Post-transcriptional Cosuppression of Ty1 Retrotransposition

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ABSTRACT

To determine whether homology-dependent gene silencing or cosuppression mechanisms underlie copy number control (CNC) of Ty1 retrotransposition, we introduced an active Ty1 element into a naïve strain. Single Ty1 element retrotransposition was elevated in a Ty1-less background, but decreased dramatically when additional elements were present. Transcription from the suppressing Ty1 elements enhanced CNC but translation or reverse transcription was not required. Ty1 CNC occurred with a transcriptionally active Ty2 element, but not with Ty3 or Ty5 elements. CNC also occurred when the suppressing Ty1 elements were transcriptionally silenced, fused to the constitutive *PGK1* promoter, or contained a minimal segment of mostly *TYA1-gag* sequence. Ty1 transcription of a multicopy element expressed from the *GAL1* promoter abolished CNC, even when the suppressing element was defective for transposition. Although Ty1 RNA and TyA1-gag protein levels increased with the copy number of expressible elements, a given element's transcript level varied less than twofold regardless of whether the suppressing elements were transcriptionally active or repressed. Furthermore, a decrease in the synthesis of Ty1 cDNA is strongly associated with Ty1 CNC. Together our results suggest that Ty1 cosuppression can occur post-transcriptionally, either prior to or during reverse transcription.

THE Ty1, -2, -3, -4, and -5 element families of *Saccharomyces cerevisiae* are composed of long terminal repeat (LTR) retrotransposons that replicate through an RNA intermediate and resemble retroviruses in many respects (Sandmeyer *et al.* 2002; Voytas and Boeke 2002). Ty elements are flanked by LTRs and are transcribed from end to end, resulting in an RNA that serves as template for both translation and reverse transcription. Translation results in the synthesis of TyA, a gag-like capsid protein, and a TyA-TyB (pol) fusion protein, which contains protease (PR), integrase, and reverse transcriptase H (RT). The structural and enzymatic proteins form a virus-like particle (VLP) within which protein maturation and reverse transcription occur. *Cis*-acting signals for Ty RNA packaging, dimer formation, and the initiation and progression of reverse transcription are present on Ty RNA. The resulting linear double-stranded cDNA enters the genome through integrase-mediated integration at preferred chromosomal sites, or, to a lesser degree, by homologous recombination with genomic elements.

How Ty elements entered the yeast genome and amplified to their present copy number is an important evolutionary question that applies to all transposable elements and their host genomes. Most laboratory strains contain 30–35 Ty1 elements, but natural isolates show a wider variation (Wilke and Adams 1992; Wilke *et al.* 1992). Many of these strains contain just a few Ty1 elements, but none harbor $>\sim$40 complete elements, which suggests that yeast possesses active mechanisms for limiting copy number. Doubling the number of Ty1 elements does not result in genome instability or obvious growth defects, indicating that Ty1 elements have not reached a critically high copy number in a laboratory strain (Boeke *et al.* 1991).

Minimizing the level of Ty1 retrotransposition is particularly important for maintaining the integrity of the yeast genome since these elements can mutate many genes, initiate genome rearrangements, and are the most abundant Ty-element family in laboratory strains. Although Ty1-element-mediated genome restructuring can result in increased fitness in response to environmental (Paquin and Williamson 1986; Dunham *et al.* 2002) or genetic challenges (Moore and Haber 1996; Teng *et al.* 1996), yeast cells usually minimize such events by modulating several steps in the Ty1 life cycle. Host genes have been identified that affect Ty1 transcription (Winston *et al.* 1984), programmed +1 frameshifting that is required to synthesize the TyA1-gag/TyB1-pol fusion protein (Farabaugh 1995), Ty1 protein processing and VLP maturation (Curcio and Garfinkel 1992; Conte *et al.* 1998), target site preference (Boeke and Devine 1998), and cDNA stability (Lee *et al.* 2000; Sundararajan *et al.* 2003). A variety of additional genes have been identified that modulate Ty1 retrotransposition, many of which are also involved in DNA repair and genome stability (Scholes *et al.* 2001).

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Ty1 elements have recently been shown to undergo a form of copy number control (CNC) called transcriptional cosuppression (Jiang 2002), which is a copy-number- and homology-dependent process used to silence repeated genes or "tame" transposable elements in many eukaryotes (Jorgensen 1995; Jensen *et al.* 1999; Whitelaw and Martin 2001). Ty1 transcriptional cosuppression is apparently unstable and is characterized by rapid switches between states where all Ty1 elements are either transcribed or shut off. Ty1 transcription is silenced in a subset of cells in a population, whereas Ty1 RNA is present in the rest. Since Ty1 RNA is very abundant and can comprise 0.1–0.8% of total RNA (Elder *et al.* 1980; Curcio *et al.* 1990), Ty1 transcripts and protein must be present in even higher amounts in many cells if transcription is silenced in certain cells.

The molecular mechanisms required for host defense against Ty1 element retrotransposition are indeed varied and far from being understood. Here, we have examined CNC of Ty1 retrotransposition using a variety of approaches aimed at defining the sequences involved and the steps in the process of retrotransposition where CNC takes place. Our mapping analyses indicate that a minimal segment of >1 kb containing mostly *TYA1-gag* sequence confers CNC on a target element. Ty1 CNC is greatly influenced by element context, copy number, and homology, but has features that differ from those required for Ty1 transcriptional cosuppression (Jiang 2002). In particular, Ty1 CNC is provoked by suppressing elements that are transcriptionally repressed or fused to the *PGK1* promoter. Although transcription of the suppressing Ty1 elements can enhance CNC, transcripts from the suppressed element are not markedly altered and retrotransposition of the suppressed elements decreases in all cases. Increasing the level of Ty1 RNA also leads to an increase in the level of TyA1-gag protein; however, a decrease in the synthesis of Ty1 cDNA is strongly associated with elements experiencing CNC. Therefore, Ty1 CNC occurs by both transcriptional and post-transcriptional cosuppression, as well as by the action of additional host defense genes.

MATERIALS AND METHODS

Genetic techniques, media, and strain construction: Yeast genetic techniques and media were used as described previously (Sherman *et al.* 1986; Guthrie and Fink 1991). The Ty1-less strains DG1768 (*MAT*α *his3-Δ200hisG ura3*), DG1929 [*MAT***a** *his3-Δ200hisG ura3* Ty1*his3-AI(96)*], and DG2196 [*MAT***a** *his3-Δ200hisG ura3 trp1* Ty1*his3-AI(96)*] were derived from strains 337 (*MAT*α *ura3 gal3* Ty1-less; Wilke and Adams 1992) and 155-5A (*MAT***a** *ura3-100* Ty1-155; kindly provided by M. Ciriacy and P. Phillipsen). The *his3-Δ200hisG* mutation was introduced into strain 337 by single-step gene disruption with plasmid pBDG652 digested with *Eco*RI and *Sph*I. Following selection for loss of the *URA3* gene, the resulting *his3-Δ200hisG* Rho$^-$ strain, DG1389, was crossed with a *kar1* mutant 8964-15A (kindly provided by G. Fink) to reintroduce functional mitochondria. The resulting strain, DG1423, was transformed with the Ty1*his3-AI* centromere plasmid pOY1 to form DG1767. Strain DG1768 is a Ura$^-$ plasmid segregant of DG1767. Strain DG1767 was crossed with 155-5A to obtain a Gal$^+$, Spo$^+$, Ty1-less, *MAT***a** strain, DG574-13D. Strain Y3056 contains a single Ty1*his3-AI(96)* element (also denoted as Ty1*his3-AI*) from the pGTy1*his3-AI* [artificial intron (AI); Curcio and Garfinkel 1991] derivative pBLR96 present in DG1768 that was induced for transposition (Boeke *et al.* 1985). Strains Y3056 and DG574-13D were crossed and then backcrossed to DG1768 six times to generate strain DG1929. Strain DG2196 was derived from DG1929 by selection with 2-amino-5-fluorobenzoic acid followed by complementation analysis to identify a *trp1* mutation (Toyn *et al.* 2000). Strains containing additional chromosomal Ty1 insertions were generated using the appropriate pGTy1 element, as described previously (Boeke *et al.* 1985). Strain DG2451 contains 20 Ty1 insertions, as estimated by Southern blotting (data not shown), and was derived from DG1768 transformed with pGTy1H3. Strain DG2353 contains *rad3-G595R* and was derived from DG1929 by two-step gene transplacement using plasmid pLAY182 digested with *Bgl*II (Bailis *et al.* 1995). Strains DG2388, DG2338, and DG2379 contain the *TRP1*-based integrating plasmids pRS404, pBDG1085 (pGTy1/404), and pBDG1095 (pGTy1-ATGfs/404), respectively, that were digested with *Bsu*36 I to target recombination to *TRP1*. Recombinants with a single copy of the integrating plasmids at *TRP1* were identified by Southern analysis using ^{32}P-labeled Ty1 and pBR322 probes.

Plasmids: Plasmids were constructed by standard techniques (Sambrook *et al.* 1989; Innis *et al.* 1990). Detailed information on plasmid constructions will be provided upon request. Plasmids pJef938 (kindly provided by J. Boeke), pGTy1H3 (Boeke *et al.* 1985), pGTy1*Neo* (Boeke *et al.* 1988b), pGTy1H3CLA (denoted pGTy1) and pGTy2-917 (Garfinkel *et al.* 1988), pGTy1H3*his3-AI* (Curcio and Garfinkel 1991), pOY1 (Lee *et al.* 1998), pLAY182 (Bailis *et al.* 1995), pRS426 (denoted 2μ vector) and pRS404 (Sikorski and Hieter 1989), pGAL-X/P (denoted pGAL; Curcio *et al.* 1988), and Ty5/2μ (Ke and Voytas 1997) are described elsewhere.

Ty1 retrotransposition: To detect spontaneous Ty1 retrotransposition events in strains bearing the Ty1*his3-AI* reporter, cells were streaked for single colonies on YEPD, supplemented SD minimal plates (*e.g.*, SD +His +Trp) or synthetic complete plates lacking uracil (SC −Ura) and incubated at 21° for 5 days. The cells were then replica plated onto SC −His or SC −His −Ura and incubated at 30° for 3–4 days. Quantitative Ty1*his3-AI* insertion rates using five or seven independent cultures per strain were determined as described previously (Curcio and Garfinkel 1991; Rattray *et al.* 2000). Galactose-induced transposition of strains containing a pGTy1 element was performed as described previously (Boeke *et al.* 1985).

Ty1 insertions at preferred target sites: Spontaneous Ty1 insertions upstream of glycine tRNA loci (Lee *et al.* 1998) were detected after growing individual colonies on YEPD plates at 30° for 3 days. A single colony was inoculated into 10 ml YEPD broth and grown for 2 days at 21° in the presence or absence of the reverse transcriptase inhibitor phosphonoformic acid (PFA; 200 μg/ml final concentration; Sigma, St. Louis; Lee *et al.* 2000). This concentration of PFA inhibited Ty1*his3-AI(96)* transposition at least 100-fold and decreased Ty1 cDNA to background level without inhibiting growth of the Ty1-less strains. Total genomic DNA was isolated from each culture and analyzed by PCR essentially as described by Lee *et al.* (1998). Primers AX020 (5′-CTATTACATTATGGGTGGT ATG-3′) and SUF16OUT (5′-GGATTTTACCACTAAACCA CTT-3′) were used to detect Ty1 insertions upstream of glycine tRNA genes. A sample of the PCR reaction was separated by agarose gel electrophoresis in the presence of ethidium

bromide (0.5 μg/ml). The gel was imaged using a Typhoon 8600 phosphorimager adjusted to detect fluorescence, as suggested by the manufacturer (Molecular Dynamics, Sunnyvale, CA). To ensure that the genomic DNA was PCR competent and as a loading control for certain experiments, DNA preparations were analyzed by PCR using primers RTLEU2./92386 (5′-ACAGCATCACCGACTTCGGTGG-3′) and LEU2/91406 (5′-AGCTATTTCTGATGTTCGTTCC-3′) that are specific to the Ty1-less *LEU2* gene.

Southern analysis of Ty1 cDNA: A single colony from each strain grown at 21° or 30° was inoculated in 10 ml of YEPD or supplemented SD liquid media and grown to saturation at 21°. Alternatively, cultures from the Ty1 cDNA half-life experiments were used before PFA was added to inhibit reverse transcription. Total genomic DNA isolated from these cultures was digested with *Pvu*II, separated on a 0.7% agarose gel, and transferred to Hybond N (Amersham, Piscataway, NJ) membrane. The ^{32}P-labeled DNA probes were made by randomly primed DNA synthesis (Amersham). Southern hybridizations were performed with probes derived from *his3-AI* or the pGTy1H3CLA *Pvu*II-*Cla*I fragment from the RT region. The intensity of the cDNA fragments was determined by phosphorimage analysis and ImageQuant 1.2 software and normalized using a common chromosomal Ty1*his3-AI(96)* junction fragment, as described previously (Lee *et al.* 1998).

Ty1 cDNA stability: The Ty1 cDNA stability assay developed by Lee *et al.* (2000) was used essentially as described. The differences were that cells were grown in supplemented SD medium and PFA was added to a final concentration of 200 μg/ml. Cells were removed at the time of PFA addition (time 0) and then at 0.5, 1, 2, 4, 6, 8.5, and 12 hr of incubation at 21°. The OD_{600} of the cultures was monitored after addition of PFA and at the same time points as cell removal to estimate the mean generation time. The decay of Ty1 cDNA was plotted as the log of the percentage of Ty1 cDNA remaining relative to the level of Ty1 cDNA at time zero *vs.* time, as described previously (Lee *et al.* 2000). Lines were generated by least-squares fit analysis and half-lives were calculated using Cricket Graph 1.2 software. R^2 values were between 0.95 and 0.975, and all half-life measurements were performed at least twice with similar results.

Northern analysis and RT-PCR: Total RNA was isolated as described previously (Schmitt *et al.* 1990) from strains grown to late log phase in 10 ml supplemented SD or YEPD liquid media at 21°. The RNA samples were separated on a 1.2% agarose/formaldehyde gel and transferred to Hybond N membrane. The ^{32}P-labeled DNA probes were made by randomly primed DNA synthesis. A 1.2-kb *Cla*I fragment from pBDG201 was used to make the *his3-AI* probe (Curcio and Garfinkel 1991). A 1.6-kb *Pvu*II-*Cla*I fragment from pGTy1H3CLA was used to make the Ty1 probe. The *PYK1* probe was made from a 1.4-kb *Eco*RI-*Xba*I fragment from pBDG502. Hybridization analysis was performed as described previously (Lee *et al.* 1998), and the signals were quantified by phosphorimage analysis using ImageQuant 1.2 software. Prior to RT-PCR, contaminating DNA was removed from the RNA preparations by treatment with RNAse-free DNase using an RNeasy kit from QIAGEN (Valencia, CA). RT-PCR was performed using a RETROscript kit from Ambion (Austin, TX), and signals were dependent on the presence of reverse transcriptase, yeast RNA, and the appropriate primers. The RT primer 7569 (5′-ATGACAGAGCAGAAAGCCC-3′) annealed with the 5′ end of the *his3-AI* gene and the Ty1*his3-AI* transcript, while the PCR primer 7568 (5′-ACGCACGGCCCCTAGGGCCTC-3′) annealed with the 3′ end of *his3-AI* and the cDNA produced by reverse transcription. Together the primers bracket the AI such that PCR products of 336- and 440-bp represent spliced and unspliced forms of the primary Ty1*his3-AI* transcript, respectively. RT-PCR primers specific to the *LEU2* gene, RTLEU2./92386 and LEU2/91406, were used in parallel reactions to amplify a fragment of 980 bp. RT-PCR products were separated by agarose gel electrophoresis and quantified by fluorescence imaging as described above.

Western analysis: Total protein was isolated from strains after growth in supplemented SD or YEPD liquid media at 21°, as described previously (Lee *et al.* 1998). Protein concentrations were determined using a dye-binding assay reagent from Bio-Rad (Hercules, CA). Proteins were separated on a preformed 10% SDS-polyacrylamide gel (Invitrogen, Carlsbad, CA) and transferred to Immobilon-P membrane (Millipore, Billerica, MA) using a semidry electroblotter. The membrane was incubated with polyclonal antisera to Ty1-VLPs (1/4000 dilution) or Hts1p (1/20,000 dilution; kindly provided by T. Mason) for 2 or 1 hr incubation, respectively, at room temperature. Detection was performed using an ECF Western blotting kit (Amersham), and signals were quantified by fluorescence imaging according to the suppliers' recommendations. To obtain signals of TyA1 proteins in the linear range, we varied the amount of total protein for certain samples. Western blots were performed under identical conditions except 10 μg of total protein was loaded per lane to detect Hts1p.

RESULTS

Ty1-less derivatives: The Ty1-less strain 337 (Wilke and Adams 1992) and derivatives DG1389 and DG1423 were analyzed for Ty element content by low-stringency Southern hybridizations. The Ty1-less strains did not contain Ty1 or Ty2 elements, and Ty3 and Ty4 elements were highly diverged, as suggested by several restriction site polymorphisms and weak hybridization signals when compared with laboratory strains. A Ty5 element probe also showed weaker hybridization with the Ty1-less strains, although the hybridization patterns were identical to those obtained with laboratory strains (data not shown). This result suggests that the Ty1-less strain 337 and its derivatives contain Ty5-like elements in the same location as a laboratory strain and supports recent work on the evolution of Ty elements in hemiascomycetous yeasts (Neuveglise *et al.* 2002).

Strain 337 required further genetic manipulation to be useful for studying Ty1 CNC. This strain lacked genetic markers but was heterothallic and mating competent. The absence of genetic markers was compounded by the fact that although 337 and its derivatives and a laboratory strain have comparable electrophoretic karyotypes (E. J. Louis, personal communication), the Ty1-less strains have diverged from a laboratory strain at the nucleotide sequence level. For example, about half of our standard PCR primers worked with the Ty1-less derivatives, gene disruption constructs generally did not target correctly, and crosses between a Ty1-less and a laboratory strain had low spore viability (data not shown). To remedy these limitations, we introduced a complete *HIS3* deletion (*his3-Δ200hisG*) by single-step gene disruption and several *trp* mutations (including *trp1*) by 2-amino-5-fluorobenzoic acid selection (Toyn *et al.* 2000). We also introduced functional mitochon-

TABLE 1

Transposition of Ty1*his3-AI* elements

Strain	Relevant genotype	Transposition rate, $\times 10^6$ (SD)	Fold change
A. DG1725	S288c (32 Ty1's), Ty1*his3-AI*/cen	0.3 (1.0)	1
DG1767	Ty1-less, Ty1*his3-AI*/cen	6.5 (1.0)	22↑
B. DG1929	Ty1-less, Ty1*his3-AI(96)*, *RAD3*	20 (9.0)	1
DG2353	*rad3-G595R*	560 (118)	28↑
C. DG1929	Ty1-less, Ty1*his3-AI(96)*	12 (3.7)	1
JC120	+14 Ty1's	0.13 (0.1)	92↓
JC121	+21 Ty1's	0.1 (0.03)	120↓
DG2464	+6 Ty1*his3-ΔMscI*	0.3 (0.05)	40↓
D. DG2411	Ty1-less, Ty1*his3-AI(96)*, vector	30 (6.2)	1
DG2276	Ty1/2μ	0.3 (0.2)	100↓
DG2274	Ty1-ATGfs/2μ	0.2 (0.06)	150↓
E. DG1981	Ty1-less, Ty1*his3-AI(96)*, vector	45 (16.4)	1
DG1980	Ty1/2μ	0.5 (0.12)	90↓
DG1986	Ty1-pbs$^-$/2μ	0.34 (0.07)	132↓
JC150	Ty1ADH ter/2μ	1.0 (0.2)	45↓
JC152	Ty2/2μ	2.0 (1.0)	22.5↓
F. DG2254	Ty1-less, Ty1*his3-AI(96)*, pGAL	38 (8.6)	1
DG2255	pGTy1	1.5 (1.1)	25↓
DG2443	pGTy1*his3-AI(96)*	1.4 (0.5)	27↓
DG2452	pBDG1177 (Δ524-815)	0.6 (0.2)	63↓
DG2462	pBDG1182 (Δ524-815, 1702-5562+LTR)	0.1 (0.06)	380↓
DG2463	pBDG1183 (Δ524-815, 1702-5562-LTR)	1.2 (0.4)	32↓
DG2279	pGTy2-917	25 (12)	1.5↓
G. DG1444	S288c, Ty1-242*his3-AI*, pGAL	0.1 (0.03)	1
DG1460	pGTy1H3	0.03 (0.004)	3.3↓
H. JC151	Ty1-less, Ty1*his3-AI(96)*, vector	36 (5.6)	1
JC158	pPGK-Ty1Δ238/ADH ter	0.3 (0.14)	120↓

Rate of His$^+$ colony formation was determined as described previously (Curcio and Garfinkel 1991; Rattray *et al.* 2000). SD, standard deviation.

dria into the resulting Ty1-less *his3-Δ200hisG* mutant and generated closely related *MAT***a** and -α strains that are Gal$^+$, Rho$^+$, and Spo$^+$.

Ty1 retrotransposition in a Ty1-less strain: To determine whether a Ty1-less strain supported Ty1 transposition, we tagged Ty1 with the retrotransposon indicator gene *his3-AI* and then measured the rate of His$^+$ formation to monitor transposition (Table 1). Surprisingly, when a centromere-based plasmid containing a Ty1 element under the control of its own promoter and *his3-AI* (Ty1*his3*-AI/cen) was introduced into a Ty1-less strain to generate DG1767, transposition increased 22-fold when compared with the same Ty1*his3-AI* element in the laboratory strain DG1725 (Table 1A). By segregation analysis of 50 independent His$^+$ colonies, we determined that all of the His$^+$ insertions in the Ty1-less strain were integrated in the genome. Since plasmid-borne His$^+$ events resulting from cDNA recombination can predominate when transpositional integration is blocked (Sharon *et al.* 1994), these results suggest that *de novo* transposition events have occurred. Southern hybridization of 18 independent His$^+$ plasmid segregants indicated that 13 of the isolates contained a single marked Ty1 insertion and 5 contained two different insertions (data not shown). Multiple spontaneous Ty*his3-AI* transposition events are rare in typical laboratory strains. We also observed that in the Ty1-less strain DG1929 (Table 1, B and C), the rate of His$^+$ formation of the genomic element, Ty1*his3-AI(96)*, was >100-fold higher than that of several representative Ty1*his3-AI* elements in laboratory strains (Curcio and Garfinkel 1991; Bryk *et al.* 1997).

In addition, by introducing the *rad3-G595R* mutation in the TFIIH helicase gene *RAD3* into a Ty1-less strain containing Ty1*his3-AI(96)*, we determined whether a Ty1 host defense gene remains active when only one

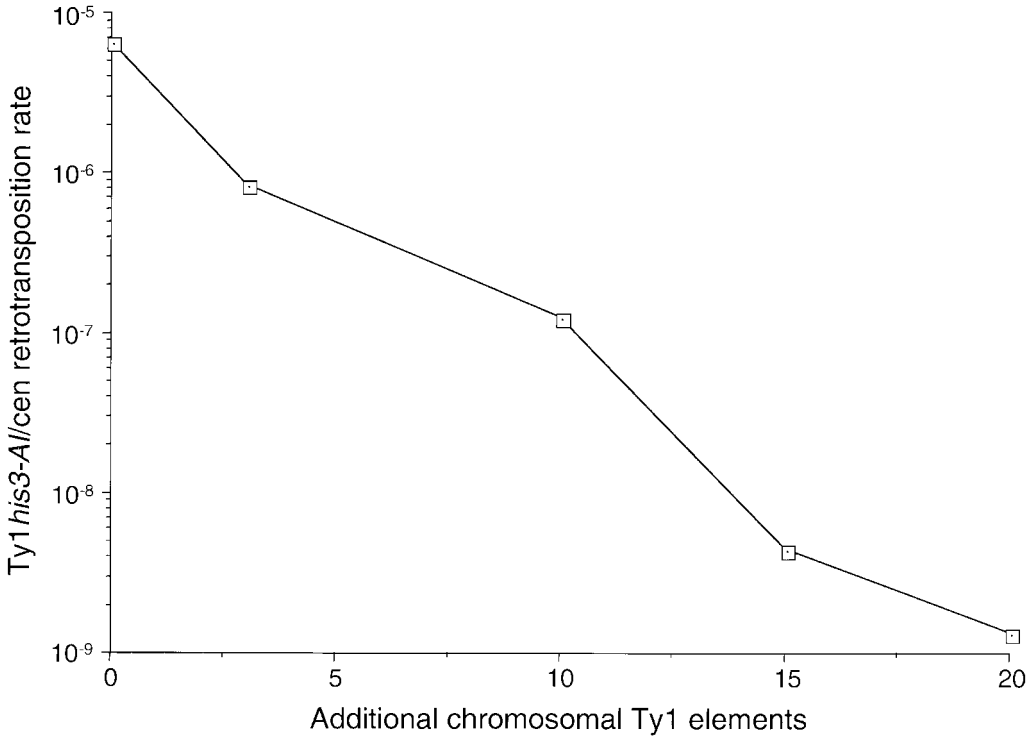

FIGURE 1.—Ty1 transposition in strains containing increasing numbers of chromosomal Ty1 elements. The Ty1-less strain DG1768 containing either pGTy1H3*Neo* or pGTy1H3 was induced for transposition and derivatives with 3 Ty1*Neo*, 10 Ty1*Neo*, 15 Ty1, or 20 (DG2451) additional Ty1 elements were recovered, as determined by Southern analysis (data not shown). The centromere-based Ty1*his3-AI* plasmid pOY1 was introduced into DG1768 and the Ty1-containing derivatives, and the rate of Ty1*his3-AI* transposition was determined by monitoring the number of His^+ colonies.

Ty1 element is present in the genome. Single-element transposition increased 28-fold in the resulting *rad3-G595R* mutant DG2353 when compared to the *RAD3* parental strain DG1929 (Table 1B). These results suggest that Ty1 retrotransposition events significantly increase when the genome contains only one element, yet transposition is still inhibited by *RAD3*.

Additional chromosomal Ty1 elements affect Ty1 transposition: We determined whether the presence of additional marked or unmarked chromosomal Ty1 elements inhibited retrotransposition of a plasmid-borne (Figure 1) or chromosomal Ty1*his3-AI* element (Table 1C). The Ty1*his3-AI*/cen plasmid was introduced into the Ty1-less strain DG1768 or derivatives with 3 Ty1*Neo*, 10 Ty1*Neo*, 15 Ty1, or 20 additional Ty1 insertions, as estimated by Southern analysis (data not shown). We observed that Ty1*his3-AI* transposition decreased in a copy-number-dependent manner over a 4800-fold range, as measured by the rate of His^+ colony formation (Figure 1). We created derivatives of a Ty1-less strain that initially lacked Ty1 elements or contained Ty1*his3-AI(96)*, but had additional copies of Ty1*Neo*, Ty1*his3-*Δ*Msc*I, or unmarked Ty1 element insertions that were introduced by induction of the appropriate pGTy1 plasmid. In this context, Ty1*his3-AI(96)* retrotransposition decreased 92- and 120-fold when cells contained an additional 14 or 21 unmarked chromosomal Ty1 elements, respectively (Table 1C). Introducing 6 Ty1*his3-*Δ*MscI* elements decreased Ty1*his3-AI(96)* retrotransposition 40-fold. Note that Ty1*his3-*Δ*MscI* is very similar to Ty1*his3-AI*, except the *his3-*Δ*MscI* allele lacks an artificial intron and instead contains a 34-bp deletion spanning the *Msc*I site where the AI is inserted (DERR and STRATHERN 1993). Together, these results suggest that Ty1 retrotransposition is sensitive to copy number and occurs regardless of whether the suppressing Ty1 elements contain genetic markers.

Multicopy Ty1 and Ty2 plasmids confer CNC: To understand further the mechanism of CNC, we determined whether increasing the copy number of a Ty1 element by placing it on a 2μ-based multicopy vector inhibited Ty1*his3-AI(96)* retrotransposition (Table 1, D and E). When a 2μ vector containing a functional Ty1 element was present in strain DG2276, Ty1*his3-AI(96)* retrotransposition decreased 100-fold over the control strain DG2411 that carried the vector plasmid. Comparable levels of inhibition were also observed when the suppressing Ty1/2μ plasmid contained *Neo*, *neo-AI*, or the spliceable *RP51* intron as Ty1 markers (data not shown). Interestingly, a suppressing plasmid-borne Ty1 element containing a frameshift mutation (ATGfs) immediately after the *TYA1* start codon (Ty1 ATGfs/2μ) decreased Ty1*his3-AI(96)* transposition 150-fold in strain DG2274. A mutant Ty1-pbs^-/2μ element lacking the (i)Met-tRNA primer-binding site in strain DG1986 or containing the *ADH1* transcriptional terminator in place of the 3′ LTR in JC150 inhibited Ty1*his3-AI(96)* transposition 132- and 45-fold, respectively. In addition, Ty1 transposition remained under CNC when a Ty1/2μ element was introduced into the *rad3-G595R* mutant (data not shown).

The specificity of Ty1 CNC was examined by monitoring the level of Ty1*his3-AI(96)*-mediated His^+ papillation in the presence of 2μ vectors containing the closely

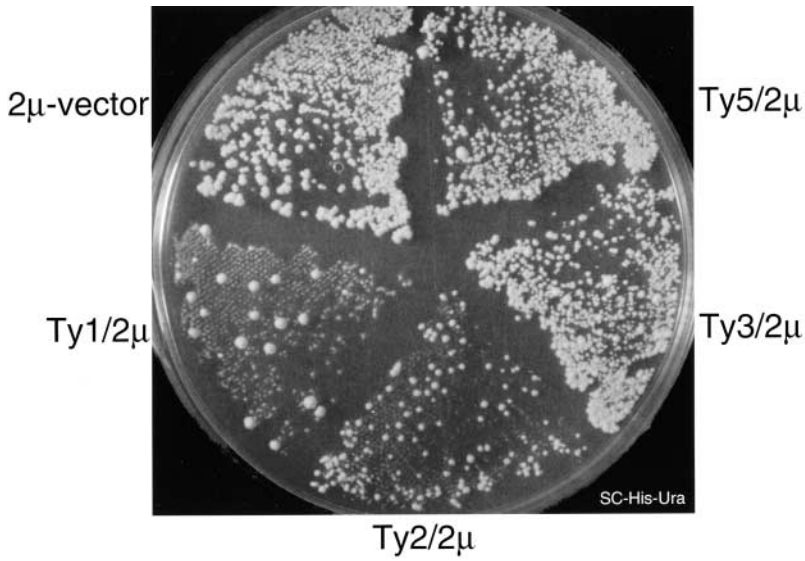

FIGURE 2.—Specificity of Ty1 copy number control. Strain DG1929 [Ty1*his3-AI(96)*] containing a 2μ-based multicopy vector (pRS426) or multicopy Ty plasmids Ty5/2μ (pNK318), Ty3/2μ (pJC376), Ty2/2μ (pJC384), or Ty1/2μ (pJC304) was streaked for single colonies on an SC−Ura plate and incubated for 5 days at 21°. Spontaneous Ty1*HIS3* transposition events were detected as His^+ papillae (CURCIO and GARFINKEL 1991), by replica plating the SC−Ura plate to SC−His −Ura medium, followed by incubation for 3 days at 30°.

related Ty2 element, as well as Ty3 or Ty5 elements (Figure 2). Cells were streaked for single colonies on synthetic complete medium lacking uracil (SC −Ura), grown at 21°, and then replica plated to SC −His −Ura. As expected, the Ty1/2μ plasmid conferred maximum CNC when compared with the 2μ vector. The closely related Ty2 element provoked a significant level of CNC but somewhat less than that provoked by a Ty1 element, whereas Ty3 or Ty5 elements conferred little if any CNC on Ty1. Strain JC152 containing the Ty2/2μ plasmid was analyzed further by determining the rate of Ty1*his3-AI(96)* transposition (Table 1E). Although the CNC conferred by a suppressive Ty2 element was less potent than that conferred by Ty1, transposition still decreased 22.5-fold. Northern hybridization analysis also suggested that Ty1, Ty2, and Ty5 elements were transcriptionally active in a Ty1-less strain; however, the Ty3 element was not (data not shown).

Native transcription of a suppressing Ty1 element is not required for CNC but Ty2 transcription is: The results presented above as well as the recent work of JIANG (2002) suggest that Ty1 transcription from its natural promoter may be required for cosuppression of Ty1 retrotransposition. Therefore, we determined whether a transcriptionally repressed pGTy1 element conferred CNC (Table 1F). Surprisingly, strain DG2255 (pGTy1) or DG2443 [pGTy1*his3-AI(96)*] inhibited Ty1 *his3-AI(96)* transposition 25- and 27-fold, respectively, when the strains were grown in medium containing the repressive carbon source glucose (Table 1F). In contrast, a competent pGTy2 element in strain DG2279 lost CNC over Ty1 when pGTy2 transcription was repressed by growth on glucose. To make sure glucose repression was active in the Ty1-less strain, we showed that pGTy1*neo-AI* or pGTy1*his3-AI* elements gave rise to very few marked transposition events ($<1 \times 10^{-8}$/cell/generation) when cells were grown on medium containing glucose.

The observation that transcriptionally silent pGTy1 elements inhibit Ty1*his3-AI(96)* retrotransposition in the Ty1-less strain encouraged us to determine whether pGTy1 also affects Ty1 transposition in a laboratory strain that harbors 32 chromosomal Ty1 elements (Table 1G). We introduced a pGTy1 element or the pGAL vector into the laboratory strain JC242, which contains the chromosomal element Ty1-242*his3-AI* (CURCIO and GARFINKEL 1991), and determined the rate of His^+ formation on glucose. As expected, the level of Ty1-242*his3-AI* transposition was much lower in the laboratory strain DG1444 containing the vector than in the Ty1-less background; however, strain DG1460 containing a transcriptionally repressed pGTy1 element decreased Ty1-242 *his3-AI* transposition 3.3-fold.

pGTy1 sequences required for CNC: The suppression conferred by pGTy1 provided a useful way to determine the minimal sequences required to inhibit chromosomal Ty1 retrotransposition in the absence of gene expression from the suppressing element. Therefore, we generated pGTy1 elements containing 5′, 3′, and internal deletions and determined whether these elements conferred CNC on Ty1*his3-AI(96)* using both qualitative (Figure 3) and quantitative transposition assays (Table 1F). Overlapping Ty1 element segments were also subcloned into a pGAL vector in the sense orientation and analyzed for CNC (data not shown). The starting point for constructing the internal deletions was obtained from earlier experiments using pPGK-Ty1 elements and from clones isolated as gene dosage suppressors. The pGTy1 sequences from +238-524 and +815-1702 (pBDG1183) were necessary and sufficient for CNC in strain DG2463 and were comparable in potency to the full-length pGTy1 element in DG2255. Most of these sequences are within *TYA1* (+338–1620) with the remainder in the R-U5 region of the 5′ LTR (+238–338) and in *TYB1* (+1621–1702). Deleting 100–200 bp more from any deletion endpoint

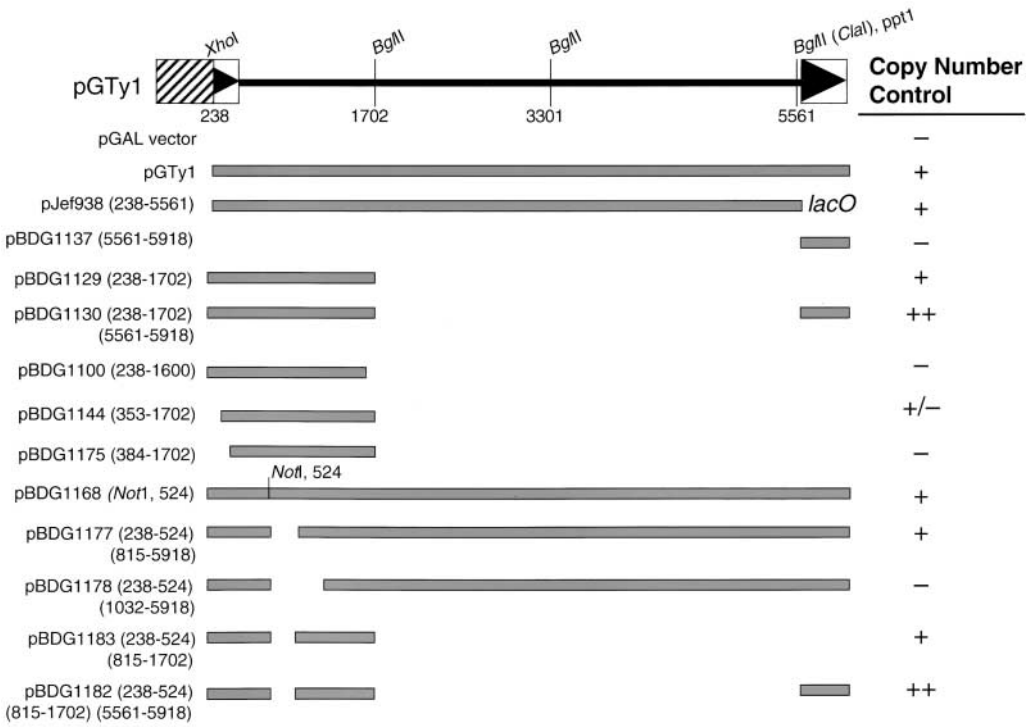

FIGURE 3.—Identifying the minimal region of pGTy1 that mediates copy number control. The *GAL1*-promoted Ty1 portion of pGTy1 is shown at the top. The *GAL1* promoter is shown as a hatched rectangle, LTRs are shown as boxed horizontal arrows, and landmark restriction sites and their nucleotide positions in Ty1H3 are noted (BOEKE *et al.* 1988a). The plus-strand initiation site 1 (ppt1) for reverse transcription is located between the *Bgl*II restriction site (nucleotide 5561) and the beginning of the 3′ LTR. Deletions are shown as gaps, and their end points are aligned with pGTy1. Sequences remaining in the deleted pGTy1 plasmids are in parentheses on the left. The pGTy1 plasmids were transformed into a Ty1-less yeast strain carrying Ty1*his3-AI(96)* and initially tested for Ty1 transposition using a qualitative papillation assay as described in Figure 2. Four transformants were tested for each plasmid. ++, strong decrease in His^+ papillation (~380-fold, Table 1F); +, decrease in His^+ papillation (~25-fold, Table 1F); +/−, weak decrease in His^+ papillation; −, no decrease in His^+ papillation.

in the pGTy1 element resulted in loss of CNC (data not shown). The 3′ LTR and plus-strand initiation site 1 (ppt1) was dispensible for CNC in a complete pGTy1 element (pJef938 and pBDG1137, Figure 3). Surprisingly, these LTR sequences enhanced the inhibitory activity of the pGTy1 element pBDG1182 in strain DG2462 >10-fold over that of the minimal element pBDG1183 in DG2463 or the full pGTy1 element in DG2255 (Table 1F). Moreover, the 380-fold decrease in Ty1*his3-AI(96)* transposition provoked by pBDG1182 was more robust than that obtained by suppressing Ty1 elements that were transcriptionally active.

Cosuppression of Ty1 transposition by elements transcribed from foreign promoters: To determine whether Ty1 transcription from the *GAL1* promoter affected Ty1 cosuppression, we analyzed galactose-induced pGTy1 elements present on 2μ- or centromere-based plasmids or integrated in the genome. Strains DG2254 (pGAL vector), DG2255 (pGTy1), DG2275 (pGTy1-ATGfs), and DG2283 (pGTy1/cen) were streaked for single colonies on SC −Ura +galactose and SC −Ura +glucose plates, incubated for 7 days at 21°, and then replica plated to SC −His −Ura plates to monitor the level of Ty1*his3-AI(96)* transposition (Figure 4). As expected, cells containing transcriptionally silent pGTy1 and pGTy1-ATGfs elements inhibited Ty1*his3-AI(96)* retrotransposition, as shown by the low level of His^+ papillation on SC −His −Ura medium, when compared to cells containing the 2μ vector or a pGTy1/cen element. However, when cells containing these plasmid-borne pGTy1 elements were induced for transcription by growth on SC −Ura +galactose, cosuppression was abolished in all cases, as shown by the high level of His^+ papillation.

The effect of increasing the level of Ty1 RNA without increasing the number of Ty1 elements was examined by constructing Ty1-less strains containing a chromosomal Ty1*his3-AI(96)* element and pGTy1/404 (DG2338), pGTy1-ATGfs/404 (DG2379), or the *TRP1*-YIp vector pRS404 (DG2388) integrated at *TRP1* and then monitoring the level of Ty1*his3-AI(96)* transposition when cells were induced with galactose (Table 2). When a wild-type pGTy1/404 element was induced for expression by growth of strain DG2338 on galactose, Ty1*his3-AI(96)* transposition increased 2.5-fold over that of the vector pRS404 in DG2388, which is reasonable considering that a multicopy pGTy1 element induced for expression increases chromosomal Ty1-242*his3-AI* transposition by 15-fold in a laboratory strain (CURCIO and GARFINKEL 1992). Induction of strain DG2379 containing pGTy1-ATGfs/404 did not alter the level of Ty1 transposition and, therefore, neither provoked nor relieved CNC. Furthermore, Northern analysis indicated that an integrated pGTy1/404 plasmid produced severalfold more Ty1 RNA than did pGTy1-ATGfs/404 (data not shown), which is expected since the Ty1-ATGfs transcript is susceptible to nonsense-mediated decay (CURCIO and GARFINKEL 1994; GONZALEZ *et al.* 2001). These

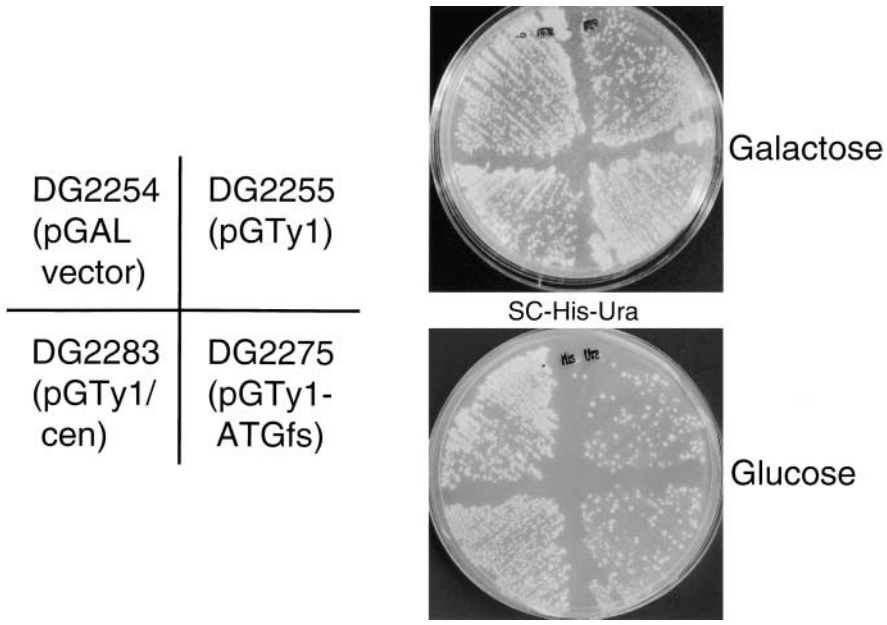

FIGURE 4.—pGTy1 induction affects Ty1 copy number control. Strains DG2254 (pGAL vector), DG2255 (pGTy1), DG2275 (pGTy1-ATGfs), and DG2283 (pGTy1/cen) were streaked for single colonies on SC −Ura with either galactose or glucose as the carbon source and incubated for 7 days at 21°. All strains contained Ty1*his3-AI(96)*. Spontaneous Ty1*HIS3* transposition events were detected as His^+ papillae by replica plating the SC −Ura plates to SC −His −Ura, followed by incubation for 3 days at 30°.

results suggest that increasing the level of either functional or nonfunctional Ty1 RNA without increasing Ty1 DNA copy number of the suppressing elements does not inhibit Ty1 transposition.

Abolishing CNC of a chromosomal Ty1 element by *GAL1*-promoted Ty1 transcription of multicopy pGTy1 or pGTy1-ATGfs elements may be a general characteristic of foreign promoters fused to the suppressing Ty1. Therefore, cells with Ty1*his3-AI(96)* and a Ty1/2μ plasmid containing the constitutive *PGK1* promoter inserted at the same position in Ty1 (nucleotide 238) as *GAL1* were analyzed for CNC (Table 1H). The pPGK-Ty1Δ238/ADH ter element also contained the *ADH1* transcriptional terminator in place of the 3′ LTR, which is not required for CNC (Table 1E; Figure 3). In contrast to transcriptional cosuppression (JIANG 2002), Ty1*his3-AI(96)* transposition decreased 120-fold in strain JC158 (pPGK-Ty1Δ238/ADH ter) when compared to strain JC151 (2μ vector; Table 1H). Northern analysis also showed that the pPGK-Ty1Δ238/ADH ter element produced more Ty1 RNA than a Ty1/2μ element expressed from the native Ty1 promoter (data not shown).

To address the possibility that growth in galactose influenced CNC, strains containing a chromosomal Ty1*his3-AI(96)* as well as multicopy Ty1 elements expressed from their native promoter (Ty1/2μ and Ty1-ADH ter/2μ) or the *PGK1* promoter (pPGK-Ty1Δ238/ADH ter), as well as a 2μ vector, were grown on SC −Ura +galactose plates and then replica plated to SC −His −Ura to monitor Ty1 transposition. Cosuppression was observed for each Ty1 element when cells were grown on galactose as the sole carbon source (data not shown), indicating that cosuppression is independent of galactose metabolism.

Global Ty1 transposition under CNC: Genomic regions upstream of tRNA genes are preferred targets for Ty1 retrotransposition (JI *et al.* 1993). To determine whether CNC affects Ty1 retrotransposition of unmarked elements in their natural chromosomal context, we utilized a PCR assay to monitor insertions of Ty1 elements in genomic regions upstream of glycine-tRNA genes (LEE *et al.* 1998) in isogenic strains containing 20 (DG2451; also see Figure 1) or 1 (DG2454) Ty1 elements (Figure 5). Two independent colonies from each strain were grown to saturation in supplemented SD medium with (+) or without (−) the reverse transcriptase inhibitor PFA and analyzed for Ty1 insertions upstream of the glycine tRNA targets. The same amount of genomic DNA was analyzed by PCR using one oligonucleotide primer that is specific for Ty1 elements (AX020) and a second oligonucleotide primer from the *SUF16* tRNA gene (SUF16OUT). All DNA samples were PCR competent, as demonstrated by control reactions with oligonucleotide primers specific to the *LEU2* gene.

TABLE 2

Ty1*his3-AI(96)* transposition in Ty1-less strains containing integrated pGTy1 elements induced for expression

Strain	Plasmid	Transposition rate, $\times 10^{-6}$, (SD)	Fold change
DG2388	pRS404	65 (17)	1.0
DG2338	pGTy1/404	160 (40)	2.5↑
DG2379	pGTy1-ATGfs/404	58 (9.4)	0.9↓

Plasmids were integrated at the *TRP1* locus in strain DG2196. Cells were then grown in YEP +galactose at 21° to induce pGTy1 transcription, and the rate of His^+ colony formation was determined as described previously (CURCIO and GARFINKEL 1991; RATTRAY *et al.* 2000). SD, standard deviation.

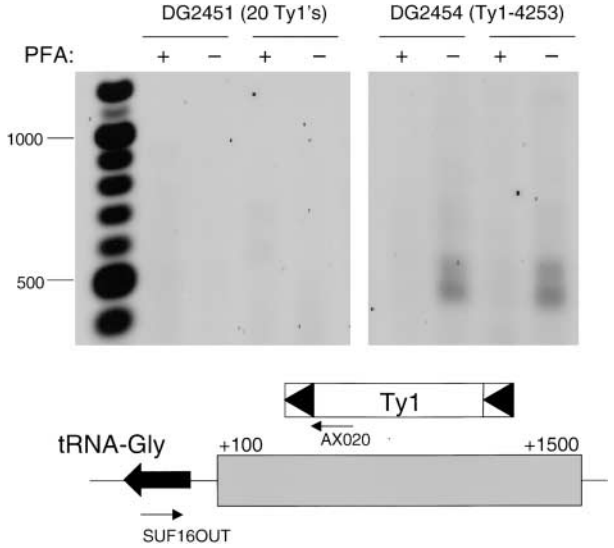

Figure 5.—Copy number control affects unselected Ty1 integration events upstream of glycine tRNA genes. Schematic representation of a typical glycine tRNA gene is at the bottom. The tRNA gene and its direction of transcription are shown by the solid arrow. Ty1 insertions can occur between ~100 and 1500 bp upstream (shaded rectangle) of one or more of the 16-glycine tRNA dispersed in the yeast genome (Lee *et al.* 1998). Oligonucleotide primers used for PCR amplifications are designated SUF16OUT and AX020, which are complementary with glycine tRNA genes and Ty1 elements, respectively. Total DNA was isolated from two independent colonies from strains DG2451 (20 Ty1's) and DG2454 (Ty1-4253) that were grown to saturation in supplemented SD liquid medium in the presence (+) or absence (−) of the reverse transcriptase inhibitor PFA (200 μg/ml) and then subjected to PCR to detect Ty1 insertions. PCR products were separated by electrophoresis on a 1.5% agarose gel in the presence of ethidium bromide and processed by fluorescence imaging. Size markers are alongside the gel.

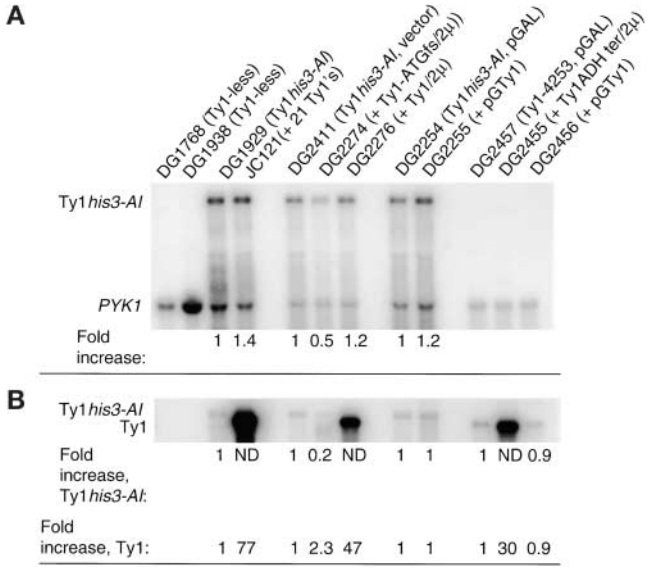

Figure 6.—Northern blot analysis of strains undergoing Ty1 copy number control. Ten-microgram quantities of total RNA from strains DG1768 (Ty1-less), DG1938 (Ty1-less), DG1929 (Ty1*his3-AI*), JC121 (+21 Ty1's), DG2411 (Ty1*his3-AI*, 2μ vector), DG2274 (+Ty1-ATGfs/2μ), DG2276 (+Ty1/2μ), DG2254 (Ty1*his3-AI*, pGAL vector), DG2255 (+pGTy1), DG2457 (Ty1-4253, pGAL vector), DG2455 (+Ty1ADH ter/2μ), and DG2456 (+pGTy1) were analyzed by Northern hybridization. ^{32}P-labeled DNA probes specific for *his3-AI*, *PYK1*, and Ty1 were used to detect (A) Ty*his3-AI* transcripts and (B) Ty*his3-AI* and Ty1 transcripts. Below each panel is the amount of Ty1*his3-AI* and Ty1 RNA relative to the *PYK1* transcript, as determined by phosphorimaging. ND, not determined.

The PCR products amplified using SUF16OUT and AX020 were separated by agarose gel electrophoresis in the presence of ethidium bromide, and the resulting gel was imaged for fluorescence.

Remarkably, strain DG2454 containing a single Ty1 element (Ty1-4253) exhibited a higher level of Ty1 transposition than did DG2451 with 20 chromosomal elements at glycine tRNA targets, as evidenced by the presence of the characteristic Ty1 "insertion ladder" (Lee *et al.* 1998) and its sensitivity to the reverse transcriptase inhibitor PFA (Figure 5). Similar results were obtained with strains containing competent multicopy Ty1 plasmids or repressed pGTy1 elements (data not shown). Together these results show that Ty1 retrotransposition at preferred regions of the genome is under CNC.

Ty1 gene expression in cells undergoing CNC: Ty1 RNA and TyA1-gag protein levels were monitored in cells undergoing CNC by Northern hybridization (Figure 6) and RT-PCR (Figure 7) and by Western blotting (Figure 8), respectively, to determine the step in the process of retrotransposition at which CNC occurs. Total RNA was isolated from strains DG1768 (Ty1-less), DG1938 (Ty1-less), DG1929 [Ty1*his3-AI(96)*], JC121 (+21 Ty1's), DG2411 [Ty1*his3-AI(96)* and a 2μ vector], DG2274 (+Ty1-ATGfs/2μ), DG2276 (+Ty1/2μ), DG2254 [Ty1*his3-AI(96)* and a pGAL vector], DG2255 (+pGTy1), DG2457 (Ty1-4253 and a 2μ vector), DG2455 (+Ty1ADH ter/2μ), and DG2456 (+pGTy1) and subjected to Northern analysis. The filters were hybridized with ^{32}P-labeled probes specific to *his3-AI*, Ty1, and *PYK1*, and the level of the Ty1 transcripts was normalized to the level of the *PYK1* transcript (Figure 6). The level of the Ty1*his3-AI(96)* transcript varied less than twofold when compared to those of the control strains, regardless of the suppressing Ty1 element present. The level of the Ty1-4253 transcript remained unchanged when challenged with the repressed pGTy1 element. However, we could not distinguish the Ty1-4253 from the Ty1ADH ter transcript using a ^{32}P-labeled Ty1 probe specific to sequences in the RT region because of their similar size and the abundance of the Ty1ADH ter transcript. We also could not clearly detect the marked Ty1*his3-AI(96)* transcript in JC121 (+21 Ty1's) and DG2276 (+Ty1/2μ) from unmarked Ty1 RNA using an RT probe in this experiment due to the large amount of unmarked Ty1 RNA present; however, after extended electrophoresis the

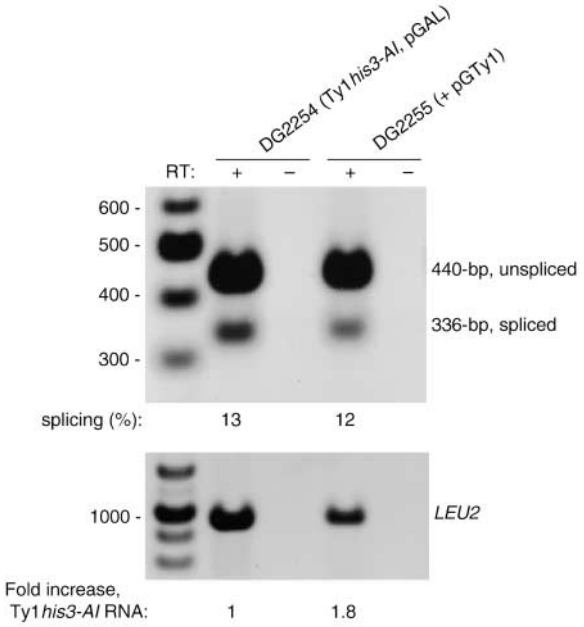

FIGURE 7.—Efficiency of Ty1*his3-AI* splicing in cells undergoing copy number control. Ten-microgram quantities of total RNA from strains DG2254 (Ty1*his3-AI*, pGAL vector) and DG2255 (+pGTy1) were analyzed by RT-PCR to determine the percentage of the Ty1*his3-AI* transcript that is spliced. Whether reverse transcriptase (RT) had been added to the reaction is denoted on the top. The sizes of the unspliced and spliced Ty1*his3-AI* RT-PCR products are on the right. A *LEU2* RT-PCR product of 980 bp was used as a loading control and is at the bottom. Refer to MATERIALS AND METHODS for the positions of the oligonucleotide primers used in the RT-PCR reactions. The splicing efficiency (%) and the relative level of Ty1*his3-AI* RNA was determined by fluorescence imaging. Size standards are on the left.

marked transcript from DG2276 was visible and remained unchanged (data not shown).

Despite the likelihood that transcriptional cosuppression is active in our strains containing multiple Ty1 elements, the level of Ty1 RNA increased from 30- to 77-fold when additional chromosomal or plasmid Ty1 elements were present in JC121, DG2276, and DG2455. The exception was DG2274, which contains the Ty1-ATGfs/2μ element. The full-length Ty1-ATGfs transcript was undetectable and a low level of a shorter Ty1 RNA was present in DG2274 (Figure 6), even though the Ty1-ATGfs/2μ element provoked strong CNC (Table 1E). Although we have not explored the loss of RNA accumulation from the Ty1-ATGfs/2μ element further, this transcript contains a premature stop codon and therefore is susceptible to nonsense-mediated decay. Furthermore, the ATGfs mutation is located in the internal enhancer region that is required for Ty1 transcription (VOYTAS and BOEKE 2002). The presence of a shorter Ty1-ATGfs RNA is also reminiscent of the Ty1 transcripts produced in an *spt3* mutant (WINSTON *et al.* 1984). However, full-length Ty1 RNA was detected when the Ty1-ATGfs element was fused to the *GAL1* promoter and induced with galactose (data not shown).

To determine whether Ty1 CNC affected RNA splicing of the Ty1*his3-AI(96)* transcript, total RNA from strains DG2254 (pGAL vector) and DG2255 (pGTy1) were analyzed by RT-PCR utilizing *HIS3* primers that bracket the AI present in *his3-AI* (Figure 7). RT-PCR products of 440 bp (unspliced) and 336 bp (spliced) should be produced from splicing of the 104-bp AI from the 6.8-kb Ty1*his3-AI(96)* transcript. Our results suggest Ty1 CNC does not alter the level of Ty1*his3-AI(96)* splicing in DG2255 (12%), when compared to the control strain DG2254 (13%). Similar splicing efficiencies were obtained from strains containing a variety of additional Ty plasmids that either did or did not confer Ty1 CNC (data not shown). In addition, the 980-bp RT-PCR product from the *LEU2* gene served both as an internal loading standard and as a control for a heterologous transcript. Using this assay, the level of Ty1*his3-AI(96)* RNA increased 1.8-fold in cells undergoing CNC, which is slightly higher than that detected by Northern analysis (Figure 6). The RT-PCR products were dependent on the presence of RT in the reaction.

Strains DG1929 [Ty1*his3-AI(96)*], JC121 (+21 Ty1's), DG2411 [Ty1*his3-AI9(96)*, 2μ vector], DG2274 (+Ty1-ATGfs/2μ), DG2276 (+Ty1/2μ), DG2254 [Ty1/*his3-AI(96)*, pGAL], DG2255 (+pGTy1), DG2457 (Ty1-4253, pGAL), DG2455 (+Ty1ADH ter/2μ), and DG2456 (+pGTy1) were also subjected to Western blot analysis to determine whether Ty1 CNC decreased retrotransposition by altering the levels of endogenous TyA1-gag proteins (Figure 8). p49-TyA1 is the primary *TYA1* translation product and p45-TyA1 is derived from p49 by cleavage near its C terminus by Ty1 PR (MERKULOV *et al.* 1996; VOYTAS and BOEKE 2002). Immunoblots containing total cell protein from these strains were prepared in parallel and incubated with polyclonal antiserum against Ty1-VLPs to detect TyA1 proteins and with antiserum against Hts1p, which is the cytoplasmic and mitochondrial histidyl-tRNA synthetase. Less total protein was analyzed from JC121 (+21 Ty1's), DG2276 (+Ty1/2μ), and DG2455 (+Ty1ADH ter/2μ) to prevent overloading the immunoblot with TyA1 proteins. The relative amounts of p49- and p45-TyA1 were determined by normalizing fluorescence image signals to that of Hts1p and correcting for the dilution.

The Ty1-less strains contained mostly processed p45-TyA1, even if only one Ty1 element was present (Figure 8). Total cell extracts from a laboratory strain usually contain about equal amounts of endogenous p49 (p58) and p45 (p54) (YOUNGREN *et al.* 1988; CURCIO and GARFINKEL 1992; LEE *et al.* 1998). Since the Ty1 elements introduced into the Ty1-less strain are derived from an active Ty1 element (BOEKE *et al.* 1985, 1988a; CURCIO and GARFINKEL 1994), the introduced elements may encode more efficient protein processing functions than the elements in a laboratory strain. The presence

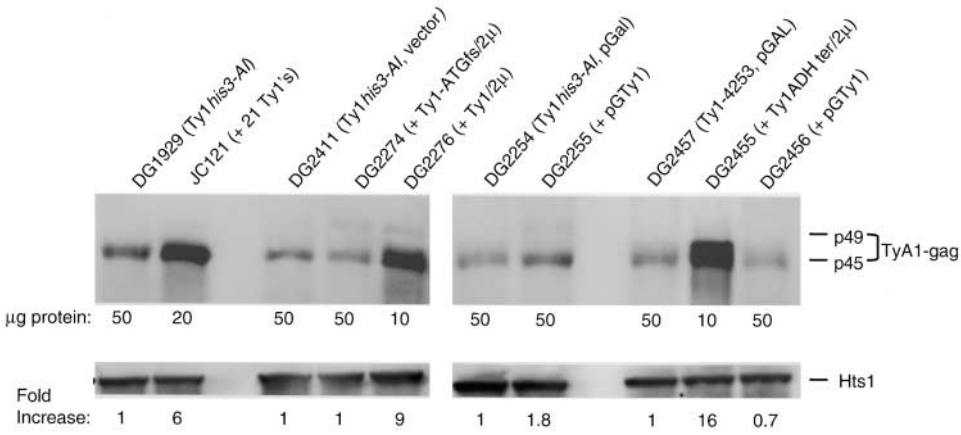

FIGURE 8.—Level of endogenous TyA1-gag proteins in cells undergoing copy number control. Total protein extracts were prepared from strains DG1929 (Ty1 *his3-AI*), JC121 (+21 Ty1's), DG2411 (Ty1*his3-AI,* 2μ vector), DG2274 (+Ty1-ATGfs/2μ), DG2276 (+Ty1/2μ), DG2457 (Ty1-4253, pGAL), DG2455 (+Ty1-ADH ter/2μ), and DG2456 (+pG Ty1) and separated by sodium dodecyl sulfate-10% polyacrylamide gel electrophoresis. The amount of protein analyzed is shown under the top panel. After transfer to Immobilon-P membrane, TyA1-gag proteins p49 and p45 were detected with Ty1-VLP polyclonal antiserum and visualized by ECF. In parallel, 10 μg of each protein sample was processed as described above but incubated with Hts1p polyclonal antiserum as a loading control. The fold increase in TyA1-gag proteins, normalized to the amount of Hts1p and dilution of each sample, was determined by fluorescence imaging and is at the bottom.

of p45 also suggests that Ty1 PR is functioning normally since a defective PR leads to an accumulation of the p49-TyA1 precursor (VOYTAS and BOEKE 2002). As the copy number of competent expressible Ty1 elements and their transcripts increase, the level of p45-TyA1 increased between 6- and 16-fold, depending on the context of the suppressing Ty1 elements.

Ty1 CNC blocks cDNA accumulation: We determined the level of unincorporated Ty1*his3-AI(96)* cDNA by Southern blot analysis using total DNA from DG2254 [Ty1*his3-AI(96)*, pGAL] and DG2255 (+pGTy1), DG-2411 [Ty1*his3-AI(96)*, vector] and DG2276 (+Ty1/2μ), and DG1929 [Ty1*his3-AI(96)*] and JC121 (+21 Ty1's) (Figure 9A). The same DNA preparations used for the zero time controls to determine the half-lives of Ty1 cDNA were analyzed here. Digestion of total DNA with *Pvu*II generated a 3.2-kb fragment containing sequences from a conserved internal *Pvu*II restriction site in Ty1*his3-AI(96)* (nucleotide 3944) to the end of the linear unincorporated cDNA (nucleotide 7118), which appeared as a distinct fragment when hybridized with a ^{32}P-labeled *his3-AI* probe. The resulting filter was quantitated by phosphorimaging. A convenient internal control is provided by the *Pvu*II fragment that contains one junction of the Ty1*his3-AI(96)* element joined to genomic DNA. In addition, DNA samples from cells treated with reverse transcriptase inhibitor PFA for the entire incubation period were included in the analysis because these cells contained very little Ty1 cDNA. When the level of Ty1*his3-AI(96)* cDNA was estimated relative to the level of the Ty1*his3-AI(96)* junction fragment, a 6-fold decrease in DG2255 (+pGTy1) and a 17-fold decrease in DG2276 (+Ty1/2μ) was observed. The level of Ty1*his3-AI(96)* cDNA from JC121 (+21 Ty1's) was very low and difficult to detect; therefore our estimate of an 88-fold decrease is essentially the same as the background measurements of the filter. However, the unmarked Ty1 cDNA from the 21 additional elements in JC121 was present and cDNA accumulation was sensitive to PFA (data not shown).

The level of unmarked Ty1-4253 cDNA in strains DG2457 (Ty1-4253, pGAL), DG2456 (+pGTy1), and DG2455 (+Ty1ADH ter/2μ) was determined to explore the possibility that Ty1 cDNA accumulation was influenced by the *his3-AI* indicator gene (Figure 9B). Here, *Pvu*II digestion generated a 2.1-kb cDNA fragment (nucleotides 3944–5918) that hybridized with a ^{32}P-labeled probe spanning most of this region of Ty1. The chromosomal junction fragment from the parental Ty1-4253 element served as an internal control. The level of cDNA decreased 8- and 17-fold in strains DG2456 (+pGTy1) and DG2455 (+Ty1ADH ter/2μ), which is similar to the results obtained using Ty1*his3-AI(96)* (Figure 9A).

We measured the decay rates of unincorporated Ty1 cDNA to determine whether CNC affected cDNA stability in strains DG2254 [Ty1*his3-AI(96)*, pGAL] and DG2255 (+pGTy1), DG2411 [Ty1*his3-AI(96)*, 2μ vector] and DG2276 (+Ty1/2μ), and DG1929 [Ty1*his3-AI(96)*] and JC121 (+21 Ty1's) (Figure 9C; Table 3). Examination of both unmarked and Ty1*his3-AI(96)* cDNA was difficult for strain DG2276 (+Ty1/2μ) and not possible for JC121 (+21 Ty1's) since the level of Ty1 *his3-AI(96)* cDNA was much reduced. Therefore, we determined the half-lives of Ty1*his3-AI(96)* cDNA for strains DG2254 (pGAL), DG2255 (+pGTy1), and DG2411 (2μ vector), unmarked Ty1 and Ty1*his3-AI(96)* cDNA for DG2276 (+Ty1/2μ), Ty1*his3-AI(96)* cDNA for DG1929 [Ty1*his3-AI(96)*], and unmarked Ty1 cDNA for JC121 (+21 Ty1's). For example, the decay rates of Ty1*his3-AI(96)* cDNA were measured in strains DG2254 [Ty1*his3-AI(96)*, pGAL] and DG2255 (+pGTy1) after addition of PFA to overnight cultures that had been diluted 1:1 with fresh supplemented SD liquid medium (Figure 9C). Aliquots of cells were removed prior to the addition of PFA (*, overnight culture) at various times after adding PFA and analyzed by Southern hybridization and

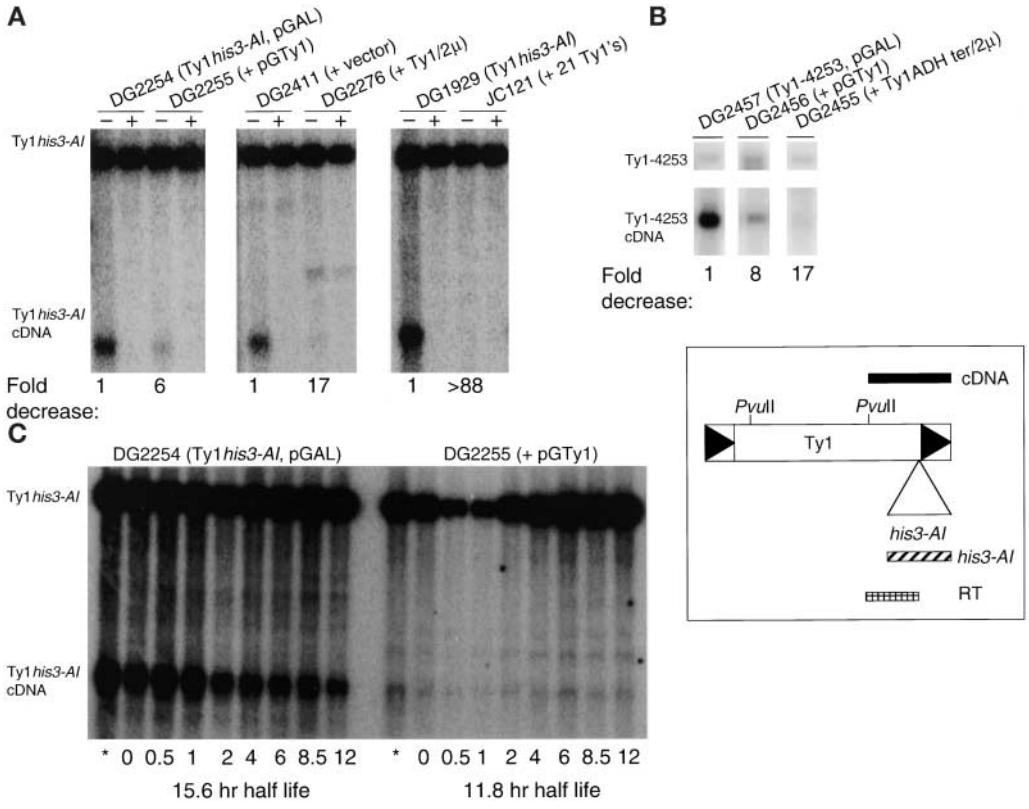

FIGURE 9.—Level and stability of Ty1 cDNA in cells undergoing copy number control. The segment of Ty1 cDNA detected by Southern blot analysis of total yeast DNA digested with *Pvu*II is shown schematically in the box. A Ty1 element is depicted along with the *Pvu*II restriction sites at nucleotide positions 475 and 3944 (BOEKE *et al.* 1988a). The solid bar represents the cDNA either with or without *his3-AI*. The positions of the *his3-AI* and Ty1 RT segments used to make the ^{32}P-labeled hybridization probes are represented by the hatched rectangles. (A) Ty1*his3-AI* cDNA levels. Total DNA was prepared after strains DG2254 (Ty1*his3-AI*, pGAL), DG2255 (+pGTy1), DG2411 (+2μ vector), DG2276 (+Ty1/2μ), DG-1929 (Ty1*his3-AI*), and JC121 (+21 Ty1's) were grown to saturation in supplemented SD medium at 21° in the presence (+) or absence (−) of the reverse transcriptase inhibitor PFA (200 μg/ml). The DNA was then digested with *Pvu*II and subjected to Southern analysis with a *his3-AI* probe. The position of the 3.2-kb Ty1*his3-AI* cDNA and the parental Ty1*his3-AI(96)* junction fragment are shown on the side. The amount of Ty1*his3-AI* cDNA relative to the parental chromosomal junction was determined by phosphorimaging and presented below the panel. (B) Ty1-4253 cDNA levels. Total DNA was prepared from strains DG2457 (Ty1-4253, pGAL), DG2456 (+pGTy1), and DG2455 (+Ty1ADH ter/2μ) that were grown to saturation in supplemented SD medium at 21°. Southern analysis was identical to that described in A, except that the Ty1-4253 cDNA was 2.1 kb in size and the probe was derived from the RT region of Ty1. (C) Stability of Ty1*his3-AI* cDNA. Southern blot analysis of Ty1*his3-AI* cDNA from strains DG2254 (Ty1*his3-AI*, pGAL) and DG2255 (+pGTy1) that were untreated (*, overnight) or treated with the RT inhibitor PFA for various lengths of time. Time intervals (in hours) after the addition of PFA are noted on the bottom. Southern hybridizations were performed as described in A. Ty1*his3-AI* cDNA half-lives, shown at the bottom, were derived from decay curves plotted on a log scale as the percentage of Ty*his3-AI* cDNA remaining relative to the level of Ty1*his3-AI* cDNA at zero *vs.* elapsed time. Also see Table 3.

phosphorimaging. The half-life of Ty1*his3-AI(96)* cDNA was 15.6 hr for DG2254 (pGAL) and 11.8 hr for DG2255 (+pGTy1). The differences in half-life could be accounted for by slightly slower mean generation time for DG2254 (pGAL; 11 hr) than for DG2255 (pGTy1; 8 hr), which was monitored during the time course (Table 3). Similarly, the modest differences in cDNA half-life calculated for DG2411 (2μ vector; 15 hr) and DG2276 (Ty1/2μ; 21 hr) could be accounted for by differences in mean generation times. Strains DG1929 [Ty1*his3-AI(96)*] and JC121 (+21 Ty1's) also had very similar Ty1 cDNA half-lives of 13 and 11.4 hr, respectively.

DISCUSSION

Ty1 cosuppression occurs at a post-transcriptional level: Our results provide evidence for a unique component of Ty1 CNC that partially affects TyA1-gag accumulation and blocks cDNA synthesis in a copy-number-dependent manner, but does not affect Ty1 RNA accumulation. Therefore, Ty1 cosuppression can also occur at a post-transcriptional level, perhaps by loss of a factor that stimulates utilization of Ty1 RNA for retrotransposition. We propose that this factor becomes limiting or more important for retrotransposition as Ty1 copy number increases and the elements undergo homology-dependent or "pairing" interactions. Our results also show similarities as well as interesting differences with Ty1 transcriptional cosuppression, another copy number and homology-dependent component of Ty1 CNC used to silence transposable elements (JIANG 2002). One of the key features distinguishing the post-transcriptional and transcriptional components of Ty1 CNC is that post-transcriptional cosuppression is provoked when the suppressing elements are transcriptionally repressed (pGTy1 elements) or fused to a constitutive foreign promoter (pPGK1-Ty1Δ238/ADH ter) whereas transcriptional cosuppression is not. We also show that

TABLE 3

Half-life of Ty1 cDNA

Strain[a]	Relevant genotype	cDNA half-life (hr)[b]	Mean generation time (hr)[c]
DG2254	pGAL vector	15.6	11
DG2255	pGTy1	11.8	8
DG2411	Vector	15.0	14
DG2276	Ty1/2μ	21.0	18
DG1929	No added Ty1's	13.0	ND
JC121	+21 Ty1's	11.4	ND

ND, not determined.

[a] All strains contain the genomic element Ty1*his3-AI(96)*.

[b] cDNA half-lives were calculated as described in MATERIALS AND METHODS and in Figure 9. The amount of Ty1 cDNA that hybridized with a ^{32}P-labeled RT probe was used to calculate cDNA half-life for strains DG1929, JC121, DG2411, and DG2276 since the level of Ty1*his3-AI(96)* cDNA was near or below our limit of detection in strains JC121 and DG2276. A *his3-AI* probe was used for strains DG2554 and DG2255.

[c] Strains were grown in supplemented SD liquid medium. Mean generation times were calculated after addition of PFA to a final concentration of 200 μg/ml.

Ty1 RNA levels increase dramatically with copy number, yet transcription of a specific element remains essentially unchanged and global retrotransposition decreases. These results are difficult to reconcile with transcriptional cosuppression being the sole CNC mechanism operating to limit Ty1 transposition.

Single-element Ty1 transposition and the role of the host defense gene *RAD3*: A single Ty1 element shows robust transpositional activity in the absence of additional Ty1 elements. Since these comparisons are between Ty1-less and laboratory strains, additional differences in genetic backgrounds may exist that influence our results; however, the Ty1*his3-AI* element used is identical. We also determined whether the previously identified *RAD3* host defense gene affected single Ty1 element transposition (LEE *et al.* 2000). *RAD3* appears to prevent Ty1 transposition through a mechanism involving degradation of the cDNA as part of the nucleotide excision repair/TFIIH complex and is related to mechanisms used to prevent short sequence recombination. Our results indicate that single Ty1 transposition is inhibited by *RAD3*, as shown by the 28-fold increase in the rate of Ty1*his3-AI(96)* transposition observed in a *rad3-G595R* mutant. These results suggest that Ty1 cosuppression is not absolutely required for *RAD3* to inhibit Ty1 retrotransposition.

Requirements for Ty1 cosuppression: Several features of this work have allowed us to consider new mechanisms for post-transcriptional cosuppression, since *S. cerevisiae* lacks the conserved pathway for RNAi found in other eukaryotes (TIJSTERMAN *et al.* 2002). Furthermore, Ty1 elements undergo transcriptional cosuppression without DNA methylation or polycomb-mediated repression (JIANG 2002). The markers routinely used to genetically tag Ty1 elements do not influence post-transcriptional cosuppression, since similar decreases in retrotransposition are obtained with suppressed elements tagged with *his3-AI* or other marker genes when compared with an unmarked element. Indeed, if *S. cerevisiae* possessed the conserved RNAi silencing pathway, the marked Ty1 transcripts would probably trigger RNAi and Ty1 mRNA destruction since these transcripts are aberrant and may contain extensive double-stranded regions.

We have observed a variety of context effects supporting the idea that Ty1-Ty1 interactions are required for cosuppression. For example, transposition decreases ~4800-fold when the target Ty1*his3-AI* element is carried on a centromere-based plasmid in a strain with 20 chromosomal Ty1 elements when compared with a strain with no additional elements. Conversely, transposition of a chromosomal Ty1*his3-AI* element decreases 100-fold when challenged with 21 chromosomal Ty1 elements or a Ty1/2μ element. Clearly, differences in the location and copy number of Ty1 elements can have a major impact on the extent of cosuppression, perhaps by influencing how accessible the elements are to each other. In addition, Ty1 cosuppression is exceptionally sensitive, since adding as few as 3 chromosomal Ty1 elements decreases transposition of the Ty1*his3-AI* element ~9-fold and resembles the copy-number sensitivity observed with the I-element retrotransposon of Drosophila (CHABOISSIER *et al.* 1998; JENSEN *et al.* 2002). It is important to note, however, that most if not all Ty1 elements in the genome are inhibited for retrotransposition as their copy number increases, even when an individual element is monitored for retrotransposition. The most compelling evidence for a global inhibition of Ty1 retrotransposition is from the glycine tRNA insertion assay, where it is evident that overall Ty1 retrotransposition decreases in strains that contain multiple transcriptionally active elements.

It will be interesting to determine whether all chromosomal Ty1 elements have an equal capacity to interact or whether there are "cold spots" where Ty1 element insertions cannot exert cosuppression. One cold spot may be in the rDNA repeats, which undergo allelic or ectopic meiotic recombination at a reduced frequency (PETES and BOTSTEIN 1977; DAVIS *et al.* 2000) and where Ty1 elements insert efficiently but are not well expressed (BRYK *et al.* 1997; SMITH and BOEKE 1997). In addition, euchromatic Ty elements undergo lower levels of ectopic recombination (KUPIEC and PETES 1988), even when cells are mutagenized with methyl methanesulfonate or ultraviolet light (KUPIEC and STEINLAUF 1997; PARKET and KUPIEC 1992). Interestingly, pGTy1 induction greatly stimulates ectopic recombination of Ty elements even when the donor element cannot synthesize cDNA (NEVO-CASPI and KUPIEC 1994). Perhaps the Ty1-Ty1 interactions required for Ty1 cosuppression prevent ectopic recombination.

The transcriptional state of the suppressing elements has diverse effects on Ty1 cosuppression and the process of retrotransposition. Native Ty1 transcription is required for transcriptional cosuppression (Jiang 2002), while our work shows that a transcriptionally silent pGTy1 element confers significant post-transcriptional cosuppression. Although *GAL1*-promoted pGTy1 transcription abolishes all cosuppression, we show that transcription of a translationally defective pGTy1-ATGfs element restores single Ty1 element transposition to a high level. In addition, wild-type pGTy1 expression limits the action of host defense genes (Conte *et al.* 1998; Lee *et al.* 1998) and results in a massive increase in processed TyB1-pol proteins, VLPs, and *de novo* transposition events (Boeke *et al.* 1985; Garfinkel *et al.* 1985; Curcio and Garfinkel 1992). Perhaps some of the striking consequences of pGTy1 "transposition induction" (Fink *et al.* 1986) are caused by disrupting the Ty1-Ty1 interactions required to establish cosuppression.

Paradoxically, fusing Ty1 to the strong constitutive *PGK1* promoter confers Ty1 CNC whereas an identical fusion to the *GAL1* promoter followed by galactose induction of the resulting pGTy1 element abolishes CNC. Since galactose itself does not mediate CNC, the opposing effects of the *PGK1*- and *GAL1*-Ty1 promoter fusions may reflect inherent differences in how Ty1 transcription affects CNC. The most obvious difference is that *PGK1*-promoted transcription is constitutive whereas pGTy1 expression is induced when galactose is present. Therefore, uncoupling *GAL1* induction from constitutive transcription may restore Ty1 CNC if induction disrupts the interactions required for CNC.

The specificity of Ty1 cosuppression further suggests that both transcription and homology dependence play an important role in minimizing Ty1 transposition. Only a transcriptionally active Ty2/2μ element inhibits Ty1 retrotransposition whereas Ty3/2μ and Ty5/2μ elements do not. However, a transcriptionally silent pGTy2 element does not inhibit Ty1 transposition. Interestingly, Ty1 and Ty2 elements share ~70% nucleotide sequence identity within the minimal region of a pGTy1 element required for post-transcriptional cosuppression, and their LTRs are highly related and cannot be defined as Ty1 or Ty2 specific (Kim *et al.* 1998; Jordan and McDonald 1999). In contrast, Ty3 and Ty5 elements, which do not confer cosuppression, share low nucleotide sequence similarity with Ty1 (data not shown). The dependence on Ty2 transcription suggests that there may be a trade-off between transcription and sequence similarity of the suppressing element when compared with the behavior of Ty1. Inhibition of Ty1 transposition by Ty2 elements also resembles other forms of post-transcriptional cosuppression. For example, the I-factor retrotransposon undergoes homology-dependent gene silencing when as little as 100 bp of the I-factor promoter is present in the suppressed copy (Jensen *et al.* 2002). However, it is unclear whether I-factor cosuppression requires transcription of the suppressing element (Chaboissier *et al.* 1998; Birchler *et al.* 1999; Jensen *et al.* 1999).

An alternative view is that Ty2/2μ expression inhibits Ty1 retrotransposition by acting as a dominant negative mutation (Herskowitz 1987). However, pGTy2 expression does not inhibit Ty1 transposition in a laboratory strain containing other Ty1 and Ty2 elements (Curcio *et al.* 1990; data not shown). Therefore, it will be interesting to determine whether Ty2 proteins are required for Ty1 CNC.

Repressed pGTy1 elements have been useful for defining the minimal sequences necessary and sufficient for post-transcriptional cosuppression. Although we have utilized a variety of approaches to identify the sequences responsible for pGTy1-mediated post-transcriptional cosuppression, the minimal elements still contain >1 kb of mostly *TYA1* sequence. Although the minimal pGTy1 element contains *cis*-acting regions required for Ty1 expression, reverse transcription, and RNA packaging (Voytas and Boeke 2002), the fact that pGTy1 elements are transcriptionally repressed suggests that these functions are not required for the suppressing elements to inhibit Ty1 transposition. However, factors required for Ty1 transcription could still play a role in the ability of the pGTy1 elements to interact if they remain bound to the element, even though pGTy1 transcription is repressed.

Surprisingly, the presence of the 3′ LTR increases post-transcriptional cosuppression of the minimal pGTy1 elements >10-fold, which also surpasses the activity of the complete pGTy1 and most other contexts where the Ty1 elements are transcribed. Yet the LTR is not required for post-transcriptional cosuppression of a full-length element nor does it have any activity by itself. These results suggest that the interactions of the complete repressed pGTy1 element are sufficient to provoke post-transcriptional cosuppression, perhaps by homology alone, and that the LTR acts to enhance the interaction when the minimal pGTy1 region is present. The LTR may contain a specific site that establishes Ty1-Ty1 interaction. In this view, the LTR may be similar to the transposon and nontransposon repeats and DNA satellites that are intimately associated with the formation and functions of heterochromatin (Henikoff 2000). Alternatively, dispersed motifs that mediate binding of a sequence-specific factor required for Ty1 post-transcriptional cosuppression could still be present in *TYA1*. The observation that a pGTy1 element significantly increases CNC in a laboratory strain containing 32 Ty1 and 13 Ty2 elements suggests that our results are not limited to an unusual Ty1-less strain background and that the capacity of laboratory strains to inhibit retrotransposition has not been exhausted. Our results extend previous work indicating that doubling the number of Ty1 elements in a laboratory strain does not destabilize the genome (Boeke *et al.* 1991).

Ty1 expression and cosuppression: Ty1 transcriptional cosuppression prevents Ty1 transcription in a subset of cells (Jiang 2002), yet our results suggest that Ty1 transcripts increase in a copy-number-dependent manner in unselected cells. The Ty1 RNA level from a specific target element remains essentially unchanged in strains containing additional chromosomal Ty1's, Ty1/2μ elements, or a silenced pGTy1 element. However, the modest decrease in Ty1*his3-AI(96)* RNA level observed in cells containing the transcriptionally and translationally defective Ty1-ATGfs/2μ element suggests the possibility that transcriptional and post-transcriptional cosuppression is hyperactive in these cells, since a similar decrease in Ty1*his3-AI(96)* RNA is not observed with the wild-type Ty1/2μ element. Ty1*his3-AI(96)* transposition also decreases 1.5-fold more in strain DG2274 (Ty1-ATGfs/2μ) than in DG2276 (Ty1/2μ), but more extensive rate experiments will be needed to determine whether this difference is significant. Taken together, our results suggest that either transcriptional cosuppression is not tightly controlled and many expressing cells exist or all Ty1 transcription is constrained to a subset of cells. If Ty1 RNA can comprise up to 0.8% of total RNA in unselected cells (Curcio *et al.* 1990), the level of expression must be dramatically higher in cells where transcriptional cosuppression has failed. We propose that post-transcriptional cosuppression evolved to prevent high levels of Ty1 retrotransposition in cells that do not undergo transcriptional cosuppression.

The level of TyA1-gag protein increases with the copy number of expressible Ty1 elements. However, the 6- to 16-fold increase in the level of TyA1 proteins is not as dramatic as the 30- to 77-fold increase in Ty1 RNA observed in strains containing multiple expressing Ty1 elements. Although this difference in protein level could be caused by a change in TyA1 stability in strains where post-transcriptional cosuppression is active, we think this is unlikely since pulse-chase immunoprecipitations show that endogenous TyA1 proteins are stable in a laboratory strain (Curcio and Garfinkel 1992). In addition, the increased level of unprocessed Ty1 proteins in pGTy1-induced cells is proportional to the increase in Ty1 RNA, suggesting that the translational capacity of Ty1 RNA has not been exceeded. These results suggest that there may be a component of Ty1 post-transcriptional cosuppression that affects translation directly or sequesters Ty1 RNA from the translation machinery. Ty1 RNA does not contain upstream AUG codons that would preclude translation initiation from the correct AUG, but *TYA1* and *TYB1* have a codon bias suggesting they are weakly expressed (Lerat *et al.* 2002). In addition, it remains to be determined whether other steps in the pathway of Ty1 gene expression, such as the efficiency of +1 translational frameshifting or the production of TyB1-pol proteins, respond to post-transcriptional cosuppression.

Ty1 post-transcriptional cosuppression blocks cDNA production: We have examined the accumulation and stability of Ty1 cDNA when all elements are located in the genome and when cells contain an expressed Ty1/2μ element and a chromosomal element or a silent pGTy1 element and a chromosomal element. In each context, there is a decrease in Ty1 cDNA level, but not in cDNA stability when post-transcriptional cosuppression is active. The levels of Ty1 cDNA and retrotransposition are also correlated, with the repressed pGTy1 elements allowing a higher level of Ty1 cDNA accumulation and retrotransposition when compared with the suppressive properties of additional chromosomal or Ty1/2μ elements. In contrast, the TFIIH helicases Rad3 and Ssl2 inhibit Ty1 retrotransposition and short sequence recombination by mechanisms involving decreases in the accumulation and stability of free DNA ends and Ty1 cDNA (Lee *et al.* 2000). Together these studies reinforce the idea that the accumulation of Ty1 cDNA is a rate-limiting step in the pathway of retrotransposition *in vitro* (Eichinger and Boeke 1990) and *in vivo* (Lee *et al.* 1998, 2000; Rattray *et al.* 2000; Scholes *et al.* 2001).

Integrating Ty1 transcriptional and post-transcriptional cosuppression: Clearly, the Ty1 transcriptional and post-transcriptional cosuppression pathways have striking similarities and differences. We propose a model to explain the related roles that transcriptional and post-transcriptional cosuppression play in Ty1 CNC (Figure 10). Ty1 cosuppression begins when the genome contains more than one element, although additional host defense genes such as *RAD3* still inhibit single-element retrotransposition. Multiple Ty1 elements interact, resulting in a cosuppressed state. For transcriptional cosuppression, a higher Ty1 element copy number may be required for transcription to be completely silenced (Jiang 2002). Since we observe significant inhibition of retrotransposition with just a few additional Ty1 copies present, perhaps post-transcriptional cosuppression begins first. Then as the Ty1 copy number increases to a level observed in laboratory strains, post-transcriptional cosuppression continues to increase, and transcriptional cosuppression becomes more active in the population. However, since Ty1 transcriptional cosuppression is characterized by rapid switches between the on and off states, post-transcriptional cosuppression and additional host defense genes may act to minimize Ty1 transposition in expressing cells, which must contain an exceptionally high level of Ty1 RNA.

How does Ty1 post-transcriptional cosuppression occur? We posit that functions required for directing Ty1 RNA into the transposition pathway become limiting or more important as Ty1-Ty1 interactions occur in the nucleus. Our results suggest that these functions act after the production of Ty1 RNA but before the completion of reverse transcription, an interval that encompasses several critical steps in the process of Ty1 retro-

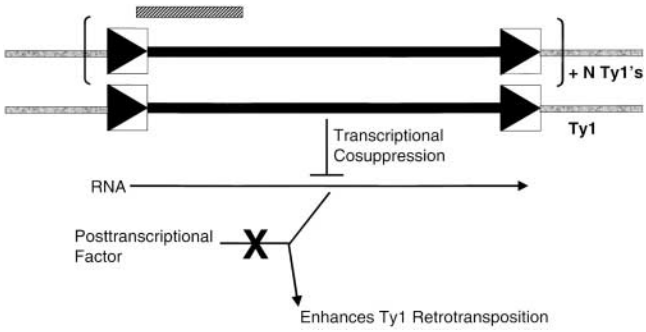

Figure 10.—The relationship between Ty1 transcriptional and post-transcriptional cosuppression. A Ty1 element is represented by a solid rectangle and an LTR by a horizontal triangle. For simplicity, two Ty1 elements are shown, but the bracketed element at the top represents multiple elements (N). The hatched rectangle represents the minimal Ty1 sequence that confers post-transcriptional cosuppression, which is greatly enhanced by the presence of a downstream LTR. When the genome contains a single Ty1 element, cosuppression pathways are inactive and only host defense genes limit Ty1 transposition (Curcio and Garfinkel 1999). As the copy number of Ty1 elements increases to a high number and the elements interact, transcriptional and post-transcriptional cosuppression is activated. Transcriptional cosuppression shuts off Ty1 transcription in a subset of cells, while post-transcriptional cosuppression limits the action (represented by the X) of a stimulatory factor with Ty1 RNA in the rest of the cells.

transposition. These include Ty1 RNA processing and nuclear export, translation to synthesize Ty1 proteins and programmed +1 frameshifting, VLP formation and correct packaging and folding of Ty1 RNA, as well as reverse transcription. It is tempting to speculate that a specific Ty1 RNA chaperone becomes critical for nuclear export as Ty1 copy number increases, since sequestering Ty1 RNA in the nucleus would explain several features of post-transcriptional cosuppression. However, the idea that Ty1-Ty1 interactions limit the action or accessibility of functions that direct Ty1 transcripts into the retrotransposition pathway will require further investigation.

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