

Single-step selection for Ty1 element retrotransposition

(*Saccharomyces cerevisiae*/reverse transcription/artificial intron)

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ABSTRACT The yeast retrotransposon Ty1 has been tagged with a reporter gene that allows selection of RNA-mediated transposition events and is applicable to the study of retroelements in other organisms. The reporter gene is a yeast *HIS3* gene interrupted by an artificial intron (AI) in the antisense orientation. The *HIS3AI* sequences were inserted into a Ty1 element such that the intron is on the sense strand of the Ty1 element; therefore, splicing and retrotransposition of marked Ty1 transcripts can give rise to His⁺ cells. Fusion of the Ty1-H3m*HIS3AI* element to the inducible *GAL1* promoter resulted in a high frequency of histidine prototrophs upon galactose induction. Moreover, spontaneous His⁺ revertants derived from strains containing genomic Tym*HIS3AI* elements are a result of retrotransposition. By using this assay, we estimated the Ty1 transposition rate to be between 3×10^{-7} and 1×10^{-5} transpositions per Ty1 element per generation. Variations in the transposition rate of individual Ty1 elements are correlated with the relative abundance of their transcripts.

Ty elements of *Saccharomyces cerevisiae* are retrotransposons that are similar to retroviral proviruses (1). Retrotransposition is a replicative process involving reverse transcription of Ty mRNA and integration of Ty cDNA into the genome (2). Ty1 elements are the most common insertional mutagen and comprise the most numerous family of the four Ty element classes, with about 25-30 copies of Ty1 per haploid genome (3, 4). Despite the fact that Ty1 RNA accounts for 1% of total yeast RNA (4), the rate of transposition is quite low (5-7). Several modulators of transposition have been described. For example, Ty transposition is stimulated at temperatures below 30°C (8), by exposure of the cells to ultraviolet irradiation or 4-nitroquinoline 1-oxide (9), or in a *rad6* mutant background (10). Mutations in the *SPT3* gene alter the initiation of Ty1 transcription (11) and abolish retrotransposition of chromosomal Ty1 elements (6). These modulators of retrotransposition were identified by their effect on the frequency of Ty insertions into specific loci and not into the genome as a whole. As a result, it can be difficult to determine whether the modulators alter Ty elements directly or the target locus (10).

A tremendous induction in the rate of Ty1 transposition is achieved by expressing an active Ty element, Ty1-H3, from the inducible *GAL1* promoter (2). The pGTy1-H3 element has been marked with selectable genes such as a bacterial gene for neomycin resistance (12) and the yeast *HIS3* gene (13). Phenotypic detection of retrotransposition events in the transposition-induction system requires loss of the pGTy plasmid. In addition, transposition of the marked Ty1 element can only be detected when it is induced to a level that exceeds the rate of homologous recombination among Ty elements (14, 15). In this paper, we describe an indicator gene, *HIS3AI*, that overcomes these limitations. This re-

porter gene is only active if marked Ty RNA is spliced prior to reverse transcription. We also demonstrate that Ty transposition may be useful for detecting agents that inhibit retroelement replication.

MATERIALS AND METHODS

Plasmid Constructions. The *HIS3AI* indicator gene was constructed by cloning a 104-base-pair artificial yeast intron (AI), contained on a *Pvu* II-*Sna*BI fragment (16), into the blunt-end *Msc* I site of *HIS3* carried on pCLA12*HIS3* (13, 17), to form pCLA12*HIS3AI*. The predicted intron-exon junctions were confirmed by DNA sequencing (18). A *HIS3AI* *Cla* I fragment was cloned into the *Cla* I site of pGTy1-H3CLA (13) in both orientations. The resulting plasmids have *HIS3AI* in either the sense or antisense orientation relative to Ty1-H3 transcription and are called pGTy1-H3m*HIS3AI* or pGTy1-H3*HIS3mAI*, respectively (Fig. 1 A and B). Plasmid pGTy1-H3m*HIS3* was made by inserting the *Cla* I fragment of pCLA12*HIS3* into the *Cla* I site of pGTy1-H3CLA (Fig. 1C).

Yeast Strains and Media. The yeast strains used are GRF167 (*MAT α* , *ura3-167*, *his3- Δ 200*, *GAL*), and an isogenic *spt3* derivative, DG789, both of which contain a complete *HIS3* deletion (2, 17). Strains JC234, JC242, JC246, and JC271 are congenic derivatives of GRF167 that contain different unspliced Tym*HIS3AI* insertions. These strains were isolated after galactose induction of plasmid pGTy1-H3m*HIS3AI* in strain GRF167 and subsequent segregation of the pGTy plasmid. Standard yeast media were prepared as described by Sherman *et al.* (19).

Transposition Efficiencies. To determine the fraction of cells that sustain a marked transposition in strains containing pGTy1-H3*HIS3AI* plasmids, cultures were inoculated at low densities and grown to saturation at 20°C in SC-ura galactose (19) to induce transposition or in SC-ura glucose (19). A portion of each culture was then plated onto SC-his glucose plates (19) and grown at 30°C to end the transposition induction and score histidine prototrophs. The cultures were titered on YEPD plates.

Southern Blot Analysis of Transposition-Induced Strains. Strains containing pGTy plasmids were grown for 5 days at 20°C on SC-ura galactose plates. Independent colonies were recovered and processed for Southern blot analysis with a randomly labeled *HIS3* or Ty probe as described (4).

Analysis of Genomic Tym*HIS3AI* Elements. Transposition rates were determined by the method of Lea and Coulson (20). Between 7 and 11 cultures inoculated with ≈ 200 cells were grown to saturation at 20°C in YEPD and then plated onto SC-his glucose medium at 30°C. Three cultures of each strain were titered on YEPD plates. Northern blot analysis was performed with excess ³²P-labeled *HIS3*, Ty1, and PYK1 RNA probes, as described (4).

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Abbreviation: AI, artificial yeast intron.

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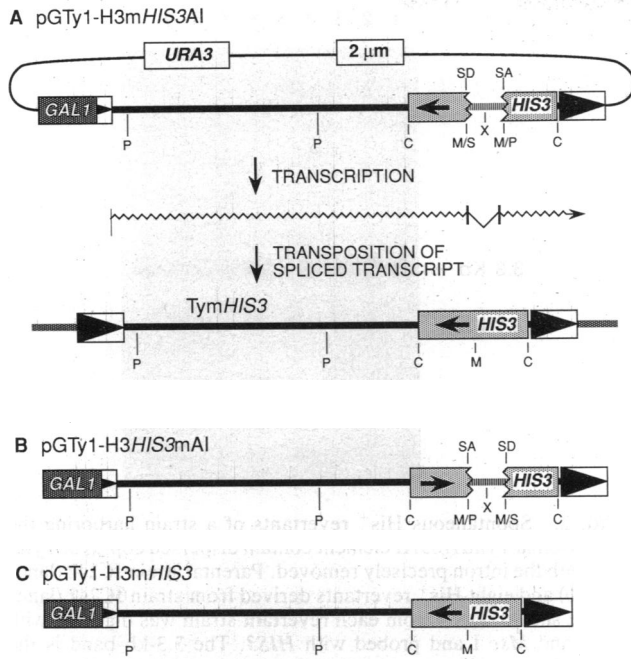


FIG. 1. Genetically marked derivatives of plasmid pGTy1-H3. (A) Structure of plasmid pGTy1-H3mHIS3AI. The boxed arrowheads represent the Ty1 long terminal repeats and the direction of Ty1 transcription. The shaded box represents the yeast *HIS3* gene and the enclosed arrow indicates its direction of transcription. AI sequences, represented by the broken line, are in the antisense orientation relative to the *HIS3* gene but in the sense orientation relative to Ty1-H3. The Ty1-H3mHIS3AI transcript is represented by the wavy line. Splicing is indicated by vertical lines in the transcript. TymHIS3 is the spliced and transposed copy of the marked element. (B) Structure of the *GAL1*-Ty1 fusion element contained in plasmid pGTy1-H3HIS3mAI. In this plasmid, the *HIS3*AI gene is transcribed in the same direction as the Ty1 element, and the intron, which is in the antisense orientation relative to both Ty1-H3 and *HIS3* transcription, is unsplicible. (C) The element contained on pGTy1-H3mHIS3. *GAL1*, yeast *GAL1* promoter; SD, splice donor; SA, splice acceptor; *URA3*, yeast *URA3* gene; 2 μ m, yeast 2- μ m origin of replication; P, *Pvu* II; C, *Cla* I; X, *Xba* I; M, *Msc* I; M/S, *Msc* I/*Sna*BI hybrid sites; M/P, *Msc* I/*Pvu* II hybrid sites.

RESULTS

Construction of a Reporter Gene for Retrotransposition. We have designed a reporter gene for retrotransposition that consists of an AI interrupting the coding sequences of *HIS3*. The intron is a portable cassette containing all of the cis-acting sequences required for splicing without any flanking exon sequences (16). The AI was inserted into the *HIS3* coding sequence in an antisense orientation, thereby inactivating *HIS3* and destroying an *Msc* I site. To form Ty1-H3mHIS3AI (the "m" indicates the presence of *HIS3* gene sequences on the minus strand of Ty1-H3), the *HIS3*AI gene was placed on the minus strand of Ty1-H3, such that transcription of *HIS3*AI is opposite of Ty transcription (Fig. 1A). Since the intron is in the antisense orientation relative to the *HIS3* transcript, it is in the correct orientation to be precisely removed from the Ty1-H3 RNA transposition intermediate by splicing. Therefore, colonies harboring transposed TymHIS3 elements can be identified by growth on medium lacking histidine. Restoration of the *Msc* I site in transposed copies of TymHIS3 can be easily detected by Southern blot analysis, confirming the presence of the correct splice junction.

As a control for DNA recombination events that confer a His⁺ phenotype, a derivative of Ty1-H3 containing *HIS3*AI in the same transcriptional orientation as Ty1-H3 (Fig. 1B) was constructed. The AI in this element, Ty1-H3HIS3mAI, is in an antisense orientation relative to both the Ty1 and

HIS3 transcripts. (The "m" signifies the presence of AI on the minus strand of Ty1-H3.) Therefore, both the starting transposon and transposed copies of Ty1-H3mHIS3AI should retain the intron and remain His⁻ if splicing of the retrotransposition intermediate is required for recreation of a functional *HIS3* gene.

Galactose Induction of pGTy1-H3mHIS3AI Transcription Results in High Levels of Retrotransposition. To determine whether *HIS3*AI was an indicator of Ty retrotransposition, pGTy plasmids containing the Ty1-H3mHIS3AI or Ty1-H3HIS3mAI elements were introduced into yeast strain GRF167 and assayed for transposition (Table 1). The transformants were phenotypically His⁻, indicating that the *HIS3*AI gene cannot confer a His⁺ phenotype in the absence of Ty1-H3 transcription. Cells were grown in liquid medium containing galactose and then plated onto SC-his glucose medium to end transposition induction and select for His⁺ colonies. After transposition induction of pGTy1-H3mHIS3AI in isogenic *SPT3* (Table 1) or *spt3* strains (data not shown), almost 2% of the cells became His⁺. Transcription of the Ty1-H3mHIS3AI element from the *GAL1* promoter is required for this high frequency of His⁺ reversion. When cells are grown on glucose, which represses pGTy transcription, His⁺ colonies appear at a frequency more than five orders of magnitude lower. The frequency of histidine prototrophs observed upon transposition induction of cells containing pGTy1-H3HIS3mAI is also more than five orders of magnitude lower than strains harboring pGTy1-H3mHIS3AI (Table 1). Therefore, splicing is required for the generation of histidine prototrophs. Thus these data show that the pGTy1-H3mHIS3AI element yields a high level of retrotransposition events in which the *HIS3* gene is recreated by splicing.

To confirm that His⁺ revertants recovered after induction of pGTy1-H3mHIS3AI contained spliced TymHIS3 transpositions, 30 His⁺ colonies were analyzed by Southern blot hybridization (data not shown). All 30 colonies contained at least one integrated TymHIS3 element with bands predicted to be present only in precisely spliced TymHIS3 elements.

Splicing Is Not Required for Ty1-H3mHIS3AI Transposition but Is Required for Generation of a His⁺ Phenotype. To determine whether the intron was ever retained during retrotransposition, 24 Ura⁺ colonies were selected after transposition induction of pGTy1-H3mHIS3AI, and 5 were His⁺. After plasmid segregation, His⁺ and His⁻ colonies were analyzed by Southern blot analysis with a *HIS3* probe (Table 2). Spliced elements were identified as those that recreated the *HIS3* *Msc* I site and lacked the intronic *Xba* I site in a *Pvu* II/*Msc* I or *Pvu* II/*Xba* I digest (Fig. 1). Elements lacking the *HIS3* *Msc* I site but retaining the intron *Xba* I site were scored as unspliced. Twenty of the 24 colonies analyzed contained genomic copies of the *HIS3*-marked element. Eighteen of these harbored between one and six copies of the unspliced TymHIS3AI element. The 5 His⁺ colonies each contained one copy of the spliced TymHIS3 element. A mean number of 1.7 marked transposition events per colony was found, and

Table 1. Transposition induction of pGTy1-H3 marked with the *HIS3*AI gene

Ty marker	Carbon source	No. His ⁺ colonies/culture	Mean transposition efficiency
mHIS3AI	Gal	549, 604, 577	1.7×10^{-2}
mHIS3AI	Glc	0, 1, 1	2.5×10^{-8}
HIS3mAI	Gal	1, 0, 1	3.2×10^{-8}

Each measurement represents the results from one of three cultures. The total number of colony-forming units was similar within each set of cultures; the average titers are (top to bottom): 3.4×10^4 , 2.7×10^7 , and 2.1×10^7 . The mean transposition efficiency is the mean fraction of total colonies assayed that are His⁺.

Table 2. Mean number of marked transposition events per induced cell

Plasmid	No. spliced elements per genome	No. unspliced elements per genome	No. total elements per genome
pGTy1-H3mHIS3AI	0.2 (5/24)	1.5 (35/24)	1.7 (40/24)
pGTy1-H3HIS3mAI	0 (0/16)	2.5 (40/16)	2.5 (40/16)
pGTy1-H3mHIS3	N/A	N/A	1.3 (26/20)

Plasmids were analyzed in yeast strain GRF167. The values given are the mean number of bands representing Ty-flanking sequence junction fragments hybridizing to a HIS3 probe on the appropriate Southern blot of transposition-induced colonies. The numbers in parentheses are the total number of junction fragment bands divided by the number of randomly selected transposition-induced colonies analyzed. N/A, not applicable.

5 of 40 (12.5%) transposed Ty elements had lost the intron. Transposition induction of cells containing pGTy1-H3-HIS3mAI resulted in 2.5 marked transpositions per isolate. All of these colonies were phenotypically His⁻, and all of the transposed TyHIS3mAI elements remained unspliced. The transposition frequencies of the Ty1-H3 derivatives marked with HIS3AI gene in both orientations were similar to the activity of the intronless pGTy1-H3mHIS3 element (Table 2) and to other marked pGTy1-H3 derivatives (12, 13). These data indicate that the intron in either orientation does not inhibit transposition, but splicing is required for histidine prototrophy.

Transposition of Chromosomal TymHIS3AI Elements Can Be Detected Phenotypically. The above analysis allowed us to determine if transposition of individual chromosomal elements could be detected. When several strains harboring unspliced TymHIS3AI elements but lacking the pGTy1-H3mHIS3AI plasmid were grown at 20°C and then replica plated to SC-his medium at 30°C, His⁺ papillae appeared. In contrast, histidine prototrophs were not detected in strains harboring chromosomal TyHIS3mAI elements. We found that the frequency of His⁺ reversion in several strains containing unspliced TymHIS3AI elements decreased more than 20-fold if the cells were grown at 30°C or 36°C relative to 20°C and that His⁺ reversion was reduced at least 100 times in isogenic *spt3* derivatives of these strains (data not shown). The dependence of His⁺ reversion on known modulators of Ty1 transposition and on intron orientation strongly suggests that we are detecting retrotransposition of genomic TymHIS3AI elements.

To extend these observations, strain JC242, a His⁻ strain harboring one unspliced TymHIS3AI element, was used to generate eight His⁺ revertants. DNA from these strains was digested with *Pvu* II and *Msc* I and analyzed by Southern blot hybridization using a HIS3 probe (Fig. 2). The parental strain contains one 3.3-kilobase (kb) band because of the absence of an *Msc* I site in the unspliced HIS3AI gene. In the His⁺ revertants, this 3.3-kb band appears unaltered, suggesting that the His⁺ phenotype does not result from rearrangement of the HIS3AI gene within the original marked Ty1 element. However, the His⁺ colonies contain two new bands indicative of replicative transposition. In every case, a 2.1-kb band expected of an internal fragment from the *Pvu* II site in Ty1 to the *Msc* I site of the HIS3 gene is seen. The *Msc* I site within the HIS3 gene demonstrates that precise splicing has occurred in the process of TymHIS3 retrotransposition. Each His⁺ revertant also contains one other band of varied size. These bands represent junction fragments extending from the *Msc* I site in HIS3 to the next *Pvu* II or *Msc* I site in flanking sequences and are also suggestive of *de novo* transposition.

Rate of Transposition of Single Genomic Ty Elements. The His⁺ reversion rate was calculated by the method of Lea and Coulson (20) for four strains containing either one, two, or five

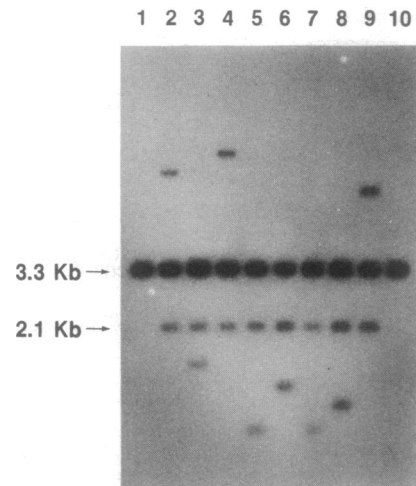


FIG. 2. Spontaneous His⁺ revertants of a strain harboring the chromosomal TymHIS3AI element contain dispersed copies of TymHIS3 with the intron precisely removed. Parental strain JC242 (lanes 1 and 10) and eight His⁺ revertants derived from strain JC242 (lanes 2-9) are shown. DNA from each revertant strain was digested with *Pvu* II and *Msc* I and probed with HIS3. The 3.3-kb band is the junction fragment between the 3' end of the TymHIS3AI element and flanking sequence DNA. The 2.1-kb band is an internal fragment of TymHIS3 from the *Pvu* II site in Ty1 to the *Msc* I site in the spliced HIS3 gene. The dispersed bands are junction fragments between the HIS3 *Msc* I site and a site in 3' flanking sequence DNA.

to six genomic copies of the TymHIS3AI elements (Table 3). The rate of His⁺ reversion in these strains varies between 3.4×10^{-9} and 1.6×10^{-7} per generation per TymHIS3AI element present in the genome. To estimate the average rate of Ty transposition, the His⁺ reversion rates were converted to transposition rates by accounting for the splicing efficiency and the effects of the marker gene on transposition. Our estimate is based on the assumption that genomic TymHIS3AI transcripts are spliced at the same frequency as those from the pGTy plasmid (Table 2). Therefore, about one-eighth of the transposition events are detected as histidine prototrophs. To determine if the presence of the HIS3AI gene decreased the level of Ty1 transposition, we compared the abilities of the unmarked pGTy1-H3 and the pGTy1-H3mHIS3AI elements to transpose in an *spt3* mutant DG789 (Table 4). These results suggest that the marker gene lowers the level of Ty transposition by a factor of 11. Therefore, the average His⁺ reversion rate was multiplied by a factor of 88 to obtain an estimated transposition rate of 3×10^{-7} and 1×10^{-5} transposition events per Ty1 element per generation.

To determine whether the 50-fold variation in transposition rates might result from differences in transcript levels of individual TymHIS3AI elements, we performed Northern blot hybridizations (Fig. 3) with total RNA from low- and

Table 3. Rate of His⁺ reversion in yeast strains containing genomic TymHIS3AI elements

Strain	No. of TymHIS3AI elements	Rate of His ⁺ reversion ($\times 10^{-7}$)	Mean rate per TymHIS3AI element ($\times 10^{-7}$)
JC234	1	0.034 \pm 0.029	0.034
JC242	1	1.6 \pm 0.5	1.6
JC246	2	1.4 \pm 0.2	0.7
JC271	5-6	9.1 \pm 3.2	1.5-1.8

Ura⁻ His⁻ colonies isolated after transposition induction of plasmid pGTy1-H3mHIS3AI in strain GRF167. The number of TymHIS3AI elements was determined by Southern blot analysis. The rate of His⁺ reversion is expressed as mutations per cell per generation (mean \pm 95% confidence interval).

Table 4. Relative transposition levels of pGTy1-H3 and pGTy1-H3mHIS3AI in *spt3* mutant DG789

Plasmid	No. of new bands hybridizing to a TyI probe	No. of colonies tested	Mean no. of transposition events
pGTy1-H3	40	16	2.5
pGTy1-H3mHIS3AI	3	13	0.23

high-reverting strains JC234 (lane 1) and JC242 (lane 2). The results show that although the amount of total Ty RNA is similar, the level of TymHIS3AI RNA is much lower in strain JC234 than JC242.

Single-Step Assay for Retrotransposition. Another application of the *HIS3AI* reporter gene is its use in a single-step test for chemical agents or conditions that effect retrotransposition. A single-step test was developed by replica plating cells containing pGTy1-H3mHIS3AI onto SC-ura galactose plates that contain limiting amounts of histidine (Fig. 4). These plates select for maintenance of the plasmid and induce transcription of the marked element. However, growth of the cells beyond a few generations cannot occur unless they become His⁺ by transposition of the spliced TymHIS3 element. Induction of pGTy1-H3mHIS3AI transcription in limiting histidine medium results in a large number of histidine prototrophs. As expected, repressing pGTy1-H3mHIS3AI transcription by plating on glucose blocks growth because there is no transposition of the marked element.

To determine whether the single-step retrotransposition test could be used to demonstrate the inhibition of transposition, induction of pGTy1-H3mHIS3AI was compared at semipermissive (30°C) or nonpermissive (37°C) temperatures (ref. 8; D.J.G., unpublished results). As shown in Fig. 3, no His⁺ revertants resulted from galactose induction of the pGTy1-H3mHIS3 element at 37°C. This is not due to temperature-dependent expression of the *HIS3* gene, since a strain containing a transposed copy of TymHIS3 grew well at 37°C.

DISCUSSION

We have reported a retrotransposition-detection system that allows for direct selection of TyI transposition events in yeast. This assay has several advantages over other transposition-detection systems used (2, 12, 13). Since the *HIS3AI*

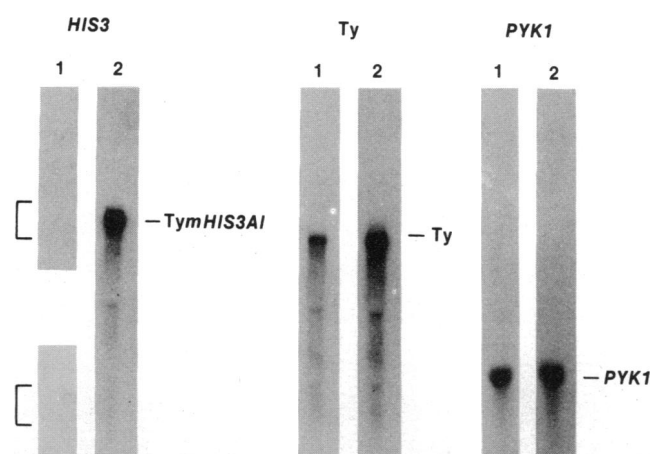


FIG. 3. Northern blot analysis of two strains each containing a single genomic TymHIS3AI element. A Northern blot containing 5 μ g of total RNA from strains JC234 (lane 1) and JC242 (lane 2) was sequentially hybridized with *HIS3*, *TyI*, and *PYK1* probes. A longer exposure of the bracketed region of lane 1, required to detect the TymHIS3AI transcript in strain JC234, is shown below lane 1.

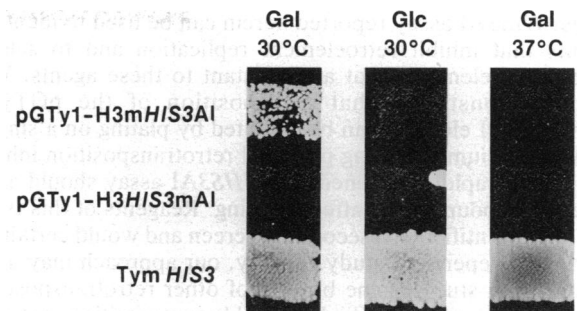


FIG. 4. Single-step assay for transposition of TyI marked with the retrotransposition reporter gene. Strains containing pGTy1-H3 marked with *HIS3AI* in the spliceable (pGTy1-H3mHIS3AI) or unspliceable (pGTy1-H3HIS3mAI) orientation or a chromosomal copy of TymHIS3 were replica plated onto SC-ura galactose (Gal) or glucose (Glc) plates containing limiting amounts of histidine (0.3 μ M) and grown at 30°C or 37°C.

reporter gene is only phenotypically activated in retrotransposed copies of the marked element, transposition can be scored in the presence of the original marked element. Moreover, selection for the transposition of chromosomal elements can be performed in the absence of insertions into specific targets. This feature should be extremely useful in identifying genes that modulate Ty transposition. Another advantage of the *HIS3AI* reporter gene is that splicing of the reverse transcript results in the creation of an *Msc I* site, allowing presence of the exact splice junction to be confirmed at the nucleotide level in a Southern blot. Finally, other rearrangements that phenotypically activate *HIS3AI* occur at a very low frequency. Recently, an indicator gene containing an antisense intron was used to mark a defective retrovirus (21). In contrast to our system, the intron contained a polyadenylation signal and was placed in untranslated sequences between the thymidine kinase promoter and neomycin-resistance gene coding sequences. This presumably created other mechanisms for gene activation. In fact, only two of the 37 G418-resistant events analyzed had a correctly generated splice junction. In comparison, all 43 His⁺ transpositions derived from pGTy or chromosomal TymHIS3AI elements have regenerated an *Msc I* site, which is indicative of correct splicing.

Our results suggest that all spliced TymHIS3 transpositions confer histidine prototrophy. This feature has allowed us to estimate the transposition rate of individual genomic TyI elements to be between 3×10^{-7} and 1×10^{-5} . Based on the rate of TyI insertions into a specific target locus, Boeke (1) has estimated that the rate of TyI transposition could be as low as 10^{-5} . However, our measurement of spontaneous TyI transposition is more direct and does not require making the untestable assumption that a particular target sequence is representative of the genome. Our estimate is based on the assumption that genomic TymHIS3AI transcripts are spliced at the same frequency as plasmid-borne transcripts (Fig. 2). Preliminary results using nuclease protection assays support this proposal (data not shown). It is also assumed that addition of the marker gene to a genomic TyI element inhibits transposition to the same degree as that of a marked pGTy element.

Three strains containing different TymHIS3AI insertions have comparable rates of transposition per marked Ty element, but the fourth strain tested has a significantly lower rate of His⁺ reversion. A likely explanation for this low rate of transposition is the comparatively low level of marked Ty transcript in strain JC234 (Fig. 4). The mechanism underlying different levels of TyI and Ty2 transcription remains to be determined (4), but it may involve either particular Ty sequences or the site of TyI insertion.

Our *HIS3AI* assay reported herein can be used to identify agents that inhibit retroelement replication and to select mutant Ty elements that are resistant to these agents. We have demonstrated that transposition of the pGTy1-H3mHIS3AI element can be detected by plating on a single growth medium, allowing potential retrotransposition inhibitors to be rapidly screened. The *HIS3AI* assay should also detect compounds that affect splicing. Reagents of this type could be identified in a secondary screen and would certainly warrant independent study. Finally, our approach may also be useful in studying the biology of other retrotransposons and retroelements for which inducible transposition systems have not been developed.

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