Fitness Effects of Ty Transposition in Saccharomyces cerevisiae

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ABSTRACT

It has been suggested that the primary evolutionary role of transposable elements is negative and parasitic. Alternatively, the target specificity and gene regulatory capabilities of many transposable elements raise the possibility that transposable element-induced mutations are more likely to be adaptively favorable than other types of mutations. Populations of Saccharomyces cerevisiae containing large amounts of variation for TyI genomic insertions were constructed, and the effects of TyI copy number on two components of fitness, yield and growth rate were determined. Although mean stationary phase density decreased with increased TyI copy number, the variance and range increased. The distributions of stationary phase densities indicate that many TyI insertions have negative effects on fitness, but also that some may have positive effects. To test directly for adaptively favorable TyI insertions, populations containing large amounts of variability for TyI copy number were grown in continuous culture. After 98–112 generations the frequency of clones containing zero TyI elements had decreased to ~0.0, and specific TyI-containing clone families had predominated. Considering that most of the genetic variation in the populations was due to TyI transposition, and that TyI insertions had, on average, a negative effect on fitness, we conclude that TyI transposition events were directly responsible for the production of adaptive mutations in the clones that predominated in the populations.

The ability of transposable elements to generate mutations, many of which may be deleterious, has been invoked to assert that the primary evolutionary role of transposable elements is negative and parasitic (DOOLITTLE and SAPIENZA 1980; ORGEL and CRICK 1980). In Drosophila, movement of P elements generally decreases fitness (FITZPATRICK and SVED 1986; MACKAY 1986), and the distribution of transposable elements among chromosomes in natural populations is consistent with the hypothesis that they have deleterious or neutral effects (CHARLESWORTH and LANGLEY 1989; MACKAY 1989). A corollary of the assertion that transposable elements have a primarily negative effect is that mechanisms have evolved to limit the number of elements in the genome. Copy number control has been shown in prokaryotes (KLECKNER 1990), and has been postulated for both yeast (BOEKE 1989) and Drosophila (CHARLESWORTH and LANGLEY 1989).

The alternative hypothesis, that transposable elements may have a positive evolutionary role in creating adaptive genetic variation, is lent support by the ability of elements to activate and deactivate neighboring genes (McCLINTOCK 1956; CAMPBELL 1981; FINNEGAN 1989). The activation of cryptic genes and the modification of gene regulation are frequently found to result from transposable element integration (WILLIAMSON, YOUNG and CIRIACY 1981; STAVENHAGEN and ROBINS 1988; LOPILATO and WRIGHT 1990). Indeed, there is a growing body of evidence that transposable elements of many types target transcriptionally active areas of the genome (SANDMEYER, HANSEN and Chalker 1990) and show a preference for the 5' regions of genes (e.g., NATSOLIS et al. 1989; WILKE et al. 1989; VOELKER et al. 1990). It is therefore possible that the mutational spectra generated by transposable elements possess a higher frequency of adaptively favorable changes, as compared to those generated by base substitutions, or by small duplications and deletions (FINNEGAN 1989). In this regard, increases in fitness coupled with transposition have been reported in both Escherichia coli (CHAO et al. 1983; CHAO and McBROOM 1985; MODI et al. 1992) and Drosophila (PASYUKOVA et al. 1986, 1988), and there is evidence that P element transposition may lead to increased variation for quantitative traits (MACKAY 1985; FRANKHAM, TORKAMANZEHI and MORAN 1991). In yeast, TyI copy number was shown to increase continuously over ~1000 generations of growth in continuous culture (WILKE, MAIMER and ADAMS 1992), and adaptive shifts in populations evolving in a glucose-limited environment were frequently accompanied by TyI copy number changes (ADAMS and OELLER 1986). These results were most easily explained by assuming that the TyI element-associated sequence alterations possess a selective advantage.

Direct evidence for the selective effects of transpos-
able elements is difficult to obtain, since the rate of transposition is on the order of the mutation rate, and the number of transposition events occurring in evolving populations will by necessity be limited. Furthermore, the genomes of many species already contain large numbers of transposable elements, and any determination of the selective roles of such elements may be complicated by a dosage effect and element heterogeneity. In this communication we report the results of experiments designed to characterize the genetic variation for fitness created by Ty1 transposition in Saccharomyces cerevisiae. Ty1 elements comprise a dispersed retrotransposon gene family, and since their first characterization (Cameron, Loh and Davis 1979), many aspects of their distribution, structure, expression and mutagenic capabilities have been well defined (Boeke 1989). They share many features with the copia-like elements in Drosophila and the proviral forms of endogenous metazoan retroviruses. We take advantage of the availability of a yeast strain containing zero Ty1 elements, and an inducible Ty1-containing plasmid which allows the generation of large amounts of genetic variation for Ty1 copy number and location. In particular we show that (i) the mean stationary phase cell density (K) decreases significantly, but that the variance in K increases significantly with increasing Ty1 copy number. The distribution of stationary phase densities indicates that the average effect of increased mutagenesis by these elements is deleterious, but that individual Ty1 transposition events have varying effects on the organism, which may be neutral or even advantageous; and (ii) despite an average deleterious effect of Ty1 transposition, clones with high numbers of newly transposed Ty1 elements were frequently selected in populations containing large amounts of genetic variation for Ty1 copy number.

MATERIALS AND METHODS

Media, growth and sampling: The defined minimal salts medium was that described by Adams and Hansche (1974) containing 2% galactose (MMgal) or 2% (w/v) glucose (MMglu) for batch cultures. When necessary uracil was added to a concentration of 20 µg/ml, and agar at a concentration of 1.7% for solid medium. YEPD medium (1% yeast extract, 2% Bacto-peptone; Difco Inc. Detroit, Michigan), and 2% glucose was used to propagate strains when selection for plasmid retention was unnecessary.

Batch cultures were grown at 30°C in a gyratory shaker at 150–200 gyrations/min. Cell densities were estimated using an electronic particle counter (Coulter Counter; Coulter Inc., Hialeah, Florida) after sonication with a Bransonsonic 1510 sonicator with a needle probe to separate clumped cells. Samples from the liquid cultures were stored at −70°C in 40% glycerol. Individual colonies were saved by first scraping them from a plate with a toothpick, and resuspending them in 1 ml MMglu plus 40% glycerol before storage at −70°C.

Strains and plasmids: Strains and plasmids are described in Table 1. The yeast strain 337, a generous gift from P. Philpipse and M. Ciriacy, was derived from a wild isolate of S. cerevisiae (Boeke 1989) containing a single element with homology to Ty1. This single Ty element was deleted, and the ura3 mutation introduced (P. Philpipse and M. Ciriacy, personal communication). The electrophoretic karyotype of 337, as determined on CHEF gels, was also found to be typical of laboratory and wild strains of S. cerevisiae (data not shown). DNA hybridization showed that strain 337 has a large number of solo deltas and a smaller number of sigma and tau elements, typical of laboratory yeast strains (data not shown). Strain 337 possesses the long-term adaptation phenotype of a gal3 mutant (Oshima 1982), being able to grow on galactose medium only after an adaptation period of 4–7 days, and was unable to grow on maltose, a pleiotropic effect of the gal3 mutation (Kew and Douglas 1976). Complementation tests with gal2 and gal3 tester strains confirmed that this strain is mutant at the gal3 locus.

The plasmid pGTy1-H3 (Boeke et al. 1985) used to induce high levels of Ty1 transposition in strain 337 was a generous gift of J. Boeke. pGTy1-H3 contains the yeast GAL1 promoter fused to a transposition competent Ty1 element, the URA3 gene as a yeast selectable marker, a 2µ origin of replication to maintain a high copy number of the plasmid in yeast, and the pBR322 origin of replication and ampicillin resistance genes.

Plasmid-containing derivatives of strain 337 were obtained by transformation with purified plasmid DNA using the lithium acetate procedure (Ito et al. 1983) and selection for uracil prototrophy. Ty1 transposition induction was carried out at either 18°C or 30°C on MMglu plates.

Serial dilution cultures: Liquid YEPD cultures (10 ml) in 25- or 50-ml Erlenmeyer flasks were inoculated with a determined total cell number ranging from 10^6 to 10^8. The cultures were then grown on a gyratory shaker at 30°C for 2–5 days. Cell densities were determined, and dilutions of the cultures were made to inoculate fresh 10-ml cultures with 1–2 × 10^6 cells/ml. Terminal cell densities ranged from 1 to 2 × 10^9 cells/ml. Every ~16 generations, samples were stored and tested for the ura^- phenotype to verify that the population had not become contaminated. In addition, at the termination of the cultures at 98 to 112 generations, the populations were tested for Ura^- and Gal^- phenotypes, and mating type. The number of generations of growth occurring in each culture was calculated from

\[ g = \ln(N_i/N_0)/\ln 2, \]

where g is the number of generations, and N0 and Ni are the terminal and initial densities of the culture, respectively.

Construction of populations containing large amounts of variation for Ty1 insertion: A 5-ml culture inoculated with CMW100 was grown at 30°C for 48 hr in MMglu. Dilutions of the culture were plated to MMgal and MMglu. Half of the MMglu plates were incubated at 18°C and half at 30°C. The MMgal plates were incubated at 30°C. One hundred individual colonies from the galactose plates at each temperature and 50 colonies from the glucose plates were picked and stored at −70°C as described above.

Each population was comprised of subclones from 11 different colonies, which had been previously stored at −70°C; 5 colonies each from the MMgal plates at 18°C and 30°C, and 1 colony from the MMglu plates. Aliquots of the stored colonies were diluted and plated to YEPD to give approximately 100 subclones. These 100 subclones from each of the 11 different stored colonies were washed from their plates with liquid YEPD medium, combined and the total density determined. From this resuspension of the
Evolution and Ty Elements

TABLE 1
Strains and plasmids

<table>
<thead>
<tr>
<th>Designation</th>
<th>Relevant characteristics</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Yeast strains</td>
<td></td>
<td></td>
</tr>
<tr>
<td>357</td>
<td>$\alpha$, ura3, gal3. Contains 0 Ty elements</td>
<td>P. PHILIPSEN and M. CIRIACY</td>
</tr>
<tr>
<td>CMWI 100</td>
<td>357 transformed with pGTyl-H3 (see below). Contains 0 genomic Ty elements</td>
<td>This work</td>
</tr>
<tr>
<td>CMWI 101</td>
<td>As 357 but contains a single genomic copy of Ty1. Constructed by induction of transposition of CMWI 100 by growth on galactose</td>
<td>This work</td>
</tr>
<tr>
<td>B. Plasmids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pGTyl-H3</td>
<td>Possesses a transposition competent copy of Ty1 fused to the GAL1 promoter</td>
<td>J. BOEKE; BOEKE et al. (1985)</td>
</tr>
<tr>
<td>pCMWI 1000</td>
<td>Ty probe plasmid containing only the internal $\alpha$ sequences from the $3'$ end of a Ty1 element. Constructed by ligating the 1.6-kb EcoRI-BgII fragment of p6-6 into pUC9 digested with EcoRI and BamHI</td>
<td>This work; LIEBMAN, SHALIT and PICOLGLOU (1981)</td>
</tr>
<tr>
<td>pNN116</td>
<td>Contains a complete Ty1 element (Ty1-D15) cloned into the HindIII site of Yip5</td>
<td>SCHERER and DAVIS (1980)</td>
</tr>
</tbody>
</table>

combined subclones, $10^5$ to $10^6$ cells were used to inoculate two 10-ml liquid YEPD cultures/population. Cells from the combined subclone culture were also plated to YEPD so that the Ty1 element distribution at generation zero could be determined. The two replicate cultures were grown separately, and serially diluted (see above) for approximately 100 generations. After the completion of 100 generations of growth, the cultures were plated to YEPD, and the distribution of Ty1 copy number estimated. A schematic outline of this procedure is shown in Figure 2.

DNA manipulations: The following procedures were used.

Plasmid and probe preparation: All plasmid and probe DNA was prepared from cultures of E. coli strain JM109 (YANISCH-PERRON, VIEIRA and MESSING 1985). E. coli transformations were performed by the CaCl$_2$ technique as described by MANDEL and HICA (1970). Large and small scale plasmid DNA preparations were adapted from the alkaline lysis procedure (BIRNBOIM and DOLY 1979). Large scale plasmid DNA preparations were further purified using p2523 columns (5Prime-3Prime Inc., West Chester, Pennsylvania) according to the company protocol. All media for the manipulation of E. coli strains were based on commonly used recipes (MANIATIS, FRITSCH and SAMBROOK 1982).

Hybridization procedures: Yeast DNA was isolated from 5- or 40-ml YEPD cultures after 1-2 days of growth (WINSTON, CHUMLEY and FINK 1983). DNA (1–5 $\mu$g) was digested with 10–20 units of the appropriate restriction enzymes (EcoRI, PstI or KmI; Boehringer Mannheim) according to the manufacturer’s specifications. DNA fragments were separated on 0.7% agarose gels in 1 × TBE (MANIATIS, FRITSCH and SAMBROOK 1982) containing 0.5 $\mu$g/ml ethidium bromide. The alkaline transfer (CHOMCZYSKI and QASBA 1984) of DNA to a nylon membrane (GeneScreen Plus; NEN, Boston, Massachusetts) was accomplished by capillary action using the “dry” blot assembly described by SMITH and SUMMERS (1980). Prehybridization and hybridization of the membranes were performed in a nonfat dry milk solution (JOHNSON et al. 1984). Posthybridization washes were carried out according to the protocols recommended by NEN. When necessary, the hybridization membranes were stripped by washing in 0.4 N NaOH for 30 min at 65$,^\circ$, followed by incubation at 65$,^\circ$ for 30 min in 0.1 $\times$ SSC (1 $\times$ SSC is 0.015 M sodium citrate plus 0.15 M sodium chloride) plus 0.1% SDS (sodium dodecyl sulfate) and then neutralized with 5 $\times$ SSC for 5 min at room temperature. Autoradiographs were exposed at $-70^\circ$ in the presence of intensifying screens. All probes were labelled with $^{32}$P by nick translation (RIGBY et al. 1977) using a kit from Boehringer Mannheim.

Ty element probes: Two different probes were used to detect genomic Ty elements. (i) The plasmid pCMWI1000 (see Table 1 and probe 1 at the bottom of Figure 1) containing only internal epsilon sequences from the $3'$ end of a Ty1 element, was constructed by ligating the 1.6-kb EcoRI-BgII fragment of p6-6 into pUC9 digested with EcoRI and BamHI. (ii) A probe for the $5'$ epsilon region of Ty1 (probe 2 at the bottom of Figure 1) was obtained by gel purification of the 3.5-kb PvuII fragment from pNN116. This fragment was eluted from agarose gels using Schleicher and Schuell (Keene, New Hampshire) NA45 DEAE cellulose paper. pNN116 contains a complete Ty1 element (Ty1-D15), cloned into the HindIII site of Yip5 (SCHERER and DAVIS 1980).

Estimation of the number of Ty1 integrations: Restriction enzyme digests, and Ty probes were chosen to maximize the resolution of the Ty1-containing fragments in the DNA hybridization spectra. The number of bands were estimated by eye, and a conservative approach was adopted when bands displayed an increased intensity of hybridization, representing several Ty-hybridizing fragments of the same size.

Assignment of clones to a family: In nearly all cases the overall hybridization patterns of individual clones either showed strong similarity (indicating members of one "family") or clear differences. Members of a family of clones having 1 to 2 Ty1 elements shared 1 or 2 Ty1 bands of the same size in common. Members of a family of subclones with 2 to 4 elements contained at least 2 bands of the same size. Clones with 5 to 7 elements were assigned to the same family, if they shared 4 or more Ty1 bands of the same size. Clones with larger numbers of Ty1 bands, classified in the same "family," shared a correspondingly large number of bands of the same size. To confirm that similar sized bands represented Ty1 integrations into the same genomic locus, the hybridization membranes were stripped and reprobed with the second Ty1 probe (see above) specific to the $5'$ epsilon region. This procedure resulted in a single case where a clone with one Ty1 element was assigned to the same family as a clone with 4 elements. All other cases met the criteria described above. This procedure is conservative;
that is the number of families present in a given population will be overestimated, since clones containing for example, 2 and 6 elements would not be assigned to the same family, when they may have in fact, been members of the same lineage.

RESULTS

Production of genetic variation for TyI transposition events: To generate high levels of genetic variation for Ty copy number and position we used the plasmid pGTY1-H3. The presence of this plasmid, which contains the yeast GAL1 promoter fused to a transposition competent TyI element, allows the induction of high levels of TyI transcription, and therefore transposition (Boeke et al. 1985) when the host strain is grown on galactose medium. Transposition of the plasmid-borne TyI element is repressed in the presence of glucose (Boeke et al. 1985). To characterize the induction of transposition, strain CMW100 (strain 337 containing zero Ty elements transformed with pGTY1-H3; see Table 1) was grown to early stationary phase in minimal glucose medium (MMglu), plated onto minimal galactose plates and incubated at 18° and at 30°. Colony viability was significantly lower than that on control MMglu plates, and those colonies which arose had scalloped margins and high levels of lethal sectoring, indicative of colony heterogeneity. Both the reduced viability, and the lethal sectoring are consistent with TyI-mediated disruption of essential genes for growth and segregational plasmid loss, and have been previously reported for other S. cerevisiae strains containing this plasmid and grown under similar inducing conditions (Boeke et al. 1985, 1988). Individual colonies from the galactose plates were then streaked on YEPD plates to obtain subclones for the determination of TyI copy number by DNA hybridization. Figure 1 shows the hybridization spectra for a sample of 10 subclones derived from one galactose induced colony grown at 18°, and for one grown at 30°. Each integrated TyI transposition into the genome gives rise to a single band (for more details see MATERIALS AND METHODS and the legend to Figure 1). It can be seen that galactose induction of CMW100 results in high levels of TyI transposition at both temperatures, but that transposition levels appear higher at 30°. The data (Table 2) for a total of 35 subclones derived from 10 colonies grown at 18° and at 30° show that the mean number of new TyI transpositions/genome is significantly higher at 30° than at 18°. Analysis of the number of genomic TyI elements in subclones from colonies of CMW100 grown on minimal glucose medium confirmed that TyI transposition was repressed on glucose.

It is also evident from Figure 1 that the subclones derived from the same galactose induced colony are related to each other. The subclones share some Ty transposition events, but many can be distinguished on the basis of unique Ty insertions. Thus, the individual subclones can be considered to be members of a genealogy generated by sequential Ty transpositions within the colony growing on galactose. A TyI transposition event resulting in disruption of an essential gene will result in a truncation of that branch of the genealogy.

The temperature/induction characteristics of CMW100 therefore make it possible to produce pools of YEPD subclones containing different numbers of newly transposed TyI elements. In a single round of growth on galactose medium at 18° and 30°, populations of yeast strains with genetic variation for TyI copy number can be produced. In the next two sections we describe experiments designed to assess the effect of TyI transposition on two components of fitness, stationary phase density and growth under serial dilution conditions.

Effect of TyI transposition on stationary phase cell density: One component of fitness which may be
important under limiting-resource conditions is the efficiency by which an organism converts that limiting resource into biomass, or cell number. Selection determined by this component of fitness has been termed K-selection (MacArthur 1962). We therefore determined the relationship between genetic variation for TyI transposition and stationary phase cell density. Individual colonies of CMW100 that had been grown under three different induction conditions (minimal glucose at 30°C—no induction, minimal galactose at 18°C, and minimal galactose at 30°C) were diluted and spread on separate YEPD plates. A single subclone from each YEPD plate was grown to stationary phase density in YEPD liquid medium at 30°C. Dilutions of these cultures were then used to inoculate a 5-ml liquid minimal glucose + uracil culture with \( \approx 3 \times 10^5 \) cells/ml. The cultures were grown at 30°C for 10 days, at which time their stationary phase cell densities were determined. The results, shown in Table 2, clearly indicate two trends. As the number of newly transposed Ty elements increases, the mean stationary phase density decreases, and the variance and range in stationary phase density increase. When TyI transposition was induced at 30°C, resulting in an average number of TyI elements of 11.20, the range of densities exceeds that for the clones with no transposition, at both ends of the distribution. That is, the maximum stationary phase density, as well as the minimum is seen in clones with a large number of TyI transpositions.

These observations indicate that TyI transpositions are, on average, deleterious. Given a large number of transposition events, the probability that at least one of these events are deleterious will therefore be high. A corollary to this interpretation is that clones containing multiple transposition events, all of which are neutral or advantageous in effect, will be rare. Clones with high numbers of TyI transposition events and with high stationary phase densities, may fall into this category. However, due to sample size limitations, and considerations of statistical significance, selectively advantageous clones with high TyI copy number are not easy to detect in this type of experimental design. We therefore grew populations containing large amounts of variation for TyI copy number in competition in serial dilution, to screen for clones with TyI transpositions conferring a selective advantage.

**Selection in populations containing genetic variation for TyI element integration**: Populations were constructed to maximize the level of variability for TyI insertions by mixing together clones that had been grown under the three different conditions of induction, growth on galactose at 18°C, growth on galactose at 30°C, and growth on glucose. Details of the construction of these populations are given in the MATERIALS AND METHODS and in Figure 2. The number of cell generations that elapsed before the inoculation of the populations was minimized to reduce as much as possible the number of mutations that occurred which were not due to TyI transposition. After induction of TyI transposition at 18°C and at 30°C, 100 cells from each of five colonies grown at the two temperatures were plated and allowed to form colonies. These colonies were then pooled, together with 100 colonies grown on glucose, in which no transposition had occurred. The populations were then inoculated with \( 1 \times 10^5 \) to \( 1 \times 10^6 \) cells. Ten independent populations, representing 10 different sets of inductions were constructed in this manner. Figure 3 shows the distribution of the number of TyI elements in these 10 populations at generation 0, as determined by DNA hybridization spectra of individual clones derived from samples of these 10 populations. The average number of TyI integration events per cell was 7.31 ± 5.29. There was no discernable difference between the populations, in the either average number of TyI elements or in the distributions of TyI.

**TABLE 2**

<table>
<thead>
<tr>
<th>Relationship between stationary cell density and TyI copy number</th>
</tr>
</thead>
<tbody>
<tr>
<td>No induction</td>
</tr>
<tr>
<td>Mean # TyI elements ± SD</td>
</tr>
<tr>
<td>N</td>
</tr>
<tr>
<td>Mean stationary cell density'</td>
</tr>
<tr>
<td>Range'</td>
</tr>
<tr>
<td>Standard deviation of stationary cell density</td>
</tr>
<tr>
<td>N</td>
</tr>
</tbody>
</table>

* Standard deviation.
  ' Sample size.
  \( \times \) Cells/ml.
  \( > \) Significantly smaller than the values for "No induction" and "Induction at 18°C" at \( \alpha < 0.01 \) as tested by the Mann-Whitney test (Conover 1971).
  \( > \) Significantly larger than the values for "No induction" and "Induction at 18°C" at \( \alpha < 0.01 \) as tested by the Siegel-Tukey test after correction for location differences (Conover 1971).
clones were assayed for the number of Tyl integration in haploid populations that clones possessing a 10% selective advantage, at the termination of the experiments, virtually every clone sampled belonged to a unique family. Thus, at generation zero, for a sample size of 17-18, the average number of families was 12.8 ± 1.6. Assignment of clones to families was aided by the use of two different Tyl probes. Probe 1 (pCMW1000) possesses homology to the ~2-kb region 3' to the EcoRI sites within Tyl elements (see Figure 1). Thus, when genomic DNA is digested with EcoRI, the size of a hybridizing band generated from a Tyl integration will include the 2 kb from the element, in addition to the genomic DNA bounded by the end of the element and the next EcoRI site 3' to the integrated Tyl element. Similarly, when the second Tyl probe (see MATERIALS AND METHODS and Figure 1), possessing homology to the region on the 5' side of the internal EcoRI sites is used, the size of the hybridizing band will include the 3.7 kb from the internal EcoRI sites to the end of the element, plus genomic DNA between the end of the element
**TABLE 3**

**Effect of selection on the number and frequencies of Ty1 families**

<table>
<thead>
<tr>
<th>Population</th>
<th>Generation 0*</th>
<th>Terminal samples*</th>
<th>Common families in terminal samples*</th>
<th>Frequency of terminal families at generation 0*</th>
<th>Concordance between replicates*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>≥13</td>
<td>8</td>
<td>3</td>
<td>2</td>
<td>0.06</td>
</tr>
<tr>
<td>B</td>
<td>≥11</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>0.06</td>
</tr>
<tr>
<td>C</td>
<td>≥14</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>0.00</td>
</tr>
<tr>
<td>D</td>
<td>≥16</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>E</td>
<td>≥13</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>0.00</td>
</tr>
<tr>
<td>F</td>
<td>≥13</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.00</td>
</tr>
<tr>
<td>G</td>
<td>≥13</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0.00</td>
</tr>
<tr>
<td>H</td>
<td>≥13</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.00</td>
</tr>
<tr>
<td>J</td>
<td>≥12</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>K</td>
<td>≥10</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.00</td>
</tr>
</tbody>
</table>

*Numbers of clone families containing >0 Ty1 elements. Sample sizes for each population were 17 or 18 clones. Therefore almost all clones at generation 0 were unique, and the estimated number at the beginning of the experiments must be considered minimum estimates.

"Numbers of clone families containing >0 Ty1 elements for both replicates.

"Number of clone families, containing >0 Ty1 elements in the terminal samples, that are common to both replicates.

"Total frequency of the clone families identified in the terminal samples, at generation 0.

"The concordance between clone families, C, is defined as

\[ C = \frac{\sum_{i=1}^{m} (n_{i1} + n_{i2})}{N}, \]

where \( n_{i1} \) and \( n_{i2} \) are the numbers of clones assayed in the first and second replicates respectively, belonging to the \( i \)th family (\( i = 1 \ldots m \)), and \( N \) is the total number of clones assayed in both replicates.

and the next 5' EcoRI site in the genome. Therefore, by using two Ty probes each integration event can, in principle, be characterized by two hybridizing bands, the length of which are specific for each site of integration.

Third, in most cases the same families of clones are selected in the two replicates of each population. Table 3 shows that the concordance between the two replicates of 7 of the 10 populations range from a low of 0.33 (populations B and C) to a high of 1.0 for population H. The distribution of the number of Ty1 elements/genome appears to be substantially different in the two replicates of population D. However, the same Ty1 integrations seen in the genome in replicate 2 also occur in clones of replicate 1, as determined by the sizes of the bands hybridizing to Ty probes specific to the 5' region and the 3' region. Thus, the dominant clones in these two replicates may belong to the same family even though, from the criteria described in the MATERIALS AND METHODS, they are classified as being in different families.

Fourth, predominant clone families in any one population were not detected in other populations. To determine if genomic Ty1 integrations had occurred in the same genomic locus in different populations, we examined the hybridization spectra obtained using the two different Ty probes (see Figure 1), for the occurrence of similar sized bands. Those populations in which clones with few Ty1 elements had predominated, did not exhibit matching bands for both probes. Although potential matching pairs of bands were observed when comparing populations in which clones with many Ty1 elements had predominated, the complexity of the hybridization spectra precluded any unequivocal assignment of identity.

Two of the 10 populations examined, J and K, exhibited a markedly different pattern of results when compared to the other eight (Table 3). In these populations, no variation for Ty1 integration was seen in the terminal samples, there was no concordance between replicates and in one of the replicates of each population, the frequency of clones containing 0 Ty1 elements was ~1.0. We conclude that adaptive mutations, independent of Ty1 transposition events, had occurred in these populations after the initiation of the replicates, and that these populations therefore provide no information on the adaptive significance of Ty transposition.

**DISCUSSION**

Elements belonging to the Ty family are widely dispersed throughout the genome of wild as well as laboratory strains of yeast (e.g., Wilke, Maimer and Adams 1992). Typically they are present in 30–35 copies in laboratory strains and comprise about 1–2% of the haploid genome, though copy number in wild strains is lower (Eibel et al. 1981). Laboratory strains appear to suffer no detrimental effects associated with Ty elements, and they are sufficiently stable such that they can be mapped by conventional genetic approaches (cf. Klein and Petes 1984). Their widespread distribution, as well as their apparent lack of
Figure 4.—Distribution of Ty1 elements in competition experiments A–H at their termination. ■, Replicate 1; □, replicate 2. Virtually no variation was present in populations J and K at their termination, and thus these data are not shown.
any associated phenotype, therefore naturally raises questions concerning their evolutionary role and effects on fitness.

Evidence that Ty1 element insertions are deleterious: The results described here document the effects of Ty transposition on components of fitness. Ty elements, in common with other mutagenic agents may be expected to generate mutations which are selectively advantageous, neutral or deleterious. The relationship between stationary phase cell density and transposition suggests that, on average, Ty1 transpositions are deleterious, and this may not be unexpected, given the ability of Ty insertions to disrupt gene function. Similar results were obtained by Boeke, Eichinger and Natsoulis (1991), who showed that yeast strains containing twice the Ty1 copy number normally found in laboratory strains exhibited both reduced growth rates and stationary phase densities.

Evidence that Ty1 element insertions are adaptively favored: Of more significance from an evolutionary perspective is evidence pointing to a neutral or positive effect of transposition on fitness. Although the mean stationary phase density decreased with increasing Ty1 copy number, there was much overlap in the distributions between the populations carrying different average numbers of Ty1 elements. Many of the individual clones from the population carrying the greatest number of Ty1 elements fell within the range of the uninduced population, indicating that, for this trait, there are many integration events which do not generate a large phenotypic effect. These data for Ty1 elements are consistent with those of Goebel and Petes (1986), who found that 70% of the random disruptions they produced in S. cerevisiae had no detectable phenotype (cf. Boeke, Eichinger and Natsoulis 1991). Similarly, Diehl and Pringle (1991), in a detailed analysis of a 34-kb region of chromosome I, reported that deletion of a number of transcribed regions of unknown function produced no obvious phenotypes.

Although the increased upper range of stationary phase density, observed with high levels of Ty1 transposition suggests an adaptive role for some Ty1 integration events, evidence that they may be selectively favored can be most clearly seen in the competition experiments. In all but four of the twenty replicates the predominant clones in the terminal samples from the populations contained between 1 and 14 Ty1 integrations/genome, and in 60% of the cases, clones containing 0 Ty1 elements had been eliminated. The differences among populations in the number of Ty1 elements/genome in the terminal samples of each population is expected, as the initial inocula for each population were derived from independent sets of transposition inductions. Thus, the spectrum of Ty1-induced mutations should be different for each population.

The overall pattern of these results strongly point to a direct selective advantage for some Ty1 transpositions. Several considerations lead us to this conclusion and allow us to effectively rule out the fortuitous occurrence of spontaneous mutations not due to Ty1 elements occurring in clones carrying Ty1 insertions ("hitchhiking"; Maynard Smith and Haigh 1974; Kaplan, Hudson and Langley 1989) as a significant factor in generating the pattern of results observed.

First, the majority of genetic variation in the initial inocula was due to Ty integration events. The number of strain manipulations prior to inoculation was kept low; only 10 colonies grown on galactose to induce Ty1 transposition were replated and the resulting colonies (1000 in total) were washed from the plates and used as part of the initial inoculum. Therefore the number of cell generations prior to inoculation of the cultures was extremely small, minimizing the possibility for the occurrence of spontaneous mutations.

In other studies where Ty transposition was induced from the same or closely related pGTy1-H3 constructs, the frequencies with which Ty transposition was responsible for reversion of the promoterless his3A4 allele (Boeke, Styles and Fink 1986), the production of lys2 mutations resulting in lysine auxotrophy, and ura3 mutations (Natsoulis et al. 1989) were 0.93, 0.62 and 0.99, respectively. Therefore the average percentage of mutations due to induced Ty transposition at these loci was close to 85%. For the experiments described here, there is evidence suggesting that the percentage of Ty-induced mutations is higher than 85%. The average number of Ty transpositions/genome in the laboratory strain used to select the ura3 mutations was 5 (Natsoulis et al. 1989), whereas the average number of Ty1 transpositions/genome in the competition populations was 7.31, and in that portion of the population induced at 30° the number was 11.2 (see Table 2).

Furthermore, there are several indications that the types of mutations caused by Ty integration are more likely to result in increases in fitness than other types of mutations. Direct and indirect evidence in both prokaryotes (Kurlandzka, Rosenzweig and Adams 1991) and eukaryotes (FinneGAN 1989; McDonald 1990, and references therein) has pointed to regulatory mutations as the primary source of the genetic variation which allows organisms to adapt to new environments. Several workers have now shown that Ty elements insert preferentially into the 5' and regulatory regions of genes (Eible and Philippsen 1984; Simchen et al. 1984; Natsoulis et al. 1989; Wilke et al. 1989). For example, Ty elements integrate into the 5' regulatory region of the lys2 gene approximately 9 times more frequently than into the central
region of the gene (Natsoulis et al. 1989). In addition, several studies have indicated that, when normal laboratory strains are challenged under conditions that demand regulatory changes in the expression of specific loci, Ty transposition is frequently responsible for mediating these regulatory changes (Paquin and Williamson 1986; Ida 1988; Toh-E et al. 1983; Errede et al. 1980). It is logical to expect that these same types of Ty-mediated changes would contribute to selective differences between the strains produced for the competition experiment.

Second, the families of clones present in the replicates of each population at their termination possess variation for TyI number and position. These families have certain TyI transpositions in common but differ for others. To explain the TyI variation within the predominating families by TyI-independent mutations, it is necessary to postulate that the selectively favored TyI-independent mutations occurred during the growth of a single colony on the galactose plates, when TyI transposition was induced. Since these colonies typically contain only $1 \times 10^6$ cells, the probability that this would occur in a majority of the populations is vanishingly small. In this regard it should be noted that the plasmid pG-TyI-H3 has no general mutagenic effect, other than that due to TyI transposition, under either induced or uninduced conditions (Boeke et al. 1985). Also, natural rates of TyI transposition and movement by recombination are too low to account for the observed variation.

Third, the same clone families are selected and predominate in both of the replicate pairs of the independent competition populations. In 7 of the 10 populations, the concordance between the two replicates ranges from 0.33 to 1.00 (see Table 3). For one population (population D), the clones selected from the two replicates show similar patterns of TyI integration, but have been classified as belonging to different families by the criteria described in MATERIALS AND METHODS. If the selective advantage of the predominant clones were due to mutations independent of the TyI transpositions, such mutations would be required to occur prior to the initiation of the replicate cultures. Given the small number of cell divisions involved in the construction of the initial inocula and the total cell number (see above), it is extremely unlikely that this would have occurred for the majority of the populations.

Fourth, all populations were initiated with a frequency of cells containing 0 Ty elements of approximately 0.10. In all but 4 of the 20 replicates from the ten populations, clones containing 0 Ty elements are either absent from the terminal samples or are present at a low frequency. However, in the absence of any positive effect of TyI-transpositions, the frequency of clones containing 0 Ty elements would increase in the population, as clones containing deleterious TyI insertions are selected against. It should be noted that cells containing no TyI elements underwent the same number of cell generations as those cells in the population in which TyI transposition had occurred. Consequently, the distribution of cells containing TyI-independent mutations was the same for all cells. We therefore conclude that TyI transpositions are directly responsible for the selective advantage of the predominant clone families.

**Distribution of selective effects of TyI insertions:**

The results of the stationary phase experiment and the competition populations both indicate that some small fraction of TyI integrations result in positive selective effects. In the case of the stationary phase experiment, one TyI containing clone out of 60 tested (Table 2) had an increased stationary phase density compared to the range of the control, and in the competition populations, individual clone families with various numbers of TyI elements were selected from the populations. In a related study, Mackay, Lyman and Jackson (1992) observed that one newly produced P element line of 94 (with an average of 3.1 elements/line) showed high viability compared to the control. The selective advantage of a clone containing a number of new transposable element integrations, occurring during a rapid burst of transposition, will naturally depend on the combination of the effects of all of the individual transpositions. Thus, selection of clones containing a number of TyI transpositions may be due to a smaller number, of selectively favored transposition events (perhaps as small as 1). The remaining transpositions may have no selective effect (neutral) or may even be deleterious as long as they do not overwhelm any selective advantage mediated by the favorable transpositions.

The characteristics of TyI transposition have now been analyzed by a number of workers [see Boeke (1989) and references therein], and these studies have led to a greater understanding of the molecular mechanisms whereby the transposition of Ty and similar elements give rise to genetic variation for fitness. The transposition of these elements into an individual gene or its vicinity can lead to mutations with a range of phenotypes not easily obtained by other mutagenic events (Garfinkel and Strathern 1991). A detailed understanding of the mechanisms alone, however, still leaves us with the knowledge of only the potential for such events to be involved in evolution. Through the experimental manipulation of populations we have obtained more evidence for a positive role of TyI elements in evolution. The work presented in this communication complements and extends those conducted at the molecular level, and together they lead to a more complete understanding of the evolutionary process.
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