MOLECULAR AND CELLULAR BIOLOGY, June 1992, p. 2813–2825
0270-7306/92/062813-13$02.00/0

Vol. 12, No. 6

Posttranslational Control of Ty1 Retrotransposition Occurs at the Level of Protein Processing

M. JOAN CURCIO AND DAVID J. GARFINKEL*

Laboratory of Eukaryotic Gene Expression, ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, P.O. Box B, Building 539, Frederick, Maryland 21702-1201

Received 30 December 1991/Accepted 26 March 1992

High-level expression of a transpositionally competent Ty1 element fused to the inducible *GAL1* promoter on a 2μm plasmid (pGTy1) overcomes transpositional dormancy in *Saccharomyces cerevisiae*. To investigate the mechanisms controlling the rate of Ty1 retrotransposition, we quantitated transposition and Ty1 gene products in cells induced and uninduced for expression of pGTy1. The increase in Ty1 transposition was 45- to 125-fold greater than the increase in Ty1 RNA effected by pGTy1 induction. Translational efficiency of Ty1 RNA was not altered in transposition-induced cells, since p190$^{TYA1\text{-}TYB1}$ protein synthesis increased in proportion to steady-state Ty1 RNA levels. Therefore, expression of a pGTy1 element increases the efficiency of Ty1 transposition at a posttranslational level. Galactose induction of pGTy1 enhanced *TYA1* protein processing and allowed detection of processed *TYB1* proteins, which are normally present at very low levels in uninduced cells. When the ability of genomic Ty1 elements to complement defined mutations in *HIS3*-marked pGTy1 elements was examined, mutations in the protease domain or certain mutations in the integrase domain failed to be complemented, but mutations in the reverse transcriptase domain were partially complemented by genomic Ty1 elements. Therefore, the activity of Ty1 elements in yeast cells may be limited by the availability of Ty1 protease and possibly integrase. These results suggest that Ty1 transposition is regulated at the level of protein processing and that this regulation is overcome by expression of a pGTy1 element.

Retrotransposons are a class of eucaryotic transposable elements that resemble retroviral proviruses in structure and mode of replication. The yeast *Saccharomyces cerevisiae* contains four distinct families of retrotransposons known as Ty1 through Ty4. Ty1, present in about 25 to 30 copies per haploid genome in typical laboratory strains, is the most numerous and recurrent insertional mutagen in yeast cells (6). Ty1 elements consist of a 5.2-kb internal domain surrounded by 338-bp long terminal repeats (LTRs). There are two partially overlapping open reading frames, called *TYA1* and *TYB1*, which are analogous to the retroviral *gag* and *pol* genes, respectively. *TYA1* encodes nucleocapsid proteins of Ty1 viruslike particles (VLPs). *TYB1* encodes a protein with homology to retroviral protease (PR), integrase (IN), reverse transcriptase (RT), and RNase H (RH). *TYB1* is expressed as a *TYA1-TYB1* fusion protein resulting from a specific +1 frameshift event occurring before the stop codon of the *TYA1* open reading frame (2). The two primary translation products of Ty1, a 58-kDa *TYA1* protein and a 190-kDa *TYA1-TYB1* protein, are processed to yield a 54-kDa *TYA1* protein, a 23-kDa PR, a 90-kDa IN, and a 60-kDa RT/RH through the action of Ty1 PR (1, 20, 31, 38). Processing of Ty1 proteins occurs in the maturing VLP and is required for Ty1 transposition (38).

To facilitate the study of Ty1 transposition, Boeke and coworkers developed a simple method for enhancing the frequency of transposition (4). The method involves fusing a Ty1 element to the inducible yeast *GAL1* promoter on a 2μm plasmid. When expression of the fusion element contained on plasmid pGTy1 is induced by growth on medium containing galactose (referred to as transposition induction [4]), transposition of the plasmid-borne element occurs at a frequency of more than one event per cell. Genomic Ty1 element transposition is also amplified during pGTy1 induction. Transposition induction results in the appearance of cytoplasmic VLPs containing RT/RH, IN, Ty1-encoded antigens, Ty1 RNA, and Ty1 DNA (16, 18). The cofractionation of these components illustrates the essential role of VLPs in the process of Ty1 retrotransposition.

In a typical haploid yeast cell, as much as 0.1 to 0.8% of total RNA is Ty1 RNA (13, 17). Despite the abundance of Ty1 RNA, Ty1 proteins and VLPs are present at very low levels, and the rate of Ty1 transposition is only 10^{-5} to 10^{-7} per Ty1 element per cell division (11). The factors involved in regulating transpositional dormancy have not been well characterized. One potential factor is the existence of defective Ty1 elements in the genome. In general, chromosomal Ty1 elements appear to be full length and not grossly rearranged. Comparison of the sequences of three Ty1 elements, including the functional element Ty1-H3, has indicated that while base substitutions are common, nonsense mutations are not (3, 10, 24). A sensitive test for the transpositional competence of a Ty1 element is to determine whether it transposes at high levels when expressed from the *GAL1* promoter on a 2μm plasmid. Genetic tagging of the pGTy1 element with the bacterial *neo* gene (7), the yeast *HIS3* gene (21), or the retrotransposition indicator gene *his3*AI (11) facilitates the analysis. To ensure that transposition of a defective pGTy1 element has not occurred by complementation from chromosomal Ty1 elements, transcription of chromosomal Ty1 elements can be inhibited by the use of an *spt3-101* strain (7, 14). The *SPT3* gene is required for the production of full-length Ty1 transcripts (36) and for the transposition of chromosomal Ty1 elements but not pGTy1 elements (7). By these methods, both functional and defective elements have been identified among Ty1 elements that transposed into selectable target loci (3, 13, 14). Among Ty1 elements that are native to the yeast genome, one transpositionally competent element has been

* Corresponding author.

TABLE 1. Strains

Strain	Genotype	Plasmid	Reference
GRF167	*MATα ura3-167 his3Δ200*		4
DG789	*MATα ura3-167 his3Δ200 spt3-101*		11
BWG1-7A	*MATa his4-519 ura3-52 leu2-3,2-112 adel-100*		23
DG531	*MATa his4-519 ura3-52 leu2-3,2-112 adel-100 spt3-101*		38
JC213	GRF167	pGTy1-H3m*his3*AI	11
JC227	*MATα ura3-167 his3Δ200* ::Ty1m*his3*AI-227		This work
JC242	*MATa ura3-167 his3Δ200* ::Ty1m*his3*AI-242		11
JC306	JC242	pGTy1-H3*neo*	This work
JC308	JC242	p*GAL1*-X/P	This work
JC310	JC242	pGTy1-H3*lacO*(*Δ3'LTR*)	This work
JC333	*MATa ura3*::*GAL1*/Ty1-H3*neo his3Δ200 trp1-289*		This work

reported (Ty1-588 [14]). Also, a recessive mutation within the IN domain that is present in a subset of genomic Ty1 elements has been described (3). These studies have shown that the genome contains both functional and defective elements, but the relative number of each is not known.

Another mechanism of controlling the Ty1 transposition rate that has been investigated is the transcriptional regulation of functional Ty1 elements. The transposition-competent genomic element Ty1-588 was marked with *neo* and shown to be well transcribed relative to several other *neo*-marked genomic Ty1 elements (14, 19). These data suggest that transposition-competent Ty1 elements are not preferentially inhibited at the level of transcription relative to defective Ty elements but that a posttranscriptional mechanism maintains their quiescence. In this report, we have continued our analysis of transpositional regulation by comparing the steps of Ty1 gene expression in transposition-induced and uninduced yeast strains. Our data suggest that Ty1 transposition is regulated at a posttranslational level. We discuss biochemical and genetic evidence showing that the levels of PR and possibly IN may limit the rate of Ty1 transposition.

MATERIALS AND METHODS

Yeast strains and media. The yeast strains used in this study are described in Table 1. Strains JC242 and JC227 are congenic derivatives of GRF167 that each contain a single genomic Ty1m*his3*AI insertion in different locations, designated ::Ty1m*his3*AI-242 and ::Ty1m*his3*AI-227, respectively. These strains were isolated after galactose induction of pGTy1-H3m*his3*AI as described previously (11). All media were prepared as described by Sherman et al. (35).

Plasmid constructions. Plasmid pGTy1-H3 (previously called pGTyH3) and derivatives marked with the yeast *HIS3* gene or bacterial *neo* gene outside of the Ty1 coding sequences have been described previously (4, 8, 21). Figure 1 is a diagram of the *GAL1*/Ty1 fusion element in pGTy1-H3*HIS3*. The figure indicates the nucleotide positions of mutations made within the Ty1-H3 element (as described below) and their locations in the *TYA1* and *TYB1* domains. Plasmid pGTy1-H3*HIS3*(*tyb-2173G*) contains a 15-nucleotide linker, d(TCGAGCTCACGCGTG), inserted into the *Sal*I site of the Ty1-H3 element at nucleotide 2173, creating a five-amino-acid in-frame insertion. Plasmid pGTy1-H3*HIS3*(*tybΔ2173*) was constructed by deleting the sequences in pGTy1-H3*HIS3*(*tyb-2173G*) between the *Sac*I site at nucleotide 2173 and the *Sac*I site at the 5′ end of the *HIS3* gene fragment inserted at Ty1-H3 position 5561. An 18-nucleotide linker containing nonsense codons in three frames, d(CTAGTCTAGACTAGAGCT), was inserted at the deletion point. Plasmid pGTy1-H3*HIS3*(*tyb-4627G*) contains a five-amino acid in-frame insertion at nucleotide 4627 made by introducing a 15-nucleotide linker, d(AGCTGAGCTCTAGAA), into the *Hin*dIII site. Plasmid pGTy1-H3*HIS3*(*tybΔ3301*) contains a deletion of Ty1 nucleotides 3301 to 5561. Using pGTy1-H3*HIS3* containing a *Sac*I linker inserted into the *Bgl*II site at nucleotide 3301 (38), we deleted the *Sac*I fragment between nucleotide 3301 and the 5′ end of the *HIS3* fragment and inserted in its place the 18-nucleotide nonsense codon linker described above. The *tyb-5463G* mutation in pGTy1-H3*neo* was made by inserting the *Sac*I linker, d(GGCGAGCTCGCC), into the *Sna*B1 site at nucleotide 5463, creating a four-codon insertion. The 5.2-kb *Xho*I-*Sac*I fragment of pGTy1-H3*neo*(*tyb-5463G*) was exchanged for the 5.3-kb *Xho*I-*Sac*I fragment of pGTy1-H3*HIS3*, resulting in a deletion of Ty1 sequences from nucleotides 5463 to 5561. The 18-nucleotide linker with

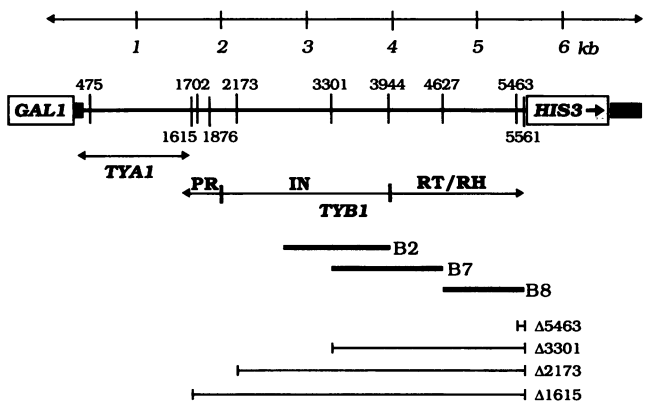

FIG. 1. Diagram of the *GAL1*-promoted Ty1 element in pGTy1-H3*HIS3*. The Ty1-H3 LTRs are represented as solid rectangles bracketing the internal ε domain, which includes the partially overlapping *TYA1* and *TYB1* open reading frames. The approximate locations of the boundaries of *TYA1* and the PR, IN, and RT/RH domains in *TYB1* are shown below. Nucleotide map positions where mutations analyzed in this study were made within the element are indicated by vertical lines. The shaded bars indicate the regions of *TYB1* that are detected by antisera B2, B7, and B8, which were raised against *trpE-TYB* fusion proteins in rabbits as described previously (20, 38). The extent of deleted *TYB1* sequences in several mutants of pGTy1-H3*HIS3* analyzed in this study is indicated by the horizontal lines. The pGTy1-H3 element marked with the yeast *HIS3* gene, bacterial *neo* gene, or *lacO* sequences at the *Bgl*II site at nucleotide 5561 were used in this study, but only *HIS3* is depicted in the figure.

amber (Am) codons in three frames described above was inserted into the *Sac*I site at the deletion point to generate pGTy1-H3*HIS3*(*tybΔ5463*). Plasmids pGTy1-H3*HIS3*(*tya-475Am*) and pGTy1H3*HIS3*(*tyb-3944Am*) were constructed by insertion of a 12-nucleotide nonsense codon linker, d(CTAGCTAGCTAG), into the *Pvu*II site at nucleotides 475 and 3944, respectively. Mutations *tyb-1702G* and *tyb-1876G*, which are four- and five-codon insertions in the PR domain, respectively, have been described previously (*Sac*I-1702 and *Mlu*I-1876 [38]). Plasmid pGTy1-H3*neo*(*tybΔ1615*) was constructed from a derivative of pGTy1-H3 that contains a linker insertion at position 1615 (*Mlu*I-1615 [38]). Sequences between the *Mlu*I site at nucleotide 1615 and the *Bgl*II site at nucleotide 5561 were deleted, and an adaptor, d(CGCGTCA TCGATGA), was inserted to construct pGTy1-H3(*tyb Δ1615*). The plasmid was marked at the *Bgl*II site with the *neo* gene contained on a *Bam*HI fragment from pGH54 (26). A derivative of pGTy1-H3 marked with a 40-nucleotide sequence of *lacO* at nucleotide 5561 (4) was used to construct pGTy1-H3*lacO*(*Δ3'LTR*), which contains a deletion from nucleotide 5561 extending 3′ into flanking plasmid sequences and including the entire 3′ LTR. Plasmids pGTy1-H3m*his3*AI (11), p*GAL1-lacZ* (4), and p*GAL1*-X/P (14) have been described previously. Plasmids were introduced into yeast strains GRF167, DG789, BWG1-7A, DG531, and JC242 by the method of Ito et al. (25).

Northern (RNA) blot analysis. Yeast strains harboring *URA3*-based plasmids were grown at 30°C in synthetic complete (SC) medium lacking uracil (SC-Ura) with 2% glucose. Strains GRF167, BWG1-7A, JC227, and JC242 were grown in SC glucose medium. The cultures were then diluted 25-fold into 20 ml of SC-Ura or SC containing 2% galactose and grown overnight at 20°C. Total RNA was isolated as described by Sherman et al. (35). As a control for quantitation, antisense *HIS3* [^{3}H]RNA was synthesized in vitro by using pGEM-HIS3, which contains an 890-bp fragment of the yeast *HIS3* gene (21) subcloned into pSP70 (Promega) as a template. In vitro transcription was performed with Riboprobe Gemini System II (Promega) by the method of Melton et al. (30). Approximately 1 μg of antisense *HIS3* [^{3}H]RNA and 10-fold dilutions were combined with 1 μg of total RNA from yeast strain GRF167 to improve banding. Denaturation of the RNA samples with glyoxal, agarose gel electrophoresis, transfer of the RNA to a nylon membrane, and hybridization to excess amounts of radiolabeled RNA probes have been described in detail elsewhere (13).

Plasmid pGEM-HIS3 was used to synthesize a ^{32}P-labeled *HIS3* RNA probe to detect the Ty1m*his3*AI and control antisense *HIS3* transcripts. Plasmids pGEM-Ty1A (13) and pGEM-PYK1, which contains a 1.8-kb *Eco*RI fragment that includes the 5′ end of the yeast *PYK1* gene cloned into pSP70, were used as templates for the transcription of antisense RNA probes to detect Ty1 and *PYK1* mRNA, respectively. To quantitate hybridization to RNA samples, a portion of the hybridization filter containing the appropriate band was excised, and liquid scintillation counting was performed in a Tri-Carb 1500 analyzer (Packard). For the 6.7-kb Ty1m*his3*AI RNA band detected by the ^{32}P-labeled *HIS3* RNA probe, the counts per minute minus the background counts per minute in two different Northern analyses were as follows: 392,800 and 401,500 cpm for strain JC213; 1,500 and 2,200 cpm for strain JC227; and 1,800 and 1,700 cpm for strain JC242.

Immunoprecipitation of Ty proteins. Yeast strains GRF167, BWG1-7A, and DG531 and derivatives harboring *URA3*-based plasmids were precultured at 30°C in SC glucose or SC-Ura glucose. Galactose induction was performed by diluting the cultures 25-fold into 50-ml cultures of yeast nitrogen base (YNB) minimal medium containing 2% galactose and the amino acids required for growth and then incubating them overnight (12 to 16 h) at 20°C. In some experiments, strains BWG1-7A and DG531 transformed with the *LEU2*-based vector YEp13 (9) were used to avoid adding leucine, which is usually contaminated with trace amounts of methionine, to the minimal medium.

For radiolabeling, the cultures were resuspended in fresh YNB galactose medium at 2.0 units of optical density at 600 nm per ml, then 200 μCi of [^{35}S]methionine was added to 1 ml of each culture, and the 1-ml cultures were incubated at 20°C. Pulse-labeling was performed for 15 min for the detection of *TYA1* proteins and 20 min for the detection of the *TYA1-TYB1* polyprotein. To examine the processing of *TYA1* proteins, the [^{35}S]methionine was chased with a 1,000-fold excess of unlabeled methionine after 15 min. Samples were then harvested immediately or at 1, 2, 4, or 8 h after the [^{35}S]methionine addition. For the detection of processed *TYB1* proteins, no chase with unlabeled methionine was used, thereby maximizing the incorporation of [^{35}S]methionine. Incorporation of [^{35}S]methionine into trichloroacetic acid-precipitable material was linear for approximately 60 min under these conditions. An 18-h incubation with [^{35}S]methionine was optimal for detecting processed *TYB1* proteins (12). The labeling was terminated by pelleting the cells, removing the supernatant, and freezing the cell pellet in a dry ice-ethanol bath. Total cell lysates were prepared under denaturing conditions, and immunoprecipitation was performed by using fixed *Staphylococcus aureus* cells (IgG Sorb; The Enzyme Center) as described by Schauer et al. (34). Rabbit antisera B2 (38), Ty1-VLP (1), and B7 and B8 (20) were used to precipitate radiolabeled Ty proteins. Immunoprecipitates were subjected to sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis according to the method of Laemmli (28). Coelectrophoresis of prestained protein markers (Rainbow markers, 14.3 to 200 kDa; Amersham) was performed to provide molecular weight standards. Gels were fixed in 30% methanol–10% acetic acid for 30 min, soaked in Amplify (Amersham) enhancing solution for 30 min, and dried under vacuum. Dried gels were exposed to XAR film (Eastman Kodak) at −70°C. Quantitation of protein bands was performed by scanning densitometry using an LKB Ultroscan XL laser densitometer and by excision of the bands from the dried gels and liquid scintillation counting.

To demonstrate that mutations in pGTy1-H3 analyzed in this study do not affect the level of Ty protein expression, galactose-induced strains harboring pGTy1-H3 with mutations *tyb-1702G*, *tyb-1876G*, *tyb-2173G*, *tybΔ1615*, *tybΔ2173*, *tybΔ3301*, and *tybΔ5463* were subjected to Western immunoblot analysis with Ty1-VLP antisera. The level of *TYA1* protein in these strains was similar to the level of *TYA1* protein in a strain expressing a wild-type pGTy1-H3 element (12, 20).

Transposition rates. The transposition rate of pGTy1-H3m*his3*AI or genomic Ty1m*his3*AI elements was determined by the method of Lea and Coulson (29). Strains JC213, JC306, JC308, and JC310, which harbor *URA3*-based plasmids, were grown at 30°C in SC-Ura glucose. Strains JC227 and JC242 were grown at 30°C in SC glucose medium. Approximately 500 cells were used to inoculate each of 9 or 11 2-ml cultures of SC-Ura or SC medium containing galactose, and the cultures were grown to saturation at 20°C. The

TABLE 2. Effect of transposition induction on Tyl*m*his3AI RNA and transposition rate

Strain	Element	Rate of His^+ prototroph formation	Ratio of transposition rates[a]	Ratio of Tyl*m*his3AI transcript levels[b]
JC227	::Tyl*m*his3AI-227	$(3.3 \pm 1.3) \times 10^{-7}$	10,000	220
JC242	::Tyl*m*his3AI-242	$(1.2 \pm 0.5) \times 10^{-7}$	28,000	225

[a] Rate of His^+ prototroph formation in strain JC213 [$(3.4 \pm 0.5) \times 10^{-3}$] divided by the rate of His^+ prototroph formation in a strain that contains a genomic Tyl*m*his3AI element (JC227 or JC242).

[b] Level of Tyl*m*his3AI RNA in strain JC213 divided by the level of Tyl*m*his3AI RNA in strain JC227 or JC242 (determined by quantitation of RNA in the Northern analysis shown in Fig. 1 and a second Northern analysis not shown).

fraction of cells sustaining a marked transposition event was determined by spreading a portion of the culture onto SC-His glucose agar and incubating it at 30°C to end transposition induction and score histidine prototrophs. The cultures were titered on YEPD plates. The number of His^+ prototrophs was determined in the entire population rather than in Ura^+ cells selected for maintenance of the plasmid so that the relative increase in His^+ prototrophs could be directly compared with the relative increase in RNA, which can be determined only for the entire population. We determined in time course experiments that linear accumulation of His^+ prototrophs begins after 3 to 4 h. Thus, the period required for induction of His^+ prototrophs is insignificant compared with the 6 days required for the culture to reach confluency.

Complementation assay. The transposition frequencies of pGTyl-H3*HIS3* and derivatives in strain GRF167 and isogenic *spt3-101* strain DG789 were determined by the following method. Cells were grown at 30°C on SC-Ura glucose agar and then used to inoculate SC-Ura medium containing 2% galactose to induce transcription of the pGTyl-H3*HIS3* elements. The cultures were grown for 2 days at 20°C. To enrich for cells that maintained the *URA3*-based plasmids, each culture was diluted 40-fold into fresh SC-Ura galactose medium and grown for 3 additional days at 20°C. Because loss of pGTyl-H3*HIS3* plasmids occurs frequently in this medium (80 to 90% of the cells in a saturated SC-Ura galactose culture are Ura^-) (4), the fraction of Ura^- cells that sustained a transposition event of the *HIS3*-marked element was determined by spreading an aliquot of each culture onto SC glucose agar containing 5-fluoro-orotic acid (5-FOA) (5) and lacking histidine (5-FOA–His). Ura^- cells were titered by spreading dilutions onto 5-FOA agar or by spreading dilutions first onto YEPD agar and then replica plating samples to SC-Ura glucose agar to determine the fraction of Ura^- colonies. The average number of His^+ Ura^- colonies divided by the total number of Ura^- colonies assayed in three to five independently grown cultures is the transposition frequency. Complementation of a pGTyl-H3*HIS3* mutant by chromosomal Tyl elements was quantitated by determining the ratio of the transposition frequency in the *SPT3* strain to the frequency in the isogenic *spt3-101* strain.

The transposition frequencies of pGTyl-H3 derivatives marked with *neo* were determined by the method of Boeke et al. (8). Briefly, strain GRF167 or DG789 containing pGTyl-H3*neo* plasmids was grown at 20°C on SC-Ura galactose agar for 5 days. Independent colonies were purified on SC-Ura glucose agar to select colonies that maintained the plasmid throughout galactose induction. Ura^- segregants were then obtained and scored for G418 resistance on YEPD agar supplemented with 200 μg of G418 (GIBCO) per ml to determine the transposition frequency.

RESULTS

Expression of pGTyl-H3m*his3*AI increases the efficiency of transposition. The effect of high-level transcription on the efficiency of Tyl transposition was quantitated by comparing the transposition rate of a *GAL1*/Tyl fusion element residing on a 2μm plasmid with the transposition rates of two different genomic Tyl elements under control of their own promoters. All three Tyl elements were marked with the *his3*AI retrotransposition indicator gene (11). The *his3*AI gene is phenotypically His^- because of an artificial intron cloned in an antisense orientation into the coding sequences of the yeast *HIS3* gene. In Tyl elements marked with the *his3*AI gene on the minus strand (Tyl*m*his3AI), the transcriptional orientation of *his3*AI is opposite that of Tyl, which places the intron in a spliceable orientation within the Tyl transcript. Consequently, the marker gene can be activated in transposed copies of the marked Tyl element by splicing of the intron during the process of retrotransposition. This process results in transposed elements that contain a recreated *HIS3* gene and render the yeast cell His^+. Therefore, the rate of transposition of Tyl*m*his3AI elements is proportional to the rate of formation of histidine prototrophs in yeast strains containing a complete deletion of the chromosomal *HIS3* locus.

The rate of formation of histidine prototrophs was determined in the galactose-induced strain JC213, which harbors plasmid pGTyl-H3m*his3*AI, as well as in strains JC242 and JC227, which contain the single marked chromosomal elements Tyl*m*his3AI-242 and Tyl*m*his3AI-227, respectively. Strains JC242 and JC227 were chosen because of their high rate of histidine prototroph formation relative to other strains harboring chromosomal Tyl*m*his3AI elements (11, 12). Following growth at 20°C in SC-Ura or SC medium containing galactose, a 10,000- to 28,000-fold-higher rate of reversion to histidine prototrophy was observed in the strain containing the pGTyl-H3m*his3*AI plasmid than in strains containing a single chromosomal *his3*AI-marked Tyl element (Table 2).

To determine whether the higher rate of His^+ reversion in the strain expressing pGTyl*m*his3AI was strictly a result of increased Tyl*m*his3AI mRNA levels, we measured the amount of Tyl*m*his3AI mRNA in each of these strains. Quantitative Northern analysis was performed with total RNA from strains JC213, JC227, and JC242 grown as described above for determining the rate of histidine prototroph formation (Fig. 2). Levels of Tyl*m*his3AI RNA were compared with those of concentration standards prepared by serial 10-fold dilution of in vitro-synthesized antisense *HIS3* RNA. With use of a sense-strand *HIS3* [^{32}P]RNA probe, hybridization to the in vitro-synthesized antisense *HIS3* RNA was linear over the 1,000-fold range of quantities tested, as determined by excision of the bands from the

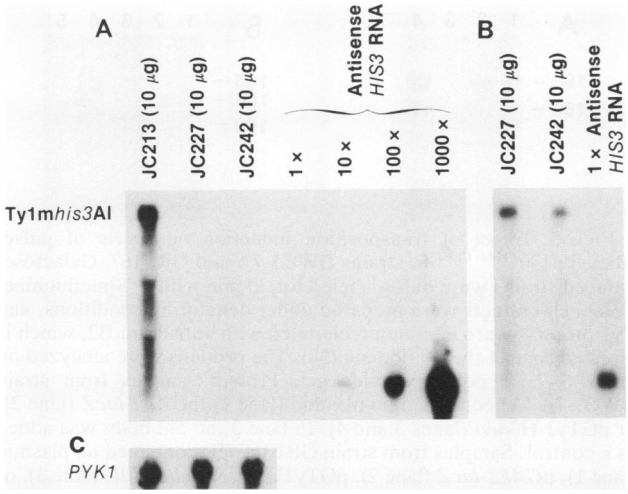

FIG. 2. (A) Quantitative comparison of Ty1m*his3*AI RNA derived from galactose-induced pGTy1 and from genomic Ty1 elements. Strains JC213 (pGTy1-H3m*his3*AI), JC227 (::Ty1m*his3*AI-227), and JC242 (::Ty1m*his3*AI-242) were grown overnight at 20°C in SC or SC-Ura medium containing galactose, and then total RNA was prepared from each strain. As a control, antisense *HIS3* [^{3}H]RNA was synthesized in vitro, and approximately 1 ng (1×), 10 ng (10×), 100 ng (100×), or 1 μg (1000×) of the RNA was combined with 1 μg of total yeast RNA as a carrier. Total RNA (10 μg) or antisense *HIS3* RNA samples were denatured with glyoxal and analyzed on a Northern blot as described in Materials and Methods. A sense-strand *HIS3* [^{32}P]RNA probe was used to detect the 6.7-kb Ty1m*his3*AI transcript as well as the 0.9-kb antisense *HIS3* RNA. (B) Segment of a longer exposure of the same autoradiograph to show the presence of the 6.7-kb Ty1m*his3*AI transcript in strains JC227 and JC242, which harbor chromosomal Ty1 elements. (C) Section of the blot that was reprobed with a *PYK1* [^{32}P]RNA probe as a control, detecting the 1.7-kb pyruvate kinase mRNA.

hybridization filter and liquid scintillation counting. By the same method, it was demonstrated that strain JC213 (pGTy1-H3m*his3*AI) had a level of Ty1m*his3*AI RNA that was 220- to 225-fold higher than those of strains JC242 and JC227, which contain chromosomal Ty1m*his3*AI elements. However, the higher level of mRNA synthesized was substantially less than the increased level of transposition that resulted from expression of pGTy1m*his3*AI. These data indicate that there was a 45- to 125-fold-greater rate of Ty1m*his3*AI transposition per Ty1m*his3*AI mRNA molecule in cells undergoing transposition induction than in cells that harbor chromosomal Ty1m*his3*AI elements (Table 2). Thus, the transposition rate of Ty1m*his3*AI is not linearly related to its mRNA concentration over this range of concentrations.

***trans* activation of genomic Ty1 transposition by pGTy1-H3 expression.** The observation that genomic Ty1 element transposition events increase when a marked pGTy1-H3 element is expressed suggests that genomic Ty1 elements are *trans* activated by transposition induction (4, 7, 13). To quantitate the level of *trans* activation of genomic Ty elements, we analyzed the effect of expressing a pGTy1-H3 element on the rate of histidine prototrophs that arise from a genomic Ty1m*his3*AI element. Strain JC242 (::Ty1m*his3*AI-242) was transformed with pGTy1-H3*neo*, which contains the transpositionally competent Ty1-H3 element, and p*GAL1*-X/P, which contains the *GAL1* promoter but lacks Ty sequences. The strains were grown at 20°C in SC-Ura galactose medium to induce expression from the p*GAL1* plasmids, and the rate

TABLE 3. *trans* activation of genomic element Ty1m*his3*AI-242 transposition

Strain	Plasmid	Rate of His$^+$ prototroph formation
JC306	pGTy1-H3*neo*	$(5.8 \pm 2.0) \times 10^{-6}$
JC308	pGAL1-X/P	$(3.9 \pm 1.9) \times 10^{-7}$
JC310	pGTy1-H3*lacO*(Δ3′*LTR*)	$(6.8 \pm 2.3) \times 10^{-6}$

of reversion to histidine prototrophy was determined by the method of Lea and Coulson (29). Histidine prototrophs resulting from transposition of the genomic Ty1m*his3*AI-242 element were formed at a 15-fold-greater rate in cells undergoing transposition induction of pGTy1-H3*neo* than in cells expressing p*GAL1*-X/P (Table 3). To determine whether transfer of the *his3*AI marker onto the plasmid Ty element by homologous recombination contributes significantly to the rate of histidine prototroph formation, we expressed pGTy1-H3*lacO*(Δ3′*LTR*), which contains the entire wild-type Ty1-H3 coding sequence but lacks the 3′ LTR. The pGTy1-H3*lacO*(Δ3′*LTR*) element lacks homology 3′ of the genomic *his3*AI sequences, thereby minimizing the possibility of homologous transfer of the marker gene. Histidine prototroph formation from Ty1m*his3*AI-242 was stimulated as well by pGTy1-H3*lacO*(Δ3′*LTR*) expression as it was by pGTy1-H3*neo* expression, indicating that our assay is specific for Ty1m*his3*AI-242 transposition (Table 3).

The data suggest that transposition of a genomic Ty1m*his3*AI element is increased approximately 15-fold by galactose induction of a transposition-competent pGTy1 element. To determine whether the *trans* activation of Ty1m*his3*AI-242 transposition resulted from an increase in mRNA transcribed from the genomic Ty1m*his3*AI element, we performed quantitative Northern blot analysis (Fig. 3). Total RNA was prepared from strains expressing p*GAL1*-X/P, pGTy1-H3*neo*, or pGTy1-H3*lacO*(Δ3′*LTR*). Steady-state levels of Ty1m*his3*AI-242 RNA remained unchanged in these three strains. Therefore, the 15-fold *trans* activation of Ty1m*his3*AI-242 transposition upon galactose induction of pGTy1-H3*neo* or pGTy1-H3*lacO*(Δ3′*LTR*) was not due to an increase in Ty1m*his3*AI-242 RNA levels.

Transposition induction does not change the efficiency of Ty1 RNA translation. Enhanced Ty1-H3m*his3*AI transposition per RNA molecule and *trans* activation of genomic Ty1

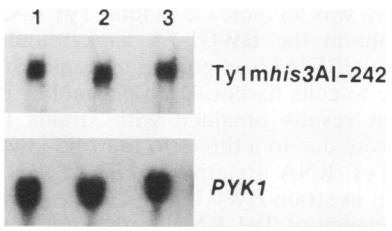

FIG. 3. Steady-state levels of Ty1m*his3*AI-242 RNA in strains expressing different p*GAL1* plasmids. Cells were grown overnight at 20°C in SC-Ura medium, conditions that result in high levels of transposition of pGTy1-H3*neo* in strain JC306. Total RNA (10 μg) prepared from galactose-induced strains JC306 (pGTy1-H3*neo*; lane 1), JC308 (p*GAL1*-X/P; lane 2), and JC310 (pGTy1H3*lacO*(Δ3′*LTR*); lane 3) was analyzed by Northern blotting. The blot was probed with a sense-strand *HIS3* [^{32}P]RNA probe to detect the Ty1m*his3*AI-242 transcript in each strain. In the bottom panel, the blot was probed with a *PYK1* [^{32}P]RNA probe as an RNA-loading control.

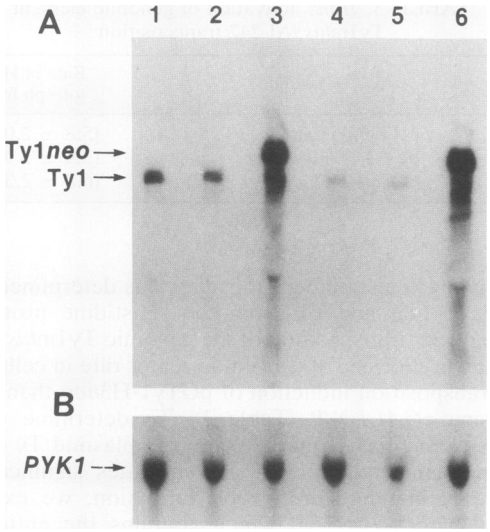

FIG. 4. Relative amounts of total Ty1 RNA in transposition-induced and uninduced strains. Strains BWG1-7A (lanes 1 to 3) and GRF167 (lane 4 to 6) containing either no plasmid (lanes 1 and 4), p*GAL1-lacZ* (lanes 2 and 5), or pGTy1-H3*neo* (lanes 3 and 6) were grown under galactose-inducing conditions as described in Materials and Methods. Total RNA was prepared from each strain, and 6-μg samples were subjected to Northern blot analysis. (A) A Ty1 [^{32}P]RNA probe was used to detect both the 5.7-kb Ty1 transcript and the 6.6-kb Ty1*neo* transcript. (B) The blot was reprobed with a *PYK1* [^{32}P]RNA probe.

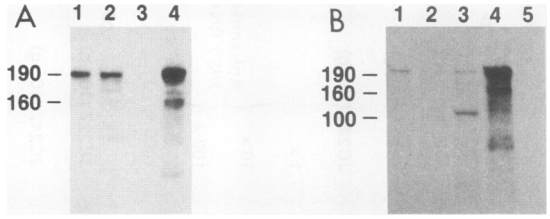

FIG. 5. Effect of transposition induction on levels of pulse-labeled p190$^{TYA1\text{-}TYB1}$ in strains BWG1-7A and GRF167. Galactose-induced strains were pulse-labeled for 20 min with [^{35}S]methionine. Total cell extracts were prepared under denaturing conditions, and Ty1 proteins were immunoprecipitated with antiserum B2, which is directed against the IN domain (38). The proteins were analyzed on an SDS–7.5% polyacrylamide gel. Protein samples from strain BWG1-7A (A) contained no plasmid (lane 1), p*GAL1-lacZ* (lane 2), or pGTy1-H3*neo* (lanes 3 and 4). In lane 3, no antibody was added as a control. Samples from strain GRF167 (B) contained no plasmid (lane 1), p*GAL1-lacZ* (lane 2), pGTy1H3*HIS3*(*tybΔ3301*) (lane 3), or pGTy1-H3*HIS3* (lanes 4 and 5). In lane 5, no antibody was added. The estimated sizes (in kilodaltons) of the Ty1 proteins, determined by coelectrophoresis of prestained protein markers, are indicated.

elements could result from more efficient translation of Ty1 RNA during transposition induction. Therefore, we compared the relative increase in the level of Ty1 RNA in cells expressing pGTy1-H3*neo* with the relative increase in the synthesis of the p190$^{TYA1\text{-}TYB1}$ polyprotein in two different strain backgrounds. The increase in total Ty1 RNA levels effected by pGTy1-H3*neo* expression was determined by Northern blot analysis of transposition-induced and uninduced strains (Fig. 4). Total RNA was isolated from galactose-induced strains BWG1-7A and GRF167, which do not contain plasmids (Fig. 4, lanes 1 and 4), and the same strains harboring p*GAL1-lacZ* (lanes 2 and 5) or pGTy1-H3*neo* (lanes 3 and 6). Ty1 RNA, including the 6.6-kb Ty1-H3*neo* RNA and the 5.7-kb unmarked Ty1 RNA, was quantitated by liquid scintillation counting of bands hybridizing to a Ty1 probe and normalized to the amount of *PYK1* RNA in each sample. There was an increase in total Ty1 RNA of approximately 5-fold in the BWG1-7A background and 15- to 20-fold in the GRF167 background in transposition-induced cells relative to cells harboring no plasmid or p*GAL1-lacZ*. The different results obtained with strains GRF167 and BWG1-7A were due to a three- to fourfold-lower amount of unmarked Ty1 RNA (relative to *PYK1* RNA) in strain GRF167 than in strain BWG1-7A.

If the efficiency of Ty1 RNA translation is improved by transposition induction, then the increase in newly synthesized p190$^{TYA1\text{-}TYB1}$ would be greater than the increase in total Ty1 RNA that results from pGTy1-H3 induction. For quantitation of newly synthesized *TYB1* protein in transposition-induced and uninduced strains, cells were grown in galactose medium and pulse-labeled for 20 min with [^{35}S]methionine. Protein was immunoprecipitated with antiserum B2 (20), which cross-reacts with Ty1 IN protein and the appropriate precursor proteins (Fig. 5). After a 20-min pulse-label, the majority of immunoprecipitated protein was p190, although a fraction of the protein was processed to the intermediate p160, especially in the strains expressing pGTy1-H3. The amount of immunoprecipitable p190 and p160 in each strain was determined both by densitometry and by excision and liquid scintillation counting of protein bands. In strain BWG1-7A, the amount of *TYB1* precursor protein in cells harboring pGTy1-H3*neo* (Fig. 5A, lane 4) was 4- to 11-fold higher than in cells with either no plasmid (lane 1) or p*GAL1-lacZ* (lane 2). Thus, the induction of *TYA1-TYB1* polyprotein synthesis was similar to the fivefold increase in Ty1 RNA. The results with strain GRF167 also indicate that galactose induction of pGTy1-H3 does not alter the efficiency of Ty1 RNA translation. There was a 14- to 40-fold increase in pulse-labeled p190 and p160 resulting from galactose induction of pGTy1-H3*HIS3* (Fig. 5B, lane 4) over the level found in galactose-induced cells containing no plasmid (lane 1), p*GAL1-lacZ* (lane 2), or pGTy1-H3*HIS3*(*tybΔ3301*) (lane 3). [The truncated 100-kDa polyprotein produced from pGTy1-H3*HIS3*(*tybΔ3301*) can be differentiated from p190 produced from endogenous Ty1 elements.] This 14- to 40-fold increase in *TYA1-TYB1* protein synthesis is comparable to the 15- to 20-fold induction of Ty1 RNA levels in strain GRF167 expressing pGTy1-H3*neo*. The increase in newly synthesized p58^{TYA1} levels also parallels that of Ty1 RNA resulting from pGTy1-H3*neo* expression in strain GRF167 (12).

The results suggest that the efficiency of Ty1 RNA translation is increased no more than twofold by galactose induction of pGTy1-H3 or considerably less than the 45- to 125-fold increase in transposition efficiency of marked Ty1 RNA. These data also suggest that the level of frameshifting from *TYA1* to *TYB1* is not increased by transposition induction, since such a change would result in elevated p190 synthesis. Furthermore, the amount of p190 synthesized from genomic Ty1 elements was comparable whether or not Ty1-H3*HIS3*(*tybΔ3301*) RNA was induced (in Fig. 5B, compare lane 1 or 2 with lane 3). This result suggests that *trans* activation of genomic Ty1 elements during transposition induction does not result from elevated synthesis of p190 from endogenous elements. The observation that transposition induction had little or no effect on the efficiency of Ty1

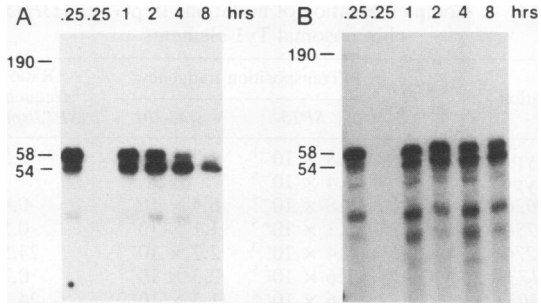

FIG. 6. Comparison of *TYA1* protein processing in transposition-induced (A) or uninduced (B) cells. Strain GRF167 containing either plasmid pGTy1-H3*neo* (A) or p*GAL1-lacZ* (B) was grown at 20°C in galactose medium. The cells were pulse-labeled by the addition of [^{35}S]methionine. After 15 min, excess unlabeled methionine was added. Samples were taken immediately (15 min) or at various times after [^{35}S]methionine addition as indicated. Ty1-VLP antiserum was used to immunoprecipitate Ty1 proteins from cell extracts prepared from radiolabeled cells. The second lane of each autoradiograph is a control lane in which no antibody was used. Immunoprecipitated proteins were analyzed by SDS-polyacrylamide gel electrophoresis on a 10% gel. The estimated sizes of the Ty1 proteins are indicated. The lower bands are due to nonspecific protein degradation of the *TYA1* proteins.

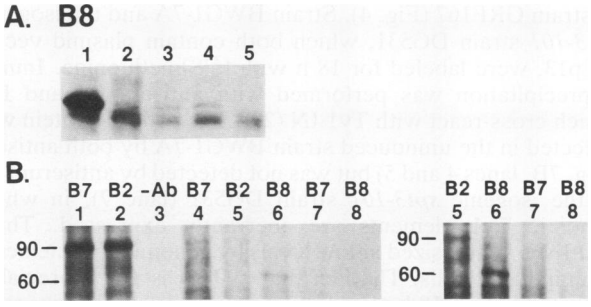

FIG. 7. Immunoprecipitation of Ty1 p60-RT/RH and p90-IN from normal yeast cells. (A) Strain GRF167 containing pGTy1-H3*HIS3* (lane 1), p*GAL1-lacZ* (lane 2), no plasmid (lane 3), or pGTy1-H3*HIS3*(*tybΔ3301*) (lanes 4 and 5) was grown at 20°C in galactose medium with [^{35}S]methionine for 18 h. In lanes 1 to 4, labeled proteins were immunoprecipitated with antiserum B8; in lane 5, no antibody was used as a control (antiserum B8 is directed against p60-RT/RH [20]). Ty1 proteins were analyzed by SDS-polyacrylamide gel electrophoresis on a 7.5% gel and then subjected to autoradiography. (B) Strain BWG1-7A containing plasmid pGTy1-H3*neo* (lanes 1 and 2) or YEp13 (lanes 3 to 6) and the isogenic *spt3-101* strain DG531 harboring YEp13 (lanes 7 and 8) were grown in galactose medium with [^{35}S]methionine for 18 h. The antiserum used to immunoprecipitate labeled proteins is indicated above each lane. In lane 3, no antibody was used. Antiserum B2 cross-reacts with p90-IN, and antiserum B7 cross-reacts with both p90-IN and p60-RT/RH (20). Ty1 proteins were analyzed by SDS-polyacrylamide gel electrophoresis on a 7.5% gel. The positions of Ty1 proteins p60 and p90 are indicated. A longer exposure of the autoradiograph of lanes 5 to 8 is included to show more clearly the presence of p90 and p60 in strain BWG1-7A (lanes 5 and 6) and the absence of these proteins in the isogenic *spt3-101* strain DG531 (lanes 7 and 8).

protein synthesis suggests that it overcomes transpositional quiescence at a posttranslational level.

Transposition induction increases the rate of *TYA1* protein processing. One posttranslational step in Ty transposition that is a potential point of regulation is the proteolytic processing of *TYA1* and *TYA1-TYB1* proteins. Ty1 PR is absolutely required for the maturation of all Ty1 proteins and for transposition (20, 38). To determine whether transposition induction affects the rate of Ty protein processing, we analyzed *TYA1* protein processing in transposition-induced and uninduced cells. Because p58^{TYA1} is synthesized at approximately 20-fold-higher levels than is p190$^{TYA1\text{-}TYB1}$, it was preferred for this analysis. Galactose-induced cells of strain GRF167 harboring either pGTy1-H3*neo* or control plasmid p*GAL1-lacZ* were pulse-labeled for 15 min with [^{35}S]methionine and chased for various periods with unlabeled methionine (Fig. 6). The results of immunoprecipitation of ^{35}S-labeled *TYA1* proteins show that the processing of p58 to p54 occurred rapidly in transposition-induced cells, resulting in 50% conversion to p54 between 1 and 2 h after [^{35}S]methionine addition and almost complete conversion after 8 h (Fig. 6A). In contrast, cells expressing p*GAL1-lacZ* appeared to process p58 more slowly, such that 50% was converted to p54 after 2 to 4 h and no more than 70% of the protein was processed to p54 after 8 h (Fig. 6B). These data suggest that the rate of processing of the *TYA1* protein is increased by galactose induction of pGTy1-H3*neo*.

Normal yeast strains contain low levels of p60-RT/RH and p90-IN. Using several antisera directed against different regions of *TYB1*, our laboratory has not detected *TYB1* proteins by immunoblot analysis of cells or purified VLPs from normal yeast strains that do not express a pGTy1 element (20, 22). Pulse-chase analysis of *TYA1-TYB1* protein processing indicated to us that the levels of processed *TYB1* proteins were undetectable or detected only after a prolonged chase in uninduced yeast cells (12). In contrast, processed *TYB1* proteins can be immunoprecipitated from transposition-induced cells 2 to 6 h after pulse-labeling (20). To demonstrate that p60-RT/RH is synthesized by endogenous Ty1 elements, we incubated yeast cells in the presence of [^{35}S]methionine at 20°C for 18 h and then subjected the cells to immunoprecipitation with antisera directed against RT/RH (Fig. 7A). Since linear incorporation of labeled methionine ceases after an incubation of approximately 1 h, the labeled methionine is expected to be chased into processed *TYB1* proteins after the 18-h incubation. As expected, p60-RT/RH was immunoprecipitated by B8 antiserum from the galactose-induced strain GRF167 that harbors pGTy1-H3*HIS3* (Fig. 7A, lane 1). Moreover, p60 was detected at lower levels in cells that harbor the control plasmid p*GAL1-lacZ* (lane 2) or have no plasmid (lane 3). The p60 protein was weakly detected by immunoprecipitation with antisera B7 and B8 in the uninduced strain BWG17A (Fig. 7B, lanes 4 and 6) but not in the isogenic *spt3-101* strain DG531 (lanes 7 and 8). These data demonstrate that processed RT/RH is produced at low levels from genomic Ty1 elements. Interestingly, expression of a pGTy1 element with a complete deletion of RT/RH [pGTy1-H3*HIS3*(*tybΔ3301*); Fig. 7A, lane 4] resulted in levels of p60 that are comparable to those seen in the absence of a plasmid, suggesting that induction of a pGTy1 element with functional *TYA1* and PR does not enhance the level of processed p60-RT/RH derived from endogenous Ty elements.

Our attempts to immunoprecipitate processed IN from the uninduced strain GRF167 after chase periods ranging from 2 to 24 h were unsuccessful, even though the p190$^{TYA1\text{-}TYB1}$ precursor protein could be detected by antiserum directed against IN (Fig. 5). We reasoned that IN might be detectable in the uninduced strain BWG1-7A, since endogenous Ty1 RNA levels are approximately three- to fourfold higher than

in strain GRF167 (Fig. 4). Strain BWG1-7A and the isogenic *spt3-101* strain DG531, which both contain plasmid vector YEp13, were labeled for 18 h with [^{35}S]methionine. Immunoprecipitation was performed with antisera B2 and B7, which cross-react with Ty1 IN (20). The p90-IN protein was detected in the uninduced strain BWG1-7A by both antisera (Fig. 7B, lanes 4 and 5) but was not detected by antiserum B7 in the isogenic *spt3-101* strain DG531 (lane 7), in which genomic Ty1 elements are aberrantly expressed. Thus, p90-IN is synthesized at low levels by genomic Ty1 elements in uninduced cells. The fact that p90-IN is not appreciably detected before 18 h after [^{35}S]methionine addition suggests that its maturation occurs very slowly in uninduced cells. We have not detected p23-PR in uninduced yeast strains, a result that was predicted by the difficulty in reproducibly detecting PR in strains induced for pGTy1 expression by using available antibodies (20).

PR mutants and certain IN mutants are not complemented by chromosomal Ty1 elements. To determine which Ty1 protein or proteins are limiting for transposition, we tested the ability of chromosomal Ty1 elements to complement mutations in different domains of the pGTy1-H3*HIS3* element. In an earlier report, we observed that galactose induction of the defective element pGTy1-H1*neo* results in a low frequency of *neo*-marked transposition events when chromosomal Ty1 elements are normally transcribed (in an *SPT3* strain) but not when transcription of genomic Ty1 elements is incorrectly initiated (in an isogenic *spt3-101* strain) (14). Apparently, gene products derived from chromosomal Ty1 elements can partially complement mutations in pGTy1 elements, resulting in transposition of the marked pGTy1 element at a detectable frequency. We have extended this type of analysis to defined mutations in different Ty1 domains by comparing the transposition of mutant pGTy1-H3*HIS3* elements in *SPT3* and isogenic *spt3-101* strains.

Plasmid pGTy1-H3*HIS3* and derivatives were introduced into the *SPT3* strain GRF167 and the isogenic *spt3-101* strain DG789. The cells were grown in SC-Ura galactose liquid medium for 2 days at 20°C, diluted into fresh SC-Ura galactose medium, and grown to saturation at 20°C. Since loss of the pGTy1 plasmid is very high in SC-Ura galactose medium (4), aliquots of each culture were tested directly to determine what fraction of Ura$^-$ cells had become phenotypically His$^+$. This fraction is referred to as the transposition frequency. Our assay for transposition appears to be quite sensitive, since the fraction of His$^+$ Ura$^-$ cells was 7,500-fold higher when the wild-type pGTy1-H3*HIS3* element was galactose induced than when its expression was repressed in glucose medium (Table 4).

The ratio of the transposition frequency in the *SPT3* background to that in the *spt3-101* background was determined following galactose induction of pGTy1-H3*HIS3* and derivatives containing different mutations (Table 4). Plasmid pGTy1-H3*HIS3* yielded an *SPT3*/*spt3-101* ratio of 1.5, indicating that the wild-type Ty1-H3 element transposes at a nearly equal frequency in the presence or absence of normal genomic Ty1 element transcription. On the other hand, the *SPT3*/*spt3-101* ratio for pGTy1-H3*HIS3* containing a five-codon insertion in RT/RH (*tyb-4627G*) was 24.5. Other mutations in the RT/RH domain, including a deletion of the 3′ end of RT/RH (*tybΔ5463*) and a nonsense codon insertion near the 5′ end of the RT/RH domain (*tyb-3944Am*), also yielded 20- to 30-fold-higher frequencies of transposition in the *SPT3* background than in the *spt3-101* background. These data indicate that chromosomal Ty1 elements can provide RT/RH to partially complement pGTy1-H3*HIS3* elements that harbor mutations in the RT/RH domain, resulting in transposition of the *HIS3*-marked element.

TABLE 4. Complementation of mutations in pGTy1-H3*HIS3* by chromosomal Ty1 elements

Mutation	Carbon source[a]	Transposition frequency[b]		Ratio of frequencies (*SPT3*/*spt3-101*)
		SPT3	*spt3-101*	
Wild type	Gal	1.8×10^{-1}	1.2×10^{-1}	1.5
Wild type	Glu	2.4×10^{-5}	ND	
tyb-1702G	Gal	2.8×10^{-5}	6.5×10^{-5}	0.4
tyb-2173G	Gal	5.3×10^{-5}	1.1×10^{-4}	0.5
tyb-4627G	Gal	5.4×10^{-3}	2.2×10^{-4}	24.5
tybΔ2173	Gal	1.6×10^{-5}	2.3×10^{-5}	0.7
tybΔ3301	Gal	3.6×10^{-4}	1.5×10^{-5}	24
tybΔ5463	Gal	3.2×10^{-4}	1.1×10^{-5}	29.1
tya-475Am	Gal	9.9×10^{-5}	1.5×10^{-4}	0.7
tyb-3944Am	Gal	9.6×10^{-5}	4.3×10^{-6}	22.3

[a] Gal, galactose; Glu, glucose.
[b] Number of His$^+$ Ura$^-$ colonies divided by the total number of Ura$^-$ colonies assayed. The number presented is the mean of measurements from three to five cultures. ND, not determined.

In contrast to mutations within RT/RH, an in-frame linker insertion in the PR domain (*tyb-1702G*) resulted in an *SPT3*/*spt3-101* ratio of less than 1 (Table 4). Furthermore, the element pGTy1-H3*HIS3*(*tya-475Am*), which has an amber codon linker inserted 180 nucleotides downstream of the translation initiation codon, failed to be complemented by chromosomal Ty1 elements (Table 4). This mutation results in a truncated *TYA1* protein and no *TYB1* protein. The results with both these mutations are consistent with the hypothesis that chromosomal Ty1 elements cannot provide PR in *trans* to complement pGTy1-H3*HIS3* mutations that affect PR function.

Analysis of mutations within the IN domain yielded discrepant results. Plasmid pGTy1-H3*HIS3*(*tybΔ3301*), in which RT/RH and the carboxy-terminal one-third of IN are deleted, has a 24-fold-higher transposition efficiency in the *SPT3* strain. On the other hand, a deletion including RT/RH and 90% of IN in pGTy1-H3*HIS3*(*tybΔ2173*) failed to transpose at a higher frequency in the *SPT3* background. Furthermore, an in-frame linker insertion near the 5′ end of the IN domain (*tyb-2173G*) was not complemented by chromosomal Ty1 elements in an *SPT3* strain. One possible interpretation of these results is that chromosomal Ty1 elements can provide IN in *trans*, but that the *tybΔ2173* and *tyb2173G* mutations affect the function of PR, which is not provided in *trans*. Another explanation is that chromosomal Ty1 elements do not complement IN-defective elements, but that the portion of IN deleted in pGTy1-H3*HIS3*(*tybΔ3301*) is nonessential. The mutations analyzed so far do not allow us to differentiate between these two explanations.

Several other mutations in pGTy1-H3 marked with *neo* were tested in a different transposition assay in which stable transfer of the marker gene is detected in independent colonies selected for having maintained the pGTy1 plasmid throughout galactose induction (Table 5). In this assay, the wild-type pGTy1-H3 element transposed in over 80% of the galactose-induced colonies tested in strain GRF167 and the isogenic *spt3-101* strain DG789. As demonstrated in the assay discussed above, the *tyb-1702G* mutation within PR was not complemented by chromosomal elements, yielding a transposition frequency of 0% (0 of 64) in the *SPT3* strain. Another in-frame linker insertion at position 1876 in the PR

TABLE 5. Complementation of mutations in pGTy1-H3*neo* by chromosomal Ty1 elements

Mutation	Transposition frequency[a] (%)	
	SPT3	*spt3-101*
Wild type	88/96 (92)	63/76 (83)
tyb-1702G	0/64 (0)	0/24 (0)
tyb-1876G	0/64 (0)	0/24 (0)
tyb-5463G	16/96 (17)	0/96 (0)
tybΔ1615	1/190 (1)	0/40 (0)

[a] Number of G418-resistant Ura$^-$ colonies divided by the total number of Ura$^-$ colonies analyzed after galactose induction of the plasmid.

domain also failed to transpose in 64 galactose-induced *SPT3* colonies analyzed, indicating that the *tyb-1876G* mutation is not complemented by endogenous Ty1 elements. A pGTy1H3*neo* derivative in which *TYA1* is intact but *TYB1* is completely deleted (*tybΔ1615*) transposed at a frequency of less than 1% (1 of 190) in the *SPT3* strain. On the other hand, a four-codon insertion in RT/RH at nucleotide 5463 that abolishes transposition in the *spt3-101* strain (0 of 96) was complemented in the *SPT3* strain, resulting in a 17% (16 of 96) frequency of transposition. These data provide further evidence that pGTy1-H3 containing mutations in RT/RH can transpose by complementation from endogenous Ty1 elements, but that pGTy1-H3 elements containing mutations in PR are not complemented by chromosomal Ty1 elements. Taken together, the results of these two assays suggest that the availability of PR and possibly IN produced from chromosomal Ty1 elements limits Ty1 transposition activity.

It is possible that particular mutations are not complemented because they have a *trans*-dominant effect on wild-type gene products produced from chromosomal Ty1 elements. If this were the case, expression of the mutant pGTy1 element would inhibit transposition of a chromosomal Ty1m*his3*AI element. We determined whether transposition of the genomic element Ty1m*his3*AI-242 in strain JC242 occurred during galactose induction of unmarked pGTy1-H3 containing mutations that fail to be complemented by chromosomal Ty1 elements (Fig. 8). The frequency of His$^+$ papillae that arose in strain JC242 during galactose induction of pGTy1-H3 containing mutations *tya-475Am*, *tyb-1702G*, and *tyb-2173G* was comparable to that observed when the plasmid vector p*GAL1*-X/P was expressed or when the complementable RT/RH mutant, pGTy1-H3(*tyb-3944Am*) is expressed. In addition, mutants pGTy1-H3*neo*(*tyb-1876G*), pGTy1-H3(*tybΔ2173*), and pGTy1-H3*neo*(*tybΔ1615*) did not inhibit His$^+$ prototroph formation in strain JC242 (data not shown). However, this qualitative assay reproduced the 15-fold increase in His$^+$ reversion that results from expression of the wild-type pGTy1-H3 element in liquid culture (Table 3). These results suggest that the mutations tested do not inhibit wild-type Ty1 gene products. The failure of mutations *tya-475Am*, *tybΔ1615*, *tybΔ2173*, *tyb-1702G*, *tyb-1876G*, and *tyb2173G* to be complemented by chromosomal Ty1 elements cannot be explained by *trans* dominance of the mutants.

Recessive mutations in TYA1, PR, and IN do not destroy the Ty1 RNA template. Xu and Boeke (37) have demonstrated that the only sequences required in *cis* for Ty1 transposition are located within and immediately adjacent to the LTRs. Although none of our mutations falls within sequences found to be required in *cis*, it is still possible that a mutation is not complemented in an *SPT3* strain because it renders the Ty1 RNA defective as a template for transposition. For this reason, we determined whether mutant pGTy1-H3*HIS3* elements transposed in cells that were induced for expression of a wild-type helper Ty1 element. Derivatives of pGTy1-H3*HIS3* were transformed into strain JC333, which contains a *GAL1*/Ty1-H3*neo* element integrated at the *URA3* locus. Mutant pGTy1-H3*HIS3* elements and the *GAL1*/Ty1-H3*neo* helper element were coinduced by growth at 20°C on SC-Ura galactose plates. Independent colonies were then analyzed for evidence of helper-mediated transposition of mutant pGTy1-H3*HIS3* elements by streaking onto 5-FOA–His glucose agar to score His$^+$ Ura$^-$ colonies. The pGTy1-H3*HIS3* elements containing mutations that were not complemented by genomic Ty1 elements, including *tya-475Am*, *tyb-1702G*, *tyb-2173G*, and *tybΔ2173*, transposed at high levels when

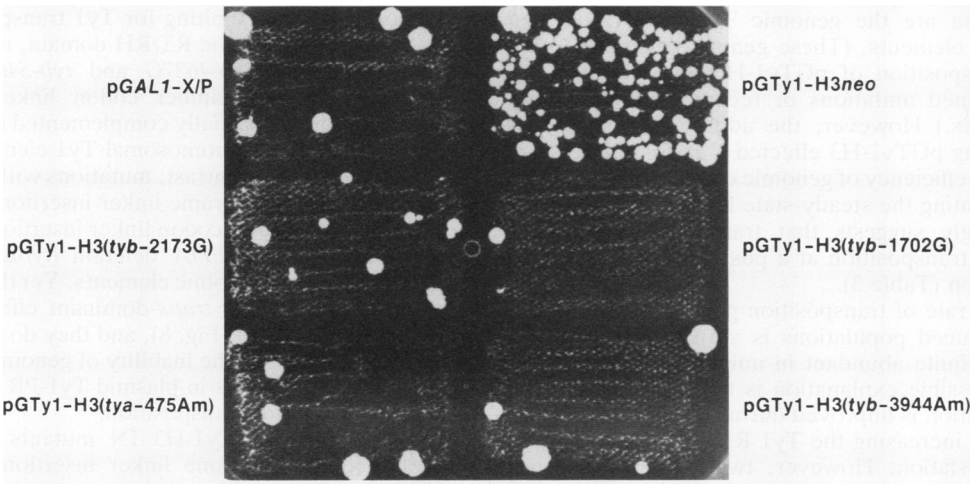

FIG. 8. Analysis of mutations in pGTy1-H3 for a *trans*-dominant effect on the transposition of the genomic element Ty1m*his3*AI-242. The vector (p*GAL1*-X/P), plasmid pGTy1-H3*neo*, and pGTy1-H3 derivatives with different mutations were transformed into strain JC242 (::Ty1m*his3*AI-242). The strains were grown on an SC-Ura 2% galactose plate for 3 days at 20°C and then replica plated to SC-His 2% glucose plates to score transposition events of the *his3*AI-marked element that result in His$^+$ prototrophs. The SC-His glucose plate is shown after incubation for 2 days at 30°C.

their expression was coinduced with that of *GAL1*/Ty1-H3*neo*. Since the *tybΔ2173* mutation adjoins *HIS3*, His^+ colonies isolated after coinduction must harbor transposition events of pGTy1-H3*HIS3*(*tybΔ2173*) that retain the mutation, demonstrating that the RNA is a functional template for retrotransposition. A fraction of the His^+ colonies resulting from helper-mediated transposition of pGTy1-H3(*tya-475Am*), pGTy1-H3(*tyb-1702G*), and pGTy1-H3(*tyb-2173G*) retained the mutation within transposed Ty1 elements, as demonstrated by Southern blot or polymerase chain reaction analysis (data not shown). These data indicate that mutations *tya-475Am*, *tyb-1702G*, *tyb-2173G*, and *tybΔ2173* do not inhibit transposition by destroying the RNA template for transposition. Since these mutations are also recessive (Fig. 8), their failure to be complemented by genomic Ty1 elements indicates that Ty1 transposition is limited by the levels of Ty1 PR and possibly IN produced by genomic Ty1 elements that is available to be provided in *trans*.

DISCUSSION

The mutagenic potential of Ty1 element transposition necessitates mechanisms to regulate it. To understand how transposition is regulated in yeast cells, we have quantitated the effect of pGTy1 expression on the rate of plasmid-borne and genomic Ty1 transposition, the level of Ty1 RNA, the efficiency of Ty1 protein synthesis, and the rate of *TYA1* processing. We have demonstrated that the efficiency of Ty1 transposition is greatly increased by transposition induction. The transposition rate of a pGTy1 element marked with the *his3*AI indicator gene was 10,000- to 28,000-fold higher than that of a relatively active genomic Ty1m*his3*AI element under control of its own promoter (Table 2). However, Ty1m*his3*AI RNA levels were only 220 to 225 times higher in strains expressing pGTy1m*his3*AI (Fig. 2). We found that the splicing efficiency of Ty1m*his3*AI RNA measured in RNase protection or polymerase chain reaction analyses is similar in strains containing a genomic Ty1m*his3*AI element and those expressing pGTy1m*his3*AI (12). Therefore, transposition induction increases the rate of Ty1 transposition per RNA molecule 45- to 125-fold. Despite the fact that the genomic elements studied transpose at relatively high levels, it may be argued that Ty1-H3 is inherently more efficient at transposition than are the genomic Ty1m*his3*AI-242 and Ty1m*his3*AI-227 elements. (These genomic elements originated from transposition of pGTy1-H3m*his3*AI, but they may have sustained mutations or recombined with other genomic elements.) However, the additional finding that galactose-inducing pGTy1-H3 effected a 15-fold increase in the transposition efficiency of genomic element Ty1m*his3*AI-242 without elevating the steady-state level of Ty1m*his3*AI-242 RNA strongly suggests that transposition induction deregulates Ty1 transposition at a posttranscriptional level of gene expression (Table 3).

The increased rate of transposition per RNA molecule in transposition-induced populations is surprising, since Ty1 RNA is already quite abundant in uninduced yeast strains (13, 17). One possible explanation is that the efficiency of Ty1 RNA translation is improved during pGTy1 expression, perhaps because increasing the Ty1 RNA levels titrates an inhibitor of translation. However, two lines of evidence suggest that the efficiency of Ty1 RNA translation does not change in transposition-induced strains. First, in the two different strain backgrounds analyzed, the relative increase in newly synthesized *TYA1-TYB1* protein in transposition-induced cells is comparable to the relative increase in steady-state Ty1 RNA levels (Fig. 5). Since a frameshift from the *TYA1* gene to the *TYB1* gene is required for expression of $p190^{TYA1\text{-}TYB1}$, these data also suggest that the efficiency of frameshifting is not altered by transposition induction. Second, galactose induction of pGTy1 elements with coding sequence mutations did not result in elevated $p190^{TYA1\text{-}TYB1}$ protein synthesized from endogenous Ty1 elements (Fig. 5B, lane 3). The unaltered efficiency of Ty1 protein synthesis during transposition induction argues against the hypothesis that pGTy1 RNA titrates a translational inhibitor. Moreover, it suggests that Ty1 transposition is inhibited by a posttranslational mechanism in normal yeast cells that is overcome by transposition induction.

The possibility that processing of Ty1 proteins, and hence maturation of Ty1 VLPs, occurs more efficiently in transposition-induced cells was investigated in pulse-chase analyses. The data indicate that processed $p54^{TYA1}$ accumulates at a faster rate in transposition-induced cells than in uninduced cells (Fig. 6). Transposition induction may also increase the rate of processing of *TYB1* proteins. The unprocessed $p190^{TYA1\text{-}TYB1}$ protein can be immunoprecipitated from pulse-labeled, uninduced yeast strains (Fig. 5), but processed p90-IN and p60-RT/RH are detected only after a prolonged chase (Fig. 7). In contrast, galactose induction of a pGTy1 element results in the accumulation of processed p60 and p90 a few hours after pulse-labeling (20). The low levels of processed p60 and p90 probably result from slow processing rather than instability, since they were detected in uninduced yeast cells approximately 17 h after the incorporation of labeled methionine had ended. Whether instability or inefficient processing of *TYB1* proteins explains their paucity in normal yeast cells, the accumulation of processed proteins is correlated with induced levels of Ty1 expression. Perhaps the concentration of $p190^{TYA1\text{-}TYB1}$ in normal yeast cells is insufficient to allow efficient processing of Ty1 proteins. In this case, galactose induction of pGTy1 may effect a certain threshold concentration of p190 that promotes protein cleavage and hence VLP maturation. Concentration dependence has been observed for the processing of in vitro-translated human immunodeficiency virus *gag-pol* precursor proteins (27).

Experiments that test the ability of genomic Ty1 elements to complement *TYB1* mutations in pGTy1-H3 identify PR and possibly IN as limiting for Ty1 transposition (Tables 4 and 5). Mutations in the RT/RH domain, including in-frame linker insertions (*tyb-4627G* and *tyb-5463G*), a deletion (*tybΔ5463*), and an amber codon linker insertion (*tyb-3944Am*), were all partially complemented in an *SPT3* strain, demonstrating that chromosomal Ty1 elements can provide RT/RH in *trans*. In contrast, mutations within or inclusive of PR, including two in-frame linker insertions (*tyb-1702G* and *tyb-1876G*), an amber codon linker insertion upstream of PR (*tya-475Am*), and a *TYB1* deletion (*tybΔ1625*), fail to be complemented by genomic elements. Yet these mutations do not appear to have a *trans*-dominant effect on a genomic Ty1m*his3*AI element (Fig. 8), and they do not inactivate the Ty1 RNA template. The inability of genomic Ty elements to complement mutations in plasmid Ty1-PR indicates that PR may be limiting for transposition.

The behavior of Ty1-H3 IN mutants is not as easily interpreted. An in-frame linker insertion within IN (*tyb-2173G*) is not complemented by endogenous Ty elements. However, processing of p58-*TYA1* occurs more slowly in this mutant than in the wild-type pGTy1-H3 element, suggesting that *tyb-2173G* creates a partial defect in PR function (12). Another element with a missense mutation in the amino

terminus of IN, *tyb-2098*, has been shown to be processing defective (3). Furthermore, a portion of the IN domain and all of the RT/RH domain are deleted in both *tybΔ2173* and *tybΔ3301*, but the *tybΔ3301* mutation is complemented in an *SPT3* strain whereas the *tybΔ2173* mutation is not. The inconsistency in this result could be explained if these IN deletions produced different phenotypes. For example, *tybΔ2173* could affect the function of PR whereas *tybΔ3301* may not. It is also possible that pGTy1-H3*HIS3*(*tybΔ3301*) encodes a functional IN but that this mutant did not transpose in an *spt3-101* strain because of the RT/RH deletion. It is interesting to note that the noncomplemented mutations *tyb-2173G* and *tybΔ2173* are within the domain that has homology to retroviral integrases, whereas the carboxy-terminal deletion of IN in *tybΔ3301* removes sequences that are quite divergent from retroviral integrases (15). More mutations within the IN domain must be analyzed to determine whether the IN protein is limiting for transposition.

We have shown that galactose induction of a pGTy1 element overcomes a block to transposition that occurs at a posttranslational level and is correlated with an increased rate of Ty1 protein processing. Furthermore, we have genetic evidence that the levels of Ty1 PR and possibly IN derived from chromosomal Ty1 elements limit the efficiency of Ty1 transposition in yeast cells. There are several mechanisms that may be involved in this regulation of transposition. First, limiting levels of PR and IN could be a result of numerous defective elements in the genome, especially ones that harbor mutations in the PR and IN domains. In this case, the 45- to 125-fold increase in transposition efficiency per Ty1 transcript effected by pGTy1 expression could result from only 1/45 to 1/125 of the genomic Ty1 transcripts being functional. Perhaps almost all genomic elements are defective, or mutant elements account for most of the Ty1 RNA in the cell. We do not favor this model, because several different transpositionally competent Ty1 elements have been identified and transpositionally competent elements are not transcriptionally repressed (12, 14, 19). While mutant elements exist in the genome and certainly play a role in determining the overall efficiency of transposition, it is unlikely that defective elements are a major factor in regulating transposition of Ty1. More genomic elements must be analyzed for transpositional competence to determine the extent of their involvement in determining the efficiency of genomic element transposition.

A second possible mechanism for controlling Ty1 transposition is an inhibitor operating at a posttranslational level. The inhibitor could be an unlinked cellular gene or mutant Ty proteins from a small number of chromosomal elements that have a dominant negative effect on transposition. Expression of a *GAL1*-promoted element would titrate the postulated inhibitor, resulting in accumulation of functional Ty1 VLPs that catalyze high levels of transposition. An inhibitor that affects Ty1 protein processing or *TYB1* protein stability could explain the relatively low levels of processed Ty1 proteins in uninduced yeast cells. Expression of an element with an RT/RH deletion but no processing defect [pGTy1-H3*HIS3*(*tybΔ3301*)] did not increase the amount of processed p60-RT/RH produced from genomic Ty1 element $p190^{TYA1\text{-}TYB1}$ precursor (Fig. 7A, lane 4). This result does not support the model that the enhanced transposition efficiency effected by pGTy1 induction results from overwhelming a Ty1 PR inhibitor. However, pGTy1-H3*HIS3*(*tybΔ3301*) expression could still increase the rate of p60-RT/RH formation, which would not be detected in our experiment. Other posttranslational mechanisms of regulation, such as inhibition of VLP formation, cDNA formation, or target site accessibility, may also be involved, and the latter two mechanisms have been suggested by the work of others (16, 32, 33).

A third model to explain the inefficient transposition of Ty1 in uninduced cells and the failure of pGTy1 PR mutants to be complemented in *trans* is that p23-PR not only is limiting but also acts preferentially in *cis* to catalyze the transposition of the RNA molecule from which it was translated. In this model, Ty1 PR associates with its own RNA, especially in the case of chromosomal elements that have a relatively low level of protein expression. This association could occur on the ribosome between the RNA and its nascent *TYA1-TYB1* polyprotein, which contains PR in its unprocessed form. Since p23-PR, p60-RT/RH, and p90-IN are all synthesized from the same precursor protein, the preferential *cis* action of PR but not RT/RH would result if p23-PR is selectively prevented from reassociating in *trans* with RNA molecules from other elements. This situation might result if PR was a relatively unstable protein, if p23-PR could only process its own polyprotein precursor, or if diffusion of p23-PR among particles is preferentially blocked. Preferential *cis* action of PR would limit the level of transposition by restricting transposition of any individual element to that which could be catalyzed by its own PR. In this model, the transposition level of a Ty1 element would be independent of the total cellular PR concentration until a certain threshold concentration of PR is reached that allows it to associate in *trans* with RNA derived from other elements. Since galactose induction of pGTy1 results in increased synthesis of Ty1 proteins, the probability that its *TYA1-TYB1* protein will associate in *trans* with Ty1 RNA from another element is expected to increase. This phenomenon probably accounts for the *trans* activation of genomic elements by galactose induction of a helper Ty1 element (Table 3).

Several observations are consistent with the hypothesis that PR preferentially associates in *cis* with the RNA that encodes it. First, expression of a pGTy1 element increases the transposition efficiency of RNA derived from the plasmid 45- to 125-fold (Table 2) but increases the transposition efficiency of genomic Ty RNA only 15-fold (Table 3). In other words, activation of transposition in *trans* is three- to eightfold lower than activation in *cis*. This observation is similar to that of Xu and Boeke (37), who found that helper-dependent transposition of deletion mutants of pGTy1-H3 is much less frequent than helper-independent transposition of functional elements. These data indicate that a Ty1 protein or proteins may act preferentially in *cis* to catalyze the transposition of the RNA that encodes it. Second, galactose induction of pGTy1-H3 stimulates transposition of genomic Ty1 elements in *trans* without increasing the amount of RNA (Fig. 3), pulse-labeled $p190^{TYA1\text{-}TYB1}$ protein (Fig. 5), or processed p60-RT/RH produced from genomic Ty1 elements (Fig. 7A, lane 4). Perhaps the *trans* activation of genomic Ty1 elements results from normally *cis*-acting *TYA1-TYB1* protein associating in *trans* with RNA from genomic elements when it is produced at high levels, rather than being a consequence of providing a limiting protein such as PR. This question can be addressed by determining whether pGTy1-H3 with mutations in any *TYB* domain is able to *trans* activate a genomic Ty1 element. The strongest evidence for *cis* action is the lack of complementation of the element pGTy1-H3*HIS3*(*tya-475Am*), in which a stop codon near the 5′ end of the mRNA results in production of a protein with less than 15% of the *TYA1*

sequences and no *TYB1* sequences. Ty1-H3*HIS3*(*tya-475Am*) RNA is presumably not sequestered in VLPs constructed from its own proteins, but the marked RNA was also not utilized by genomic Ty1 element proteins as a template for transposition (Table 4). However, transposition of the marked RNA occurs when a helper *GAL1*/Ty1 fusion element is expressed, demonstrating that Ty1-H3(*tya-475Am*) is a functional template for transposition. These data suggest that a Ty1 protein (or proteins) derived from genomic elements associates preferentially in *cis* with the RNA that encodes it rather than in *trans* with RNA derived from other Ty1 elements. Further work using genomic Ty1 elements marked with the transposition indicator gene will be useful in clarifying the mechanism of posttranslational regulation of Ty1 element transposition.

ACKNOWLEDGMENTS

We gratefully acknowledge the helpful comments of S. Brown, T. Burkett, A. Rein, J. Strathern, G. Sharon, and A. Arthur, the assistance of M. Powers in oligonucleotide synthesis, and the assistance of P. Hall in preparation of the manuscript.

This research was sponsored by the National Cancer Institute under contract N01-CO-74101 with ABL.

REFERENCES

1. **Adams, S. E., J. Mellor, K. Gull, R. B. Sim, M. F. Tuite, S. M. Kingsman, and A. J. Kingsman.** 1987. The functions and relationships of Ty-VLP proteins in yeast reflect those of mammalian retroviral proteins. Cell **49**:111–119.
2. **Belcourt, M. F., and P. J. Farabaugh.** 1990. Ribosomal frameshifting in the yeast retrotransposon Ty: tRNAs induce slippage on a 7 nucleotide minimal site. Cell **62**:339–352.
3. **Boeke, J. D., D. Eichinger, D. Castrillon, and G. R. Fink.** 1988. The *Saccharomyces cerevisiae* genome contains functional and nonfunctional copies of transposon Ty1. Mol. Cell. Biol. **8**:1431–1442.
4. **Boeke, J. D., D. J. Garfinkel, C. A. Styles, and G. R. Fink.** 1985. Ty elements transpose through an RNA intermediate. Cell **40**:491–500.
5. **Boeke, J. D., F. Lacroute, and G. R. Fink.** 1984. A positive selection for mutants lacking orotidine 5′-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. Mol. Gen. Genet. **197**:345–346.
6. **Boeke, J. D., and S. B. Sandmeyer.** 1991. Yeast transposable elements. *In* J. R. Broach, J. Pringle, and E. Jones (ed.), The molecular and cellular biology of the yeast *Saccharomyces*: genome dynamics, protein synthesis, and energetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., p. 193–261.
7. **Boeke, J. D., C. A. Styles, and G. R. Fink.** 1986. *Saccharomyces cerevisiae SPT3* gene is required for transposition and transpositional recombination of chromosomal Ty elements. Mol. Cell. Biol. **6**:3575–3581.
8. **Boeke, J. D., H. Xu, and G. R. Fink.** 1988. A general method for the chromosomal amplification of genes in yeast. Science **239**: 280–282.
9. **Broach, J. R., J. N. Strathern, and J. B. Hicks.** 1979. Transformation in yeast: development of a hybrid cloning vector and isolation of the *CAN1* gene. Gene **8**:121–133.
10. **Clare, J., and P. J. Farabaugh.** 1985. Nucleotide sequence of a yeast Ty element: evidence for an unusual mechanism of gene expression. Proc. Natl. Acad. Sci. USA **82**:2828–2833.
11. **Curcio, M. J., and D. J. Garfinkel.** 1991. Single-step selection for Ty1 element retrotransposition. Proc. Natl. Acad. Sci. USA **88**:936–940.
12. **Curcio, M. J., and D. J. Garfinkel.** Unpublished results.
13. **Curcio, M. J., A.-M. Hedge, J. D. Boeke, and D. J. Garfinkel.** 1990. Ty RNA levels determine the spectrum of retrotransposition events that activate gene expression in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **220**:213–221.
14. **Curcio, M. J., N. J. Sanders, and D. J. Garfinkel.** 1988. Transcriptional competence and transcription of endogenous Ty elements in *Saccharomyces cerevisiae*: implications for regulation of transposition. Mol. Cell. Biol. **8**:3571–3581.
15. **Doolittle, R. F., D.-F. Feng, M. S. Johnson, and M. A. McClure.** 1989. Origins and evolutionary relationships of retroviruses. Q. Rev. Biol. **64**:1–30.
16. **Eichinger, D. J., and J. D. Boeke.** 1988. The DNA intermediate in yeast Ty1 element transposition copurifies with virus-like particles: cell-free Ty1 transposition. Cell **54**:955–966.
17. **Elder, R. T., T. P. St. John, D. T. Stinchcomb, and R. W. Davis.** 1980. Studies on the transposable element Ty1 of yeast. I. RNA homologous to Ty1. Cold Spring Harbor Symp. Quant. Biol. **45**:581–584.
18. **Garfinkel, D. J., J. D. Boeke, and G. R. Fink.** 1985. Ty element transposition: reverse transcriptase and virus-like particles. Cell **42**:507–517.
19. **Garfinkel, D. J., M. J. Curcio, S. D. Youngren, and N. J. Sanders.** 1988. The biology and exploitation of the retrotransposon Ty in *Saccharomyces cerevisiae*. Genome **31**:909–919.
20. **Garfinkel, D. J., A.-M. Hedge, S. D. Youngren, and T. D. Copeland.** 1991. Proteolytic processing of *pol-TYB* proteins from the yeast retrotransposon Ty1. J. Virol. **65**:4573–4581.
21. **Garfinkel, D. J., M. F. Mastrangelo, N. J. Sanders, B. K. Shafer, and J. N. Strathern.** 1988. Transposon tagging using Ty elements in yeast. Genetics **120**:95–108.
22. **Garfinkel, D. J., and S. D. Youngren.** Unpublished results.
23. **Guarente, L., R. R. Yocum, and P. Gifford.** 1982. A *GAL10-CYC1* hybrid yeast promoter identifies the *GAL4* regulatory region as an upstream site. Proc. Natl. Acad. Sci. USA **79**:1173–1181.
24. **Hauber, J., P. Nelbock-Hochstetter, and H. Feldman.** 1985. Nucleotide sequence and characteristics of a Ty element from yeast. Nucleic Acids Res. **13**:2745–2758.
25. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153**:163–168.
26. **Joyce, C. M., and N. D. F. Grindley.** 1984. Methods for determining whether a gene of *Escherichia coli* is essential: application to the *polA* gene. J. Bacteriol. **158**:636–643.
27. **Krausslich, H.-G., H. Schneider, G. Zybarth, C. A. Carter, and E. Wimmer.** 1988. Processing of in vitro-synthesized *gag* precursor proteins of human immunodeficiency virus (HIV) type 1 by HIV proteinase generated in *Escherichia coli*. J. Virol. **62**:4393–4397.
28. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:68–85.
29. **Lea, D. E., and C. A. Coulson.** 1949. The distribution of the numbers of mutants in bacterial populations. J. Genet. **49**:264–285.
30. **Melton, D. A., P. A. Kreig, M. R. Rebagliati, T. Maniatis, K. Zinn, and M. R. Green.** 1984. Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. Nucleic Acids Res. **12**:7035–7056.
31. **Muller, F., K.-H. Bruhl, K. Freidel, K. V. Kowallik, and M. Ciriacy.** 1987. Processing of Ty1 proteins and formation of Ty1 virus-like particles in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **207**:421–429.
32. **Muller, F., W. Laufer, U. Pott, and M. Ciriacy.** 1991. Characterization of products of TY1-mediated reverse transcription in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **226**:145–153.
33. **Picologlou, S., N. Brown, and S. W. Liebman.** 1990. Mutations in *RAD6*, a yeast gene encoding a ubiquitin-conjugating enzyme, stimulate retrotransposition. Mol. Cell. Biol. **10**:1017–1022.
34. **Schauer, I., S. Emr, C. Gross, and R. Schekman.** 1985. Invertase signal and mature sequence substitutions that delay intercompartmental transport of active enzyme. J. Cell Biol. **100**:1664–1675.
35. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1986. Laboratory course manual for methods in yeast genetics. Cold Spring

Harbor Laboratory Press, Cold Spring Harbor, N.Y.
36. **Winston, F., K. J. Durbin, and G. R. Fink.** 1984. The *SPT3* gene is required for normal transcription of Ty elements in S. cerevisiae. Cell **39:**675–682.
37. **Xu, H., and J. D. Boeke.** 1990. Localization of sequences required in *cis* for yeast Ty1 element transposition near the long terminal repeats: analysis of mini-Ty1 elements. Mol. Cell. Biol. **10:**2695–2702.
38. **Youngren, S. D., J. D. Boeke, N. J. Sanders, and D. J. Garfinkel.** 1988. Functional organization of the retrotransposon Ty from *Saccharomyces cerevisiae*: Ty protease is required for transposition. Mol. Cell. Biol. **8:**1421–1431.