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Genetics

Ty-mediated gene expression of the *LYS2* and *HIS4* genes of *Saccharomyces cerevisiae* is controlled by the same *SPT* genes

(insertions/suppressors/transposons)

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ABSTRACT **Five Ty insertion mutations were isolated at the *LYS2* locus of *Saccharomyces cerevisiae*. Genetic and physical analyses show that four Ty insertions are in the 5′ noncoding region of *LYS2* and one is within the structural gene. Three of these Ty elements have been cloned and characterized. The Ty mutations differ from each other in restriction pattern, phenotypic effects on *LYS2*, reversion frequency, and the nature of reversion events. *Spt2* and *spt3* mutations, known to suppress Ty insertions and their solo δ derivatives at *HIS4*, can also suppress at least one of the Ty insertions (Ty*61*) at *LYS2* and can also suppress the Lys⁻ phenotype of a solo δ derivative of another Ty insertion (Ty*128*) at *LYS2*. These results demonstrate that *spt* mutations can suppress Ty and δ mutations at both *HIS4* and *LYS2*, suggesting that they are general for their effects on Ty and δ elements.**

Transposable (Ty) elements of *Saccharomyces cerevisiae* are repeated elements about 6 kilobase pairs (kb) in length that have direct repeats of about 300 base pairs (bp) at their ends (δ sequences) (1). Standard *S. cerevisiae* strains have about 35 Ty elements occurring naturally in the haploid genome (1). Considerable homology exists between different Tys, even though most elements have a unique restriction map. Solo δ sequences, unassociated with the complete Ty element, are present in about 100 copies in the genome (1). These δ sequences are thought to arise by recombination between the δ repeats of intact Ty elements. δ–δ recombination has been documented for Ty elements inserted at *HIS4* (2) and at *ADH2* (3).

Several mutations in *S. cerevisiae* result from alteration in expression of a gene by the insertion of a Ty element in the regulatory region of that gene (4). Such insertion mutations generally result in one of two opposite effects—inhibition of gene expression or constitutive high-level gene expression. The phenotypes of Ty insertion mutations can be affected by unlinked genes. For example, many Ty insertions that result in constitutive gene expression are under control of the mating type locus of the cell (5–7). Recently, mutations have been characterized in seven genes (*SPT* genes) that suppress the His⁻ phenotype of some Ty insertions and their solo δ derivatives in the 5′ noncoding region of the *HIS4* gene (8).

In this paper we report on the isolation and characterization of Ty insertions at the *LYS2* locus. We have found that *spt2* and *spt3* mutations can suppress the Lys⁻ phenotype of Ty and δ insertions at *LYS2*, suggesting that suppression by *spt* mutations is not specific to the *HIS4* gene.

MATERIALS AND METHODS

Yeast Strains. The four principal strains used for the isolation of *lys2* mutations are listed in Table 1. Strains carrying *spt* mutations used in crosses were L1426 [*MATα*, *his4-912δ*, *spt2-150(rad4)*, *ade2-1*, *ura3-52*, *cry-1*] and L1386 [*MATα*, *his4-917δ*, *spt3-101*, *leu2-1*, *ade2-1*]. Lys⁺ revertants were crossed to *SPT*⁺ strains 7517-2A [*MATa*, *his4-912δ*, *cry-1*, *can1*] and 6461-9B [*MATα*, *lys2-1*, *trp1-1*, *can1*]. The Spt⁻ phenotype was scored by following suppression of *his4-912δ* or *his4-917δ* or by UV sensitivity of *spt2-150* strains, which contain a deletion spanning *SPT2* and the neighboring *RAD4* gene (8). We have changed the designation *SPM* to *SPT* (8).

Media and Genetic Methods. Standard procedures and media were used (9). Mutants that were able to grow on α-aminoadipate medium were isolated according to Chatoo *et al.* (10) with the following modifications. Cells were grown overnight and spread on YEPD plates to form 50–100 colonies per plate. After 2–3 days these plates were replicated onto α-aminoadipate medium and incubated 5–6 days at 30°C, when papillae could be seen in most colony replicas. From each colony replica a single papilla was streaked and a single colony of each was used for further analysis.

Plasmids and Bacteria. The plasmid pSL42-2 (Fig. 1) was constructed by S. C. Falco (unpublished), who generously provided both the plasmid and the restriction map for this study. pSL42-2 consists of an *Eco*RI–*Hin*dIII 4.7-kb fragment, which contains the *LYS2* gene, inserted between the *Eco*RI and *Hin*dIII sites of YIp5 (11). We used *Escherichia coli* HB101 as the recipient in bacterial transformations.

Screening for *LYS2* Insertions. To detect chromosomal rearrangements among the mutants we employed a rapid screening method, combining different mutant strains into a single pool. To make each pool we grew separate 1.2-ml liquid cultures from eight strains and mixed them for a rapid DNA preparation. A sample of DNA from each pool was digested by the restriction endonuclease *Xho* I and analyzed by Southern hybridization, using plasmid pSL42-2 as the probe (Fig. 1). When an altered band appeared in any of the pools, the eight constituent members were grown again in individual 10-ml cultures and DNA was prepared separately for each. Additional Southern analysis revealed which of the mutants carried the rearrangement in the *LYS2* region.

Southern Analysis of Yeast DNA. Yeast DNA was prepared by a scaled-down procedure (12) adapted from a previously described method (13). Southern hybridization analysis was done as previously described (14, 15). pSL42-2 was used as a hybridization probe for both *LYS2* and *URA3* yeast sequences. Plasmid B161, which contains the *Bgl* II 1.6-kb fragment of Ty*1* (2) cloned in the *Bam*HI site of pBR322 was used as a probe for Ty sequences.

Cloning of Ty Insertions at *LYS2*. The insertions were cloned by the integration and excision method (13, 15). Yeast mutants were transformed (16) with the plasmid pSL42-2. which had been linearized with the enzyme *Hpa* I,

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Abbreviations: kb, kilobase pair(s); bp, base pair(s).

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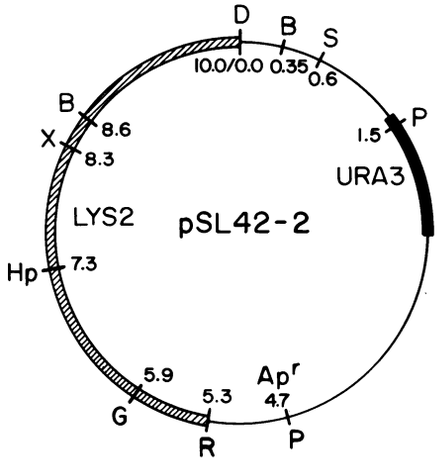

FIG. 1. Structure of plasmid pSL42-2. This 10-kb plasmid was constructed by S. C. Falco. It consists of a *LYS2* 4.7-kb fragment of *S. cerevisiae* DNA inserted between the *Hin*dIII and *Eco*RI sites of YIp5. Restriction sites: B, *Bam*HI; D, *Hin*dIII; G, *Bgl* II; Hp, *Hpa* I; P, *Pst* I; R, *Eco*RI; S, *Sal* I; X, *Xho* I.

and transformants were selected as Ura$^+$ colonies. The plasmid DNA was linearized by cutting at the *Hpa* I site in *LYS2* to enrich for integration of the plasmid at the *LYS2* locus (17). DNA from 5 ml of each transformant was prepared as described earlier and resuspended in 100 μl of TE buffer (10 mM Tris/1 mM EDTA, pH 7.6). Twenty microliters of the DNA was digested at 37°C with *Eco*RI for 30 min or 3 hr (partial or complete digestions, respectively), incubated at 65°C for 10 min to destroy the *Eco*RI activity, and ligated at 15°C overnight in a 500-μl volume with T4 DNA ligase. Ten or 50 μl of the ligation mix was used to transform *E. coli* HB101 and transformants were selected on LB plates (18) with 100 μg of ampicillin per ml.

Biochemicals. All restriction enzymes and T4 DNA ligase were purchased from New England BioLabs. Ampicillin and α-aminoadipate were purchased from Sigma.

RESULTS

Isolation of Ty Insertions at *LYS2*. Greater than 90% of mutants able to grow on α-aminoadipate medium contain a mutation in the *LYS2* gene (10). This allows easy isolation of a large number of independent *lys2* mutations. The *lys2* mutants can then be screened by Southern hybridization analysis for Ty insertions and other rearrangements.

Using the α-aminoadipate selection, we isolated 856 independent, spontaneous mutations that permit growth on α-aminoadipate medium (Table 1). The mutants show a variety of phenotypes when tested on medium without lysine: lysine prototrophy, lysine auxotrophy, and temperature conditional prototrophy (heat- or cold-sensitive growth). Those mutants that are still lysine prototrophs are presumably only partially deficient in *LYS2* (or *LYS5*) function.

The screening method described in *Materials and Methods* identified six rearrangements at or near the *LYS2* gene. Four were found among Lys$^-$ mutants isolated from strain SR36 (*lys2-61*, *lys2-128*, *lys2-173*, and *lys2-201*) and two were found among Lys$^-$ mutants of 9487-9A (*lys2-901* and *lys2-902*). No rearrangements were found among Lys$^-$ mutants of the other two strains. In the six mutants with rearrangements of the *LYS2* region, the *Xho* I restriction fragment from the Lys$^+$ parent that hybridizes to the *LYS2* probe was replaced by one longer or two shorter *Xho* I restriction fragments.

Southern Mapping of Rearrangements. DNA from the six strains with the *LYS2* rearrangements was prepared and the *LYS2* restriction pattern was analyzed by Southern hybridization using the *LYS2* probe pSL42-2. This analysis showed that four of the mutants contain insertions of ≈6 kb located in the *Eco*RI–*Bgl* II segment of the *LYS2* gene, a fifth has an insertion of similar size in the region between the *Bgl* II and *Hpa* I sites, and the sixth has a deletion of at least 7 kb, including 1 kb of the *LYS2* gene (Fig. 2). The restriction site patterns of the new insertions at *LYS2* are similar to those of known Ty elements. The five new insertions will be referred to as Ty*61*, Ty*128*, Ty*173*, Ty*901*, and Ty*902*.

The mutation *lys2-201* appears to be a deletion based on its inability to revert to Lys$^+$, its failure to recombine with 99 of 491 *lys2* mutations, and its altered Southern pattern. The positions of the five Ty insertions in Fig. 2 were verified by mitotic recombination tests with *lys2-201*. The Ty insertion *lys2-902* was the only one that gave Lys$^+$ recombinants with *lys2-201*.

Cloning of New Tys and Characterization of Plasmids. The three Ty elements Ty*61*, Ty*128*, and Ty*173* were cloned as described in *Materials and Methods*. The restriction maps of the Ty elements (Fig. 2) were obtained by Southern analysis and analysis of the cloned yeast segments.

Southern hybridization analysis of restriction digests of the plasmids shows that they contain Ty elements. The plasmid B161, which contains the *Bgl* II 1.6-kb fragment of Ty*1* (2) inserted into the *Bam*HI fragment of pBR322, was used as the probe. The clones show strong hybridization of the *Bgl* II 1.6-kb fragment from the plasmids thought to carry any one of the three Ty elements next to *LYS2* and to B161 (authentic Ty), but not to pBR322. In digests with other enzymes, fragments carrying segments of the *Bgl* II fragment hybridize with B161, as expected. The relative positions of the 1.6-kb fragments in the Tys suggest that Ty*128* has the same transcriptional orientation as *LYS2*, whereas Ty*61* and Ty*173* have the opposite orientation.

Phenotypes of Rearrangements and Their Suppression by *spt* Mutations. The strains carrying *lys2-128*, *lys2-173*, *lys2-901*, and *lys2-902* are auxotrophic for lysine, whereas those with *lys2-61* have a cold-sensitive Lys$^-$ auxotrophy (they grow well on medium without lysine at 30°C and 37°C, but not at 20°C). Strains containing *lys2-61* give rise to many Lys$^+$ revertants at 20°C after 5 days of incubation, whereas *lys2-128* and *lys2-173* are extremely stable at all temperatures (Table 2). Four Lys$^+$ revertants obtained from a strain with *lys2-61* were examined by Southern analysis and were found to have a *LYS2* region with the same restriction pattern as the *lys2-61* parent, suggesting that reversion to Lys$^+$ in-

Table 1. Lysine auxotrophy of spontaneous mutants obtained as α-aminoadipate utilizers

Strain	Genotype	Growth on lysine-free medium					
		−	±	HS	CS	+	Total
SR36	*MAT***a** *his4-912 ura3-52*	205	22	4	7	18	256
7615-2C	*MAT***a** *his4-912 ura3-52 trp1-1*	264	19	4	0	41	328
9487-9A	*MAT***a** *his4-912 leu2-3 rad52-1*	84	0	6	37	1	128
7531-9B	*MAT***a** *his4-912 leu2-3 rad52-1*	100	4	7	27	6	144

HS and CS mean heat-sensitive and cold-sensitive growth, respectively, the two extreme temperatures used being 37°C and 20°C.

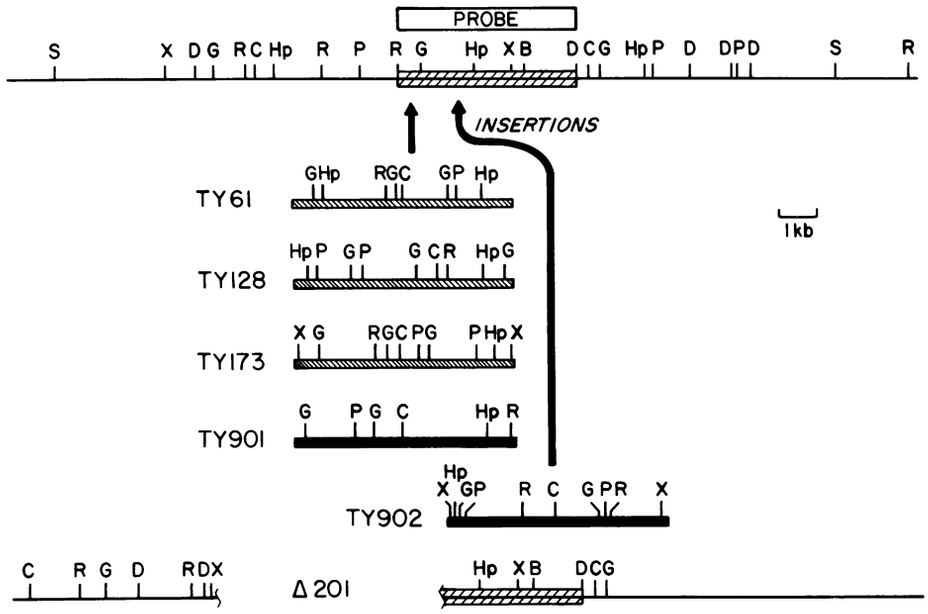

FIG. 2. Rearrangements found among *lys2* mutants. Most of the restriction sites were determined by Southern analysis of genomic digests, but some information was obtained from restriction digests of plasmids. The hatched bars are the Ty insertions found in strain SR36, the blocked bars are the two Tys in strain 9487-9A, and *Δ201* is the deletion found in the mutant carrying *lys2-201* (derived from SR36). Restriction sites are abbreviated as in Fig. 1 and C designates *Cla* I. The arrows mark the positions of insertions.

volved an unlinked suppressor rather than a rearrangement at the *LYS2* gene. Because suppression of Ty-mediated mutations at *HIS4* has been shown to result from mutations in any one of seven unlinked *SPT* genes (8), we decided to determine whether mutation in any of the known *SPT* genes was responsible for the reversion of strains carrying Ty*61* at *LYS2*.

Fifty-four Lys^+ revertants of *lys2-61* were subjected to complementation and dominance tests to determine whether they carried *spt* mutations. Forty-two of the Lys^+ revertants were recessive. Of these, 18 failed to complement *spt2-1* and 1 failed to complement *spt3-101*. The remainder presumably carry mutations in other *SPT* genes (8) or are Ty gene convertants (7). Twelve of the Lys^+ revertants are dominant and are most likely dominant *SPT2* alleles [79 of 169 mutations previously isolated in this gene are dominant (8)]. The one putative *spt3* mutation has been confirmed as an allele of *spt3* by tetrad analysis. These results demonstrate that mutations in *spt2* and *spt3* can suppress *lys2-61*.

A more detailed study of the suppression of the *LYS2* rearrangements was accomplished by construction of double mutants with two characterized *spt* mutations: *spt2-150*, a deletion, and *spt3-101*, a frameshift mutation (unpublished data). The double mutants *lys2-61 spt2-150* and *lys2-61 spt3-101* show partial suppression of the lysine auxotrophy at 20°C. These strains grow considerably better than their *lys2-61* SPT^+ parent, but not as well as LYS^+ strains, whether spt^- or SPT^+. There is no evidence for suppression of the lysine auxotrophy of *lys2-128*, *lys2-173*, *lys2-901*, *lys2-902*, or *lys2-201* by either *spt2-150* or *spt3-101*.

Table 2. Spontaneous reversion from lysine auxotrophy to prototrophy of several rearrangements at *LYS2* (number of revertants per 10^{10} cells)

Rearrangement	*SPT*	*spt2-150*
Δ201	0	0
Ty*61**	2×10^6	—
Ty*128*	2	41
Ty*173*	18	258
Ty*901*	0	54
Ty*902*	0	0

Temperature of incubation was 30°C, unless otherwise indicated.
*Incubation temperature, 20°C.

Reversion of Ty Insertions in SPT^+ and *spt2-150* Strains. The original mutations caused by Ty*128*, Ty*173*, Ty*901*, and Ty*902* are very stable, reverting to Lys^+ at frequencies ranging from $<1 \times 10^{-10}$ to 1.8×10^{-9} (Table 2). However, when associated with the mutation *spt2-150*, the frequency of Lys^+ revertants increased significantly for Ty*128*, Ty*173*, and Ty*901* (Table 2).

Revertants from *lys2-128* and *lys2-173* were characterized by Southern analysis and by genetic analysis of crosses to LYS^+ or $lys2^-$ strains. DNA was prepared from 21 Lys^+ revertants of strain 9665-1A (*MAT***a**, *lys2-128*, *his4-912*, *spt2-150*) and from 13 revertants of strain 9667-1D (*MAT***a**, *lys2-173*, *his4-912*, *spt2-150*). DNA samples were digested with *Xho* I or with *Hin*dIII and subjected to Southern analysis. The revertants of *lys2-128* appear to result from excision of most of Ty*128*. The *LYS2* chromosomal segments of two of the revertants were cloned as described for the Tys. The *Eco*RI–*Bgl* II fragment in the cloned segment was 960 bp long instead of the original 630 bp found in pSL42-2, showing an insertion of 330 bp, as expected from the presence of a solo δ. Confirmation of the presence of a solo δ came from hybridization of this clone to the δ-containing probe S1 (2). The revertants of *lys2-173* appear to be more complex and heterogenous: some show a replacement of Ty*173* by a Ty with a different restriction pattern, whereas others show two *LYS2* regions, one with a new Ty near the *LYS2* gene and the other with the original Ty*173* insertion in the *LYS2* region. The latter class could result from unequal crossing-over between Ty*173* and another Ty on the same chromosome. Both reversion events, therefore, could have occurred by recombination or gene conversion (or both) between Ty*173* and other Tys. Events of this type have been documented at *HIS4* (7, 19).

Tetrad analysis of crosses of four Lys^+ revertants of *lys2-128 spt2-150* to a *LYS2* SPT^+ strain showed one or two lys^- progeny in some of the tetrads, demonstrating that the mutations at *LYS2* were auxotrophic when separated genetically from *spt2-150*. The Lys^+ revertants of *lys2-173 spt2-150* proved more refractory to genetic analysis. In crosses of 10 revertants with a *lys2-1* SPT^+ strain, 9 showed very poor

Table 3. Effects of *spt* mutations on auxotrophy of Ty-mediated mutations at *LYS2* and *HIS4*

Gene	Insertion orientation		*SPT*	*spt2*	*spt3*
LYS2	Ty*61*	←	CS	+	+
	Ty*128*	→	−	−	−
	δ128	→	−	+	+
	Ty*173*	←	−	−	−
HIS4	Ty*912*	→	−	−	−
	δ912	→	CS	+	+
	Ty*917*	←	−	+	+
	δ917	←	−	−	+

The 5′ end of the gene is to the right of the insertions. + denotes prototrophy; −, auxotrophy; CS is auxotrophy at low temperature and prototrophy at high temperature.

spore viability, suggesting chromosome rearrangements and aneuploidy. Nevertheless, in six of the nine crosses Lys^- progeny were obtained that were not *lys2-1* (as evidenced by mitotic recombination analysis with the deletion *lys2-201*). The presence of these Lys^- progeny suggests that the prototrophy of the revertants containing the Ty rearrangements or conversions is dependent upon suppression by *spt2*$^-$.

Suppression of a *lys2-128δ* mutation was studied in greater detail by construction of *lys2-128δ spt2-150* and *lys2-128δ spt3-101* double mutants. In both cases, the *spt* mutations suppress the Lys^- phenotype, demonstrating suppression of this δ insertion by both *spt2* and *spt3* mutations.

DISCUSSION

We have isolated five new Ty insertions at the *LYS2* locus of *S. cerevisiae*. Five additional Ty insertions at *LYS2* have been found by H. Eibel and P. Philippsen (20). Those found by Eibel and Philippsen all map between the *Eco*RI and *Bgl* II sites covered by the 4.7-kb cloned gene (Fig. 2), just outside the 5′ end of the coding region. Four of our Tys map in the same 0.6-kb segment, whereas the fifth, Ty*902*, maps inside the gene, ≈1.1 kb to the right of the *Bgl* II site. The distribution of Tys at *LYS2* appears to be nonrandom, because 9 of 10 map just outside the 5′ coding region. This distribution is similar to that of Tys at *HIS4*, *ADR2*, and other genes of yeast (1). The only other Ty insertion mutation besides Ty*902* identified to be within the coding region of a gene is Ty*52*, an insertion within the *URA3* gene (21).

The Tys we characterized are different from each other in restriction sites, in orientation, in the Lys^- phenotype of the mutants carrying them, and in the frequency and nature of Lys^+ revertants. The differences in the Lys^- phenotype could result from the position of the insertion relative to the coding region, from the variability in Ty structure that exists among the 30–35 different elements in the yeast genome, or from a combination of position, structure, and orientation.

Table 3 shows a comparison between insertion mutations at *LYS2* with those at *HIS4*. At both genes auxotrophy results from insertions in the 5′ noncoding regions, regardless of the orientation of the Ty element. Ty*128* at *LYS2* is very similar to Ty*912* at *HIS4*. Both elements revert by δ–δ recombination and the resulting solo δ in each case is suppressed by both *spt2* and *spt3*. We also found that the Ty*61* insertion at *LYS2* is suppressed by *spt2* and *spt3* mutations, similar to Ty*917* at *HIS4* (8). Thus, mutations resulting from four different Ty elements, two at *HIS4* and two at *LYS2*, are suppressed by the same *spt* mutations.

The suppression of independent Ty-mediated mutations at two different loci by the same *spt* mutations shows that the action of the *spt* genes is independent of the function of the genes near which the Tys insert. This result suggests that the *SPT* gene products interact in some way with δ and Ty elements to exert their phenotype. Suppression of the yeast insertion mutations is analogous to what has been observed in *Drosophila melanogaster* in the case of gypsy insertion mutations and their suppression by *su(Hw)*, the suppressor of Hairy wing (21).

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