A Rare tRNA-Arg(CCU) That Regulates Ty*1* Element Ribosomal Frameshifting Is Essential for Ty*1* Retrotransposition in *Saccharomyces cerevisiae*

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ABSTRACT

Translation of the yeast retrotransposon Ty*1* *TYA1(gag)-TYB1(pol)* gene occurs by a +1 ribosomal frameshifting event at the sequence **CUU AGG C**. Because overexpression of a low abundance tRNA-Arg(CCU) encoded by the *HSX1* gene resulted in a reduction in Ty*1* frameshifting, it was suggested that a translational pause at the AGG-Arg codon is required for optimum frameshifting. The present work shows that the absence of tRNA-Arg(CCU) affects Ty*1* transposition, translational frameshifting, and accumulation of mature TYB1 proteins. Transposition of genetically tagged Ty*1* elements decreases at least 50-fold and translational frameshifting increases 3–17-fold in cells lacking tRNA-Arg(CCU). Accumulation of Ty*1*-integrase and Ty*1*-reverse transcriptase/ribonuclease H is defective in an *hsx1* mutant. The defect in Ty*1* transposition is complemented by the wild-type *HSX1* gene or a mutant tRNA-Arg(UCU) gene containing a C for T substitution in the first position of the anticodon. Overexpression of *TYA1* stimulates Ty*1* transposition 50-fold above wild-type levels when the level of transposition is compared in isogenic *hsx1* and *HSX1* strains. Thus, the *HSX1* gene determines the ratio of the TYA1 to TYA1-TYB1 precursors required for protein processing or stability, and keeps expression of *TYB1* a rate-limiting step in the retrotransposition cycle.

THE *Saccharomyces cerevisiae* retrotransposon Ty*1* is a mobile genetic element that replicates via an RNA intermediate (reviewed by BOEKE and SANDMEYER 1991; GARFINKEL 1992). The transposition cycle of Ty*1* elements resembles several important steps in the replication of retroviruses. Ty*1* protein maturation by Ty*1*-protease (PR) and reverse transcription take place within Ty*1* virus-like particles (Ty*1*-VLPs), which appear to be absolutely required for the transposition process. The Ty*1* genome contains two genes, *TYA1* and *TYB1*, which correspond to the *gag* and *pol* genes of retroviruses, respectively (CLARE and FARABAUGH 1985). As with certain retroviral *pol* genes (reviewed by HATFIELD *et al.* 1992), expression of *TYB1* requires programmed ribosomal frameshifting (CLARE, BELCOURT and FARABAUGH 1988). Ribosomal frameshifting solves two problems encountered in the life cycle of a retrovirus or retrotransposon. First, since catalytic Pol proteins, such as reverse transcriptase/ribonuclease H (RT/RH) and integrase (IN), are usually found in much lower amounts than the structural Gag proteins, requiring a frameshift event for *pol* expression is an effective strategy of gene regulation. Second, since Pol proteins function within a particle, creating a Gag-Pol fusion protein by frameshifting delivers Pol proteins to the correct compartment.

The TYA1-TYB1 fusion protein is synthesized by a +1 frameshifting event in the TYA1 sequence **CUU AGG C** (BELCOURT and FARABAUGH 1990). Ribosomal pausing at a rare AGG-arginine codon and slippage of a leucyl-tRNA from CUU to UUA are required for frameshifting. A single-copy tRNA-Arg(CCU) gene that recognizes the AGG codon is located on chromosome *X* (GAFNER, DE ROBERTIS and PHILIPPSEN 1983). BELCOURT and FARABAUGH (1990) have shown that overexpression of the tRNA-Arg(CCU) gene reduces Ty*1* frameshifting. Ty*1* transposition is also reduced when the level of the tRNA-Arg(CCU) is increased (XU and BOEKE 1990). These results suggest that the low abundance of tRNA-Arg(CCU) promotes frameshifting. Recently, we have identified this tRNA gene as the *HSX1* gene involved in the heat shock response (KAWAKAMI *et al.* 1992). Even though there is only one copy of the *HSX1* gene (GAFNER, DE ROBERTIS and PHILIPPSEN 1983), an *hsx1* disruption mutant is viable. Apparently, the AGG codons normally decoded by the single-copy *HSX1* gene are decoded by another tRNA [probably by the near-cognate tRNA-Arg(UCU)

TABLE 1

Yeast strains

Strain	Genotype	Plasmid	Source or reference
DMy51	*MATα ura3-167 his3Δ200 leu2Δ trp1Δ1 GAL*	pGTy*1*A-B*neo* (pD109)	This work
DMy94	*MAT***a** *ura3-52 his3Δ200 lys2 trp1-289 GAL*		This work
JC287	*Matα ura3-167 his3Δ200 leu2GB* Ty*1*-146::*lacZ* Ty*1*m*his3*AI-263 *GAL*		M. J. CURCIO
JC344	*MATα ura3-167 his3Δ200 leu2GB* Ty*1*-146::*lacZ* Ty*1*m*his3*AI-270 *GAL*		M. J. CURCIO
KK156	JC287; *hsx1::LEU2*		This work
KK157	JC344; *hsx1::LEU2*		This work
KK240	*MAT***a** *ura3 his3 leu2 trp1 hsx1::HIS3*		This work
KK242	*MAT***a** *ura3 his3 leu2 trp1*		This work
KD198-16A	*MAT***a** *his4Δ5 ura3 arg11 GAL*		K. J. DURBIN
DG1301	JC344	p*GAL1-lacZ*	This work
DG1302	JC344	pGTy*1*-H3*neo*	This work
DG1305	KK157	p*GAL1-lacZ*	This work
DG1306	KK157	pGTy*1*-H3*neo*	This work
DG1333	JC344	pGTy*1*-H3*neo::SacI-1702*	This work
DG1334	KK157	pGTy*1*-H3*neo::SacI-1702*	This work
DG1344	JC344	pGTyA1*neo*(*PGK1* ter.)	This work
DG1347	KK157	pGTyA1*neo*(*PGK1* ter.)	This work

gene]. In this paper, we describe the effects of an *hsx1* disruption mutant on Ty*1* frameshifting, transposition and protein processing.

MATERIALS AND METHODS

Yeast strains, plasmids, general genetic methods and media: The strains used for the Ty*1* transposition assays are listed in Table 1. Strains KK240 (*MAT***a** *ura3 his3 leu2 trp1 hsx1::HIS3*) and KK242 (*MAT***a** *ura3 his3 leu2 trp1*) were used to test Ty*1* frameshifting. These strains were derived from an *hsx1::HIS3/HSX1* diploid strain (KAWAKAMI *et al.* 1992).

The plasmids pMB38-9merWT and pMB38-9merFusion contain the frameshift heptamer fused to *Escherichia coli lacZ* gene in the +1 *TYB1* reading frame and the 0 *TYA1* reading frame, respectively (BELCOURT and FARABAUGH 1990). The plasmid pMB38-9merFusion(w/o AGG) contains the AGG-less 0 reading frame [GAT CCG CTG ACA **CTT GGC** CAT GAG GTA C (the frameshift region is highlighted)] fused to *lacZ*. The plasmid pKK67 was constructed by cloning the 230 base-pair (bp) wild-type *HSX1* DNA, amplified by polymerase chain reaction (PCR) (SAIKI *et al.* 1985) into the *URA3*-based centromere-plasmid YCp50 (ROSE, *et al.* 1987). The plasmid pKK68 carrying the mutant *hsx1*(*Mlu*I*) gene was constructed by digestion of the plasmid pKK67 with *Mlu*I, fill-in synthesis with Klenow DNA polymerase, and ligation to a *Sal*I linker. The *hsx1::HIS3* and *hsx1::LEU2* disruption alleles were constructed by modifying the same *Mlu*I restriction site and ligation to a *Cla*I fragment containing the *HIS3* gene, or an *Mlu*I-*Cla*I fragment containing the *LEU2* gene (kindly provided by P. ROGAN). The plasmid pKK69 was constructed by cloning the PCR-amplified 112-bp wild-type *SUP201-0* gene (THIREOS, PENN and GREER 1984; MORISHITA and UNO 1991) into the *URA3*-based centromere-plasmid pRS316 (SIKORSKI and HIETER 1989). The plasmid pKK71 carrying the *SUP201-0-1*(*CCU*) gene was constructed by digestion of plasmid pKK69 with *Mlu*I and *Bam*HI and ligation to a 63-bp synthetic double-stranded DNA containing the C for T substitution at 3′ base of the anticodon. The *Eco*RI-*Bam*HI DNA fragments containing the mutant and wild-type tRNA genes were prepared from plasmids pKK67, pKK68, pKK69 and pKK71, and subcloned into the *TRP1*-based centromere-plasmid pRS314 (SIKORSKI and HIETER 1989). These subcloning procedures generated plasmids pKK73 (derived from plasmid pKK67), pKK74 (from pKK68), pKK75 (from pKK69), and pKK76 (from pKK71). The plasmid pGTy*1*A-B*neo* (also known as plasmid pD109), with the Ty*1* frameshift correctly removed (BELCOURT and FARABAUGH 1990), was constructed from a transposition-competent pGTy*1*-H3/Ty*1*-912 hybrid plasmid by oligonucleotide-bridge mutagenesis (MANDECKI 1986). The frameshift mutation and tRNA sequences were confirmed by chain-terminating DNA sequencing (SANGER, NICKLEN and COULSON 1977) using Sequenase 2.0 (U.S. Biochemical Corp.). The plasmid pGTyA*1neo* (PGK ter.), kindly provided by P. ROGAN, was constructed by replacing almost all of the pGTy*1*-H3 TYB1 gene (from a *Bgl*II site located at position 1702 to the end of the element) with the bacterial neo gene and the *PGK1* transcriptional terminator. Standard techniques were used for all molecular cloning procedures (SAMBROOK, FRITSCH and MANIATIS 1989).

The *hsx1::HIS3* and *hsx1::LEU2* disruption mutants were constructed by single-step gene disruption (ROTHSTEIN 1991). Plasmids were introduced into cells using the transformation procedure of ITO *et al.* (1983). All yeast media and standard genetic techniques were those described by ROSE, WINSTON and HIETER (1990).

Transposition assays: Ty*1*m*his3*AI and Ty*1*m*ade2*AI transposition assays were performed as described previously (CURCIO and GARFINKEL 1991, 1992), and will be presented briefly here. For detecting spontaneous Ty*1*m*his3*AI transposition events, liquid cultures were inoculated at low densities (about 2×10^3 cells/ml) and grown to saturation at 20° in YPD or in SC-ura (glucose). A portion of each culture was spread on SC-his or SC-his-ura (glucose) plates and incubated at 30°. The cultures were titered on YPD or SC-ura (glucose) plates. For detecting chromosomal Ty*1*m*his3*AI transposition events in the presence of a pGTy*1* helper plasmid, cells were grown on SC-ura (galactose) plates for 7 days at 20°, or an overnight SC-ura (glucose) liquid culture was diluted 50-fold into SC-ura (galactose) liquid medium and incubated with aeration for

3 days at 20°. Ty*1*m*his3*AI transposition events were detected as His$^+$ papillae by replica plating cells from the SC-ura (galactose) to SC-his-ura (glucose) plates, followed by incubation at 30° for 3 days. To determine the number of Ty*1*m*his3*AI or Ty*1*m*ade2*AI transposition events in galactose-grown liquid cultures, the cells were concentrated, spread on several SC-his-ura (glucose) or SC-ade-ura (glucose) plates, and incubated at 30° for 3–5 days. Cells were titered on SC-ura (glucose) plates. Ty*1neo* and Ty*1*A-B*neo* transposition events were detected as described previously (BOEKE, XU and FINK 1988; CURCIO, SANDERS and GARFINKEL 1988) with the following minor modifications. Diploid strains were constructed by mating strains KD198-16A with strains DG1302 or DG1306, or by mating strains DMy51 and DMy92 (Table 1). The resulting diploids were induced for Ty*1* transposition on SC-ura (galactose) plates as described above. After segregation of the pGTy*1neo* plasmid from the strains, the level of resistance to the antibiotic G418 (Gibco) was determined by growth on YPD plates containing a final G418 concentration of 500 μg per ml (for diploids derived from mating strain KD198-16A with strains G1302 or DG1306) or 75 μg per ml (for diploid derived from mating strain DMy51 with DMy94).

Ty*1* RNA levels and Ty*1*m*his3*AI splicing efficiency: We isolated total RNA from *hsx1* and *hsx1::LEU2* strains by established procedures (CURCIO, SANDERS and GARFINKEL 1988; ROSE, WINSTON and HIETER 1990). Northern analysis was used to analyze Ty*1* RNA levels (CURCIO, SANDERS and GARFINKEL 1988; CURCIO and GARFINKEL 1992), and reverse transcription-PCR (RT-PCR) was used to estimate Ty*1*m*his3*AI RNA splicing efficiency (WANG, DOYLE and MARK 1989). The total amount of RNA transferred to hybridization membranes was estimated by staining with NAQ-STAIN, a reversible fluorescein-based stain developed by Integration Separation Systems. Transcripts from the *PYK1* and *ACT1* genes were used as internal loading standards. RNA sequences that span the region where the artificial intron (AI) was inserted in *HIS3* (CURCIO and GARFINKEL 1991) were amplified using the *HIS3*-specific oligonucleotide primers CTCCACGCGCCAGTAGGGCC (for DNA amplification) and ATGACAGAGCAGAAAGC CC (for reverse transcription and DNA amplification). The amplified products were separated by agarose gel electrophoresis through a 2% NuSieve/1% SeaKem (FMC Bioproducts) gel, stained with ethidium bromide, and photographed. The resulting negatives were scanned using an LKB Ultroscan XL enhanced laser densitometer. Relative splicing efficiencies were estimated by the amount of the amplified products. The splicing efficiency is defined as the amount of 334-bp spliced product over the amount of spliced plus 438-bp unspliced products.

Immunoblot analysis: Total yeast protein isolation, polyacrylamide gel electrophoresis, protein transfer, and antibody reactions were performed as described previously (YOUNGREN *et al.* 1988; GARFINKEL *et al.* 1991). Antibodies were added in at least 10-fold excess, as determined by titration experiments. Ty*1*-VLP antibodies were previously shown to react with TYA1 and TYA1-TYB1 precursor proteins, but not with TYB1 proteins (ADAMS *et al.* 1987; YOUNGREN *et al.* 1988; GARFINKEL *et al.* 1991). Ty*1*-VLP antibodies did not show a dramatic difference in avidity for TYA1 *vs.* TYA1-TYB1 precursor proteins, as determined by titration experiments (A.-M. HEDGE and D. J. GARFINKEL, unpublished results). Ty*1*-VLPs were isolated by the method of EICHINGER and BOEKE (1988), except the final continuous sucrose gradient was omitted. Equal amounts of protein (approximately 20 μg per lane) were loaded onto SDS-8% polyacrylamide gels. Protein concentrations were verified by staining gels run in parallel with Coomassie blue. Cross-reactivity of immunoblotted proteins with antisera that recognize the mature proteins p54-TYA1 (Ty*1*-VLP antiserum; ADAMS *et al.* 1987; YOUNGREN *et al.* 1988), p90-Ty*1*-IN (B2 antiserum; YOUNGREN *et al.* 1988), p60-Ty*1*-RT/RH (B8 antiserum; GARFINKEL *et al.* 1991), and their respective precursor proteins were detected using the ECL chemiluminescent detection system (Amersham).

Ty*1* frameshifting efficiency: β-Galactosidase assays and the efficiency of Ty*1* frameshifting were determined as described previously (BELCOURT and FARABAUGH 1990). Briefly, six transformants of each plasmid were each assayed in triplicate for β-galactosidase activity. The frameshifting efficiency is measured by determining the ratio of β-galactosidase activity produced from the construct requiring a +1 frameshift to express *lacZ* (pMB38-9merWT) to that of a construct in which the upstream and downstream genes are fused in frame [pMB38-9merFusion and pMB38-9merFusion(w/oAGG)].

The efficiency of Ty*1*-H3 frameshifting was also estimated from immunoblot analysis. Strains DG1333 (pGTy*1*-H3*neo::SacI-1702, HSX1*) and DG1334 (pGTy*1*-H3*neo::SacI-1702, hsx1::LEU2*) were constructed by transforming the plasmid pGTy*1*-H3*neo::SacI-1702*, which contains a Ty*1*-PR mutation (YOUNGREN *et al.* 1988), into strains JC344 and KK157, respectively (Table 1). Total protein isolated from galactose-grown cultures of strains DG1333 and DG1334 was analyzed by immunoblotting using Ty*1*-VLP antiserum. To determine the ratio of p58-TYA1 to p190-TYA1-TYB1 protein, exposures of the resulting blots were scanned using a laser densitometer. The efficiency of Ty*1* frameshifting equals the amount of p190-TYA1-TYB1 protein divided by the total amount of p58-TYA1 plus p190-TYA1-TYB1 protein.

RESULTS

Ty*1* transposition is inhibited in an *hsx1* disruption mutant: We determined whether a disruption mutation of *HSX1* affects Ty*1* transposition using two assays that monitor transposition of chromosomal elements marked with the *his3*AI retrotransposition indicator gene (CURCIO and GARFINKEL 1991), as well as by monitoring the transposition of plasmid-borne pGTy*1neo* and pGTy*1made2*AI elements (BOEKE *et al.* 1985; BOEKE, XU and FINK 1988; M. J. CURCIO and D. J. GARFINKEL, unpublished results). The *his3*AI gene is a yeast *HIS3* gene interrupted by an artificial intron (AI) in the antisense orientation. The *his3*AI sequences are inserted in a Ty*1* element at a unique restriction site located between the *TYB1* gene and the downstream long terminal repeat, such that the intron is on the sense strand of the Ty*1* element. Placement of marker genes at this position of a Ty*1* element does not severely inhibit transposition. Since splicing and retrotransposition of the marked Ty RNA gives rise to His$^+$ cells, the relative efficiency of Ty*1*m*his3*AI transposition can be monitored by plating cells on media lacking histidine. An *ade2*AI retrotransposition indicator gene has also been developed (M. J. CURCIO and D. J. GARFINKEL, unpublished results).

First, the relative efficiency of Ty*1*m*his3*AI trans-

TABLE 2

Ty*1*m*his3*AI transposition in an *hsx1* disruption mutant

Genotype	Ty*1*m*his3*AI	His$^+$ colonies/ total cells ($\times 10^7$)	Relative transposition efficiency
HSX1	Ty*1*m*his3*AI-263	25/1.6	2.0×10^{-6}
		46/1.6	
		40/1.8	
		36/1.9	
		30/1.9	
hsx1::LEU2	Ty*1*m*his3*AI-263	0/2.3	2.7×10^{-8}
		0/2.3	
		1/2.2	
		2/2.2	
		0/2.1	
HSX1	Ty*1*m*his3*AI-270	28/1.6	2.0×10^{-6}
		34/1.6	
		22/1.4	
		32/1.4	
		36/1.6	
hsx1::LEU2	Ty*1*m*his3*AI-270	3/1.7	3.8×10^{-8}
		0/2.3	
		0/2.4	
		0/2.1	
		1/1.8	

The Ty*1*m*his3*AI-263 element is present in *HSX1* strain JC287 and *hsx1::LEU2* strain KK156. The Ty*1*m*his3*AI-270 element is present in *HSX1* strain JC344 and *hsx1::LEU2* strain KK157. Each measurement represents the results of one of five independent cultures. The relative transposition efficiency is the mean fraction of total colonies that are His$^+$. To estimate the efficiency of Ty*1* transposition, the relative transposition efficiency should be multiplied by a factor of 8, to account for the splicing efficiency of the Ty*1*m*his3*AI transcript, and by a factor of 11, to account for the effect of introducing the *his3*AI marker gene into a Ty*1* element (CURCIO and GARFINKEL 1991).

TABLE 3

Ty*1*m*his3*AI-270 transposition in *hsx1* mutant KK157 containing plasmid copies of tRNA genes

Plasmid (genotype)	His$^+$ colonies/ total colonies ($\times 10^6$)	Relative transposition efficiency
pKK67 (*HSX1*)	17/5.3	6.4×10^{-6}
	58/2.3	
	16/3.6	
	18/5.3	
	23/4.1	
pKK68 [*hsx1*(*MluI**)]	0/4.5	$<4.9 \times 10^{-8}$
	0/4.6	
	0/4.4	
	0/2.3	
	0/4.5	
pKK69 [*SUP201-0*(*UCU*)]	0/5.7	$<3.5 \times 10^{-8}$
	0/6.5	
	0/5.2	
	0/5.6	
	0/5.9	
pKK71 [*SUP201-0-1*(*CCU*)]	4/7.5	1.1×10^{-6}
	3/5.6	
	7/5.3	
	11/5.9	
	8/6.1	

The Ty*1*m*his3*AI-270 element is present in the *hsx1::LEU2* strain KK157. The designated plasmids were introduced into strain KK157 and single transformants were chosen for further analysis. Refer to Table 2 for more information.

position in isogenic *HSX1* and *hsx1::LEU2* strains containing single marked chromosomal elements Ty*1*m*his3*AI-263 or Ty*1*m*his3*AI-270 was determined (Table 2). These unspliced Ty*1*m*his3*AI elements were identified after galactose-induction of a strain containing plasmid pGTy*1*-H3m*his3*AI, and are present at different chromosomal locations (CURCIO and GARFINKEL 1991). There was a 53- or 74-fold decrease in the efficiency of Ty*1*m*his3*AI-263 or Ty*1*m*his3*AI-270 transposition, respectively, as monitored by the number of His$^+$ colonies in a *hsx1::LEU2* mutant background. The transposition defect in the *hsx1::LEU2* mutant KK157 was complemented by a low copy number plasmid carrying the wild-type *HSX1* gene (pKK67), but not by a plasmid carrying a mutant *hsx1*(*M1uI**) gene (pKK68) (Table 3).

The second transposition assay depends upon the ability of a pGTy*1* helper plasmid to stimulate transposition of a genomic Ty*1*m*his3*AI element in trans (CURCIO and GARFINKEL 1992). Expression of the pGTy*1*-H3 helper plasmid increases the frequency of genomic Ty*1*m*his3*AI transposition about 100-fold (CURCIO and GARFINKEL 1992; M. J. CURCIO and D. J. GARFINKEL, unpublished results). The pGTy*1*-H3*neo* helper plasmid (BOEKE, XU and FINK 1988) or the control plasmid p*GAL1-lacZ* (BOEKE *et al.* 1985) were introduced into isogenic strains JC344 (*HSX1*) and KK157 (*hsx1::LEU2*) that also contain the chromosomal Ty*1*m*his3*AI-270 element. Ty*1* transposition was induced by growing the cells on SC-ura (galactose) plates and spliced Ty*1*m*HIS3* transposition events were detected by replica plating onto SC-his-ura (glucose) plates (Figure 1). The *HSX1* strain DG1301 (containing the p*GAL1-lacZ* control plasmid) gave rise to a few transposition events, while the *HSX1* strain DG1302 (containing the pGTy*1*-H3*neo* helper plasmid) gave rise to hundreds of transposition events. In contrast, no Ty*1*m*HIS3* transposition events were present in the *hsx1::LEU2* strains DG1305 and DG1306, even though strain DG1306 contains a pGTy*1*-H3*neo* helper plasmid that was induced for transposition. Since the *hsx1::LEU2* mutation is recessive (Table 3), we showed that the pGTy*1*-H3*neo* helper plasmid is transposition-competent by testing pGTy*1*-H3*neo* transposition in an *hsx1::LEU2/HSX1* diploid strain (Table 4).

Several controls were performed to determine whether the *hsx1* mutation directly affected the Ty transposition process or whether the *hsx1* mutation affected RNA splicing or Ty RNA levels. The splicing efficiency of the Ty*1*m*his3*AI-270 transcript varied

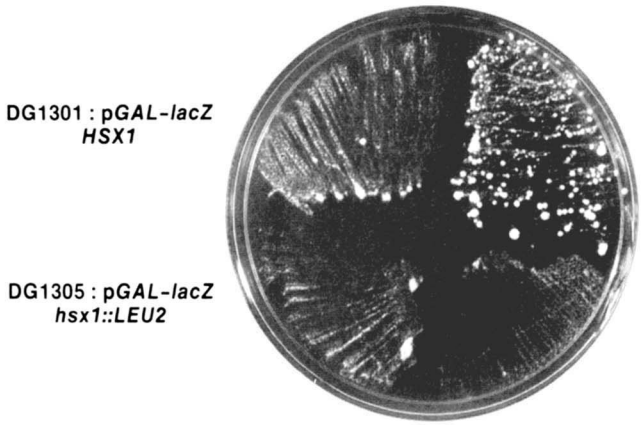

FIGURE 1.—Ty*1*m*his3*AI-270 transposition in an *hsx1* mutant background. Strains DG1301, DG1302, DG1305 and DG1306 contain the genomic Ty*1*m*his3*AI-270 element. The relevant plasmids and status of the *HSX1* gene are shown alongside the strains. These strains were tested for transposition by growing cells on SC-ura (galactose) plates for 7 days at 20°, replica plating to SC-his-ura (glucose), and incubating the replicas for 3 days at 30°.

TABLE 4

Ty*1neo* transposition in *hsx1/HSX1* diploid strains

Relevant genotype[a]	Relative transposition efficiency (%)[b]
HSX1/HSX1	42 (15/36)
hsx1::LEU2/HSX1	47 (16/34)

[a] Homozygous *HSX1/HSX1* diploids were obtained by mating strains DG1302 and KD198-16A. Heterozygous *hsx1::LEU2/HSX1* diploids were obtained by mating strains DG1306 and KD198-16A.

[b] In this transposition test, the transposition efficiency is the number of G418^r, Ura$^-$ plasmid segregants divided by the total number of Ura$^-$ plasmid segregants.

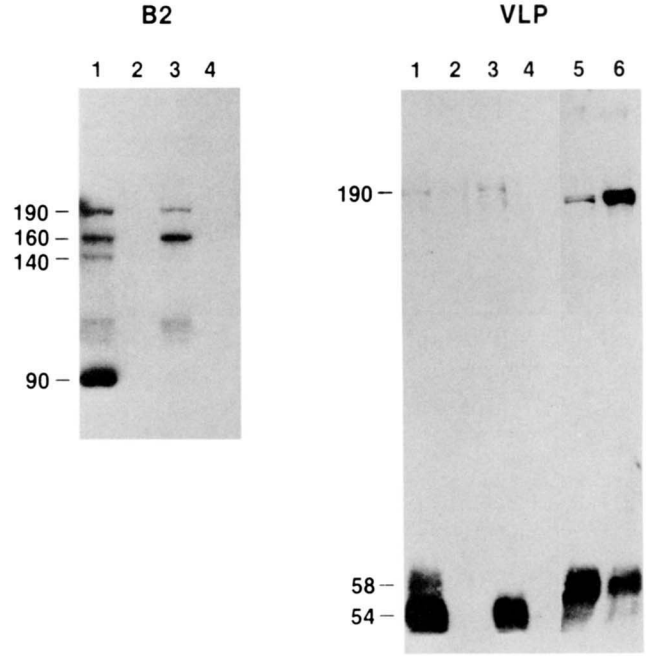

FIGURE 2.—Immunoblot analysis of Ty*1* proteins from an *hsx1* mutant background. Strains DG1302 (*HSX1*, pGTy*1*-H3*neo*; lane 1), DG1301 (*HSX1*, p*GAL-lacZ*; lane 2), DG1306 (*hsx1::LEU2*, pGTy*1*-H3*neo*; lane 3), DG1305 (*hsx1::LEU2*, p*GAL-lacZ*; lane 4), DG1333 (*HSX1*, pGTy*1*-H3*neo::SacI-1702*; lane 5), and DG1334 (*hsx1::LEU2*, pGTy*1*-H3*neo::SacI-1702*, lane 6) were induced for transposition by growth in SC-ura (galactose) medium and total protein was isolated for immunoblot analysis. Proteins were separated by electrophoresis on an SDS-8% polyacrylamide gel, transferred to a nitrocellulose membrane, and cross-reacted with B2 and VLP antisera. The B2 antiserum detects p90-Ty*1*-IN and its precursors. The VLP antiserum detects p54 and p58, which are VLP structural proteins derived from TYA1, as well as p190-TYA1-TYB1. The minor bands observed between p90-Ty*1*-IN and p140-TYB1 are probably caused by cellular proteolysis because they are present in immunoblots prepared from a Ty PR mutant (S. D. YOUNGREN and D. J. GARFINKEL, unpublished results). Ty*1* protein size estimates (in kilodaltons) are indicated.

from 12 to 20% in both *HSX1* or *hsx1::LEU2* strains as determined by RT-PCR. These splicing efficiencies agree with previous results where it was shown that about 12% of the Ty*1*m*his3*AI transposition events had lost the AI by splicing (CURCIO and GARFINKEL 1991). However, the overall Ty*1* and Ty*1*m*his3*AI-270 RNA levels were between 2- and 8-fold lower in an *hsx1::LEU2* mutant background when compared with *ACT1*, *PYK1* RNA or rRNA levels, although these differences were not completely reproducible.

To determine whether this moderate decrease in the level of Ty RNA could account for the more than 50-fold reduction in Ty*1* transposition, we assayed the level of pGTy*1*-H3*made2*AI retrotransposition (M. J. CURCIO and D. J. GARFINKEL, unpublished results) in an *hsx1::LEU2* mutant. In collateral experiments, the level of pGTy*1* expression in an *hsx1::LEU2* mutant was determined by immunoblotting (see below). The efficiency of Ty*1*m*ade2*AI transposition was reduced almost 70-fold in an *hsx1::LEU2* mutant background, while the level of *GAL1*-promoted Ty*1* proteins remained unchanged in the mutant (Figure 2). A similar decrease in transposition was also observed when an *HSX1* strain containing a pGTy*1*A-B*neo* plasmid with a mutation that corrects the frameshift was galactose-induced. Taken together, these results suggest that neither inhibition of splicing nor the lower concentration of chromosomal Ty*1* or Ty*1*m*his3*AI RNA can completely account for the reduction of Ty*1* transposition in an *hsx1::LEU2* mutant. Previous analyses have shown that increased expression of tRNA-Arg(CCU) (*HSX1*) negatively regulates Ty*1* transposition (XU and BOEKE 1990). Our results indicate that the *HSX1* gene is required for transposition of Ty*1* elements.

Mature TYB1 proteins do not accumulate in an *hsx1* disruption mutant: To further investigate the inhibition of Ty*1* transposition by *hsx1::LEU2*, we compared the levels and processing of Ty*1*-encoded proteins in isogenic *HSX1* and *hsx1* disruption strains (Figure 2). Total protein was isolated from strains DG1302 (*HSX1*, pGTy*1*-H3*neo*; lane 1), DG1301 (*hsx1*, p*GAL1-lacZ*; lane 2), DG1306 (*hsx1::LEU2*, pGTy*1*-H3*neo*; lane 3), and DG1305 (*hsx1::LEU2*, p*GAL1-lacZ*; lane 4) that were induced with galactose. The proteins were separated on SDS-polyacrylamide gels and immunoblotted. The resulting filters were reacted with B2 antiserum, which reacts with the full-length 190-kilodalton (kD) TYA1-TYB1 precursor protein, the 160-kD and 140-kD processing intermediates, and mature 90-kD Ty*1*-IN (GARFINKEL *et al.* 1991) or Ty*1*-VLP antiserum, which reacts with the 58-kD TYA1 precursor protein and the mature 54-kD TYA1 product (ADAMS *et al.* 1987; MULLER *et al.* 1987; YOUNGREN *et al.* 1988). Wild-type protein patterns were observed when the *HSX1* strain DG1302 was analyzed with B2 or Ty*1*-VLP antiserum (lane 1), or with an antiserum (B8) that detects p60-Ty*1*-RT/RH (B. FAIOLA and D. J. GARFINKEL, data not shown). As expected, strains DG1301 (lane 2) and DG1305 (lane 4) containing the heterologous expression plasmid p*GAL-lacZ* had very low levels of Ty*1* proteins (GARFINKEL *et al.* 1985; CURCIO and GARFINKEL 1992).

The *hsx1::LEU2* strain DG1305 (Figure 2, lane 3) displayed a different protein pattern when reacted with B2 and Ty*1*-VLP antisera. Essentially wild-type levels of the 190-kD TYA1-TYB1 precursor protein and 160-kD processing intermediate were detected using B2 antiserum. However, very little of the 140-kD precursor or 90-kD IN protein was detected. Similar results were obtained when an antiserum (B8) that detects RT/RH was used: the 190-kD and 160-kD TYB1 precursor proteins were present at wild-type levels, but the 140-kD precursor and the 60-kD Ty*1* RT/RH protein were barely detectable (B. FAIOLA and D. J. GARFINKEL, data not shown). When TYA1 proteins were analyzed with Ty*1*-VLP antiserum, normal levels of mature p54-TYA1 protein were observed in an *hsx1* mutant, but very little full-length p58-TYA1 precursor was detected even after extended exposure of the filter. Furthermore, similar protein patterns were observed when partially purified Ty*1*-VLPs were reacted with B2, B8, or Ty*1*-VLP antisera (B. FAIOLA and D. J. GARFINKEL, data not shown). These results suggest that the transposition defect observed in *hsx1* mutants is related to aberrant protein processing.

Ty*1* frameshifting increases in an *hsx1* disruption mutant: We tested whether the observed transposition defect in the *hsx1* mutant resulted from abnormal frameshifting using two different frameshifting assays. In the first assay, the *HSX1* strain KK242 and *hsx1::HIS3* mutant strain KK240 were transformed with pMB38-9merFusion and pMB38-9merWT plasmids in which the 0 (*TYA1*) and +1 (*TYA1-TYB1*) reading frames and *lacZ* are fused, respectively (Table 5). β-Galactosidase activity was determined from at least six different transformants of each plasmid and Ty*1* frameshifting efficiencies were calculated as described (see MATERIALS AND METHODS; BELCOURT and FARABAUGH 1990). A frameshifting efficiency of 35% was obtained in an *HSX1* background, which is comparable to published values (BELCOURT and FARABAUGH 1990). In contrast, the *hsx1::HIS3* disruption resulted in 91% frameshifting. The frameshifting efficiency was restored to 35% by a low copy number plasmid carrying the wild-type *HSX1* gene (pKK73; Table 6).

We also determined the Ty*1* frameshifting efficiency by quantitating the ratio of the unprocessed p58-TYA1 precursor to the p190-TYA1-TYB1 precursor in *HSX1* and *hsx1::LEU2* strains DG1333 and DG1334, respectively (Figure 2, lanes 5 and 6). To insure that unprocessed precursor proteins accumulated during the galactose induction, strains DG1333 and DG1334 contained a pGTy*1*-H3 plasmid with a well characterized Ty*1*-PR mutation, pGTy*1*-

TABLE 5

Translational frameshifting in an *hsx1* mutant

Relevant genotype	Frameshift site	β-Galactosidase units	Frameshifting efficiency (%)
HSX1	9merWT	2400	
	9merFusion	6800	35
	9merFusion(w/o AGG)	8900	27
hsx1::HIS3	9m34WT	5100	
	9merFusion	5600	91
	9merFusion(w/o AGG)	6100	84

Strains KK242 (*HSX1*) and KK240 (*hsx1::HIS3*) were transformed with plasmids pMB-9merWT, pMB38-9merFusion, and pMB38-9merFusion(w/o AGG). β-Galactosidase activities are the averages from six independent transformants. The frameshift efficiency is defined as the β-galactosidase activity of the 9merWT divided by the β-galactosidase activity of either the 9merFusion or the 9merFusion(w/o AGG) (BELLCOURT and FARABAUGH 1990).

TABLE 6

Translational frameshifting in an *hsx1* mutant KK240 containing plasmid copies of tRNA genes

Plasmid genotype	Frameshifting efficiency (%)
pKK73 (*HSX1*)	35
pKK74 [*hsx1*(*MluI**)]	98
pKK75 [*SUP201-0*(*UCU*)]	90
pKK76 [*SUP201-0-1*(*CCU*)]	65

Plasmids were introduced into strain KK240 (*hsx1::HIS3*) by transformation. Refer to Table 5 for experimental details.

H3*neo::SacI-1702* (YOUNGREN *et al.* 1988; GARFINKEL *et al.* 1991; CURCIO and GARFINKEL 1992). Proteins were analyzed by immunoblotting using Ty*1*-VLP antiserum, which recognizes TYA1 proteins and the 190-kD TYA1-TYB1 precursor protein (ADAMS *et al.* 1987; MULLER *et al.* 1987; YOUNGREN *et al.* 1988), and frameshifting efficiencies were calculated by densitometry (see MATERIALS AND METHODS).

The *HSX1* strain DG1333 (Figure 2, lane 5) showed the pattern of unprocessed 58-kDa and 190-kDa proteins expected from a Ty*1*-PR mutant (ADAMS *et al.* 1987; MULLER *et al.* 1987; YOUNGREN *et al.* 1988). A frameshifting efficiency of about 3% was obtained from densitometric scans of various exposures of the immunoblot. In contrast, the *hsx1::LEU2* strain DG1334 (Figure 2, lane 6) had much more of the 190-kD TYA1-TYB1 precursor and slightly less of the 58-kD TYA1 precursor than the *HSX1* parent strain DG1333 (Figure 2, lane 5). The *hsx1::LEU2* disruption mutant had a frameshifting efficiency of about 50%, which is about 17-fold higher than in an *HSX1* background. The overall level of Ty*1* protein also appeared to be similar in the *HSX1* or *hsx1* mutant backgrounds. These results suggest that the absence of tRNA-Arg(CCU) enhances ribosomal pausing at AGG and slippage of the leucyl-tRNA from CUU to UUA. Furthermore, the regulation of frameshifting by the *HSX1* gene is essential for Ty*1* transposition. The reduction in transposition in an *hsx1* mutant may be caused by a defect in protein processing that results from an aberrant stoichiometry of Ty proteins.

The capacity to translate an AGG codon does not limit β-galactosidase synthesis in an *hsx1* mutant: The *lacZ* fusion gene in the pMB38-9merFusion plasmid has only one AGG codon and it is located at the fusion site (BELCOURT and FARABAUGH 1990). That AGG codon is missing in the pMB38-9merFusion(w/oAGG) *lacZ* fusion gene. Therefore, the effect of a single AGG codon on β-galactosidase synthesis was determined in an *hsx1::HIS3* mutant. Interestingly, β-galactosidase activities in the *hsx1::HIS3* mutant or the *HSX1* parental strain harboring the pMB38-9merFusion and the pMB38-9merFusion(w/oAGG) plasmids were similar (Table 5). These results suggest that the capacity to translate the AGG codon does not limit β-galactosidase synthesis in an *hsx1* mutant. However, we do not know how the AGG is translated in an *hsx1* mutant. Since haploid cells contain more than eight tRNA-Arg(UCU) genes (BECKMANN, JOHNSON and ABELSON 1977), it is possible that tRNA-Arg(UCU) decodes AGG codons by near-cognate recognition when tRNA-Arg(CCU) is absent (YOKOYAMA *et al.* 1985).

Complementation of *hsx1* by a tRNA suppressor *SUP201-0-1(CCU)*: Although tRNA-Arg(UCU) may decode AGG codons, excess tRNA-Arg(UCU) does not inhibit frameshifting (BELCOURT and FARABAUGH 1990). This may be because of sequence or structural differences between tRNA-Arg(UCU) and tRNA-Arg(CCU) (Figure 3). Alternatively, the information needed to regulate Ty*1* frameshifting may reside within the anticodon. To determine if the CCU anticodon is sufficient to regulate Ty*1* transposition (Table 3) and frameshifting (Table 6), we constructed a low-copy-number plasmid carrying a mutant tRNA-Arg gene that has a CCU instead of a UCU anticodon. The *SUP201-0-1(CCU)* anticodon mutation was introduced into the *SUP201-0* tRNA-Arg(UCU) gene (THIREOS, PENN and GREER 1984; MORISHITA and UNO 1991), by oligonucleotide mutagenesis (refer to MATERIALS AND METHODS). Functionally active tRNAs were synthesized from these plasmids because a plasmid carrying the same 112-bp segment of DNA with a *SUP201* nonsense suppressor complemented the *cyr1-2* UGA allele (MORISHITA and UNO 1991; K. KAWAKAMI and Y. NAKAMURA, unpublished results).

To determine if *SUP201-0-1(CCU)* could suppress the transposition defect imposed by an *hsx1* mutation, strain KK157 containing Ty*1*m*his3*AI-270 and *hsx1::LEU2* was transformed with the suppressor plasmid pKK71 [*SUP201-0-1(CCU)*] or the parental plasmid pKK69 [*SUP201-0(UCU)*]. The level of Ty*1* transposition was partially restored when the pKK71 [*SUP201-0-1(CCU)*] plasmid was present in the *hsx1::LEU2* mutant (Table 3). This result suggests that the CCU anticodon can regulate transposition.

An *hsx1::HIS3* mutant strain KK240 harboring plasmids pMB38-9merWT or pMB38-9merFusion was transformed with plasmids pKK75 [*SUP201-0(UCU)*] and pKK76 [*SUP201-0-1(CCU)*] and frameshifting efficiencies were analyzed in these transformants (Table 6). The *SUP201-0-1(CCU)* mutant tRNA resulted in an intermediate level of frameshifting. Interestingly, frameshifting in the pKK76 [*SUP201-0-1(CCU)*] transformant was higher (65%) than in the pKK73 [*HSX1*; tRNA-Arg(CCU)] transformant (35%). This result is consistent with the lower level of transposition of the pKK71 [*SUP201-0-1(CCU)*] transformant (1.1×10^{-6}) when compared to the pKK67 [*HSX1*; tRNA-Arg(CCU)] transformant (6.4×10^{-6}; Table 3). Therefore, although *SUP201-0-1(CCU)* can partially regulate Ty transposition and frameshifting, it does not work as well as tRNA-Arg(CCU) encoded by *HSX1*. Other aspects of *SUP201-0-1(CCU)* expression or structure may prevent full complementation of the *hsx1* mutation. These results also suggest that base pairing at the third position of the second codon in the frameshift heptamer is essential for regulating Ty*1* transposition and frameshifting.

Increasing TYA1 expression restores Ty*1* transposition in an *hsx1* mutant: Our results indicate that more of TYA1-TYB1 fusion protein is translated in

```
SUP201-0  GCUCGCGUGGCGUAAUGGCAACGCGUCUGACUUCUAAUCAGAAGAUUAUGGGUUCGACCCCCAUCGUGAGUG
          * **  ********** *********  ** **** ****** * ********  *** *  **  *
HSX1      GUUCCGUUGGCGUAAUGGUAACGCGUCUCCCCUCCUAAGGAGAAGACUGCGGGUUCGAGUCCCGUACGGAACG
                            MluI
```

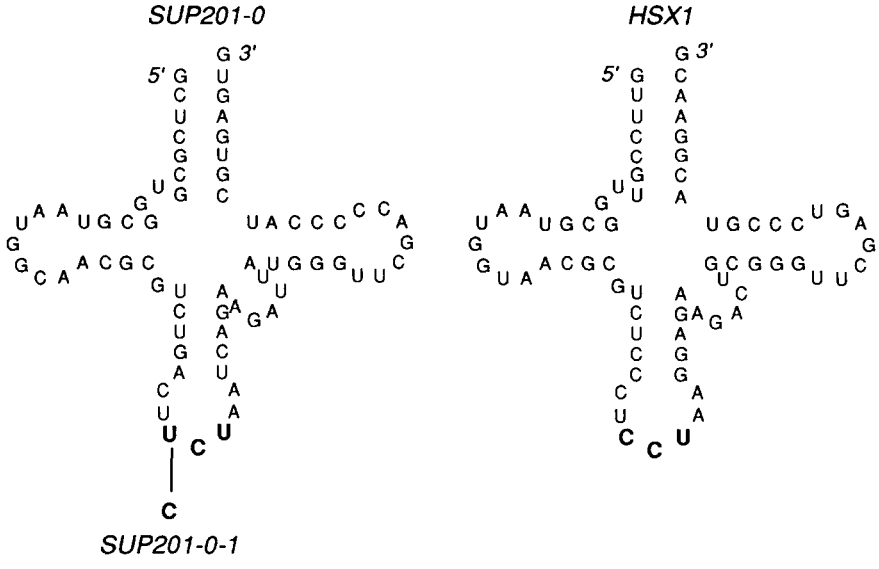

FIGURE 3.—Comparison of *SUP201-0* and *HSX1*. Nucleotide sequences of the *SUP201-0* and *HSX1* tRNAs are shown. Identical nucleotides are indicated by asterisks. Anticodons are in bold lettering. The clover leaf structure of the *SUP201-0* and *HSX1* genes, and the *SUP201-0-1(CCU)* mutation are also shown.

the *hsx1* disruption mutant, and that this altered stoichiometry of TYA1 to TYA1-TYB1 precursor proteins may inhibit Ty*1* transposition. Therefore, providing more TYA1 protein should restore Ty*1* transposition in an *hsx1* mutant background. To test this idea, the efficiency of Ty*1* transposition was determined with strains DG1344 (*HSX1*) and DG1347 (*hsx1::LEU2*) containing the helper plasmid pGTyA*1neo* (*PGK1* ter.), and strains DG1301 (*HSX1*) and DG1305 (*hsx1::LEU2*) containing the heterologous expression plasmid p*GAL-lacZ*. The helper plasmid pGTyA*1neo* (*PGK1* ter.) contains a complete *TYA1* gene, about 25 codons of N-terminal *TYB1* sequence, the *neo* marker gene, and a transcriptional terminator from the *PGK1* gene in place of the downstream long terminal repeat. Liquid cultures of strains DG1301, DG1305, DG1344 and DG1347 were galactose-induced and transpositions of the chromosomal Ty*1*m*his3*AI-270 element were selected on SC-his-ura (glucose) medium (Table 7). Although expression of the helper plasmid pGTyA1*neo* (*PGK1* ter.) did not markedly affect Ty*1* transposition in an *HSX1* strain, expression of *TYA1* stimulated Ty*1* transposition 50-fold more in an *hsx1* strain than in an *HSX1* strain. In addition, galactose-induction of pGTyA*1neo* (*PGK* ter.) did not affect the level of full-length Ty*1* RNA in an *HSX1* or *hsx1* disruption strain (B. FAIOLA and D. J. GARFINKEL, data not shown). As expected, the level of the TyA*1neo* (*PGK* ter.) transcript was the same in the *HSX1* and *hsx1::LEU2* mutant strains. These results indicate that expression of pGTyA*1neo* (*PGK1* ter.) stimulates Ty*1* transposition in an *hsx1* mutant background by restoring the proper stoichiometry of TYA1 to TYA1-TYB1 precursor proteins.

TABLE 7

Effect of pGTyA*1neo*(*PGK1* ter.) expression on Ty*1*m*his3*A1-270 transposition in an *hsx1* mutant

Strain	Relevant genotype	Relative transposition efficiency
DG1301	*HSX1*, p*GAL1-lacZ*	1.3×10^{-7}
DG1344	*HSX1*, pGTyA*1neo*(*PGK1* ter.)	4.8×10^{-7}
DG1305	*hsx1::LEU2*, p*GAL1-lacZ*	$<3.7 \times 10^{-9}$
DG1347	*hsx1::LEU2*, pGTyA*1neo*(*PGK1* ter.)	2.4×10^{-5}

These strains contain the genomic Ty*1*m*his3*AI-270 element. Relative transposition efficiencies were determined from liquid cultures grown in SC-ura (galactose) as described in MATERIALS AND METHODS. The relative transposition efficiency is the number of His^+, Ura^+ colonies divided by the number of Ura^+ colonies. Each measurement represents the mean of four cultures. The total number of colony-forming units was similar within each set of cultures. Refer to Table 2 for more information.

DISCUSSION

Our study reveals that the *HSX1* gene is necessary for Ty*1* transposition because elimination of this gene causes a significant transposition defect. Our work also shows that Ty*1* translational frameshifting increases dramatically in an *hsx1* disruption mutant. The *hsx1* mutant defects in frameshifting and Ty*1* transposition are completely complemented by the wild-type *HSX1* gene and partially complemented by the

mutant *SUP201-0-1(CCU)* gene, while no complementation occurs with the *SUP201-0(UCU)* gene (Tables 3 and 6). Therefore, at least some of the information required for Ty*1* frameshifting is provided by the CCU anticodon. The partial complementation activity of the mutant *SUP201-0-1* tRNA-Arg(CCU) suggests two possibilities. First, *SUP201-0-1(CCU)* may be expressed at a lower level than *HSX1*, thus directly affecting the level of tRNA-Arg(CCU) available for frameshifting. Second, *SUP201-0-1(CCU)* may not recognize the AGG codon within the context of the frameshift heptamer as well as *HSX1*, since the *SUP201-0-1 and HSX1* tRNA genes differ by 20 nucleotide changes (Figure 3). RAFTERY and YARUS (1987) have shown that the structure of the proximal anticodon stem affects efficiency of a tRNA suppressor of *E. coli* and suggested that it is a part of the extended anticodon. The 2-bp difference in the anticodon stem between *SUP201-0-1* and the *HSX1* tRNAs may result in the altered AGG codon recognition activity of the *SUP201-0-1* tRNA.

Both −1 and +1 frameshifting mechanisms used by a variety of RNA viruses, retroviruses, and retrotransposons apparently require a translational pause for optimum efficiency (reviewed by Hatfield *et al.* 1992). For example, the translational pause in retroviral −1 frameshifting is created by a pseudoknot located a few nucleotides downstream of the frameshift, whereas Ty*1* +1 frameshifting uses a the rare tRNA-Arg(CCU). Our results are consistent with the +1 frameshifting model proposed by BELCOURT and FARABAUGH (1990). According to this model, the increase in +1 frameshifting results from a longer translational pause in an *hsx1* mutant created by the absence of tRNA-Arg(CCU). The longer translational pause regulates translation of *TYB1-pol* by allowing more time for the tRNA-Leu to slip from the 0-frame CUU codon in *TYA1* to the +1-frame UUA codon in *TYB1*.

Two different approaches were used to estimate the increase in frameshifting that occurs in an *hsx1* disruption mutant. First, frameshifting was measured using the minimal heptamer sequence with *lacZ* as a reporter gene (BELCOURT and FARABAUGH 1990). The absence of tRNA-Arg(CCU) increased frameshifting as measured by β-galactosidase activity about 3-fold. Second, frameshifting was measured by immunoblotting using Ty*1*-VLP antiserum and a Ty*1*-PR mutant defective in protein processing. The increase in frameshifting at the **CUU-AGG-C** sequence leads to accumulation of slightly less p54-TYA1 protein and much more p190-TYA1-TYB1 fusion protein. Using this assay, frameshifting increased about 17-fold in an *hsx1* background.

We estimate that Ty*1* frameshifting occurs at about a 3% efficiency in an *HSX1* background by immunoblotting. In other words, 3% of ribosomes translating the *TYA1-gag* open reading frame undergo a +1 frameshift and continue translating the *TYB1-pol* open reading frame. It is somewhat surprising that the Ty*1* frameshifting efficiency of 3% is about 5–10-fold lower than that obtained by lacZ fusion analysis. It is possible that we have underestimated the Ty*1* frameshifting efficiency obtained from immunoblotting because of an inability to detect the p190-TYA1-TYB1 precursor protein. However, control experiments suggest that p58-TYA1 and p190-TYA1-TYB1 are transferred at about the same rate under the immunoblotting conditions used in this study, bind to Ty*1*-VLP antiserum with comparable affinities, and have similar turnover rates (A.-M. HEDGE and D. J. GARFINKEL, unpublished results; CURCIO and GARFINKEL 1992). There may also be differences in translation rates of *lacZ* in yeast, or in the placement of the frameshift heptamer relative to the start of translation that contribute to this apparent discrepancy (P. J. FARABAUGH, unpublished results).

The Ty*1* frameshifting efficiency of 3% obtained by immunoblot analysis is comparable to the efficiencies obtained from several viral systems that utilize different mechanisms for translation of the pol gene. Retroviruses that utilize programmed ribosomal frameshifting or read-through suppression undergo translational suppression at an efficiency of about 5% (reviewed by HATFIELD *et al.* 1992). Yeast Ty*3* retrotransposons have a +1 frameshifting efficiency of about 4%, even though these elements use a different frameshifting site (KIRCHNER, SANDMEYER and FORREST 1992) and mechanism than Ty*1* or Ty*2* elements (P. J. FARABAUGH, unpublished results). In addition, the yeast L-A double-stranded RNA virus undergoes −1 frameshifting to express its *pol* gene at an efficiency of about 2% (DINMAN, ICHO and WICKNER 1991). Even though the molecular mechanisms underlying these expression strategies are quite different, a certain ratio of "structural" (Gag) proteins to "catalytic" (Gag-Pol) proteins may be a general requirement for formation of a transposition/replication-competent particle.

Immunoblot analysis suggested that a processing defect of the TYA1-TYB1 fusion protein is related to the lower level of Ty*1* transposition in an *hsx1* disruption mutant. The protein cleavages required to form p54-TYA1 and the p160 processing intermediate still occur, while the proteolytic cleavage required to convert the p160 processing intermediate to p23-PR and the p140 processing intermediate apparently do not (Figure 4). Since formation of p140-TYB1 is defective, it follows that low amounts of mature IN and RT/RH are detected in an *hsx1::LEU2* mutant. Perhaps Ty*1*-PR is not completely activated when more of the TYA1-TYB1 fusion protein is produced. Alternatively, normal amounts of p140, IN and RT/RH

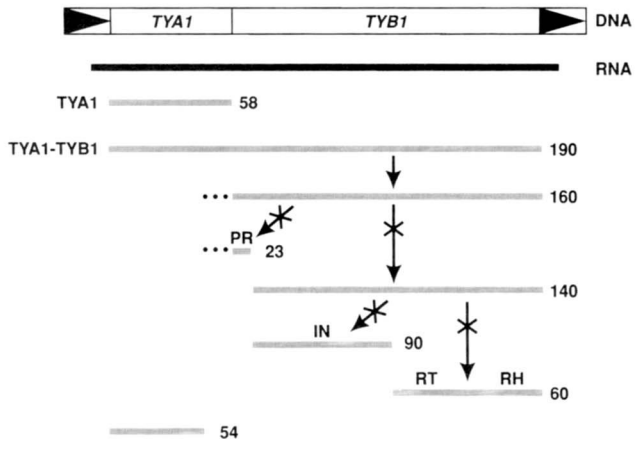

FIGURE 4.—Scheme for TYB1 protein processing in an *hsx1* mutant (modified from GARFINKEL *et al.* 1991). p190-TYA1-TYB1, the 190-kD product of the *gag-TYA1* and *pol-TYB1* genes, is cleaved near the frameshift region (the vertical line separating *TYA1* and *TYB1*). This proteolytic cleavage releases p160-TYB1, which is normally cleaved to form Ty*1*-PR (23 kD) and p140-TYB1. Cleavage of p140-TYB1 produces IN (90 kD) and RT/RH (60 kD). The dotted lines indicate that p160 and p23 may be encoded by both *TYA1* and *TYB1*. The arrows show that neither the p140-TYB1 percursor nor mature p90-Ty*1*-IN and p60-Ty*1*-RT/RH accumulate in an *hsx1* mutant. Also shown is the p58-TYA1 precursor and p54 processed product, which are the major structural components of Ty*1*-VLPs. In an *hsx1* mutant, we detect p54-TYA1 but not the p58-TYA1 precursor.

may be synthesized, but are rapidly degraded because of an *hsx1*-dependent defect in Ty*1*-VLP assembly.

To prove that aberrant protein stoichiometry is the major reason for the block in Ty*1* transposition in an *hsx1* disruption mutant, we showed that a pGTy*1* plasmid expressing just the *TYA1* gene not only restores Ty*1* transposition in an *hsx1::LEU2* mutant, but stimulates transposition to a level 50-fold higher than is observed in an *HSX1* strain. We also showed that overexpression of *TYA1* does not alter the level of Ty*1* RNA in an *hsx1* mutant. These results suggest that overexpression of *TYA1* enhances the utilization of Ty*1* RNA as a transposition template by rebalancing the level of TYA1 and TYA1-TYB1 proteins required to make transposition-competent Ty*1*-VLPs in an *hsx1* mutant, even though the absolute level of Ty*1* RNA is somewhat lower in the *hsx1* mutant. Furthermore, since *GAL1*-promoted Ty*1* transposition decreases about 70-fold without a concomitant decrease in *GAL1*-promoted Ty*1* protein levels in an *hsx1* disruption mutant, whatever effect the *hsx1* mutation has on Ty*1* RNA levels is limited to chromosomal Ty*1* elements. These results suggest that the *hsx1* mutation may affect chromosomal Ty*1* RNA accumulation, but we have not investigated this idea further.

The stimulation of Ty transposition that occurs in an *hsx1* mutant when *TYA1* is overexpressed supports and extends previous biochemical and genetic studies that identified the availability of Ty*1*-PR, which is encoded by *TYB1*, as a rate-limiting step in the Ty*1* retrotransposition cycle (CURCIO and GARFINKEL 1992). Since more TYA1-TYB1 precursor protein is made in an *hsx1* mutant, the availability of TYA1 protein becomes rate-limiting under these conditions. Therefore, a specific ratio of TYA1 to TYA1-TYB1 precursor proteins is required to form fully processed Ty*1* proteins and functional Ty*1*-VLPs.

Several retrovirus, retrotransposon and endogenous viral mutants in which *gag* and *pol* have been artificially fused are defective in particle formation, replication and infectivity. For example, fusion of *gag* and *pol* genes blocks production of infectious Moloney murine leukemia virus (FELSENSTEIN and GOFF 1988) and human immunodeficiency virus (PARK and MORROW 1992). In Moloney murine leukemia virus, the Gag-Pol precursor protein is produced, but neither protein processing nor particle formation occurs. In human immunodeficiency virus, the Gag-Pol protein is produced and processed, but particles do not form. A protein processing and transposition defect similar to the one created in an *hsx1* mutant is observed when *TYA1* and *TYB1* are fused by deleting one base at the frameshift site of a pGTy*1* plasmid and transposition is galactose-induced in an *HSX1* strain. Preliminary experiments suggest that Ty*1*-VLPs are formed in an *hsx1* mutant (B. FAIOLA and D. J. GARFINKEL, unpublished results) or when just the TYA1-TYB1 fusion protein is expressed (J. D. BOEKE and D. J. GARFINKEL, unpublished results). Recently, a Ty*3* *GAG3-POL3* fusion mutant has been analyzed for defects in transposition and Ty*3*-VLP formation using a pGTy*3* expression system (KIRCHNER, SANDMEYER and FORREST 1992). The fusion mutant is transposition-defective, but can be rescued by coexpression of *GAG3* or just the capsid domain of *GAG3*. Protein processing of GAG3 capsid protein and Ty*3*-IN is altered in the mutants, as is individual Ty*3* protein and Ty*3*-VLP yield. Optimal ribosomal frameshifting and the proper Gag to Gag-Pol protein ratio are also required for L-A virus propagation in yeast (DINMAN and WICKNER 1992). Therefore, Ty*1* and Ty*3* elements seem to be unique in that some particle assembly can take place when excess Gag-Pol precursor protein is synthesized (KIRCHNER, SANDMEYER and FORREST 1992) when only Gag protein is synthesized (ADAMS *et al.* 1987; BURNS *et al.* 1992), or when PR-dependent protein processing is blocked (ADAMS *et al.* 1987; MULLER *et al.* 1987; YOUNGREN *et al.* 1988; KIRCHNER and SANDMEYER 1993).

In summary, our work has identified an essential role for *HSX1* in Ty*1* frameshifting and transposition. This is one of a small but growing collection of cellular genes required for Ty*1* transposition that act post-transcriptionally (reviewed by BOEKE and CHAPMAN 1991; GARFINKEL 1992). The additional defects of an

hsx1 disruption mutant (KAWAKAMI *et al.* 1992) may allow us to select second-site suppressors that restore Ty*1* transposition without affecting Ty*1* frameshifting mediated by tRNA-Arg. These suppressors may identify additional cellular genes involved in Ty*1* frameshifting or Ty*1*-VLP assembly.

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