Ty3 Transposes in Mating Populations of Yeast: A Novel Transposition Assay for Ty3

Philip T. Kinsey[1] and Suzanne B. Sandmeyer

Department of Microbiology and Molecular Genetics, College of Medicine, University of California, Irvine, California 92717

Manuscript received November 4, 1993
Accepted for publication September 16, 1994

ABSTRACT

Ty3 is a retrotransposon of *Saccharomyces cerevisiae* that integrates just upstream of the transcription initiation site of genes transcribed by RNA polymerase III. Ty3 transcription is pheromone-inducible in haploid cells and is mating-type regulated in diploid cells. The specificity of Ty3 integration was exploited in the design of a novel target into which transposition of Ty3 elements could be selected. The target plasmid contains divergently oriented tRNA genes with 19 base pairs separating the two tRNA gene coding sequences. An inactive ochre suppressor $tRNA^{Tyr}$ gene with a modified transcription initiation region was used as the selectable marker and a $tRNA^{Val}$(AAC) gene was used to direct Ty3 integration into the transcription initiation region of the suppressor $tRNA^{Tyr}$ gene. Integration of Ty3 activated expression of the suppressor tRNA gene, which resulted in suppression of ochre nonsense alleles *ade2-101o* and *lys2-1o* and allowed cell growth on selective medium. Based on the activity of this target, Ty3, under control of a galactose-inducible promoter and present on a high copy-number plasmid, was estimated to transpose into the genome at a rate of 5.6×10^{-3} per cell division. We show here that induction of Ty3 transcription from its natural promoter results in transposition. Ty3 elements in strains of the **a** or α mating-type transposed efficiently to target plasmids in cells of the opposite mating-type. Thus, natural transposition of Ty3 is regulated temporally to occur in mating populations.

RETROTRANSPOSONS are found in the genomes of most eukaryotic organisms where they are propagated passively via genomic replication. Retrotransposons can be mobilized to new regions of the genome by reverse transcription of the RNA copy and integration of the resultant cDNA (reviewed by BOEKE and SANDMEYER 1991). The potentially lethal consequences of retrotransposon mobilization must be restricted to achieve a balance between mobility and cell viability, which results in propagation of the retrotransposon. In yeast this control includes transcription repression (ELDER *et al.* 1980; CLARK *et al.* 1988; BILANCHONE *et al.* 1993), post-transcriptional (CLARE *et al.* 1988; XU and BOEKE 1991) and post-translational mechanisms (CURCIO and GARFINKEL 1992). Position-specific integration and integration into noncoding regions in which cellular gene expression is not disrupted are other mechanisms by which the detrimental consequences of transposition are minimized (KINSEY and SANDMEYER 1991; JI *et al.* 1993). Here we discuss an additional point of control of retrotransposon mobility: restriction of retrotransposon transcription induction which acts to limit transposition to a discrete window of the yeast life cycle.

The retrotransposon Ty3 is present in one to five copies per haploid genome in standard laboratory strains of *Saccharomyces cerevisiae* (CLARK *et al.* 1988). Ty3 sequence and genome organization indicate that it is a member of the gypsy class of retrotransposons and that it is more closely related to animal retroviruses than to other yeast retrotransposons (HANSEN *et al.* 1988; HANSEN and SANDMEYER 1990). The 4.7-kbp internal domain of Ty3 contains two overlapping open reading frames (ORFs), known as *GAG3* and *POL3*, that encode the structural and catalytic proteins of Ty3, respectively (HANSEN *et al.* 1988; HANSEN and SANDMEYER 1990). The Ty3 ORFs are translated with a +1 frameshift mechanism (FARABAUGH *et al.* 1993) into a single polyprotein (KIRCHNER *et al.* 1992) from which the structural and catalytic proteins are processed by the Ty3 encoded protease (KIRCHNER and SANDMEYER 1993). The internal domain is bound by direct, long terminal repeats (LTRs) of 340 bp known as sigma elements. Sigma elements are also found as single entities at ~30 copies per haploid genome (DEL REY *et al.* 1982; SANDMEYER and OLSON 1982). Sequences that confer haploid-specific and pheromone-inducible expression upon genes whose products are required for mating are present within the Ty3 LTRs. Haploid cells have very low levels of Ty3 transcripts except when exposed to pheromone from cells of the opposite mating type and diploid cells do not contain detectable levels of Ty3 transcripts (VAN ARSDELL *et al.* 1987; CLARK *et al.* 1988; BILANCHONE *et al.* 1993).

Corresponding author: Suzanne B. Sandmeyer, Department of Microbiology and Molecular Genetics, College of Medicine, University of California, Irvine, California 92717.

[1] *Present address:* Institute of Molecular Biology, University of Oregon, Eugene OR 97403.

Integration of Ty*3* was demonstrated to occur within the transcription initiation regions of different classes of genes transcribed by RNA pol III (tRNA, 5S rRNA and the U6 snRNA genes; CHALKER and SANDMEYER 1992). However, it is the population of tRNA genes that act as the primary genomic target for Ty*3* integration (CHALKER and SANDMEYER 1990). The tRNA gene class is distinguished by the *box A* and *box B* internal promoter elements (reviewed in GEIDUSCHEK and TOCCHINI-VALENTINI 1988). These regions of the tRNA gene coding sequence direct binding of transcription factor TFIIIC (BAKER *et al.* 1987; FABRIZIO *et al.* 1987), which subsequently directs binding of TFIIIB (SEGALL *et al.* 1980; LASSAR *et al.* 1983; KASSAVETIS *et al.* 1989) to the 5′-flanking region of the tRNA gene in a position-specific manner (KASSAVETIS *et al.* 1990). TFIIIB bound to the DNA template is sufficient to direct RNA pol III to initiate multiple rounds of transcription (KASSAVETIS *et al.* 1990). Although sequences present in the 5′-flanking regions of tRNA genes from several organisms have been demonstrated to influence tRNA gene expression (SPRAGUE *et al.* 1980; DEFRANCO *et al.* 1981; SHAW and OLSON 1984), no consensus DNA binding sequences for TFIIIB have been defined. Ty*3* integration into a target tRNA gene requires functional tRNA gene promoter elements, and it appears that transcription factors are bound upstream of the gene at the time of integration. Nevertheless, the competitiveness of a tRNA gene as a Ty*3* integration target does not correlate with its rate of transcription (CHALKER and SANDMEYER 1992, 1993). The presence of Ty*3* upstream of a tRNA gene is compatible with tRNA gene expression (KINSEY and SANDMEYER 1991). These observations suggested that an inactive tRNA gene target could be developed in which the tRNA gene could be activated by Ty*3* transposition.

Previously, transposition of Ty*3* was detected by mobilization of a genetically marked, transpositionally incompetent Ty*3* by a wild-type helper Ty*3* element (CHALKER and SANDMEYER 1990). Although useful for characterization of Ty*3* transposition targets, this qualitative assay was limited in that the transposition properties of native Ty*3* elements could not be directly analyzed. Here we report the development of a novel target plasmid into which Ty*3* integration can be selected. This direct selection obviates the requirement for selection against the presence of donor plasmids, allows transposition of unmarked Ty*3* elements to be quantitated and allows transposition products to be recovered easily for subsequent analysis. We used this assay to determine the rate of Ty*3* transposition and characterize transposition of Ty*3* in mating populations.

MATERIALS AND METHODS

Strains and culture conditions: Culturing of *Escherichia coli* was carried out by standard methodologies (MANIATIS *et al.* 1982). Transformation of *E. coli* was by electroporation (DOWER *et al.* 1988). Plasmid DNAs were maintained in *E. coli* strain HB101 (*F⁻ hsdS20* [r_B^-, m_B^-] *recA13 leuB6 ara-14 proA2 lacY1 galK2 rpsL20* [Sm^r] *xyl-5 mtl-1 supE44* λ⁻). Single-stranded DNA used for site-directed oligonucleotide mutagenesis (KUNKEL 1985) was prepared from *E. coli* strain RZ1032 (*lysA* [*61–62*] *thi-1 relA1 spoT1 dut-1 ung-1* [tet^r] *supE44*), as described by International Biotechnologies, Inc. Strains of *S. cerevisiae,* described in Table 1, were cultured as described by SHERMAN *et al.* (1986) and transformed by the method of SCHIESTL and GIETZ (1989).

Plasmid constructions: The tRNA genes of target-plasmid pCH2bo19V (Table 1) were a tRNA^{Val}(AAC) gene, isolated in an earlier study (CHALKER and SANDMEYER 1990) and a *SUP2* gene (*sup2b*) marked by the addition of 6 bp within the intron, which was previously described (KINSEY and SANDMEYER 1991). An *Eco*RI restriction site (shown below in bold) was engineered by site-directed oligonucleotide mutagenesis at position −6 relative to the tRNA^{Val}(AAC) gene-coding sequence with the oligonucleotide: 5′-CCGTTATTCAACAATTAAG**GAATTC**TGGTTTCGTGG-3′. Two modifications were made to the *sup2b* gene by site-directed mutagenesis: (1) the anticodon (GUA) was altered to render the gene an ochre suppressor allele (UUA, the reverse-complementary sequence of which is underlined below) with the oligonucleotide: 5′-TCTCAAGATTTCGTAGGTTACCTGATAAATTAAAGTCTTGCGCC-3′ and (2) a polypyrimidine tract was substituted for the transcription initiation region of the gene, from position −3 to −16, relative to *sup2b* coding sequence, with the oligonucleotide: 5′-CCGAGAGTCGAAGAGAAGGGA**GAATTC**GAAATATGTTC-3′. The tRNA genes were joined at *Eco*RI sites (shown in bold), which immediately flanked the tRNA^{Val}(AAC) gene-coding sequence and the polypyrimidine tract of the *sup2bo* gene, with the result that 19 bp separated the two tRNA gene-coding sequences. The four base pairs adjacent to the tRNA^{Val}(AAC) gene-coding sequence were changed by site-directed mutagenesis to eliminate the *Eco*RI restriction site and to extend the length of the polypyrimidine tract to position −19 relative to the *sup2bo* gene using the oligonucleotide: 5′-CGAAACCTCTCTTCCCTTCTCTTCG-3′. The divergent tRNA genes, present on a 1250-bp *Eco*RI-*Bam*HI fragment, were cloned into a pIBI20 (International Biotechnologies, Inc.) plasmid vector into which the *HIS3* gene had been inserted on a 880-bp *Bam*HI-*Pst* I fragment (derived from pDG201, a gift from D. GARFINKEL, Frederick Cancer Research Center, Frederick, MD). A 2.8-kbp *Eco*RI fragment that contained the *ARS 1/CEN 4* element was added to this clone to complete the construction of plasmid pCH2bo19V. For some assays where it was desirable to monitor transposition into the target plasmid independent of the site of insertion (CHALKER and SANDMEYER 1992, and see below), two modifications of the target plasmid were required. To maintain the plasmid at high copy-number, the *Eco*RI fragment containing the *ARS 1/CEN 4* sequences was replaced with a 2.1-kbp *Eco*RI fragment containing the 2μ replication sequence. Because suppressor tRNA genes are lethal in high copy, the anticodon of the ochre suppressor tRNA carried in this plasmid was reverted to wild type by site-directed mutagenesis with the oligonucleotide: 5′-CGCAAGACTGTAATTTATCAGG-3′ (anticodon region underlined). This plasmid was named pEH2b19V. To determine the relative usage of the divergent tRNA gene target to that of a single tRNA gene, a single *sup2b* gene (present on a 1.0-kbp *Bam*HI fragment) was inserted into plasmid pEH2b19V, at the *Bam*HI site ~1 kbp downstream from and in the same orientation as the *sup2b* gene present as part of the divergent tRNA gene target. This plasmid was named pEH2b19V2b.

TABLE 1

***Saccharomyces cerevisiae* strains and plasmids**

Strain	Genotype[a]	Source
yDLC221	*MATa ura3-52 his3-Δ200 ade2-101o lys2-1o leu1-12 can1-100o gal3*	CHALKER and SANDMEYER (1990)
yVB114	*MATα ura3-52 his3-Δ200 ade2-101o lys2-1o leu1-12 can1-100o trp1-Δ901 gal3 ΔTy3*	BILANCHONE *et al.* (1993)
yVB115	*MATa/MATα ura3-52 his3-52 his3-Δ200 ade2-101o lys2-1o leu1-12 can1-100o trp1-Δ901 gal3 ΔTy3*	BILANCHONE *et al.* (1993)
yTM444	*MATa ura3-52 his3-Δ200 ade2-101o lys2-1o leu1-12 can1-100o ΔTy3*	T. MENEES (personal communication)
yTM441	*MATa ura3-52 his3-Δ200 ade2-101o lys2-1o leu1-12 can1-100o trp1::hisG ΔTy3*	T. MENEES (personal communication)
Plasmid	**Relevant markers**	**Source**
pCH2bo19V	*CEN4, HIS3,* divergent tRNA gene target with ochre suppressor	This work
pEH2b19V	*2μ, HIS3,* divergent tRNA gene target without ochre suppressor	This work
pEH2b19V2b	*2μ, HIS3,* divergent tRNA gene target without ochre suppressor, additional *sup2b* gene	This work
pDLC348	*CEN4, URA3, amp*$^-$, galactose-inducible Ty3-1 marked with *neo*^{r}	CHALKER and SANDMEYER (1992)
pEGTy3-1	*2μ, URA3,* galactose-inducible Ty3-1	HANSEN *et al.* (1988)
pEUTy3-1	*2μ, URA3,* Ty3-1	This work
pCUTy3-1	*CEN4, URA3,* Ty3-1	This work

[a] Alleles that are suppressible by a $tRNA^{Tyr}$ ochre suppressor are indicated with an "*o*."

For some experiments the source of Ty3 transcripts was a Ty3 element in which the *GAL1-10* upstream activating sequence was fused upstream of the site of transcription initiation. This Ty3 element occurred on pEGTy3-1, a high copy number plasmid described previously (HANSEN *et al.* 1988). The source of Ty3 transcripts in experiments with the native Ty3 element under pheromone control (see below) was plasmid pEUTy3-1. This plasmid contains the following: wild-type Ty3-1 (HANSEN *et al.* 1988) on a 6.4-kbp *Eco*RI-*Hin*dIII fragment, the *URA3* gene present on a 1.0-kbp *Hin*dIII fragment and the 2μ replication sequence present on a 2.1-kbp *Eco*RI fragment cloned into the polylinker of vector pIBI21 (International Biotechnologies, Inc.). A low copy version of pEUTy3-1, pCUTy3-1, was created by replacing the 2μ replication sequence with the *ARS 1/CEN 4* sequence described above.

Selection for Ty3 transposition: Cells transformed with the pCH2bo19V and pEGTy3-1 plasmids were grown in synthetic complete (SC) −Ura −His −Arg +canavanine (80 mg/l) medium containing dextrose to mid-log phase (OD_{600} 0.6 to 0.8, ~1.0×10^7 cell per ml). SC medium contained dextrose unless a different carbon source is specified in parentheses. Where a different carbon source is specified, that carbon source was substituted for dextrose. Canavanine is a toxic arginine analogue that can be transported by cells that have the arginine permease encoded by the *CAN1* gene. Because the cells used in this experiment had *can1-100o,* an allele that contains a suppressible ochre nonsense mutation, growth of cells containing spontaneously arising ochre suppressor mutations is inhibited. Cultures were harvested, washed twice in one-half volume sterile water and suspended to 1×10^7 cells per ml. One million cells (0.1 ml) were applied to SC −Ura −His and to SC (galactose) −Ura −His media and incubated at 30° for 29 and 48 hr, respectively (Figure 2). These times of incubation allow the same number of cell divisions on the different media. The number of viable cells was determined by assaying colony formation from 150 cells plated onto rich medium. On average, 69% of the cells plated resulted in colony growth. Cells were replicaplated from SC −Ura −His and SC (galactose) −Ura −His media onto SC −Ade −Lys to terminate induction of Ty3 transcription and select for Ade^+ Lys^+ colonies. The genomic *ade2-101o* and *lys2-1o* mutations are suppressed when *SUP2bo* is expressed. SC −Ade −Lys plates were incubated at 30° for 4–5 days at which time colonies were picked to a master plate for further analysis.

Plasmid DNA from Ade^+ Lys^+ colonies was assayed by Southern blot analysis to detect target plasmids that contained newly transposed Ty3 elements. Colonies were picked into 2 ml SC −Ade −Lys or SC −His medium and grown overnight. Cells were harvested, washed, treated with zymolyase and lysed. Cellular debris was pelleted by centrifugation and the nucleic acids, present in the supernatant were separated by agarose gel electrophoresis and subjected to Southern blot analysis using a random-primer labeled (FEINBERG and VOGELSTEIN 1983, 1984) *HIS3* gene-specific probe. Target plasmids that contained integrated Ty3 elements were distinguished from native target plasmids by slower mobility of intact plasmids through the agarose gel. To characterize Ty3 integration sites by nucleotide sequence analysis, Ade^+ Lys^+ colonies were patched onto SC −Ade −Lys +5FOA (1 g/l) medium to select for cells that had lost the *URA3*-marked

plasmid (BOEKE *et al.* 1984) pEGTy3-1. Target plasmids were isolated from yeast by the method of HOFFMAN and WINSTON (1987), amplified in *E. coli* and prepared (MANIATIS *et al.*1982) for analysis. Plasmid DNAs were sequenced by the method of SANGER *et al.* (1977) using the Sequenase enzyme (U.S. Biochemicals).

Helper/donor assay for Ty*3* transposition: Transposition events that were not selected on the basis of insertion site were monitored using a genetically marked element. An integrated galactose-inducible Ty*3* element (present in strain yDLC221) was used to supply protein functions in *trans* to allow the transposition of a galactose-induced Ty*3* donor element (present on the *URA3*-marked plasmid, pDLC348; see Table 1), which is transpositionally incompetent due to the insertion of the bacterial *neo* gene within Ty*3* coding sequence. This plasmid does not carry the bacterial *bla* gene that confers the Amp^r phenotype (CHALKER and SANDMEYER 1992). Target plasmids (pEH2b19V; see Table 1) into which donor Ty*3* DNA had transposed were collected essentially as previously described (CHALKER and SANDMEYER 1992). Transposition was induced by growth of yDLC221, containing plasmids pDLC348 and pEH2b19V, on SC (galactose) −Ura −His medium for 4–5 days. Single colonies were spread as patches onto YPD medium that contained G418 (0.7 g/l) and were incubated for 48 hr to allow loss of pDLC348, but retention of the *neo*-marked Ty*3* element. The patches were replicaplated onto YPD medium that contained 5FOA (1 g/l) and G418 (0.7 g/l) to select for cells that had lost the *URA3*-marked plasmid pDLC348 but retained the *neo*-marked Ty*3* element. Cosegregation of the His$^+$ and G418^r phenotypes (conferred by pCH2b19V and the *neo*-marked donor Ty*3* element, respectively) was assessed on SC −His medium that contained G418 (0.7 g/l). Target plasmids were isolated from cells grown in this medium and were used to transform *E. coli* to both ampicillin and kanamycin resistance. The sites of Ty*3* integration were analyzed as described above.

Statistical analysis of Ty*3* transposition rate: Strain yTM441 cells carrying plasmids pCH2bo19V and pEGTy3-1 were grown to a cell density of 9×10^5 cells per ml in SC (raffinose) −Ura −His medium. This culture was diluted 10-fold into 96 tubes of SC (galactose) −Ura −His medium. The cultures were grown to a cell density of $\sim 1.2 \times 10^7$ cells per ml. The cultures were then diluted to 8.4×10^5 cells per ml and 100 ml of the diluted cells were spotted onto SC −Ade −Lys plates. The plates were incubated for 4 days at which time the number of Ade$^+$ Lys$^+$ colonies was determined. To determine the Ty*3* transposition rate in an actively growing culture, the starting culture for this analysis was grown in raffinose-containing medium to minimize the lag in attaining logarithmic growth.

Mating-induced transposition: Strain yTM444 (*MAT***a**), yVB114 (*MAT*α) (see Table 1) were transformed separately with pEUTy3-1 or pCH2bo19V. Transformed cells were grown separately in SC −Ura or SC −His medium to mid-log phase, harvested and washed, and the cell number of the cultures was determined. Haploid cells containing pCH2bo19V were mixed in 10-fold excess with cells containing pEUTy3-1 (2×10^8 and 2×10^7 cells, respectively). The mixed cultures of cells were plated onto YPD plates and incubated for 24 hr. In these experiments, diploid cells were selected by replicaplating onto SC −Ura −His plates and by incubating for 24 hr. Diploid cells containing target plasmids into which Ty*3* had transposed were selected by replicaplating onto SC −Ade −Lys plates and were incubated for 4–5 days. Three kinds of control experiments were performed. In the first, a galactose-inducible Ty*3* element (on pEGTy3-1) was substituted for the pheromone-inducible Ty*3* element (on pEUTy3-1) (Table 4, experiments 9 and 10). In the second, one yTM444 culture was transformed with pEUTy3-1 and one culture with pCH2bo19V. The two transformants were co-cultured for the experiment. In the third experiment, yVB115 (diploid) cells in which Ty3 expression is repressed by mating-type control, carried both pEUTy3-1 and pCH2bo19V. Control experiments in which both plasmids were present in a single strain were performed as described above except that between 7×10^6 and 1×10^7 cells were applied directly to SC −Ura −His medium. Target plasmids for all experiments were analyzed as described above. Mating efficiency was determined essentially as described by SPRAGUE (1991).

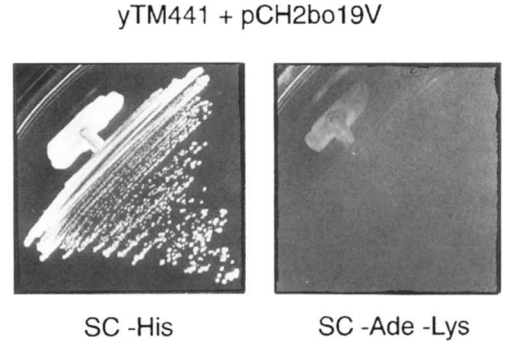

FIGURE 1.—Growth of cells that contain target plasmid pCH2bo19V. Strain yTM441 was transformed with the *HIS3*-marked plasmid pCH2bo19V and selected on SC −His medium. These cells were streaked onto SC −His medium and SC −Ade −Lys medium. Transformed cells grow on −His medium, but not on −Ade −Lys medium that requires suppression of the *ade2-101o* and *lys2-1o* alleles that contain ochre nonsense mutations. Prior to Ty*3* integration into the appropriate position in the target plasmid, the suppressor is inactive.

RESULTS

Design of the selection for Ty*3* transposition: Ty*3* integrates into the yeast genome just upstream of the transcription initiation sites of different tRNA genes. Previous experiments demonstrated that insertion of Ty*3* and sigma sequences upstream of tRNA gene transcription initiation sites does not disrupt expression of the adjacent tRNA gene (KINSEY and SANDMEYER 1991). Because RNA pol III initiates transcription predominantly with purine nucleotides, the expression of tRNA genes can be reduced by substituting pyrimidine nucleotides for the purine nucleotides within the transcription initiation region (CHALKER and SANDMEYER 1992). These characteristics of Ty*3* integration and RNA pol III transcription were used in the design of a novel, plasmid-based target into which Ty*3* transposition could be selected.

The target plasmid contains divergently oriented tRNA genes, *sup2bo*, a gene for an ochre-suppressor tRNA^{Tyr}, and a gene for a tRNA^{Val}(AAC), separated by 19 bp and a polypyrimidine tract from position −3 to −19 with respect to the *sup2bo* gene coding sequence. This sequence, along with apparent steric hindrance between the two genes for transcription factor binding, maintains expression of *sup2bo* below phenotypically de-

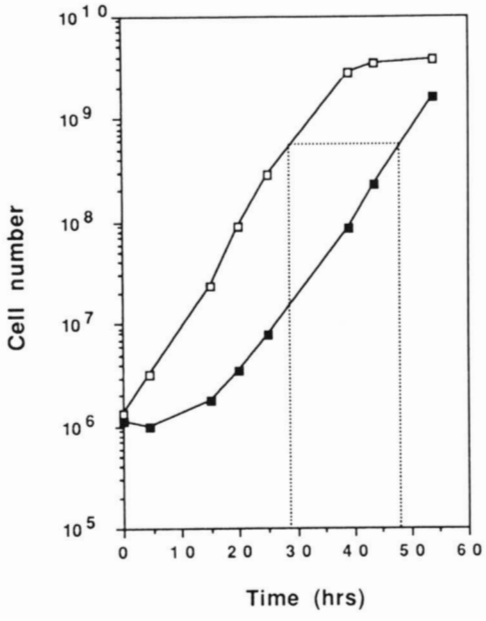

FIGURE 2.—Growth of cells on SC and SC (galactose) −Ura −His plates. Strain yTM441 cells, transformed with plasmids pCH2bo19V and pEGTy3-1, were grown to mid-log phase in SC -Ura -His medium. The cultures were harvested and washed, and cell numbers were determined as described in MATERIALS AND METHODS. The cells were diluted in H_2O and 1×10^6 cells were applied, by vacuum, to 8.2-cm diameter nitrocellulose filters (0.45 mm, Schleicher and Schuell) in two 7-ml aliquots. The filters were applied to plates containing SC −Ura −His (□) or SC (galactose) −Ura −His (■) media and incubated. At various time points, cells were washed from the filters and total cell numbers were determined. The dashed lines indicate incubation times of the cultures on different media that result in the same number of cells.

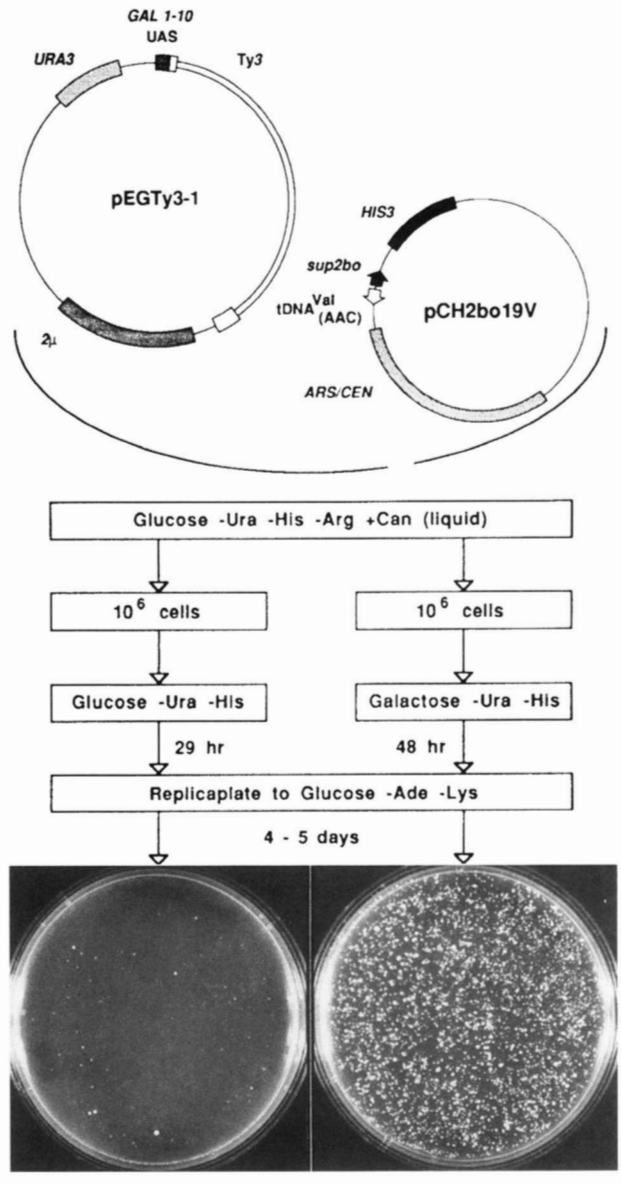

FIGURE 3.—Selection for position-specific transposition of Ty3. The target (pCH2bo19V) and Ty3-source (pEGTy3-1) plasmids are depicted schematically at the top of the figure. Integration of Ty3 into the pCH2bo19V target plasmid results in activation of the *sup2bo* suppressor gene. This results in suppression of the *ade2-101o* and *lys2-1o* nonsense alleles in the strain and allows growth on SC -Ade -Lys medium. The transposition regime is summarized and examples of colony growth on SC −Ade −Lys medium are shown.

tectable levels (see Figure 1). Because only 19 bp separate the coding sequences of the two genes, the transcription initiation regions overlap. The $tRNA^{Val}$(AAC) gene is positioned to target Ty3 integration upstream of the $tRNA^{Val}$(AAC) transcription initiation site and into the transcription initiation region of *sup2bo*. The Ty3 element originally used in the selection was under control of a galactose-inducible promoter. Therefore, Ty3 transcription and transposition could be induced by growth of cells on SC (galactose) medium. The insertion of Ty3 sequence within the divergent tRNA gene target was designed to relieve the steric hindrance for transcription factor binding and provide the purine nucleotides necessary for transcription initiation and phenotypically detectable expression of *SUP2bo*. The host cell background included ochre nonsense alleles of *LYS2* (*lys2-1o*) and *ADE2* (*ade2-101o*). Expression of the ochre suppressor gene could therefore be monitored by growth on −Ade −Lys medium (Figure 3). Note that when expression of the ochre suppressor $tRNA^{Tyr}$ gene is phenotypically undetectable (as it is in the native target plasmid) the gene is referred to as *sup2bo*; when expression of that gene can be detected phenotypically, it is referred to as *SUP2bo*. This selection scheme restricted detectable transposition events to those targeted by the $tRNA^{Val}$(AAC) gene, although events targeted by *sup2bo* were also expected to occur.

Induction of Ty3 transcription results in transposition and activation of suppressor tRNA gene expression: Cells were transformed with plasmids pCH2bo19V and pEGTy3-1 (Table 1). Transcription of Ty3, under control of the *GAL1-10* upstream activating sequence,

TABLE 2

Dependence of Ade$^+$Lys$^+$ colonies of Ty*3* expression

Experiment[a]	Carbon source	Ade$^+$ Lys$^+$ colonies[b]
1	Glucose	11, 10, 24, 23
	Galactose	643, 650, 480, 362
2	Glucose	0, 0, 0, 0
	Galactose	55, 134, 114, 406
3	Glucose	4, 10, 9, 25
	Galatose	80, 113, 483, 334

[a] Independent transformants of yTM441 with plasmids pCH2bo19V and pEGTy3-1.

[b] As described in MATERIALS AND METHODS, 1×10^6 cells containing pCH2bo19V and pEGTy3-1 plasmids were plated onto selective media containing glucose (repressing) or galactose (inducing) medium. Cultures were subsequently replicaplated into SC -Ade -Lys medium to select for suppressor-containing cells.

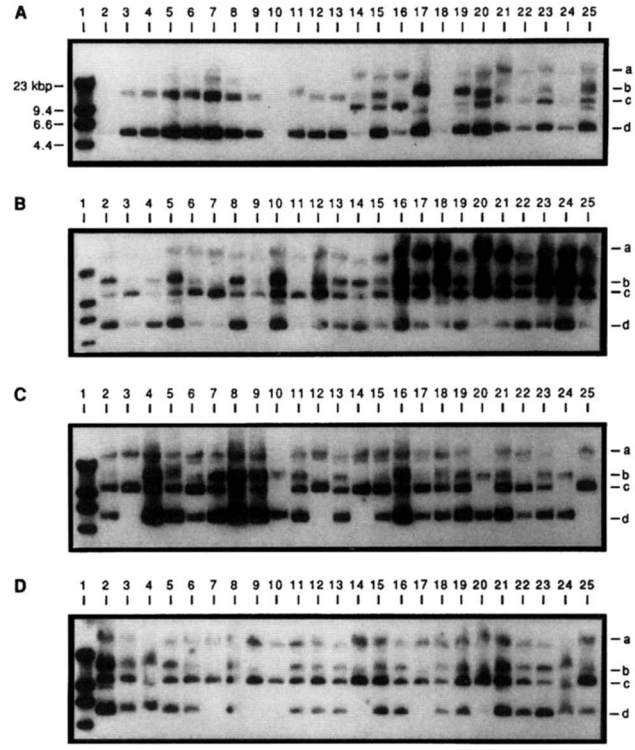

FIGURE 4.—Southern analysis of Ty*3* transposition into the target plasmid. (A–D) Lane 1 represents the four largest molecular weight species of λ *Hin*dIII size markers (23, 9.4, 6.6 and 4.4 kbp). Lanes 2–13 (A) represent uncut plasmid DNAs isolated from independent Ade$^+$ Lys$^+$ colonies that originated from cells grown on SC −Ura −His medium. Bands b and d correspond to nicked and supercoiled forms of the native-target plasmid, respectively. Lanes 14–25 (A) and lanes 2–25 (B–D) represent independent Ade$^+$ Lys$^+$ colonies that originated from cells grown on SC(galactose) −Ura −His medium. Bands a and c correspond to nicked and supercoiled forms of uncut target plasmid into which Ty*3* has transposed.

was induced by plating 1×10^6 cells onto SC (galactose) −Ura −His medium. As a control, 1×10^6 cells of the same culture were plated onto SC −Ura −His medium. After periods of growth to attain similar numbers of cell divisions (see Figure 2), the SC (galactose), and SC −Ura −His plates were replicaplated onto SC −Ade −Lys plates to select for cells in which *SUP2bo* expression was activated by integration of Ty*3* into pCH2bo19V. Cells containing activated *SUP2bo* genes were detected as colonies growing on the selective medium after 4–5 days. Several hundred colonies grew on the SC −Ade −Lys medium from cultures grown on SC (galactose) medium, whereas only a few colonies grew on the SC −Ade −Lys medium from control cultures grown on SC medium (Figure 3, Table 2).

Target plasmids were isolated from Ade$^+$ Lys$^+$ colonies and assessed for integration products. An example of this analysis is shown in Figure 4. Plasmid DNAs from Ade$^+$ Lys$^+$ cells were separated in agarose gels, transferred to nitrocellulose filters and hybridized to a *HIS3*-specific probe. Target plasmids that contained Ty*3* elements were expected to increase in size from 9.4 to 14.8 kbp, which could be distinguished from native target plasmids on the basis of reduced mobilities of the intact plasmids through the agarose gel (Figure 4). The hybridization patterns shown in lanes 2–13 of Figure 4A are indicative of native target plasmids. These DNA samples were prepared from Ade$^+$ Lys$^+$ colonies that originated from control cultures grown on SC −Ura −His medium. The b and d bands correspond to nicked- and supercoiled-circular DNA, respectively. The hybridization patterns of DNA from cultures grown on SC (galactose) −Ura −His medium shown in lanes 14–25 of Figure 4A and lanes 2–25 of Figure 4, B–D, are indicative of mixtures of native target plasmids and target plasmids of significantly increased molecular weight that indicated integration of Ty*3* into the target plasmid. In a separate analysis of 90 samples, >95% (86/90) of the Ade$^+$ Lys$^+$ colonies contained target plasmids with integrated Ty*3* elements. Because target plasmids that did not contain Ty*3* elements were not discriminated against in this assay, they were often retained in the Ade$^+$ Lys$^+$ cells. Greater than 80% of the samples analyzed in this experiment contained two forms of the *CEN4*-marked target plasmid. Thus, at least two copies of the native target plasmid were present in nearly all cells prior to integration of Ty*3*. Target plasmids were isolated (see MATERIALS AND METHODS) and a subset was subjected to nucleotide sequence analysis to confirm that the increase in size was due to acquisition of a Ty*3* element (refer to Figure 5, discussed below).

Reverse primer extension using an oligonucleotide that specifically annealed to the *sup2bo* (described in KINSEY and SANDMEYER 1991) intron demonstrated that Ty*3* transposition into the target plasmid resulted in

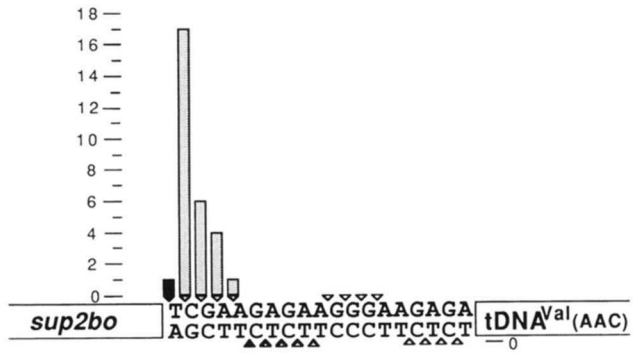

FIGURE 5.—Positions of Ty3 insertion under selection for suppressor tRNA gene expression. Coding sequences of the divergent tRNA genes of the target plasmid are represented by open boxes and are labeled. The nucleotide sequence of the region between the divergent tRNA genes is shown. The positions and number of independent integration events that were isolated at those positions are represented by the location and height of the bars, respectively. The bars shown in stiple represent integration events that occurred 16–19 bp upstream of the coding sequence of the $tRNA^{Val}$(AAC) gene. The stipled triangles shown below the nucleotide sequence represent the extent of the 5-bp duplication of insertion site sequence that occurred as a result of the transposition events at positions 16–19 bp upstream of the $tRNA^{Val}$(AAC) gene coding sequence. The black bar represents a single integration event that occurred at −20 bp relative to the $tRNA^{Val}$(AAC) gene coding sequence. The black triangle indicates the extent of the 5-bp duplication of insertion site sequence that occurred as a result of the transposition event represented by the black bar. Open triangles shown above and below the nucleotide sequence represent the boundaries of the 5-bp insertion site sequences predicted to be duplicated in transposition events that occurred 16–19 bp upstream of the *sup2bo* gene.

the initiation of *SUP2bo* transcription within Ty3 sequence. The transcription initiation sites for the wild-type *sup2b* gene are at −10 (major) and −12 (minor) (KINSEY and SANDMEYER 1991). Total RNA was isolated from cells (ELDER *et al.* 1983) carrying a native target plasmid and from cells carrying a target plasmid into which Ty3 had inserted and was present 19-bp upstream of $tRNA^{Val}$(AAC) coding sequence. The initiation site for *SUP2bo* transcription in the plasmid containing the Ty3 insertion was mapped to position −10, relative to *SUP2bo* coding sequence, which corresponds to an adenine nucleotide 4 bp within Ty3 sequence (data not shown). Overexposure of the autoradiograph also revealed extension products from RNA samples isolated from cells containing only the native target plasmid. This indicated that there was leaky expression of *sup2bo* on the native target plasmid. Nevertheless, cells that bore this plasmid lacked the suppressor phenotype (Figure 1). This result validated the design of the genetic selection for the site-specific transposition of Ty3.

After induction of Ty3 transcription in cells grown on SC (galactose) −Ura −His medium, 4/90 of the resulting Ade^+ Lys^+ colonies contained target plasmids with no detectable, stably integrated Ty3 elements. Target plasmids were isolated from over 50 independent Ade^+ Lys^+ colonies (from several independent experiments) arising from cells grown on SC −Ura −His medium and analyzed by restriction enzyme digestion. None of these were found to contain Ty3 insertions. We reasoned that there were several possible sources of these Ade^+ Lys^+ colonies. First, new mutations, recombination or gene conversion between plasmid and genomic sequences could have resulted in a genomic $tRNA^{Tyr}$ gene with an ochre-suppressing anticodon. Second, sequence changes or small insertions within the tRNA gene transcription initiation region of the target plasmid or sequence changes within the tRNA gene promoter elements might have allowed increased expression of the *sup2bo* gene. Third, new mutations could enhance the expression of the plasmid-borne debilitated suppressor. Intragenic reversion of the genomic alleles *ade2-101o* and *lys2-1o* (Table 1) was considered unlikely due to the low combined reversion rate expected for the two independent mutations.

The source of the suppressor phenotype in the control (uninduced) class of Ade^+ Lys^+ colonies was investigated. Ade^+ Lys^+ cells arising from growth on SC −Ura −His medium were cured of plasmids. Five of five independent Ade^+ Lys^+ isolates from these control glucose-grown cultures displayed the Ade^+ Lys^+ phenotype after being cured of the target plasmid. This indicated that the primary source of Ade^+ Lys^+ colonies from control cultures was genomic mutation.

To determine whether the suppressor phenotype of cells grown on SC (galactose) −Ura −His medium was plasmid dependent, Ade^+ Lys^+ colonies were streaked onto rich medium to allow loss of the target plasmid. Four cases in which there were Ade^+ Lys^+ colonies in the absence of evidence from the cracking gel of a Ty3 insertion were analysed in more detail. Two of these colonies retained the Ade^+ Lys^+ phenotype in the absence of the plasmid and so probably had acquired a genomic suppressor. Two colonies cosegregated suppressor activity together with the plasmid. Restriction enzyme digestion and sequence analysis showed that they had acquired a sigma element within the region of most Ty3 insertions (data not shown). Whether these products resulted from integration of single sigma elements or recombination between the two sigma elements that flanked newly integrated Ty3 elements is unknown.

Ty3 transposition events in the target plasmid have the characteristics of genomic transposition events: Although the divergently oriented tRNA genes of the target plasmid did serve as a target for Ty3 integration, the effect of this organization of target genes on Ty3 integration was unknown. For instance, TFIIIB and TFIIIC have been shown to bend the tRNA gene tem-

plate near the initiation site (LEVEILLARD *et al.* 1991). Competition between the two tRNA gene promoters for transcription factor binding was expected because of overlap of the TFIIIB binding region of each gene with the TFIIIC binding region of the other gene. Heterologous proteins bound to the region occupied by TFIIIB have been shown to result in elimination of tRNA gene transcription (DINGERMANN *et al.* 1992) or significant decreases in tRNA gene transcription concomitant with changes in positions of transcription factor binding and sites of transcription initiation (LEVEILLARD *et al.* 1993). Each of these circumstances were potential sources of irregular integration events within the divergent tRNA gene target plasmid.

Insertions of Ty*3* into the divergent tRNA gene target were analyzed to determine whether this target functioned similarly to a single tRNA gene target. Characteristics of typical Ty*3* integrations include: (1) a 5-bp direct repeat of target sequence flanking the integrated Ty*3* element, (2) Ty*3* sequence located 16–19 bp upstream of the tRNA gene coding sequence and (3) Ty*3* oriented such that the direction of transcription of Ty*3* and the adjacent tRNA gene are divergent in approximately two-thirds of the insertions (SANDMEYER *et al.* 1988). The results of the sequence analysis of 29 independent transposition products obtained through the selection procedure are presented in Figure 5. Sequences, flanking both ends of the Ty*3* elements, were analyzed and 5-bp direct repeats were demonstrated for all insertions. Ty*3* sequence was located 16–19 bp upstream of the base pair that encodes the first nucleotide of the mature $tRNA^{Val}$(AAC) in 28 of 29 independent insertions. In one isolate, the position of Ty*3* sequence was 20 bp upstream of the $tRNA^{Val}$(AAC) gene. Ty*3* elements were integrated in the more common orientation in 16 of the 29 transposition products (data not shown). However, both the positions of insertion and short direct repeats were the same as for genomic insertions. This argued that selected insertions had important fundamental properties in common with genomic insertions.

The selection for Ty*3* transposition detects a subset of transposition events: To estimate the total number of transposition events that occurred in the genome from those detected by the selection, additional information was required. Although Ty*3* integration sites within the divergent tRNA gene target displayed the characteristics of natural integration events, the target activity of the divergent tRNA gene relative to that of a single tRNA gene was unknown. Also, because transposition events targeted by the *sup2bo* gene of the target were not predicted to be detected phenotypically, it was unknown to what extent such events occurred. As mentioned in the Introduction, the helper/donor assay for Ty*3* transposition (CHALKER and SANDMEYER 1992, see MATERIALS AND METHODS) was useful for determining the competitive ability of different Ty*3* integration targets (present on the same plasmid) to be used as sites for Ty*3* integration. This assay, which did not depend on expression of *sup2bo* and therefore the position of Ty*3* insertion, was used to determine the usage of the divergent tRNA gene target relative to that of a single tRNA gene. In addition, it was used to determine the percentage of transposition events into the divergent tRNA gene target actually detected by the selection procedure.

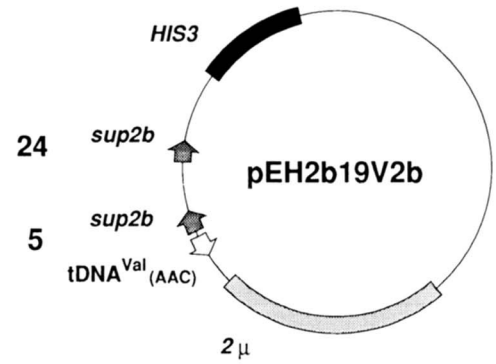

FIGURE 6.—Divergent- *vs.* single-tRNA gene target utilization. The plasmid used in the competition assay is shown with yeast sequences indicated. The bold numerals adjacent to the different targets indicate the number of independent transposition events that were detected at these targets out of 29 integrations into the plasmid when no selection for the site of integration was imposed.

Plasmid pCH2bo19V was modified for use in the helper/donor assay by converting it to a high-copy number plasmid and reverting the ochre suppressing anticodon to wild type (pEH2b19V, Table 1). This plasmid system was based on that described by CHALKER and SANDMEYER (1993); it allowed collection of plasmids containing Ty*3* insertions independent of the position of integration. A high-copy number plasmid was useful for identification of *neo*-marked Ty*3* integrations. The suppressor tRNA gene was converted to wild type to avoid the presence of a potentially detrimental high-copy number gene for a suppressor tRNA. To determine the usage of the divergent tRNA gene target relative to that of a single tRNA gene, a single *sup2b* gene was inserted into pEH2b19V ~1 kbp downstream of, and in the same orientation as, the *sup2b* gene present as part of the divergent tRNA gene target. This plasmid was named pEH2b19V2b and is schematized in Figure 6. Independent Ty*3* transposition products of a *neo*-marked, donor Ty*3* element into this plasmid were obtained. Target plasmids were isolated, positions of Ty*3* transposition were determined by restriction digestion analysis and the target of integration was deduced (data not shown). The results of this experiment are presented in Figure 6. Of 29 independent transposition events into this plasmid, 5 occurred within the diver-

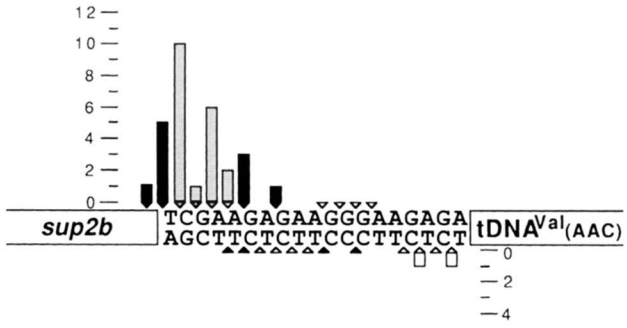

FIGURE 7.—Positions of Ty*3* insertion with no selection for suppressor tRNA gene expression. Coding and intergenic sequences of the divergent tRNA genes of the target plasmid are represented as in Figure 5. The distribution and position of independent integration events are represented by the height and location of the bars, respectively. The bars shown in stiple represent integration events that occurred 16–19 bp upstream of the coding sequence of the $tRNA^{Val}$(AAC) gene. The stipled triangles shown below the nucleotide sequence represent the extent of the 5-bp duplication of insertion site sequence that occurred as a result of the transposition events at positions 16–19 bp upstream of the $tRNA^{Val}$(AAC) gene coding sequence. The bars shown in black represent integration events that occurred outside of the natural window of insertion sites from positions 16–19 bp upstream of the $tRNA^{Val}$(AAC) gene-coding sequence. The triangles shown in black represent the extent of the 5-bp duplication of insertion site sequence that occurred at positions indicated by the black bars. Open bars represent the distribution and position of independently isolated integration events that occurred 16–19 bp upstream of *sup2b*. Open triangles shown above and below the nucleotide sequence represent the boundaries of the 5-bp insertion site sequence duplicated from transposition events that occurred at these positions.

gent tRNA gene target. The remaining 24 events occurred at the single tRNA gene locus.

Plasmid pEH2b19V was used to determine the percentage of transposition events that occur within the divergent tRNA gene target that are not likely to activate suppressor expression and thus not likely to be detected by the selection for transposition. Transposition products of a *neo*-marked, donor Ty*3* element into pEH2b19V were collected and Ty*3* insertion sites were determined by nucleotide sequence analysis. These results are presented in Figure 7. Of 31 independent Ty*3* transposition events analyzed, 24 (77%) occurred within the discrete window of integration sites (16–20 bp upstream of $tRNA^{Val}$(AAC) coding sequence) that allowed recovery by the selection procedure for the divergent tRNA gene target. As evidenced by their detection in the selection, Ty*3* integration events at these positions activate *SUP2bo* expression. Of the seven integrations into positions not detected in the selection, one integration occurred at position −21 relative to $tRNA^{Val}$(AAC) coding sequence. Three integrations (10% of the total) occurred at position −15 relative $tRNA^{Val}$(AAC) coding sequence. One integration occurred at position −13; the gene that acted as the target for this insertion could not be determined. The remaining two insertions occurred at positions consistent with the *sup2b* gene acting as the target for integration and were therefore predicted to be too far upstream to activate *SUP2bo* expression. From this analysis, we concluded that at least 77% of the transposition events within the divergent tRNA gene target activate *sup2bo* gene expression sufficiently to be phenotypically detectable. It is formally possible that integrations at positions −21 and −15 relative to the $tRNA^{Val}$(AAC) gene activate *SUP2bo* expression to detectable levels but occurred too infrequently to have been included in the sample analyzed (Figure 4). In this case, up to 90% of the transposition events within the divergent tRNA gene target activate *sup2bo* gene expression sufficiently to be detected phenotypically.

TABLE 3

Ty*3* transposition rate determined by P(0)

Carbon source	P(0)[a]	Mutation rate to Ade^+ Lys^+[b]	TCF[c]	Transposition rate
Galactose	62/96	$5.2 \pm 0.9 \times 10^{-6}$	1110	$5.8 \pm 1 \times 10^{-3}$

[a] Number of independent samples containing zero Ade^+ Lys^+ colonies per the number of samples tested.

[b] Values are mean ± SE; SE was determined by the method of LEA and COULSON (1949).

[c] Transposition Correlation Factor: the estimated number of genomic Ty*3* transposition events that occurred per Ade^+ Lys^+ colony.

Ty*3* transposition rate: The P(0) method of LURIA and DELBRUCK (1943) was used to determine the mutation rate to the Ade^+ Lys^+ phenotype (Table 3). Cultures containing galactose to induce transposition were inoculated with 9×10^4 cells per ml and allowed to grow to 1.2×10^7 cells per ml. A portion of this culture containing 8.4×10^4 cells was plated onto each of 96 plates of SC −Ade −Lys medium. Sixty-two of the 96 plates did not show growth. A mutation rate to Ade^+ Lys^+ of $5.2 \pm 0.9 \times 10^{-6}$ per division was calculated. This rate was then used to estimate the number of Ty*3* transpositions into the genome per generation. To estimate the transposition rate from the number of Ade^+ Lys^+ colonies, the number of *CEN* plasmids, the activity of the divergent tRNA gene target relative to genomic tRNA genes and the relationship between the number of plasmid-directed transposition events and the number of Ade^+ Lys^+ colonies were considered. Because *CEN* plasmids with and without insertions were recovered from colony isolates, the number of target plasmids per cell was estimated as two (Figure 4). Comparison of a single target to the divergent target by the replica plating assay showed that the single target was used five times more frequently (Figure 6). There are an estimated 360 tRNA genes per haploid genome

(GUTHRIE and ABELSON 1982). If it is assumed that a plasmid-borne tRNA gene mimics the Ty*3* targeting activity of a chromosomal tRNA gene and that transposition is proportional to the number of targets, then each insertion in the target plasmid reflects 5/2 × 360, or 900 genomic transposition events. Data from plasmid target assays showing the percentage of Ade$^+$ Lys$^+$ colonies that contained Ty*3* or sigma insertions (95%) and the percentage of insertions into the target plasmids that resulted in Ade$^+$ Lys$^+$ colonies (at least 77%) were used to estimate the number of transposition events into the plasmid from the number of Ade$^+$ Lys$^+$ colonies. Thus, (0.95 insertions that activate *SUP2bo* expression per Ade$^+$ Lys$^+$ colony) × (one divergent target insertion per 0.77 insertions that activate *SUP2bo* expression) = 1.23, the number of divergent target insertions per Ade$^+$ Lys$^+$ colony. The product of the relative use of the genomic target to the divergent plasmid target (900) and the insertions represented per Ade$^+$ Lys$^+$ colony (1.23) is 1110. The product of this factor (1110) and the rate of mutation to Ade$^+$ Lys$^+$ ($5.2 \pm 0.9 \times 10^{-6}$) per cell division is a transposition rate of $5.8 \pm 1.0 \times 10^{-3}$ transposition events per cell division.

Ty*3* transposes in populations of mating yeast: Exposure of haploid yeast to the mating pheromone of the opposite cell type results in alterations in gene expression that lead to cellular differentiation and arrest of the cell cycle in G1 in preparation for mating (reviewed in SPRAGUE and THORNER 1992). Ty*3* transcription is induced 30- to 40-fold by the mating pheromone α-factor and is repressed by mating-type control (VAN ARSDELL *et al.* 1987; CLARK *et al.* 1988; BILANCHONE *et al.* 1993). This pattern of Ty*3* transcription suggested that Ty*3* transposition occurs naturally during mating. To test that hypothesis, cells that lacked endogenous Ty*3* elements but which contained plasmid-borne Ty*3* under control of its natural promoter were exposed to α-factor and assessed for transposition using a modification of the selection scheme described above (see MATERIALS AND METHODS). Transposition of Ty*3* into the target plasmid was verified by Southern blot analysis of target plasmids isolated from Ade$^+$ Lys$^+$ cells.

To determine whether transposition could be produced under physiological conditions of induction, transposition of Ty*3* was assessed in mating yeast (see MATERIALS AND METHODS). *MAT***a** (yTM444) and *MAT*α (yVB114) cells carrying plasmids pEUTy3-1 and pCH2bo19V, respectively, were grown separately, mixed and plated onto rich medium to allow mating. These strains had no endogenous Ty*3* elements. Cells that contained target plasmids into which Ty*3* had transposed were selected from diploid isolates as described in MATERIALS AND METHODS. The reciprocal experiment, in which pEUTy3-1 was carried in the *MAT*α strain and pCH2bo19V was carried in the *MAT***a** strain, was also performed. In each case, the cells carrying the target plasmid were present at 10-fold the level of cells carrying the Ty*3* element. Mating efficiencies of cells carrying pEUTy3-1 were ~35% for experimental cultures and were 24 and 56% for control experiments 9 and 10, respectively. The results from these experiments are summarized in Table 4. Control populations in which (1) the Ty*3* element of mating cells was not inducible by pheromone (Table 4, experiments 9 and 10) and (2) the target plasmid and the plasmid-borne pheromone-inducible Ty*3* element were present in either *MAT***a** or (3) *MAT***a**/*MAT*α cells (Table 4, experiments 11 and 12, respectively) displayed no evidence of transposition. Although the number of Ade$^+$ Lys$^+$ colonies from experimental cultures fluctuated, they were at least 10-fold above those of control cultures (experiments 9–12). The fluctuation is presumably due to the two replicaplating steps, in which the transfer of cells is variable. Acquisition of Ty*3* by the target plasmids was confirmed by Southern blot analysis (as described above) of the size of intact target plasmids isolated from the diploid Ade$^+$ Lys$^+$ colonies. As in the case of the results from the selection for transposition in haploid cells, ~90% of the Ade$^+$ Lys$^+$ colonies yielded target plasmids that contained complete Ty*3* elements. Nucleotide sequence analysis of the Ty*3* integration sites from a subset of these target plasmids confirmed that those events displayed the characteristics of genomic Ty*3* transposition events as described above (data not shown). Thus, Ty*3* was shown to transpose under the control of its natural promoter and in either haploid cell type during a discrete window of the yeast life cycle: mating.

DISCUSSION

We have described a direct selection for Ty*3* transposition events into a novel plasmid-based target. This procedure exploits the position specificity of integration displayed by Ty*3* and obviates the previous requirement of a helper-Ty*3* element to complement transposition of a genetically marked, transpositionally defective Ty*3* donor element. Insertions of Ty*3* into the target plasmid were flanked by the characteristic 5-bp direct repeat and occurred primarily at the positions observed for insertions upstream of genomic tRNA genes. This assay was used to estimate the Ty*3* transposition frequency and to demonstrate that transposition of Ty*3* occurs naturally in mating populations of cells.

The distribution of integration sites in the divergent tRNA gene target determined without *sup2bo* activation was biased toward those targeted by the tRNA^{Val}(AAC) gene (28/30 positions in which the target tRNA gene could be deduced). Although target activity is not a direct function of sequence or pol III activity, it has been shown to depend on the ability of a gene to bind transcription factors (CHALKER and SANDMEYER 1992).

TABLE 4

Ty3 transposition induced by mating

Experiment	Plasmid carried in			Ade$^+$ Lys$^+$ colonies[a]
	*MAT***a** cells	*MAT*α cells	*MAT***a**/α cells	
1	pEUTy3-1	pCH2bo19V	—	16, 8
2	pEUTy3-1	pCH2bo19V	—	23, 30
3	pEUTy3-1	pCH2bo19V	—	17, 31, 5, 7
4	pCH2bo19V	pEUTy3-1	—	33, 34, 7, 0
5	pCH2bo19V	pEUTy3-1	—	39, 34, 0
6	pCH2bo19V	pEUTy3-1	—	45, 56, 0
7	pCH2bo19V	pEUTy3-1	—	35, 23, 0, 7, 15
8	pCH2bo19V	pEUTy3-1	—	32, 18, 16, 0, 39
9	pCH2bo19V	pEGTy3-1	—	0, 0, 4
10	pCH2bo19V	pEGTy3-1	—	1, 5, 0
11	pCH2bo19V	—	—	0, 0, 0, 0
	pEUTy3-1	—		
12	—	—	pCH2bo19V	0, 0, 0, 0
	—	—	pEUTy3-1	

[a] Each number represents the Ade$^+$ Lys$^+$ colonies present on separate SC −Ade −Lys plates.

Recent studies of the U6 snRNA gene have also shown that factors are bound upstream of the target site at the time of integration (CHALKER and SANDMEYER 1993). Therefore, the most simple explanation of the observed tRNA gene bias is the relative ability of the divergently oriented tRNA genes to bind transcription factors in their respective 5′-flanking regions. The 5′-flanking sequence for each gene contains the 19-bp intergenic region. In addition, for the *sup2bo* gene it is comprised of tRNA^{Val}(AAC) coding sequence and for the tRNA^{Val}(AAC) gene, the *sup2bo* coding sequence. However, this juxtaposition of the genes means that TFIIIB binding for each gene, which is mediated by TFIIIC, must occur on the TFIIIC binding region of the opposing tRNA gene. Sequences in the 5′-flanking region are known to influence tRNA gene expression (SPRAGUE *et al.* 1980; DEFRANCO *et al.* 1981; SHAW and OLSON 1984), presumably by influencing TFIIIB interaction with the DNA. Thus, differences in the tRNA gene 5′-flanking sequences could serve as a source of integration site bias in the divergent tRNA gene target based on TFIIIB interactions with the templates. Consistent with this supposition, expansion of the spacing of the divergent tRNA genes, which was predicted to relieve the overlap between TFIIIC and TFIIIB binding regions, resulted in a relaxation of the bias of integration events at the tRNA^{Val}(AAC) gene. A variant of target plasmid pEH2b19V was also used in conjunction with the helper/donor assay to generate a set of nonselected Ty3 insertions. The variant target plasmid, pEH2b25V, contained an additional 6 bp or a total of 25 bp between the two tRNA gene coding sequences that extended the length of the polypyrimidine/polypurine tract between the two genes. In this analysis, approximately one-third (5/16) of the independently isolated transposition products displayed Ty3 elements in positions consistent with the *sup2b* gene acting as the target for integration (data not shown).

Detection of Ty3 transposition events with the plasmid target revealed that Ty3 transposition occurred at nearly one transposition event per 100 cell divisions (0.6×10^{-2}) (Table 3) when Ty3 was expressed from a galactose-inducible promoter on a high copy-number plasmid. This rate of Ty3 transposition refines an earlier rough estimate of Ty3 transposition that used the same Ty3-containing plasmid, pEGTy3-1 (HANSEN *et al.* 1988) to generate genomic Ty3 transposition events. In that case, 50 isolates of each of 10 colonies, grown on galactose-containing medium for 10 days and which had subsequently lost plasmid pEGTy3-1, were assayed for transposition by colony hybridization. Approximately 7% (6.6×10^{-2}) of the isolates from each galactose-grown colony showed evidence of having acquired a genomic Ty3 element. The current study represents a refinement that more accurately reflects the activity of Ty3 in growing cells by yielding a rate of transposition derived from statistical analysis of a large number of independent cultures grown over a short period of time.

Transposition of Ty1 and Ty2 yeast copialike elements distantly related to Ty3 is not expected to occur at elevated frequencies under the conditions used in our experiments. Endogenous Ty1 elements are neither naturally galactose inducible nor pheromone inducible. In addition, expression of Ty3 does not stimulate or complement transposition of Ty1 to high levels (CHALKER and SANDMEYER 1992). Although Ty1 could transpose into the target and activate expression of the suppressor tRNA gene, activation requires integration within a region of <10 bp upstream of the tRNA gene. The preference of Ty1 for tRNA genes is observed only over 500- to 1-kb regions (JI *et al.* 1993).

Our measurements allow a rough comparison of the transposition frequency of a galactose-inducible Ty*3* element to that of a similarly regulated Ty1 element (CURCIO and GARFINKEL 1991). A *HIS3* gene carrying an artificial antisense-intron was inserted into a Ty*1* element so that the intron was in the sense orientation with respect to the Ty*1* transcript. Induction of Ty*1* transcription from a *GAL1* promoter and splicing of the intron from the Ty*1* genomic RNA allowed *HIS3* expression upon integration of the marked element. When this marked-Ty*1* element was present on a high copy-number plasmid the resulting frequency of Ty*1* transposition was estimated at greater than one transposition event per cell division (CURCIO and GARFINKEL 1991), ~200-fold higher than that of Ty*3*. Because little is understood about processes other than transcription, which may control rate-limiting steps in retrotransposition, it may be instructive to consider, qualitatively, factors that differentially influence transposition of these two classes of yeast elements. One obvious functional difference that could account for a difference in transposition rates is the insertion of Ty*3* with a high degree of specificity into a genomic target comprised of ~360 tRNA genes (CHALKER and SANDMEYER 1990). Ty*1* transposition exhibits less specificity and theoretically results in a correspondingly greater target size, potentially approaching the size of the genome itself. Depending on the extent to which target size is limiting for transposition, this could explain several orders of magnitude difference in transposition rates between induced Ty*1* and Ty*3* elements. It is important to note, however, that the effective Ty*1* target size is probably not four or five orders of magnitude larger than the number of potential Ty*3* genomic targets, as it would be if Ty*1* displayed no bias (JI *et al.*, 1993). It is therefore possible that the gross regions of genomic accessibility for Ty*1* and Ty*3* are similarly set, by chromatin structures induced by the pol III transcription machinery in the case of Ty*1* and by the pol III transcription complex itself in the case of Ty*3*.

In contrast to transposition rates in cells carrying inducible fusion elements, transposition rates in cells carrying the natural complement of Ty*1* and Ty*3* elements, are actually quite low. Ty*1* transcript levels under these conditions are nevertheless high and the low rate of transposition appears to result in part at least from limited availability of active particle components (CURCIO and GARFINKEL 1992). Ty*3* is present in lower-copy number than Ty*1* and transcript levels are extremely low in the absence of pheromone induction, even in haploid cells. Thus, coarsely approximate similarities in transposition rates between these different classes of elements may be set by independent mechanisms.

Under the transcriptional control of its natural promoter, Ty*3* was shown to transpose efficiently in mating populations of yeast. The pheromonal induction of Ty*3* has two interesting ramifications with respect to temporal coordination of transposition with the cell cycle: (1) Ty*3* transcription is induced as the cell cycle is arrested in G1 in preparation for mating and (2) this profile of Ty*3* transcription regulation limits mobilization of Ty*3* to the time near diploidization. Induction of Ty*3* transcription as cells are arrested in G1 is particularly interesting in light of results from several studies that show incomplete retroviral replication in nondividing cells (FRITSCH and TEMIN 1977; VARMUS *et al.* 1977; CHEN and TEMIN 1982). HIV infection of quiescent lymphocytes is blocked during reverse transcription but this block is reversed by mitogenic stimulation (ZACK *et al.* 1990, 1992). Whether Ty*3* reverse transcription and integration occur in G1-arrested cells is an interesting question not addressed by these experiments. However, once cells have mated, *de novo* Ty*3* transcription and transposition is repressed by mating-type control and thus blocked in diploid cells (CLARK *et al.* 1988; BILANCHONE *et al.* 1992). Therefore, our results demonstrated that the limited induction of Ty*3* transcription that occurs as cells are arrested in G1 is sufficient to support transposition of Ty*3* after the cells have mated.

Strict temporal regulation of some other eukaryotic mobile elements is documented. In fact several elements are mobilized exclusively in haploid or gametic cells. Ty*1* is transcribed in haploid yeast, but is repressed in diploids by mating-type control (ELDER *et al.* 1980). Pheromones do not induce expression of Ty*1* as occurs for Ty*3*. However, Ty*1* particles are destablized during α-factor arrest (XU and BOEKE 1991). *P* elements of Drosophila transpose only in germ line cells where the 2–3 intron of the *P* element RNA is spliced to encode a functional transposase (LASKI *et al.* 1986). Likewise, *LINE* transposition in germ line tissue has been deduced (DOMBROWSKI *et al.* 1991) and expression of these elements is elevated in germ line tissue (MARTIN and BRANCIFORTE 1993). Our experiments demonstrated that Ty*3* can transpose at high rates and identified a brief time in the yeast cell life cycle when this transposition occurs naturally. Because of the lack of an extracellular phase in the retrotransposon life cycle, persistence of the retrotransposon is dependent upon survival of the cell and stringent regulation of transposition may be important. Ty*3* transposition is repressed by mating-type control in diploid cells. It is further controlled through its position-specificity for tRNA genes where influences on gene expression is limited (KINSEY and SANDMEYER 1991). However, loss of this exquisite specificity could prove lethal to both cell and retrotransposon. In eukaryotic cells, including yeast that exist primarily as diploid organisms, linking retrotransposition to germ line or haploid regulated expression may represent an economical mode of restricting genomic damage.

We thank DOUGLAS CHALKER for helpful discussions and DENIS GUTTRIDGE for technical assistance. This work was supported by U.S. Public Health Service grant GM-33281 to S.B.S. from the National Institutes of Health (NIH). P.T.K. was supported by a predoctoral training grant from the NIH (GM-0713417).

LITERATURE CITED

BAKER, R. E., S. CAMIER, A. SENTENAC and B. D. HALL, 1987 Gene size differentially affects the binding of yeast transcription factor tau to two intragenic regions. Proc. Natl. Acad. Sci. USA **84:** 8768–8772.

BILANCHONE, V. W., J. A. CLAYPOOL, P. T. KINSEY and S. B. SANDMEYER, 1993 Positive and negative regulatory elements control expression of the yeast retrotransposon Ty*3*. Genetics **134:** 685–700.

BOEKE, J. D., F. LACROUTE and G. R. FINK, 1984 A positive selection for mutants lacking orotidine-5′-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. Mol. Gen. Genet. **197:** 345–346.

BOEKE, J.D., and S.B. SANDMEYER, 1991 Yeast transposable elements, pp.193–261 in *The Molecular and Cellular Biology of the Yeast Saccharomyces cerevisiae,* edited by J. R. BROACH, E. W. JONES and J. PRINGLE. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

CHALKER, D. L., and S. B. SANDMEYER, 1990 Transfer RNA genes are genomic targets for de novo transposition of the yeast retrotransposon Ty*3*. Genetics **126:** 837–850.

CHALKER, D. L. and S. B. SANDMEYER, 1992 Ty*3* integrates within the region of RNA polymerase III transcription initiation. Genes Dev. **6:** 117–128.

CHALKER, D. L., and S. B. SANDMEYER, 1993 Sites of RNA polymerase III transcription initiation and Ty*3* integration at the U6 gene are positioned by the TATA box. Proc. Natl. Acad. Sci. USA **90:** 4927–4931.

CHEN, I. S. Y., and H. M. TEMIN, 1982 Establishment of infection by spleen necrosis virus: inhibition in stationary cells and the role of secondary infection. J. Virol. **41:** 183–191.

CLARE, J. J., M. BELCOURT and P. J. FARABAUGH, 1988 Efficient translational frameshifting occurs within a conserved sequence of the overlap between the two genes of a yeast Ty*1* transposon. Proc. Natl. Acad Sci. USA **85:** 6816–6820.

CLARK, D. J., V. W. BILANCHONE, L. J. HAYWOOD, S. L. DILDINE and S. B. SANDMEYER, 1988 A yeast sigma composite element, Ty*3*, has properties of a retrotransposon. J. Biol. Chem. **263:** 1413–1423.

CURCIO, M. J., and D. J. GARFINKEL, 1991 Single-step selection for Ty*1* element retrotransposition. Proc. Natl. Acad. Sci. USA **88:** 936–940.

CURCIO, M. J., and D. J. GARFINKEL, 1992 Posttranslational control of Ty*1* retrotransposition occurs at the level of protein processing. Mol. Cell. Biol. **12:** 2813–2825.

DEFRANCO, D., S. SHARP and D. SOLL, 1981 Identification of regulatory sequences contained in the 5′-flanking region of Drosophila lysine $tRNA_2$ genes. J. Biol. Chem. **256:** 12424–12429.

DEL REY, F. J., T. F. DONAHUE and G. R. FINK, 1982 Sigma, a repetitive element found adjacent to tRNA genes of yeast. Proc. Natl. Acad. Sci. USA **79:** 4138–4142.

DINGERMANN, T., U. FRANK-STOLL, H. WERNER, A. WISSMANN, W. HILLEN *et al.,* 1992 RNA polymerase III catalyzed transcription can be regulated in *Saccharomyces cerevisiae* by the bacterial tetracycline repressor-operator system. EMBO J. **11:** 1487–1492.

DOMBROWSKI, B. A., S. L. MATHIAS, E. NANTHAKUMAR, A. F. SCOTT, and H. H. KAZAZIAN, JR., 1991 Isolation of an active human transposable element. Science **254:** 1805–1808.

DOWER, W. J., J. F. MILLER and C. W. RAGSDALE, 1988 High efficiency transformation of *E. coli* by high voltage electroporation. Nucleic Acids Res. **16:** 6127–6145.

ELDER, R. T., T. P. ST. JOHN, D. T. STINCHCOMB and R. W. DAVIS, 1980 Studies on the transposable element Ty*1* of yeast. I. RNA homologous to Ty*1*. Cold Spring Harbor Symp. Quant. Biol. **45:** 581–584.

ELDER, R. T., E. Y. LOH and R. W. DAVIS, 1983 RNA from the yeast transposable elementTy*1* has both ends in the direct repeats, a structure similar to retrovirus RNA. Proc. Natl. Acad. Sci. USA **80:** 2432–2436.

FABRIZIO, P., A. COPPO, P. FRUSCOLONI, P. BENNEDETTI, G. DI SEGNI *et al.,* 1987 Comparative mutational analysis of wild-type and stretched $tRNA^{Leu}$ gene promoters. Proc. Natl. Acad. Sci. USA **84:** 8763–8767.

FARABAUGH, P. J., H. ZHAO, S. PANDE and A. VIMALADITHAN, 1993 Translational frameshifting expresses the *POL3* gene of retrotransposon Ty*3* of yeast. Cell **74:** 93–103.

FEINBERG, A. P. and B. VOGELSTEIN, 1983 A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132:** 6–13.

FEINBERG, A. P. and B. VOGELSTEIN, 1984 Addendum. Anal. Biochem. **137:** 266–267.

FRITSCH, E. F., and H. M. TEMIN, 1977 Inhibition of viral DNA synthesis in stationary chicken embryo fibroblasts infected with avian retroviruses. J. Virol. **24:** 461–469.

GEIDUSCHEK, E. P., and G. P. TOCCHINI-VALENTINI, 1988 Transcription by RNA polymerase III. Annu. Rev. Biochem. **57:** 873–914.

GUTHRIE, C., and J. ABELSON, 1982 Organization and expression of tRNA genes in *Saccharomyces cerevisiae,* pp. 487–528 in *The Molecular Biology of the Yeast Saccharomyces,* edited by J. STRATHERN, E. W. JONES and J. R. BROACH, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

HANSEN, L. J., and S. B. SANDMEYER, 1990 Characterization of a transpositionally active Ty*3* element and identification of the Ty*3* integrase protein. J. Virol. **64:** 2599–2607.

HANSEN, L. J., D. L. CHALKER and S. B. SANDMEYER, 1988 Ty*3*, a yeast retrotransposon associated with tRNA genes, has homology to animal retroviruses. Mol. Cell. Biol. **8:** 5245–5256.

HOFFMAN, C. S., and F. WINSTON, 1987 A ten-minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation into *Escherichia coli.* Gene **57:** 267–272.

JI, H., D. P. MOORE, M. A. BLOMBERG, L. T. BRAITERMAN, D. F. VOYTAS *et al.,* 1993 Hotspots for unselected Ty*1* transposition events on yeast chromosome III are near tRNA genes and LTR sequences. Cell **73:** 1007–1018.

KASSAVETIS, G. A., D. L. RIGGS, R. NEGRI, L. H. NGUYEN and E. P. GEIDUSCHEK, 1989 Transcription factor IIIB generates extended DNA interactions in RNA polymerase III transcription complexes on tRNA genes. Mol. Cell. Biol. **9:** 2551–2566.

KASSAVETIS, G. A., B. R. BRAUN, L. H. NGUYEN and E. P. GEIDUSCHEK, 1990 *S. cerevisiae* TFIIIB is the transcription initiation factor proper of RNA polymerase III, while TFIIIA and TFIIIC are assembly factors. Cell **60:** 235–245.

KINSEY, P. T. and S. B. SANDMEYER, 1991 Adjacent pol II and pol III promoters: transcription of the yeast retrotransposon Ty*3* and a target tRNA gene. Nucleic Acids Res. **19:** 1317–1324.

KIRCHNER, J. and S. B. SANDMEYER, 1993 Proteolytic processing of Ty*3* proteins is required for transposition. J. Virol. **67:** 19–28.

KIRCHNER, J., S. B. SANDMEYER and D. B. FORREST, 1992 Transposition of a Ty*3 GAG3-POL3* fusion mutant is limited by availability of capsid protein. J. Virol. **66:** 6081–6092.

KUNKEL, T. A., 1985 Rapid and efficient site-specific mutagenesis without phenotypic selection. Proc. Natl. Acad. Sci. USA **82:** 488–492.

LASKI, F. A., D. C. RIO and G. M. RUBIN, 1986 Tissue specificity of Drosophila *P* element transposition is regulated at the level of mRNA splicing. Cell **44:** 7–19.

LASSAR, A. B., P. L. MARTIN, and R. G. ROEDER, 1983 Transcription of class III genes: formation of preinitiation complexes. Science **222:** 740–748.

LEA, D. E., and C. A. COULSON, 1949 The distribution of the numbers of mutants in bacterial populations. J. Genet. **49:** 264–285.

LEVEILLARD, T., G. A. KASSAVETIS and E. P. GEIDUSCHEK, 1991 *Saccharomyces cerevisiae* transcription factors IIIB and IIIC bend DNA of the $tRNA^{Gln}$ gene. J. Biol. Chem. **266:** 5162–5168.

LEVEILLARD, T., G. A. KASSAVETIS and E. P. GEIDUSCHEK, 1993 Repression and redirection of *Saccharomyces cerevisiae* tRNA synthesis from upstream of the transcriptional start site. J. Biol. Chem. **268:** 3594–3603.

LURIA, S. E., and M. DELBRÜCK, 1943 Mutations of bacteria from virus sensitivity to virus resistance. Genetics **28:** 491–511.

MANIATIS, T., E. F. FRITSCH and J. SAMBROOK, 1982 Molecular clon-

ing: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor Press, NY.

MARTIN, S. L., and D. BRANCIFORTE, 1993 Synchronous expression of *LINE-1* RNA and protein in mouse embryonal carcinoma cells. Mol. Cell. Biol. **13:** 5383–5392.

SANDMEYER, S. B., and M. V. OLSON, 1982 Insertion of a repetitive element at the same position in the 5′-flanking regions of two dissimilar yeast tRNA genes. Proc. Natl. Acad. Sci. USA **79:** 7674–7678.

SANDMEYER, S. B., V. W. BILANCHONE, D. J. CLARK, P. MARCOS, G. F. CARLE *et al.*, 1988 Sigma elements are position-specific for many different yeast tRNA genes. Nucleic Acids Res. **16:** 1499–1515.

SANGER, F., S. NICKLEN and A. R. COULSON, 1977 DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:** 5463–5467.

SCHIESTL, R. H., and R. D. GIETZ, 1989 High efficiency transformation of intact yeast cells using single stranded nucleic acids as a carrier. Curr. Genet. **16:** 339–346.

SEGALL, J., T. MATSUI and R. G. ROEDER, 1980 Multiple factors are required for the accurate transcription of purified genes by RNA polymerase III. J. Biol. Chem. **255:** 11986–11991.

SHAW, K. J., and M. V. OLSON, 1984 Effects of altered 5′-flanking sequences on the *in vivo* expression of a *Saccharomyces cerevisiae* $tRNA^{Tyr}$ gene. Mol. Cell. Biol. **4:** 657–665.

SHERMAN, F., G.R. FINK and J.B. HICKS, 1986 *Methods in Yeast Genetics.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

SPRAGUE, G. F., JR., 1991 Assay of yeast mating reaction. Methods Enzymol. **194:** 77–93.

SPRAGUE, G. F., JR., and J. W. THORNER, 1992 Pheromone response and signal transduction during the mating process of *Saccharomyces cerevisiae,* pp. 657–744 in *The Molecular and Cellular Biology of the Yeast Saccharomyces cerevisiae,* edited by J. R. BROACH, E. W. JONES and J. PRINGLE. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

SPRAGUE, K. U., D. LARSON and D. MORTON, 1980 5′ Flanking sequence signals are required for activity of silkworm alanine tRNA genes in homologous *in vitro* transcription systems. Cell **22:** 171–178.

VAN ARSDELL, S. W., G. L. STETLER and J. THORNER, 1987 The yeast repeated element sigma contains a hormone-inducible promoter. Mol. Cell. Biol. **7:** 749–759.

VARMUS, H. E., T. PADGETT, S. HEASLEY, G. SIMON and J. M. BISHOP, 1977 Cellular functions are required for the synthesis and integration of avian sarcoma virus-specific DNA. Cell **11:** 307–319.

XU, H., and J. D. BOEKE, 1991 Inhibition of Ty*1* transposition by mating pheromones in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **11:** 2736–2743.

ZACK, J. A., S. J. ARRIGO, S. R. WEITSMAN, A. S. GO, A. HAISLIP and I. S. Y. CHEN, 1990 HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. Cell **61:** 213–222.

ZACK, J. A., A. M. HAISLIP, P. KROGSTAD and I. S. Y. CHEN, 1992 Incompletely reverse-transcribed human immunodeficiency virus type 1 genomes in quiescent cells can function as intermediates in the retroviral life cycle. J. Virol. **66:** 1717–1725.

Communicating editor: F. WINSTON