JOURNAL OF BACTERIOLOGY, Sept. 1984, p. 1065–1067
0021-9193/84/091065-03$02.00/0

Vol. 159, No. 3

Deletion of Plasmid Sequences During *Saccharomyces cerevisiae* Transformation

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Received 14 May 1984/Accepted 16 June 1984

***Saccharomyces cerevisiae* was transformed with DNA by the lithium acetate method. Mutation of nonselected markers on the transforming vector was observed at a frequency several orders of magnitude higher than spontaneous mutation frequencies. These mutations were shown to be deletions. Linearization of the vector before transformation stimulated deletion formation.**

The manipulation of DNA in yeasts has been greatly facilitated in recent years by the development of yeast transformation systems. The transformation of yeast spheroplasts was first described by Hinnen et al. (2) in 1978, and recently, a method of transforming intact cells by treatment with lithium acetate has been developed (3). Very little is known, however, about the mechanism of DNA uptake or passage to the nucleus or about the effects of this process on the transforming DNA. We observed that nonselected markers were lost at a low frequency during transformation. In light of recent findings that DNA transfected into mammalian cells is subject to mutation as a result of the transfection process itself (1, 5, 6; J. S. Lebkowski, R. B. DuBridge, E. A. Antell, K. S. Greisen, and M. P. Calos, Mol. Cell. Biol., in press), we wished to determine whether a similar phenomenon occurred during *Saccharomyces cerevisiae* transformation.

S. cerevisiae YNN281 (**a** *Δtrp1 Δhis3-200 ura3-52 ade2-101 lys2-801*) was transformed by treatment with lithium acetate with the vectors YRp17ia and YCp19 (Fig. 1) and selected for the Trp$^+$ marker. Mutation of the plasmid was scored in three nonselected markers: the *S. cerevisiae URA3* gene and the bacterial *lacI* and tetracycline resistance (*tet*) genes. The *S. cerevisiae* Trp$^+$ transformants were tested for the presence of the Ura$^+$ marker by replica plating. DNA was prepared (7) from a pooled culture of the *S. cerevisiae* Trp$^+$ transformants and returned to *Escherichia coli*. These transformants were scored for mutations in *lacI* and *tet*. The scoring of Ura$^-$ mutations gives the only direct measure of frequency, and therefore, the observed mutation frequencies in this gene (Table 1) most accurately reflect the actual frequencies at which mutations occur on the transforming vector.

Plasmids extracted from Trp$^+$ Ura$^-$ transformants were examined directly by gel electrophoresis and Southern blotting. Some mutant plasmids were returned to *E. coli* for additional analysis. The eight Ura$^-$ mutants of YRp17ia (U1 through U8) were demonstrated to contain deletions, ranging in size from ca. 1 to 5 kilobases (kb). Six of these plasmids, isolated by rapid lysis of *S. cerevisiae* (9), are shown in Fig. 2. Four of the eight mutant plasmids (U2, U3, U4, U6) could not be recovered in *E. coli*, presumably due to deletion of the pBR322 origin of replication. The four plasmids that were recovered in *E. coli* (U1, U5, U7, U8) were further analyzed, and the restriction maps of these deletions are presented in Fig. 3. The 13 Ura$^-$ mutants of YCp19 were also determined to contain large deletions by Southern blotting and by restriction analysis of the five plasmids that were recovered in *E. coli* (data not shown). All 21 of these deletions were distinct.

Mutations in the *lacI* and *tet* genes of YRp17ia were scored in strain MC1061 F′Δ150 (*hsrR hsdM*$^+$ *araD* Δ[*ara-leu*] Δ*lac*[*IPOZY*] *galU galK rpsL* F′Δ*lacIZ-150 kan*). This strain was transformed with DNA prepared from a pooled culture of the *S. cerevisiae* Trp$^+$ transformants and selected for ampicillin resistance on media containing the indicator 5-bromo-4-chloro-3-indolyl-β-D-galactoside. Colonies containing a plasmid with a wild-type *lacI* gene were white on 5-bromo-4-chloro-3-indolyl-β-D-galactoside medium; those containing a plasmid with a mutation in the *lacI* gene were blue (1). Of 30,104 ampicillin-resistant colonies, 18 were *lacI* (0.06%). The mutations represent two independent deletions (L1 and L2) of ca. 1.5 and 2.5 kb. One of these (L1) is identical, as determined by restriction mapping, to U7. The restriction maps of L1 and L2 are presented in Fig. 3. Mutations in the *tet* gene were scored by replica plating ampicillin-resistant colonies to plates containing tetracycline. Of 240 colonies, 3 were Tet$^-$ (1.25%). Plasmids from these colonies were found to contain a deletion of ca. 4.5 kb, which appears to be identical to U1.

The observed deletion frequency for nonselected markers varies with their proximity to the selected *ARS1* region of the plasmid which is required for replication in *S. cerevisiae* (9). The closely linked *lacI* gene underwent deletion at ca. 10% the frequency of the less tightly linked *URA3* gene in YRp17ia or YCp19. When *S. cerevisiae* was transformed with YRp17 (Fig. 1) by selecting for the Ura$^+$ marker, the frequency of Ura$^+$ Trp$^-$ colonies was ca. 10-fold lower than the frequency of Trp$^+$ Ura$^-$ colonies when Trp$^+$ is selected (data not shown). In mammalian cells, the observed mutation frequency of *lacI* is lowered when it is flanked by selected sequences. Presumably, these lower observed frequencies are due to failure to detect deletions that affect the selected sequence as well as the tightly linked nonselected sequence.

The observed deletions appear to be generated during the process of *S. cerevisiae* transformation. When *S. cerevisiae* was transformed with full-length YRp17 supercoiled and open circular plasmid, which was extracted from agarose gels, we obtained Trp$^+$ Ura$^-$ colonies at the same frequency as that for DNA that was not size selected. Therefore, these deletions did not pre-exist in the population of plasmid molecules obtained from *E. coli*. Also, Trp$^+$ Ura$^+$ *S. cerevisiae* transformants did not segregate Trp$^+$ Ura$^-$ cells on

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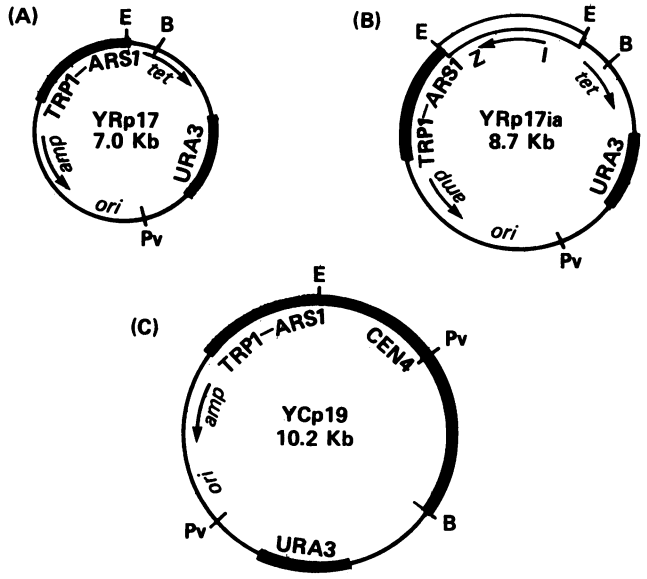

FIG. 1. *S. cerevisiae* transformation vectors. The plasmids are drawn to scale, and the arrows indicate the direction of transcription. The heavy line represents *S. cerevisiae* DNA, the single line represents pBR322 sequences, and the open line represents the *lac* fragment. Abbreviations: E, *Eco*RI; B, *Bam*HI; Pv, *Pvu*II. (A) YRp17 has been previously described (8). (B) YRp17ia was constructed by inserting a 1.7-kb *lac* fragment (1), which contains the *lacI* gene and the beginning of the *lacZ* gene, into YRp17. The a denotes the orientation of the *lac* fragment. (C) YCp19 has been previously described (8).

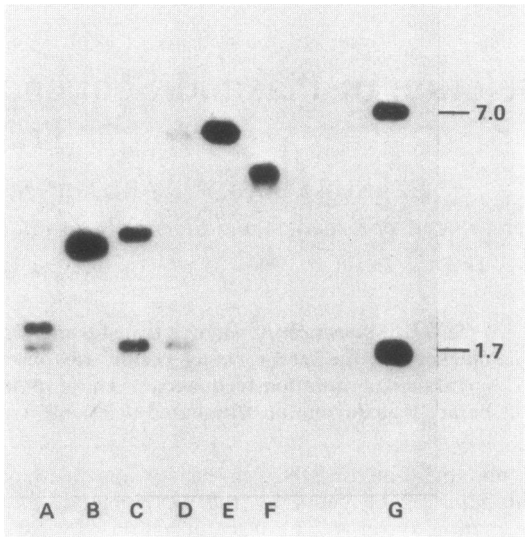

FIG. 2. Autoradiograph of *Eco*RI-digested derivatives of YRp17ia isolated by rapid lysis of *S. cerevisiae*. The Southern blot was probed with ^{32}P-labeled YRp17ia. The hybridized filters were exposed to XAR-5 film for 15 h. Lane A, U2; lane B, U3; lane C, U4; lane D, U5; lane E, U7; lane F, U8, lane G, YRp17ia. Size standards on the right are given in kb.

mitotic passage at frequencies comparable to those generated during transformation.

We and others have observed that transfection of linear vectors into mammalian cells stimulated the frequency with which those vectors suffered deletions (5; J. S. Lebkowski and M. P. Calos, unpublished data). Plasmid YRp17ia was linearized at the *Bam*HI site (Fig. 1), which lies close to the 5′ end of the *tet* gene, and was used to transform *S. cerevisiae*. An increase in the mutation frequencies of the Ura (Table 1) and Tet markers was observed. Of the resulting 10 Ura$^-$ plasmids, 3 could be recovered in *E. coli*. These were demonstrated by restriction enzyme analysis to contain deletions ranging in size from ca. 1.5 to 3 kb. The other seven Ura$^-$ plasmids, which could not be recovered in *E. coli*, were not examined; they most likely contain deletions that extend into the pBR322 origin. Small deletions upon transformation of *S. cerevisiae* with vectors linearized

TABLE 1. Observed mutation frequencies

Vector	No. of transformants	No. of Ura$^-$	Frequency of mutation (%)
YRp17ia	1,559	8	0.5
YCp19	1,146	13	1.1
*Bam*HI-cut linear YRp17ia	243	10	4.1

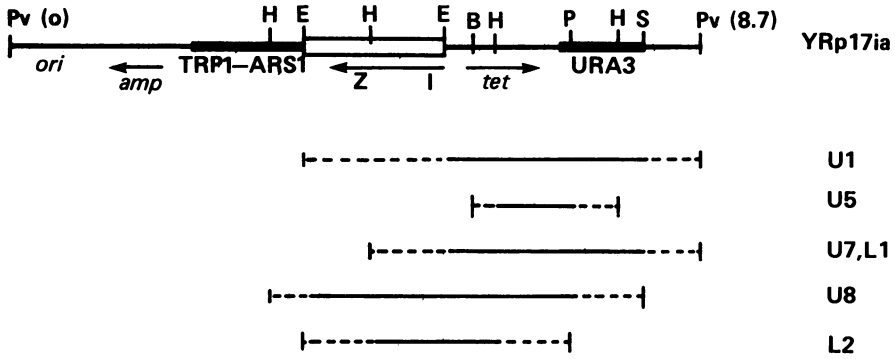

FIG. 3. Restriction maps of deletions of YRp17ia drawn to scale. The wild-type plasmid is represented by the conventions described in the legend to Fig. 1 but is shown linearized at the *Pvu*II site. In the deletion maps, the solid line indicates DNA that is known to be deleted through the restriction sites flanking it; the dashed line indicates DNA which may be deleted, but the precise endpoints have not been determined; vertical bars indicate those restriction sites that have been preserved. Abbreviations: E, *Eco*RI; B, *Bam*HI; Pv, *Pvu*II; H, *Hinc*II; P, *Pst*I; S, *Sma*I.

in bacterial sequences has previously been observed (4). Our results indicate that the production of large deletions is also stimulated.

Mutations in the *tet* gene were scored in *E. coli* as described above. Of 700 ampicillin-resistant colonies examined, 149 were Tet^- (21.3%). Plasmids from 36 of these were analyzed. The *Bam*HI site is contained within a 934-base-pair *Bgl*I-*Eco*RI fragment; the size of this fragment was not detectably altered in 25 of the mutant plasmids. The *Bam*HI site, however, was no longer present. These plasmids must contain either very small deletions or point mutations at this site. The remaining 11 mutant plasmids represent two independent large deletions of ca. 2.5 and 3.5 kb.

The frequencies of mutation of the Ura and Tet markers in the experiments presented here are similar to the mutation frequencies of nonselected markers during mammalian transfection (1, 5, 6; Lebkowski et al., in press). In addition to deletions, however, point mutations and, less frequently, duplications and insertions are observed during transfection of mammalian cells. We did not observe these types of mutations during transformation of *S. cerevisiae*, although they may occur at a low level relative to deletion formation. The mechanisms by which these mutations are generated are presently unknown. Linearization of a plasmid stimulates the formation of deletions spanning the cleavage site. This observation suggests that linearization may be a step in the production of deletions of circular molecules. Mutation of transfected DNA has been observed in primate, rodent, and yeast cells. It is probably a general feature of eucaryotic transformation and should be recognized in the planning and interpretation of experiments in which these procedures are utilized.

S.C. and C.M. were supported by Public Health Service training grants T32 GM-07790-05 and T32 GM 07599-06 from the National Institute of Health. This work was funded by Public Health Service grant CA-33056 from the National Cancer Institute and grant 83-E-101 from the Chicago Community Trust/Searle Scholars Program, which were awarded to M.P.C., and by American Cancer Society grant NP-286A awarded to R.W.D.

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