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Genetics

Rapid mapping of antigenic coding regions and constructing insertion mutations in yeast genes by mini-Tn*10* "transplason" mutagenesis

(λgt11/immunoscreening/antibody probes/transposon mutagenesis)

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ABSTRACT **A "transplason" mutagenesis procedure was developed for the dual purposes of low resolution mapping of antigenic coding regions (using transpo*sons*) and constructing insertion mutations in yeast genes (by *transpla*cement). Mini-Tn*10* transposon derivatives containing both *Escherichia coli* and yeast selectable markers have been constructed. These elements are used to mutagenize λgt11 clones that express foreign antigens in *E. coli*. The transposition events are first selected in *E. coli*, and the effect of these insertions on antigen expression is used to locate the antigenic coding regions on the cloned DNA. Insertion mutations located within a desired yeast sequence are then substituted for the genomic copies by one-step gene transplacement. This provides a powerful method for rapidly mapping antigenic coding sequences of cloned genes and inactivating these genes in yeast to help determine their function. Several examples using this technique are presented.**

There are many techniques for cloning genes. Once a clone containing a gene of interest is isolated, the coding sequences must be identified. In addition, in yeast and bacteria, a mutated gene can be readily substituted for the genomic copy to help determine the function of the gene *in vivo* (for examples, see refs. 1–3).

A rapid method for isolating genes is to use λgt11 expression libraries and antibody probes (4–6). λgt11 is a bacteriophage vector that contains the *lacZ* gene of *Escherichia coli* for the expression of foreign DNA inserts as β-galactosidase fusion proteins. A recombinant DNA library is first constructed in λgt11 and foreign antigens are expressed in *E. coli* from the DNA inserts. The antigens produced are transferred to nitrocellulose filters and detected with antibody probes. This technique is called immunoscreening.

In general, cDNA libraries are used for immunoscreening. However, for organisms of small genome size, such as yeast (1.4×10^7 base pairs), genomic libraries have been constructed and screened (M.S. and R.W.D., unpublished results; ref. 7). These have the advantage that all sequences of the genome can be screened independently of their level of expression in the organism of interest. However, in yeast, >50% of the clones isolated by immunoscreening the genomic library do not synthesize β-galactosidase fusion proteins. Instead, the foreign antigens were expressed and not fused to β-galactosidase, as was observed in the isolation of yeast genes encoding topoisomerase II, ubiquitin, and the examples described below (2, 8). In some cases, the expression of these genes is still *lacZ* dependent and, presumably, the *lacZ* promoter is used for expression (Fig. 1, case II). Alternatively, expression can be *lacZ* independent and insert sequences can serve as transcriptional and translational signals in *E. coli* (case III). In cases in which β-galactosidase fusion proteins are not identified, it can be particularly difficult to locate the coding sequences.

To map coding sequences on λgt11 clones, a mini-Tn*10* (mTn*10*) "transplason" mutagenesis scheme was developed. This procedure uses transposon insertions to rapidly identify antigen coding sequences and can be applied to λgt11 clones containing DNA from any source. In addition, because the mTn*10* transplasons contain yeast selectable markers, yeast genes containing transplason insertions can be substituted for the genomic copy to determine the effect of mutating the gene *in vivo*. Independently, H. Seifert and F. Heffron (personal communication) have developed a similar procedure for mutagenizing plasmid clones with Tn*3* transposons containing yeast selectable markers. The procedure described below is designed for mutagenesis of phage clones, particularly λgt11 clones, and allows the rapid mutagenesis of newly cloned yeast genes.

MATERIALS AND METHODS

Bacterial and Yeast Strains. The following *E. coli* strains were used: BNN91, Δ*lacZ hflA150 strA* (4); NM430, *hsdR lacZ* (amber) (9); MC1061(p3), F⁻ *hsdR Δara-leu7697 araD139* Δ(*lac*)*3 galU-galK*⁻ *rpsL* (str^R) containing the p3 plasmid [RPI amp^R (amber) tet^R (amber) *tra*] (10); BNN45, *hsdR514* (r⁻,m⁺) *supE44 supF58 B1*⁻ *met*⁻ (11); BNN114 was derived from RB132 (12–15) by isolating amp^S colonies that lost the plasmid, pNK217, which contains a mTn*10* transposon (13). BNN114 genotype, F⁻ Δ*lac rpsL gal* chr::Tn*10*Δ4HH104 (overproduces Tn*10* transposase) is described (12, 13).

The yeast strain used was YNN293 × YNN294; **a**/α *ura3-52/ura3-52, lys2-801/lys2-801, ade2-101/ade2-101, trp1-Δ901/+, his3-Δ200/+, tyr1/+* (derived from M. Johnston's strains, this laboratory). It was derived from S288C.

Clones and Antibody Probes. The following yeast clones were used: λgt11·RNA polymerase, λ102, was isolated by J. Kelly, A. Greenleaf, and I. R. Lehman. λgt11·spindle pole antigen clones and λEMBL3A·spindle pole antigen clone, λ3, which contains a 15-kilobase (kb) yeast insert were isolated by M.S. and R.W.D. A λgt11·ribonucleotide reductase clone producing a fusion protein was isolated by S.E. and R.W.D. A description of all these clones and the polyclonal antibody probes used to isolate them will be published elsewhere. Immunoscreening protocols can be found in ref. 6.

Construction of Tn*10* Transplason Mutagenesis Vectors. mTn*10*/*URA3*/*tet*^{R} was constructed by cloning the 1.1-kb *Hin*dIII *URA3* fragment of YEp24 (16) into the *Hin*dIII site

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Abbreviations: X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactoside; IPTG, isopropyl β-D-thiogalactopyranoside; kb, kilobases.

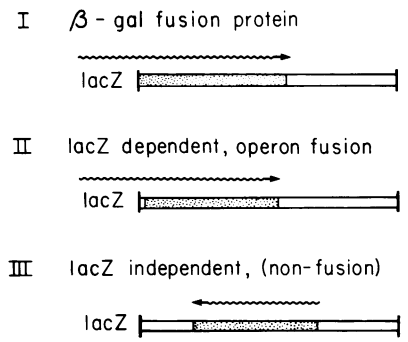

FIG. 1. Antigen expression from λgt11 genomic clones. Expression of foreign antigens from λgt11 genomic clones can either be as β-galactosidase fusion proteins (I), or not fused to β-galactosidase (II and III). Expression of antigens as nonfusion proteins is either *lacZ*-dependent and *lacZ* promoter sequences are required (II) or *lacZ*-independent where insert sequences probably serve for transcription and translation (III). Stippled region indicates protein coding sequences and wavy arrow indicates RNA.

of pNK217 (12–14). mTn*10/URA3/supF* was constructed by excising the internal *Hpa* I fragment of the mTn*10/URA3/tet*^{R} vector and replacing it with the 0.2-kb *Eco*RI *supF* fragment [source, J. Reichardt; originally from πVX(10)] containing ends filled in with DNA polymerase I large fragment (17). mTn*10/TRP1/kan*^{R} was made by ligating a *Hin*dIII/*Bam*HI fragment (S.E., unpublished results) containing the *neo*^{R} (*kan*^{R}) gene of Tn*5* (18) together with the *Eco*RI (made flush with the large fragment of DNA polymerase I)/*Bgl* II-cut fragment of YRp12 (19) containing the *TRP1* gene into *Hin*dIII/*Hpa* I-cut pNK217. All constructions are contained on pBR322-type plasmids and are present in an *E. coli* strain, BNN114, that overproduces the Tn*10* transposase.

Transposition Mutagenesis of λgt11 and EMBL3A Clones. λgt11 clones were mutagenized with mTn*10/URA3/tet*^{R} and mTn*10/TRP1/kan*^{R} as follows: 0.5-ml cultures of *E. coli* strains carrying the mutagenesis vectors were grown in LB medium (11) ampicillin (50 μg/ml)/0.2% maltose/5 mM $MgSO_4$, to OD_{600} = 0.4 (≈2 × 10^8 cells). These were infected with 4 × 10^8 λgt11 phage (multiplicity of infection, 2), and incubated with shaking for 2 hr at 37°C. Cells were lysed by the addition of several drops of chloroform and incubated for 20 min at 37°C. The lysate was cleared twice of cells and debris by centrifugation in an Eppendorf centrifuge for 5 min and stored over 100 μl of chloroform.

Cleared lysate (100 μl; 2–10 × 10^8 phage) is then mixed with 0.4 ml of BNN91 culture grown to saturation on LB medium/0.2% maltose/5 mM $MgSO_4$ incubated for 25 min at room temperature, plated on LB plates containing either 15 μg of tetracycline per ml to select Tet^R lysogens or 25 μg of kanamycin per ml to select for Kan^R lysogens (11). Plates were incubated at 30°C. Colonies (2–10 × 10^4) appear after ≈20 hr. Lysogens can be further checked by testing for growth at 30°C and at 42°C. Cells containing λgt11 clones, which has *cI857* (a *cI* gene that encodes a temperature-sensitive repressor), will not grow at 42°C. Under these conditions, >95% of the colonies do not grow at 42°C. The frequency of transposition events detected (≈10^{-4} per phage produced) is similar to values observed by others (12–15).

Selection of mTn*10/URA3/supF* transpositions was carried out as follows: λgt11 clones and EMBL3A clones (≈10^4 phage per 90-mm LB plate) were plated on 0.1 ml of a saturated culture of the mTn*10/URA3/supF* mutagenesis strain grown on LB medium/0.2% maltose/ampicillin (50 μg/ml). Plates were incubated at 37°C for 8 hr. Phage were extracted from the plate by using 2.5 ml of λdil (20 mM Tris·HCl, pH 8.0/10 mM $MgSO_4$) and incubating with shaking for 2 hr at room temperature or overnight at 4°C. Chloroform-treated lysates were next plated on a nonsuppressing host (NM430) to select suppressor-containing phage. Phage were extracted from these cells in λdil as described above and suppressor-containing phage were identified by either of two methods: (*i*) Plating on a *lacZ* amber host NM430 in the presence of 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal) and isopropyl β-D-thiogalactopyranoside (IPTG) to identify blue plaques or (*ii*) infecting MC1061 (p3) cells that contain a plasmid bearing *tet*^{R} (amber) and *amp*^{R}(amber) genes, and selecting for both *tet*^{R} and *amp*^{R} lysogens (reversion frequency of both Tet^R and Amp^R genes, 10^{-10} to 10^{-12}). This was performed in a fashion analogous to that described above for selecting for λgt11::mTn*10/URA3/tet*^{R} or λgt11::mTn*10/TRP1/kan*^{R} lysogens.

Preparation of Mutagenized Phage and Phage DNA. Phage were prepared from individual lysogens by growing 5-ml cultures at 30°C in L broth/5 mM $MgSO_4$/0.2% maltose to OD_{600} = 0.5 (see ref. 11). Tubes were shifted to 44°C for 15 min with vigorous shaking to induce lysogens and then incubated at 37°C for 2 hr. Cultures were lysed by the addition of $CHCl_3$ and incubated for an additional 15 min at 37°C. "Libraries" of transposon mutagenized phage can be prepared by mixing the collection of lysogens and using the mixture to inoculate the starting culture.

Quick preparations of phage DNA were prepared as follows (11): Phage stocks (5 ml) were centrifuged at 6000 × *g* for 5 min to remove cellular debris. Supernatants were treated with RNase A (1 μg/ml)/DNase I (0.2 μg/ml), and incubated for 60 min at 0°C. Phage were precipitated by the addition of 0.35 ml of 5 M NaCl and 0.5 g of PEG-4000 and incubated overnight at 4°C. Phage were recovered by centrifugation in a JA20 rotor at 10,000 rpm for 15 min at 2°C. All supernatant was discarded. The pellet was resuspended in 0.3 ml of λdil and extracted with an equal volume of chloroform. Sixty microliters of 5 M NaCl and 15 μl of 0.5 M EDTA were added and the solution was extracted successively with an equal volume of phenol, phenol/chloroform (1:1), and chloroform alone. The sample was precipitated with 0.75 ml of 95% ethanol at 4°C for 2–12 hr. The DNA was recovered by centrifugation, rinsed once with 70% ethanol, dried, and resuspended in 50 μl of double-distilled H_2O, yielding ≈5 μg of λ DNA. Restriction mapping analysis of mutagenized phage was performed with *Eco*RI and *Hin*dIII according to standard conditions (17).

Yeast Transplacement (20). Miniprep λgt11 clone DNA (2–3 μg) was digested with enzymes that cut in the yeast sequences flanking the transposon. For the λgt11·RNA polymerase clone, λ102, *Bgl* II, which cuts near each end of the insert, was used. *Eco*RI partial digests have also been used successfully.

The resulting DNA fragments were transformed into diploid yeast cells by using the LiAc procedure (21). Approximately 10–50 transformants were obtained for each clone. λgt11·RNA polymerase was transformed by A. Greenleaf into YNN293 × YNN294. Substitution at the RNA polymerase locus was confirmed by genomic Southern analysis (17). For the mTn*10/URA3/tet*^{R} transplacements using RNA polymerase clones and other clones, gene conversion at the *ura3-52* locus has not been observed (eight transformants tested). For mTn*10/TRP1/kan*^{R} transplacements, gene conversion at the *trp1-Δ901* locus is not possible because all of the homologous *TRP1* sequences have been deleted in this strain (P. Hieter and R.W.D., unpublished results; ref. 22).

Construction of a Yeast EMBL3A Genomic DNA Library. Yeast DNA prepared from YNN259, *a ura3-52, his3-Δ200, lys2-801, ade2-101, met*$^-$ (gift of M. Johnston, Washington University) was partially digested with *Sau*3A and size-fractionated on a sucrose gradient (17). One microgram of 13- to 19-kb fragments (average size, ≈15 kb) was cloned into 1

μg of EMBL3A (9) cleaved with *Bam*HI and *Eco*RI and was then ethanol-precipitated to prevent ligation of the internal fragments. The ligated DNA was packaged and amplified on BNN45 according to standard protocols (17). The library contains a total of 2×10^6 independent recombinants and <5% EMBL3A vector.

RESULTS

mTn*10* Mutagenesis Vectors. mTn*10* transposons (13) were used to construct a new type of mutagenesis vector. These are called mTn*10*/*URA3*/*tet*^{R} (4.2 kb), mTn*10*/*URA3*/*supF* (2.1 kb), and mTn*10*/*TRP1*/*kan*^{R} (3.2 kb) and are shown in Fig. 2. Their relevant features are (*i*) they all contain the 70-base-pair terminal sequences of Tn*10* (12–14); (*ii*) each contains an *E. coli* selectable marker, *tet*^{R}, *supF*, or *kan*^{R}; (*iii*) they also contain the *URA3* or *TRP1* gene for selection of the inserted copy in yeast (see below); and (*iv*) they are present on high copy number pBR322-type plasmids and in an *E. coli* strain that overproduces Tn*10* transposase (12–14). Because of their use for both transpo*son* mutagenesis in *E. coli* and for *transpla*cement of genomic sequences in yeast, they are called transplasons.

Mutagenesis of λgt11 Clones. The overall strategy for transplason mutagenesis of λgt11 clones and constructing insertion mutations in yeast genes is illustrated in Fig. 3. λgt11 clones containing yeast inserts were first grown on the mTn*10* mutagenesis strains through one or more rounds of infection. Transposition events of the mTn*10*s into the clones were then selected. These events were detected at 10^{-4} per phage produced. mTn*10*/*URA3*/*tet*^{R} and mTn*10*/*TRP1*/*kan*^{R} insertions were obtained by infecting an *hflA* (*h*igh *f*requency *l*ysogeny) *E. coli* strain and selecting for Tet^R or Kan^R lysogens. mTn*10*/*URA3*/*supF* transpositions were selected

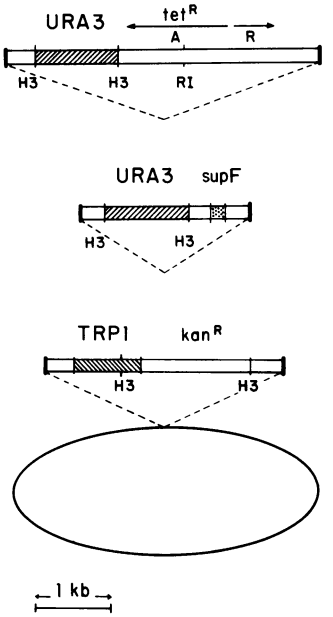

FIG. 2. mTn*10* transplason mutagenesis vectors. Top to bottom: mTn*10*/*URA3*/*tet*^{R} (4.2 kb), mTn*10*/*URA3*/*supF* (2.1 kb), mTn*10*/*TRP1*/*kan*^{R}(3.2 kb). Each contains a yeast-selectable marker, *URA3* or *TRP1*, an *E. coli*-selectable marker, *tet*^{R}, *supF*, and *kan*^{R}, and the 70-base-pair terminal sequences of Tn*10* (indicated by the thick bars at the transplason termini). All are contained on high copy number pBR322-type plasmids (oval at the bottom) and in an *E. coli* strain that overproduces Tn*10* transposase (12–14). *tetR* and *A* genes encode the structural gene for tetracycline resistance and its repressor. The position of the *Eco*RI (RI) and *Hin*dIII (H3) sites is indicated. More detailed maps of these elements are available on request.

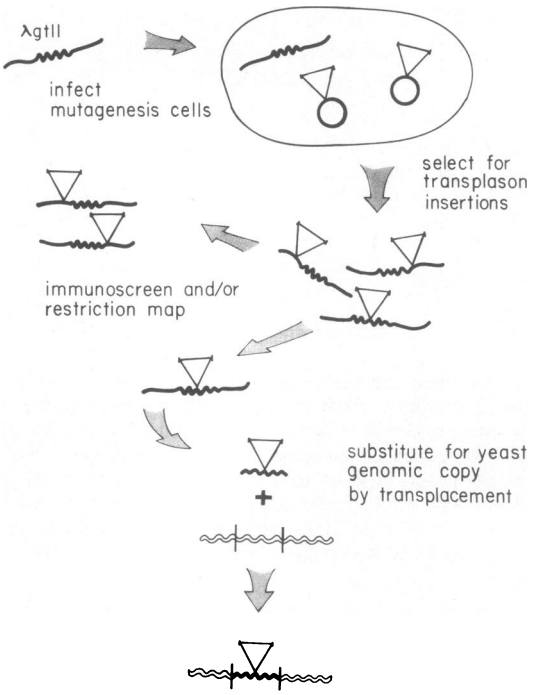

FIG. 3. Transplason mutagenesis scheme for λ clones. λgt11 (or EMBL3a) clones were mutagenized as described and transplason insertions were selected. Transplasons (▽) located in the gene of interest (〰) are identified by restriction mapping and/or immunoscreening. Finally, the mutated copy is substituted for the genomic copy (≈) to determine the effect of mutating the gene *in vivo*.

by plating on a nonsuppressing *E. coli* host. λgt11 carries *S100*, an amber mutation in the *S* lysis gene so that only phage that have reverted (10^{-5} to 10^{-6} per phage) or acquired a suppressor will form plaques. In the four cases tested (Table 1), 30–60% of the transposition events occur in the 3- to 4-kb cloned DNA inserts as judged by restriction mapping analysis. Insertions of the 4.2-kb mTn*10*/*URA3*/*tet*^{R} transplason into a λgt11 clone containing a 4.0-kb insert increases the phage size to 52.9 kb, which is 109% that of wild-type λ DNA. Nonetheless, these phage form medium-sized plaques in the presence of 5 mM Mg^{2+} ion.

Mapping Antigenic Coding Regions Using mTn*10* Transplason Mutagenesis. After transplason mutagenesis, clones were screened with antibody probes to map the antigenic coding regions. The effect of the insertions on the level of

Table 1. Fraction of transpositions located in cloned DNA insert

Mutagenesis transposon	Yeast clone	Transpositions in insert per phage tested	Insert size, kb
mTn*10*/*URA3*/ *tet*^{R}	Mitochondrial RNA polymerase, λ102	6/9	4.0
mTn*10*/*URA3*/ *tet*^{R}	Spindle pole antigen gene, λ2.2	4/12	3.4
mTn*10*/*URA3*/ *supF*	Spindle pole antigen gene, λ2.2	5/8	3.4
mTn*10*/*TRP1*/ *kan*^{R}	Ribonucleotide reductase gene, λ2-1	4/10	3.2

The first three cases record the number of transpositions located in the yeast insert/random phage chosen. In the fourth case, a phage containing ≈500 base pairs of open reading frame fused to β-galactosidase was mutagenized and immunoscreened for insertions that exhibit no immunoscreening signal. It is expected that the 6/10 insertions that lie outside the insert are located in *lacZ*.

antigen expression was determined by immunoscreening λ plaques. A correlation between the position of the insertion and the effect on the immunoscreening signal was used to locate the antigen coding region.

To test this method, mTn*10/URA3/tet*^{R} transplason mutagenesis was performed on a λgt11 clone encoding a mitochondrial yeast RNA polymerase activity (isolated by J. Kelly and I. R. Lehman, personal communication; see below) and on a yeast gene that encodes a protein immunologically related to mammalian spindle pole antigens (M.S. and R.W.D., unpublished data). The λgt11·mt RNA polymerase clone contains a 4.0-kb yeast insert encoding a 120- to 140-kDa nonfusion protein (A. Greenleaf and I. R. Lehman, personal communication; Fig. 4.) Most of this insert is protein coding sequence, except for the terminal 0- to 400-base-pair sequences. After mTn*10/URA3/tet*^{R} mutagenesis, six of nine randomly chosen phage had transposons located in the yeast DNA insert (Table 1). Four of these transposons are shown in Fig. 4, and two (nos. 1 and 2) appear by restriction mapping analysis to be located in an identical position. Based on the size of the polymerase protein produced in *E. coli*, all of these transposons must lie in protein coding sequences. Immunoscreening of clones 2, 3, and 4 showed that two (clones 2 and 3) have reduced immunoscreening signals, while the third (clone 4) had near maximal levels of signal (λgt11·polymerase control without transposons produced similar signals to clone 4). Data presented below demonstrate that the Tn*10* transplason insertions in a coding sequence completely eliminate the downstream expression in that coding sequence, regardless of the orientation of the insertion. These data suggest that the RNA polymerase gene is transcribed from left to right, as shown, and that most of the antigenic coding sequences lie in the left two-thirds of the clone. In summary, insertions located in protein coding sequences reduce the antigen signal detected with a polyclonal antiserum.

As another example of this technique, the antigenic coding sequences were mapped for a yeast clone, λ2.2, which was isolated with human anti-spindle pole autoantibodies (M.S. and R.W.D., unpublished results). Expression of antigens from this clone is *lacZ* independent, and the location of the coding region is unknown. A library of 10^5 mTn*10/URA3/tet*^{R} insertions was generated on λ2.2 containing a 3.4-kb yeast DNA insert. Four hundred mutagenized phage were screened with antibody probes. Eight phage that exhibited reduced or no immunoscreening signals were chosen and retested along with four other randomly chosen phage that contained transposons in their yeast insert. The position of each transposon was determined by restriction mapping analysis. The immunoscreening and insertion position results are shown in Fig. 5. Six insertions, which lie in four unique positions on the left end of the insert, all show no immunoscreening signal. Three of these insertions are within 50 base pairs of the *lacZ*/yeast DNA boundary. The insertion at position 7 results in a very weak immunoscreening signal. Numbers 8, 9, 10, and 11 exhibit 70–100% the immunoscreening signal of the parental phage, λ2.2. Number 12 exhibits full-level expression. These data, therefore, suggest that most of the antigenic determinant coding regions lie in the 800-base-pair region between numbers 7 and 8, and that this gene is transcribed from left to right. Alternative explanations are discussed below. Note that the mTn*10/URA3/tet*^{R} insertions 1–6 completely eliminate downstream antigen expression in *E. coli*, regardless of the orientation of the transposon. A similar result was obtained for another yeast clone, λ1.1, isolated with anti-spindle pole antibodies, which makes a 160-kDa β-galactosidase fusion protein. All (5/5) insertions that completely eliminated λ1.1 immunoscreening signals were located in the *lacZ* gene. Thus, insertions located in coding sequences can eliminate downstream antigen expression.

One surprising result in the two examples described above is that the mTn*10* transplasons inserted with a preferred orientation. Four out of four transpositions in the yeast RNA polymerase clone were in one orientation relative to *lacZ*; for the spindle pole antigen clone, λ2.2, 11/12 insertions were in the opposite orientation.

Creating Transplason Mutations in Yeast Genes. A further advantage of this technique is that the mutagenized yeast clones can be directly substituted for the genomic copies, thereby inactivating the gene *in vivo*. This is done by one-step

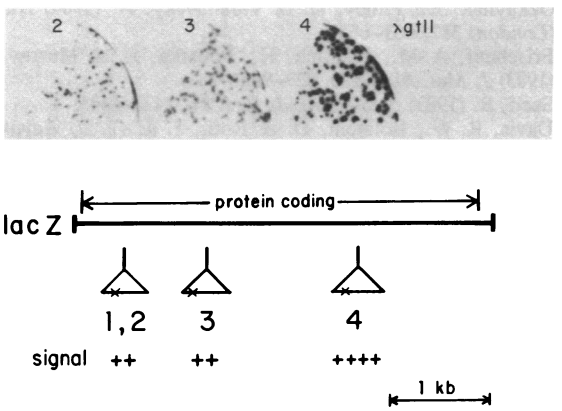

FIG. 4. Effect of mTn*10* transplason insertions on immunoscreening signals for a yeast mitochondrial RNA polymerase clone. A λgt11 clone with a 4.0-kb yeast insert coding a RNA polymerase gene was mutagenized with mTn*10/URA3/tet*^{R}. This clone has been described by A. Greenleaf, J. Kelly, and I. R. Lehman (personal communication). The protein produced from λgt11·mt RNA polymerase in *E. coli* is not fused to β-galactosidase, and it is not known whether its expression is *lacZ* dependent. Four phage with insertions located within the protein coding region were chosen and plaques were immunoscreened with anti-yeast RNA polymerase antibody. Each spot represents a plaque that is producing antigen, which is detected with antibody probes and ^{125}I-labeled protein A. The parental polymerase clone without insertion elements produces immunoscreening signals similar to that of clone 4. The insertions (not drawn to scale) are all oriented such that the *URA3* gene, indicated by the X, is located on the left.

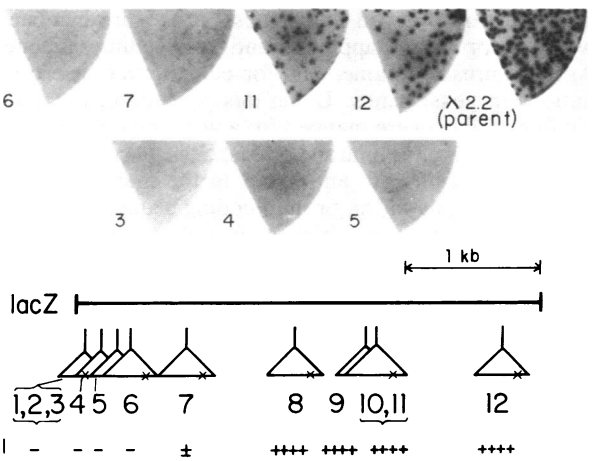

FIG. 5. Transplason mutagenesis on λ2.2—a λgt11·spindle pole antigen clone. λ2.2 contains a 3.4-kb yeast DNA insert, and its antigen expression in *E. coli* is *lacZ* independent. This clone was mutagenized mTn*10/URA3/tet*^{R}. Four random insertions, 3, 8, 9, and 12, and eight other phage that exhibited reduced or no immunoscreening signals were chosen and retested by immunoscreening as described in the text. The immunoscreening signal for clone 11 is slightly reduced relative to clone 12 and the parental clone, and it is difficult to see in this figure. Numbers 8 and 9 gave similar signals to that of number 11 (data not shown). All but number 4 were oriented such that the *URA3* gene, indicated by the X, is on the right.

gene transplacement (20). Mini-preparations of yeast DNA are cut on either, or both, sides of the transplason-containing insert with a restriction enzyme (usually *Eco*RI), and the resulting fragments are used to transform diploid Ura$^-$ or Trp$^-$ yeast. Selection for Ura$^+$ or Trp$^+$ colonies yields transformants where the inserted gene replaces one of the *in vivo* copies. The diploid cells are sporulated and tetrads dissected to determine the effect of the insertion on cell viability. If the gene is essential, two Ura$^-$ (or Trp$^-$) spores and two dead spores are obtained.

For the case of the yeast mitochondrial RNA polymerase λgt11 clones, all three mTn*10*/*URA3*/*tet*^{R} insertions 2, 3, and 4, were substituted for genomic copies in a Ura$^-$ yeast strain (A. Greenleaf, J. Kelly, and I. R. Lehman, personal communication). Sporulation and dissection revealed that all four spored tetrads segregated two wild type/two petite colonies, where the petites were all Ura$^+$. Thus, the RNA polymerase gene is required for a mitochondrial function. The phenotype of this mutant will be described elsewhere (A. Greenleaf, J. Kelly, and I. R. Lehman, personal communication).

Constructing Insertion Mutations in Yeast EMBL3A Clones. A yeast genomic DNA library was prepared in which 15-kb fragments of *Sau*3A-digested DNA were cloned into the bacteriophage vector EMBL3A (described in *Materials and Methods*). EMBL3A contains amber mutations in the *A* and *B* genes (9). Reversion of both amber mutations was determined to occur at a frequency of $<10^{-11}$. Transplason mutagenesis of EMBL3A clones can be performed by first growing clones on a mTn*10*/*URA3*/*supF* transplason mutagenesis strain and then plating on a nonsuppressing host to select phage that have acquired a *supF* gene. This procedure was performed for a yeast clone with a 15-kb insert encoding a protein antigenically related to mammalian spindle pole. After selecting transplason insertions, all phage produced blue plaques when plated on an *E. coli* strain containing a *lacZ* (amber) gene in the presence of IPTG and X-Gal, indicating that they had acquired a suppressor. Thus, clones isolated from the EMBL3A library can be rapidly mutagenized by this process.

DISCUSSION

A mTn*10* transplason mutagenesis procedure has been developed for rapid mapping of antigenic coding sequences of λgt11 expression clones and for constructing insertion mutations in yeast genes. Using this procedure, the antigenic coding regions were mapped for a yeast mitochondrial RNA polymerase·λgt11 clone and a yeast clone encoding an antigen that crossreacts with antibodies to the mammalian spindle pole. Insertions in the protein coding sequence give rise to reduced immunoscreening signals with a polyclonal antiserum. This suggests that the original clone encodes multiple epitopes and that only some epitopes are expressed when the coding sequences are disrupted. However, other interpretations are also possible. Transpositions that give reduced or no signals may simply affect mRNA or protein stability or could have a long-distance effect on transcription or translation. Insertions can also lie in a protein coding sequence outside the antigen coding region and affect antigen presentation by altering the secondary structure of that protein. Analysis will be further complicated if a polyclonal antiserum principally recognizes only one epitope, similar to that of a monoclonal antibody, or a limited number of epitopes. It is therefore useful to isolate many insertions throughout a cloned DNA insert, which is easy to accomplish. A correlation of the position of the insertion with the effect on the immunoscreening signal can suggest an approximate location of an antigen coding region. This can be tested further by RNA mapping and/or DNA sequence analysis. All relevant insertions can be substituted for genomic DNA copies. Insertion mutations were constructed in yeast for an RNA polymerase gene, demonstrating that it had a mitochondrial function.

The key features of this procedure are as follows: (*i*) It is fast and simple. (*ii*) It can be applied directly to newly isolated λgt11 clones without subcloning or detailed restriction analysis. This is particularly useful when multiple genes are isolated from a library screen. In yeast, the entire genome can be screened to identify coding regions that react with a given antibody. Each gene identified in such a screen can be inactivated to examine its role *in vivo*. (*iii*) It should be possible to apply this procedure to map the sequences encoding the epitope recognized by a monoclonal antibody. (*iv*) A series of transplason insertions can be used to generate a series of truncated proteins in yeast and may identify functional domains in proteins. (*v*) Transplasons inserted adjacent to a gene of interest can be accomplished without recloning and used as linked selectable markers for genetic mapping and strain construction.

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