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Genetic Mapping of Ty Elements in *Saccharomyces cerevisiae*

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We used transformation to insert a selectable marker at various sites in the *Saccharomyces cerevisiae* genome occupied by the transposable element Ty. The vector CV9 contains the *LEU2*$^+$ gene and a portion of the repeated element Ty1-17. Transformation with this plasmid resulted in integration of the vector via a reciprocal exchange using homology at the *LEU2* locus or at the various Ty elements that are dispersed throughout the *S. cerevisiae* genome. These transformants were used to map genetically sites of several Ty elements. The 24 transformants recovered at Ty sites define 19 distinct loci. Seven of these were placed on the genetic map. Two classes of Ty elements were identified in these experiments: a Ty1-17 class and Ty elements different from Ty1-17. Statistical analysis of the number of transformants at each class of Ty elements shows that there is preferential integration of the CV9 plasmid into the Ty1-17 class.

The family of repeated sequences in *Saccharomyces cerevisiae* called the Ty sequences has been described by Cameron et al. (4). Their study suggested that the chromosomal location of a particular Ty element can vary between different strains of *S. cerevisiae* and that the appearance of a Ty element at a site previously shown to be lacking one is due to a transposition of a Ty element into that site. Subsequently, several groups have shown that Ty elements can arise at new positions in the yeast genome (10, 27, 35). This type of event will result in a net increase in the number of Ty elements in the genome. The frequency of these transposition events is difficult to measure because their detection usually requires a change in the expression of a gene in the vicinity of the site of transposition of the Ty element. Most laboratory strains contain 30 to 40 Ty elements (4, 9, 14), and this number appears to be relatively stable.

The number of Ty elements has been estimated by restriction endonuclease digestion and Southern hybridization of genomic DNA. Although these results show a wide distribution in the sizes of fragments that hybridize with a Ty probe, in the absence of cytological evidence there are no data that can describe the distribution of the 30 to 40 Ty elements among the 17 chromosomes of the *S. cerevisiae* genome. The fact that the pattern of hybridization remains relatively stable suggests that the rate of change in the overall distribution is quite low. Thus, it should be feasible to place the Ty elements on the genetic map.

In their initial studies on *S. cerevisiae* transformation, Hinnen et al. (17) reported that 5 of 42 transformants analyzed after transformation of *S. cerevisiae* strain AH22 with pYeLeu10 (containing the *S. cerevisiae LEU2*$^+$ gene) were at chromosomal locations other than the *LEU2* locus. The reason for this observation was not clear at the time, although the presence of a repeated yeast sequence on the plasmid was suggested (16). Subsequent studies have shown that a fragment of a Ty element near the *LEU2* gene is present on the plasmid pYeLeu10 (18). It seems likely that these five transformants resulted from integration of pYeLeu10 at different Ty sites through an interaction between the Ty sequence on the plasmid and the Ty sequences on the *S. cerevisiae* chromosome.

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The particular Ty element adjacent to the *LEU2* gene carried on the pYeLeu10 plasmid is called Ty1-17 (18). Comparisons of this Ty element with the Ty1 element described by Cameron et al. (4) by restriction enzyme mapping and electron microscopic heteroduplex analyses have shown that there are two large regions of sequence divergence between Ty1 and Ty1-17. The substitutions are sufficiently different that a 1.6-kilobase-pair (kbp) *Cla*I fragment from one substitution sequence in Ty1-17 does not cross-hybridize to the Ty1 elements in a Southern hybridization (14).

We used the CV9 vector to insert the selectable *LEU2*$^+$ gene into Ty elements to mark these elements genetically and thus permit their mapping. The segment of the Ty1-17 element that is carried on the CV9 vector (Fig. 1) is derived from the region in common with other Ty1 elements (18) and is contained in the most conserved sequence of the Ty elements (28, 34a). We found that the transformation event is a result of a reciprocal recombination event between a chromosomal Ty element and the fragment of Ty1-17 present on CV9. By examining the type of Ty elements in which the CV9 vector has integrated, it should be possible to determine whether the plasmid exhibits preferential integration at the Ty1-17 class of Ty elements.

MATERIALS AND METHODS

Strains. The strains used in these experiments are listed in Table 1. Independent transformants of strain AH22 with CV9 are numbered as AH22(CV9)-1 through AH22(CV9)-49. Derivatives of these transformants in a *MAT*α background instead of the *MAT***a** genotype of AH22 were obtained by crossing the transformants to strain 2262. *MAT*α *LEU2*$^+$ spore isolates were used in crosses between two different transformants. The vector CV9 is shown in Fig. 1. Recombinant vectors used in this study are listed in Table 2.

Genetic analysis. Standard genetic analysis techniques were used (22).

Media. YPD medium was used for nonselective growth (30). Galactose utilization was scored on YP medium with 1.5% galactose, and the petite phenotype markers were scored on YP medium with 3% glycerol. Nutritional markers were scored on synthetic complete media lacking the appropriate supplement. Canavanine sulfate was added at 60 μg/ml to

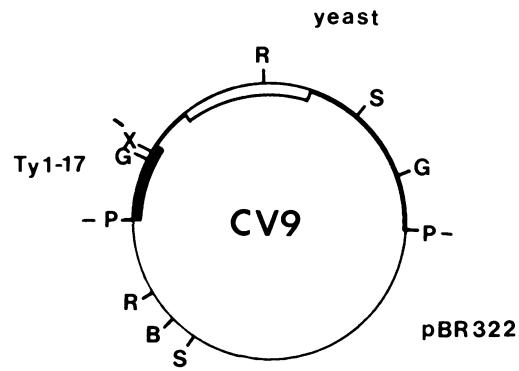

FIG. 1. Map of CV9 vector. The vector CV9 is composed of a 4.5-kbp *Pst*I insert containing the *LEU2*$^+$ gene inserted into the *Pst*I site of pBR322. The coding sequence for the *LEU2* gene is represented by the open box. The fragment of the adjacent Ty1-17 sequence included in CV9 is represented by the black box and is ca. 820 base pairs. The dark lines represent *S. cerevisiae* sequences, and the thin lines represent pBR322 sequences. Positions of the indicated restriction endonuclease sites were determined from published sequences (2) and sequences of the *Sal*I-*Xho*I fragment containing the *LEU2*$^+$ gene (A. Andreadis, personal communication). Abbreviations: P, *Pst*I; G, *Bgl*II; X, *Xho*I; R, *Eco*RI; S, *Sal*I; B, *Bam*HI. Figure is drawn to scale.

synthetic complete plates lacking arginine to score canavanine resistance.

Isolation of DNA. *S. cerevisiae* nuclear DNA was isolated on cesium chloride gradients containing Hoechst dye 33258 (34). Plasmid DNA was isolated from cesium chloride gradients containing ethidium bromide (6). Plasmid DNA fragments were isolated from agarose gels by the method of Chen and Thomas (5).

***S. cerevisiae* colony hybridization.** *S. cerevisiae* colony hybridization was performed according to Hinnen et al. (17).

Southern analysis. Restriction enzyme digestions and ligation reactions were performed according to the conditions recommended by the supplier. Approximately 5 μg of *S. cerevisiae* nuclear DNA was used in each reaction. The resulting fragments were separated by agarose gel electrophoresis on horizontal gel slabs ranging from 0.5% to 0.7%, run at 1 V/cm for 36 to 40 h. DNA fragments were transferred to nitrocellulose (32) and hybridized to DNA probes labeled with ^{32}P by the nick-translation reaction (29).

RESULTS

Types of transformants. Forty-nine independent transformants were obtained by transforming strain AH22 with the vector CV9 to *LEU2*$^+$. These were first analyzed by yeast colony hybridization with radiolabeled pBR322 and by tetrad analysis of crosses of the transformants to the *LEU2*$^+$ strain HK3A. The data obtained allowed us to group the transformants as type I, II, or III events according to Hinnen et al. (17). Type I transformants contain the CV9 vector integrated into chromosome III at the *LEU2* locus. DNA from such transformants hybridizes to pBR322. The type I transformants give 4$^+$:0$^-$ segregation for *LEU2*$^+$ when crossed to wild type. Twenty-two transformants were in this class. Type II transformants result from the CV9 vector integrating at a chromosomal location different from the *LEU2* locus. These transformants also contain pBR322 sequences in chromosomal DNA. However, when crossed to a *LEU2*$^+$ strain, deviations from 4:0 segregation of *LEU2*$^+$ are seen. If the site of integration is at a centromere-linked site other than chromosome III (the chromosome containing the *LEU2* locus), then equal numbers of 4:0 and 2:2 tetrads will be recovered, and the frequency of 3:1 tetrads will be less than two-thirds. This results from independent assortment of the *LEU2* gene on chromosome III and the *LEU2*$^+$ gene brought in by the CV9 vector to another linkage group. If this second linkage group carries the *LEU2*$^+$ gene at a site that is not centromere linked, then two-thirds of the segregations will be 3:1. Additional data were necessary to analyze transformants carrying the CV9 vector integrated at sites on chromosome III other than the *LEU2* locus. Half (25) of the transformants recovered were of the type II category.

TABLE 1. Strains used in experiments

Strain	Genotypes	Source
W224-1C	α *leu2-1 ura3 lys1-1 his6 met14 pet8*	S. Klapholz
W224-1D	α *leu2-1 ura3 lys1-1 his6 met14 pet8 aro7*	S. Klapholz
W223-9A	α *gal1 trp1 his2 cdc14 arg4 his6 asp5 pet17*	S. Klapholz
W230-12A	α *ade1 leu1 met3 lys7 met4 cyc3 cyc2 his6*	S. Klapholz
W85-8B	**a** *ade2-1 leu1-12 lys2-1 met4-1 trp5-2 ura1-1 can1-100*	S. Klapholz
W87-4D	α *ade2-1 leu1-12 lys2-1 met4-1 trp5-2 ura1-1 can1-100*	S. Klapholz
S90-2D	**a** *ade1 lys1 lys7 his2 his6 met3 gal7 tyr2*	S. Klapholz
S90-2C	α *ade1 lys1 lys7 his2 his6 met3 gal7 tyr2*	S. Klapholz
JG49-20A	**a**/α *HO ade1 leu1 his2 met14 trp1 ura3 gal*	S. Klapholz
2262	α *ade1 ura1 gal1 his5 lys11 leu2*	T. Petes
AH22	**a** *his4-519 leu2-3 leu2-112 can1*	G. Fink
HK3A	α *trp1 ade6 met13 tyr7*	H. Klein
HK180-4A	α *leu1 leu2 his4 met4 ade1*	This study
HK181-2B	α *leu2 pet8 lys1 ura3 his6*	This study
HK182-8D	α *his4 his6 leu1 leu2 met3 met4 lys7 ade1*	This study
HK183-6C	α *gal1 his2 his4 his6 arg4 asp5 pet17 cdc14 leu2*	This study
HK201-3D	α *leu2 cdc4*	This study
HK202-2A	α *thr4 ade6 leu2 ura1 can1*	This study
HK204-23D	α *leu2 ura3 pet8 lys9 his2 his4 met14*	This study
HK205-5D	α *leu2 lys9 pet8 met4*	This study
HK206-13C	α *cdc8 leu2 ade1 can1 met1 ura1*	This study

Type III transformants contain a *LEU2*$^+$ gene at the *LEU2* locus but do not contain pBR322 sequences and presumably are the result of a gene conversion event between the CV9 *LEU2*$^+$ sequence and the chromosomal *LEU2* gene (17). Two transformants belonged to this class. These transformants contained a *LEU2*$^+$ gene at the *LEU2* locus but showed no evidence of plasmid integration and were not analyzed further.

Physical analysis. (i) Type II transformants are not integrat-

TABLE 2. Recombinant plasmid and phage lambda[a]

Vector	Insertion	Source
CV9	*Pst* fragment containing *LEU2*$^+$ gene	J. Hicks
λgtKG17	*Eco*RI fragment containing Ty1-17	J. Carbon
pHK18	*Xho*I fragment of Ty1-17	This study

[a] All the plasmid insertions are carried in pBR322 (3).

ed at *LEU2*. In addition to the analysis described above, restriction enzyme digestion and Southern blotting analysis were used to confirm that the type II transformants were integrated into the chromosomal DNA at sites different from the *LEU2* locus. The possible types of chromosomal configurations are shown in Fig. 2. Figure 2A represents the chromosome III region flanking the *LEU2* gene. The Ty1-17 element to the left of the *LEU2* gene is contained within two *Xho*I sites and carries an internal *Bam*HI site (18) characteristic of this class of Ty elements. Figure 2B shows the chromosome resulting from integration of the CV9 vector at a Ty element elsewhere in the genome that is of the Ty1-17 subclass; that is, at a Ty element that contains the *Bam*HI site characteristic of Ty1-17. The chromosome structure depicted in Fig. 2C results from integration of the CV9 vector at a different kind of Ty element, one lacking the *Bam*HI site. These three classes can be distinguished by Southern blotting analysis.

Chromosomal DNA was isolated from each transformant and treated with *Bam*HI. After agarose gel electrophoresis and Southern transfer, the filters were probed with ^{32}P-labeled pBR322 and *LEU2* sequences (20). All of the type I transformants showed a loss of the chromosomal *LEU2* band and the presence of at least two new bands (e.g., Fig. 3). Some transformants also showed a band the size of the linear CV9 vector, the result of tandem multiple insertions of CV9. The upper band, common to all type I transformants, is the *Bam*HI fragment that extends into the adjacent Ty1-17 sequence and is indicative of the Ty1-17 class of Ty elements. In contrast to the type I transformants, none of the type II transformants showed a loss of the chromosomal *LEU2* band (Fig. 3). Again, some transformants have multiple insertions and contain a *Bam*HI fragment that is the same size as the linear CV9 vector. In addition, each lane contained a third band of hybridization, marked by the arrowheads. These bands result from insertion of the CV9 vector at a chromosomal location other than the *LEU2* locus. These

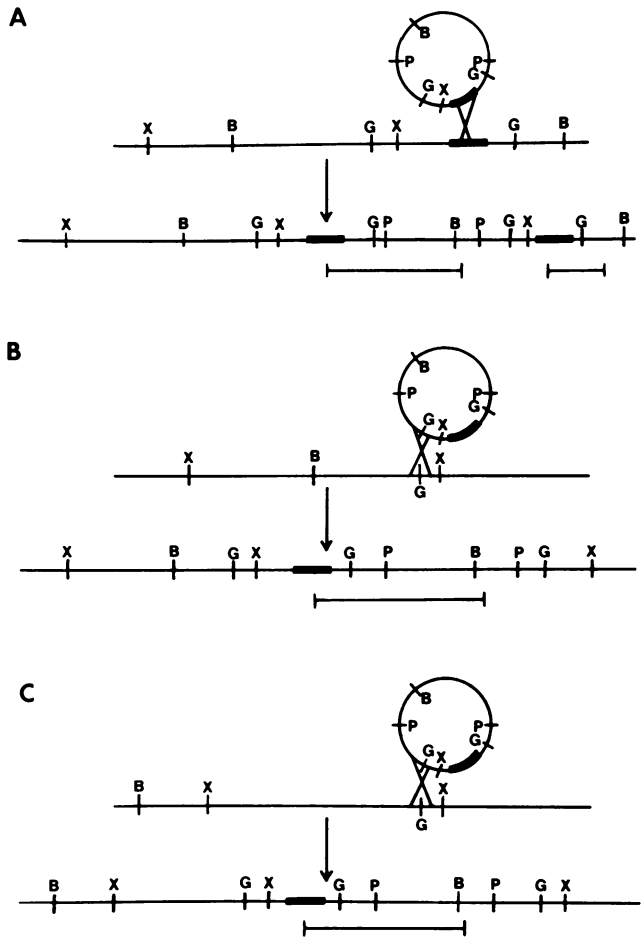

FIG. 2. Schematic drawing of different integration sites of the CV9 vector and the resulting transformants. (A) Integration at the *LEU2* locus, type I transformant. (B) Integration at a Ty1-17 class of Ty sequence, type II transformant. (C) Integration at a Ty element other than the Ty1-17 subclass, type II transformant. The dark line indicates the *LEU2* gene. Abbreviations: X, *Xho*I; B, *Bam*HI; G, *Bgl*II; P, *Pst*I. The lines underneath the resulting chromosomes indicate the region of homology covered by the radioactive probes used in subsequent experiments. Figure is not drawn to scale.

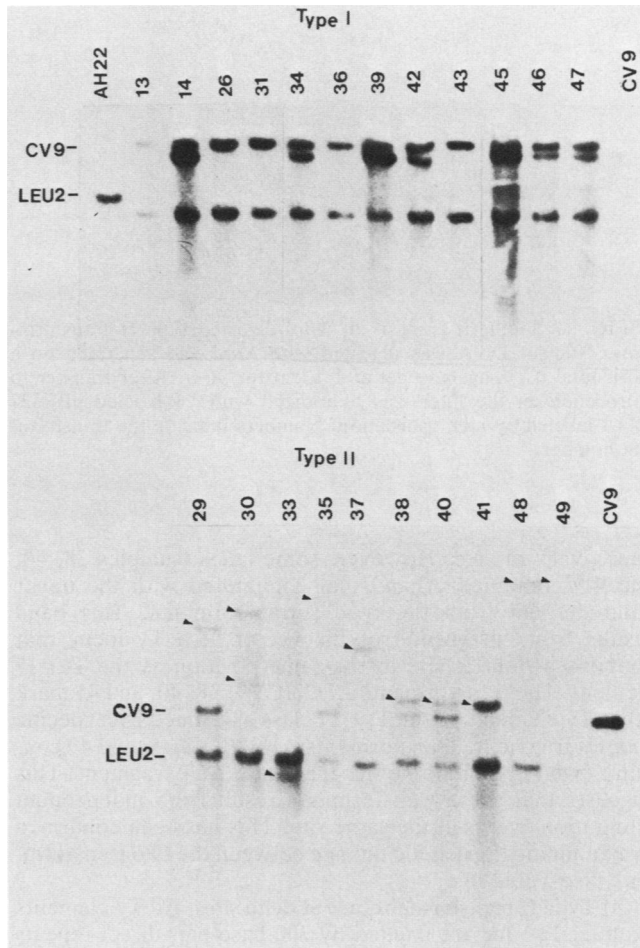

FIG. 3. Southern analysis of type I and type II transformants after *Bam*HI digestion. Nuclear DNA was isolated from the different transformants and treated with *Bam*HI restriction endonuclease. The fragments were separated on a horizontal 0.7% agarose gel at 1 V/cm for 36 h. After transfer to nitrocellulose, the filters were hybridized with a [^{32}P]DNA probe from the large *Eco*RI fragment of CV9. Shown are the parental strain (AH22), different transformants indicated by the transformant number, and the CV9 vector. The positions of the chromosome III *Bam*HI fragment containing the *LEU2* gene and the linear CV9 vector are indicated. The arrowheads in the lower panel indicate the position of one of the junction fragments of the CV9 insert in type II transformants (Fig. 2).

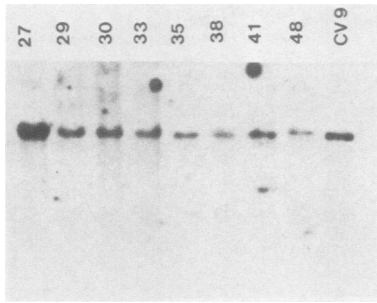

FIG. 4. Southern analysis of *Xho*I-digested type II transformants. Nuclear DNA was digested with *Xho*I and separated on a horizontal 0.7% agarose gel at 1 V/cm for 36 h. After transfer to nitrocellulose, the filter was hybridized with ^{32}P-labeled pBR322 DNA labeled by nick translation. Numbers indicate the transformant number.

bands vary in size. However, some lanes (samples 38, 40, and 41) contained a band that comigrated with the upper band derived from the type I transformants. This band results from integration of the vector at a Ty locus that contains a *Bam*HI site in the same position as the Ty1-17 element. The transformants AH22(CV9)-38, 40, and 41 mark other Ty elements of the Ty1-17 class as defined by a specific *Bam*HI fragment. Transformants AH22(CV9)-48 and 49 contain a band in common other than the *LEU2* fragment. This suggests that these transformants result from independent integration events at the same site. This has been confirmed by examining the genetic linkage between the two transformants (see Table 4).

(ii) Type II transformants are at delta sites. All Ty elements examined so far are flanked by 300-base-pair direct repeats called delta sequences (4, 12, 15). Delta sequences can be found in the *S. cerevisiae* genome independent of the rest of the Ty sequence (4). Most, but not all, of the delta sequences contain an *Xho*I site. Although the data presented in Fig. 3 suggest that at least some of the transformants, those showing the characteristic Ty1-17 *Bam*HI band, are integrated at Ty sites, we performed two other restriction enzyme analyses to substantiate our claim that the CV9 vector was indeed integrated at Ty sites. Since the transformation was presumably the result of homologous recombination between the delta and small Ty1-17 fragment on CV9 and an endogenous Ty-delta sequence, we anticipated that the chromosomal delta at the point of integration would contain an *Xho*I site as did the delta sequence on CV9. Figure 4 shows the result of an *Xho*I treatment of DNA from several transformants hybridized with a pBR322 probe. Only one band of hybridization is seen, and this band is the same size as the linear CV9 vector. This result was seen with all but one of the type II transformants (number 20) and was the expected result (Fig. 2).

(iii) Type II transformants are at Ty-delta sites. The fragment of Ty sequence on the CV9 vector contained a *Bgl*II site (Fig. 2). This restriction site has been conserved in all Ty sequences documented to date (34a). To show that the integration event had occurred at a Ty-delta sequence and not only at a solo delta sequence, DNAs from the transformed strains were treated with *Bgl*II and analyzed by Southern blots. The probe used was ^{32}P-labeled pBR322 and *LEU2* sequences. Most of the transformants contained only two strong bands of hybridization, and these bands comigrated with the *Bgl*II bands derived from CV9 (Fig. 5). This is the expected result if the sequence adjacent to the vector insertion contains a *Bgl*II site in the same location as the *Bgl*II site in the Ty sequence carried on CV9 (Fig. 2). Transformants AH22(CV9)-32 and 44 showed the same pattern as CV9 (H. L. Klein, data not shown). Transformants AH22(CV9)-10, 20, 29, and 37 require additional explanation. All of these transformants contained an additional band of hybridization, and transformant AH22(CV9)-10 contained two bands. Cloning of the sequences adjacent to the integration of transformant AH22(CV9)-10 has shown that there is a Ty element at the site of integration (Klein, unpublished data). This transformant contains multiple tandem insertions of CV9 with a rearrangement in one integrated copy of the plasmid. This rearrangement gives rise to the extra bands seen in Fig. 5 (Klein, unpublished data). The remaining transformants, AH22(CV9)-20, 29, and 37, have not been analyzed further.

(iv) Number of Ty1-17-like elements in strain AH22. Studies determining the number of Ty elements in different labora-

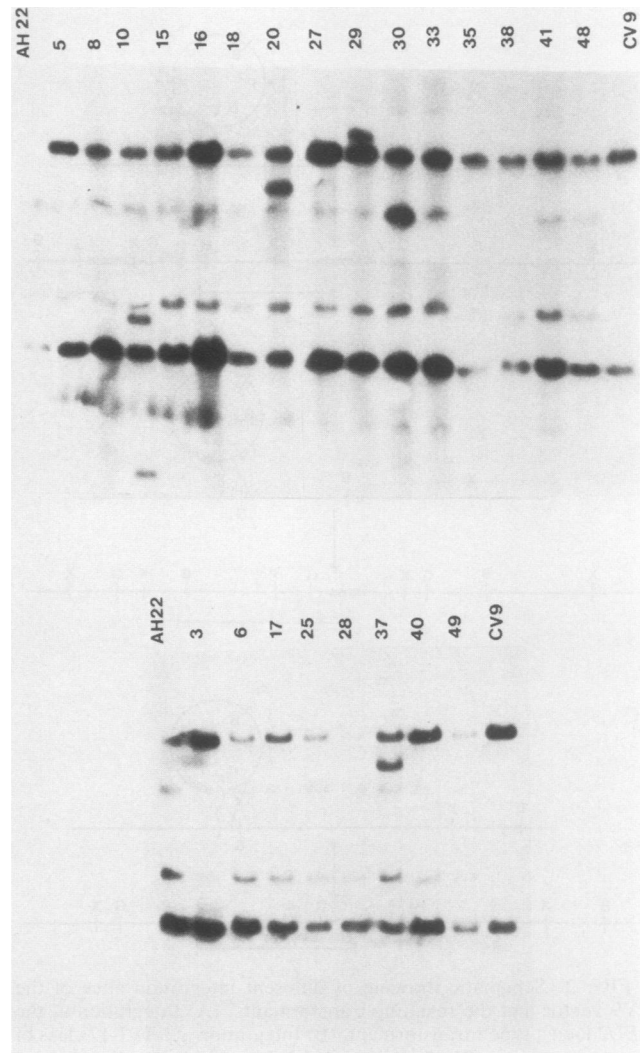

FIG. 5. *Bgl*II digestion of type II transformants. Nuclear DNA was treated with *Bgl*II and separated on a horizontal 0.7% agarose gel at 1 V/cm for 36 h. After transfer to nitrocellulose, the filter was hybridized to a ^{32}P-labeled large *Eco*RI fragment of CV9 (Fig. 2). Numbers indicate the transformant number.

tory strains of *S. cerevisiae* have shown that there is wide variation in this value (4, 9, 14). Between 4 and 40 Ty elements have been counted in various strains. Roeder et al. (26) have estimated that there are six members of the Ty1-17 class of Ty elements in a strain derived from S288C. We wished to determine the number of the Ty1-17 class of Ty elements in the strain used as the recipient in the transformation experiments described in this report. Using an analysis similar to that of Roeder et al. (26), we subcloned the *Xho*I fragment from λgtKG17 (Table 2) into the *Sal*I site of pBR322 to form pHK18. This plasmid was used as a source of the *Cla*I fragment unique to the Ty1-17 class of Ty elements (18, 26). Using the *Cla*I fragment radioactively labeled with ^{32}P as a probe to Southern blots of restriction enzyme-treated AH22 DNA, we obtained the results shown in Fig. 6.

The *Xho*I digest shows that with the *Cla*I fragment used as a probe, most of the Ty1-17 class of Ty elements are of equal size, most likely the result of the *Xho*I sites in two delta sequences that flank the Ty element. Most of this band is abolished by additional treatment with *Bam*HI and yields a fragment of ca. 3.2 kbp in length, indicated by the lower arrowhead (Fig. 6). This size of fragment corresponds to the anticipated fragment resulting from the Ty1-17 elements (18). *Bam*HI and *Hin*dIII cut once in Ty1-17, whereas no *Eco*RI site is present (18). However, the *Cla*I fragment used as a probe in these experiments is homologous to only one of the two halves of the Ty element resulting from *Bam*HI or *Hin*dIII digestions. Therefore, the last three lanes of Fig. 6 can be used to estimate the number of the Ty1-17 class of Ty elements in strain AH22, a derivative of S288C. These data give an estimate of ca. 12 Ty1-17 elements in AH22.

Number of sites of vector insertion. The tetrad segregation patterns obtained when a type II transformant is crossed to a *LEU2*$^+$ strain could result from integration of the CV9 vector at more than one site. To determine the number of genetically distinguishable sites in which the CV9 vector is carried in each transformant, all of the type II transformants were crossed to a *leu2*$^-$ strain (strain 2262). Tetrad analysis of the resulting diploids showed 2:2 segregation for the *LEU2* marker in every case. This result shows that the vector CV9 is at one genetic locus in each transformant and it is stably integrated. This was confirmed in several cases by colony hybridization and the detection of 2:2 segregation for pBR322 in those tetrads. Although the CV9 vector is at one site genetically, these data do not address the question of multiple tandem insertions of CV9. This is discussed below.

Centromere linkage and mapping of type II transformants. Among the 25 type II transformants, 11 showed centromere linkage, as indicated by less than 67% 3:1 tetrads in crosses to a *LEU2*$^+$ strain. Initially, 10 tetrads were analyzed to determine centromere linkage. This was then confirmed in crosses with centromere markers in which 25 to 50 tetrads were scored (Table 3). The transformants were mapped by crosses to strains containing centromere-linked markers for every chromosome (Table 1). The 11 transformants define six genetic loci, shown in Table 3 and Fig. 7. In those cases in which two independent transformants are mapped to the same site, only one transformant was used for extensive tetrad analysis. This approach seems justified, as the linkage between the two transformants is very tight, often showing no recombination events in 100 tetrads (Table 4).

The placement of each transformant on the proper chromosome arm with respect to the centromere marker is unambiguous, except in transformants AH22(CV9)-3 and 25. These map to chromosome VI; however, the second-division segregation frequency is too low to permit placement on VI right arm (R) or VI left arm (L) definitively. Cloning of the centromeric region of chromosome VI has shown the presence of a Ty sequence centromere proximal to *cdc4* (P. Philippsen, personal communication), suggesting that the AH22(CV9)-3 and 25 transformants may be located on this arm.

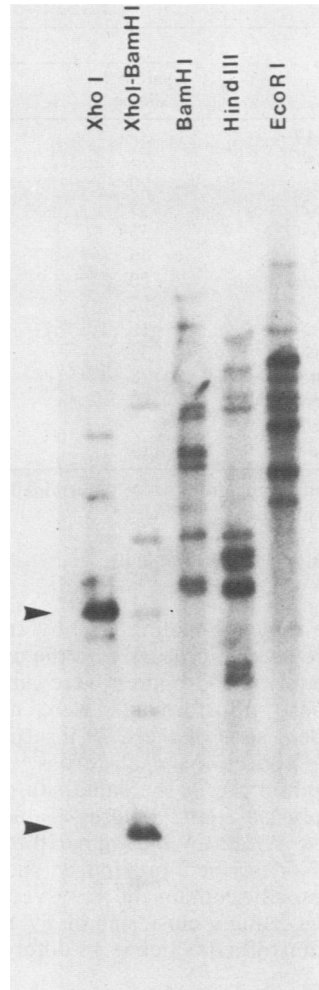

FIG. 6. Number of Ty1-17 Ty elements in AH22. Nuclear DNA from strain AH22 was digested with the restriction enzymes as indicated, and the fragments were separated on a horizontal 0.7% agarose gel at 1.2 V/cm for 40 h. After transfer to nitrocellulose, the filter was hybridized with a ^{32}P-labeled *Cla*I fragment of Ty1-17 inserted as an *Xho*I fragment in pBR322 to form pHK18. After hybridization and washing, the filter was exposed to X-ray film for 18 h without intensifying screens. The upper arrowhead indicates the 5.6-kbp *Xho*I fragment, and the lower arrowhead indicates the 3.2-kbp *Bam*HI-*Xho*I fragment of the Ty1-17 class.

It has been reported that chromosomes XIV and XVII form a single linkage group (19). As transformants AH22(CV9)-17 and 28 are located on chromosome XIV, it was possible to test for linkage of these transformants with markers previously mapped to chromosome XVII. The linkage of AH22(CV9)-28 with *met4* confirms this new chromosomal linkage (Table 3).

Recombination groups of centromere-unlinked transformants. The number of different locations represented by the remaining 14 transformants was determined by pairwise crosses and random spore analysis. Transformants were

TABLE 3. Linkage of transformants with mapped markers

Gene pair	Tetrad types			Map distance (centimorgans)	% of second-division segregations[a]
	Parental ditype	Nonparental ditype	Tetratype		
AH22(CV9)-32–*MAT*	32	0	5	6.8	27.0
AH22(CV9)-32–*thr4*	23	0	23	25.0	
AH22(CV9)-3–*his2*	20	0	19	24.4	0
AH22(CV9)-25–*his2*	5	1	5	31.8	ND[b]
AH22(CV9)-37–*cdc4*	33	0	2	2.9	0
AH22(CV9)-6–*leu1*	46	0	11	9.6	5.4
AH22(CV9)-15–*leu1*	20	0	6	11.5	6.5
AH22(CV9)-40–*leu1*	22	0	4	7.7	20.8
AH22(CV9)-17–*pet8*	16	0	8	16.7	33.3
AH22(CV9)-28–*lys9*	33	2	54	33.3	36.4
AH22(CV9)-28–*pet8*	28	0	16	18.2	
AH22(CV9)-28–*met4*	20	3	40	46.0	
AH22(CV9)-48–*aro7*[c]					
AH22(CV9)-49–*aro7*	19	4	52[d]	50.7	69.9

[a] Second-division segregations were determined with *pet8* and either *trp1*, *leu1*, or *met3* as centromere markers.
[b] ND, Not determined.
[c] See Table 4.
[d] Chi-square analysis shows $P < 0.01$.

recovered in the opposite mating type by crosses to strain 2262. These were used in crosses with the other transformants. Approximately 1,000 spores were analyzed in each cross. Since these transformants were not centromere linked, independent sites of the CV9 insertion should give ca. 25% *leu2*$^-$ spores in each cross. Transformants AH22(CV9)-5 and 38 are at the same site or very closely linked, giving only 1.6% *leu2*$^-$ spores (Table 5). The cross AH22(CV9)-18 × AH22(CV9)-27 gave 0% *leu2*$^-$ spores. Physical analysis described previously shows that these transformants actually contain the CV9 vector at different Ty elements, suggesting a clustering of Ty elements at this site. These 14 transformants define 12 different sites (Table 5).

Correlation of different Ty1-17-like elements with chromosomal sites of Ty. The *Eco*RI lane of Fig. 6 showed the best distribution of the Ty1-17 class of Ty elements in AH22. Insertion of the CV9 vector at one of these sites should disrupt the Ty element. *Eco*RI digestion and Southern analysis of the Ty1-17 class of Ty elements should show the loss of a particular band, and this band should vary in the different transformants. Moreover, all transformants that map to the same site by genetic analysis should be missing the same band in the display of the Ty1-17 class of Ty elements. This analysis is shown in Fig. 8. The arrowheads mark the changes, either the loss of a band or the appearance of a novel band. No changes could be detected in transformants AH22(CV9)-8, 16, or 44. Transformant AH22(CV9)-10 does not carry the CV9 vector inserted at a Ty element of the Ty1-17 class of Ty elements and showed no change in the pattern of the Ty1-17 elements. The remaining transformants shown all displayed some change in the pattern. Transformants AH22(CV9)-3 and 25 map to the same site on chromosome VI and showed the loss of the same Ty band. Similarly, transformants AH22(CV9)-5 and 38 and transformants AH22(CV9)-6, 15, and 40 each form one genetic site and showed the identical change in Ty distribution within the group. Clearly, not all losses of a band and acquisitions of a novel band can be detected in this analysis. However, the fact that the same changes were seen in different members of each group strongly suggests that specific Ty elements can be identified and that these transformants are really integrated at Ty elements.

Is there preferential integration at Ty1-17-like elements? We have estimated that there are approximately 35 Ty elements in our isolate of strain AH22 (data not shown). Of the 25 independent transformants described here, 12 result from integration of the CV9 vector at a number of the Ty1-17 class of Ty elements. Excluding the ambiguous transformant AH22(CV9)-20, the Southern analyses show that the remaining 24 transformants are at delta sites and that at least 22 of these also contain a Ty element adjacent to the delta sequence. We wished to determine whether the 12 successes (integrations at the Ty1-17 class of Ty elements) of 24 trials (integrations at Ty elements), when 12 of the 35 Ty elements in the genome are of the Ty1-17 class, are indicative of preferential integration at the Ty1-17 class. We exclude the Ty1-17 element adjacent to *LEU2* in these calculations as we cannot determine whether the class I transformants result from homologous recombination and integration by the Ty1-17 or the *LEU2* sequence or both. Therefore, we consider the probability of $P(k \geq 12)$, where k is the number of successes and p is the frequency of Ty1-17 class of Ty elements in the genome. Using a normal approximation of the binomial distribution (24):

$$z_1 = d_1 \left[\frac{1 + qg\left(\frac{S}{np}\right) + pg\left(\frac{T}{nq}\right)}{(n + 1/6)pq} \right]^{1/2}$$

where $k = 12$, $n = 24$, $p = 11/34$ (the Ty1-17 element adjacent to *LEU2* has been excluded in this frequency), and $q = 23/34$; $s = k - 1$, $S = s + 1/2$, $T = n - s - 1/2$, $d_1 = S + 1/6 - (n + 1/3)p$; and $g(x) = (1 - x^2 + 2x\ln x)/(1 - x)^2$, we found $P(k \geq 12) = 0.0548$. This number is an upper value and will be decreased if n, the number of trials (integrations) at Ty elements, is lowered. This number means that there is a 5.5% probability of getting 12 or more integrations at the Ty1-17 class of Ty elements in 24 trials when 11 of 34 Ty elements in the genome are members of the Ty1-17 class (excluding the Ty1-17 at *LEU2*). $P(k \geq 12)$ will also be reduced if p is reduced by underestimating the total number of Ty elements or by overestimating the number of Ty1-17-like Ty elements in the genome. The value of $P(k \geq 12)$ is

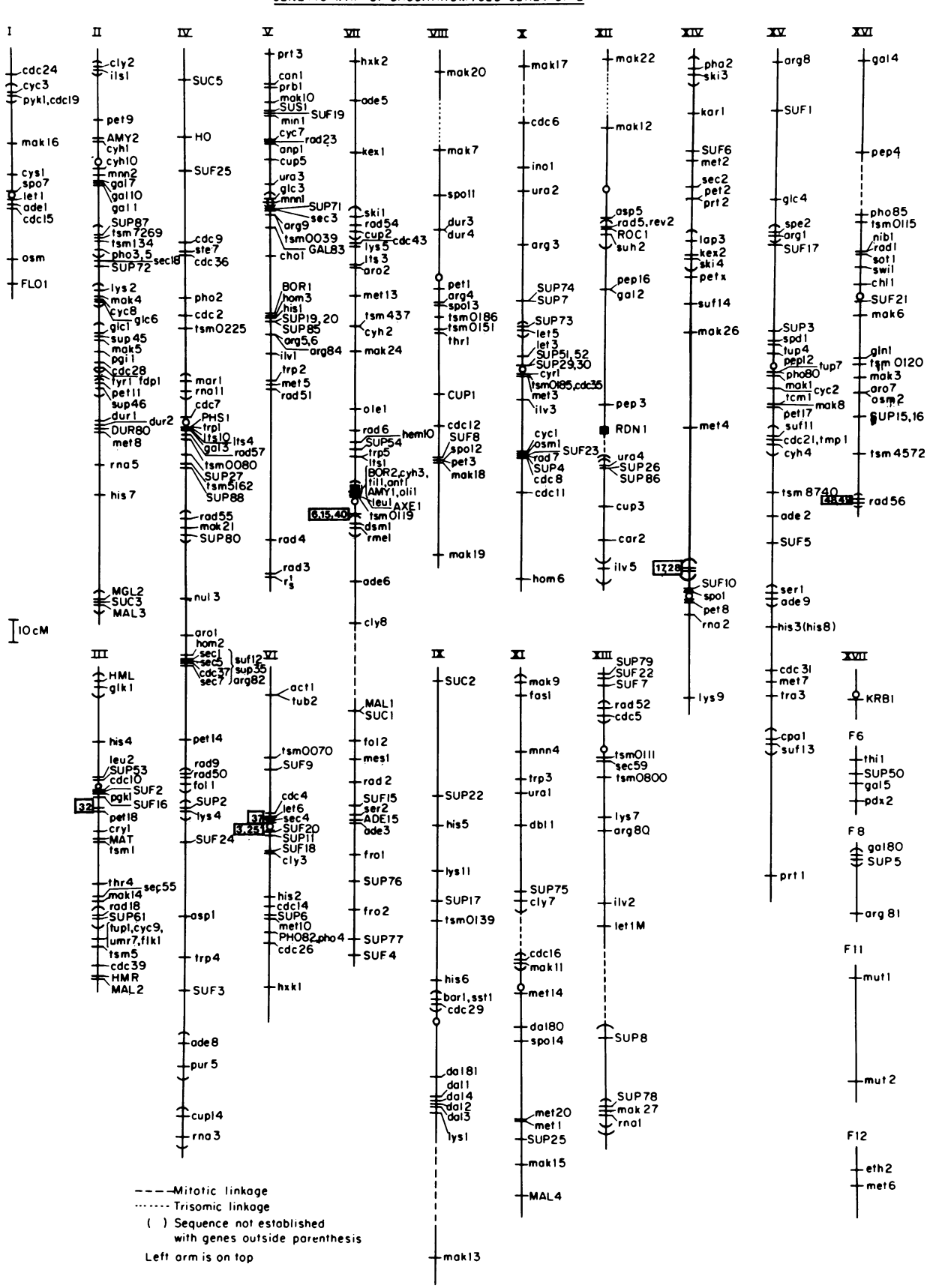

FIG. 7. Genetic map of *S. cerevisiae* showing the mapped Ty sequences in AH22. The mapped locations of several transformants are shown by the transformant number to the left of the chromosome on the current genetic map (23). Six sites are indicated: one each on chromosomes III, VII, XIV, and XVI and two on chromosome VI.

TABLE 4. Linkage between different transformants

Transformant pair	Tetrad types (PD:NPD:T)[a]
AH22(CV9)-3 × AH22(CV9)-25	103:0:0
AH22(CV9)-6 × AH22(CV9)-15	109:0:0
AH22(CV9)-6 × AH22(CV9)-40	66:0:0
AH22(CV9)-17 × AH22(CV9)-28	88:0:7
AH22(CV9)-48 × AH22(CV9)-49	28:0:0
AH22(CV9)-41 × AH22(CV9)-44	16:4:26[b]

[a] PD, Parental ditype; NPD, nonparental ditype; T, tetratype.
[b] Chi-square analysis shows PD:NPD is significantly different from 1:1 at $P = 0.01$.

suggestive of preferential integration at the Ty1-17 class of Ty elements.

Further calculations have shown that the distribution of the integration events at different genetically defined sites is random and that no hot spots for integration at different Ty1-17 elements could be found.

DISCUSSION

We used integrative transformation to insert a selectable marker, the *LEU2*$^+$ gene, at Ty elements in the yeast genome. The first question to address is whether the class II transformants are really integrated at Ty elements. All of the Southern mapping data are consistent with integration events occurring at Ty sites, except with transformant AH22(CV9)-20. In the cases in which only one copy of the CV9 vector has been integrated at a particular site, the preservation of a *Bgl*II site at that site is very strong evidence of a Ty sequence. In most of the cases in which the site of integration appears to be at the Ty1-17 class of Ty elements, one of the family of Ty1-17 bands can be seen to have shifted as a result of the integration (Fig. 8). Moreover, all transformants that map to the same genetic site show the identical change in the Ty1-17 profile. In those cases that do not represent integrations at the Ty1-17 class of Ty sequences, a novel junction band is seen in Southern analysis of *Bam*HI digestions (Fig. 3). Those transformants that map to the same site genetically have a junction fragment of the same size. This suggests that the integration of the CV9 vector is not occurring randomly but rather is the result of a homologous recombination event between the vector DNA and the chromosome.

Using the *LEU2*$^+$ marker as a tag for Ty sequences, we were able to map six Ty sites in strain AH22. In addition, there is a Ty1-17 adjacent to the *LEU2* gene, giving a total of seven mapped Tys. We also grouped the remaining transformants into 12 distinct sites of Ty elements in strain AH22. These sites are dispersed throughout the genome in what appears to be a random fashion. In this regard, *S. cerevisiae* is similar to other organisms in which the distribution of repeated DNA sequences has been studied. Repeated DNA sequences have been described in the *Drosophila* (8, 25, 33, 37) and human (1, 21) genomes. These sequences are dispersed throughout the genome, although some clustering has been suggested to occur in the 1.9-kbp *Hin*dIII repeat in the human genome (21).

We found two examples of pairs of Ty elements in our study. Transformants AH2(CV9)-18 and 27 do not recombine (Table 5). However, Southern analysis of the *Bam*HI junction fragments shows that AH22(CV9)-18 has a band characteristic of the Ty1-17 class, whereas AH22(CV9)-27 does not (Table 6), suggesting two very closely linked Ty elements. Transformants AH22(CV9)-17 and 28 are both on chromosome XIV and show similar linkage to the *pet8* marker (Table 3). When these two transformants are crossed, they map 3.7 centimorgans apart (Table 4). Southern analysis after *Bam*HI digestion has shown that the transformants give junction fragments of different sizes (data not shown), supporting the tetrad data. Farrelly and Butow (13) have recently reported two closely spaced Ty elements in the nuclear genome of S288C. This strain also contains a tandem duplication of two similar, but not identical, Ty elements near the *MAT* locus on chromosome III (S. Roeder, personal communication).

Although most laboratory strains of *S. cerevisiae* contain 30 to 40 Ty elements (28, 34a), the distribution of these elements varies among different strains. Similarly, the number of members of any given repeated DNA family in *D. melanogaster* other than P elements appears to be constant. However, great variation is seen in the chromosomal distribution of these sequences between different strains of a single species (8, 33), giving rise to the term nomadic DNA (37). The reason for this variability in location, yet lack of detection of movement, is unknown. Polymorphisms in the chromosomal locations of Ty sequences have been observed (4, 7, 13). Recently, *S. cerevisiae* chromosome III has been extensively examined for the presence of Ty elements. Two Ty sequences have already been described on chromosome III: Ty1-17 adjacent to *LEU2* and Ty1-161 9 kbp from *PGK1* (18). We have reported here a transformant, AH22(CV9)-32,

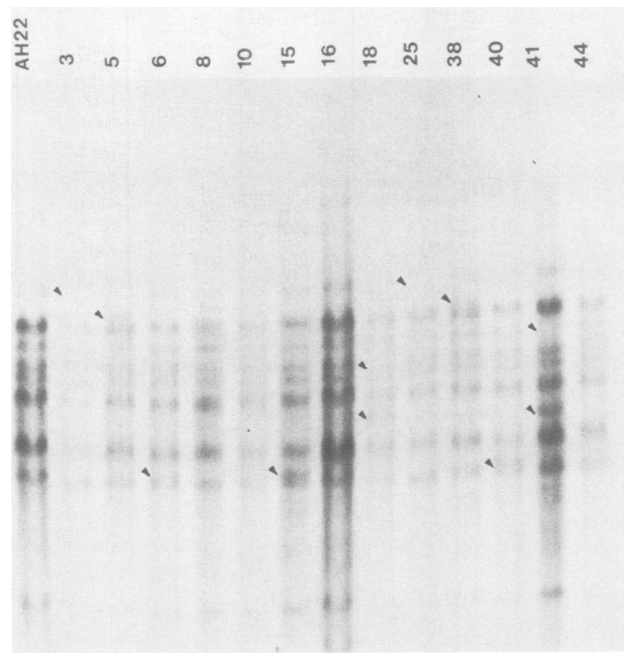

FIG. 8. Changes in the pattern of Ty1-17 bands in some type II transformants. Nuclear DNAs from AH22 and several transformants were treated with *Eco*RI restriction endonuclease, and the fragments were separated on a horizontal 0.5% agarose gel at 1.2 V/cm for 40 h. After transfer to nitrocellulose, the filter was hybridized to the *Cla*I fragment of pHK18 labeled with ^{32}P. The arrowheads indicate positions of band changes, either as the loss of a band or the appearance of a new band when compared with the pattern of the AH22 lane. Numbers indicate the transformant number. The filter was exposed to X-ray film for 20 h without intensifying screens.

TABLE 5. Random spore analysis of pairwise crosses between non-centromere-linked transformants, shown as percentage of *leu*$^-$ spores

Transformant no.	Transformant no.													
	5	8	10	16	18	20	27	29	30	33	35	38	41	44
5	0													
8	15	0.1												
10	28	24	0.1											
16	16	21	24	0										
18	17	19	19	12	0.1									
20	17	20	22	32	12	0.18								
27	17	17	26	12	0	ND[a]	0							
29	13	21	22	13	14	14	ND	0						
30	23	17	20	16	12	16	ND	15	0.3					
33	19	17	20	19	13	12	ND	10	14	0				
35	18	14	22	17	19	15	ND	15	17	12	0.1			
38	1.6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.1		
41	17	16	22	16	14	17	ND	13	20	8	16	ND	0	
44	11	14	10	17	11	14	ND	17	15	16	18	ND	9	0.3

[a] ND, Not determined.

that maps between *MAT* and *CEN3*. A tandem duplication of Ty sequences has been found 8 centimorgans from *MAT* (Roeder, personal communication). These Tys are not allelic with AH22(CV9)-32 but, rather, map 10 centimorgans apart (Klein, unpublished data), suggesting another site of Ty sequences on chromosome III. J. Larkin and J. Woolford (personal communication) have found a Ty sequence centromere proximal to the *CRYI* locus. It is not yet known whether this represents yet another site of a Ty element. The presence or absence of some of these Ty sequences has been examined in different *S. cerevisiae* strains through the use of various recombinant DNA libraries. Two different types of polymorphisms have been seen. Some strains contain a Ty sequence and the associated delta sequences, whereas other strains only contain a single delta sequence at that site. In other cases, no evidence of a Ty or delta sequence can be found (C. Newlon, personal communication). The right arm of chromosome III between *CEN3* and *MAT* appears to be especially polymorphic, according to this study and other data (J. Larkin, C. Newlon, S. Roeder, and J. Woolford, personal communication).

Several aspects of the genetic behavior of the transformants deserve comment. First, it is expected that 25% of the random spores should be *leu2*$^-$. The data in Table 5 show values ranging from 9 to 28%. However, this most likely is the result of an underestimation of the number of *leu2*$^-$ colonies. Further experiments have shown that there is no preferential segregation or recovery of the *LEU2*$^+$ spores in the pairwise crosses in transformants by tetrad analysis. Other mapping procedures, including the 2 μ DNA mapping technique (11) and the methyl benzimidazole-2-yl-carbamate-induced chromosome loss (36), have been tried unsuccessfully on three different transformants, all of which are not linked to any centromere by conventional mapping methods. It is not clear whether Ty sequences behave aberrantly in these analyses or whether these methods failed because the sites we tried to map are very far out on the chromosome arms.

We estimated by Southern analysis that strain AH22 contains 12 Ty1-17 sequences (Fig. 6 and 8). The genetics of the different transformants suggest that there are at least nine different Ty1-17 elements, including Ty1-17 adjacent to *LEU2* (Table 6). In a similar analysis, others have concluded that a related strain, S288C, contains only six Ty1-17 sequences (14, 26). This discrepancy could be due to inadequate resolution of very closely spaced bands on an agarose gel or could indeed reflect a difference in Ty classes between these two strains. This is rather unlikely, as AH22 is a derivative of S288C, and Ty elements do not appear to change or transpose very rapidly in unselected cultures. Nonetheless, the possibility that a difference exists between these two strains in the Ty1-17 class population cannot be eliminated.

Electron microscopic heteroduplex studies (18) have shown that regions of the Ty1-17 element form a homoduplex with the Ty1 element of Cameron et al. (4). The portion

TABLE 6. Type of Ty element marked by CV9 vector

Transformant no.	Ty1-17 subclass	Single or multiple copy[a]	Genetic location
3	+	M	VI R, same as 25
5	+	M	Unlinked, same as 38
6	+	M	VII R, same as 15, 40
8	+	M	Unlinked
10	−	M	Unlinked
15	+	M	VII R
16	+	M	Unlinked
17	−	M	XIV R, same as 28
18	+	M	Unlinked, same as 27
20	−	S	Unlinked
25	+	S	VI R
27	−	M	Unlinked, same as 18
28	−	S	XIV R
29	−	M	Unlinked
30	−	S	Unlinked
32	−	M	III R
33	−	S	Unlinked
35	−	M	Unlinked
37	−	S	VI R
38	+	M	Unlinked, same as 5
40	+	M	VII R
41	+	S	Unlinked
44	+	S	Unlinked
48	−	S	XVI L, same as 49
49	−	S	XVI L

[a] S, Single insertion of CV9; M, multiple tandem insertions of CV9.

of Ty1-17 that is carried on the CV9 vector is in a region that forms a homoduplex with Ty1. Statistical analysis of the transformants we recovered using CV9 suggests that we are seeing preferential integration at the Ty1-17 class of Ty elements. This preferential integration suggests that there are regions of microheterogeneity between Ty1-17 and Ty1 that cannot be detected with an electron microscope but are sufficient to bias the reciprocal recombination reaction involved in the integration of the CV9 vector. Using plasmids containing *S. cerevisiae* rDNA, Smolik-Utlaut and Petes (31) have also observed that microheterogeneity between interacting sequences can result in preferential integration.

We used integrative transformation to insert a selectable marker at Ty elements. A vector that contains both a Ty sequence that is repeated many times in the *S. cerevisiae* genome and a unique selectable marker was integrated by homology at several sites of these repeated elements. We were able to map 7 sites of this repeated sequence (including the sequence adjacent to *LEU2*) with the selectable *LEU2*$^+$ gene and defined 12 additional sites of the Ty element. The genetic data show that these sites are dispersed throughout the yeast genome.

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LITERATURE CITED

1. **Adams, J. W., R. E. Kaufman, P. J. Kretschner, M. Harrison, and A. W. Neinhuis.** 1980. A family of long reiterated DNA sequences, one copy of which is next to the human beta globin gene. Nucleic Acids Res. **8**:6113–6128.
2. **Andreadis, A., Y.-P. Hsu, G. B. Kohlhaw, and P. Schimmel.** 1982. Nucleotide sequence of yeast LEU2 shows 5′-noncoding region has sequences cognate to leucine. Cell **31**:319–325.
3. **Bolivar, R., R. L. Rodriguez, P. J. Greene, M. C. Betlachk, H. L. Heyneker, H. W. Boyer, J. H. Crosa, and S. Falkow.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.
4. **Cameron, J. R., E. Y. Loh, and R. W. Davis.** 1979. Evidence for transposition of dispersed repetitive DNA families in yeast. Cell **16**:739–751.
5. **Chen, C. W., and C. A. Thomas, Jr.** 1980. Recovery of DNA segments from agarose gels. Anal. Biochem. **101**:339–341.
6. **Clewell, D., and D. Helinski.** 1970. Properties of a supercoiled deoxyribonucleic acid-protein relaxation complex and strand specificity of the relaxation event. Biochemistry **9**:4428–4440.
7. **Dobson, M. J., S. M. Kingsman, and A. J. Kingsman.** 1981. Sequence variation in the LEU2 region of the *Saccharomyces cerevisiae* genome. Gene **16**:133–139.
8. **Dowsett, A. P., and M. W. Young.** 1982. Differing levels of dispersed repetitive DNA among closely related species of Drosophila. Proc. Natl. Acad. Sci. U.S.A. **79**:4570–4574.
9. **Eibel, H., J. Gafner, A. Stotz, and P. Philippsen.** 1980. Characterization of the yeast mobile element Ty1. Cold Spring Harbor Symp. Quant. Biol. **45**:609–617.
10. **Errede, B., T. S. Cardillo, F. Sherman, E. Dubois, J. Deschamps, and J.-M. Wiame.** 1980. Mating signals control expression of mutations resulting from insertion of a transposable repetitive element adjacent to diverse yeast genes. Cell **22**:427–436.
11. **Falco, S. C., Y. Li, J. R. Broach, and D. Botstein.** 1982. Genetic properties of chromosomally integrated 2μ plasmid DNA in yeast. Cell **29**:573–584.
12. **Farabaugh, P. J., and G. R. Fink.** 1980. Insertion of the eukaryotic transposable element Ty 1 creates a 5 bp duplication. Nature (London) **286**:352–356.
13. **Farrelly, F., and R. A. Butow.** 1983. Rearranged mitochondrial genes in the yeast nuclear genome. Nature (London) **301**:296–301.
14. **Fink, G., P. Farabaugh, G. Roeder, and D. Chaleff.** 1980. Transposable elements (Ty) in yeast. Cold Spring Harbor Symp. Quant. Biol. **45**:575–580.
15. **Gafner, J., and P. Philippsen.** 1980. The yeast transposon Ty 1 generates duplications of target DNA insertion. Nature (London) **286**:414–418.
16. **Hicks, J. B., A. Hinnen, and G. R. Fink.** 1978. Properties of yeast transformation. Cold Spring Harbor Symp. Quant. Biol. **43**:1305–1313.
17. **Hinnen, A., J. B. Hicks, and G. R. Fink.** 1978. Transformation of yeast. Proc. Natl. Acad. Sci. U.S.A. **75**:1929–1933.
18. **Kingsman, A. J., R. L. Gimlich, L. Clarke, A. C. Chinault, and J. Carbon.** 1981. Sequence variation in dispersed repetitive sequences in *Saccharomyces cerevisiae*. J. Mol. Biol. **145**:619–632.
19. **Klapholz, S., and R. E. Esposito.** 1982. Chromosomes XIV and XVII of *Saccharomyces cerevisiae* constitute a single linkage group. Mol. Cell. Biol. **2**:1399–1409.
20. **Klein, H. L., and T. D. Petes.** 1981. Intrachromosomal gene conversion in yeast. Nature (London) **289**:144–148.
21. **Manuelidis, L., and P. A. Biro.** 1982. Genomic representation of the HindIII 1.9kb repeated DNA. Nucleic Acids Res. **10**:3221–3229.
22. **Mortimer, R. K., and D. C. Hawthorne.** 1969. Yeast genetics, p. 286–460. *In* A. H. Rose and J. S. Harrison (ed.), The yeasts, vol. 1. Academic Press, Inc., New York.
23. **Mortimer, R. K., and D. Schild.** 1982. Genetic map of Saccharomyces cerevisiae, p. 639–650. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast Saccharomyces: metabolism and gene expression. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **Peizer, D. B., and J. W. Pratt.** 1968. A normal approximation for binomial, F, beta and other common, related tail probabilities. J. Am. Stat. Assoc. **63**:1416–1456.
25. **Potter, S. S., W. J. Brorein, P. Dunsmuir, and G. M. Rubin.** 1979. Transposition of elements of the 412, copia and 297 dispersed repeated gene families in Drosophila. Cell **17**:415–428.
26. **Roeder, G. S., P. Farabaugh, D. Chaleff, and G. R. Fink.** 1980. The origin of gene instability in yeast. Science **209**:1375–1380.
27. **Roeder, G. S., and G. R. Fink.** 1980. DNA rearrangements associated with a transposable element in yeast. Cell **21**:239–249.
28. **Roeder, G. S., and G. R. Fink.** 1982. Transposable elements in yeast, p. 299–329. *In* J. A. Shapiro (ed.), Mobile genetic elements. Academic Press, Inc., New York.
29. **Schachat, F. W., and D. S. Hogness.** 1973. Repetitive sequences in isolated Thomas circles from *Drosophila melanogaster*. Cold Spring Harbor Symp. Quant. Biol. **38**:371–381.
30. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1981. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
31. **Smolik-Utlaut, S., and T. D. Petes.** 1983. Recombination of plasmids into the *Saccharomyces cerevisiae* chromosome is reduced by small amounts of sequence heterogeneity. Mol. Cell Biol. **3**:1204–1211.
32. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
33. **Strobel, E., P. Dunsmuir, and G. M. Rubin.** 1979. Polymorphisms in the chromosomal locations of elements of the 412, copia and 297 dispersed repeated gene families in Drosophila. Cell **17**:429–440.

34. **Williamson, D. H., and D. J. Fennel.** 1975. The use of fluorescent DNA binding agents for detecting and separating yeast mitochondrial DNA. Methods Cell Biol. **12**:335–351.
34a. **Williamson, V. M.** 1983. Transposable elements in yeast. Int. Rev. Cytol. **83**:1–25.
35. **Williamson, V. M., E. T. Young, and M. Ciriacy.** 1981. Transposable elements associated with constitutive expression of yeast alcohol dehydrogenase II. Cell **23**:605–614.
36. **Wood, J. S.** 1982. Mitotic chromosome loss induced by methyl benzimidazole-2-yl-carbamate as a rapid mapping method in *Saccharomyces cerevisiae*. Mol. Cell Biol. **2**:1080–1087.
37. **Young, M. W.** 1979. Middle repetitive DNA: a fluid component of the Drosophila genome. Proc. Natl. Acad. Sci. U.S.A. **76**:6274–6278.