Doubling Ty1 Element Copy Number in Saccharomyces cerevisiae: Host Genome Stability and Phenotypic Effects

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ABSTRACT

Haploid yeast strains bearing approximately double the normal number of Ty1 elements have been constructed using marked GAL/Ty1 fusion plasmids. The strains maintain their high transposon copy number and overall genome structure in the absence of selection. The strains bearing extra Ty1 copies are surprisingly similar phenotypically to the parental strain. The results suggest that the limit to transposon copy number, if any, has not been reached. When these strains are crossed by wild-type strains (*i.e.*, bearing the normal complement of Ty1 elements) or by strains of opposite mating type also bearing excess Ty1 elements, normal to very slightly reduced spore viability is observed, indicating that increasing the extent of transposon homology scattered around the genome does not result in significant increases in frequency of ectopic reciprocal recombination. The results suggest that yeast cells have evolved mechanisms for coping with excess transposon copies in the genome.

TRANSPOSABLE elements inhabit the genomes of essentially all organisms. Their ubiquity suggests that they have coexisted with their hosts for millennia. Clearly, in a homeostatic genome, the rate of accumulation of transposon copies by transposition and other events must be balanced by an equal rate of loss of copies. Ty1 is a well studied retrovirus-like transposon that transposes through an RNA intermediate (BOEKE *et al.* 1985; BOEKE 1989). We have investigated the effects of introducing extra Ty1 elements into the genome of the host by making use of pGTy1 plasmids, which consist of the yeast *GAL1* promoter fused to an active Ty1 element, Ty1-H3, in place of the Ty's own promoter. The transposition of this element is controllable by the carbon source on which yeast bearing it are grown; galactose induces transposition to rates 20–100-fold above normal levels (BOEKE *et al.* 1985; BOEKE, STYLES and FINK 1986). The transposon can be marked with a variety of selectable markers (BOEKE, XU and FINK 1988; GARFINKEL *et al.* 1988). Repeated cycling of a transposition-inducing regimen results in the generation of strains bearing ever-increasing numbers of Ty1 elements. We have investigated the mitotic and meiotic properties of some of these strains.

MATERIALS AND METHODS

Media: YPD, YPGE and SD media were prepared as described (SHERMAN, FINK and HICKS 1986). SC-ura plates (lacking uracil) containing glucose or galactose (2%) as carbon source were identical to SD plates except that 2 g/liter of a mixture [consisting of all 20 amino acids (2 g each

except 4 g leucine) and adenine sulfate (0.5 g)] was added prior to autoclaving.

Strains: Yeast and bacterial strains used are described in Table 1. Plasmids pGTy1-H3*lacO* (= pJEF896) (BOEKE *et al.* 1985; BOEKE, STYLES and FINK 1986) and pGTy1-H3-*neo* (= pJEF1105) (BOEKE, XU and FINK 1988) have been described elsewhere.

Construction of sets of strains ("lineages") differing in the number of genomic Ty1 elements: Two Gal⁺ strains of yeast, GRF167 (*MATα ura3-167 his3Δ200*) and BWG1-7a (*MATa ura3-52 his4-519 ade1-100 leu2-3,112*) were transformed with the recombinant plasmids pGTy1-H3-*lacO* and pGTy1-H3-*neo*, respectively, producing yeast strains JB442 and JB516, respectively. These two strains were the parental strains from which two lineages of strains bearing various numbers of extra Ty1 elements were derived. Strain JB442 served as the parental strain for lineages L1–L12; JB516 was the parent of lineages L13–L24. The lineages were produced as follows.

The steps below comprise one cycle of transposition induction. For each of the two parental strains, 12 independent colonies were streaked onto two SC-ura/galactose plates divided into 6 pie-shaped sectors (for a total of 24 sectors). These were incubated for 5 days at 22°. The single (isolated) colony in each sector nearest to the center of the plate was picked and restreaked onto a similar sector of an SC-ura/glucose plate and grown at 30° for 2 days. The colonies from each sector closest to the center of the plate were again selected and saved as L1 . . . 24–1. The number following the L indicates the lineage number, the number following the hyphen indicates the cycle number. At this stage, cells from colonies L1 . . . 24–1 were (1) streaked out on YPD to allow spontaneous plasmid curing, giving rise to Ura⁻ colonies, identified by replica plating to SC-ura plates, and designated L1 . . . 24-1C; and (2) used as parents in a second cycle of transposition as just outlined.

The 24 lineages produced consisted of five such cycles of transposition and the strains generated, L1 . . . 24-1 through L1 . . . 24-5, as well as L1 . . . 241C through

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TABLE 1

Yeast and bacterial strains

Strain	Genotype	Plasmid	Source
BWG1-7a	MATa ade1-100 his4-519 leu2-3,112 ura3-52		L. GUARENTE
GRF167	MATα his3Δ200 ura3-167		G. FINK
F762	MATa trp1Δ1 ura3-52		J. THOMAS
JB442	=GRF167	pGTy1-H3-lacO	This work
JB516	=BWG1-7a	pGTy1-H3-neo	This work
L1 . . . 12-1 . . . 5c	=GRF167		This work
L13 . . . 24-1 . . . 5c	=BWG1-7a		This work

L1 . . . 24–5C, were all saved for further analysis as frozen (−80°) cultures.

Orthoganol field alternating gel electrophoresis (OF-AGE) analysis: Cells were grown at 30° in 100 ml of YPD overnight and chromosome-sized DNA prepared in agarose essentially as described (SCHWARTZ and CANTOR 1984) with the following modifications. Cells were washed twice with 12 ml 50 mM EDTA, pH 7.5 and resuspended in 1–2 ml 50 mM EDTA, pH 7.5 (total volume of resuspended cells is about 2 ml). The cell suspension was placed in a 60-mm Petri dish alongside 0.7 ml of a solution consisting of a mixture of 10 mg Zymolyase 100T in 10 ml SCE (1 M Sorbitol, 0.1 M sodium citrate, 60 mM EDTA, pH 5.6) and 0.5 ml β-mercaptoethanol. Then 3.3 ml of 1% low-melting point agarose in 125 mM EDTA pH 7.5 (at 42°) was added and quickly mixed with the other solutions until homogeneous and then allowed to gel. 3.3 ml of a solution consisting of 25 ml of 0.5 M EDTA, pH 9.0, 0.6 ml of 0.5 M Tris-HCl, pH 8.0 and 2.1 ml β-mercaptoethanol was overlayed on the surface of the plate and it was incubated in a sealed bag overnight at 37°. The overlay was poured off and replaced with 3.3 ml of a solution containing: 25 ml 0.5 M EDTA, pH 9.0, 28 mg of proteinase K, and 2.8 ml 10% (w/v) Sarkosyl. Incubation was at 50–55° overnight. The plate was chilled and the overlay removed by aspiration. The agarose was transferred to a 250 ml beaker containing 15 ml 0.5 M EDTA, pH 9.0, and washed overnight at 4°, then this was replaced by 4 washes of 15 min each in 50 mM EDTA, pH 9.0. Gels were poured on frosted glass plates and consisted of 30 ml conventional (for analytical electrophoresis) or low-melting point (for preparative gels) 1% agarose in 0.5 × TBE buffer (2.8 g boric acid, 5.4 g Tris base, 0.4 g disodium EDTA per liter water). Electrophoresis was for 14 hr at 270 V with a 40-sec pulse time at 11° for separation of all chromosomes except *IV* and *XII*. Chromosomes *IV* and *XII* were separated on the same type of gel, but electrophoresis was for 60 hr at 120 V with a 5-min pulse time. Gels were stained 30′ in gel buffer containing 0.5 μg/ml ethidium bromide and destained (if necessary) in gel buffer for 1–2 hr.

Southern blotting: Southern blotting and probing with a *lacO* probe was as described (BOEKE *et al.* 1985). The *neo* probe was the *Bam*HI fragment of plasmid pGH54 (JOYCE and GRINDLEY 1984) labeled by the random hexamer priming method (FEINBERG and VOGELSTEIN 1983). The Ty1-specific probe was the *Pvu*II-*Pst*I fragment (nucleotides 476–1142) from Ty1-H3 (BOEKE *et al.* 1988) labeled by the random hexamer priming method.

Analysis of mitotic stability of the newly transposed Ty1 copies: Single colonies were picked from strains L1-5C, 2-5C, 8-5C and 10-5C into 10 ml YPD medium and grown for 1 week at 30°. Aliquots of 3.5 μl of cells were then rediluted into 10 ml fresh YPD and grown for 1 week

at 30°. This process was repeated for a total of four such growth cycles. Cells from the fourth growth cycles and the parental strains were streaked onto fresh YPD plates and four single colonies (a–d) were picked from each culture and analyzed by Southern blotting with a *lacO* probe as described above.

Growth rate measurements: The number of cells in YPD and SD + uracil (200 μM) and histidine (33 μM) cultures grown at 30° were measured as A_{600} and plotted on semi-logarithmic paper. Long-term mitotic growth studies were carried out by growing pairs of cultures in YPD; cultures were sequentially diluted exactly 20-fold (to approximately 0.1 A/ml from approximately 2.0 A/ml) three times. The cells remained in log phase throughout this experiment.

Meiotic analysis: Diploids were selected by complementing auxotrophies in the parental strains; purified diploids were then sporulated on sporulation medium supplemented with 100 μM uracil. Incubation was at 20° overnight and then at 30° for 2–4 days. Sporulation efficiency was monitored by phase contrast microscopy. Tetrads were dissected on fresh YPD plates and grown at 30°.

RESULTS

A cycling procedure for obtaining yeast strains with very high Ty1 copy numbers: We have previously shown that yeast strains bearing pGTy1-H3 plasmids (and their genetically marked derivatives) suffer multiple transposition events after growth on galactose under conditions selective for the plasmid (*i.e.*, on medium lacking uracil). These elements transposed are derived from the Ty1 element carried by the plasmid and, to a lesser extent, from chromosomal copies of Ty1 (BOEKE *et al.* 1985; BOEKE 1989). Growth on SC-ura galactose medium for 5 days at 22° results in incorporation of a mean of 2–5 copies of marked Ty elements per haploid genome. Ty1 elements marked with a small 40-bp marker (*Escherichia coli lacO*) transpose at a somewhat higher average frequency than do elements marked with a larger intact gene marker (1000 bp) such as the bacterial *neo* gene (BOEKE, XU and FINK 1988). We have modified this simple procedure to enable multiple rounds of transposition to be induced as outlined in Figure 1 and described in detail in MATERIALS AND METHODS. Strains are colony purified after each round of transposition-induction. With this procedure, sets of strains related to each other by a single cycle of transposition, and deriving from a common parent, can be pro-
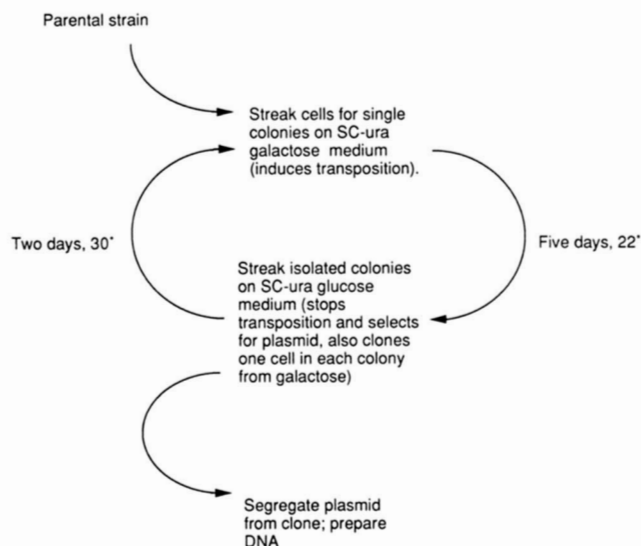
Parental strain

Streak cells for single
colonies on SC-ura
galactose medium
(induces transposition).

Two days, 30°

Five days, 22°

Streak isolated colonies
on SC-ura glucose
medium (stops
transposition and selects
for plasmid, also clones
one cell in each colony
from galactose)

Segregate plasmid
from clone; prepare
DNA

FIGURE 1.—Method for obtaining strains with very high Ty*1* transposon copy numbers.

duced. Twenty-four such "lineages" were constructed by this procedure. Lineages 1–12 were derived from parental strain JB442, a *MAT*α strain bearing plasmid pGTy*1*-H3-*lacO*, and lineages 13–24 were derived from JB516, a *MAT*a strain containing pGTy*1*-H3-*neo*. After one cycle of transposition, the chosen clone from lineage 1 was designated L1-1, the second L1-2, etc. All subsequent work was performed on cloned derivatives of these strains that had been cured of the pGTy*1*-H3 plasmid by mitotic segregation and subsequent screening for Ura⁻ colonies by replica plating (designated L1-1C, L1-2C, etc.). Lineage 6 was abandoned when it was determined that the pGTy plasmid had integrated into a chromosome in clone L6-1.

We wished to determine whether transposon numbers would increase linearly with each cycle or reach an equilibrium value. DNA was extracted from strains L1-1C to L1-5C, L2-1C to L2-5C, L3-1C to L3-5C and L12-1C to 12-5C, digested with a restriction enzyme (*Hin*dIII) that should give rise to a single *lacO*-containing restriction fragment for each transposon copy integrated into the genome of that strain, and electrophoretically separated on an agarose gel. The DNA was transferred to nitrocellulose and probed with radioactive *lacO* DNA (Figure 2). It can be seen that as the number of cycles of transposition induction increases, the number of hybridizing fragments increases nearly linearly; the average increase in copy number is 4.6 per cycle (Table 2). There is a slight decrease in copy number per cycle in cycles 4 and 5, but this is probably not significant. In each lineage and at every cycle, at least two new bands can be seen. Lineage 12 is unusual in that in cycles 2, 4 and 5, loss of one or two specific bands is seen in three cycles (Figure 2, Table 2). Qualitatively similar results were obtained with the *neo*-marked Ty elements in lineages 13 and 14 (not shown). For all lineages, strains from

A

Pvu*II* EcoRI Pvu*II* Hin*d*III lacO

Ty*1* probe lacO probe

B

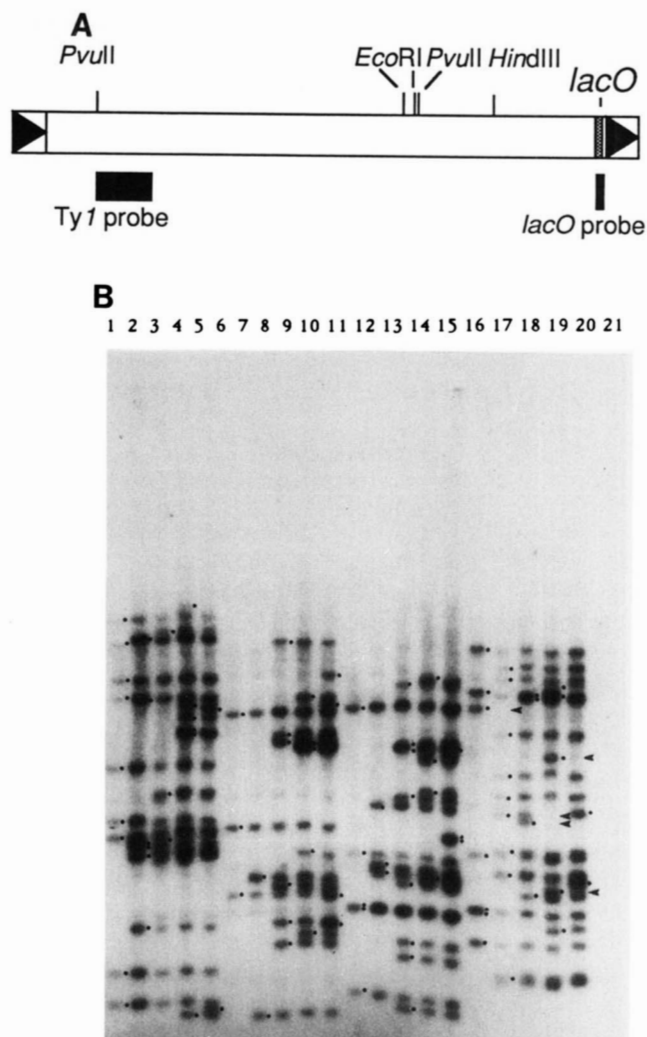1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

FIGURE 2.—Southern analysis of lineages. (A) Map of *lacO*-marked Ty*1* element, indicating restriction sites and DNA probes referred to in this paper. (B) DNA extracted from clones isolated after each of five cycles of transposition induction was digested with *Hin*dIII, electrophoretically separated, transferred to nitrocellulose, and probed with a *lacO* probe (BOEKE *et al.* 1985) for four "lineages" and for the parental strain. Lanes 1–5, L1-1C–L1-5C; lanes 6–10, L2-1C–L2-5C; lanes 11–15, L3-1C–L3-5C; lanes 16–20, L12-1C–L12-5C; lane 21, parental strain (GRF167). Dots are new bands that occurred during that cycle; arrowheads are bands lost during that cycle.

the first and fifth cycle were analyzed by the Southern blotting procedure outlined above and were found to be similar to the smaller set of strains just described.

From the above Southern blotting data, we estimated the minimum total number of marked Ty transposition events that had occurred in the 22 lineages for which there were good hybridization data. The collection of strains carried a minimum of 422 new transposition events in their chromosomes. The mean copy number of marked Ty*1* elements in L1 . . . 12-5C strains was 23 and in L13 . . . 24-5C strains was 13.3. These figures are probably underestimates because of a conservative approach to dealing with bands that were present in higher than molar

TABLE 2

Copy number increase of marked Ty*1* elements per cycle

Lineage No.	Cycle					Mean per cycle ± SD
	1	2	3	4	5	
1	9	6	3	6	2	5.2 ± 2.77
2	3	2	6	5	4	4.0 ± 1.58
3	5	3	8	4	3	4.6 ± 2.07
12	7	9 (−1)[a]	4	5 (−2)	2 (−2)	4.4 ± 3.21
Aggregate mean						4.6 ± 0.50

[a] Negative numbers in parentheses indicate bands missing from their position in the previous cycle. The numbers given indicate the number of new marked copies (or losses of marked copies) that occurred during that cycle of transposition. See Figure 2 for raw data. The total number of new transposition events is probably an underestimate, because of conservatism applied when doublets (which may in fact be higher order multiplets) were encountered. SD, standard deviation.

ratio and presumably represented multiple transposition events, and because transpositions resulting in very high molecular weight restriction fragments might be missed. Moreover, this estimate applies only to copies of marked elements. We have previously shown that induction of transposition by a pGTy*1* plasmid results in the mobilization of chromosomal (unmarked) Ty*1* elements in addition to the mobilization of plasmid-derived Ty*1* elements. Mobilization of unmarked elements in this study was much less frequent however, than had been observed previously (BOEKE *et al.* 1985; BOEKE, STYLES and FINK 1986) in studies using different host strains (data not shown). Some of the difference in the ratio of unmarked/marked elements in the two studies can be attributed to the fact that in this study, the proportion of marked RNA increases as strains accumulate genomic copies of marked Ty elements in the later stages of the experiment (the previous studies used only a single cycle of galactose induction).

Phenotypic characterization: The 23 fifth cycle strains (L1 . . . 24-5C strains) were examined for phenotypic differences from the parental strain. Surprisingly, the strains differed very little from the parents. Only L3-5C, L15-5C, and L22-5C differed in colony morphology from the parents; L3-5C showed increased apparent lethal sectoring of the colonies (*i.e.*, the colony edges were not entire but scalloped in appearance) and a slightly darker colony color than did its parent, and L15-5C and L22-5C were petite. Because the strains were grown on SC-ura medium, which is supplemented with all amino acids plus adenine, we expected to find auxotrophic mutants among our collection. However, we would not be able to detect mutations in the uracil or histidine biosynthetic pathways for L1-12 or in the uracil, adenine, histidine or leucine pathways for L13-24 strains by replicaplating to appropriately supplemented minimal (SD) plates, due to preexisting auxotrophies in the parental
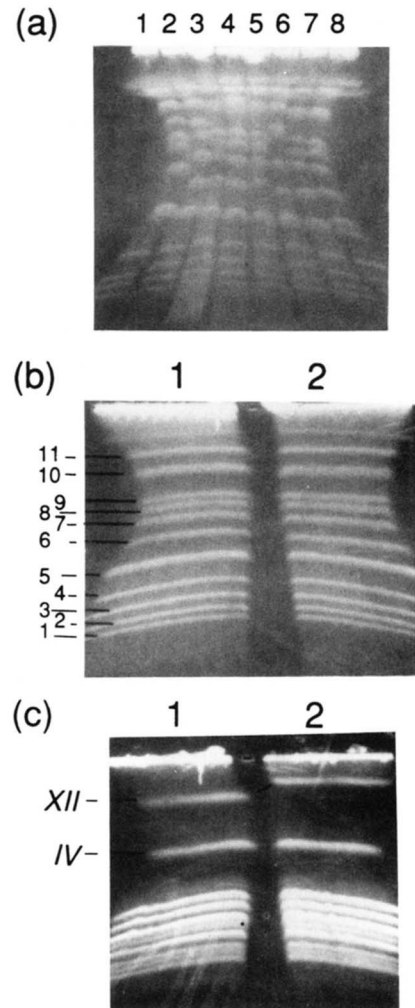

FIGURE 3.—OFAGE analysis of strains bearing excess Ty*1* elements. (a) Analytical gel. Lane 1, L5-5C,; lane 2, L7-5C; lane 3, L8-5C; lane 4, parental (GRF167); lane 5, L1-5C; lane 6, L2-5C, lane 7, L3-5c; lane 8, L4-5C. (b) Preparative gel for Figure 4, showing resolution of smaller chromosomes. Lane 1, L5-5C, lane 2, wild type (GRF167). (c) Preparative gel for Figure 4, resolving the larger chromosomes. Chromosomes *XII* and *IV* are indicated. Lane 1, L1-5C; lane 2, wild type (GRF167).

strains. We found no new auxotrophies in any of the 23 fifth-cycle strains. Mating tests with all of the strains failed to indicate any sterility mutations, and all were capable of growth at high (37°) and low (11°) temperatures.

OFAGE analysis: Several of the fifth-cycle strains were analyzed for differences in chromosomal DNA size compared to the parental strains (Figure 3). Marked differences in chromosomal DNA mobility are observed, sometimes in more than one band, in nearly every strain checked carefully. Even perturbations of electrophoretic mobility of the very large chromosomes are observed. Chromosome *XII* (which contains the rDNA repeat units) of strain L1-5C migrates faster than its parental counterpart under the pulsed field gel conditions used. Two-dimensional
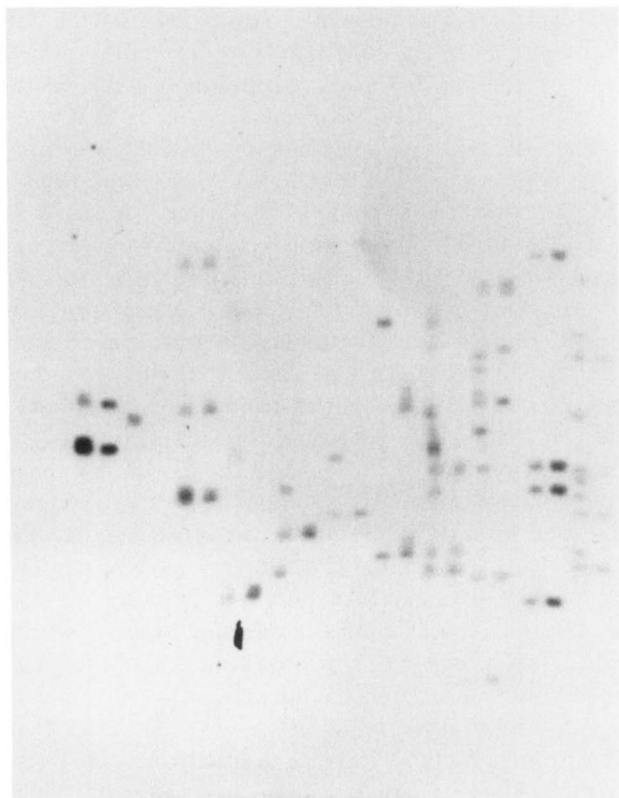
1 2 3 4 5 6 7 8 9 10 11 *IV XII*

FIGURE 4.—Two-dimensional Southern analysis of strain L4-5C. Each pair of lanes contains DNA from L5-5c and wild type, in that order. Each band from the preparative low-melting point agarose OFAGE gels (Figure 3, b and c) was excised, melted, and the DNA was digested with EcoRI. Lanes 1–11 indicate bands 1–11 from Figure 3b; lanes IV and XII indicate chromosomes IV and XII from Figure 3c.

TABLE 3

Chromosomal analysis of Ty1 elements in strains with many excess copies of Ty1 elements

Band No.	Presumed chromosome(s)	GRF 167	L4-5c		L1-5c		Mean gain per chromo-some[a]
			Gains	Losses	Gains	Losses	
1	I	0	2	0	0	0	1
2	VI	0	2	0	0	0	1
3	III	2	1	0	1	0	1
4	IX	0	NT[b]	NT	1	0	1
5	V, VIII	3	4	0	1	0	1.25
6	XI	1	2	0	3	0	2.5
7	X	1	2	1	2	0	2
8	XIV	2	1	0	1	0	1
9	II	2	2	1	1	1	1.5
10	XIII, XVI	3	3	0	5	0	2
11	VII, XV	6	4	0	5	1	2.25
(A)	IV	4	NT	NT	0	0	0
(B)	XII	4	4	0	6	0	5
Total		30	≥27	≥2	26	2	

[a] Per five cycles of transposition.

[b] NT, not tested. The numbers given indicate the number of marked copies of Ty1 on the indicated chromosome, i.e., the number of copies gained (or lost) after five cycles of transposition relative to the parental strain, GRF167.
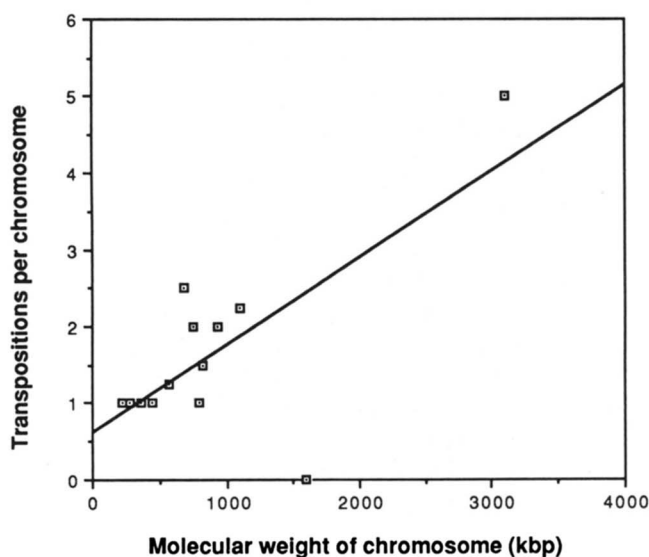

FIGURE 5.—Number of insertions per chromosome as a function of chromosome molecular weight.

analysis (see below) indicates that chromosome *XII* of L1-5C contains six new copies of Ty1.

Counting transposon numbers: The very high copy numbers and the mixture of marked and unmarked elements obtained with this procedure gave rise to problems in accurately counting them by conventional genomic Southern blotting. To circumvent this, we effected a two-dimensional separation of the Ty1 elements as follows. Preparative low-melting-point agarose OFAGE gels were run using two different conditions to allow clean preparative separation of all chromosomal DNA molecules from strain L5-5C and its parent, GRF167 (Figure 3) (some chromosomal DNAs are obtained as doublets). The chromosomal DNAs were then separately excised from the gel and digested with EcoRI, and each digested band was separated electrophoretically on a conventional 0.6% agarose gel. The DNA was transferred to nitrocellulose and probed with the Ty1-specific probe (Figure 4). Bands were counted to determine the number of new Ty1 copies present in the DNA. From this analysis, we found that there are 26 new copies of Ty1 in L5-5C. Similar analysis of L4-5C showed that it con-

tains at least 27 new copies of Ty1 (Table 3). The new Ty1 element copies are distributed more or less randomly among the chromosomes, with insertion on the larger chromosomes being favored, presumably due to larger target size (Figure 5).

Mitotic stability of newly transposed Ty1 elements in strains with very high transposon copy numbers: The large increase in Ty1 copy number might be expected to destabilize the haploid yeast genome significantly; it might allow for an unacceptably high amount of mitotic recombination among the dispersed repeats. It also seemed possible that there might exist special mechanisms to eliminate excess
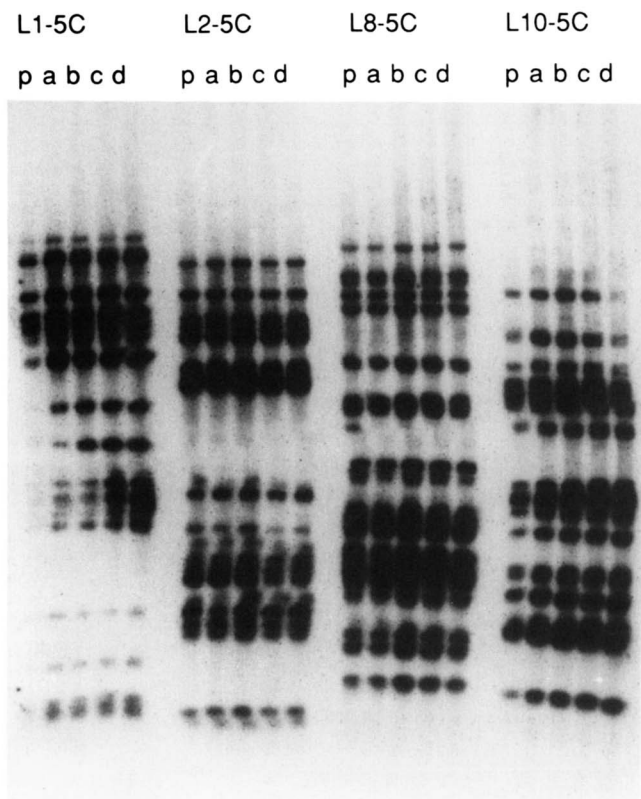
L1-5C   L2-5C   L8-5C   L10-5C

p a b c d   p a b c d   p a b c d   p a b c d

FIGURE 6.—Mitotic stability of new Ty*1* copies. Strains L1-5C, L2-5C, L8-5C and L10-5C were evaluated for mitotic stability by extensive growth (see text). Four random progeny colonies (a–d) were selected from each culture and compared to the parental strains (p). DNA was extracted and analyzed as in Figure 2.

copies of Ty*1* elements once their number exceeded some limit. To examine these possibilities, we performed a long-term growth test on strains L15C, L2-5C, L8-5C and L10-5C. A single colony of each strain was inoculated into 10 ml of YPD broth and grown at 30° to stationary phase. The culture was diluted approximately 3000-fold and again grown to stationary phase at 30° in YPD. The latter procedure was repeated two more times, and cells were streaked onto a YPD agar plate and allowed to form single colonies at 30°. The genomic DNA of four single colonies deriving from each culture (designated a–d) was then analyzed by *Hind*III restriction and Southern blotting with a *lacO* probe as described above. As can readily be seen in Figure 6, the pattern of hybridizing bands remains essentially unchanged after approximately 45 generations in all of the clones analyzed. In one case (L8-5C), one band is missing in all four progeny colonies. The submolar nature of this band in parental L8-5C suggests that L8-5C is probably not a pure culture; this band probably represents a transposition event that occurred during the growth of the original L8-5C colony. Therefore the subpopulation of cells lacking the extra copy probably outgrew the subpopulation containing it. A similar Southern blotting experiment using enzyme *Pvu*II (not shown) revealed

that colony L2-5C-a lacked one hybridizing band; colonies L2-5C-b . . . d were indistinguishable from the parent, as were all of the strains derived from the other three lineages studied. Assuming we could identify losses or restriction site changes in 80% of the bands on the gel, this suggests a loss rate of about one transposon loss per 14,000 "Ty*1* copy-generations" (0.8 × 25 marked Ty*1* copies × 45 generations × 4 original strains × 4 subclones analyzed per strain). This *lacO* marker loss could result either from loss of a transposon copy by LTR-LTR recombination or other deletion events, or through a gene conversion event in which the marked element is the recipient and an unmarked copy is the donor. This frequency of *lacO* marker loss is very similar to the frequency of movement of Ty*1*-912 (marked with *URA3*) by gene conversion to other Ty*1* element copies in the genome, reported to be about 10^{-4}(ROEDER, SMITH and LAMBIE 1984). Even higher frequencies of Ty-associated marker loss have been reported in other strains (PICOLOGLOU *et al.* 1988). Thus the frequency of marker loss observed is within the realm of such marker loss frequencies as have been reported for normal yeast strains.

We note that there were occasional cases of Ty*1*-hybridizing bands being lost during the cycles of transposition (Figures 2 and 4, Tables 2 and 3). This result is difficult to interpret definitively, but there are at least three reasonable explanations for these apparent loss events. It should be noted that the loss events illustrated in Figure 2 and Table 2 represent loss of the *lacO* marker, and not necessarily the whole Ty*1* element, and thus could reflect loss by deletions or by conversions as outlined above. The loss events in Figure 4 and Table 3 cannot represent such conversion events as the probe in this case is an internal Ty*1* fragment, although other explanations must also be considered (see below). (1) Some of these loss events may actually reflect new insertions into the same restriction fragment occupied by the Ty*1* that was "lost"–this requires that a new band of different size should be gained simultaneously–candidates for this scenario include the loss events in lanes 17–21 of Figure 2. These of course would not represent real loss events at all, but rather changes in the restriction fragment size resulting from additional insertions. (2) Loss events may occur under transposition-inducing conditions at higher frequency than under normal mitotic growth conditions. M. KUPIEC (personal communication) has observed that overproduction of Ty*1* proteins results in an increase in gene conversion events involving Ty*1* elements, so this is not an unreasonable possibility. Further experiments are needed to definitively show whether or not this is the case. (3) Finally, some apparent loss events may result for trivial reasons, similar to the "loss" event described above
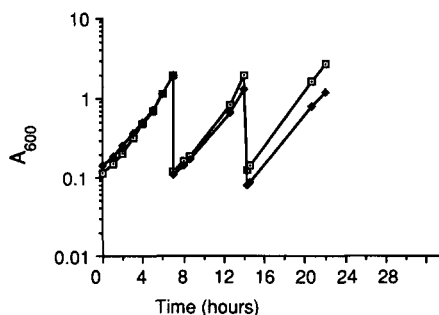
FIGURE 7.—Growth curves. Strains L1-5C (filled diamonds) and GRF167 (the parental strain, open squares) were separately inoculated into YPD medium and diluted 20-fold into fresh prewarmed medium when GRF167 reached an A_{600} of approximately 2.0 three times.

for strain L8-5C. Such trivial loss events during the cycling process could occur if only a subpopulation within a colony contained a given transposon copy. During the subcloning step in the following cycle, choice of a clone deriving from a cell that happened to lack that transposon copy would result in its apparent loss.

Growth properties of strains with very high transposon copy numbers: The doubling times of strains L1-5C, L2-5C, L3-5C, L4-5C and L5-5C were compared to that of the parent, GRF167. Doubling times in both rich (YPD) and minimal (SD + histidine + uracil) media were measured. The calculated doubling times of these strains were only slightly longer than that of the parental strain (data not shown). In confirmation of the difference in doubling time, is the finding that when the parental strain was inoculated at a slightly lower density than were the strains with excess Ty elements, the absorbance of the culture of the parental strain often caught up with and then exceeded that of the strains with excess Ty elements; the reverse was never observed. Also, the absorbances of stationary phase cultures of the parental strain were always slightly higher (data not shown). The growth rates of GRF167 and L1-5C were compared carefully by growing them in log phase for about 24 hr as described in MATERIALS AND METHODS (Figure 7). The slightly faster growth rate of the parental strain is very obvious in this experiment (T_d of 100 vs. 112 min).

Meiotic properties of strains with very high transposon copy numbers: Although the strains described above were obviously selected for mitotic viability, they were never selected for the ability to undergo meiosis successfully. We analyzed the meiotic capabilities of some of the strains bearing excess Ty*1* elements when crossed with their wild-type parents and with each other. The data in Table 4 show that spore viability is actually somewhat higher in a cross of two strains bearing excess transposon copy numbers than in the corresponding parent × parent cross, indicating that transposon copy number increase *per se* does not

TABLE 4

Meiotic analysis of strains with many extra Ty elements

Cross number(s)	MATa parent	MATα parent	Percent viability	No. of tetrads
186, 198, 211	BWG1-7a	GRF167	87	65
208	L13-5c	L12-5c	94	88

result in decreased meiotic capability. The pattern of spore lethality was carefully studied in each cross made and was inconsistent with the presence of preexisting large deletions, large inversions or reciprocal translocations in the parental strains; spore lethality followed a random pattern. Appropriate segregation of the auxotrophic and mating-type loci was observed in all such crosses tested; we could detect no significant differences in gene conversion frequencies of the markers scored.

DISCUSSION

We have constructed a bank of 23 strains containing large numbers of excess copies of Ty*1* elements. This study clearly demonstrates that at least 25 extra Ty*1* elements can be supported by the yeast genome with little trouble. Construction of such strains was accomplished by simply cycling cells containing marked pGTy*1*-H3 plasmid derivatives through a regimen that induces high levels of Ty*1* transposition in the cells. In strains L1 . . . 12-5C, which received five cycles of transposition-induction, there are about twice the number of Ty*1* elements normally found in the genome; most of these are marked with a *lacO* hybridization marker. Somewhat lower Ty*1* copy numbers are found in strains L13 . . . 24-5C, which contain elements marked with the larger *neo* gene. The rather uniform number of elements added to the genome with each transposition cycle suggests that much higher transposon copy numbers could be attained by continuing to cycle the cells through the transposition regimen. Because target sites, in the form of disruptable DNA, are abundant in the genome (GOEBL and PETES 1986) and may be unlimited (the number of Ty elements, which can serve as target sites for transposition, is ever-increasing), the upper limit to transposon copy number that can be tolerated may greatly exceed the levels studied here.

Strains with excess Ty*1* elements show surprisingly little phenotypic variation from parental strains: Obviously, the collection of strains studied was under selection for viability during the entire experiment, but every effort was made to pick colonies in an unbiased way, always choosing the single colony in each sector that was closest to the center of the plate, regardless of color, shape or size. The collection of strains was screened for a variety of mutant phenotypes. Most of the strains were indistinguishable

from the parent strain in terms of colony morphology, nutritional requirements, growth at extreme temperatures, mating competence and ability to respire. Of the entire collection, only two were petite, none were sterile, none were temperature sensitive or cold sensitive and none were auxotrophic for a wide range of metabolites. Considering that well over 400 insertions are represented in this collection of strains, we expected to find about four auxotrophs if transposition were random and 1% of the genome could mutate to auxotrophy. GOEBL and PETES (1986) made a study of the phenotypic effects of randomly generated gene disruptions. Plasmids bearing fragments of randomly selected single-copy yeast genomic DNA were selected and screened for those bearing unique sites for certain retriction enzymes. A selectable marker gene was inserted at these sites and the disrupted restriction fragments were used to replace the wild-type restriction fragments corresponding to them in diploid cells. These cells heterozygous for randomly positioned disruption mutations were then sporulated. They recovered one auxotroph in 29 such insertions, as well as four recessive lethals (which obviously would not have been recovered in our selection for viability) and two *pet* mutants. They obtained similar results with Tn*3* insertions made in *E. coli* into yeast DNA, except that no further auxotrophs were obtained. Thus there may be a slight bias against Ty*1* transposition into coding regions of yeast chromosomes, although these clearly can occur (ROSE and WINSTON 1984; NATSOULIS *et al.* 1989; WILKE *et al.* 1989). However, the small numbers of events on which this speculation is based will require much more extensive study to confirm or refute.

Growth rates: Growth rate measurements indicated only small differences in mitotic growth rates between strains bearing large numbers of excess Ty elements and parental strains. One of the strains with excess Ty*1* elements, whose growth rate was checked very carefully, was indeed found to grow at a slightly slower rate (doubling time 112 min) than its parent (doubling time 100 min) (Figure 7). Obviously, in the wild even small differences in growth rate could result in enormous selection biases, and these biases may help to contribute to keeping Ty*1* copy number under control to some degree. The changes in doubling time observed are probably due to insertions that reduce but do not eliminate expression of essential genes, or inactivate genes whose products are important for maximal growth rates. Alternatively, it may be that the presence of extra Ty*1* element copies *per se* is responsible for the growth difference. In any case, it appears that, under laboratory conditions, there is not heavy selection against Ty*1* insertions into the genome. Indeed, it has been observed that Ty*1* element copy number is higher in laboratory strains than in

wild strains (EIBEL *et al.* 1981), although the evolution of Ty*1* element copy number and genomic position during the conversion of a "wild" strain into a "lab" strain has not yet been directly monitored.

Large numbers of Ty*1* insertions are relatively stably maintained in the genome: It might be expected that increasing the number of patches of homologous DNA in the form of Ty*1* elements scattered widely throughout the genome would destabilize it. Alternatively, it might be imagined that the cell had evolved a special mechanism to rid its genome of excess transposon copies when their copy number exceeded some limit. We can find no evidence for the latter, and little evidence for loss of significant numbers of Ty*1* element copies during mitotic growth. All but one of about 100 copies present in four strains were stable through about 45 generations. This is good news for those interested in using Ty*1* elements as a means of building strains with very high copy numbers of gene(s) of interest inserted throughout the genome; such strains will probably be much more stable than strains bearing episomal plasmids.

Meiotic reciprocal recombination between chromosomal Ty elements: We examined the meiotic competence of a pair of strains bearing excess copy numbers of Ty*1* elements. We observed no significant diminution of spore viability when strains bearing excess copies were intercrossed. In fact, in the cross for which a large number of tetrads was dissected, spore viability was actually somewhat higher than in the parent × parent cross. Some fluctuation in spore viability in other crosses of this type (done on a much smaller scale) was observed. Since poor viability is expected in certain strains bearing insertions that disrupt genes whose function is important for efficient germination, this is not particularly surprising. The result of the cross presented in Table 4 shows that it is possible to approximately double the number of Ty*1* copies in the genome without reducing spore viability. It is unlikely that the Ty*1* insertions are lost in the heterozygous diploid state; VINCENT and PETES (1989) have shown that mitotic gene conversion events favor the retention of the Ty-bearing allele when heterozygous strains bearing point mutations and Ty insertion mutations in the same gene undergo mitotic gene conversion.

The paucity of effect of excess Ty*1* elements on meiosis is significant, because the transposon copies should provide many additional potential sites for gene conversion events associated with reciprocal recombination events that lead to lethal chromosome aberrations including deletions, inversions, and translocations. Such ectopic recombination, when it occurs between heteroalleles of normal yeast genes residing at different chromosomal loci, is surprisingly frequent, although it varies considerably according to

TABLE 5

Meiotic ectopic recombination frequencies

Gene segment	Length (kb)	Position[a] Copy A	Copy B	Method[b]	Frequency[c]	Crossing over frequency[d]	Reference
HIS3	1.8	*XV*	*IX*	U	5×10^{-3}	ND	JINKS-ROBERTSON and PETES (1985)
URA3	5.5	*V*	*XV*	H	5×10^{-5}	0.43	JINKS-ROBERTSON and PETES (1986)
LEU2	2.7	*III*	*III*	H			LICHTEN, BORTS and HABER (1987)
		LEU2	*HIS4*		$6-9 \times 10^{-3}$	0.46	
		LEU2	*MAT*		$2-10 \times 10^{-3}$	ND	
		LEU2	*HML*		$1.4-5.5 \times 10^{-3}$	ND	
		III	*V*		$0.4-2 \times 10^{-3}$	0.22	LICHTEN, BORTS and HABER (1987)
HIS4	5.0	*III*	*VIII*	U	5×10^{-2}	0.40	NAG and PETES (1990)

[a] Chromosomes are indicated; for intrachromosome *III* experiments, loci on chromosome *III* are indicated. Copy A, normal gene position; copy B, ectopic position.

[b] U, conversion events in unselected tetrads; H, selected conversion events between heteroalleles (selected by random spore platings on selective medium).

[c] Frequencies from "H" experiments are underestimates of recombination frequencies because only prototrophic recombinants are detected.

[d] Corrected to account for presumed loss of inviable meiotic products.

the length of homology available, the gene studied, and the position at which the gene being studied is inserted (these data are summarized in Table 5) (JINKS and PETES 1985, 1986; LICHTEN, BORTS and HABER 1987). If, for illustration, we assume that ectopic recombination frequency is on average 5×10^{-3} per pair of Ty*1* elements, and 50% of these result in crossing over, then a genome with 25 Ty*1* elements should generate one ectopic crossover in 75% (300 pairs $\times$ 5 $\times$ 10^{-3} per pair $\times$ 0.5 crossovers) of the tetrads. Clearly, this would result in a massive spore lethality which is not observed.

Previous authors have suggested three possible mechanisms for the lack of ectopic recombination between Ty elements. (1) Sequence heterogeneities among different Ty element copies may be sufficient to block recombination between ectopic Ty copies while allowing allelic (and presumably identical) copies to recombine. (2) Ty elements have accumulated in regions of the yeast genome that are "cold" for recombination, and their retention in recombinationally active regions of the host genome has been actively selected against by cycles of sporulation and mating. (3) There is a mechanism that specifically inhibits Ty/Ty recombination. This model is based on the observations that mutations in the *EDR1* gene give rise to an increase in recombination events involving Ty elements (ROTHSTEIN 1984). Recently, *EDR1* (*TOP3*) has been shown to encode a new type I topoisomerase (WALLIS *et al.* 1989). (4) Finally, it should be kept in mind that the events or factors that initiate recombination, rather than the number of homologous sites available, may limit recombination frequency.

The sequence heterogeneity hypothesis is not well supported by our data, since most of the new copies of Ty*1* inserted into the genome in this study derive from the same parental element, Ty*1*-H3. Our pre-

vious study (BOEKE, STYLES and FINK 1986) has shown that the majority of transposition events deriving from such a parental element are indistinguishable from the parental element in sequence (determined by checking for the presence/absence of a small number of restriction enzyme sites). However, a minority of progeny Ty*1*-H3 elements differ extensively from the parental copy at certain sites, raising the possibility that minor differences escaped detection in the majority of the elements. Consideration of the probable cause of the extreme heterogeneity observed in a minority of progeny elements suggests that the latter argument is unlikely. Our experiments strongly suggest that, as is the case in retroviruses, two Ty*1* RNAs are packaged per particle, and that the heterogeneous progeny elements arise from "heterodimer" genomes consisting of one plasmid-derived (marked) and one chromosomally derived (unmarked, and presumably heterogeneous) RNA; jumping between these two templates during minus-strand reverse transcription would generate hybrid progeny reverse transcripts (COFFIN 1979; BOEKE, STYLES and FINK 1986). A relatively high proportion of the Ty*1*-VLPs in our strains should contain "homodimer" genomes where both RNAs are plasmid-derived, because the pGTy*1*-H3 transcript is 2–10 times more abundant than are the chromosomal Ty*1* transcripts combined (BOEKE, EICHINGER and FINK 1988; CURCIO *et al.* 1990). Consequently, reverse transcripts derived from such "homodimer" VLPs should be completely homologous to Ty*1*-H3 (except by virtue of rarer misincorporation errors).

Similarly, the second hypothesis, that Ty*1*s have historically accumulated in regions of low meiotic recombination activity due to constant selection for meiotic competence, seems very unlikely in light of our results. Throughout our experiment, there was

no selection for meiotic capability, yet the strains retained high spore viability.

By elimination, the third and fourth possibilities, that there is a special mechanism for reducing the frequency of ectopic recombination events involving Ty elements, or that recombination frequencies are limited by the number of initiation events rather than by amount of homology, seem most likely. KUPIEC and PETES (1988) have shown that meiotic ectopic recombination of Ty elements in normal laboratory strains occurs at much lower frequencies than would be expected based on ectopic recombination frequencies observed between *his3* and *ura3* heteroalleles. Surprisingly, the frequency of allelic recombination between Ty elements is within the normal range, so that inhibition of ectopic recombination is not achieved by general inhibition of recombination within Ty sequences. Thus these experiments, in agreement with most published data, are most consistent with the interpretation that there is a mechanism in meiotic yeast cells that limits crossing over between Ty elements residing at different chromosomal loci. Whether this mechanism is an active one, that suppresses recombination directly, or a passive one, such as a lack of ectopic recombination-initiating *cis* sequences within Ty*1* elements, remains to be demonstrated.

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