# Transposon Tagging Using Ty Elements in Yeast

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ABSTRACT

We have used the ability to induce high levels of Ty transposition to develop a method for transposon mutagenesis in *Saccharomyces cerevisiae*. To facilitate genetic and molecular analysis, we have constructed *GAL1*-promoted Ty*H3* or Ty*917* elements that contain unique cloning sites, and marked these elements with selectable genes. These genes include the yeast *HIS3* gene, and the plasmid PiAN7 containing the Tn*903 NEO* gene. The marked Ty elements retain their ability to transpose, to mutate the *LYS2*, *LYS5*, or *STE2* genes, and to activate the promoterless *his3Δ4* target gene. Ty elements containing selectable genes are also useful in strain construction, in chromosomal mapping, and in gene cloning strategies.

MOBILE genetic elements have been used as powerful genetic tools in such diverse organisms as bacteria (KLECKNER, ROTH and BOTSTEIN 1977), maize (MCCLINTOCK 1965), Drosophila (RUBIN and SPRADLING 1982), mice (reviewed by GRIDLEY, SORIANO and JAENISCH 1987), and nematodes (EIDE and ANDERSON 1985). We have developed a method for transposon mutagenesis in *Saccharomyces cerevisiae* using the native yeast transposon Ty for two reasons. These elements cause interesting and novel mutations (reviewed by ROEDER and FINK 1983), and Ty provides an alternative to the Tn*3*- and Tn*10*-based shuttle mutagenesis systems (SEIFERT *et al.* 1986; HUISMAN *et al.* 1987). The bacterial systems require that insertional mutagenesis of cloned yeast sequences occur in *Escherichia coli*. The mutagenized clones are then introduced into yeast for further analysis. In contrast, Ty element mutagenesis can be directly incorporated into mutant searches in yeast without any intermediate steps.

There are two related families of yeast Ty elements, Ty*1* and Ty2, which share extensive homology at the nucleotide and amino acid sequence level (WARMINGTON *et al.* 1985; FULTON *et al.* 1985; STUCKA, HAUBER and FELDMANN 1986), and have similar properties. Recently, a third Ty element group, called Ty*3* or sigma composite elements, has been found in *S. cerevisiae* (CLARK *et al.* 1988). For Ty*1* elements, it has been shown that transposition proceeds via an RNA intermediate, and resembles the process of retroviral reverse transcription and integration (BOEKE *et al.* 1985; GARFINKEL, BOEKE and FINK 1985; MELLOR *et al.* 1985). Ty2 and Ty*3* elements also show the structural features of retrotransposons (WARMINGTON *et al.* 1985; CLARK *et al.* 1988), and therefore, it is likely that the mechanisms of Ty*1*, Ty2, and Ty*3* transposition are similar overall.

Insertion of a Ty*1* or Ty2 element within the coding sequence of a gene usually disrupts its function. These mutations are generally quite stable and do not revert. However, Ty element insertions in the promoter region of yeast genes have novel effects on gene expression and are generally unstable mutations. Some Ty-induced mutations, called *ROAM* mutations, overproduce their corresponding gene product and respond to mating-type signals (ERREDE *et al.* 1980). Ty sequences appear to be responsible for the *ROAM* effect (ERREDE *et al.* 1985; ERREDE, COMPANY and HUTCHISON 1987; ROEDER, ROSE and PERLMAN 1985; RATHJEN, KINGSMAN and KINGSMAN 1987). Ty insertion into a promoter can also result in gene inactivation (CHALEFF and FINK 1980; ROEDER and FINK 1980). Reversion events that alter gene function usually involve rearrangement of the inserted Ty or mutations in extragenic suppressor genes (CIRIACY and WILLIAMSON 1981; WINSTON *et al.* 1984). Therefore, Ty-induced promoter mutations can give insight into both the regulation and function of a particular gene.

Two major obstacles had to be overcome before Ty elements could be routinely used as a mutagen in yeast. In normal yeast strains even under optimal conditions, transpositional movement of Ty elements occurs at a low frequency ($10^{-7}$ to $10^{-8}$ at a specific locus) (SCHERER, MANN and DAVIS 1982; PAQUIN and WILLIAMSON 1984; GIROUX *et al.* 1988). In addition, all common laboratory yeast strains contain about 25–30 Ty*1* elements, and about 10–15 Ty2 elements per

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haploid genome (CAMERON, LOH and DAVIS 1979; KINGSMAN *et al.* 1981; D. GARFINKEL and J. BOEKE, unpublished results). Therefore, if a gene is mutated by Ty insertion, the relevant Ty must be identified against a background of numerous other copies of the element.

A system for studying Ty transposition has been developed that overcomes these problems (BOEKE *et al.* 1985). When a genetically marked Ty*1* element (Ty*H3*) is fused to the controllable yeast *GAL1* promoter on a high copy plasmid (pGTy*H3*), addition of galactose induces high levels of transposition of both the marked Ty*H3* element and genomic Ty elements. Markers ranging in size from a 40-bp *lacO* (BOEKE *et al.* 1985) fragment to selectable genes of 1 kb (BOEKE, XU and FINK 1988) have been used successfully in Ty*H3*.

In the present work, the *GAL1*-promoted-Ty delivery system has been modified for transposition mutagenesis in yeast. We have constructed pGTy plasmids containing either a Ty*1* (*H3*) or a Ty2 (Ty*917*) element that contain a unique cloning site. These Ty elements are easier to tag with foreign sequences than earlier derivatives. Marked Ty elements retain their ability to transpose, and to mutate various target genes. If a Ty element contains a bacterial replicon, transpositions can be directly recovered from yeast. Further genetic and molecular analysis of strains containing marked transpositions indicate that Ty tagging is a useful technique.

## MATERIALS AND METHODS

**Yeast strains, general genetic methods and media:** The yeast strains used in this study are described in Table 1. To describe the genotypes of strains carrying marked transpositions, we adopted the nomenclature used for bacterial transposable elements. For example, the designation *lys2-941*::Ty*H3HIS3* refers to a strain carrying a particular Ty*H3HIS3*-induced mutation at the *LYS2* locus. Additional marked transpositions in the genome will be designated as ::Ty*H3HIS3*. Standard yeast genetic procedures for crossing, sporulation, tetrad analysis, and complementation testing were followed as described previously (MORTIMER and HAWTHORNE 1969; SHERMAN, FINK and HICKS 1986). All media were made as described previously (SHERMAN, FINK and HICKS 1986).

**Construction of the marked pGTy plasmids:** The plasmid pGTy*H3* was described previously (BOEKE *et al.* 1985) and is diagrammed in Figure 1. To construct pGTyH3CLA, the single *Cla*I site present at nucleotide 3580 of the Ty*H3* coding sequence (BOEKE *et al.* 1988) was removed by oligonucleotide-directed mutagenesis. A restriction fragment containing Ty*H3* sequence from a *Kpn*I site at nucleotide 3505 to a *Hin*dIII site at nucleotide 4627 was subcloned into a derivative of pZ152 (ZAGURSKY and BERMAN 1984) that has its *Eco*RI site replaced by a *Kpn*I site (kindly provided by C. MCGILL). To induce the mutation, an oligonucleotide d(TTCACCTTCAATTGATGCTTCTCCAC) lacking the *Cla*I site (the mutated site is underlined, and contains a *T* instead of a *C* in the third position of the *Cla*I recognition sequence) was synthesized (PRI, NCI-Frederick Cancer Research Facility, Frederick, MD). TyH3 encoded proteins remain unchanged because the mutation was made in the third position of a codon, ATC, that specifies isoleucine. ATT and ATC codons are each used numerous times in the Ty coding sequence. Standard procedures for oligonucleotide phosphorylation, annealing, DNA synthesis, and subsequent screening of clones were used to produce the desired C→T transition (ZOLLER and SMITH 1983). The loss of the *Cla*I site was confirmed by DNA sequencing (SANGER, NICKLEN and COULSON 1977), and the 1122 bp *Kpn*I-*Hin*dIII segment was subcloned back into pGTyH3. We then introduced a *Bgl*II-*Cla*I adapter d(GATCTATCGATA) into the *Bgl*II site (nucleotide 5561) of the *Cla*I-minus pGTyH3 derivative to generate pGTyH3CLA.

The plasmid pGTy917 contains the Ty element that causes the *his4-917* mutation (ROEDER *et al.* 1980). The construction of this plasmid will be described elsewhere (M. CURCIO, N. SANDERS and D. GARFINKEL, unpublished data). The pGTy*917* plasmid has many of the same features as pGTy*H3*, except it has a unique *Bgl*II site in a position where foreign DNA can be introduced without affecting transposition (Figure 1).

Three markers were cloned into pGTy*H3* or pGTy*917* (Figure 1); the *NEO* gene from Tn903 (OKA, SUGISAKI and TAKANAMI 1981), the miniplasmid PiAN7 (SEED 1983) containing the *NEO* gene (π*N*), or the yeast *HIS3* gene (STRUHL 1985). The *NEO* gene, originally from the plasmid pGH54 (generously provided by N. GRINDLEY), contains nucleotides 1082–2038 and is flanked by *Bam*HI sites (JOYCE and GRINDLEY 1984). The plasmid pGTy*H3NEO* (formal plasmid designation is pJEF1105) (BOEKE, XU and FINK 1988) was generously provided by J. BOEKE. To construct π*N*, the *Bam*HI fragment containing the *NEO* gene was subcloned into the *Bam*HI site of PiAN7. To construct pGTy*917*π*N*, the π*N* plasmid was linearized with *Bgl*II, and cloned into the *Bgl*II site of pGTy*917*. In the plasmid pGTy*917*π*ND*, both Ty*917* and *NEO* are transcribed in the same direction. In plasmid pGTy*917*π*NI*, Ty*917* and *NEO* are transcribed in opposite directions. During the construction of π*N* and pGTy*917*π*N*, we noticed that *E. coli* cells harboring these plasmids formed small colonies. This was not pursued further, but it is possible that the expression of the PiAN7 *supF* gene on a high copy plasmid is deleterious to cells.

In an attempt to remove sequences that might inhibit Ty transposition, such as transcriptional terminators, the yeast *HIS3* gene was modified in two ways. First, an artificial *Bam*HI site was introduced at nucleotide −170 of the *PET56-HIS3-DED1* gene region (STRUHL 1985). The details of this oligonucleotide-directed mutagenesis will be given elsewhere (C. MCGILL and J. STRATHERN, unpublished data). Second, the *HIS3* gene was subcloned as a *Bam*HI to *Pst*I segment into the adapter plasmid pCLA12, which contains the pUC12 polylinker flanked by *Cla*I sites (HUGHES *et al.* 1987). These manipulations resulted in a *HIS3* gene with only small amounts of flanking sequence. The plasmid pGTy*H3HIS3* was constructed by subcloning the *Cla*I fragment containing *HIS3* from pCLA12*HIS3* into pGTy*H3CLA*. In plasmid pGTy*H3HIS3*, transcription of Ty*H3* and *HIS3* is in the same direction.

**Nucleic acid manipulations:** Rapid plasmic isolation, standard cloning methodologies, restriction enzyme analysis, agarose gel electrophoresis, and DNA filter hybridizations (SOUTHERN 1975) were done as described by MANIATIS, FRITSCH and SAMBROOK (1982). Intact yeast chromosomal DNA was isolated by the method of CARLE and OLSON (1985), and separated on a 1% agarose gel using a CHEF electrophoretic system (CHU, VOLLRATH and DAVIS 1986). The gels were run at 200 V (constant voltage) for at least

**TABLE 1**

**Yeast strains**

| Strain | Genotype[a] | Plasmid | Source |
|---|---|---|---|
| GRF167 | *MATα his*Δ*200 ura3-167 GAL* | | G. FINK |
| YH8 | *MATα his3*Δ*200 ura3-167 trp1*Δ*1 leu2*Δ | | J. BOEKE |
| JB183 | *MATa his3*Δ*200 ura3-52 trp1-289 lys2 GAL* | pAB100 | |
| DG545 | JB183 | pAB100, pGTy*H3NEO* | |
| DG662 | YH8 | pGTy*H3HIS3* | |
| DG776 | *MATa arg4-17 his3 tyr7-1 trp1*Δ*1 GAL* | | |
| DG788 | *MATa ade6 his3 tyr7-1 trp1*Δ*1 GAL* | | |
| DG799 | GRF167 | pGTy*917πND* | |
| DG801 | GRF167 | pGTy*917πNI* | |
| DG818 | *MATα his3*Δ*200 ura3-167 trp1*Δ*1 leu2*Δ *lys2-923*::Ty*H3HIS3 GAL* | | |
| DG819 | *MATα his3*Δ*200 ura3-167 trp1*Δ*1 leu2*Δ *lys2-941*::Ty*H3HIS3 GAL* | | |
| DG820 | *MATα his3*Δ*200 ura3-167 trp1*Δ*1 leu2*Δ *lys2-952*::Ty*H3HIS3*::Ty*H3HIS3*[1] | | |
| DG821 | *MATα his3*Δ*200 ura3-167 trp1*Δ*1 leu2*Δ *lys2-956*::Ty*H3HIS3* | | |
| DG822 | *MATa his3*Δ*200 ura3-167 trp1*Δ*1 leu2*Δ *lys2-955*::Ty*H3HIS3*::Ty*H3HIS3* | | |
| DG823 | *MATα his3*Δ*200 ura3-167 trp1*Δ*1 leu2*Δ *lys2-966*::Ty*H3HIS3*::Ty*H3HIS3* | | |
| DG824 | *MATα his3*Δ*200 ura3-167 trp1*Δ *leu2*Δ *lys5-973*::Ty*H3HIS3*::Ty*H3HIS3*[1] | | |
| DG837 | GRF167::Ty*917πND* | | |
| DG838 | GRF167::Ty*917πNI* | | |
| DG839 | GRF167::Ty*917πNI* | | |
| DG840 | GRF167::Ty*917πND* | | |
| DG197-1A | *MATα leu2*Δ *trp1 ade6 his3*Δ*200 ura3-167* | | |
| DG197-1B | *MATα trp1 tyr7-1 his3*Δ*1* | | |
| DG197-1C | *MATa trp1 lys2-956*::Ty*H3HIS3 his3*Δ*200* | | |
| DG197-1D | *MATa leu2*Δ *trp1 tyr7-1 ade6 lys2-956*::Ty*H3HIS3 his3*Δ*1 ura3-167* | | |
| DG201-2A | *MATα trp1*Δ*1 lys5-973*::Ty*H3HIS3 ura3-167 arg4-17 his3* | | |
| DG201-14C | *MATa trp1*Δ*1 lys5-973*::Ty*H3HIS3 tyr7-1 arg4-17 his3* | | |
| GRY354 | *MATa his3*Δ*1 leu2-3,2-112 trp1-289 ste2-217*::Ty*H3HIS3 ura3 GAL* | | |
| JSS56-11B | *MATa his3*Δ*1 leu2-3,2-112 trp1-289 ura3 GAL* | | |
| GRY458 | JSS56-11B | pGTy*H3HIS3* | |
| MFM2-4-12B | *MATα ade6 ste2-217*::Ty*H3HIS3 tyr7-1 ura3* | | |
| JSS68-1C | *MATα ade6 his3 try7-1 ura3* | | |
| JSS102-1B | *MATα ade2-101 his3*Δ*200 leu2*Δ *ura3-52* | | |
| JSS102-2B | *MATa ade2-101 his3*Δ*200 leu2*Δ *ura3-52* | | |
| BWG1-7A | *MATa his4-519 ura3-52 leu2-3,2-112 ade1-100 GAL* | | L. GUARENTE |
| D550-2C | *MATa lys2* | | G. FINK |
| 17/14 | *MATa his1* | | G. FINK |
| 17/17 | *MATα his1* | | G. FINK |
| D551-1A | *MATα lys2* | | G. FINK |
| 699 | *MATa lys5 trp1* | | F. SHERMAN |
| 696 | *MATα lys5* | | F. SHERMAN |

[a] The designation ::Ty*H3HIS3* refers to a strain carrying a Ty*H3HIS3* transposition. When appropriate, the number of additional Ty*H3HIS3* copies in the genome are designated by a superscripted number.

40 h at 11°. The switching time was 82 sec. The CHEF system was made by CBS Scientific Co. (Del Mar, California). Prior to transfer, the gels were placed over a shortwave UV source for 5 min.

For cloning experiments, DNA was introduced into competent *E. coli* DH5 cells supplied by BRL Laboratories (Gaithersburg, Maryland). For plasmid recovery from yeast, DNA was introduced into competent *E. coli* strain HB101 (MANDEL and HIGA 1970). Plasmids were introduced into yeast by the spheroplast transformation procedure of HINNEN, HICKS and FINK (1978), or the lithium acetate procedure of ITO *et al.* (1983). Total yeast DNA was prepared by the method of HOLM *et al.* (1986). Prior to transposition-induction (see below), the marked pGTy plasmids were

recovered from yeast, and analyzed for a set of restriction sites diagnostic for Ty*H3*, Ty*H3CLA*, or Ty*917*. This minimized the chance of losing the relevant Ty as a result of homologous recombination with a chromosomal element. These events can occur during or soon after yeast transformation (J. BOEKE and D. GARFINKEL, unpublished results). The Ty*H3* restriction sites checked were *Bgl*II, *Fnu*dII, *Hha*I, *Hin*dIII, *Hpa*I, *Sal*I, *Sca*I, *Sfa*NI and *Xho*I. The Ty917 restriction sites checked were *Cla*I, *Dra*I, *Hin*dIII, *Nde*I, *Nhe*I, *Sca*I, *Sph*I and *Xho*I. The exact position of these sites is known from the DNA sequence of Ty*H3* (BOEKE *et al.* 1988) and Ty917 (P. FARABAUGH, personal communication). $^{32}$P-Labeled hybridization probes were made by randomly primed DNA synthesis of purified restriction fragments (FEINBERG and VOGELSTEIN 1984).

**Recovery of Ty*917πN* transpositions from yeast:** Total DNA isolated from strains containing either the Ty*917πND* or the Ty*917πNI* transpositions was cleaved using *Pst*I or *Sac*I, the DNA was ligated under conditions to promote recircularization, and plasmids were recovered as kanamycin resistant transformants in *E. coli.* Usually, the cells from an entire transformation were plated on one or two LB plates containing 25 μg of kanamycin (Sigma, St. Louis, Missouri) per ml.

To aid in cloning Ty-induced mutations caused by Ty*HIS3* or Ty*NEO* elements, we constructed *URA3*-based integrating vectors containing the *NEO* or *HIS3* genes.

**Transposition assay:** The transposition assay using Ty*H3* or Ty*917* marked with selective genes was a modification of the original (BOEKE *et al.* 1985; BOEKE, XU and FINK 1988). Yeast cells containing the relevant *URA3*-based plasmid were induced for transposition by growth on SC-ura plates containing galactose (Sigma) at 2% final concentration for 5 days (BOEKE *et al.* 1985) at 22° (PAQUIN and WILLIAMSON 1984). Colonies from the induction plates were restreaked for single colonies on SC-ura plates containing glucose (the *GAL1* promoter is strongly catabolite repressed in the presence of glucose), and then a single colony was grown nonselectively on YPD plates to allow for loss of the plasmid. Ura$^-$ segregants were identified by replica plating to SC-ura plates or to media containing 5-fluoroorotic acid (BOEKE, LACROUTE and FINK 1984). Transposition events were detected by the ability of the Ura$^-$ segregants to grow on SC-his plates if *HIS3* was the marker, or YPD plates containing the antibiotic G418 (GIBCO Laboratories, Grand Island, New York) at a final concentration of 100 or 200 μg/ml if *NEO* was the marker. The correct G418 concentration required for selection must be determined empirically for each strain. Transpositions were scored after 1 day of incubation at 30° if *HIS3* is the Ty marker, or after 2 days if *NEO* is the marker. The transposition efficiency is defined as the number of G418$^R$ or His$^+$, Ura$^-$ segregants divided by the total number of Ura$^-$ segregants.

**Selections for Ty-induced mutations at specific target genes:** (1) *his3Δ4*: His$^+$ revertants caused by activation of the promoterless *his3Δ4* gene were selected as described previously (BOEKE *et al.* 1985). (2) *LYS2* or *LYS5*: Selection of L-α-aminoadipate (Sigma) resistant mutants (CHATTOO *et al.* 1979) was done as described previously (BOEKE *et al.* 1985), except cells were induced for transposition for 5 days at 22° before the colonies were printed to selective medium. (3) *STE* genes: Selection for mutants resistant to α-pheromone was done essentially as described (HARTWELL 1980). After transposition-induction, cells were washed from the plates, 0.15 ml of the suspension (about $10^6$ cells) was spread onto several YPD plates containing 1 μg of α-pheromone (Sigma) per ml, and the plates were incubated at 30°. The resulting mutants were analyzed for marked Ty-induced mutations as described in the text. A detailed description of this mutant analysis will be presented elsewhere (M. MASTRANGELO, K. WEINSTOCK, B. SHAFER, D. GARFINKEL and J. STRATHERN, unpublished data).

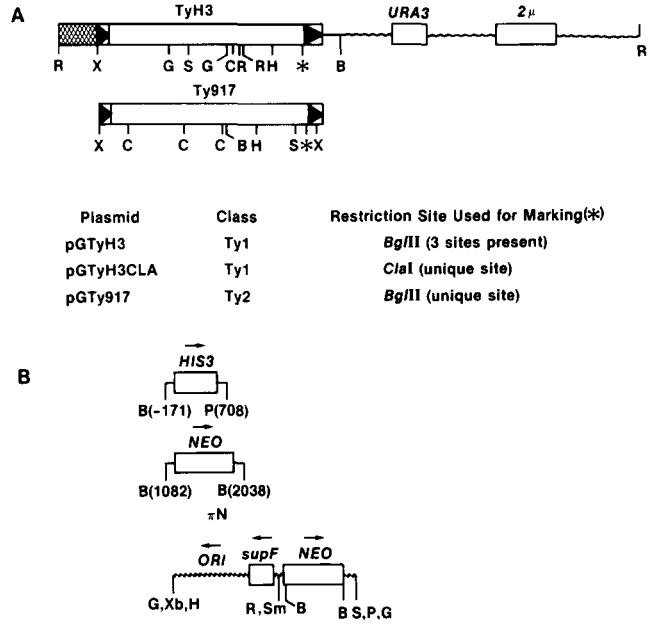


FIGURE 1.—Restriction maps of the pGTy*H3* and pGTy*917* plasmids (A), and marker genes (B) (the maps are not drawn to scale). Boxed segments represent yeast or bacterial genes; the arrows point in the direction of transcription. The marker genes and the Ty elements are drawn in the same transcriptional orientation. The wavy line represents pBR322 sequences. Restriction sites are abbreviated as follows (not all restriction sites are presented): B, *Bam*HI; C, *Cla*I; G, *Bgl*II; H, *Hin*dIII; P, *Pst*I; R, *Eco*RI; S, *Sal*I; Sm, *Sma*I; X, *Xho*I; Xb, *Xba*I. (A) Organization of pGTyH3 and pGTy917. The hatched box represents the *GAL1* promoter sequence. The boxed arrows are the Ty LTR sequences. In the pGTy plasmids, either Ty*H3* and Ty*917* is fused to the *GAL1* promoter at a *Xho*I site within the 5′ LTR. The Ty*H3* segment is derived from a Ty transposition into plasmid pNN162 (BOEKE *et al.* 1985). The Ty*H3CLA* segment is derived from pGTy*H3* using oligonucleotide-directed mutagenesis (refer to MATERIALS AND METHODS). The Ty*917* segment is derived from plasmid phis917 (kindly provided by G. FINK). The 2μ plasmid origin-containing segment is designated by a box. The (*) represents the restriction site just inside the 3′ LTR where the marker genes are inserted. (B) The marker genes used to construct the plasmids pGTy*H3HIS3*, pGTy*H3NEO* (BOEKE, XU and FINK 1988), and pGTy917πN. The numbers in parentheses indicate the nucleotide positions in the *HIS3* gene (STRUHL 1985), or in the Tn*903* element for the *NEO* gene (OKA, SUGISAKI and TAKANAMI 1981; JOYCE and GRINDLEY 1984). The plasmid πN contains the Tn*903 NEO* gene. The arrows above the *HIS3*, *NEO*, and *supF* genes indicate the direction of transcription. The arrow above the origin of replication (ORI) in the plasmid πN indicates the direction of DNA replication.

## RESULTS AND DISCUSSION

**pGTy plasmids:** We created a derivative of pGTyH3 that makes it easier to insert a variety of foreign sequences (Figure 1). Since the plasmid pGTyH3 contains sites for most common restriction endonucleases, including three *Bgl*II sites, inserting a polylinker in the permissible *Bgl*II site at nucleotide

5561 was of little value. Instead, we constructed the plasmid pGTyH3CLA, which contains a unique *Cla*I restriction site placed at the *Bgl*II site. A *Cla*I site was chosen because we could remove the only *Cla*I site in the plasmid without changing the Ty protein sequence, and a *Cla*I adaptor plasmid pCLA12 has been developed as an intermediate vector (HUGHES *et al.* 1987). This plasmid is useful because it contains the polylinker array from pUC12 flanked by *Cla*I sites. Virtually any segment of DNA can be converted to a fragment with *Cla*I ends, and then inserted into the *Cla*I site of pGTyH3CLA.

The second pGTy plasmid contains the Ty917 element. This element is the causative agent of the *his4-917* promoter mutation (ROEDER *et al.* 1980). We chose Ty917 as an additional mutagen for several reasons. When overexpressed, marked Ty917 derivatives transpose with a higher efficiency than a similarly marked TyH3 element (M. CURCIO, N. SANDERS and D. GARFINKEL, unpublished data). Ty*917* belongs to the Ty*2* structural class of Ty elements, whereas Ty*H3* is a Ty*1* element. Although Ty*1* and Ty*2* elements share homology at the nucleotide and amino acid sequence level (WARMINGTON *et al.* 1985; FULTON *et al.* 1985; STUCKA, HAUBER and FELDMANN 1986), they persist as separate retrotransposon families in yeast. It is possible that Ty*H3* and Ty*917* have different functional properties, such as different insertion site specificities, or different mutagenic effects on target genes (ROEDER, ROSE and PERLMAN 1985). Furthermore, the pGTy917 plasmid is easy to tag with foreign sequences because it contains a single *Bgl*II site in the correct position.

We marked the Ty elements with a truncated yeast *HIS3* gene (pGTy*H3HIS3*), or the *Escherichia coli* miniplasmid $\pi$N (pGTy*917*$\pi$*N*) (Figure 1). We also used a marked version of pGTyH3 containing the *NEO* gene (pGTy*H3NEO*) that recently has been shown to tranpose in yeast (BOEKE, XU and FINK 1988). The yeast *HIS3* gene present in Ty*H3HIS3* allowed direct selection in *his3* mutant strains. The *NEO* gene confers dominant resistance to the antibiotic G418 in yeast (JIMENEZ and DAVIES 1980), and to neomycin and kanamycin in *E. coli.* The Ty*917*$\pi$*N* element could be used to recover any Ty*917*$\pi$*N*-induced mutation directly, since it contains sequences required for selection and replication in *E. coli.*

**Experimental approach:** Most of the Ty tagging experiments presented here were done with Ty*H3HIS3*. The generalized protocol shown here can be adapted to a variety of mutant selections and screens (Figure 2). The plasmid pGTy*H3HIS3* was introduced into yeast cells by transformation (HINNEN, HICKS and FINK 1978; ITO *et al.* 1983). For Ty mutagenesis using pGTy*H3HIS3*, the recipient strain must be *GAL* because Ty*H3HIS3* is fused to the *GAL1* promoter, and mutant at the *ura3* and *his3* loci in order to maintain the pGTy*H3HIS3* plasmid and to detect marked transpositions. The transformant was grown in the presence of galactose to induce Ty*H3HIS3* transposition, and then the relevant mutant screen or selection was done. The plasmid pGTy*H3HIS3* was segregated from the putative mutants, and the mutants were analyzed for marked transpositions into the relevant loci. Strains containing a marked transposition remained $His^+$ (or resistant to the antibiotic G418 if *NEO* is the marker gene) after loss of the pGTy plasmid. A useful first step in the characterization of mutants has been to cross the putative Ty*H3HIS3*-induced mutants with an appropriate strain, and then do tetrad or random spore analysis. This cross indicates if the mutant contains multiple marked transpositions, and if the mutant phenotype is linked to a Ty*H3HIS3* insertion. If multiple marked mutations are present, backcrosses are done to isolate the marked Ty-induced mutation.

Mutations can be caused by unmarked Ty elements and by other spontaneous events as well as by Ty*H3HIS3*. The background of unwanted mutations depends on the particular screen or selection. In the limited number of tests we have done, it appears that the highest fraction of Ty-induced mutations occur in selections for gene activation.

**Transposition of Ty elements carrying selectable markers:** The transposition efficiencies of Ty*H3HIS3* and Ty*917*$\pi$*N* were initially determined using randomly selected colonies (Table 2). In strain DG662, 50% of the cells contained at least one Ty*H3HIS3* transposition in the genome. Hybridization analysis using a radiolabeled *HIS3* probe showed that randomly selected $His^+$ derivatives of strain DG662 contained from 1 to 4 copies of Ty*H3HIS3* per cell with an average number of 1.9. Similar results were obtained if the *HIS3* gene was inserted into pGTy*H3CLA* in the opposite orientation (data not shown). The hybridization patterns suggested that Ty*H3HIS* transposed into many different sites in the genome. The relative efficiency of Ty*H3HIS3* transposition was also determined in cells that were induced for transposition, and then selected for mutations in the *LYS2* or *LYS5* genes (CHATTOO *et al.* 1979), or in certain *STE* genes involved in the response to $\alpha$-factor (HARTWELL 1980). Using either selection, about 40% of the mutants contained at least one Ty*H3HIS3* transposition (Table 2).

The transposition efficiency of Ty*917* containing the 1830-bp $\pi$N plasmid was determined. These plasmids were introduced into strain GRF167, and the resulting transformant strains DG799 and DG801 were tested for transposition. Regardless of the orientation of the $\pi$N miniplasmid, both marked elements transposed with an efficiency of over 80%

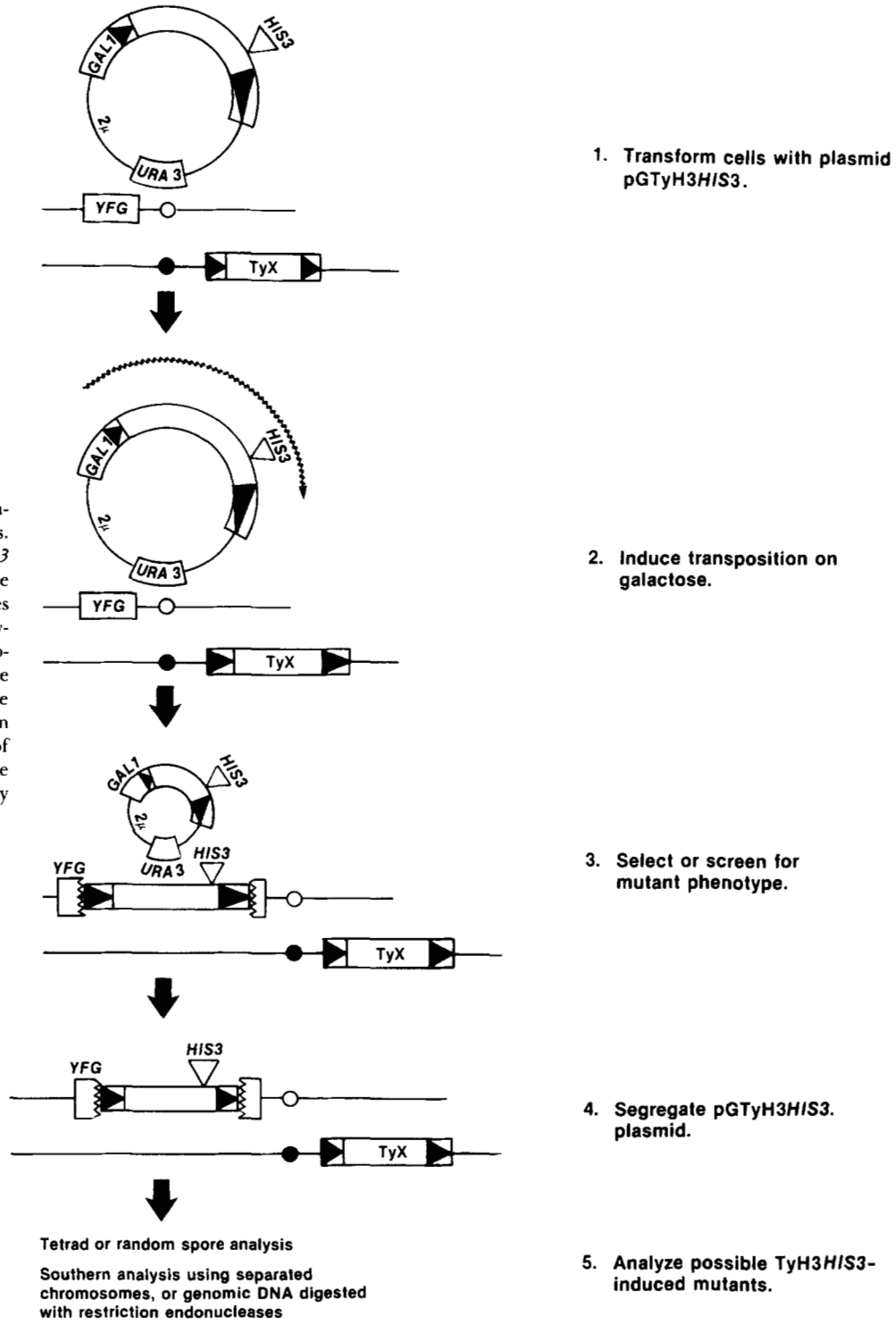


FIGURE 2.—General steps for mutagenesis using marked Ty elements. The symbols for the pGTy*H3HIS3* plasmid and its structure are the same as in Figure 1. Yeast chromosomes are represented below the pGTy-*H3HIS3* plasmid. On the chromosomes is a hypothetical target gene *YFG*, and a native element Ty*X*. The wavy line represents the transcription of Ty*H3HIS3* after the addition of galactose. Mutations in *YFG* can be caused by Ty*H3HIS3*, Ty*X* or by other spontaneous events.

(Table 2). Hybridization analysis of total genomic DNA isolated from G418 resistant strains indicated that no gross rearrangements of πN or Ty*917* occurred during transposition (data not shown).

**Recovery of Ty*917*πN transpositions:** To determine if Ty*917*πN-induced mutations can be cloned directly, we used the πN replicon present on Ty*917* to recover several random Ty*917*πN transpositions in *E. coli*. Total DNA isolated from strains containing either the Ty*917*πND or the Ty*917*πNI transpositions was cleaved using either *Pst*I or *Sac*I, the DNA was ligated under conditions to promote recircularization, and plasmids were recovered as kanamycin-resistant transformants in *E. coli*. The plasmids rescued using *Pst*I were analyzed further. The only *Pst*I site in Ty*917*πN is located immediately adjacent to one of the *Bgl*II sites that bracket the πN miniplasmid (Figure 1). Depending on the orientation of the πN plasmid

**TABLE 2**

**Transposition efficiency of marked Ty elements**

| Strain | Plasmid | Selection[a] | Marker length (nucleotides) | Transposition efficiency (%)[b] | Copies per genome[c] |
|---|---|---|---|---|---|
| DG662 | pGTy*H3HIS3* | None | 750 | 26/52 (50) | 1.9 (28/15) |
| DG662 | pGTy*H3HIS3* | α-aa | 750 | 60/150 (40) | 1.6 (31/19) |
| GRY458 | pGTy*H3HIS3* | α-Factor | 750 | 26/60 (43) | ND |
| DG799 | pGTy*917πND* | None | 1830 | 38/42 (88) | 2.3 (16/7) |
| DG801 | pGTy*917πNI* | None | 1830 | 40/46 (87) | 1.0 (4/4) |

[a] None: after transposition-induction, random colonies were analyzed. α-aa: after transposition-induction, cells resistant to L-α-aminoadipate were selected for analysis. α-Factor: after transposition-induction, cells resistant to α-factor were selected for analysis.

[b] The transposition efficiency is the number of G418$^R$ or His$^+$, Ura$^-$ segregants divided by the total number of Ura$^-$ segregants analyzed. The transposition efficiency of Ty*H3HIS3* is about the same in colonies that were chosen randomly or if a mutant selection was done first.

[c] Measured as the number of bands hybridizing with an appropriate probe on a genomic Southern filter. The fraction in parenthesis is the total number of bands counted, divided by the total number of His$^+$ or G418$^R$, Ura$^-$ colonies analyzed. ND: not determined.

within Ty*917*, yeast sequences on either side of the integration site were recovered in *E. coli*. As expected, Ty*917πND* transpositions from strains DG837 and DG840 yielded πN plasmids containing almost all of the marked Ty, and genomic sequences 5′ to the transposition. Ty*917πNI* transpositions from strains DG838 and DG839 yielded plasmids containing the 3′ Ty long terminal repeat (LTR), and 3′ flanking sequences.

To determine whether rearrangements occurred during the cloning of the Ty*917πN* transpositions, DNA blots were prepared using *Pst*I-digested genomic DNA from strains containing Ty*917πN* element transpositions and *Pst*I-digested plasmids recovered from the relevant insertions. A $^{32}$P-labeled *NEO* probe hybridized with a transposition specific *Pst*I fragment of the same size in both the genomic and plasmid blots (Figure 3). This result suggests that the rescued plasmids are accurate circular forms of the genomic DNA. Restriction analysis of the recovered plasmids with *Pst*I and *Xho*I also suggests the rescued Ty*917πN* sequences are not rearranged (data not shown). *Pst*I digests resulted in a single unique fragment, indicating that no additional *Pst*I fragments above about 500 bp were present in a clone. *Xho*I digests produced fragments of the sizes expected from the positions of the *Xho*I sites in the Ty LTRs, and in the *NEO* gene of Ty*917πN*.

We recovered the single transpositions present in strains DG838, DG839, and DG840 (Figure 3). From the several insertions present in strain DG837, two were recovered in bacteria. We would expect to recover the other insertions if more *E. coli* colonies were analyzed. However, it is possible some insertions will be difficult to rescue by this technique. These results suggest that the same manipulations can be used to rescue genes tagged by Ty*917πN*.

**Transposition of marked Ty elements into specific target genes:** To determine the usefulness of Ty*H3HIS3* and Ty*H3NEO* elements as mutagens, we analyzed marked transpositions into the following target genes: a plasmid-borne promoterlesss *HIS3* gene

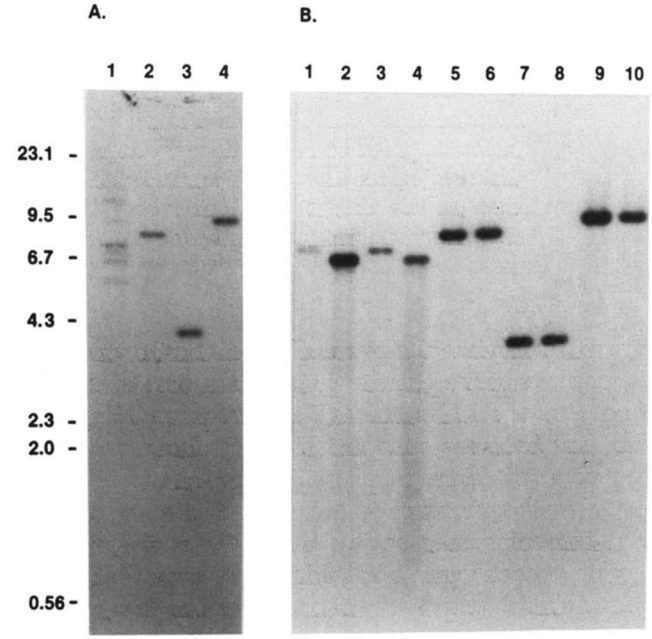


FIGURE 3.—Hybridization of plasmids recovered from Ty*917πN* transpositions in the yeast genome. Total genomic DNA was isolated from strains DG837 and DG840, which contain Ty*917πND* transpositions, and strains DG838 and DG839, which contain Ty*917πNI* transpositions (panel A). Plasmid DNA was isolated from various *E. coli* transformants harboring rescued Ty insertions (panel B). Total DNA and plasmid DNA samples were digested with *Pst*I, separated by electrophoresis under identical conditions on 0.7% gels, and transferred to nitrocellulose filters according to the method of SOUTHERN (1975). The resulting filters were annealed with a radiolabeled *NEO* probe from plasmid pGH54 (JOYCE and GRINDLEY 1984). The faint 6.3-kb band is caused by hybridization between the plasmid pBR322, which is present in the *NEO* probe and the endogenous yeast plasmid 2μ (data not shown). (A) Southern hybridization of yeast DNA isolated from strains DG837 (lane 1), DG840 (lane 2), DG838 (lane 3), and DG839 (lane 4). (B) Southern hybridization of plasmid DNA isolated from various *E. coli* transformants harboring rescued Ty insertions of strain DG837 (lanes 1–4), DG840 (lanes 5 and 6), DG838 (lanes 7 and 8), and DG839 (lanes 9 and 10). Bacteriophage λ DNA digested with *Hin*dIII was included as a size marker alongside the blots.

TABLE 3

**Transpositions of marked Ty elements into specific target genes**

| Strain | Marker | Target gene | Ty fraction (%)[a] | Number of marked Ty elements/ total number Ty elements (%)[b] |
|---|---|---|---|---|
| DG545 | *NEO* | *his3Δ4* | 54/54 (100) | 8/54 (15) |
| DG662 | *HIS3* | *LYS2* | 17/52 (33) | 6/17 (35) |
| DG662 | *HIS3* | *LYS5* | ND | 1/1 |
| GRY458 | *HIS3* | *STE2*[c] | 2/26 (7.7) | 1/2 |

[a] The total number of mutants that are caused by a marked or unmarked Ty insertion divided by the total number of mutants analyzed. ND = not determined.

[b] The total number of mutants caused by a marked transposition divided by the total number of Ty-induced mutants at the locus.

[c] α-Pheromone resistance. Note that Ty-induced mutations can occur at a variety of genes involved in the response to α-pheromone. In this experiment, only the *STE2* gene was monitored for insertions.

(*his3Δ4*), the *LYS2* or *LYS5* genes, and genes involved in the response to α-pheromone.

***his3Δ4*:** Reversion of a plasmid-borne promoterless *HIS3* gene can occur by Ty insertions that restore *HIS3* expression (SCHERER, MANN and DAVIS 1982), thus creating a *ROAM* mutation. In the present work, we wanted to determine whether Ty*H3* marked with the 956 bp *NEO* gene could activate *his3Δ4* with the same efficiency as Ty*H3* marked with the much shorter 40 nucleotide *lacO* sequence (BOEKE *et al.* 1985). The plasmid pGTyH3NEO was introduced into the same parental strain, JB183, that was used in the earlier studies (BOEKE *et al.* 1985), and the resulting transformant DG545 was induced for transposition by addition of galactose. Plasmid DNA from 54 $His^+$ revertants was analyzed in *E. coli*, and all of the revertants were found to be caused by Ty insertional activation of *his3Δ4* (Table 3). Fifteen percent of the Ty insertions (8/54) were marked with *NEO*. This percentage of TyH3*NEO* transpositions is similar to the 22% (8/37) (BOEKE *et al.* 1985), and 16% (40/247) (BOEKE, STYLES and FINK 1986) obtained with Ty*H3lacO* in earlier studies.

***LYS2*:** To demonstrate Ty*H3HIS3* insertional mutagenesis into a chromosomal target, we induced transposition in strain DG662 and then selected 150 L-α-aminoadipate resistant colonies. We expected to obtain mostly *lys2* mutants (≥90%), and a few *lys5* mutants (CHATTOO *et al.* 1979). This positive selection has been used successfully to recover Ty insertions at *LYS2* (EIBEL and PHILIPPSEN 1984; SIMCHEN *et al.* 1984) in normal cells (1–5% are Ty-induced mutations), and in cells induced for transposition (about 30–40% are Ty-induced, see below) (BOEKE *et al.* 1985). Of the 60 $His^+$, L-α-aminoadipate resistant mutants we isolated, 52 failed to complement a *lys2* tester strain. To detect insertions at *LYS2*, filter hybridizations were done with DNA isolated from these 52 mutants using the cloned *LYS2* gene as a probe (data not shown). DNA from 17 of the 52 *lys2* mutants (33%) showed characteristic alterations that were indicative of Ty insertions (Table 3). The percentage of Ty insertions at *LYS2* in $His^-$ cells was not determined because we were specifically looking for mutants caused by Ty*H3HIS3*. In similar studies with cells overexpressing pGTyH3*lacO*, Ty transposition causes about 36% (8/22) of the *lys2* mutations picked at random (BOEKE *et al.* 1985).

To identify *lys2* mutations caused by Ty*H3HIS3*, duplicate DNA blots were prepared from the 17 strains described above. If Ty*H3HIS3* transposed into *LYS2*, both a *HIS3* and *LYS2* probe should hybridize with the same *Bam*HI junction fragment. Six of 17 mutants (35%) showed this type of hybridization pattern, indicating that Ty*H3HIS3* caused the mutation (Figure 4). This compares favorably with the 20% efficiency obtained in earlier studies with Ty*H3lacO* (J. BOEKE, C. STYLES, D. GARFINKEL and G. FINK unpublished results).

**Chromosomal assignment of Ty*H3HIS3*-induced *lys2* mutations:** An advantage of Ty mutagenesis is that the mutation is physically and genetically tagged with a unique sequence. This feature, coupled with the rapid development of electrophoretic systems that separate yeast chromosomes, should allow any mutation caused by a marked transposition to be assigned to a chromosome. To test this application, we analyzed the chromosomes from the parental strain YH8 and from two strains containing single Ty*H3HIS3* insertions at *LYS2* using DNA filter hybridization (Figure 5). In the *lys2* mutants DG819 and DG821, a *HIS3* or a *LYS2* specific probe hybridized with a chromosome band of the same mobility. Since the *LYS2* gene has been genetically mapped, this band should be chromosome *II* (MORTIMER and SCHILD 1981). CHU, VOLLRATH and DAVIS (1986) also have assigned chromosome *II* to this band using the same CHEF electrophoretic system (Contour-clamped Homogeneous Electric Field) as is shown in Figure 5.

***LYS5*:** Of the 60 $His^+$, L-α-aminoadipic acid resistant mutants we isolated, 8 failed to complement a *lys5* tester strain. Hybridization analysis indicated that at least half of these mutants contained multiple Ty*H3HIS3* transpositions (data not shown), but a *LYS5* probe was not available to aid us in checking for insertions at *LYS5*. To determine if a Ty*H3HIS3* insertion occurred at *LYS5* in any of these mutants, we looked at the segregation of the marked Ty*H3HIS3* transpositions, and the *lys5* mutation in crosses (see below).

**α-Pheromone-resistant mutants (*STE2*):** Intensive mutant searches using this selection have identified several genes involved in mating, and cell-type control (HARTWELL 1980; WHITEWAY and SZOSTAK 1985). In the hope of identifying new loci, we have begun to analyze a large number of $His^+$, α-pheromone-resist-

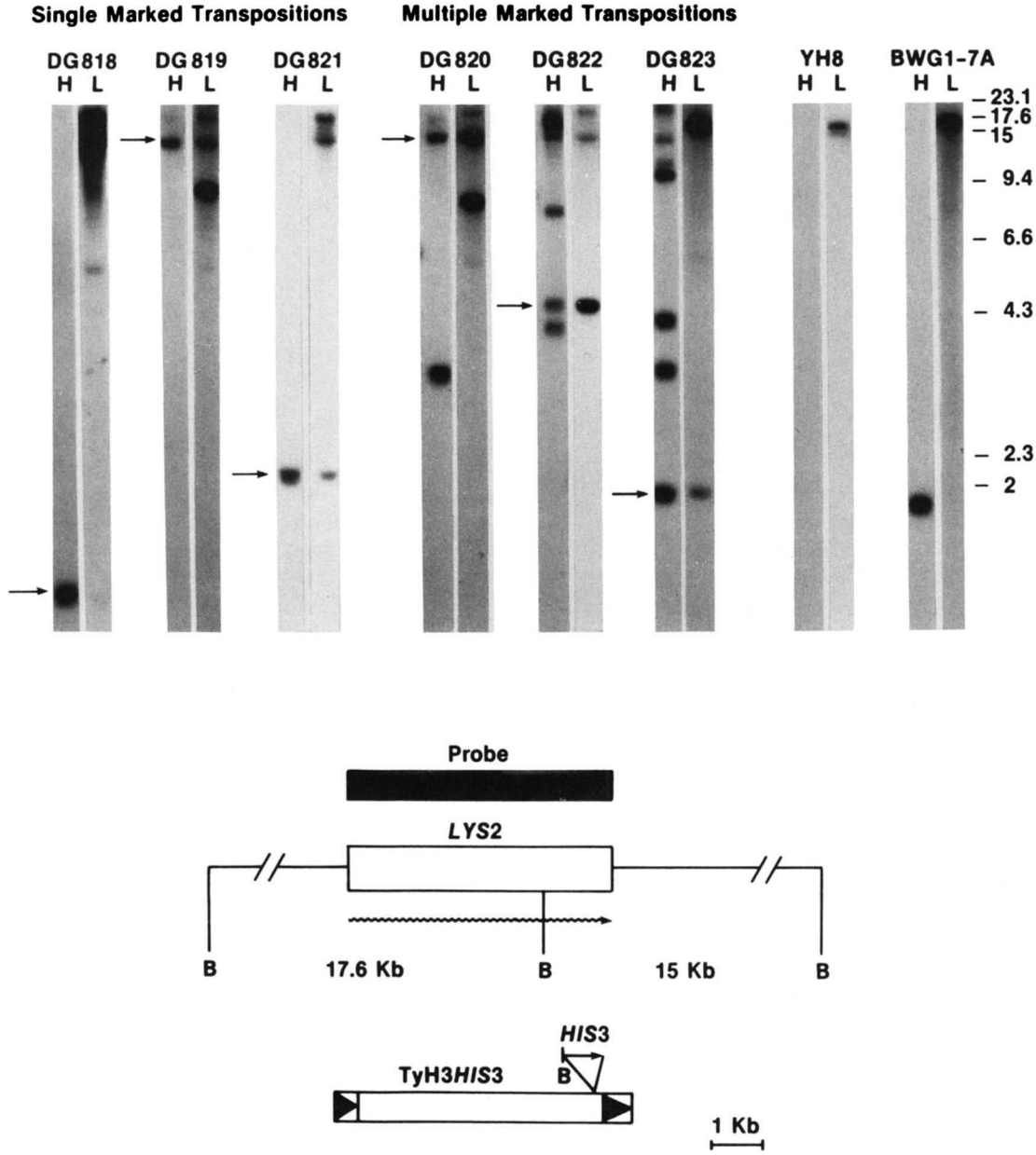


FIGURE 4.—Genomic hybridization of Ty*H3HIS3*-induced *lys2* mutants. Strains DG818–DG823 are *lys2* mutants made in strain YH8. Strain BWG1-7A is a *HIS3* control strain. Total DNA was prepared from these strains, and digested with *Bam*HI (*Bam*HI restriction sites are abbreviated as B). The samples were split in half and separated by electrophoresis on two 0.7% agarose gels under identical conditions, and then blotted. The resulting filters were annealed with a radiolabeled *HIS3* (H) or *LYS2* (L) specific probe. The *LYS2* hybridization probe, represented by the solid rectangle, is from plasmid pSL42-2 (kindly provided by C. FALCO). The *HIS3* probe is the *Cla*I fragment isolated from the plasmid p12CLA*HIS3*. There is a single *Bam*HI site in the *LYS2* gene (EIBEL and PHILIPPSEN 1984), and a single *Bam*HI site that defines 5′ end of the *HIS3* gene inserted in Ty*H3HIS3* (shown at the bottom). If Ty*H3HIS3* mutated *LYS2*, both probes should hybridize with a novel *Bam*HI fragment that results from the transposition event. The arrows point to Ty*H3HIS3*/*LYS2* junction fragments that hybridize with both probes. Three of the Ty-induced mutants contain a single Ty*H3HIS3* transposition (strains DG818, DG819 and DG821), and three have additional Ty*H3HIS3* transpositions elsewhere in the genome (strains DG820, DG822 and DG823). In strain DG818, the Ty insertion is within 200 nucleotides of the *Bam*HI site in *LYS2*, and as a result, the *Bam*HI junction fragment weakly hybridizes with a *LYS2* probe. Size standards appear alongside the blots. The 17.6- and 15-kb *Bam*HI fragments are from the *LYS2* gene; the rest of the fragments are *Hin*dIII fragments from bacteriophage λ. The *LYS2* gene is represented by the open rectangle, and the wavy line shows the direction of *LYS2* transcription.

ant mutants that were exposed to the novel mutagen Ty*H3HIS3*. As a preliminary screen, 26 of the mutants were checked for Ty*H3HIS3* insertions into an expected target gene *STE2*, which encodes the α-pheromone receptor (JENNESS, BURKHOLDER and HARTWELL 1983). The mutants were initially analyzed by DNA filter hybridization using a *STE2*-specific probe. Of the 26 strains, DNA isolated from one strain, GRY354, displayed a hybridization pattern suggestive of a Ty*H3HIS3*-induced mutation, and another strain contained an unmarked Ty-induced mutation. To confirm the presence of the Ty*H3HIS3*-

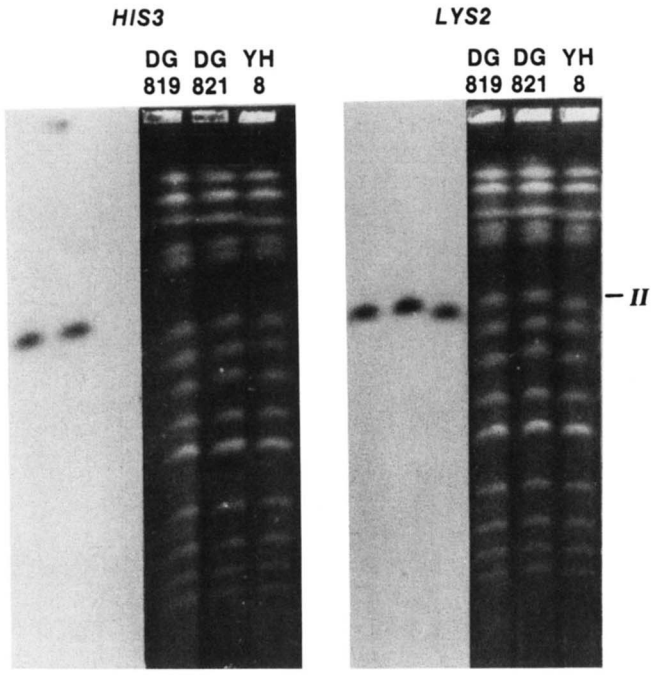


FIGURE 5.—Chromosomal assignment of Ty*H3HIS3*-induced *lys2* mutants. Yeast chromosomal DNA was prepared from the *lys2* mutants DG819 and DG821, and the parental strain YH8. Duplicate samples of chromosomal DNA were separated on a 1% agarose gel using a CHEF electrophoretic system. DNA from the gel whose ethidium bromide staining pattern is shown on the right was transferred to nitrocellulose, and the resulting blots were cut into two strips. One strip was hybridized with a *HIS3* specific probe, and the other with a *LYS2* probe as described in Figure 4. Strain YH8 contains a complete deletion of the *HIS3* gene, *his3Δ200* (STRUHL 1985). The position of chromosome *II*, which contains the *LYS2* gene, is indicated alongside the gel.

induced mutation, duplicate filters were prepared using *Bam*HI digests of total DNA isolated from the mutant GRY354 and the parental strain JSS56-11B (Figure 6). Each filter was hybridized with either a *HIS3* or a *STE2* specific probe. Two fragments hybridized with a $^{32}$P-labeled *HIS3* probe; a 1.6-kb fragment containing the mutant chromosomal *his3Δ1* allele, and a 2.4 kb fragment corresponding to the TyH3*HIS3* transposition. A 2.4-kb fragment also hybridized with a *STE2* probe, suggesting this *Bam*HI fragment contains a Ty*H3HIS3/STE2* junction from the transposition.

Since Ty insertion is known to turn-on genes, a mechanism for producing an α-pheromone resistant mutant would be activating the silent *HMLα* locus. To determine if this type of mutant was present in the collection, we screened α-pheromone resistant *ste* mutants for insertions into *HMLα*, and found that several were caused by Ty*H3HIS3* insertions (data not shown). Therefore, Ty*H3HIS3* can activate a repressed gene. The molecular and genetic analysis of this interesting mutant class will appear elsewhere (M. MASTRANGELO, K. WEINSTOCK, B. SHAFER, D. GARFINKEL, and J. STRATHERN, unpublished data).

**Genetic analysis of Ty*H3HIS3*-induced mutants:** Each mutation caused by the marked Ty*H3HIS3* element should carry a functional *HIS3* gene genetically

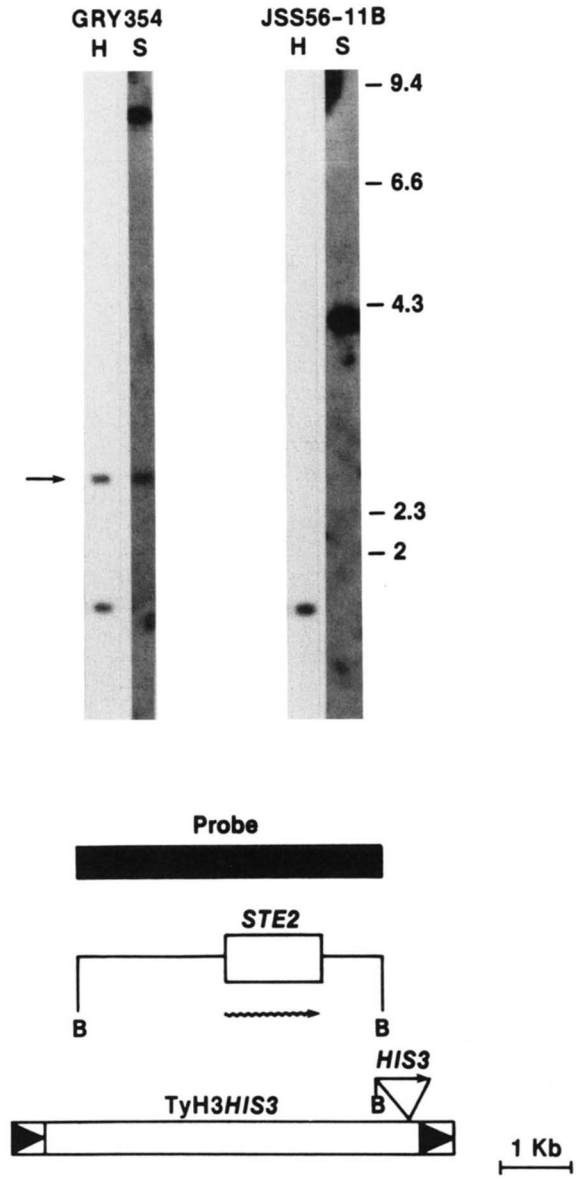


FIGURE 6.—Genomic hybridization of a Ty*H3HIS3*-induced *ste2* mutant. Strain GRY354 is a *ste2* mutant made in strain JSS56-11B. Total DNA was prepared from these strains, and digested with *Bam*HI (*Bam*HI restriction sites are abbreviated as B). The samples were split in half and separated by electrophoresis on two 0.7% agarose gels under identical conditions, and then blotted. The resulting filters were hybridized with a radiolabeled *HIS3* (H) probe described in Figure 4, or a *STE2* (S) probe from plasmid YCpSTE2B (NAKAYAMA, MIYAJIMA and ARAI 1985). The *STE2* gene occupies approximately 1.4 kb of a 4.2-kb *Bam*HI restriction fragment, and a single *Bam*HI site defines the 5′ end of the *HIS3* gene inserted in Ty*H3HIS3* (shown at the bottom). If a Ty*H3HIS3* insertion mutated *STE2*, both probes should hybridize with a common *Bam*HI junction fragment containing *HIS3* and *STE2* specific sequences. The arrow points to a Ty*H3HIS3/STE* junction fragment that hybridizes with both probes. The *his3Δ1* mutation is present on a 1.6-kb *Bam*HI fragment. Size standards appear alongside the blots. The solid rectangle is the *STE2* probe. The *STE2* gene is represented by the open rectangle, and the wavy line shows the direction of *STE2* transcription.

**TABLE 4**

**Tetrad analysis of single Ty*H3HIS* transpositions**

| A. Cross | Target gene | Gene pair | Ascus type[a] PD | NPD | T |
|---|---|---|---|---|---|
| DG×195 (DG788 × DG819) | *LYS2* | *lys2-941/HIS3* | 20 | 0 | 0 |
| DG×197 (DG788 × DG821) | *LYS2* | *lys2-956/HIS3* | 18 | 0 | 0 |
| DG×227 (DG776 × DG818) | *LYS2* | *lys2-923/HIS3* | 14 | 0 | 0 |
| DG×211 (JSS102-2B × DG201-2A) | *LYS5* | *lys5-973/HIS3* | 8 | 0 | 0 |
| DG×215 (DG201-14C × JSS102-1B) | *LYS5* | *lys5-973/HIS3* | 10 | 0 | 0 |

| B. Cross[b] | Target gene | Tetrads analyzed[c] | Spore phenotype **a**,His$^+$ | **a**,His$^-$ | $\alpha$,His$^+$ | $\alpha$,His$^-$ | Ste$^-$,His$^+$ | Ste$^-$,His$^-$ |
|---|---|---|---|---|---|---|---|---|
| MFM2-1 | *STE2* | 16 | 0 | 16 | 16 | 16 | 16 | 0 |
| MFM2-4 | *STE2* | 15 | 0 | 17 | 17 | 13 | 13 | 0 |
| MFM92 | *STE2* | 21 | 0 | 23 | 23 | 19 | 19 | 0 |

[a] PD, Parental ditype; NPD, nonparental ditype; T, tetratype. Only tetrads with four viable spores were included. These asci showed 2:2 segregation for both markers.

[b] The diploid strains MFM2-1 and -4 were obtained from different rare matings between strains GRY354 and JSS68-1C. The parental strains in cross MFM92 were JSS56-11B × MFM2-4-12B.

[c] Only tetrads with four viable spores were included. Three tetrad types were observed in these crosses. A total of 5 tetrads were 2 $\alpha$,His$^-$: 0 **a**: 2 Ste$^-$,His$^+$, 9 tetrads were 2 $\alpha$,His$^+$: 2 **a**,His$^-$: 0 Ste$^-$, and 38 tetrads were 1 $\alpha$,His$^+$ and 1 $\alpha$,His$^-$: 1 **a**,His$^-$: 1 Ste$^-$,His$^+$.

linked to the new mutation. To determine if Ty*H3HIS3* was tightly linked to the mutated target gene, tetrad analysis was done with Ty-induced mutants. Two types of strains were investigated: one where the marked-Ty was the only copy of Ty*H3HIS3* in the genome, and another where there were two copies of Ty*H3HIS3* in the genome.

**Single transpositions of Ty*H3HIS3*:** Three independent Ty*H3HIS3*-induced mutants at *LYS2*, and the Ty*H3HIS3*-induced mutant at *STE2*, were mated with the appropriate *his3* mutant strains and the resulting diploids were sporulated (Table 4). As expected, the *HIS3* gene now segregated as a gene tightly linked to the mutated *lys2* target gene (Table 4A). Also, the structure of several TyH3*HIS3* transpositions at *LYS2* and *STE2* remained unchanged throughout meiosis (data not shown).

Segregation of the marked *ste2* mutation was followed in two sets of crosses (Table 4B). In the first cross, rare diploids were selected in matings between the **a**, Ty*H3HIS3*-induced *ste2* mutant and an appropriate $\alpha$-strain. In two independently selected diploids (Table 4B, crosses MFM2-1 and MFM2-4), an **a**-specific sterile segregated among the progeny [**a**-specific sterility is a characteristic of *ste2* mutants (HARTWELL 1980)]. All of the sterile segregants were His$^+$, indicating that the **a**-specific sterile cells were the result of a Ty*H3HIS3* insertion. Furthermore, the absence of His$^+$, **a**-maters in the progeny suggests that a suppressor mutation was not selected in the rare matings needed to form the diploids.

A second cross was done where an $\alpha$, *ste2* mutant (obtained in cross MFM2-4 described above) was mated with an appropriate **a** strain (Table 4B, cross MFM92). Diploids were formed at a normal frequency in the cross because *STE2* is an **a**-specific gene. No **a**, His$^+$ segregants appeared in the progeny, and all **a**-specific steriles were His$^+$. These results confirm that the *HIS3* gene was tightly linked to the *ste2-217*::Ty*H3HIS3* mutation.

**Multiple transpositions of Ty*H3HIS3*:** Multiple unlinked Ty*H3HIS3* transpositions should assort independently during meiosis. As a result, the ratio of His$^+$:His$^-$ segregants should increase as the number of unlinked Ty*H3HIS3* transpositions increases. For example, if two unlinked copies of Ty*H3HIS3* are present in the genome, the ratio of His$^+$:His$^-$ progeny should be 3:1, if three copies are present the ratio should be 7:1, etc. To test these predictions, we analyzed the segregation pattern of the *lys2* mutant DG820, which appeared to contain two Ty*H3HIS3* transpositions; one at *LYS2* and another elsewhere in the genome (Figure 4). Strains DG820 and DG788 were crossed, and the resulting diploid was sporulated (Table 5, cross DG×198). In 13 tetrads, the ratio of His$^+$:His$^-$ segregants approached 3:1 (38:14), and there were no His$^-$, Lys$^-$ segregants. These results suggest there are two unlinked Ty*H3HIS3* insertions in the genome, and one of these is the *lys2-952*::Ty*H3HIS3* mutation.

In the absence of a hybridization probe, the *lys5* mutants were analyzed genetically to determine if any were caused by Ty*H3HIS3* transposition. When crossed with strain DG788, 7 of the 8 *lys5* mutants were not marked by Ty*H3HIS3* because His$^-$, Lys$^-$ segregants appeared among the progeny (data not shown). One cross involving strain DG824 showed a different segregation pattern (Table 5, cross DG×201). Even though two *HIS3* genes were segregating among the progeny, there was an association

TABLE 5

**Tetrad analysis of Ty*H3HIS3*-induced *lys2* or *lys5* mutants that contain an additional marked transposition**

| Cross[a] | Target gene | Tetrads analyzed[b] | Spore phenotype | | | | His⁺:His⁻[c] |
|---|---|---|---|---|---|---|---|
| | | | His⁺,Lys⁺ | His⁺,Lys⁻ | His⁻Lys⁺ | His⁻Lys⁻ | |
| DG×198 | *LYS2* | 13 | 12 | 26 | 14 | 0 | 38:14 (2.7:1) |
| DG×201 | *LYS5* | 32 | 33 | 64 | 31 | 0 | 97:31 (3.1:1) |

[a] The parental strains used in cross DG×198 were DG788 and DG820. The parental strains used in cross DG×201 were DG788 and DG824.

[b] Only tetrads with four viable spores were included. The two Ty*H3HIS3* insertions in strains DG820 and DG824 were unlinked. The parental ditype (2 His⁺: 2 His⁻): nonparental ditype (4 His⁺: 0 His⁻): tetratype (3 His⁺: 1 His⁻) ratios were 2:1:10 in cross DG×198 and 4:5:23 in DG×201.

[c] The total number of His⁺:His⁻ spores present.

between the *lys5* mutation and one copy of the *HIS3* gene. Southern analysis of strain DG824 also indicated it had suffered two Ty*H3HIS3* transpositions (data not shown). To isolate the *lys5-973*::Ty*H3HIS3* mutation from the other marked transposition, several His⁺, Lys⁻ progeny were chosen from tetrads where His⁺:His⁻ segregated 3:1 (tetratype pattern), and these cells were put through a second cross. The 2:2 segregation in crosses involving strains DG201-2A and DG201-14C (Table 4A, crosses DG×211 and DG×215) confirms that Ty*H3HIS3* caused the *lys5-973*::Ty*H3HIS3* mutation in the absence of another Ty*H3HIS3* transposition. The ability to tag the *LYS5* gene with Ty*H3HIS3* demonstrates that we can identify a marked mutation by genetic analysis alone.

**Other applications and modifications:** We constructed and tested pGTy plasmids containing a unique cloning site. A variety of sequences can now be used to genetically tag an element as long as the markers do not inhibit transposition. This feature should be useful in strain constructions, and for genetic mapping. pGTy plasmids that are easy to manipulate can also simplify the construction of sophisticated Ty element vectors, which stably amplify and express useful genes in yeast (BOEKE, XU and FINK 1988).

Using the pGTy system, Ty-induced mutations are caused by both native chromosomal elements and by marked elements. It would be advantageous to reduce the background created by unmarked Ty elements. In this study, about 12% (6/52) of the *lys2* mutants are caused by TyH3*HIS3*, and about 21% (11/52) are caused by unmarked chromosomal Ty elements. It should be possible to decrease the number of chromosomal Ty transpositions by inducing transposition in an *spt3* mutant background. The *SPT3* gene is required for transposition of chromosomal elements, but transposition of the *GAL1*-promoted Ty elements is unaffected (BOEKE, STYLES and FINK 1986).

Although many new Ty insertions appear in the genome after transposition induction, little is known about their location. Ty elements marked with a selectable gene and a bacterial replicon can be used to recover these "random" transpositions. By choosing a restriction endonuclease that does not cleave the marked Ty, we should be able to recover complete transpositions along with the Ty target site. DNA sequence analysis of the target site can then be carried out by using oligonucleotide primers homologous to the termini of the Ty. The properties of the flanking yeast sequences may help us understand what sequences and regions of the genome make good targets.

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