High frequency excision of Ty elements during transformation of yeast

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ABSTRACT
Yeast (Saccharomyces cerevisiae) transposons (Ty elements) are excised from up to 20% of supercoiled plasmids during transformation of yeast cells. The excision occurs by homologous recombination across the direct terminal repeats (deltas) of the Ty element, leaving behind a single delta in the transforming plasmid. Only the initial transforming plasmid is susceptible to excision, and no high frequency excision is observed in plasmids that have become established in transformed cells or in plasmids that are resident in cells undergoing transformation. High frequency excision from plasmids during yeast transformation is not specific for Ty elements and can be observed with other segments of plasmid DNA bounded by direct repeats. The frequency of Ty excision from supercoiled plasmids is greatly reduced when the host yeast cells contain the rad52 mutation, a defect in double-strand DNA repair. When linear or ligated-linear plasmid DNAs containing a Ty element are used for transformation, few or no excision plasmids are found among the transformant colonies. These results suggest that when a yeast cell is transformed with a supercoiled plasmid, the plasmid DNA is highly susceptible to homologous recombination for a short period of time.

INTRODUCTION
Yeast (S. cerevisiae) transposons (Ty elements) consist of a 5.6 kbp central fragment (epsilon) bracketed by 0.3 kbp direct terminal repeats (deltas) and occur in 30-50 copies per genome (1,2). Transposition of Ty elements occurs through an RNA intermediate and reverse transcriptase (3), leaving behind an intact parent transposon in the DNA. The primary Ty element transcript spans epsilon and portions of each delta and accounts for about 5% of total cellular poly(A)RNA in haploid cells but is greatly reduced in a/a diploid cells (4). Insertion of a Ty element results in a 5 bp duplication of the target DNA, however the insertion site appears to be independent of the DNA sequence (5). Many Ty elements have been found in the promoter regions of genes (6) where they may cause increased or decreased expression of the adjacent gene (7,8). Where insertion of a Ty element results in over-expression of the adjacent gene, the effect on gene expression often is
greatly reduced in a/a diploids along with the level of Ty transcription (9,4). Ty insertions that increase gene expression are always oriented such that the transposon and the affected gene are transcribed in opposite directions (7,10).

Ty elements can also undergo excision by homologous recombination across the deltas to eliminate epsilon and parts of the deltas, leaving behind a single delta in the host DNA (11,5). Yeast genomes typically contain about 100 of these deltas (intact and degenerating) probably marking the sites of previous transposon excision. In growing yeast cells, genomic Ty elements are relatively stable with an excision frequency of $10^{-5}$ to $10^{-6}$ per cell (8,7). We have found that under conditions of yeast cell transformation, plasmid-borne Ty elements undergo an initial excision at high frequency. Those Ty that survive the initial high rate of excision then assume a stability similar to that of genomic Ty elements. The initial high frequency excision is dependent on the topology of the plasmid DNA.

MATERIALS AND METHODS

Strains and Media

*S. cerevisiae* strains XS852-23C[cir*](a trpl leu2-3 leu2-112 cdc10) and AH22[cir*](a leu2-3 leu2-112 his4-519 can1) have been described (12). *S. cerevisiae* strain D78-3D(a his3-11 his3-15 leu2-3 leu2-112 can1 rad52-1) was obtained from J. Szostak (13) and was cured of its endogenous 2 micron plasmid as described (12). All the yeast strains used in these experiments are designated [cir*] to indicate that they lack the endogenous 2 micron plasmid DNA. The use of [cir*] strains is essential with recombinant plasmids containing the entire 2 micron DNA to prevent recombination catalyzed by the gene products of the endogenous plasmid.

Yeast YPD media contains per liter: 10g yeast extract, 20g peptone and 20g glucose with 30g of agar added for plates. Yeast minimal media contains per liter: 6.7g yeast nitrogen base without amino acids, 20g glucose, 100mg of each required base or amino acid and 30g of agar for plates. Antibiotic G418 sulfate (Geneticin) was from Grand Island Biological at 493 μg G418/mg of solid material. The amounts referred to in this paper are the actual concentrations of the antibiotic, not the amount of solid material. G418 was added to YPD after the media was autoclaved by dissolving the antibiotic in several mls of water and adding it to warm media just before pouring plates.

Transformation of Yeast and Selection of Transformants

Yeast cells were transformed with plasmid DNAs using the lithium acetate
procedure (14) with several modifications. The cells were grown in minimal media to early log phase (Absorbance at 660 nm of 1) and treated with cations at a cell concentration of $5 \times 10^8$ cells/ml. All manipulations were done at 25°C in 1.5 ml Eppendorf tubes and no carrier DNA was used in the transformations. Typical yields for supercoiled plasmid DNAs were 50-100 transformants per µg of DNA. Transformed yeast cells were first selected on minimal media by plasmid complementation of the host cell leu2 mutation. After four days of growth at 25°C, the Leu⁺ transformants were picked or replica-plated onto YPD plates containing 500 µg of G418 per ml. This two step selection was used because G418 selection does not work on minimal media and the transformation frequency is low when the initial selection is done on YPD plates containing G418 (15). Therefore all plasmids used include both the LEU2 gene and a gene conferring resistance to G418 as selectable markers in yeast. Yeast-to-bacteria plasmid shuttles, screening of bacterial transformants, preparation of total DNA from yeast and copy number Southern have been described (12,16).

**Functional Elements Used in Plasmid Construction**

Plasmid constructions used the 2.2 kbp SalI-XhoI fragment containing the yeast LEU2 gene (17,18) inserted at the SalI site of pBR322. The yeast centromere CEN3 is on a 2 kbp HindIII-BamHI fragment (19) used to replace the HindIII-BamHI fragment of pBR322. The phosphotransferase gene from Tn5 is on a 1.2 kbp BglII-SalI fragment obtained from the plasmid pKC7 (20). The promoter fragment is a 0.6 kbp BglII-BamHI fragment of DNA from Chlamydomonas reinhardtii selected for its ability to give weak resistance to G418 in yeast. The 2 micron plasmid Form B was used and was opened at the EcoRI site located within the FLP gene. This configuration is necessary to prevent intra- and intermolecular recombination of the plasmids across the inverted repeats of the 2 micron DNA catalyzed by the product of the FLP gene (21). All the plasmids constructed are 'shuttle' plasmids since they are capable of replication and selection in both *E. coli* and *S. cerevisiae*.

**RESULTS**

**Plasmids Designed to Indicate Their Copy Number**

We constructed a yeast plasmid system that allows us to determine relative copy number of the plasmid by observing growth of the host cell on antibiotic medium. Plasmid GT60 (Fig. 1) contains a yeast centromere (CEN3), a selectable marker (LEU2), and the entire yeast 2 micron plasmid (Form B) opened at the EcoRI site located within the FLP gene. It also contains the
FIGURE 1. Plasmids designed to indicate their copy number. The thin lines are pBR322 DNA, the thick lines are yeast DNA. The open segment is the phosphotransferase gene and the hatched segment is the C. reinhardtii DNA that functions as the promoter for the phosphotransferase gene. The dashed lines indicate the insertion of Tyl and the wavy lines indicate the direction of transcription for the transposon and the phosphotransferase gene. X/S indicates a junction formed by XhoI-SalI ends and Bg/B is a BglII/BamHI junction. The diagram shows only those restriction sites used in construction or required to demonstrate orientation of fragments. Plasmid GT60 containing the Tyl element is designated pGT60A. Plasmid GT60 is a low copy number plasmid, pGT61 is a high copy number plasmid.

The phosphotransferase gene from the bacterial transposon Tn5 attached to a fragment of C. reinhardtii DNA that functions as a weak promoter in yeast. The promoter was inserted at the BglII site located 30 bp 5' of the phosphotransferase coding region (22) to replace the bacterial promoter which is nonfunctional in yeast. The centromere restrains the copy number of pGT60 to about one per cell whereas a similar plasmid (GT61) that lacks the CEN3 sequence (Fig. 1) is driven to high copy number by the 2 micron DNA (12) thereby increasing the copy number of the phosphotransferase gene.

TABLE 1. G418 resistance of yeast cells alone or containing various plasmids. Tests for G418 resistance were done in yeast strains XSB52-23C and AH22 by streaking cells with or without plasmids onto YPD agar containing the indicated amounts of G418. The (+) indicates growth, a blank indicates no growth. Plasmids with names ending with an 'A' contain the Tyl insertion in the promoter region of the phosphotransferase gene.

<table>
<thead>
<tr>
<th>PLASMID</th>
<th>COPY NUMBER</th>
<th>100</th>
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<th>200</th>
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<td></td>
</tr>
<tr>
<td>pGT60</td>
<td>low</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pGT61</td>
<td>high</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
</tr>
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<td>+</td>
<td>+</td>
<td>+</td>
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FIGURE 2. Copy number determination of various plasmids in yeast. The autoradiograph shows a Southern blot of total yeast DNA isolated from transformants, digested with SalI and hybridized with a nick-translated LEU2 fragment. The plasmid (pl) and chromosomal (ch) LEU2 bands are indicated. Yeast cells (XSB52-23C) contained the following: (a) no plasmid, (b) pGT60, (c) pGT60A, (d) the excision form of pGT60A, (e) pGT61. The Ty1 in pGT60A contains a SalI site, hence this plasmid contains two SalI sites and SalI digestion yields a relatively small plasmid LEU2 band (lane c).

Yeast cells are sensitive to the antibiotic G418, however the product of the phosphotransferase gene inactivates G418 by phosphorylation, thereby rendering yeast cells resistant to this antibiotic (23). Yeast cells transformed by the low copy number plasmid GT60 are sensitive to levels of G418 greater then 150 μg/ml, but are slightly more resistant than cells without plasmid (Table 1). However when the CEN3 sequence is absent as in pGT61 or inactivated, the plasmid is driven to high copy number by the 2 micron DNA and host yeast cells become resistant to more than 500 μg G418/ml (Table 1). Hence the relative copy number of the transforming plasmid can be rapidly determined by testing the transformed cells for growth on YPD media containing G418.

A Plasmid Mutation Increases Expression of the Phosphotransferase Gene

Among yeast cells transformed with pGT60, one transformant was obtained that was resistant to a G418 concentration of 500 μg/ml. Plasmids from this transformant were shuttled to E. coli and colonies resistant to ampicillin were screened. Restriction enzyme mapping of the plasmid showed that an insertion of about 6 kbp of DNA had occurred in the region between the cloned...
centromere and the phosphotransferase gene (Fig. 1). Further restriction analysis showed the insert was identical in its restriction map to Ty1 (24,1) a yeast transposon. Plasmid GT60 containing the Ty1 insert was designated pGT60A.

We suspected that the Ty1 element might have inactivated the cloned centromere located less than 1 kbp from the insertion site (Fig. 1), thereby allowing the plasmid to go to high copy number and making the host cell resistant to G418. To test this hypothesis, total yeast DNA was prepared from the transformant and digested with SalI, an enzyme that has a single site within pGT60 but no site within the LEU2 gene. The digests were separated on a 0.8% agarose gel for Southern analysis and probed with a nick-translated 2.2 kbp SalI-XhoI fragment containing the LEU2 gene (Fig. 2). Comparison of the intensity of plasmid and chromosomal LEU2 bands indicates that pGT60A has the low copy number typical of pGT60, and hence the G418 resistance of yeast cells transformed by pGT60A is not due to increased copy number of the plasmid.

An alternative explanation for the effect of the Ty1 insertion in pGT60 was that it made the host cell resistant to G418 by increasing the expression of the phosphotransferase gene. Overexpression of an adjacent gene is a common effect of Ty insertion in yeast (2), although neither the phosphotransferase gene nor its promoter is of yeast origin. Further restriction mapping however showed that the Ty1 was inserted about 100 bp from the initiation codon of the phosphotransferase gene and therefore probably within the promoter region. We also deduced the orientation of the Ty1 insert from its restriction map to determine the direction of Ty1 transcription (10) with respect to that of the phosphotransferase gene on the plasmid. As shown in Figure 1, the orientation of the insert is such that the direction of Ty transcription is opposite to that of the phosphotransferase gene, a situation found in every case where overexpression of the adjacent gene results from Ty insertion (10). To confirm that the effect of the Ty1 insert was overexpression of the phosphotransferase gene, we used the fact that transcription of Ty1 and its effect on gene overexpression is greatly reduced in a/a diploid cells (9). Diploid yeast cells constructed by mating strains XSB52-23C and AH22 were transformed with pGT60A and Leu+ transformants were tested for resistance to G418. As expected for a condition of increased expression due to Ty insertion, the diploids containing pGT60A were all sensitive to 500 ug G418/ml. This result shows that haploid yeast cells containing pGT60A are resistant to G418 because the Ty1 insertion causes overexpression of the
phosphotransferase gene, even though neither the gene nor its promoter is yeast DNA.

Transformation with pGT60A DNA Yields Excision Plasmids

As described in Materials and Methods, we used a two step procedure for yeast transformation, first selecting Leu^+ transformants and then testing these for G418 resistance. When yeast strain XS852-23C was transformed with pGT60A using this procedure, 18 of 100 Leu^+ transformants tested were found to be sensitive to G418. This was surprising since the original yeast transformant containing pGT60A did not yield any colonies sensitive to G418 and the plasmids used in transformation appeared homogeneous on agarose gels. Transformant colonies that were Leu^+ but sensitive to G418 were grown in minimal media, plasmids were shuttled to E. coli and transformant colonies were screened. Restriction mapping indicated that each had an identical deletion of the Ty1 element such that a 330 bp insert containing a XhoI site remained behind. Comparison of restriction fragment sizes indicated that this remaining insert was identical to the delta sequences at the ends of Ty1. In each case, all the plasmids shuttled from a given transformant colony contained an identical excision if the colony was sensitive to G418 or were identical to pGT60A if the colony was resistant to the antibiotic. This suggests that an excision of the Ty1 element occurs from some of the pGT60A plasmids upon transformation into a yeast cell. Moreover the excision must occur only in the initial transforming plasmid since all progeny cells of a given transformant contain either intact or excised plasmids, never a mixture of the two.

Excision of Ty1 Is Not Strain or Plasmid Dependent

We suspected that the unique chromatin structure of centromeric DNA (25) combined with the proximity of CEN3 to the insertion (Fig. 1) might be responsible for the high frequency excision of the transposon. Therefore several additional plasmids were constructed to determine if the excision frequency of Ty1 was related to either the CEN3 or 2 micron DNAs present on plasmid GT60A (Fig. 3). Plasmid GT61A is a derivative of pGT60A obtained by removal of the CEN3 fragment. Plasmid GT64A is also a derivative of pGT60A obtained by deletion of the 2 micron DNA and insertion of the 0.6 kbp XhoI fragment containing ARS2 (16) into the SalI site adjacent to the LEU2 gene. The functional yeast origin of replication (ARS2) was required to replace the deleted 2 micron origin. Hence plasmids GT60A, GT61A and GT64A contain identical constructions spanning the Ty1 element and the phosphotransferase gene.
Yeast strains XS852-23C and AH22 were transformed with these plasmids and the Leu+ transformants were tested for resistance to G418. For plasmids pGT60A and pGT64A, 8-20% of the Leu+ transformants were sensitive to G418 (Table 2). Since GT61A is a high copy number plasmid (it lacks a centromere sequence), we expected that any excision forms of this plasmid would continue to be resistant to G418. When Leu+ transformants obtained with pGT61A were tested for resistance to G418, all the transformants were resistant as expected, however about 20% of the transformants grew more slowly than the others in the presence of the antibiotic. Plasmids were shuttled from several of the pGT61A transformants that grew slowly in the presence of G418 and from pGT60A and pGT64A transformants that were sensitive to G418. Restriction mapping of the recovered plasmids showed that all the plasmids contained the identical excision of Ty1, leaving behind a delta sequence. The relatively slow growth of the pGT61A excision plasmids in the presence of G418 probably results from the delta sequence that is left behind in the promoter region.

TABLE 2. Excision frequency of Ty1 from supercoiled plasmids. Yeast strains XS852-23C and AH22 were transformed with the plasmids indicated and 200 Leu+ transformants obtained with each plasmid and strain were tested on YPD media containing 500 μg of G418/ml. The (+) indicates growth, the (-) indicates no growth. (*) Transformants containing the excision form of pGT61A remain resistant to G418 due to the high copy number of the plasmid, however these transformants grow more slowly than those containing an intact pGT61A.

<table>
<thead>
<tr>
<th>PLASMID</th>
<th>SIZE</th>
<th>COPY NUMBER</th>
<th>EXCISION FREQUENCY</th>
<th>G418 RESISTANCE</th>
</tr>
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<tr>
<td>pGT60A</td>
<td>22.2</td>
<td>low</td>
<td>20%</td>
<td>+</td>
</tr>
<tr>
<td>pGT61A</td>
<td>20.2</td>
<td>high</td>
<td>18%</td>
<td>+</td>
</tr>
<tr>
<td>pGT64A</td>
<td>16.5</td>
<td>low</td>
<td>8%</td>
<td>+</td>
</tr>
</tbody>
</table>

2996
following excision of the Ty element. Formation of the excision plasmids from pGT61A and pGT64A indicates that neither the CEN3 DNA nor the 2 micron DNA is required for excision of the Ty element during transformation. The excision frequency may be related to the size of the plasmid since a consistently higher frequency was obtained with the larger plasmids (Table 2).

As noted above, a/a diploid yeast cells transformed with intact pGT60A remain sensitive to G418 due to suppression of the Ty1 effect on the phosphotransferase gene, making it impossible to test for these excision plasmids in diploids. Our results with pGT61A in haploids however suggested that Ty excision from pGT61A might be detected in diploids on the basis of growth rate. Diploid yeast cells constructed by mating X5852-23C and AH22 were transformed with pGT61A and the Leu+ transformants were tested for resistance to G418 as before. As expected, all the Leu+ transformants were resistant to G418 due to the high copy number of the plasmid, however a few of these grew slowly on the antibiotic plates. Analysis of the plasmids shuttled to bacteria showed that the slow growers represented excision plasmids whereas the fast growers contained intact pGT61A. These results suggest that the excision of Ty1 during transformation also occurs in diploid cells and is not affected by the reduction in Ty-mediated overexpression of the phosphotransferase gene.

**Resident Plasmids are Not Excised during Host Cell Transformation**

We have shown above that excision of Ty elements occurs at high frequency from plasmids used to transform yeast cells. It is not clear, however, if transforming DNA is more susceptible to homologous recombination, or if the process of transformation stimulates homologous recombination. To investigate this question, yeast strain X52-23C was transformed with plasmid GT60A and several transformants containing intact (non-excised) plasmids were selected. These transformants were in turn supertransformed with a yeast plasmid (YRp7) that contains the TRP1 gene (26) and Leu+ Trp+ cotransformants were selected. These cotransformants were tested on YPD media containing G418 to determine if the resident pGT60A had undergone Ty excision during supertransformation of the host cell. In all cases, 100% of the cotransformants were resistant to G418, indicating that none of the resident pGT60A had undergone excision during supertransformation of the host cell with YRp7. These results suggest that only the transforming DNA is susceptible to the high frequency excision that we have observed.

**High Frequency Excision is Not Specific for Ty Elements**

The excision of Ty elements leaves behind a single copy of the direct
terminal repeat, indicating that excision occurs by homologous recombination across the repeats. This suggests that any direct repeats in plasmid DNA might yield the same excision during transformation. To test this possibility we have used pGT45, a CEN3-ARS2-LEU2-2 micron plasmid similar to pGT44 (12) but having the small EcoRI fragment of 2 micron DNA inverted. Hence the normal 599 bp inverted repeats of 2 micron become direct repeats separated by about 2 kbp of unique DNA in pGT45. Yeast strain X52-23C was transformed with pGT45 and Leu<sup>+</sup> transformants were selected. Plasmids from individual colonies were shuttled to bacteria, isolated, digested with EcoRI and analyzed on agarose gels. The results showed that 14% of the yeast transformants contained plasmids that had undergone homologous recombination across the direct repeats in the 2 micron DNA. This suggests that high frequency excision may occur by recombination across any direct repeats during transformation of yeast, and there is no specific requirement for the delta sequences of a Ty element.

**Excision Frequency is Greatly Reduced by the rad52 Mutation**

The product of the RAD52 gene in yeast functions in mitotic and meiotic recombination (27). It has been shown that the rad52 mutation blocks chromosomal deletions that normally result from delta-delta recombination in yeast (28). We therefore analyzed the effect of the rad52 mutation on the plasmid Tyl excisions that we had observed. Yeast strain D78-3D containing the rad52 mutation was first cured of its endogenous 2 micron plasmid and the resulting strain was transformed with plasmids containing the Tyl insert and the Leu<sup>+</sup> transformants were tested for sensitivity to G418. The frequency of excision was greatly reduced but was not completely eliminated in the presence of the rad52 mutation. The excision frequency for the plasmids in strain D78-3D was about 0.5%, an approximate twenty-fold reduction when compared to that of yeast strains lacking the rad52 mutation. This suggests that the RAD52 gene product is primarily responsible for the formation of excision plasmids but may not be an absolute requirement.

**Supercoiled Plasmids Facilitate Excision of Tyl**

It has been shown that plasmids linearized by restriction enzymes that leave complementary ends are efficiently religated following transformation into yeast cells (29,30). We have used this method to determine if the supercoiled state is essential for the plasmid Tyl excision that we observe. Our plasmids all contain a unique SacI (GAGCT<sup>C</sup>) recognition site located within the _C. reinhardtii_ DNA about 0.4 kbp from the site of Tyl insertion. Since the SacI site is not located within yeast DNA and no carrier DNA was
TABLE 3. Effects of plasmid topology on Ty excision. Yeast strains XSB52-23C and AH22 were transformed with equal aliquots of plasmids in the form indicated, and each value was obtained by testing 200 Leu\(^+\) transformants on YPD plates containing 500 \(\mu\)g of G418/ml. The transformants that are sensitive to G418 are those in which the Ty1 has been excised from the plasmid.

<table>
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<th>PLASMID FORM</th>
<th>TRANSFORMANTS SENSITIVE TO G418</th>
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<tbody>
<tr>
<td></td>
<td>XSB52-23C</td>
</tr>
<tr>
<td>Linear</td>
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</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Ligated-linear</td>
<td>1%</td>
</tr>
<tr>
<td>Supercoiled</td>
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</table>

used in our transformation procedure, the transformants obtained with linear plasmids opened at the SacI site should primarily result from religation of the linear DNA (29,31). Equal aliquots (15 \(\mu\)g) of supercoiled plasmids GT60A and GT64A were treated either with SacI, with SacI followed by DNA ligase or with no enzyme and a portion of each was analyzed on an agarose gel. In each case, digestion and/or ligation appeared complete as judged by band mobility on the gels.

Yeast strains XSB52-23C and AH22 were transformed with linear, ligated-linears and supercoiled plasmids GT60A and GT64A. The linear and supercoiled plasmids each yielded about 50 Leu\(^+\) transformants per \(\mu\)g of input DNA compared to about 20 transformants per \(\mu\)g of ligated-linear DNA. For each plasmid form and strain, 100 Leu\(^+\) transformants were tested for resistance to G418. As shown in Table 3, the linear and the ligated-linear plasmids yielded a few or no transformants that were sensitive to G418, indicating that Ty excisions rarely occurred in these plasmid forms. Supercoiled plasmids carried through the identical procedures but without addition of SacI or DNA ligase yielded the typical high frequency excision of Ty1 (Table 3). Plasmids from some of the transformants obtained with linear and ligated-linear DNAs were shuttled to bacteria, isolated and analyzed with restriction enzymes. In all cases, the restriction map was identical to that of the transforming plasmid and the SacI site was intact. These findings indicate that plasmids must be in the supercoiled state to undergo the high frequency excision of Ty elements that we have observed.

DISCUSSION

Our results show that the yeast transposon Ty1 is excised from up to 20\% of supercoiled plasmid DNAs during the initial stages of yeast transformation.
We observed this excision by transforming yeast cells with plasmids containing two selectable yeast markers; the LEU2 gene and a G418-resistance gene whose expression is dependent upon a Tyl element. Our selection scheme of first isolating Leu+ transformants and then testing these for resistance to G418 detected some colonies that were Leu+ but sensitive to G418. Plasmids isolated from these unexpected transformants had lost the Tyl element but retained a delta sequence at the insertion site. The consistent form of the plasmid (either excised or intact) among progeny of individual transformants suggests that only the initial transforming plasmid is subject to high frequency of excision, and only for a short period prior to replication of the transformed cell. In addition, few excision plasmids were found when yeast cells were transformed by nonsupercoiled plasmids. These results can most easily be explained if supercoiled plasmids undergo a change in topology shortly after transformation into a yeast cell.

We have shown that high frequency excision during transformation of yeast also occurs when the DNA is bounded by direct repeats that are not delta sequences. In addition, we have demonstrated that the excision is dependent on the RAD52 gene. The simplest interpretation of these results is that we are observing normal homologous recombination in the yeast cell, but that plasmid DNA is more susceptible to recombination for a short period following its entry into the cell. We have not observed any excision from Ty plasmids that are resident in yeast cells during transformation by other plasmids. Therefore, it is unlikely that the excision we have observed is due to any general stimulation of homologous recombination resulting from the transformation procedure. The easiest explanation is that the transforming DNA is more susceptible to homologous recombination and this condition changes rapidly after it enters the cell. Such a change might consist of the incoming plasmid DNA being organized into nucleosomal subunits in the cell.

REFERENCES