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Recombinational Instability of a Chimeric Plasmid in *Saccharomyces cerevisiae*

MALCOLM S. WHITEWAY† AND ASAD AHMED*
Department of Genetics, University of Alberta, Edmonton, Alberta, Canada T6G 2E9

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Wild-type strains of *Saccharomyces cerevisiae* exhibit mitotic recombination between the chimeric plasmid TLC-1 and the endogenous 2μ circle that involves sequence homologies between the two plasmids that are not acted on by the 2μ circle site-specific recombination system. This generalized recombination can be detected because it separates the *LEU2* and *CAN1* markers of TLC-1 from each other through the formation of a plasmid containing only the *S. cerevisiae LEU2* region and the 2μ circle. This derivative plasmid is maintained more stably during vegetative growth than TLC-1, and strains which carry it frequently lose the endogenous 2μ circle. Therefore, TLC-1 can provide a convenient selection for [cir^0] cells. Formation of this new plasmid is greatly reduced, but not eliminated, in strains containing the *rad52-1* mutation. This indicates that generalized mitotic recombination between plasmid sequences utilizes functions required for chromosomal recombination in *S. cerevisiae*.

Most laboratory strains of *Saccharomyces cerevisiae* contain an endogenous plasmid commonly referred to as the 2μ circle (4). Although this plasmid has not yet been shown to carry out an essential function in yeast cells, its properties have made it suitable for studies on yeast DNA replication (15, 16, 29) and site-specific recombination (5). The origin of replication of this plasmid has also been introduced into a number of yeast cloning vectors (6, 24, 25) to allow their autonomous, high-copy-number replication.

One of the cloning vectors that contains the 2μ circle origin of replication is YEp13 (6). This plasmid also includes the yeast β-isopropyl malate dehydrogenase (*LEU2*) gene. A derivative of YEp13 has been constructed which contains the yeast arginine permease (*CAN1*) gene (6). This plasmid (TLC-1) is unstable, because *S. cerevisiae* strains which contain it can form derivatives which have lost the *CAN1* gene while retaining the *LEU2* gene. Such derivatives can be identified because the *CAN1* gene confers dominant sensitivity to the arginine analog canavanine (28), so that loss of the gene from the plasmid results in a canavanine-resistant phenotype in a *can1* host. This separation of plasmid markers is due to recombination between TLC-1 and the endogenous 2μ circle and involves sequences that are not part of the site-specific recombination system that operates on the inverted repeat sequences. We have used this marker separation to investigate the role of the *RAD52* gene product on generalized mitotic recombination between autonomously replicating plasmids in *S. cerevisiae*.

MATERIALS AND METHODS

Strains. *S. cerevisiae* strains MSW28-10Cα (*leu2-3,2-112 his3-11,3-15 can1-100 ura3-1 trp5-2*) and MSW152-1Aa (*leu2-3,2-112 his1-7 can1-100 ura3-1 rad52-1*) were constructed for this study from strain GRF18α obtained from G. R. Fink. The *rad52-1* strain E053-6Dα (*rad52-1 arg4-17 ade2-1 lys1-1 his1-7*) was supplied by S.-K. Quah. Strain YT6-2-1Lα (*leu2-3,2-112 his4-419 can1* [cir^0]) (11) was from V. Mackay, and strain SS101a (*pet*$^-$) (16) was obtained from D. Livingston. *E. coli* strain JF1754 (*hsdR lac gal metB hisB leuB*) (23) was from D. Bendiak.

Media. Complete medium (YEPD) was 2% peptone, 1% glucose, and 1% yeast extract. Supplemented minimal medium (SC) was 2% glucose and 0.67% yeast nitrogen base without amino acids, supplemented with (per liter) 40 mg of lysine and leucine, 30 mg of adenine and tryptophan, and 20 mg of histidine, methionine, and uracil. SC − leucine lacked leucine, and canavanine medium was SC or SC − leucine containing 60 mg of canavanine sulfate per liter. Sporulation medium was 1% potassium acetate, 0.25% yeast extract, and 0.1% glucose, with additional supplements as for SC. All solid media contained 2% agar. Regeneration agar was 2 M sorbitol, 3% agar, and 1% YEPD, with additional supplements as for SC − leucine.

Plasmids and probes. Plasmid TLC-1 (6) was obtained from J. Hicks, and pBR322 (3) was obtained from J. Calvo. The *LEU2*-specific probe contained the *Eco*RI-*Sal*I fragment of the *LEU2* region of TLC-1 inserted between the *Eco*RI and *Sal*I sites of pBR322. The 2μ probe specific for sequences not part of TLC-1 was obtained by electroelution of the 1.3-kilobase (kb) *Hin*dIII fragment of the 2μ circle. This fragment encompasses 2μ circle (A form) coordinates 1017 to 2331, whereas TLC-1 contains coordinates 0 to 939 and 2407 to 3714 of the sequence determined by Hartley and Donelson (12). The source of the 1.3-kb fragment was plasmid pTM2, which contains the A form of the 2μ circle from SS101 inserted at the *Eco*RI site of pBR322.

DNA purification and restriction. *S. cerevisiae* DNA was purified by chloroform-isoamyl alcohol extraction of lysed spheroplasts (8). Plasmid DNA from *E. coli* was purified by cesium chloride-ethidium bromide centrifugation (26). Restriction enzymes were purchased from Bethesda Research Laboratories and used according to the manufacturer's instructions.

Yeast methods. Yeast crosses and dissection of asci were performed by using standard techniques (22). Yeast transformation (13) followed the protocol of J. D. Beggs (2).

Southern analysis. Agarose gel electrophoresis was performed on horizontal slab gels (17). Nick translations, Southern blotting, and hybridization in 50% formamide were performed as described previously (9).

* Corresponding author.
† Present address: Division of Cancer Genetics, Dana-Farber Cancer Institute and Department of Biological Chemistry, Harvard Medical School, Boston, MA 02115.

TABLE 1. Effect of *RAD52* and 2μ plasmid on the formation of *can1 LEU2* derivatives

Strain	Genotype	Frequency ($\times 10^{-6}$)[a]
MSW28-10C(TLC-1)	*RAD52*$^+$ [cir$^+$]	1,500[b]
YT6-2-1L(TLC-1)	*RAD52* [cir^0]	150[c]
MSW152-1A(TLC-1)	*rad52-1* [cir$^+$]	7[d]

[a] *can1 LEU2* derivatives per cell. Mean values are given.
[b] Range, 3,300–340.
[c] Range, 610–0.
[d] Range, 8.8–1.8.

Bacterial transformation. *E. coli* cells made competent by $CaCl_2$ treatment (7) were used for transformation.

RESULTS

Instability of TLC-1. The *LEU2* and *CAN1* markers of plasmid TLC-1 are physically linked (6). When chromosomal *can1 leu2* strains containing TLC-1 were plated on medium containing canavanine, derivatives which had lost the *CAN1* marker were selected. Only 1 in 1,000 of these derivatives retained the *LEU2* marker (Table 1). These infrequent *can1 LEU2* strains can be selected on medium that contains canavanine but lacks leucine.

Five independent *can1 LEU2* derivatives were studied. These derivatives lacked the pBR322 sequence found in TLC-1. Bulk DNA isolated from these strains did not contain homology to nick-translated pBR322 and also failed to transform *E. coli* cells to ampicillin resistance. However, these derivatives did contain low-molecular-weight DNA species with homology to a probe specific for the *LEU2* region of the yeast chromosome. These plasmids were estimated to be about 10.5 kb, which is considerably smaller than the 15-kb TLC-1 molecule.

S. cerevisiae strains which contained the smaller plasmids were more stable for the *LEU2* phenotype than the strains that contained TLC-1. The instability of TLC-1 was ca. 5×10^{-2} per cell per generation, whereas that of the smaller plasmids was ca. 1×10^{-2} per cell per generation, as calculated from an equation for the determination of high mutation rates (1).

Involvement of the 2μ circle in TLC-1 instability. Formation of *can1 LEU2* derivatives was reduced in strains which lacked endogenous 2μ circles ([cir^0]). Strain YT6-2-1L(TLC-1) generated *can1 LEU2* derivatives at a frequency of about 2 in 10,000 (Table 1). Three independent *can1 LEU2* derivatives obtained in the [cir^0] background were tested, and all contained a mitotically stable *LEU2* marker, which suggests that they resulted from recombination between the *LEU2* regions of TLC-1 and the chromosome.

The formation of the smaller *LEU2* plasmid in the *can1 LEU2* derivatives has, therefore, been detected only in [cir$^+$] strains. Reciprocal recombination involving each of the two regions of homology between TLC-1 and the 2μ circle, or conversions of large regions of nonhomology, can generate a plasmid containing an entire 2μ circle plus the *LEU2* region (Fig. 1). This plasmid has a predicted size of 10.7 kb and lacks all pBR322 sequences. The plasmids in the *can1 LEU2* derivatives that hybridized to the probe specific for 2μ circle sequences that are not contained in TLC-1 (Fig. 2A) also hybridized to the *LEU2*-specific probe (Fig. 2B). This is consistent with the smaller *LEU2* plasmids having arisen through recombination between TLC-1 and the 2μ circle. All five derivative plasmids contained a 4.15-kb *Eco*RI fragment with homology to the probe (Fig. 3). This fragment is also found in TLC-1, so the *LEU2* region is found in the same position (relative to the 2μ circle sequences) in TLC-1 as in the derivative plasmids. This suggests that the derivative plasmids arise by homologous recombination between TLC-1 and the 2μ circle and not by illegitimate recombination events during which the *LEU2* marker is inserted at different sites within the 2μ circle. In addition to the 4.15-kb fragment, the derivative plasmid preparations contained a 5.8-kb *Eco*RI fragment with homology to the *LEU2* probe. This fragment results from interconversion of the derivative plasmid about the inverted repeat sequences. This provides further evidence that the derivative plasmids consist of a 2μ circle containing the *LEU2* region from TLC-1 inserted at the *Pst*I site.

Only two of the five *can1 LEU2* derivatives contained the DNA species characteristic of the endogenous 2μ circle (Fig. 2A). Therefore, selection for *can1 LEU2* cells seems to generate a subpopulation enriched for [cir^0] cells.

Effect of *rad52-1*. The formation of *can1 LEU2* derivatives is greatly reduced in strains which contain the *rad52-1* mutation. Strain MSW152-1A(TLC-1) generated *can1 LEU2* derivatives at a frequency of less than 1 in 100,000 (Table 1), and this reduced frequency cosegregated with the *rad52-1*

FIG. 1. Recombination between TLC-1 and the 2μ circle. (a) Plasmids TLC-1 and 2μ circle. (b) Alignment of the two plasmids at their regions of homology. A double cross-over or a conversion event can separate the *CAN1* and *LEU2* sequences. (c) Recombinationally derived 2μ-LEU2 hybrid (pLEU2). This plasmid exists in two forms resulting from recombination between inverted repeats present on the molecule. Restriction sites for *Eco*RI, *Pst*I, and *Sal*I are indicated by the letters E, P, and S, respectively. Sequences from pBR322 are shown in black, those from the yeast *CAN1* region are shown in white, those from the yeast *LEU2* region are stippled, and those from the 2μ circle are hatched. The inverted repeat sequences of the 2μ circle are crosshatched.

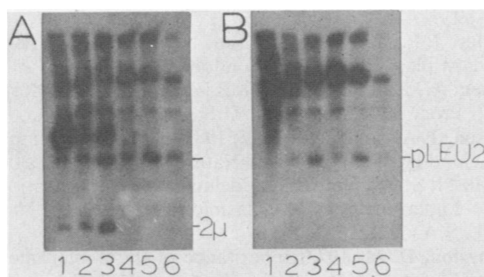

FIG. 2. Autoradiograph of a Southern blot of unrestricted bulk yeast DNA hybridized with (A) the 2μ probe and (B) the *LEU2* probe. Lane 1 contains DNA from strain MSW28-10C(TLC-1), and lanes 2 through 6 contain DNA from *can1 LEU2* derivatives of this strain. The sequences that hybridize both probes are from the recombinationally derived *LEU2* plasmid, whereas the 2μ-specific bands found in lanes 1, 2, and 3 represent the endogenous 2μ circle. Only the supercoiled form of the plasmids is labeled; the slower moving bands are from relaxed, linear, and multimeric forms of the plasmids. Samples were run in 1% agarose gels for 16 h at 20 V. Direction of migration is to the bottom.

mutation in two complete tetrads in which all four spores received a copy of TLC-1.

Five independent *can1 LEU2* strains derived in a *rad52-1* background have been studied. Three of these strains contained plasmids that were indistinguishable from TLC-1 except that they did not contain a functional *CAN1* gene. DNA from these strains hybridized to probes for both pBR322 and *LEU2* and could be used to generate Amp^r $LeuB^+$ transformants of *E. coli*. In addition, the plasmids were identical in size to the TLC-1 plasmid as judged by agarose gel electrophoresis, and displayed stabilities characteristic of the original TLC-1 plasmid. These strains presumably arose by mutations at the *CAN1* gene of TLC-1. It has been reported that the *rad52-1* mutation can enhance the spontaneous mutation rates at a number of loci (27).

The two remaining strains contained plasmids similar to the *LEU2* plasmid formed by generalized recombination between TLC-1 and the 2μ circle in the RAD^+ strain. These plasmids lacked pBR322 sequences but contained *LEU2* sequences and were smaller and more stable than TLC-1. Therefore, although generalized mitotic recombination between TLC-1 and the 2μ circle was greatly reduced in strains containing the *rad52-1* mutation, it was not eliminated.

DISCUSSION

Plasmid TLC-1 (6) contains both the *CAN1* and *LEU2* genes of *S. cerevisiae*. *S. cerevisiae* strains which contain the endogenous 2μ circle as well as TLC-1 can, however, lose the *CAN1* marker even when selection is applied for the *LEU2* gene. This loss is a consequence of recombination between TLC-1 and the 2μ circle which physically separates the *CAN1* and *LEU2* genes. This recombination process involves sequences that are not part of the site-specific recombination system of the 2μ circle, which acts on a limited region within the inverted repeats of the plasmid (5). Therefore, the process that separates the *CAN1* and *LEU2* markers must involve generalized recombination functions.

The instability of TLC-1 is greatly reduced in strains which contain the *rad52-1* mutation. This suggests that the *RAD52* gene product is involved in generalized mitotic recombination between sequences on autonomously replicating plasmids, such as the chromosomal sequences (19).

Recent evidence shows that, although the *RAD52* gene product is necessary for conversions between chromosomal duplications, it is not required for reciprocal recombination involving such duplications (14, 21). However, some special events that generate reciprocal exchanges, such as chromosomal integration of linearized plasmids (18) and UV-induced sister chromatid exchange (20), also require the *RAD52* gene product. In the present study, only the derivative *LEU2* plasmid can be identified genetically, so it is not possible to determine whether this plasmid arose by conversion or reciprocal recombination. Because the derivative plasmid can be formed either by a reciprocal or a nonreciprocal event, the present experiment may be identifying either *RAD52*-dependent conversion between plasmids or a further example of *RAD52*-dependent reciprocal exchange.

Although the formation of the derivative *LEU2* plasmid is greatly reduced in *rad52-1* strains, it is not eliminated. Because other recombination events, such as conversion between chromosomal duplications (14) and integration of linearized plasmids (18) are reduced (but not eliminated) in *rad52-1* strains, it is possible that the *rad52-1* allele is leaky. Until a defined null allele of the *RAD52* locus is generated, interpretation of the effects of the *RAD52* gene product through the *rad52-1* phenotype must remain tentative. If the *rad52-1* allele is nonleaky, the low frequency of derivative plasmids may be caused by *RAD52*-independent reciprocal recombination. Alternatively, these plasmids may be generated by another process, such as a low frequency of site-specific recombination system-initiated events being resolved outside the normal sequences acted on by this system. However, any derivative plasmids generated by *RAD52*-independent processes represent only a small fraction of the total derivative plasmids formed in RAD^+ strains, since the majority arise by *RAD52*-dependent generalized recombination between plasmids.

A number of practical considerations arise from these observations on recombination between plasmids in *S. cerevisiae*. Because genes cloned at the *Bam*HI site of YEp13 can be separated from the *LEU2* marker by recombination, genes that are detrimental to yeast cells when carried on a multicopy plasmid can be selected against in YEp13-derived clone banks maintained in *S. cerevisiae* by selection for the *LEU2* marker. In addition, a stable derivative of TLC-1

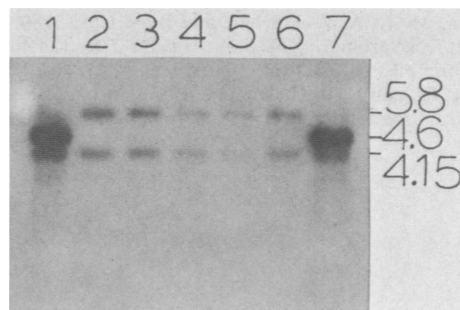

FIG. 3. Autoradiograph of a Southern blot of *Eco*RI-restricted bulk yeast DNA hybridized with the *LEU2* probe. Lanes 1 and 7 contain DNA from strain MSW28-10C(TLC-1), and lanes 2 through 6 contain DNA from *can1 LEU2* derivatives of this strain. The intense 4.6-kb band in lanes 1 and 7 is the *Eco*RI fragment with homology to the pBR322 part of the probe. The 4.15- and 5.8-kb bands are the *Eco*RI fragments with homology to the *LEU2* region of the probe. These two bands (in lanes 2 through 6) result from the interconversion of the 2μ-*LEU2* hybrid about its inverted repeats. Gel electrophoresis conditions were the same as those described in the legend to Fig. 2.

needs to be constructed before this vector can be used for positive selection of cloned fragments in *S. cerevisiae* (6). In particular, removal of the smaller 2μ circle fragment flanking the *LEU2* region should eliminate the recombinational instability documented in this work. Finally, because selection of *can1 LEU2* derivatives of *S. cerevisiae* strains containing TLC-1 generates a subpopulation highly enriched for [cir^0] cells, this plasmid is a good choice for use in the selection of [cir^0] strains by *S. cerevisiae* transformation (10, 11).

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