silencing at telomeres and the response to DNA damage.

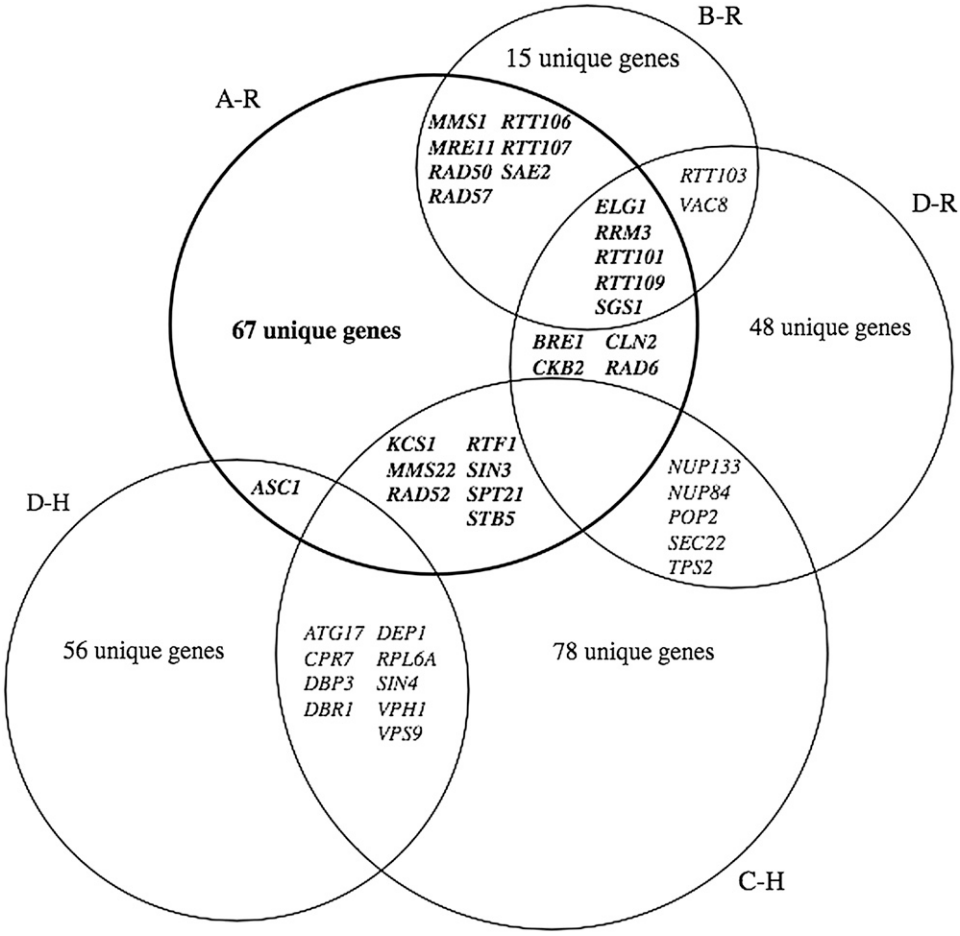

FIGURE 3.—Identification of common and unique Ty modulators. Abbreviations are defined in supplemental Figure S1 and in Figure 2. The shaded circle represents Ty1 restriction genes identified in this work (A-R). No genes in common were found when B-R and C-H, and B-R and D-H, were compared. Also refer to MAXWELL and CURCIO (2007) for further comparative analyses of Ty1 (B-R and C-H) and Ty3 (D-R and D-H) modulators.

There were few cellular processes that restricted Ty1 but were required for Ty3 mobility (compare A-R and D-H). This probably reflects the large number of genes involved in RNA metabolism and protein trafficking that are required for Ty3 and in certain instances Ty1 mobility (GRIFFITH *et al.* 2003; IRWIN *et al.* 2005; MAXWELL and CURCIO 2007). Together, these results indicate that both element-specific and shared cellular processes affect Ty1 and Ty3 movement. Our results also suggest the possibility that similar element–host interactions exist with Ty1/Copia and Ty3/Gypsy family members present in other organisms.

Common and unique cellular modulators obtained in different genetic screens: We cross-referenced the four screens for Ty1 or Ty3 modulators to determine the number of identical genes identified in each screen (Figure 3). As mentioned above, over half of the restriction genes identified by SCHOLES *et al.* (2001) were reisolated here, including *RTT101*, *RTT106*, *RTT107*, and *RTT109*. *BRE1*, *CKB2*, *CLN2*, *ELG1*, *RAD6*, *RRM3*, *RTT101*, *RTT103*, *RTT109*, *SGS1*, and *VAC8* restricted both Ty1 and Ty3 mobility, forming a core set of retrotransposon restriction genes and functions. In particular, *BRE1* and *RAD6* are responsible for ubiquitinating histone H2B, additional histone modifications (DOVER *et al.* 2002; NG *et al.* 2002; SUN and ALLIS 2002), and transcription elongation by RNA polymerase II (XIAO *et al.* 2005). *RAD6* is also implicated in determining Ty1 insertion-site preference at several loci (KANG *et al.* 1992; LIEBMAN and NEWNAM 1993; HUANG *et al.* 1999). *RRM3* may inhibit Ty1 cDNA recombination (SCHOLES *et al.* 2001), and *SGS1* minimizes multimeric Ty1-integration events (BRYK *et al.* 2001). Therefore, it will be important to determine whether a given restriction gene affects the same process during Ty1 and Ty3 retrotransposition.

Seven genes were identified that restricted Ty1 mobility in our systematic screen yet were required for Ty1 mobility in the screen performed by GRIFFITH *et al.* (2003) (Figure 3; *KCS1*, *MMS22*, *RAD52*, *RTF1*, *SIN3*, *SPT21*, and *STB5*). Although there were several differences in the way the screens were performed, including cell type and incubation temperature, a major reason for this discrepancy in the data was that different Ty1 mobility assays and secondary tests were employed. GRIFFITH *et al.* (2003) used a Ty1*HIS3* element expressed at a high level from the *GAL1* promoter carried on an episomal plasmid (termed a pGTy1 element), whereas we used a chromosomal Ty1*his3-AI* element expressed from its natural promoter that will yield a phenotypic signal only if reverse transcription of Ty1-*HIS3* mRNA occurs. Expression of a pGTy1 element overrides post-translational and copy number control

mechanisms (Curcio and Garfinkel 1992; Garfinkel *et al.* 2003) and, therefore, may have biased the genes identified in the Griffith *et al.* (2003) screen. Conversely, the Ty1*his3-AI* mobility assay is very sensitive with a dynamic range over several orders of magnitude (Curcio and Garfinkel 1991). Since *GAL1*-promoted Ty1 and native Ty1 RNA levels were not determined in the helper mutants identified by Griffith *et al.* (2003), Ty1 expression may have been altered in certain mutants, even though *GAL1* expression apparently remained unchanged, as judged by a qualitative assay using a *GAL1-lacZ* reporter. A control for the level of DNA recombination between pGTy1*HIS3* and chromosomal Ty1 sequences or the internally deleted *his3-Δ1* locus was also not included, which could be the reason for identifying the DNA repair and recombination gene, *RAD52*, as a Ty1 helper (Griffith *et al.* 2003). Finally, four of the Ty1 restriction genes in conflict have been described independently (*RAD52*) (Rattray *et al.* 2000) or confirmed by gene disruption (*KCS1*, *RTF1*, and *SIN3*) in a strain (JC3787) containing a different chromosomal Ty1*his3-AI* insertion (supplemental Table S1).

Although 31 genes were isolated in more than one of the Ty modulator screens, a large number of unique genes were identified in the various screens (Figure 3). Isolation of 160 and 104 unique modulators of Ty1 and Ty3 retrotransposition, respectively, suggests that the life cycles of these elements may indeed have different genetic requirements, which is consistent with several differences in their mode of retrotransposition. For example, Ty1 elements can mutate cellular genes and their transcripts accumulate to very high levels (Voytas and Boeke 2002), whereas Ty3 elements do not mutate cellular genes and Ty3 transcription and retrotransposition are induced by mating pheromones (Sandmeyer *et al.* 2002). Ty1 and Ty3 also utilize different targeting strategies at their preferred sites of insertion upstream of tRNA genes. Another possibility is that different factors involved in the same biological process (supplemental Figure S1 and Figure 2) modulate Ty1 and Ty3 retrotransposition. For example, *BRE1* and *RAD6* restrict Ty1 and Ty3 mobility; however, several genes composing the Paf complex, which interact with *BRE1* and *RAD6*, restrict Ty1 but apparently do not modulate Ty3 mobility (Aye *et al.* 2004; Irwin *et al.* 2005). *GAL*-Ty3 expression may have also biased the mutants identified in the screen performed by Irwin *et al.* (2005).

Alternatively, the large number of different Ty modulators identified in the various screens may act through a few common pathways having many inputs. Support for this idea is evident from recent results indicating that loss of any one of 19 genome integrity genes stimulates Ty1 transposition by activating S-phase checkpoints caused by an increase in intrinsic DNA-damage or -replication blocks (Curcio *et al.* 2007). Therefore, epistasis analysis between additional Ty1 and Ty3 restriction genes and S-phase DNA checkpoint genes will further define the genetic pathways restricting transposition. How S-phase checkpoint activation stimulates Ty retrotransposition remains to be determined, but multiple steps in the process of retrotransposition and its control may be involved.

Cellular genes restrict Ty1*his3-AI* mobility to varying degrees: the chromatin/transcription group: The frequency of Ty1*his3-AI*-mediated His^+ events was determined for 33 Ty1 restriction mutants chosen for further analysis (Figure 1) that have defects in chromatin/transcription, stress response, and miscellaneous functions (Table 2, supplemental Table S1). The chromatin/transcription gene deletions conferred an increase in Ty1*his3-AI* mobility ranging from 5- to 275-fold. Interestingly, several members of the Paf transcription complex were identified as Ty1 restriction genes. Deletion of *CDC73*, *LEO1*, *PAF1*, and *RTF1* enhanced Ty1*his3-AI* mobility 16- to 101-fold. The Paf complex is required for transcription elongation, 3′-end formation (Squazzo *et al.* 2002; Rondon *et al.* 2004; Penheiter *et al.* 2005; Sheldon *et al.* 2005), and histone H2B ubiquitination (Ng *et al.* 2003; Wood *et al.* 2003b; Xiao *et al.* 2005), where it acts in concert with Bre1p and Rad6p (Hwang *et al.* 2003; Wood *et al.* 2003a). *RAD6* was previously identified as a Ty1 modulator (Picologlou *et al.* 1990) and was also detected in our screen (supplemental Table S1). In addition, Ty1*his3-AI* mobility increased 34-fold in a *bre1*Δ mutant (Table 2), which was also detected in our screen. Genes required for histone acetylation by the NuA4 complex (*EAF3*) (Reid *et al.* 2004), histone chaperone activity (*ASF1*) (Schwabish and Struhl 2006), histone deacetylation by the Rpd3-Sin3 complex (Kadosh and Struhl 1998), histone gene transcription by *SPT21* (Dollard *et al.* 1994), and one of the histone H3 subunit genes, *HHT1*, restricted Ty1 mobility from 5- to 131-fold. It is surprising that deleting *RPD3* or *SIN3* increased Ty1 mobility 131- or 5-fold, respectively, since mutations in these genes usually confer similar phenotypes (Stillman *et al.* 1994; Kasten *et al.* 1996). Disrupting *RPD3* and *SIN3* in the wild-type strain JC3787 (supplemental Table S1) recapitulated the different levels of Ty1 mobility observed in the original *rpd3*Δ and *sin3*Δ strains, suggesting that suppressor mutations in additional Ty1 modulator genes were not present in the original mutants.

Functional relationships between members of the chromatin/transcription genes identified here and in other studies were also observed. For example, Rtt109p, which was initially identified by Scholes *et al.* (2001), has recently been shown to promote genome stability by acetylating histone H3 K56 in association with Asf1p (Driscoll *et al.* 2007), a Ty1 modulator identified in our screen. Asf1p also interacts with members of the chromatin assembly factor (CAF-1) and the HIR complex (Green *et al.* 2005), both of which have been implicated in restricting Ty1 transposition (supplemental Table S1) (Qian *et al.* 1998). In addition, a previously

TABLE 2

Effects of deleting Ty1 restriction genes on Ty1 mobility and *CAN1* mutagenesis

Gene deleted[a]	Ty1*his3-AI* mobility × 10^{-5} (SD)	Ty1 mobility, fold increase	*SUF16* insertions[a]	Ty1 RNA, fold change	Ty1*his3-AI* RNA, fold change	Ty1 cDNA, fold change	Can^R level[b]
Wild type	0.16 (0.03)[c]	1	[d]	1	1	1	0
Chromatin/transcription							
ARG82	23 (11)	144	[e]	4.4	16	13.9	0
ASF1	5 (0.8)	31	[e]	5.2	1.6	5	+
BRE1	5.4 (0.6)	34	[f]	1.8	1.4	4.3	+
CDC73	2.6 (0.3)	16	[f]	1.2	1.4	2	0
CSE2	5 (0.2)	31	[e]	1.7	1.3	4.2	0
EAF3	1 (0.1)	6	[d]	1	1.5	1	0
ELF1	4 (0.4)	25	[e]	1	1	1	+
HHT1	1.2 (0.1)	8	[f]	3	2.6	1	0
LEO1	4.2 (0.4)	26	[f]	1	1	3	+
PAF1	16.2 (7.0)	101	[e]	2.8	2.8	4.3	+
RPD3	21 (1.4)	131	<WT[g]	0.6	1.3	1	0
RTF1	2.8 (0.6)	17.5	[e]	1.5	1.3	2.5	+
SIN3	0.8 (0.1)	5	<WT[g]	0.5	1	1.4	0
SOH1	5.6 (0.9)	35	[d]	2	2.6	2.7	0
SPT21	2.7 (0.5)	17	[f]	3.4	3	3	0
SPT5#[h]	44 (10)	275	[e]	2.8	8.4	2.5	+
SRB5	7 (0.4)	44	[e]	2.8	4.2	5	+
Stress response							
ASC1	4.4 (3.1)	27.5	[d]	1.5	1.5	4.8	0
IPK1	3.4 (0.3)	21.3	[e]	5	2.8	4	0
KCS1	2.4 (0.43)	15	[f]	3.7	2.4	3.8	0
MMS22	1.5 (0.3)	9.4	[f]	2	1	4.2	0
PBS2	0.5 (0.11)	3	[d]	1	1.3	2	+
SSK2	0.7 (0.02)	4.4	[d]	1	1.3	2	0
SSK22	0.6 (0.08)	3.8	[d]	1	2	1	0
Miscellaneous							
AGP3	1.3 (1.0)	8	[f]	1	1.7	1	0
ALR2	0.7 (0.13)	4.4	[d]	0.5	1	0.6	0
BEM4	2.2 (0.6)	13.8	[f]	2	2.6	1	0
BUD27	38 (1.4)	237.5	[f]	0.8	6.3	0.5	0
CKB2	9.7	60.6	[f]	1.6	1.8	0.7	0
CLN2	1 (0.2)	6.3	[d]	1.4	2.6	1.7	0
SIC1	1.6 (0.01)	10	[f]	2	2.4	2.4	0
Uncharacterized							
YPL183C (*RTT10*)	5 (0.2)	31.3	[f]	1.7	2.4	1.9	0

[a] Mutants analyzed in parallel for insertions at *SUF16* are underlined (supplemental Figure S2).
[b] Mutations that confer a mutator phenotype at the *CAN1* locus. 0, wild-type level of Can^R; +, increased level of Can^R. Also refer to Figures 4 and 5 and to supplemental Tables S4–S6.
[c] Average of 13 trials.
[d] Wild-type level of insertions as judged by intensity of the banding pattern upstream of *SUF16*.
[e,f] Relative increase in the intensity of the banding pattern.
[g] Relative decrease in the intensity of the banding pattern. WT, wild type (also refer to supplemental Figure S2).
[h] *YML009W-B* overlaps *SPT5*, designated as *SPT5#*.

identified Ty1 restriction gene, *RTT106* (Scholes *et al.* 2001), encodes another histone chaperone involved in heterochromatin silencing along with CAF-1 (Huang *et al.* 2007).

Genes encoding subunits of the RNA polymerase II Mediator complex (Dotson *et al.* 2000; Lewis and Reinberg 2003; Guglielmi *et al.* 2004) modulate Ty1 mobility. We identified three Mediator genes, *CSE2*, *SOH1*, and *SRB5* that restrict Ty1 mobility from 31- to 44-fold (Table 2), while Scholes *et al.* (2001) identified *MED1* and a viable allele of *NUT2* that also restrict Ty1 mobility. Griffith *et al.* (2003) identified three other Mediator subunit genes, *SIN4*, *SRB8*, and *SSN2* that are required for Ty1 mobility. The Mediator subunits that help or restrict Ty1 mobility are correlated with Mediator modules that activate or repress transcription (Dotson

et al. 2000; Lewis and Reinberg 2003; Guglielmi *et al.* 2004). Ty1 helper proteins Srb8p and Ssn2p, and Sin4p contribute to the Cdk8 and Tail modules, respectively; the Ty1 restriction protein Srb5p is present in the Head module; and the restriction proteins Cse2p, Med1p, Nut2p, and Soh1p are associated with the Middle module. Together our results show that genes involved in histone dynamics and transcription restrict Ty1 mobility.

Two genes chosen for further study are involved in transcription elongation and pre-mRNA processing. Deletion of the transcription elongation gene *ELF1* (Prather *et al.* 2005) increased Ty1 mobility 25-fold (Table 2, supplemental Table S1). Deletion of the dubious ORF, *YML009W-B*, probably created a viable deletion allele of *SPT5* (designated *SPT5#*), an essential gene involved in transcription elongation of RNA polymerase I and II and processing of pre-mRNA and rRNA (Hartzog *et al.* 1998; Lindstrom *et al.* 2003; Schneider *et al.* 2006). This deletion elevated Ty1 mobility 275-fold. *ELF1* is synthetically lethal with *SPT4*, *-5*, and *-6*, as well as with members of the Paf complex (Prather *et al.* 2005). Elf1p also associates with casein kinase 2 and a regulatory subunit of casein kinase 2, Ckb2p, was identified in our screen. Therefore, several of the Ty1 restriction genes identified in our screen have genetic or physical interactions with *ELF1*, including *SPT5*, genes composing the Paf complex, and *CKB2*.

We chose one pathway-specific gene regulator, *ARG82*, to analyze further because Ty1 mobility increased 144-fold in an *arg82*Δ mutant. Arg82p is a multifunctional protein with inositol polyphosphate multikinase activity and is a component of the ArgR repressor that cooperates with diverse sequence-specific transcription factors to control transcription of arginine-, phosphate-, and nitrogen-responsive genes (Odom *et al.* 2000; Yoon *et al.* 2004; York 2006). However, the Arg82p kinase activity is not required for regulation of arginine gene expression in yeast (Dubois *et al.* 2000).

Ty1 restriction genes involved in stress responses: Genes involved in various types of stress responses restricted Ty1 mobility 3- to 144-fold, including those affecting osmotic challenge through the high-osmolarity glycerol (HOG) pathway (*PBS2*, *SSK2*, and *SSK22*) (Hohmann 2002), ribosome-associated signal transduction (*ASC1*) (Nilsson *et al.* 2004; Valerius *et al.* 2007), ionizing radiation (*MMS22*) (Baldwin *et al.* 2005), and inositol signaling (*ARG82*, *IPK1*, and *KCS1*) (Odom *et al.* 2000; Shears 2000; Dubois *et al.* 2002; Auesukaree *et al.* 2005; York 2006) (Table 2, supplemental Table S1). Although genes composing the HOG pathway modestly restricted Ty1 mobility, our results extend the work of Conte *et al.* (2000) who showed that inactivation of the HOG pathway stimulates Ty1 transposition by precociously activating the haploid invasive-growth pathway. Genes involved in inositol phosphate metabolism also restricted Ty1 mobility. As mentioned above, *ARG82* is a potent Ty1 restriction gene and has roles in both gene regulation and inositol metabolism. Deletion of *IPK1*, which encodes a nuclear inositol 1,3,4,5,6-pentakisphosphate 2-kinase involved in a variety of cellular processes including mRNA export and telomere maintenance (York *et al.* 2005; Alcazar-Roman *et al.* 2006; York 2006), increased Ty1 mobility ~21-fold. Deletion of *KCS1*, which encodes an inositol hexakisphosphate required for resistance to salt stress, cell wall integrity, vacuolar morphogenesis, and phosphate regulation (Dubois *et al.* 2002; Auesukaree *et al.* 2005), enhanced Ty1 mobility 15-fold. A variety of genes involved in DNA double-strand break repair and genome maintenance restrict Ty1 retrotransposition (Maxwell and Curcio 2007). Here we analyzed *MMS22*, a gene that interacts with previously identified Ty1 modulators *MMS1* (*RTT108*), *RTT101*, and *RTT107* to repair DNA damage associated with DNA replication (supplemental Table S1) (Scholes *et al.* 2001; Baldwin *et al.* 2005). Ty1 mobility increased >9-fold when *MMS22* was deleted, which is comparable to the Ty1 mobility observed in an *rtt107* mutant (11-fold), but is lower than that obtained in *mms1* (75-fold) or *rtt101* (60-fold) mutants (Scholes *et al.* 2001).

Ty1 restriction genes involved in miscellaneous functions: We chose genes involved in several additional cellular processes such as transport of amino acids (*AGP3*) and magnesium ions (*ALR2*), cell polarity (*BEM4* and *BUD27*), cell-cycle progression (*CDC40*, *CLN2*, and *SIC1*), and protein phosphorylation by casein kinase 2 (*CKB2*) to learn more about the diversity of pathways that restrict Ty1 mobility (Table 2, supplemental Table S1). In particular, deletion of *BUD27*, *CDC40*, or *CKB2* dramatically increased Ty1 mobility ~237-, 87-, and 60-fold, respectively. *BUD27* encodes a prefoldin protein chaperone involved in bud-site selection, nutrient signaling, and gene expression controlled by the TOR kinase (Gstaiger *et al.* 2003). *CDC40* encodes a pre-mRNA splicing factor required for cell-cycle progression at the G_1/S and G_2/M transitions (Kaplan and Kupiec 2007). As mentioned above, *CKB2* encodes a β-regulatory subunit of casein kinase 2, a Ser/Thr protein kinase with wide-ranging roles in cell growth, the cytoskeleton, DNA checkpoint activation, and transcription (Ghavidel *et al.* 1999; Ahmed *et al.* 2002; Prather *et al.* 2005; Guillemain *et al.* 2007).

Although we recovered several uncharacterized Ty1 restriction genes (supplemental Table S1; *YLR282C*, *YPL183C*, *YGR110W*, *YJR142W*, *YBR239C*, and *YEL008W*), only *YPL183C* (*RTT10*) was analyzed further because deleting *RTT10* increased Ty1 mobility 31-fold (Table 2), which is more than that from the other uncharacterized genes recovered in our screen. Rtt10p may be present in a complex with the tRNA methyltransferase Trm7p (Pintard *et al.* 2002; Krogan *et al.* 2004, 2006), which was also identified here (supplemental Table S1).

Integration at preferred sites upstream of *SUF16* increases in many Ty1 restriction mutants: To determine

whether deletion of a Ty1 restriction gene influenced *de novo* retrotransposition events, spontaneous Ty1 insertions upstream of a preferred tRNA target, the *SUF16* locus on chromosome III (Ji *et al.* 1993), were monitored using a qualitative PCR assay (Table 2, supplemental Figure S2 at http://www.genetics.org/supplemental/). Three independent colonies per strain from 33 Ty1 restriction mutants were chosen for PCR analysis using one primer containing Ty1 RT sequence and one primer from *SNR33*, which is adjacent to *SUF16* (supplemental Figure S2). Ty1 transposition events several hundred base pairs upstream of and in the same transcriptional orientation as *SUF16* were detected after separation of the PCR products by agarose-gel electrophoresis, staining with ethidium bromide, and phosphorimage analysis. The intensity of the PCR products indicated that *de novo* Ty1-integration events were elevated when compared with the pattern and intensity observed with the wild-type strain DG2122. To assess the variation inherent in this Ty1-integration assay, 12 representative restriction mutants were analyzed in parallel, and comparable integration patterns and intensities were obtained when compared with those of the original mutants (supplemental Figure S2). Amplification of the *CPR7* gene served as a PCR control (data not shown).

Most Ty1 restriction mutants showed a concomitant increase in Ty1*his3-AI* mobility and integration events upstream of *SUF16*, with *arg82*Δ, *asf1*Δ, *cse2*Δ, *elf1*Δ, *paf1*Δ, *rtf1*Δ, *spt5*Δ#, and *srb5*Δ mutants in the chromatin/transcription group and an *ipk1*Δ mutant in the stress-response group showing the largest increases (Table 2 and supplemental Figure S2). We did not observe a change in the pattern of Ty1-integration events upstream of *SUF16* in any of the restriction mutants, suggesting that Ty1*his3-AI* mobility faithfully monitored Ty1 retrotransposition and that normal targeting preferences at *SUF16* were maintained. Since *CDC40* encodes a splicing factor, the increase in integration events at *SUF16* in this mutant also suggests that alterations in splicing of the Ty1*his3-AI* intron cannot account for the increase in Ty1 mobility. However, Ty1*his3-AI* mobility increased much more than the level of Ty1 integration at the *SUF16* locus in the prefoldin mutant *bud27*Δ, the histone deacetylase subunit mutant *rpd3*Δ, the ribosome-associated regulatory mutant *asc1*Δ, and the transcriptional Mediator subunit mutant *soh1*Δ (Table 2 and supplemental Figure S2). As mentioned above, homologous recombination between Ty1 cDNA and chromosomal elements may increase in these restriction mutants and, if so, is consistent with the hyperrecombination phenotype observed in a *soh1* mutant (Fan and Klein 1994). Other possibilities are that the differences in Ty1*his3-AI* mobility and *SUF16* insertion levels reflect a biased insertion orientation or novel insertion sites in the genome.

Most restriction genes act post-transcriptionally to inhibit Ty1 mobility: Total RNA isolated from the 33 mutants as well as from the wild-type strain DG2122 was subjected to Northern hybridization using a ^{32}P-labeled probe from the Ty1 RT region (Table 2, supplemental Figure S3 and supplemental Table S2 at http://www.genetics.org/supplemental/). The Ty1 probe also detects the Ty1*his3-AI* transcript from pBJC573 due to the size increase resulting from the presence of *his3-AI* (data not shown). The level of Ty1 transcripts was normalized to that of the 18S and 28S rRNAs, as determined by phosphorimage analysis. Ty1 RNA increased less than threefold in 28 of the 33 restriction mutants while the level of Ty1 RNA increased threefold or more in 5 mutants. Surprisingly, the level of Ty1 RNA increased less than threefold in cells lacking the Mediator subunit genes identified in our screen (*CSE2*, *SOH1*, and *SRB5*) or elsewhere (*MED1* and *NUT2*) (Scholes *et al.* 2001). These results suggest that the Mediator complex may act indirectly by influencing expression of other Ty1 modulator genes or restricts Ty1 mobility post-transcriptionally. The levels of Ty1 and Ty1*his3-AI* transcripts usually showed similar changes in abundance in most restriction mutants; however, the levels of Ty1 and Ty1*his3-AI* RNAs were markedly different in *arg82*Δ, *asf1*Δ, *srb5*Δ, *spt5*Δ#, *bud27*Δ, and *ipk1*Δ mutants. Two altered patterns were observed. The level of the Ty1*his3-AI* transcript increased more than the level of Ty1 RNA in strains lacking *ARG82*, *SPT5#*, *SRB5*, or *BUD27*, while the converse occurred in the absence of *ASF1* or *IPK1*. The differences in Ty1 and Ty1*his3-AI* transcript levels in these mutants were not pursued further, but may result from exogenous signals from the *HIS4* promoter region that influence transcription of the Ty1*his3-AI* element integrated at *HIS4* (Silverman and Fink 1984). In addition, both element-based sequence polymorphisms and chromosomal context differences can influence transcription of resident Ty1 elements (Morillon *et al.* 2002).

The level of Ty1 RNA increased 3.7- to 5-fold in *arg82*Δ, *ipk1*Δ, and *ksc1*Δ mutants, suggesting that transcription or stability of Ty1 RNA is regulated by inositol phosphate metabolism or signaling to a downstream process. Alternatively, Ty1 transcription may be under the control of the ArgR repressor. Deletion of *ASF1*, the histone H3 subunit gene, *HHT1*, or a regulator of histone gene transcription, *SPT21*, increased the levels of Ty1 RNA 3- to 5.2-fold. These results suggest that genes involved in chromatin transactions negatively regulate the level of Ty1 RNA. Relief of chromatin-based repression of Ty1 transcription also occurs in cells lacking histone 2A subtype genes *HTA1* and *HTB1* (Hirschhorn *et al.* 1992; Morillon *et al.* 2002; Todeschini *et al.* 2005). Taken together, our results suggest that although most Ty1 restriction mutations affect Ty1 mobility at a post-transcriptional level, deletion of certain genes increases the level of genomic Ty1 RNA or affects the levels of Ty1 and Ty1*his3-AI* differentially.

Diverse cellular genes affect the level of Ty1 cDNA: We determined the level of unincorporated Ty1 cDNA

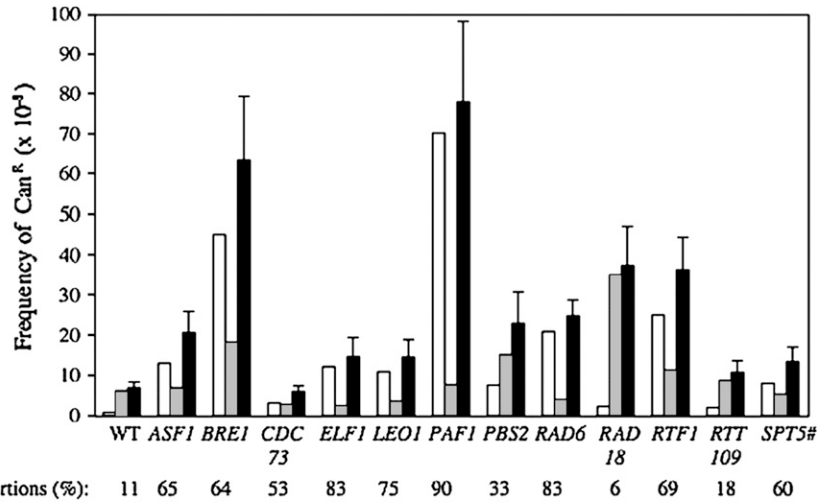

FIGURE 4.—Frequency estimate of Ty1- and non-Ty1-induced Can^R mutations. Open bars, frequency of Can^R mutations caused by Ty1 insertion; cross-hatched bars, frequency of Can^R mutations caused by other mutational events; solid bars, overall frequency of Can^R mutations. Standard deviations are above the solid bars. On the bottom is the fraction of Can^R mutations caused by Ty1. Also refer to supplemental Tables S4 and S5.

in the 33 restriction mutants and the wild-type strain DG2122 by Southern hybridization (Table 2, supplemental Figure S4 and supplemental Table S3 at http://www.genetics.org/supplemental/). Digestion of total DNA with *Pvu*II generates a characteristic 2-kb fragment containing sequences from a conserved internal *Pvu*II restriction site in Ty1 (nucleotide 3944) to the end of the linear unincorporated cDNA (nucleotide 5918) (CONTE *et al.* 1998; LEE *et al.* 1998) (supplemental Figure S4). A ^{32}P-labeled probe spanning part of this region of Ty1 was hybridized with the resulting membranes, and the 2-kb Ty1 cDNA fragment was quantified by phosphorimage analysis. The *Pvu*II fragments containing preexisting Ty1-genomic DNA junctions provide internal loading controls.

When the levels of unincorporated Ty1 cDNA in the restriction mutants and wild type were compared, there was <3-fold increase in Ty1 cDNA in 20 of the 33 restriction mutants, while a ≥3-fold increase was observed in 13 mutants (Table 2, supplemental Figure S4 and supplemental Table S3). The relatively modest elevation in Ty1 cDNA exhibited in a variety of restriction mutants suggests that the increase in cDNA may not entirely account for the change in Ty1 mobility. For example, when the Paf subunit genes *CDC73*, *RTF1*, and *LEO1* or the functionally related transcription elongation gene *ELF1* were deleted, Ty1 RNA and cDNA levels increased ≤3-fold, yet Ty1 *his3-AI* mobility increased 16- to 26-fold, and insertions at *SUF16* were also more frequent. Perhaps Ty1 cDNA is more efficiently utilized for retrotransposition in these mutants.

The 13 Ty1 restriction mutants showing threefold or more additional cDNA covered a variety of cellular functions. Certain mutants, such as *arg82*Δ, *asf1*Δ, *ipk1*Δ, *kcs1*Δ, and *spt21*Δ, had an elevated level of Ty1 RNA and cDNA, suggesting that an increase in Ty1 gene expression results in a higher level of Ty1 mobility. Several Ty1 restriction mutants, such as *bre1*Δ and *paf1*Δ, showed moderate increases in Ty1 RNA or cDNA and large increases in Ty1 mobility and insertions at *SUF16* (Table 2, supplemental Figure S2), again raising the possibility that cDNA utilization might be enhanced during the process of retrotransposition. Conversely, deletion of *ASC1* resulted in an increase in Ty1 cDNA and Ty1 *his3-AI* mobility, but *SUF16* insertions and Ty1 RNA remained at wild-type levels. These results can be explained by an elevation in cDNA recombination in the *asc1*Δ mutant, although a change in *his3-AI* splicing and Ty1 insertion preference remain formal possibilities.

Ty1 restriction genes that alter insertional mutagenesis and target-site preference at *CAN1*: Certain Ty1 restriction genes minimize transposition into coding sequences of genes, as first illustrated by the E2 ubiquitin conjugating gene, *RAD6* (LIEBMAN and NEWNAM 1993). Therefore, we determined whether any of the 33 Ty1 restriction mutants as well as several additional candidates from the larger collection (supplemental Table S1) possessed a mutator phenotype at the arginine permease gene *CAN1* (Table 2), where resistance to the amino acid analog canavanine (Can^R) occurs when *CAN1* is defective. Deletion of the Paf complex subunit genes *PAF1*, *LEO1*, and *RTF1*; the histone chaperone *ASF1*; the transcription elongation genes *ELF1* and *SPT5#*; the HOG pathway protein kinase gene *PBS2*; and the ubiquitin-metabolism genes *BRE1*, *RAD6*, and *RAD18* increased the frequency of Can^R from 2- to 11-fold when cells were grown at a permissive temperature for Ty1 retrotransposition (20°) (PAQUIN and WILLIAMSON 1984) (Figure 4, supplemental Table S4 at http://www.genetics.org/supplemental/). Unlike in the Paf complex mutants *leo1*Δ, *paf1*Δ, and *rtf1*Δ, the frequency of Can^R remained unchanged in a *cdc73*Δ mutant. Deleting *RTT109*, which encodes the histone H3 K56 acetylase and interacts with Asf1p (DRISCOLL *et al.* 2007), also did not alter the frequency of Can^R.

The fraction of Ty1-induced *can1* mutations was determined by PCR analysis using oligonucleotide primers specific to *CAN1* and Ty1 (Figure 4, supplemental Table S4). Reactions with primers that flank *CAN1* either amplified a 2283-bp product indicative of the wild-type

CAN1 locus or did not amplify a wild-type product. The mutant DNA samples that failed to amplify a wild-type *CAN1* product were analyzed with a primer specific to Ty1 and overlapping primers in opposite orientations located at the start of the *CAN1* coding region. In the wild-type strain DG2122, ~11% (4/36) of the *can1* mutations were caused by Ty1 insertion while the rest were caused by mutations that did not dramatically alter the size of the 2283-bp PCR product (supplemental Table S4), which was comparable to the fraction of *can1* mutations resulting from endogenous Ty1 insertions obtained previously in wild-type strains (Wilke *et al.* 1989; Picologlou *et al.* 1990; Liebman and Newnam 1993; Qian *et al.* 1998). The efficiency of Ty1 insertional mutagenesis at *CAN1* ranged between 6.25 and 90% in different restriction mutants. There was a dramatic increase in the fraction of Ty1-induced *can1* mutations in the strains lacking *PAF1* (90%), *ELF1* (83%), or *RAD6* (83%). Solo-LTR insertions, most likely caused by a Ty1 insertion followed by intraelement LTR–LTR recombination (Sutton and Liebman 1992), and putative deletion events occurred in the Ty1 restriction mutants at about the same level as had been observed in wild-type strains (Wilke *et al.* 1989; Picologlou *et al.* 1990; Liebman and Newnam 1993; Rinckel and Garfinkel 1996; Qian *et al.* 1998) (supplemental Table S5 at http://www.genetics.org/supplemental/).

Knowing the fraction of *can1* mutations resulting from Ty1 retrotransposition events allowed us to estimate the increase in Ty1 mutagenesis *vs.* non-Ty1 mutagenesis in the restriction mutants (Figure 4, supplemental Table S4). Although several mutational spectra are observed, the results suggest that most of the mutator activity observed at *CAN1* in the Ty1 restriction mutants is caused by Ty1 insertional mutagenesis and is not due to other mutational events. For example, Ty1-induced mutations at *CAN1* increased 90-fold in a *paf1*Δ mutant, whereas other mutational events remained at the wild-type level. Deletion of *BRE1* showed a modest elevation in non-Ty1 events of ~3-fold when compared to the >57-fold increase in Ty1 mutagenesis, while deletion of *PBS2* showed 2.5-fold more non-Ty1 events and a moderate ~10-fold increase in Ty1 insertions. Deletion of *ELF1* increased Ty1 mutagenesis by almost 16-fold, but surprisingly, non-Ty1-induced mutations decreased 5-fold when compared with the wild-type strain DG2122. It will be interesting to determine whether *elf1*Δ's non-Ty1 "antimutator" phenotype occurs elsewhere in the genome. Conversely, deleting *RAD18* increased the frequency of non-Ty1 *can1* mutations 5.6-fold, which is expected from previous work (Quah *et al.* 1980), while Ty1 insertions increased only 3-fold. Ty1*his3-AI* mobility was also higher in a *rad18*Δ mutant (supplemental Table S1), suggesting that insertions into preferred targets upstream of genes transcribed by RNA polymerase III or cDNA recombination may occur more often when *RAD18* is deleted.

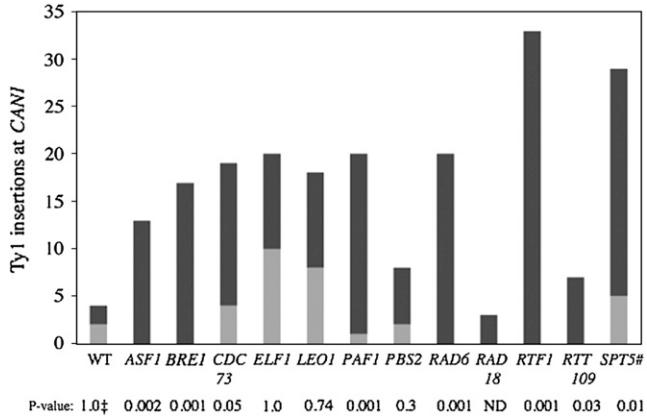

Figure 5.—Ty1 insertions in the *CAN1* promoter region *vs.* the coding sequence. Shaded bars, Ty1 insertions in the *CAN1* promoter region; solid bars, Ty1 insertions in the *CAN1* coding sequence. On the bottom are Ty1 restriction genes that were analyzed. *P*-values were obtained by comparing the distribution of promoter *vs.* coding sequence insertions in WT and Ty1 restriction mutants (‡, refer to materials and methods). Also refer to supplemental data for more information on the spectrum of Can^R mutations (supplemental Tables S4 and S5), the orientation of the Ty1 insertions (supplemental Table S6), and DNA sequence analysis of Ty1 insertions when *PAF1* was deleted (supplemental Figure S5).

Ty1 insertions at *CAN1* show a strong preference for the *CAN1* promoter region, with ~50% of Ty1 insertions occurring in a 317-bp window upstream and 50% in the 1773-bp open reading frame (Wilke *et al.* 1989; Picologlou *et al.* 1990; Liebman and Newnam 1993; Rinckel and Garfinkel 1996; Qian *et al.* 1998), which is similar to the distribution of Ty1 insertions at other genes such as *LYS2* and *URA3* (Eibel and Philippsen 1984; Simchen *et al.* 1984; Natsoulis *et al.* 1989). PCR analysis using *CAN1* primers specific to the beginning of the coding sequence allowed us to determine if the Ty1 insertion-site preference for the *CAN1* promoter region observed in wild-type cells was altered in the Ty1 restriction mutants (Figure 5, supplemental Table S5). There was a striking change in insertion-site preference in strains lacking *ASF1*, *BRE1*, *CDC73*, *PAF1*, *RTF1*, *RTT109*, and *SPT5#*, as well as *RAD6*. Between 78 and 100% of the Ty1 insertions occurred in the coding sequence of *CAN1* in these mutants, suggesting that Ty1 targeting was now random within the *CAN1* interval monitored in our analysis. Similar insertion patterns have also been observed in strains lacking *RAD6* (Liebman and Newnam 1993) and also when *CAC3*, which encodes a CAF-1 subunit (Ach *et al.* 1997), and *HIR3* were both deleted (Huang *et al.* 1999). The sizes of Ty1-*CAN1* PCR products suggested that Ty1 insertions occurred throughout *CAN1* (data not shown). In addition, sequence analysis of 15 Ty1 transposition events obtained in a *paf1*Δ mutant confirmed the change in Ty1 target-site preference (supplemental Figure S5 at http://www.genetics.org/supplemental/). Although

the fraction of Ty1-induced *can1* mutations increased from 3- to 7.5-fold in strains lacking *ELF1*, *LEO1*, or *PBS2*, the insertion-site preference remained the same (Figure 5). Taken together, these results suggest that certain defects in Paf complex function or transcriptional elongation fail to protect *CAN1* coding sequence from Ty1 insertional mutagenesis.

Almost all of the Ty1 insertions (28/29, supplemental Table S6 at http://www.genetics.org/supplemental/) within the promoter region were oriented such that Ty1 and *CAN1* transcription were in the same direction, as expected from previous work showing that adjacent gene activation occurs when Ty1 and target gene transcription occur in opposite directions (see review by Voytas and Boeke 2002). To address the possibility that a selection bias occurred with the coding-sequence insertions, tetrad analysis was performed after mating a given Ty1 restriction mutant with a wild-type strain (DG3027) containing a spontaneous Ty1 insertion in the promoter region of *CAN1* [*can1-26(Ty1)*, 126 bp from the start of the *CAN1* coding sequence], which is a common site for Ty1 integration in wild-type cells (Rinckel and Garfinkel 1996). Tetrad analysis (7–11 tetrads/Ty1 restriction mutant) of diploids derived from DG3027 and *asf1*Δ::*KanMX*, *cdc73*Δ::*KanMX*, *leo1*Δ::*KanMX*, *paf1*Δ::*KanMX*, *rtf1*Δ::*KanMX*, *rtt109*Δ::*KanMX*, and *spt5*#Δ::*KanMX* mutants showed 2:2 segregation for Can^R and $G418^R$ typical of unlinked markers, indicating that the restriction mutations do not suppress the Ty1-induced promoter mutation *can1-26(Ty1)*. The orientation of the Ty1 insertions in the *CAN1* coding sequence was not affected in the restriction mutants (supplemental Table S6).

Protecting the yeast genome from Ty1 insertional mutagenesis: The analyses of Ty1 insertions at *SUF16* and *CAN1* in different restriction mutants suggest that multiple pathways protect the yeast genome from insertional mutagenesis (Figures 4 and 5, Table 2, supplemental Figure S2). However, a prominent pathway identified in this work involves genes encoding the Paf complex subunits Cdc73p, Paf1p, Leo1p, and Rtf1p and Rad6p and Bre1p (Figure 6). Analyses of these Ty1 restriction genes suggests that H2B ubiquitination or possibly additional but as yet unidentified Rad6p substrates are required to minimize Ty1 insertions not only at sites upstream of genes transcribed by RNA polymerase II or III but also within protein-coding sequences. Therefore, it will be interesting to determine whether the process of Ty1 retrotransposition is restricted by *htb-K123R*, a mutation in histone H2B that prevents ubiquitination by Rad6p/Bre1p (Robzyk *et al.* 2000; Hwang *et al.* 2003; Wood *et al.* 2003a). Since H2B-K123 ubiquitination is required for H3-K4 methylation by Set1p and H3-K79 methylation by Dot1p (Ng *et al.* 2002; Sun and Allis 2002), Ty1 target-site preference may be modulated by histone H3 methylation events. *SET1* is also required to repress Ty1 transcription of

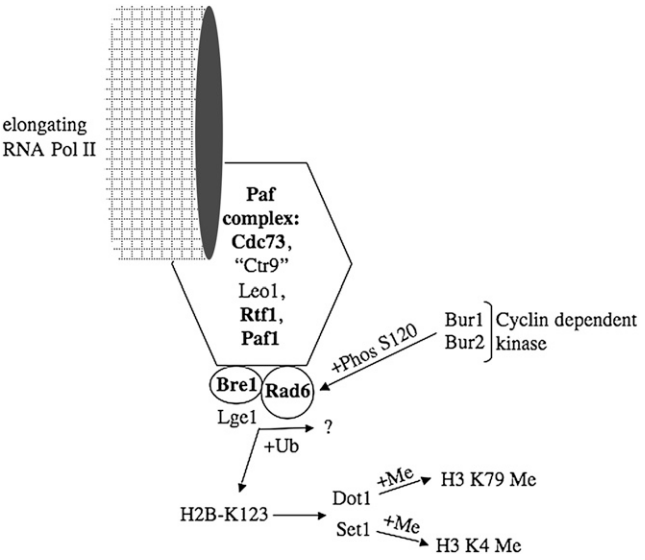

Figure 6.—Relationship between ubiquitination of proteins involved in transcription elongation by RNA polymerase II and by Ty1 transposition. Protein names (in boldface type) required for restricting Ty1 transposition and maintaining target site preference at *CAN1* include the Paf complex subunits Cdc73, Paf1, and Rtf1 and the Rad6-Bre1 ubiquitination (+Ub) complex that modifies histone H2B on K123. Rad6-Bre1 may also ubiquitinate additional proteins that have not been identified. The Paf1 complex subunit protein Leo1 is required for restricting Ty1 transposition but is not required for target-site preference at *CAN1*, and Ctr9 was not analyzed. The Bur1-Bur2 cyclin-dependent protein kinases (+Phos) and the histone H3 methylases (+Me) Dot1 and Set1 were not identified in our screen for Ty1 restriction genes, but may affect Ty1 target preference.

elements inserted in silent regions of the genome (Bryk *et al.* 2002). Rad6p undergoes phosphorylation by the Bur1p-Bur2p cyclin-dependent protein kinase on S120, which is required for full Rad6p-Bre1 ubiquitination of H2B (Wood *et al.* 2005), and raises the possibility that Rad6p 120S phosphorylation restricts Ty1 retrotransposition. In addition, our results suggest that Rad6p-Rad18p-mediated ubiquitination of PCNA (Bailly *et al.* 1997; Hoege *et al.* 2002) does not protect yeast coding sequences from Ty1 transposition, because deleting *RAD18* does not markedly alter the level of Ty1 insertional mutagenesis or preference at *CAN1*. Proteins functionally related to the Paf1 complex were also detected in our screen. In particular, the transcription elongation proteins Spt4p/Spt5p stimulate association of the Paf complex with elongating RNA polymerase II (Qiu *et al.* 2006). Therefore, partially deleting *SPT5* may increase Ty1 retrotransposition and insertions into *CAN1* coding sequences by inhibiting Paf complex function.

How does disrupting the Paf complex/Bre1p-Rad6p pathway stimulate Ty1 retrotransposition and mutagenesis throughout the genome? Our results suggest that the Paf/Bre1p-Rad6p pathway restricts Ty1 transposition at multiple steps. Since the level of Ty1 RNA increases less than threefold in strains lacking *BRE1*,

CDC73, *LEO1*, *PAF1*, and *RTF1* as well as remaining unchanged in a *RAD6* mutant (Picologlou *et al.* 1990), the Paf/Bre1p-Rad6p pathway may restrict Ty1 transposition post-transcriptionally. However, the cDNA level increases more than fourfold in a *PAF1* or a *BRE1* mutant, suggesting that an increase in Ty1 reverse transcription or cDNA stability contributes to the increase in Ty1 transposition. It will be interesting to determine whether defects in the Paf/Bre1p-Rad6p pathway increase Ty1 transposition via activation of an S-phase checkpoint (Curcio *et al.* 2007). Target-site selection is another step in the process of Ty1 retrotransposition that is restricted by the Paf/Bre1p-Rad6p pathway. We propose that even though regions upstream of genes transcribed by RNA polymerase III are considered hotspots for Ty1 integration, favorable insertion sites remain limiting in wild-type cells. In the absence of Paf/Bre1p-Rad6p, aberrant histone transactions create more favorable target sites on a genomic scale. However, the nature of the target sites revealed by compromising the Paf complex/Bre1p-Rad6p function remains unknown. Determining the full spectrum of Ty1-integration sites throughout the genome when histone H2B ubiquitination is blocked may provide further insights on target-site preference and genome protection.

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