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 TyH3 or Ty917 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 Tn903 NEO gene. The marked Ty elements retain their ability to transpose, to mutate the LYS2, LYS3, or STE2 genes, and to activate the promoterless his3ΔA 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, Sorianno 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 Tn3- and Tn10-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 mutated 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, Ty1 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 Ty3 or sigma composite elements, has been found in S. cerevisiae (Clark et al. 1988). For Ty1 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 Ty3 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 Ty1, Ty2, and Ty3 transposition are similar overall.

Insertion of a Ty1 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 (Giracy 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 Ty1 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 Ty1 element (TyH3) is fused to the controllable yeast GAL1 promoter on a high copy plasmid (pGTyH3), addition of galactose induces high levels of transposition of both the marked TyH3 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 TyH3.

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 Ty1 (H3) or a Ty2 (Ty917) 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-s is a useful technique.

**CONSTRUCTION OF THE MARKED pGTy PLASMIDS:** The plasmid pGTyH3 was described previously (BOEKE et al. 1985) and is diagrammed in Figure 1. To construct pGTyH3CLA, the single ClaI site present at nucleotide 3580 of the TyH3 coding sequence (BOEKE et al. 1988) was removed by oligonucleotide-directed mutagenesis. A restriction fragment containing TyH3 sequence from a KpnI site at nucleotide 3505 to a HindIII site at nucleotide 4627 was subcloned into a derivative of pZ152 (ZAGURSKY and BERMAN 1984) that has its EcoRI site replaced by a KpnI site (kindly provided by C. MCGILL). To induce the mutation, an oligonucleotide d(TTCACCTTGAATTGATGCTTCTCCAC) lacking the ClaI site (the mutated site is underlined, and contains a T instead of a C in the third position of the ClaI 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 ClaI site was confirmed by DNA sequencing (SANGER, NICKLEN and COULSON 1977), and the 1122 bp KpnI-HindIII segment was subcloned back into pGTyH3. We then introduced a BglII-ClaI adapter d(GATACATCGATA) into the BglII site (nucleotide 5561) of the ClaI-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 pGTy917 plasmid has many of the same features as pGTyH3, except it has a unique BglII site in a position where foreign DNA can be introduced without affecting transposition (Figure 1).

Three markers were cloned into pGTyH3 or pGTy917 (Figure 1); the NEO gene from Trn903 (OKA, SUGISAKI and TAKANAMI 1981), the miniplasmid pIAN7 (SEED 1983) containing the NEO gene (nN), 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 BamHI sites (JOYCE and GRINDLEY 1984). The plasmid pGTyH3NEO (formal plasmid designation is pEF1105) (BOEKE, XU and FINK 1988) was generously provided by J. BOEKE. To construct nN, the BamHI fragment containing the NEO gene was subcloned into the BamHI site of pIAN7. To construct pGTyH3NEO, the nN plasmid was linearized with BglII, and cloned into the BglII site of pGTy917. In the plasmid pGTyH3NEO, both Ty917 and NEO are transcribed in the same direction. In plasmid pGTyH3NEO, the Ty917 and NEO are transcribed in opposite directions. During the construction of nN and pGTy917nN, 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 BamHI site was introduced at nucleotide 170 of the PET36-HIS3-DED1 gene region (STRUHL 1985). The details of this oligonucleotide-directed mutagenesis will be given elsewhere (C. Mcgill and J. STRATHERN, unpublished data). After the HIS3 gene was subcloned as a BamHI to PstI segment into the adapter plasmid pCLA12, which contains the pUC12 polylinker flanked by ClaI sites (HUGHES et al. 1987). These manipulations resulted in a HIS3 gene with only small amounts of flanking sequence. The plasmid pGTyH3HIS3 was constructed by subcloning the ClaI fragment containing HIS3 from pCLA12HIS3 into pGTyH3CLA. In plasmid pGTyH3HIS3, transcription of TyH3 and HIS3 is in the same direction.

**Nucleic acid manipulations:** Rapid plasmid isolation, standard cloning methodologies, restriction enzyme analysis, agarose gel electrophoresis, and DNA filter hybridizations (SOUTHERN 1975) were done as described by MANIATIS, FRITSCHE and SAMBUCK (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, VOLLRAUTH and DAVIS 1986). The gels were run at 200 V (constant voltage) for at least
Ty Elements as Insertional Mutagens

### TABLE 1

#### Yeast strains

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<tr>
<th>Strain</th>
<th>Genotype</th>
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<th>Source</th>
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<td></td>
<td>pAB100, pGTYH3HIS3</td>
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<td>pGTYH3HIS3</td>
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<tr>
<td>696</td>
<td>MATα lys5</td>
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</table>

*The designation ::TyH3HIS3 refers to a strain carrying a TyH3HIS3 transposition. When appropriate, the number of additional TyH3HIS3 copies in the genome are designated by a superscripted number.

40 h at 11°C. 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 HICA 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 TyH3, TyH3CLA, or Ty917. 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 TyH3 restriction sites checked were BglII, FnuDI, HhaI, HindIII, HpaI, SalI, SacI, SfiI, and XhoI. The Ty917 restriction sites checked were ClaI, DraI, HindIII, NdeI, NheI, SacI, SphI, and XhoI. The exact position of these sites is known from the DNA sequence of TyH3 (BOEKE et al. 1988) and Ty917 (P. FARABAUGH, personal communication). 32P-Labeled hybridization probes were made by randomly primed DNA synthesis of purified restriction fragments (FEINBERG and VOGELSTEIN 1984).

Recovery of Ty917\(\pi\)/N transpositions from yeast: Total DNA isolated from strains containing either the Ty917\(\pi\)/ND or the Ty917\(\pi\)/N transpositions was cleaved using PstI or SalI, 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 \(\mu\)g of kanamycin (Sigma, St. Louis, Missouri) per ml.

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

Transposition assay: The transposition assay using TyH3 or Ty917 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, LACROYTE and FINK 1984). Ty transposition events detected by the ability of the Ura\(^{+}\) segregants to grow on SC-ura 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 \(\mu\)g/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 marker, or after 2 days if NEO is the marker. The transposition efficiency is defined as the number of G418\(^{+}\) or His\(^{+}\), Ura\(^{+}\) segregants divided by the total number of Ura\(^{+}\) segregants.

Selections for Ty-induced mutations at specific target genes: (1) hisA34: His\(^{+}\) revertants caused by activation of the promoterless hisA34 gene were selected as described previously (BOEKE et al. 1985). (2) LYS2 or LYS3: Selection of U-\(\alpha\)-aminoacidipate (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 \(\alpha\)-phero- mone 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 \(\mu\)g of \(\alpha\)-phero- mone (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. WERNSTOCK, B. SHAFER, D. GARFINKEL and J. STRATHERN, unpublished data).

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 BglII sites, inserting a polylinker in the permissible BglII site at nucleotide
plasmid pGTyH3CLA, which contains a unique ClaI restriction site placed at the BglII site. A ClaI site was chosen because we could remove the only ClaI site in the plasmid without changing the Ty protein sequence, and a ClaI adaptor plasmid pCLAl2 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 ClaI sites. Virtually any segment of DNA can be converted to a fragment with ClaI ends, and then inserted into the ClaI 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). Ty917 belongs to the Ty2 structural class of Ty elements, whereas TyH3 is a Ty1 element. Although Ty1 and Ty2 elements share homology at the nucleotide and amino acid sequence level (Warrington et al. 1985; Fulton et al. 1985; Stucka, Hauber and Feldmann 1986), they persist as separate retrotransposon families in yeast. It is possible that TyH3 and Ty917 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 BglII site in the correct position.

We marked the Ty elements with a truncated yeast HIS3 gene (pGTyH3HS3), or the Escherichia coli miniplasmid pNE (pGTy917pNE) (Figure 1). We also used a marked version of pGTyH3 containing the neo gene (pGTyH3NEO) that recently has been shown to transpose in yeast (Boeke, Xu and Fink 1988). The yeast HIS3 gene present in TyH3HS3 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 Ty917pNE element could be used to recover any Ty917pNE-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-H3HS3. The generalized protocol shown here can be adapted to a variety of mutant selections and screens (Figure 2). The plasmid pGTyH3HS3 was introduced into yeast cells by transformation (Hinnen, Hicks and Fink 1978; Ito et al. 1983). For Ty mutagenesis using pGTyH3HS3, the recipient strain must be GAL because TyH3HS3 is fused to the GAL1 promoter, and mutant at the ura3 and his3 loci in order to maintain the pGTyH3HS3 plasmid and to detect marked transpositions. The transformant was grown in the presence of galactose to induce TyH3HS3 transposition, and then the relevant mutant screen or selection was done. The plasmid pGTyH3HS3 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 TyH3HS3-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 TyH3HS3 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-H3HS3. 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 TyH3HS3 and Ty917pNE were initially determined using randomly selected colonies (Table 2). In strain DG662, 50% of the cells contained at least one TyH3HS3 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 TyH3HS3 per cell with an average number of 1.9. Similar results were obtained if the HIS3 gene was inserted into pGTyH3CLA in the opposite orientation (data not shown). The hybridization patterns suggested that TyH3HS3 transposed into many different sites in the genome. The relative efficiency of TyH3HS3 transposition was also determined in cells that were induced for transposition, and then selected for mutations in the LYS2 or LYS3 genes (Chattoo et al. 1979), or in certain STE genes involved in the response to α-factor (Hartwell 1980). Using either selection, about 40% of the mutants contained at least one TyH3HS3 transposition (Table 2).

The transposition efficiency of Ty917 containing the 1830-bp pNE 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 pNE 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 pGTyH3HIS3 plasmid and its structure are the same as in Figure 1. Yeast chromosomes are represented below the pGTyH3HIS3 plasmid. On the chromosomes is a hypothetical target gene YFG, and a native element TyX. The wavy line represents the transcription of TyH3HIS3 after the addition of galactose. Mutations in YFG can be caused by TyH3HIS3, TyX or by other spontaneous events.

1. Transform cells with plasmid pGTyH3HIS3.

2. Induce transposition on galactose.

3. Select or screen for mutant phenotype.

4. Segregate pGTyH3HIS3 plasmid.

5. Analyze possible TyH3HIS3-induced mutants.

(Table 2). Hybridization analysis of total genomic DNA isolated from G418 resistant strains indicated that no gross rearrangements of πN or Ty917 occurred during transposition (data not shown).

Recovery of Ty917πN transpositions: To determine if Ty917πN-induced mutations can be cloned directly, we used the πN replicon present on Ty917 to recover several random Ty917πN transpositions in E. coli. Total DNA isolated from strains containing either the Ty917πND or the Ty917πNI transpositions was cleaved using either PstI or SacI, the DNA was ligated under conditions to promote recircularization, and plasmids were recovered as kanamycin-resistant transformants in E. coli. The plasmids rescued using PstI were analyzed further. The only PstI site in Ty917πN is located immediately adjacent to one of the BglII sites that bracket the πN miniplasmid (Figure 1). Depending on the orientation of the πN plasmid
within Ty917, yeast sequences on either side of the integration site were recovered in E. coli. As expected, Ty917*ND transpositions from strains DG837 and DG840 yielded *N plasmids containing almost all of the marked Ty, and genomic sequences 5' to the transposition. Ty917*NI 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 Ty917* transpositions, DNA blots were prepared using PstI-digested genomic DNA from strains containing Ty917* element transpositions and PstI-digested plasmids recovered from the relevant insertions. A 32P-labeled NEO probe hybridized with a transposition specific PstI 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 PstI and XhoI also suggests the rescued Ty917* sequences are not rearranged (data not shown). PstI digests resulted in a single unique fragment, indicating that no additional PstI fragments above about 500 bp were present in a clone. XhoI digests produced fragments of the sizes expected from the positions of the XhoI sites in the Ty LTRs, and in the NEO gene of Ty917*N.

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 Ty917*.

**Transposition of marked Ty elements into specific target genes:** To determine the usefulness of TyH3HIS3 and TyH3NEO elements as mutagens, we analyzed marked transpositions into the following target genes: a plasmid-borne promoterless HIS3 gene

---

**TABLE 2**

**Transposition efficiency of marked Ty elements**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid</th>
<th>Selection*</th>
<th>Marker length (nucleotides)</th>
<th>Transposition efficiency (%)</th>
<th>Copies per genome*</th>
</tr>
</thead>
<tbody>
<tr>
<td>DG662</td>
<td>pGTyH3HIS3</td>
<td>None</td>
<td>750</td>
<td>26/52 (50)</td>
<td>1.9 (28/15)</td>
</tr>
<tr>
<td>DG662</td>
<td>pGTyH3HIS3</td>
<td>a-aa</td>
<td>750</td>
<td>60/150 (40)</td>
<td>1.6 (31/19)</td>
</tr>
<tr>
<td>GRY458</td>
<td>pGTyH3HIS3</td>
<td>a-Factor</td>
<td>750</td>
<td>26/60 (43)</td>
<td>ND</td>
</tr>
<tr>
<td>DG799</td>
<td>pGTy917*ND</td>
<td>None</td>
<td>1830</td>
<td>38/42 (88)</td>
<td>2.3 (16/7)</td>
</tr>
<tr>
<td>DG801</td>
<td>pGTy917*ND</td>
<td>None</td>
<td>1830</td>
<td>40/46 (87)</td>
<td>1.0 (4/4)</td>
</tr>
</tbody>
</table>

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

* The transposition efficiency is the number of G418* or His*, Ura- segregants divided by the total number of Ura* segregants analyzed. The transposition efficiency of TyH3HIS3 is about the same in colonies that were chosen randomly or if a mutant selection was done first.

* 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*, Ura- colonies analyzed. ND: not determined.

**Figure 3.**—Hybridization of plasmids recovered from Ty917* transpositions in the yeast genome. Total genomic DNA was isolated from strains DG837 and DG840, which contain Ty917*ND transpositions, and strains DG838 and DG839, which contain Ty917*NI 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 PstI, 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 pH54 (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 X DNA digested with HindIII was included as a size marker alongside the blots.
bridizations were done with DNA isolated from these
LYSZ transposition in strain DG662 and then selected 150
activation of his3A4 (Table 3). Fifteen percent of the
has been used successfully to recover Ty insertions at
TyH3lacO in earlier studies.

The total number of Ty insertions (8/54) were marked with NEO. This
percentage of TyH3NEO transpositions is similar to
the 22% (8/37) (BOEKE et al. 1985). Of the 17 mutants using the cloned
tester strain. To detect insertions at L-a-aminoadipate resistant colonies. We expected to
obtain mostly
mutations, and in cells induced for transposition (about
30-40% are Ty-induced, see below) (BOEKE et al. 1985). Of the 60 His+, L-a-aminoadipic acid resistant mutants we isolated, 52 failed to complement a lys2
tester strain. To detect insertions at LYSS2, 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 TyH3HIS3. In similar studies with cells
overexpressing pGTyH3lacO, Ty transposition causes
about 36% (8/22) of the lys2 mutations picked at random (BOEKE et al. 1985).

To identify lys2 mutations caused by TyH3HIS3, duplicate DNA blots were prepared from the 17
strains described above. If TyH3HIS3 transposed into
LYS2, both a HIS3 and LYS2 probe should hybridize
with the same BamHI junction fragment. Six of 17
mutants (35%) showed this type of hybridization pattern,
indicating that TyH3HIS3 caused the mutation
(Figure 4). This compares favorably with the 20% efficiency obtained in earlier studies with TyH3lacO
(J. BOEKE, C. STYLES, D. GARFINKEL and G. FINK
unpublished results).

Chromosomal assignment of TyH3HIS3-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
casted 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 TyH3HIS3 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, VOLL-
RATH and DAVIS (1986) also have assigned chromo-
some II to this band using the same CHEF electropho-
etic system (Contour-clamped Homogeneous Electric Field) as is shown in Figure 5.

LYS5: Of the 60 His+, L-a-aminoadipic acid resistant
mutations picked at LYS5, 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 TyH3HIS3 insert-
ion occurred at LYS5 in any of these mutants, we
looked at the segregation of the marked TyH3HIS3
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; WHITETAY and SZOSTAK 1985). In
the hope of identifying new loci, we have begun to
analyze a large number of His+, α-pheromone-resist-

### Table 3

<table>
<thead>
<tr>
<th>Strain</th>
<th>Marker</th>
<th>Target gene</th>
<th>Ty fraction (%)</th>
<th>Number of marked Ty elements/total number Ty elements (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DG545</td>
<td>NEO</td>
<td>his3Δ4</td>
<td>54/54 (100)</td>
<td>8/54 (15)</td>
</tr>
<tr>
<td>DG662</td>
<td>HIS3</td>
<td>LYS2</td>
<td>17/52 (33)</td>
<td>6/17 (35)</td>
</tr>
<tr>
<td>DG662</td>
<td>HIS3</td>
<td>LYS3</td>
<td>ND</td>
<td>1/1</td>
</tr>
<tr>
<td>GRY458</td>
<td>HIS3</td>
<td>STE2</td>
<td>2/26 (7.7)</td>
<td>1/2</td>
</tr>
</tbody>
</table>

* 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.

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 TyH3 marked with
the 956 bp NEO gene could activate his3Δ4 with the
same efficiency as TyH3 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 TyH3NEO transpositions is similar to
the 22% (8/37) (BOEKE et al. 1985), and 16% (40/
247) (BOEKE, STYLES and FINK 1986) obtained with
TyH3lacO in earlier studies.

**LYS2:** To demonstrate TyH3HIS3 insertional muta-
genesis into a chromosomal target, we induced
transposition in strain DG662 and then selected 150
L-a-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-a-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
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(HARTWELL 1980; WHITETAY 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 TyH3HIS3-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 BamHI (BamHI 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 ClaI fragment isolated from the plasmid p12CLAHIS3. There is a single BamHI site in the LYS2 gene (Ebel and Philippson 1984), and a single BamHI site that defines 5' end of the HIS3 gene inserted in TyH3HIS3 (shown at the bottom). If TyH3HIS3 mutated LYS2, both probes should hybridize with a novel BamHI fragment that results from the transposition event. The arrows point to TyH3HIS3/LYS2 junction fragments that hybridize with both probes. Three of the Ty-induced mutants contain a single TyH3HIS3 transposition (strains DG818, DG819 and DG821), and three have additional TyH3HIS3 transpositions elsewhere in the genome (strains DG820, DG822 and DG823). In strain DG818, the Ty insertion is within 200 nucleotides of the BamHI site in LYS2, and as a result, the BamHI junction fragment weakly hybridizes with a LYS2 probe. Size standards appear alongside the blots. The 17.6- and 15-kb BamHI fragments are from the LYS2 gene; the rest of the fragments are HindIII 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 TyH3HIS3. As a preliminary screen, 26 of the mutants were checked for TyH3HIS3 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 TyH3HIS3-induced mutation, and another strain contained an unmarked Ty-induced mutation. To confirm the presence of the TyH3HIS3-
induced mutation, duplicate filters were prepared using *BamHI* 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 *³²P*-labeled *HIS3* probe; a 1.6-kb fragment containing the mutant chromosomal *his3Δ1* allele, and a 2.4 kb fragment corresponding to the TyH3HIS3 transposition. A 2.4-kb fragment also hybridized with a *STE2* probe, suggesting this *BamHI* fragment contains a TyH3HIS3/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 TyH3HIS3 insertions (data not shown). Therefore, TyH3HIS3 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 TyH3HIS3-induced mutants:**

Each mutation caused by the marked TyH3HIS3 element should carry a functional *HIS3* gene genetically
Ty Elements as Insertional Mutagens

TABLE 4
Tetrad analysis of single TyH3HIS transpositions

<table>
<thead>
<tr>
<th>A. Cross</th>
<th>Target gene</th>
<th>Gene pair</th>
<th>Ascus type*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>PD</td>
</tr>
<tr>
<td>DG×195 (DG788 × DG819)</td>
<td>LYS2</td>
<td>lys2-941/HIS3</td>
<td>20</td>
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<tr>
<td>DG×197 (DG788 × DG821)</td>
<td>LYS2</td>
<td>lys2-956/HIS3</td>
<td>18</td>
</tr>
<tr>
<td>DG×227 (DG776 × DG818)</td>
<td>LYS2</td>
<td>lys2-923/HIS3</td>
<td>14</td>
</tr>
<tr>
<td>DG×211 (JSS102-2B × DG201-2A)</td>
<td>LYS3</td>
<td>lys3-973/HIS3</td>
<td>8</td>
</tr>
<tr>
<td>DG×215 (DG201-14C × JSS102-1B)</td>
<td>LYS3</td>
<td>lys3-973/HIS3</td>
<td>10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>n. Cross</th>
<th>Target gene</th>
<th>Tetrads analyzed†</th>
<th>a,His+</th>
<th>a,His-</th>
<th>α,His+</th>
<th>α,His-</th>
<th>Ste-,His+</th>
<th>Ste-,His-</th>
<th>Ste+,His+</th>
<th>Ste+,His-</th>
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<td>MFM2-1</td>
<td>STE2</td>
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<td>16</td>
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<td>19</td>
<td>19</td>
<td>19</td>
<td>19</td>
<td>0</td>
</tr>
</tbody>
</table>

* PD, Parental ditype; NPD, nonparental ditype; T, tetatype. Only tetrads with four viable spores were included. These asci showed a 2:2 segregation for both markers.
† 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 JSS56-118 × MFM2-4-12B.
‡ Only tetrads with four viable spores were included. Three tetrad types were observed in these crosses. A total of 5 tetrams were 2 a,His+: 0 a: 2 Ste-,His+, 9 tetrads were 2 a,His+: 2 a,His-: 0 Ste+, and 38 tetrads were 1 a,His+ and 1 a,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 TyH3HIS3 in the genome, and another where there were two copies of TyH3HIS3 in the genome.

Single transpositions of TyH3HIS3: Three independent TyH3HIS3-induced mutants at LYS2, and the TyH3HIS3-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 TyH3HIS3 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, TyH3HIS3-induced ste2 mutant and an appropriate a 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 TyH3HIS3 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 a, 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::TyH3HIS3 mutation.

Multiple transpositions of TyH3HIS3: Multiple unlinked TyH3HIS3 transpositions should assort independently during meiosis. As a result, the ratio of His+:His- segregants should increase as the number of unlinked TyH3HIS3 transpositions increases. For example, if two unlinked copies of TyH3HIS3 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 TyH3HIS3 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 TyH3HIS3 insertions in the genome, and one of these is the lys2-952::TyH3HIS3 mutation.

In the absence of a hybridization probe, the lys5 mutants were analyzed genetically to determine if any were caused by TyH3HIS3 transposition. When crossed with strain DG788, 7 of the 8 lys5 mutants were not marked by TyH3HIS3 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
between the lys5 mutation and one copy of the HIS3 gene. Southern analysis of strain DG824 also indicated it had suffered two TyH3HIS3 transpositions (data not shown). To isolate the lys5-973::TyH3HIS3 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 DGX211 and DGX215) confirms that TyH3HIS3 caused the lys5-973::TyH3HIS3 mutation in the absence of another TyH3HIS3 transposition. The ability to tag the LYS5 gene with TyH3HIS3 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 TyH3HIS3, 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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LITERATURE CITED


Challeff, D. T., and G. R. Fink, 1980 Genetic events associated

TABLE 5

Tetrad analysis of TyH3HIS3-induced lys2 or lys5 mutants that contain an additional marked transposition

<table>
<thead>
<tr>
<th>Cross*</th>
<th>Target gene</th>
<th>Tetrads analyzed</th>
<th>Spore phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>His⁺,Lys⁻</td>
</tr>
<tr>
<td>DGX198</td>
<td>LYS2</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>DGX201</td>
<td>LYS3</td>
<td>52</td>
<td>33</td>
</tr>
</tbody>
</table>

* The parental strains used in cross DGX198 were DG788 and DG820. The parental strains used in cross DGX201 were DG788 and DG824.

+ Only tetrads with four viable spores were included. The two TyH3HIS3 insertions in strains DG820 and DG824 were unlinked. The parental diploid (2 His⁺: 2 His⁻): nonparental diploid (4 His⁺: 0 His⁻): tetratype (3 His⁺: 1 His⁻) ratios were 2:1:10 in cross DGX198 and 4:5:23 in DGX201.

+ The total number of His⁺:His⁻ spores present.


Cloning vectors that yield high levels of single-stranded DNA for rapid DNA sequencing. Gene. 27: 183–191.


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