MOLECULAR AND CELLULAR BIOLOGY, Sept. 1988, p. 3571–3581
0270-7306/88/093571-11$02.00/0

Vol. 8, No. 9

Transpositional Competence and Transcription of Endogenous Ty Elements in *Saccharomyces cerevisiae*: Implications for Regulation of Transposition

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Received 21 March 1988/Accepted 7 June 1988

Transposition of Ty elements in the yeast *Saccharomyces cerevisiae* occurs through an RNA intermediate. Although Ty RNA accounts for 5 to 10% of the total polyadenylated RNA in a haploid cell, the transposition frequency is only 10^{-7} to 10^{-8} per gene. To determine whether Ty elements native to the yeast genome are transpositionally competent, two elements were fused to the *GAL1* promoter and tested for their ability to transpose. These native elements, Ty1-588 and Ty2-117, transposed at high levels when the *GAL1* promoter was induced. Three Ty's identified as spontaneous transpositions in specific target genes were also tested. Of these three, Ty2-917 and the previously characterized element Ty1-H3 were shown to be transpositionally competent. The third element, Ty1-H1, was transposition defective. In addition, we marked the chromosomal copy of Ty1-588 with the *NEO* gene and demonstrated that Ty1-588*NEO* was actively transcribed in yeast cells. Ty1-588*NEO* transcription was regulated by the *SPT3* and *MAT* loci in the same manner as that observed for Ty's collectively. These results indicate that the yeast genome contains functional Ty elements. The presence of a transpositionally competent, actively transcribed element suggests that regulation of Ty transposition occurs at a posttranscriptional level.

Three classes of retrotransposons have been identified in the yeast *Saccharomyces cerevisiae*, called Ty1, Ty2, and Ty3. Ty1 and Ty2 elements are highly related, although they contain two distinct regions of sequence divergence (24). These elements consist of 334- to 338-base-pair (bp) long terminal repeats, or delta (δ) elements, surrounding a 5.3-kilobase (kb) internal region called epsilon (ε). Ty3 is a recently identified element consisting of direct repeats, called sigma elements, flanking a 4.7-kb internal region (9). Numerous structural similarities exist between Ty elements and eucaryotic retroviruses. Ty sequence analysis suggests that there are regions homologous to retroviral *gag* and *pol* genes (8, 21, 40, 43). A functional relationship between Ty elements and retroviruses was demonstrated in experiments that showed that transposition of Ty elements occurred through an RNA intermediate (2).

There are approximately 35 Ty1 and Ty2 elements per haploid genome. RNA from these elements is very abundant, accounting for an estimated 5 to 10% of total polyadenylated RNA in the haploid cell (11). Ty transposition, on the other hand, occurs at a very low frequency, approximately 10^{-7} to 10^{-8} per gene per generation (4, 18, 30, 36). To date, only one transpositionally competent element has been described (2). This element, Ty1-H3, was originally isolated as a spontaneous transposition into the *his3Δ4* gene. Fusion of Ty1-H3 to the *GAL1* promoter carried on a 2μm-based plasmid induces a 20- to 100-fold increase in the frequency of Ty transposition (2). Induction of transposition is accompanied by a large increase in cytoplasmic viruslike particles containing reverse transcriptase activity (16).

Several mutant elements have been described that do not induce high levels of transposition when expressed from the *GAL1* promoter (1, 48). Boeke et al. (1) characterized a missense mutation, designated *tyb-2098*, in the consensus integrase region of Ty1-173, an element isolated as an insertional mutation in the *LYS2* gene (38). It appears that the *tyb-2098* mutation is carried in several Ty elements native to the yeast genome (1). Other mutations made in the consensus protease region of *TyB* abolish transposition of the *GAL1*-promoted element (48).

Total cellular Ty transcription is regulated by several host genes, including *MAT*, *SPT*, *ROC*, and *STE* genes (7, 10, 11, 45). Transcriptional regulation by *MAT* and the *SPT3* gene have been characterized in detail. These studies have shown that there is a 20-fold decrease in total Ty RNA in a/α cells compared with a or α cells (11). In *spt3* mutants, full-length Ty transcripts are abolished, and the only Ty transcripts present are initiated 800 bp downstream of the wild-type start site (47). Transposition of chromosomal Ty elements is also absent in *spt3* mutants. However, transcription of Ty1-H3 that is induced by the *GAL1* promoter results in high levels of transposition in *spt3-101* mutants (4).

A phenotypic assay for transposition of Ty elements fused to the *GAL1* promoter has recently been described (5). This method involves marking the element at the extreme 3′ end of the ε region, outside the coding sequences, with a selectable gene such as the bacterial neomycin phosphotransferase gene (*NEO*). Ty1-H3*NEO* transposes at a high frequency when it is expressed from the *GAL1* promoter. In this paper, we used this assay to determine whether several Ty1 and Ty2 elements are competent to induce high levels of transposition. Each element was fused to the *GAL1* promoter carried on a 2μm-based plasmid and marked with the *NEO* gene. If the element tested is competent for transposition, galactose induction of the pGTy*NEO* plasmid will result in transposition of the *NEO*-marked element into the yeast genome at a high efficiency. These experiments reveal that several Ty1 and Ty2 elements encode functional gene products and *cis*-acting sequences required for transposi-

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tion. Two of the transposition-competent elements, Ty1-588 and Ty2-117, are native to the yeast genome. We have demonstrated that the endogenous Ty1-588 is actively transcribed.

MATERIALS AND METHODS

Plasmids. The pGTy*NEO* plasmids (Fig. 1A) were constructed by insertion of a restriction fragment containing Ty sequences from the 5′ δ *Xho*I site to a suitable cloning site downstream of the 3′ δ sequence into a p*GAL1-Xho*I vector. The vector used was the 2μm-based plasmid pCGE329 (2) (provided by J. Schaum and J. Mao, Collaborative Research) or pGAL-X/P, except in the case of pGTy1-588(G)*NEO* (see below). pGAL-X/P is a modified version of pCGE329 with a *Sac*I-*Pvu*I-*Eco*RI-*Bam*HI polylinker inserted 3′ to the *Xho*I site. The insert of each pGTy*NEO* plasmid is indicated in Fig. 1B. The *NEO* gene, contained on a *Bam*HI fragment from pGH54 (23), was cloned into each Ty at the *Bgl*II site at a map position of approximately 5600. This *Bgl*II site is at the extreme end of the ε region, 3′ to *TyB* coding sequences, and has been shown to be a permissive site for insertion of foreign sequences and genes (2, 5; D. Garfinkel, M. Mastrangelo, N. Sanders, B. Shafer, and J. Strathern, submitted for publication). In all cases, the transcriptional orientation of the *NEO* gene relative to the Ty was the same. Construction of pGTy1-H3 (2) and the *NEO*-marked derivative pGTy1-H3*NEO* (5) has been described previously. The plasmid pGTy1-H3*NEO*(3301) contains an insertion of the *NEO* gene at the *Bgl*II site at map position 3301 of Ty1-H3 (5).

The plasmid pGTy1-H1*NEO* is derived from pH1, an insertion of Ty1-H1 into the *his3Δ4* gene in plasmid pNN162 (J. Boeke, D. Garfinkel, and G. Fink, unpublished results). A 6.0-kb fragment from the *Xho*I site within the 5′ δ to the *Bam*HI site 100 bp downstream of the 3′ δ (Fig. 1B) was cloned as contiguous *Xho*I-*Kpn*I and *Kpn*I-*Bam*HI fragments into pCGE329.

The Ty1-588(S) element analyzed here is derived from the yeast genomic clone contained on pNF4000 and obtained from E. Friedberg (31). The plasmid pNF4000 is a member of the genomic library made from strain DBY939, which is isogenic with the wild-type strain S288C (6). The plasmid pGTy1-588(S)*NEO* contains the fragment extending from the *Xho*I site in the 5′ δ of Ty1-588(S) to the *Bam*HI site downstream of Ty1-588(S) in unique-sequence yeast genomic DNA (Fig. 1B). It was cloned as contiguous *Xho*I-*Kpn*I and *Kpn*I-*Bam*HI fragments, using a unique *Kpn*I site within the Ty1-588(S) element, into pGAL-X/P.

The element Ty2-917 is derived from the *his4-917* mutation, which resulted from transposition of the element to a site 72 bp upstream of the *HIS4* transcription start site. The transcriptional orientation of Ty2-917 is opposite to that of the *HIS4* gene (15, 33). The insert of pGTy2-917*NEO* extends from the *Xho*I site in the 5′ δ to the *Bgl*II site in the *HIS4* promoter region (Fig. 1B). It was cloned into pGAL-X/P as contiguous *Xho*I and *Xho*I-*Bgl*II fragments.

Ty2-117 is a genomic element found just centromere-distal to the *LEU2* gene on chromosome III (24). The element is derived from plasmid R338, which is a genomic subclone of Ty2-117 contained on an *Eco*RI fragment. R338 was obtained from G. Fink and G. S. Roeder. Plasmid pGTy2-117*NEO* was constructed by cloning contiguous *Xho*I-*Sal*I and *Sal*I-*Eco*RI fragments from the 5′ δ *Xho*I site to the *Eco*RI site in the *LEU2* gene into the vector pGAL-X/P (Fig. 1B).

The integrating vector YIp331-588 was constructed by insertion of the 0.5-kb *Pst*I-*Hin*dIII fragment flanking the genomic Ty1-588(S) element at the 3′ end into the yeast integrating vector YIp331. This vector, a derivative of YIp5 containing the polylinker of pUC18, was obtained from R. Gaber. A second integrating vector, YIp331-588*NEO*, was made by insertion of the 3.9-kb *Eco*RI-*Hin*dIII fragment of pGTy1-588(S)*NEO* cloned into YIp331. The insert of YIp331-588*NEO* extends from the 3′ *Eco*RI site (at a Ty1 map position of approximately 3920) of Ty1-588(S)*NEO* to the *Hin*dIII site downstream of the element (Fig. 1B).

The element Ty1-588(G) was obtained by integration and eviction (46) from strain GRF167, as described in detail later. To construct pGTy1-588(G)*NEO*, the evicted element was subcloned into the p*GAL1* vector from the *Xho*I site within its 5′ δ to a *Hin*dIII site outside of the element on the 3′ side. This insert was cloned as contiguous *Xho*I-*Kpn*I and *Kpn*I-*Hin*dIII fragments by using a unique *Kpn*I site within the Ty1-588(G) element. The vector used, pCGS42, is a derivative of pBR322 containing the *URA3* gene and the yeast 2μm origin of replication. Plasmid pCGS42 was obtained from G. Fink. The 7-kb *Eco*RI-*Hin*dIII fragment of pCGS42 was ligated to the 0.8-kb *Eco*RI-*Xho*I fragment of pCGE329 containing the *GAL1* promoter and the *Xho*I-*Kpn*I and *Kpn*I-*Hin*dIII fragments of Ty1-588(G).

Yeast strains and media. The *S. cerevisiae* strains used are listed in Table 1. Genotypes listed in brackets indicate integrated sequences, while those listed in parentheses refer to 2μm-based plasmids. Strain YH8 is an isogenic *trp1Δ1 leu2Δ* derivative of GRF167 obtained from H. Xu and J. Boeke. Strain DG531 is isogenic to strain BWG1-7A except that it carries *spt3-101* (48). Strain DG791 is an isogenic *spt3-101* derivative of strain DG733, constructed as described previously for strain DG531 (48).

The *MAT***a** *cry1* strain GRY340, kindly provided by J. Strathern, was used to obtain mitotic segregants that had become homozygous at the *MAT* locus (20). The *MAT***a**/α diploid JC46 was obtained by mating strains GRY340 and DG733. JC46 was placed onto YPD medium containing cryptopleurine (0.5 μg/ml). Cry^r colonies were then tested for homozygosis to *MAT***a**/**a** by mating tests with *MAT***a** and α tester strains 17/14 and 17/17, respectively.

Standard yeast genetic procedures of crossing, sporulation, and tetrad analysis were followed (37). All tetrads analyzed contained four viable spores. Yeast media were prepared as described by Sherman et al. (37).

Transposition assay. To determine the efficiency of transposition induced by an element expressed from a pGTy*NEO* plasmid, we used the assay developed by Boeke et al. (5). Strain BWG1-7A or the isogenic *spt3-101* derivative DG531 was transformed with the appropriate pGTy*NEO* plasmid by the procedure of Ito et al. (22), and Ura$^+$ transformants were selected. To induce transcription from the *GAL1* promoter, transformants were grown on synthetic complete medium minus uracil (SC-ura) containing 2% galactose at 20°C. After 5 days, independent colonies were then restreaked for single colonies on SC-ura–2% glucose to abolish transcription from the *GAL1* promoter and to select cells that retained the pGTy*NEO* plasmid throughout galactose induction. Colonies were then grown on nonselective YPD plates to allow segregation of the plasmid. Ura$^-$ segregants were identified by replica plating to SC-ura plates or to medium containing 5-fluoro-orotic acid (3). Ura$^-$ segregants that sustained a transposition of the *NEO*-marked Ty were identified by replica plating to YPD plates containing 200 μg of G418 (a neomycin analog; Gibco Laboratories) per ml. The transpo-

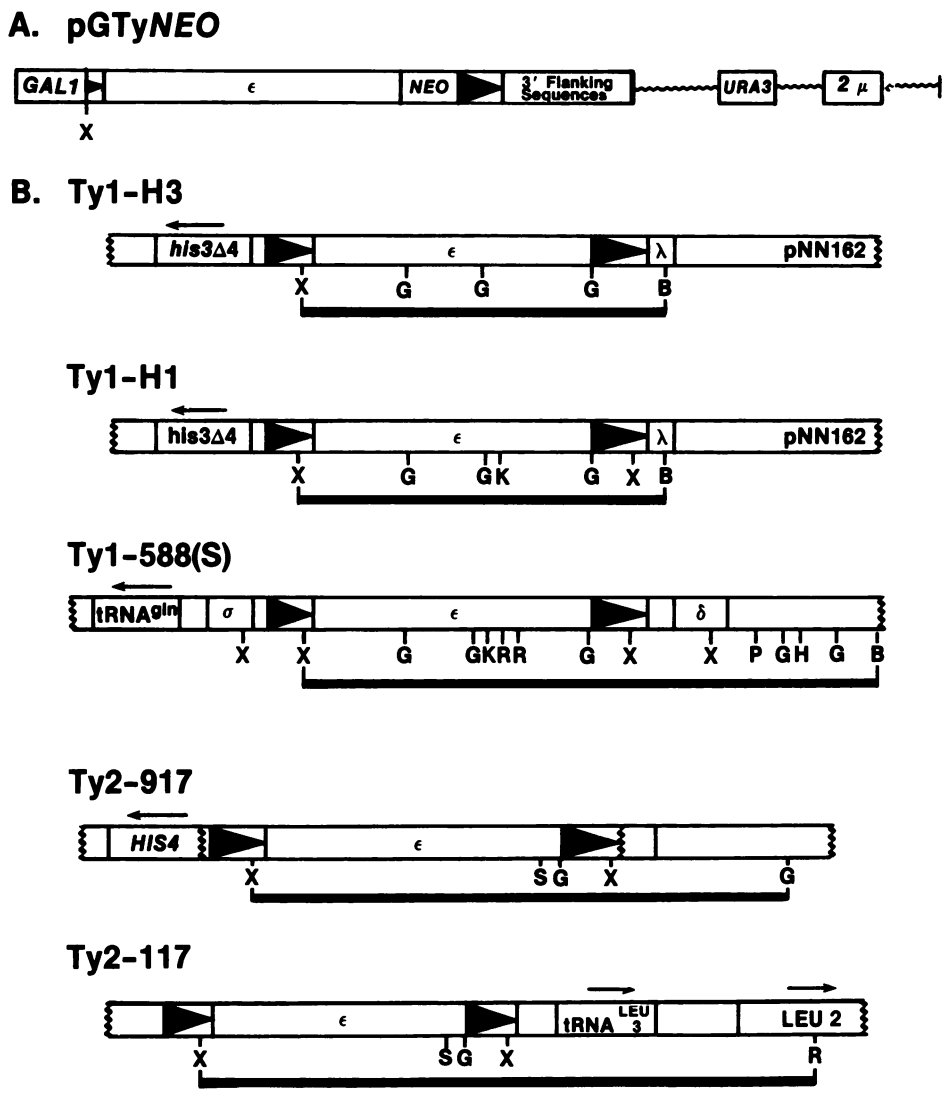

FIG. 1. Construction of pGTy*NEO* plasmids. (A) Diagram showing the general structure of a pGTy*NEO* plasmid. The boxed segment labeled *GAL1* represents the *GAL1* promoter element. A Ty is represented as two arrowheads flanking a boxed segment labeled epsilon (ε). The arrowheads represent δ elements and indicate the direction of transcription. The restriction site *Xho*I is abbreviated X and indicates the location of the *GAL1* promoter fusion to the Ty element within its 5′ δ. The boxed segment labeled *NEO* represents the neomycin phosphotransferase gene derived from pGH54 (23). The *NEO* gene, contained on a *Bam*HI fragment, was cloned into the conserved *Bgl*II site found at a map position of approximately 5600 in each element. The boxed segment labeled 3′-flanking sequences indicates the genomic or plasmid sequences flanking the Ty element in the DNA from which it is derived. Each element is cloned into a p*GAL1-Xho*I vector from its 5′ δ *Xho*I site to a convenient restriction enzyme site found within the 3′-flanking sequences. The wavy line represents pBR322 sequences, and the boxed segments labeled *URA3* and 2μ represent the yeast *URA3* and the origin-containing segment of the yeast 2μm plasmid, respectively. (B) Restriction maps of various Ty elements and flanking plasmid or genomic sequences into which they are inserted. The sequences of each Ty element and 3′-flanking sequences that were cloned into the p*GAL1-Xho*I vector are indicated by the dark bar below each map. Restriction sites are abbreviated as follows: X, *Xho*I; G, *Bgl*II; B, *Bam*HI; K, *Kpn*I; R, *Eco*RI; P, *Pst*I; H, *Hin*dIII; S, *Sal*I. The direction of transcription of flanking yeast genes is indicated by arrows above the boxed segments, and the gene designation (when known) is located within the box. pNN162 is a *CEN* plasmid with bacteriophage lambda sequences (λ) upstream of the promoterless *his3Δ4* gene (36) into which Ty1-H3 and Ty1-H1 are inserted. The box labeled δ is a solo δ element. The restriction maps are not drawn to scale. See Materials and Methods for a detailed description of the derivation of each element.

sition efficiency is defined as the fraction of independent Ura$^-$ segregants analyzed that became G418^r.

Eviction and transplacement of Ty1-588. To analyze the endogenous Ty1-588, we first confirmed the presence of Ty1-588 in strains BWG1-7A and GRF167 by cloning the element and then marked the element in the genome with *NEO* by transplacement (46). To initiate these constructions, the 0.5-kb *Pst*I-*Hin*dIII restriction fragment at the 3′ end of Ty1-588(S) in the genomic clone derived from pNF4000 was used as a probe in Southern blot analysis of genomic DNA from strains BWG1-7A and GRF167. Southern hybridization demonstrated that this fragment flanking Ty1-588 was single-copy DNA (data not shown). Next, the *Pst*I-*Hin*dIII fragment was cloned into a yeast integrating vector, and the resulting plasmid, called YIp331-588 (described in Plasmids section), was linearized at the unique *Bgl*II site within the insert. *Bgl*II-digested YIp331-588 was used to transform strain BWG1-7A and a derivative of strain GRF167 known as

TABLE 1. Yeast strains

Strain	Genotype	Reference or source
BWG1-7A	*MAT***a** *ade1-100 ura3-52 leu2-3,112 his4-519 GAL*$^+$	19
DG531	*MAT***a** *ade1-100 ura3-52 leu2-3,112 his4-519 spt3-101 GAL*$^+$	48
DG397	BWG1-7A(pGTy1-H3*NEO*)	
DG496	BWG1-7A(pGTy1-588(S)*NEO*)	
DG485	BWG1-7A(pGTy1-H1*NEO*)	
JC23	BWG1-7A(pGTy2-117*NEO*)	
DG481	BWG1-7A(pGTy2-917*NEO*)	
JC83	BWG1-7A(pGTy1-588(G)*NEO*)	
DG692	DG531(pGTy1-H3*NEO*)	48
JC17	DG531(pGTy1-588(S)*NEO*)	
JC22	DG531(pGTy1-H1*NEO*)	
JC16	DG531(pGTy2-117*NEO*)	
JC19	DG531(pGTy2-917*NEO*)	
GRF167	*MAT*α *his3*Δ*200 ura3-167 GAL*$^+$	2
YH8	*MAT*α *his3*Δ*200 ura3-167 trp1*Δ*1 leu2*Δ *GAL*$^+$	H. Xu and J. Boeke
DG803	GRF167(pGTy2-917*NEO*)	
DG704	*MAT***a** *ade1-100 ura3-52 leu2-3,112 his4-519 GAL*$^+$ Ty1-588{*URA3*}	YIp331-588 integrant
DG705	*MAT*α *his3*Δ*200 ura3-167 trp1*Δ*1 leu2*Δ *GAL*$^+$ Ty1-588{*URA3*}	YIp331-588 integrant
DG731	*MAT***a** *ade1-100 ura3-52 leu2-3,112 his4-519* Ty1-588*NEO GAL*$^+$	
DG733	*MAT*α *his3*Δ*200 ura3-167* Ty1-588*NEO GAL*$^+$	
DG791	MATα *his3*Δ*200 ura3-167 spt3-101* Ty1-588*NEO GAL*$^+$	
GRY340	*MAT***a**{*URA3*} *cry1 ade2 his3*Δ*200 ura3-52 trp1*Δ*1 leu2*Δ*1 lys2-8*Δ*1 tyr7-1 GAL*$^+$	J. Strathern
JC46	*MAT***a**{*URA3*}/*MAT*α *cry1*/+ *ade2*/+ *his3*Δ*200*/*his3*Δ*200 ura3-52*/*ura3-167 trp1*Δ*1*/+ *leu2*Δ*1*/+ *lys2-8*Δ*1*/+ *tyr7-1*/+ +/Ty1-588*NEO GAL*$^+$/*GAL*$^+$	DG733 × GRY340
JC53	*MAT***a**{*URA3*}/*MAT***a**{*URA3*} *cry1*/*cry1 ade2*/+ *his3*Δ*200*/*his3*Δ*200 ura3-52*/*ura3-167 trp1*Δ*1*/+ *leu2*Δ*1*/+ *lys2-8*Δ*1*/+ *tyr7-1*/+ +/Ty1-588*NEO GAL*$^+$/*GAL*$^+$	Cry^r derivative of JC46
L2370	*MAT*α *pet18 trp1*Δ*1 ura3-52*	I. Chiu and G. R. Fink

YH8. Ura$^+$ transformants were selected. To evict YIp331-588 and the adjacent Ty1-588 as a single restriction fragment, genomic DNA from transformants DG704 and DG705 was digested with *Xba*I, which cuts once in the vector sequences 3′ to the bacterial origin of replication and does not cut within the Ty1 sequences. *Xba*I-digested genomic DNA was ligated and used to transform *Escherichia coli* HB101 to ampicillin resistance. The *E. coli* transformants obtained yielded plasmid DNA with a restriction digestion pattern diagnostic of a Ty1 element, confirming the presence of Ty1-588 in both yeast strains BWG1-7A and GRF167.

The elements Ty1-588(B) (in strain BWG1-7A) and Ty1-588(G) (in strain GRF167) were marked with the *NEO* gene by transplacement of YIp331-588*NEO*, which contains the 3′ end of Ty1-588(S)*NEO* from the 3′ *Eco*RI site and 3′ flanking sequences. (See Fig. 2 for diagram of transplacement and Plasmids section for description of YIp331-588*NEO*.) To direct recombination, YIp331-588*NEO* was linearized at the unique *Bgl*II site within the 3′-flanking sequences (29) and used to transform strains BWG1-7A and GRF167 (22). Integration of YIp331-588*NEO* next to the genomic copy of Ty1-588 should result in a duplication of the 3′ end of Ty1-588 bracketing the *URA3* gene (Fig. 2C). We selected for cells that lost the plasmid sequences via recombination by plating Ura$^+$ G418^r transformants on medium containing 5-fluoro-orotic acid (3). Transplacement of the *NEO* gene in Ura$^-$ recombinants derived from strains GRF167 and BWG1-7A were scored on YPD plates containing G418 (100 and 200 μg/ml, respectively). Ura$^-$ G418^r colonies were subjected to Southern blot analyses with *NEO* sequences and the *Pst*I-*Hin*dIII fragment from the 3′-flanking sequences of Ty1-588(S) as probes (data not shown). This analysis confirmed the formation of a chromosomal Ty1-588(B)*NEO* locus in strain DG731 and a Ty1-588(G)*NEO* locus in strain DG733. When strains DG731 and DG733 were crossed to G418^s strains, segregation of G418^r and G418^s was 2:2 for all tetrads involving strain DG731 and for 30 of 31 tetrads involving strain DG733. These data demonstrate the transplacement of *NEO* at a single locus in each strain.

Southern blot analysis with oligonucleotide probes. Plasmid DNA samples were digested with *Bgl*II, subjected to gel electrophoresis in 1% agarose, and transferred to Hybond-N nylon membranes according to the manufacturer's instructions (Amersham). Prehybridization and hybridization of the filters were performed as described by Geliebter et al. (17). The oligonucleotides used as probes were kindly provided by J. Boeke (1). One is a 20-mer from the *tyb-2098* region of Ty1-173, called 173-2. A second oligomer probe, 173-4, contains wild-type *TyB* sequences from the same region of Ty1-H3. Oligomers were end labeled with [γ-^{32}P]ATP and polynucleotide kinase and then added directly to the hybridization mixture. The membranes were washed three times in 0.15 M NaCl–0.015 sodium citrate–0.1% sodium dodecyl sulfate at 51°C and then exposed to X-Omat AR film (Kodak) with an intensifying screen.

Northern (RNA) blot analysis. Isolation of total cellular RNA was performed essentially as described by Sherman et al. (37). RNA samples were denatured with glyoxal (39), separated by agarose gel electrophoresis, and transferred to Hybond-N nylon filters (Amersham). The following restriction fragments were used as hybridization probes: the *Bam*HI insert of pGH54 containing the *NEO* gene; the 750-bp *Pst*I fragment of Ty1-588 from the *TyA* region (which recognizes Ty1 but not Ty2 sequences; J. Curcio, S. Youngren and D. Garfinkel, unpublished results); and the yeast pyruvate kinase gene contained on a 6.7-kb *Hin*dIII fragment in plasmid pFR2 (kindly provided by A. Hinnebusch).

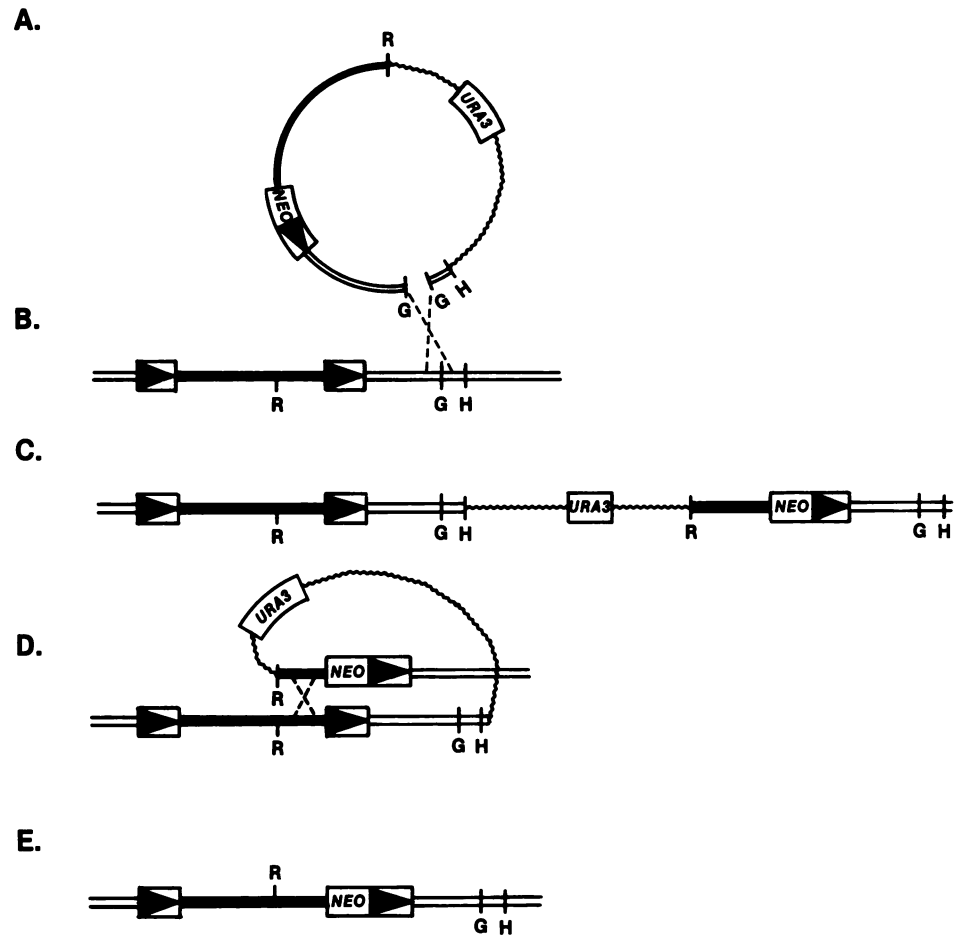

FIG. 2. Transplacement of Ty1-588*NEO*. (A) Integrating plasmid YIp331-588*NEO*, containing the 3′ end of Ty1-588(S)*NEO* from the *Eco*RI site (R) in ε to the *Hin*dIII (H) site in the 3′-flanking sequences. The plasmid is linearized at the *Bgl*II (G) site within unique-sequence DNA and used to transform yeast strains BWG1-7A and GRF167. (B) Genomic copy of Ty1-588. Linearized YIp331-588*NEO* will preferentially recombine with homologous sequences flanking Ty1-588 in the genome (29). (C) Structure of the Ty1-588 region after integration of YIp331-588*NEO*. (D) Loop out of the plasmid sequences can be accomplished by a recombination event between Ty1-588 and the 3′ end of Ty1-588*NEO*. If the event occurs between the *Eco*RI (R) site and the *NEO* gene, as diagrammed, a transplacement of the *NEO* gene will occur, as shown in panel E.

RESULTS

Transpositional competence of Ty1 and Ty2 elements. Several Ty elements were tested for their ability to induce high levels of transposition when expressed from the *GAL1* promoter. Two classes of Ty elements were studied: the first class, referred to as "native" elements, are defined as those chromosomal elements that have been identified by genomic cloning rather than by causing an insertional mutation. The elements Ty1-588, which was mapped in this paper, and Ty2-117, which resides adjacent to the *LEU2* structural gene, are representatives of this class. The second class of elements, designated "spontaneous transpositions," were identified as insertional mutations into selected target genes. This class includes Ty1-H3 and Ty1-H1, both of which were found to activate the promoterless *his3Δ4* gene by insertion (2). We have also studied Ty2-917, which inserted into the *HIS4* locus to create the *his4-917* mutation (33).

In order to investigate the transpositional competence of these elements, a pGTy*NEO* plasmid was constructed for each Ty studied (Fig. 1). Each element was fused to the *GAL1* promoter at the 5′ δ *Xho*I site so that the δ promoter sequences were replaced by the *GAL1* promoter. This *GAL1*-Ty fusion carried on a high-copy-number plasmid was marked at the conserved *Bgl*II site at the 3′ end of the Ty with the *NEO* gene. The *GAL1* promoter was then induced by growth of yeast strains containing the pGTy*NEO* plasmids on galactose, a procedure that is subsequently referred to as transposition induction. The transposition frequency of *NEO*-marked elements was determined by scoring transposition-induced colonies for growth on medium containing G418 (Table 2).

Previous results have demonstrated that galactose induction of pGTy1-H3*NEO* results in high levels of transposition (5). In this work, galactose induction of Ty1-H3*NEO* RNA rendered almost every induced cell resistant to G418 by virtue of a transposition of the *NEO*-marked element. Ty1-H3*NEO* transposition was dependent on growth on galactose and on placement of the *NEO* gene at the permissive *Bgl*II site outside of coding sequences. These data support the notion that the background level of recombination between Ty1-H3*NEO* and endogenous elements resulting in chromosomal integration of the *NEO* gene is negligible.

Nothing is known about the transpositional competence of native Ty elements. To determine whether a native Ty1

TABLE 2. Transpositional competence of various Ty elements

Plasmid[a]	Transposition efficiency (%)[b]
pGTy1-H3*NEO*	67/71 (94)
pGTy1-H3*NEO* (uninduced)[c]	0/10 (0)
pGTy1-H3*NEO*(3301)[d]	0/81 (0)
pGTy1-588(S)*NEO*	48/54 (89)
pGTy1-H1*NEO*	13/103 (14)
pGTy2-917*NEO*	40/40 (100)
pGTy2-117*NEO*	28/41 (68)

[a] Plasmids were introduced into strain BWG1-7A by the transformation procedure of Ito et al. (22). Transposition induction and subsequent analysis are described in Materials and Methods.

[b] Transposition efficiency is the number of G418^r Ura$^-$ segregants divided by the total number of Ura$^-$ plasmid segregants analyzed.

[c] In the uninduced control, the transformant of pGTy1-H3*NEO* was grown at 20°C for 5 days on SC-ura medium containing 2% glucose instead of galactose.

[d] In the plasmid pGTy1-H3*NEO*(3301), the *NEO* gene was cloned into the *Bgl*II at position 3301, interrupting *TyB* coding sequences, instead of the *Bgl*II site at position 5561.

element can induce high levels of transposition, the element Ty1-588 was tested (Table 2). This Ty was derived from yeast strain DBY939 (isogenic with the wild-type strain S288C [6]) and is called Ty1-588(S). Galactose induction of pGTy1-588(S)*NEO* resulted in marked transpositions in 89% of the colonies, equalling the activity of Ty1-H3*NEO*. This is the first demonstration that a native Ty element is transpositionally competent.

Furthermore, our data demonstrate that Ty2 elements were as competent as Ty1 elements in our assay. pGTy*NEO* plasmids were constructed from the spontaneous transposition Ty2-917 and the native element Ty2-117. Transposition induction resulted in transposition in 100 and 68% of the colonies analyzed, respectively.

Southern analysis was performed on 12 randomly selected colonies of strain BWG1-7A containing plasmid pGTy2-117*NEO* following transposition induction. The two G418^s colonies tested showed no hybridization to a radiolabeled *NEO* probe, while the 10 G418^r colonies contained between 1 and 4 copies of Ty2-117*NEO*. The average number of *NEO*-marked transposition events per cell was 1.7 (Table 3). This is slightly lower than the average 2.6 copies of Ty1-H3*NEO* observed by Boeke et al. (5) in the same strain background.

Southern analysis was also performed after transposition induction of the *SPT3* strain GRF167 containing the plasmid pGTy2-917*NEO*. The average number of copies of Ty2-917*NEO* seen in 46 transposition-induced colonies was 6.8 (Table 3). The 45 G418^r colonies tested contained between 2 and 15 copies of the *NEO*-marked element. The strain background of these colonies analyzed by Southern hybridization was different from that of the pGTy2-917*NEO* transformants analyzed in the transposition induction assay (Table 2) and that of other pGTy*NEO* transformants analyzed by Southern hybridization (Table 3). However, it is unlikely that the high frequency of Ty2-917*NEO* transposition reflects strain differences, because the average number of transpositions of Ty1-H3*NEO* in strains BWG1-7A and GRF167 was approximately equal (3.0 copies per colony in strain BWG1-7A and 2.0 copies per colony in strain GRF167). These results demonstrate that Ty2-917 is a highly competent element when it is transposition induced.

TABLE 3. Average number of transposition events resulting from transposition induction of various Ty elements

Plasmid	Relevant genotype	No. of *NEO*-marked copies[a]/no. of colonies analyzed	Copies/genome
pGTy2-117*NEO*	*SPT3*	20/12	1.7
pGTy2-917*NEO*	*SPT3*[b]	314/46	6.8
pGTy2-117*NEO*	*spt3-101*	21/12	1.8

[a] Determined by counting the number of bands hybridizing to a *NEO* probe on a Southern blot. Colonies analyzed were randomly selected.

[b] Transformant of *SPT3* strain GRF167.

TABLE 4. Transpositional competence of various Ty elements in an *spt3-101* strain

Plasmid[a]	Transposition efficiency (%)[b]
pGTy1-H3*NEO*	28/36 (78)
pGTy1-588(S)*NEO*	32/47 (68)
pGTy1-H1*NEO*	0/84 (0)
pGTy2-917*NEO*	45/45 (100)
pGTy2-117*NEO*	35/40 (88)

[a] Plasmids were transformed into strain DG531, an isogenic *spt3-101* derivative of strain BWG1-7A.

[b] See Table 2, footnote *b*.

The Ty1 spontaneous transposition Ty1-H1 was less active than any of the other Ty1 and Ty2 elements tested (Table 2). The transposition efficiency of pGTy1-H1*NEO* in the *SPT3* strain BWG1-7A was only 14%, which is five to seven times lower than the efficiency seen for other elements. Southern blot analysis performed on 18 G418^s pGTy1-H1*NEO*-induced colonies showed that none of these colonies contained copies of Ty1-H1*NEO*, demonstrating that the low transposition efficiency was not a result of cryptic Ty1-H1*NEO* transpositions that fail to express the *NEO* gene (data not shown).

Transposition in *spt3-101* strains. It is possible that transpositional recombination (4) or genetic complementation between a *GAL1*-promoted Ty and the ensemble of native elements accounts for the transpositional activity of the elements described above. To investigate these possibilities, the transpositional competence of the *GAL1*-promoted elements in an *spt3-101* mutant background was determined. In *spt3-101* mutants, only aberrant Ty transcripts are produced from endogenous elements, which probably accounts for the absence of transposition of chromosomal elements (4, 48). However, transposition induction of pGTy1-H3*NEO* in *spt3-101* mutants results in high levels of transposition of the *NEO*-marked element (4). In our analysis, Ty1-H3*NEO*, Ty1-588(S)*NEO*, Ty2-117*NEO*, and Ty2-917*NEO*, which were active in the *SPT3* strain BWG1-7A, transposed with efficiencies ranging from 68 to 100% when tested in the isogenic *spt3-101* strain DG531 (Table 4). These transposition efficiencies are equivalent to the levels of activity seen in the wild-type strain.

The number of Ty2-117*NEO* transpositions in 12 randomly selected colonies following transposition induction in an *spt3-101* mutant background was determined by Southern hybridization with a radiolabeled *NEO* probe. Two G418^s colonies contained no copies of Ty2-117*NEO*, while 10 G418^r colonies contained between 1 and 5 copies of Ty2-117*NEO*. The average number of Ty2-117*NEO* transpositions was the same in both the *SPT3* and *spt3-101* strains (Table 3). These data demonstrate that native Ty1 and Ty2 elements, as well as spontaneous Ty1 and Ty2 transpositions, contain all the functions necessary for efficient transposition in the absence of complementation by endogenous Ty elements.

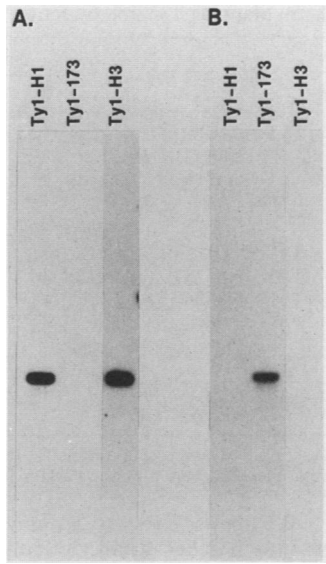

FIG. 3. Defective element Ty1-H1 does not have the *tyb-2098* mutation. Plasmids containing Ty1-H1 (pGTy1-H1*NEO*), Ty1-173 (pGS49, provided by G. Fink) or wild-type element Ty1-H3 (pGTy1-H3*NEO*) were digested with *Bgl*II. Half of each digest was used to prepare two Southern blots. The blots were probed with (A) the oligomer 173-4, containing wild-type sequences surrounding map position 2098 of Ty1-H3, and (B) the oligomer 173-2, containing sequences surrounding map position 2098 of Ty1-173, including the *typ-2098* mutation described by Boeke et al. (1).

In contrast, *GAL1*-promoted Ty1-H1*NEO* failed to transpose in 84 *spt3-101* colonies tested (Table 4). It is unlikely that this failure is caused by a defect in *NEO* gene expression, because the *NEO* gene carried on the other four elements tested confers G418^r in *spt3-101* mutants. Furthermore, mating 40 G418^s colonies to an Spt3$^+$ strain did not result in resistance to G418 in any of the Spt3$^+$ diploids, even after an extended incubation period. Since the *spt3-101* mutation is recessive (45), the diploid cells would become G418^r if *NEO* expression is under *SPT3* control. In numerous other strains containing Ty*NEO* transpositions, G418^r is a dominant trait. Thus, the failure of Ty1-H1*NEO* to transpose in the *spt3-101* strain suggests that the element contains a mutation that renders it transposition defective. In addition, the low levels of Ty1-H1*NEO* transposition observed in an *SPT3* strain (Table 2) probably require complementation between the *GAL1*-promoted Ty1-H1*NEO* element and the endogenous Ty elements.

Defective element Ty1-H1 contains a novel mutation. Further analysis was performed on Ty1-H1 to determine the nature of the lowered transposition frequency observed with this element. Ty1-H1 contained no large deletions or insertions detectable by restriction enzyme analysis (data not shown). RNA synthesized from pGTy1-H1*NEO* in galactose-induced cells appears to be the same length and accumulates to the same levels as that from pGTy1-H3*NEO* (S. Youngren and D. Garfinkel, unpublished results). Heteroduplex analysis between Ty1-H1 and Ty1-H3 demonstrated that these elements did not contain any mismatches of 100 bp or larger (data not shown).

The only naturally occurring transposition-defective element that has been characterized to date is Ty1-173, which contains a point mutation at nucleotide 2098 (1). The *tyb-2098* mutation drastically lowers transposition of the *GAL1*-promoted Ty1-173 element. To determine whether Ty1-H1 contains this mutation, we performed Southern analysis of pGTy1-H1*NEO* DNA with two 20-nucleotide oligomer probes that contain either Ty1-H3 wild-type or Ty1-173 mutant sequences (1). Figure 3 demonstrates that the 1.6-kb *Bgl*II fragment from positions 1700 to 3300 of Ty1-H1 hybridized to the wild-type oligomer, 173-4. On the other hand, Ty1-H1 sequences failed to hybridize to the 173-2 oligomer containing the *tyb-2098* mutation. These results suggest that Ty1-H1 does not contain the *tyb-2098* mutation present in Ty1-173. Therefore, the mutation in Ty1-H1 that inhibits transposition is probably located elsewhere in the element.

Ty1-588 is transcriptionally active. To determine whether a native, transpositionally competent Ty element is transcribed in yeast cells, the chromosomal Ty1-588 element in strains BWG1-7A and GRF167 was marked with the *NEO* gene by transplacement so that its transcript could be easily differentiated from those of other elements. The integrating plasmid used, YIp331-588*NEO*, contains the 3′ end of Ty1-588(S)*NEO* beginning at the *Eco*RI site at map position 3920. This fragment of Ty1-588(S)*NEO* was chosen because it lies downstream of any sequences reported to be involved in Ty transcription (13, 14, 26, 32, 35), and therefore would be unlikely to modify the transcription detected from the endogenous elements. Standard transplacement techniques (Materials and Methods) were used to generate derivatives of strains BWG1-7A (strain DG731) and GRF167 (strain DG733) that contain Ty1-588*NEO* (Fig. 2).

RNA from strains DG731 and DG733 and the parental strains BWG1-7A and GRF167 was analyzed by Northern hybridization with a radiolabeled Ty1-specific probe (Fig. 4). In addition to the 5.7-kb Ty1 transcript seen in RNA from isogenic parental and Ty1-588*NEO* strains, the Ty1 probe hybridized to a slightly larger transcript in the Ty1-588*NEO*

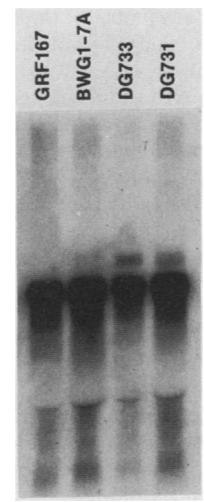

FIG. 4. Northern blot analysis of Ty1 RNA in isogenic parental and Ty1-588*NEO* transplacement strains. Total RNA (20 μg) was isolated from parental strains GRF167 and BWG1-7A, strain DG733 (an isogenic Ty1-588*NEO* derivative of GRF167), and strain DG731 (an isogenic Ty1-588*NEO* derivative of BWG1-7A). The RNA was subjected to Northern blot analysis (see Materials and Methods) and probed with a radiolabeled *Pst*I fragment from the Ty1-588 *TyA* region, which recognizes Ty1 but not Ty2 sequences (J. Curcio, S. Youngren, and D. Garfinkel, unpublished results). The strongly hybridizing band present in all four lanes is the 5.7-kb Ty1 transcript, while the higher-molecular-weight band in the two lanes on the right is the 6.6-kb Ty1-588*NEO* transcript.

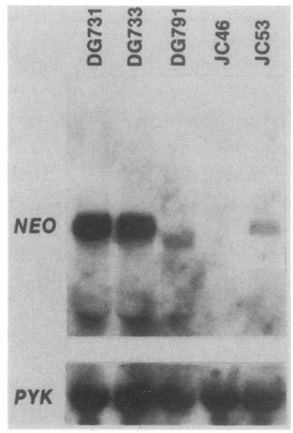

FIG. 5. Ty1-588*NEO* transcription in *MAT***a**/α and *spt3-101* strains. Northern hybridization was performed with 20 μg of total RNA from each of the following strains: DG731 and DG733, which are Ty1-588*NEO* transplacement strains; DG791, an isogenic *spt3-101* derivative of DG733; JC46, a *MAT***a**/α diploid from a cross between DG733 and tester strain GRY340; and JC53, a *MAT***a**/**a** derivative of JC46. The probe used is indicated to the left of each panel. In the top panel, the *NEO* gene was used as a probe to detect the Ty1-588*NEO* transcript, which is 6.6 kb when it is full length. In the bottom panel, the same blot was probed as a control with the radiolabeled yeast *PYK* gene, detecting the 1.7-kb pyruvate kinase mRNA.

strains DG731 and DG733. This 6.6-kb band was the expected size of a full-length Ty1-588*NEO* transcript, approximately 0.9 kb larger than the transcript of unmarked Ty1 elements due to the presence of *NEO* sequences. The 6.6-kb transcript was also detected when RNA from strains DG731 and DG733 was probed with ^{32}P-labeled *NEO* sequences (Fig. 5, lanes 1 and 2). The levels of Ty1-588*NEO* transcript present in strains DG731 and DG733 were approximately equal. These data demonstrate that the native element Ty1-588, marked in a manner that does not interfere with transposition and residing in its normal chromosomal location, is transcribed in two different yeast strains.

The Ty1-588(S) element tested in the transposition induction assay (Tables 2 and 4) is derived from a different strain (DBY939) than the Ty1-588 elements shown to be transcriptionally active [Ty1-588(G) in strain GRF167 and Ty1-588(B) in strain BWG1-7A]. To compare Ty1-588 elements derived from different strains, one of the transcriptionally active elements, Ty1-588(G), was isolated from the yeast genome, and a pGTy1-588(G)*NEO* plasmid was constructed. The plasmids pGTy1-588(S)*NEO* and pGTy1-588(G)*NEO* were compared by restriction enzyme analysis for polymorphisms that are commonly found among Ty1 elements (34, 44; J. Boeke and D. Garfinkel, unpublished results). No restriction site polymorphisms between the two elements were detected (data not shown). The transpositional competence of pGTy1-588(G)*NEO* was tested by using the transposition induction protocol. Of the 34 colonies analyzed from an *SPT3* strain, 31 were rendered resistant to G418 by virtue of one or more transposition events of the *NEO*-marked element. In addition, Ty1-588(G)*NEO* transposed at high levels in an isogenic *spt3-101* strain (data not shown). Therefore, the element Ty1-588(G)*NEO* is as efficient as Ty1-588(S)*NEO* in catalyzing high levels of transposition. These results demonstrate that Ty1-588(S) and Ty1-588(G) are highly related, if not identical, elements. Furthermore, the data indicate that the native element, Ty1-588(G), is both transpositionally competent and actively transcribed.

TABLE 5. Mapping Ty1-588 by tetrad analysis

Parental strains	Segregating markers	Tetrad[a]			cM[b]
		PD	NPD	T	
DG731, DG705	Ty1-588(B)*NEO*, Ty1-588(G){*URA3*}	12	0	0	
DG733, DG704	Ty1-588(G)*NEO*, Ty1-588(B){*URA3*}	11	0	0	
DG731, DG705	Ty1-588(B)*NEO*, *MAT*	11	0	1	
DG733, DG704	Ty1-588(G)*NEO*, *MAT*	10	0	1	
DG733, L2370	Ty1-588(G)*NEO*, *MAT*	13	0	6	
		34	0	8	9.5
DG733, L2370	Ty1-588*NEO*, *pet18*	18	0	1	2.6
DG733, L2370	*MAT*, *pet18*	12	0	7	18.4

[a] PD, Parental ditype; NPD, nonparental ditype; T, tetratype.
[b] Centimorgans (cM) were calculated by the Perkins formula (30a).

Regulation of Ty1-588*NEO* transcription. Previous work has demonstrated that transcription of Ty elements in yeast cells is under control of the *SPT3* locus (45, 47) and the *MAT* locus (11, 12). To determine whether Ty1-588*NEO* transcription is regulated by the *SPT3* gene, we constructed an isogenic *spt3-101* derivative of the Ty1-588(G)*NEO* strain DG733. In addition, *MAT***a**/α and **a**/**a** derivatives of DG733 were constructed to test transcriptional control by the *MAT* locus. Ty1-588*NEO* RNA in these strains was analyzed by Northern blot analysis with radiolabeled *NEO* sequences as a probe (Fig. 5). The *spt3-101* mutation had a dramatic effect on Ty1-588*NEO* RNA. No full-length 6.6-kb transcript was seen, but there was a transcript 0.5 to 1.0 kb shorter that was present at reduced levels (Fig. 5, lane 3). Winston et al. (47) demonstrated that in *spt3-101* mutants, transcription of full-length Ty RNA was abolished, but a transcript 0.8 kb shorter at the 5′ end was synthesized. Qualitative regulation of Ty1-588*NEO* transcription by the *SPT3* locus appeared to be identical to that observed for Ty's collectively.

In contrast to the *SPT3* locus, the *MAT* locus had a quantitative effect on Ty transcription that reduced the level of total Ty transcript in **a**/α cells to 1/20 the level seen in haploids or **a**/**a** or α/α diploids (11). Similarly, a large reduction in the level of Ty1-588*NEO* transcript was seen in the *MAT***a**/α diploid by Northern analysis (Fig. 5, lane 4). These experiments show that no Ty1-588*NEO* transcript was detectable even after overexposure of the autoradiogram (data not shown). The transcript reappeared in the isogenic *MAT***a**/**a** strain, which was heterozygous for Ty1-588*NEO* (Fig. 5, lane 5). Quantitative scanning densitometry suggested that the level of Ty1-588*NEO* RNA in the *MAT***a**/**a** strain was at least three times lower per gene copy than in the *MAT*α strain. This measurement was normalized to the amount of hybridization observed with the pyruvate kinase gene probe. Furthermore, the amount of Ty1-588*NEO* RNA in a *MAT*α/α strain homozygous for Ty1-588*NEO* was also approximately three times lower per gene copy (data not shown). Therefore, transcription of Ty1-588*NEO* in *MAT***a**/**a** and *MAT*α/α cells was not completely restored to the levels seen in haploid cells.

Mapping of Ty1-588*NEO*. Tetrad analysis was performed to determine whether Ty1-588 was in the same location in both strains BWG1-7A and GRF167 (Table 5). The Ura$^+$ G418^s strains DG704 and DG705 were obtained by integration of the *URA3*-based plasmid YIp331-588 into the 3′-flanking sequences of Ty1-588 in strains BWG1-7A and YH8

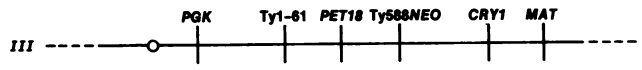

FIG. 6. Partial genetic map of chromosome III, showing position of Ty1-588. By tetrad analysis, Ty1-588 is linked to *MAT* and tightly linked to *pet18* on the right arm of chromosome III. The map is not drawn to scale.

(a *leu2Δ trp1Δ1* isogenic derivative of strain GRF167), respectively. Strains DG704 and DG705 were crossed to strains DG733 or DG731, which are Ty1-588*NEO* strains derived from strains GRF167 and BWG1-7A, respectively. In all tetrads analyzed, G418^r did not cosegregate with Ura$^+$ (Table 5). Failure to detect a recombinant in 23 tetrads analyzed suggests that Ty1-588 resides in the same chromosomal location in both strains GRF167 and BWG1-7A.

In the course of genetic analysis, we discovered that Ty1-588*NEO* was linked to the *MAT* locus (Table 5). Our tetrad data indicate that they were 9.5 centimorgans (cM) apart on the right arm of chromosome III. Ty1-588*NEO* was also tightly linked to *pet18* (2.6 cM), which suggests that Ty1-588 is located centromere-proximal to *MAT*. Furthermore, the distance between *MAT* and Ty1-588*NEO* (9.5 cM) was less than the *MAT-pet18* distance of 18.4 cM obtained in this analysis and the *MAT-pet18* distance of 12.5 cM reported previously (28). These results place Ty1-588 centromere-distal to *pet18*. A partial genetic map of the right arm of chromosome III with the proposed location of Ty1-588 is shown in Fig. 6. The element Ty1-61 has previously been mapped to this region of chromosome III (24). However, Ty1-61 is centromere proximal to *pet18* and is easily differentiated from Ty1-588 because it contains a 1.2-kb insertion. The *pet18* mutation is a multigene deletion that may result from intramolecular recombination between Ty elements (41). It appears that the *PET18* locus is bracketed on either side by Ty elements, Ty1-61 on the centromere-proximal side and another Ty on the centromere-distal side (42). Ty1-588 may be the same element as the Ty1 element found to be flanking *PET18* on the centromere-distal side. In addition, it may be the same Ty1 element as the one mapped 6.8 cM from *MAT* by Klein and Petes (25).

DISCUSSION

Using recently developed tools to mark Ty elements with selectable genes and assay the elements phenotypically for transpositional competence (1, 5, 48), we have begun to characterize the factors involved in regulation of Ty element transposition. Our results indicate that the yeast genome contains native Ty1 and Ty2 elements that are functional. These elements, typified by Ty1-588 and Ty2-117, individually encode all of the gene products and *cis*-acting control sequences needed to catalyze their own transposition. Furthermore, we have demonstrated that Ty1-588 is transcriptionally active. These results indicate that regulation of Ty expression may occur at a posttranscriptional level to limit the transposition of functional native elements in the yeast cell.

The potentially mutagenic nature of Ty element insertions and the elevated levels of genome rearrangement that might result from an increase in Ty copy number probably explain the selective advantage of inhibiting transposition. Thus, it is not entirely surprising that although 5 to 10% of the total polyadenylated RNA is Ty, the frequency of transposition is very low. However, it is enigmatic that overexpression of a single functional Ty element, which increases the cellular concentration of Ty RNA two- to fourfold (S. Youngren and D. Garfinkel, unpublished results) produces a 20- to 100-fold increase in transposition frequency (1, 2). Three hypotheses to explain these observations have been considered. The first is that all of the elements native to the yeast genome are defective for transposition. In this scenario, rare spontaneous transposition would result from complementation between elements in the genome or a recombinational event during reverse transcription to form a functional template for transposition (4). Second, the yeast genome might consist of both functional and nonfunctional elements, but only the defective elements are transcribed. Transposition-competent elements would be silent or poorly transcribed. Boeke et al. (1) have proposed that there might be weak and strong δ element promoters that are shuffled in front of functional and nonfunctional coding sequences by homologous recombination among elements. Evolutionary selection would favor weak promoters associated with functional elements. The third hypothesis is that the expression of functional elements is inhibited at a posttranscriptional level. The fact that transposition induction of a pGTy construct is accompanied by an estimated 20-fold increase in *TyB*-encoded proteins supports this hypothesis (16). Posttranscriptional control might involve inhibition of translational initiation, inhibition of the frameshifting event necessary for *TyB* synthesis (8, 27), or a rapid turnover of Ty proteins.

Our data refute the hypothesis that all genomic Ty elements are defective. We have demonstrated that four of the five elements tested transpose at high levels when induced from the *GAL1* promoter (Table 2). Prior to this work, only one element that was isolated as a spontaneous transposition was shown to be active (2). Therefore, it remained possible that Ty elements isolated on the basis of their transposition into a target gene were the only functional elements in the genome. This is the first demonstration that elements such as Ty1-588 and Ty2-117, which are native to the yeast genome and not selected on the basis of a recent transposition, are also transpositionally competent. In addition, this is the first demonstration that Ty2 elements are transpositionally competent in the transposition induction system.

A strong argument can be made against the possibility that the high levels of transposition observed are entirely dependent on complementation between the plasmid-borne element and endogenous elements. First of all, mutant elements such as Ty1-173 (1) and Ty1-H1 (Table 2) are significantly less active in inducing transposition in an *SPT3* strain than the transposition-competent elements, suggesting that an element must be completely functional to transpose at high levels. More importantly, the elements that were transpositionally competent in a wild-type strain were equally competent in an *spt3-101* mutant (Table 4). This is compelling evidence that Ty1-H3, Ty1-588, Ty2-917, and Ty2-117 each encode all of the functions involved in efficient transposition.

The fifth element tested, Ty1-H1, was active at five- to sevenfold reduced levels in the *SPT3* strain and completely failed to transpose in an isogenic *spt3-101* strain. Our data suggest that the failure of Ty1-H1 to transpose at high levels is not a result of reduced levels of Ty1-H1*NEO* RNA in galactose-induced cells or to cryptic transposition events of the Ty1-H1*NEO* element. Preliminary experiments suggest that *TyB* proteins produced from Ty1-H1 are incorrectly processed and that the reverse transcriptase activity associated with Ty1-H1 viruslike particles is abnormally low (S. Youngren and D. Garfinkel, unpublished results). The fact that Ty1-H1*NEO* transposes at low levels in the *SPT3* strain but not in the *spt3-101* strain may indicate that transposition

can occur by complementation with endogenous elements in an *SPT3* background. However, this complementation is weak, because it does not result in the high levels of transposition seen when a functional element such as Ty1-H3 is transposition induced (5).

Our data also suggest that regulation of Ty transposition does not occur by repressing transcription of functional Ty elements, at least in the case of the native element Ty1-588. When marked with the *NEO* gene, the Ty1-588 element in strains BWG1-7A and GRF167 produced 6.6-kb full-length Ty*NEO* transcripts seen by Northern hybridization with either a Ty1 or a *NEO* probe (Fig. 4 and 5). Preliminary evidence in our laboratory suggests that transcription of Ty1-588*NEO* in these strains is quite strong compared with transcription of random Ty1 elements marked with *NEO* (J. Curcio and D. Garfinkel, unpublished results).

The possibility that the Ty1-588 genomic transcript is incorrectly initiated and therefore a defective transpositional template has not been eliminated. Such a defect may not be detected by Northern analysis. In this case, the galactose-induced transcript might promote transposition, whereas the genomic transcript might not. In the case of pGTy1-H3, *GAL1*-promoted transcripts were shown to be identical to transcripts synthesized from native elements by primer extension (A. Bystrom, J. Boeke, D. Garfinkel and G. Fink, unpublished results). However, it remains possible that transcription of the endogenous Ty1-588 element is initiated at a different site from most of the other genomic elements.

Transcription of Ty1-588 is controlled by the constitution of both the *SPT3* and *MAT* loci in a similar fashion to total Ty transcription (Fig. 5). However, one potentially interesting difference between the regulation of Ty1-588 and total Ty transcription was observed. The level of Ty1-588*NEO* per gene copy was approximately threefold lower in the *MAT***a**/**a** strain cells tested than in the *MAT*α strain. In the case of total Ty transcription, however, the levels seen in haploid *MAT***a** or *MAT*α cells, reduced by 95% in *MAT***a**/α cells, is completely restored to unrepressed levels in *MAT***a**/**a** or *MAT*α/α diploids (11). Thus, the data suggest that Ty1-588 transcription may be quantitatively regulated by factors other than the constitution of the mating type locus. This type of effect becomes noticeable only when transcripts from a particular Ty can be differentiated from the bulk of the Ty RNA. To investigate this potential difference, we are currently analyzing both total Ty transcription and the transcription of other chromosomal elements marked in the same fashion in our strains.

The results presented here suggest that the Ty1-588 elements from strains DBY939, GRF167, and BWG1-7A are actually the same element and that Ty1-588 is located in the same place in the genome of these strains. Integration of phenotypic markers at the Ty1-588 locus allowed us to map the element to a position 9.5 cM centromere-proximal to the *MAT* locus and 2.6 cM centromere-distal to the *pet18* locus, on the right arm of chromosome III (Fig. 6). Ty1-588 mapped to the same position in both strains BWG1-7A and GRF167 (Table 5). In addition, Ty1-588(B) and Ty1-588(G) were cloned by virtue of their having the same unique 3′-flanking sequences as Ty1-588(S), the element derived from strain DBY939. No quantitative or qualitative differences in transpositional competence or transcriptional activity were detected between elements from different strains.

We have demonstrated that the yeast genome contains a transposition-competent Ty1 element that appears to be correctly transcribed and regulated. These results support the hypothesis that regulation of transposition of Ty1 elements at least occurs at a posttranscriptional level. Two working models can be proposed to explain our observations. The first proposes that the yeast genome contains a mixture of functional and nonfunctional native Ty elements, many of which are actively transcribed, but the levels of Ty proteins are low because of a transposition inhibitor. This inhibitor is titrated by expression of a *GAL1*-promoted Ty element, allowing Ty proteins to accumulate in the cell and catalyze transposition. Expression of a *GAL1*-promoted mutant element also titrates the inhibitor. For this reason, certain mutant elements such as Ty1-H1 can transpose by complementation with endogenous elements. It is not likely that this transposition inhibitor interacts with and is titrated by Ty RNA. This would be inconsistent with the fact that expression of *GAL1*-promoted Ty elements containing in-frame mutations in the protease region apparently abolishes transposition in *SPT3* cells (48). However, the inhibitor may work at the level of Ty protein processing or viruslike particle formation.

A second possibility is that the majority of Ty elements in the cell encode proteins which are defective for transposition and that only 10% of the Ty RNA is derived from functional elements. Transposition of this functional Ty RNA (which would still account for 0.5 to 1% of polyadenylated RNA) might then be regulated by inefficient translation of the Ty RNA or by instability of the Ty proteins. In this case, the two- to fourfold induction of Ty RNA from a functional element fused to the *GAL1* promoter would constitute a 20- to 40-fold increase in functional RNA. This induction in functional RNA would be consistent with the induction seen in TyB proteins and transposition. The fact that four of the five Ty elements tested were transpositionally competent and that at least one competent element was actively transcribed make this model seem unlikely. However, further analysis of individual genomic elements will be necessary to disprove this model.

ACKNOWLEDGMENTS

This research was sponsored by the National Cancer Institute under contract NO1-CO-74101 with Bionetics Research, Inc. M.J.C. was supported by National Research Service Award 1F32GM12295-01.

We are grateful to R. Fishel, J. Strathern, and D. Higgins for their comments on the manuscript, M. Powers (PRI, NCI-FCRF, Frederick, Md.) for oligonucleotide synthesis, and S. Lucas for preparation of the manuscript.

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