
The role of recombination and *RAD52* in mutation of chromosomal DNA transformed into yeast

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ABSTRACT

While transformation is a prominent tool for genetic analysis and genome manipulation in many organisms, transforming DNA has often been found to be unstable relative to established molecules. We determined the potential for transformation-associated mutations in a 360 kb yeast chromosome III composed primarily of unique DNA. Wild-type and *rad52* *Saccharomyces cerevisiae* strains were transformed with either a homologous chromosome III or a diverged chromosome III from *S.carlsbergensis*. The host strain chromosome III had a conditional centromere allowing it to be lost on galactose medium so that recessive mutations in the transformed chromosome could be identified. Following transformation of a *RAD*⁺ strain with the homologous chromosome, there were frequent changes in the incoming chromosome, including large deletions and mutations that do not lead to detectable changes in chromosome size. Based on results with the diverged chromosome, interchromosomal recombinational interactions were the source of many of the changes. Even though *rad52* exhibits elevated mitotic mutation rates, the percentage of transformed diverged chromosomes incapable of substituting for the resident chromosome was not increased in *rad52* compared to the wild-type strain, indicating that the mutator phenotype does not extend to transforming chromosomal DNA. Based on these results and our previous observation that the incidence of large mutations is reduced during the cloning of mammalian DNA into a *rad52* as compared to a *RAD*⁺ strain, a *rad52* host is well-suited for cloning DNA segments in which gene function must be maintained.

INTRODUCTION

Since the original experiments of Avery and McLeod, it has been realized that DNA transformation provides a useful means for the introduction of genetic material as well as genetic manipulation of the host. Prominent recent examples of the relevance to human health include opportunities for gene therapy and the characterization of the human genome by cloning

chromosomal fragments into model prokaryotes and eukaryotes. However, the state of transforming DNA as compared to *in vivo* chromosomal material would suggest that it is particularly vulnerable to alterations and this is exemplified by results with human DNAs cloned into yeast.

Systems developed in the yeast *Saccharomyces cerevisiae* that are based on artificial chromosome vectors (YACs) have provided the means for isolating large DNAs (1), up to several megabases of mammalian chromosomal material (2–11). This has proven especially important in the Human Genome Project where large fragments are used to map the human genome. Many investigations with transforming DNA require that its integrity be maintained. Once cloned as a YAC, the DNA can be manipulated using homologous recombination in yeast and subsequently introduced into mammalian cells using different transfection procedures (12–16). There are several examples of genes cloned into YACs that were subsequently found to be functional when transferred to mammalian cells, including G6PD (13), HPRT (17), GART (18).

Thus, there is sufficient fidelity in the YAC cloning of mammalian DNA to allow physical mapping of chromosomes as well as transfer of functional material to mammalian cells. However, there are many reports of mammalian DNA in YACs being altered during transformation and subsequent propagation. For example, 10–60% of clones in existing human YAC libraries have co-cloned DNA sequences (chimeras arising from different regions of the genome) (9, 19–27). Furthermore, internal rearrangements and deletions appear to be common in YACs (19, 21, 27–30).

Alterations of cloned DNAs in YACs appear to occur primarily during transformation based on results with model plasmids and YACs. Rearrangements and deletions involving repeats within plasmid DNA are commonly observed during yeast transformation (31–34). The levels are much higher than spontaneous recombination frequencies during mitotic growth. Recently we have shown that when mitotically stable model YACs were retransformed into yeast, nearly 30% had large internal deletions (35).

Recombination is considered to be an important source of altered YACs. Deleted forms of YACs have been proposed to result from recombination between multiple repeated sequences

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common in mammalian DNAs. The high level of transformation-associated deletions in YACs can be reduced over 10-fold when the host is a recombination-deficient strain deleted for the *RAD52* gene (35, 36). Chimeric YACs in mammalian libraries (20) could also arise by recombination of co-transformed molecules, presumably between repeated sequences. Transformation experiments with pairs of differently marked homologous and non-homologous human YACs demonstrated that the level of interYAC recombination was significantly reduced in a recombination-deficient *rad52* host strain (Larionov *et al.*, in preparation). The low yield of chimeric YAC clones in a recently constructed library within a *rad52* host strain was probably due to reduced opportunities for recombination (37). Recombination also appears to be a source of YAC instability during mitotic propagation. Internal instability within some human YACs is decreased in *rad52* as compared to isogenic *RAD*⁺ strains (35, 36, 38, 39).

While the integrity of cloned human DNA can be improved by reducing recombination, the use of *rad52* strains could be problematic. During mitotic growth, the incidence of spontaneous intragenic (small) mutations including missense and frameshift mutations is several-fold greater in *rad52* mutants as compared to *RAD*⁺ strains (40, 41). Given the many activities of the *RAD52* gene (42, 43), it is possible that mutation rates in transforming DNA could also be elevated in *rad52*. With this in mind, we have investigated the consequences of transformation on large, essentially unique DNAs when transferred into *rad52* and *RAD*⁺ strains. In this way we could establish the minimal consequences of transformation on chromosomal size material in terms of mutations arising from recombination versus other factors.

A model system for detecting mutations in chromosomal size DNA molecules during yeast transformation was developed. Specifically the yeast *S.cerevisiae* chromosome III was transformed into a wild-type and a *rad52* mutant. The host strain contained a conditionally stable resident chromosome III that could be easily eliminated; this allowed changes in the transformed chromosome to be detected readily. We also transformed strains with a diverged chromosome III from *S.carlsbergensis*, which was expected to greatly reduce opportunities for recombination (44–47).

When the host is a *RAD*⁺ strain, large alterations are common in transforming chromosome III and they appear to result from interchromosomal recombination with the resident chromosome. The alterations are greatly reduced if the transforming chromosome is diverged or if the host is *rad52*. Since the frequency of small mutations in the chromosomal size DNAs during transformation is not increased in the *rad52* mutant, a *rad52* host is a good host for cloning YACs containing functional genes.

MATERIALS AND METHODS

Media

S.cerevisiae cells were grown on complete medium (YPD and YPG) or synthetic selective medium (SD) lacking either leucine, uracil, threonine or histidine, depending on the gene under selection. Medium containing 5-fluoro-orotic acid was used to select for *ura3* mutants and medium with canavanine was used to select for strains containing *can1* mutations. The media has been described by Sherman *et al.* (48).

Yeast strains

Three strains containing a chromosome III with a conditional centromere, YVL6, YVL6- Δ 1 and YVL10, were used as hosts for retransformation experiments involving chromosomal size DNA. In these strains the centromeric sequence in the chromosome III was substituted by a *GAL1*–*CEN3* cassette using a one-step gene replacement technique (49). The centromere substitution vector *GALCEN3* was described by Hill and Bloom (50). The genotype of YVL6 is *MAT α* , *leu2- Δ 1*, *lys2-801*, *ade1*, *his3- Δ 200*, *trp1- Δ 1*, *met14*, *ura3-52*, *GALCEN3::URA3*. An isogenic strain with a complete deletion of the *RAD52* gene, YVL6- Δ 1 (*MAT α* , *rad52- Δ 1::TRP1*, *leu2- Δ 1*, *lys2-801*, *ade1*, *his3- Δ 200*, *met14*, *trp1- Δ 1*, *ura3-52*, *GALCEN3::URA3*) was made by a one-step gene replacement technique using a *SalI* digest of the *RAD52* disruption plasmid p52BLAST (obtained from E.Perkins). The deletion of the *RAD52* gene was confirmed by blot-hybridization and radiation sensitivity of the transformants. YVL10 is a *MAT α* derivative of YVL6 obtained by switching the mating type using a *HO*-containing *CEN* plasmid. The *S.cerevisiae* chromosome III in strains YVL6 and YVL6- Δ 1 is 340 kb.

Two yeast strains were used as donors of chromosome III in retransformation experiments. The *S.cerevisiae* strain YVL49 (*MAT α* , *LEU2*, *ura3-52*, *ade2-101*, *trp1- Δ 1*, *lys2-801*) contains a 360 kb *S.cerevisiae* chromosome III. The differences in size between *S.cerevisiae* chromosomes III in donor and recipient strains result from differences in the size of the right arms. The right arm of chromosome III is highly polymorphic and differences between strains relate to the presence or absence of Ty elements and delta sequences derived from them (51). In the second *S.cerevisiae* strain SDP86-4A (*MAT α* , *LEU2*, *his4-53*, *ura3-52*, *ade2-101*, *trp1-hisG*, *ilv1-92*) the *S.cerevisiae* chromosome III was substituted by a 360 kb chromosome III from *S.carlsbergensis*. This and strain SDP113 (*MAT α* *his4::arg4* *chal leu2- Δ 1* *ilv1-92*) used for complementation tests were obtained from S.Priebe.

Transformation experiments

YVL6, YVL6- Δ 1 and YVL10 cultures were made competent using a high-frequency spheroplast transformation protocol (52) with minor modifications (53). Spheroplasts were prepared with Zymolyase 100T (ICN) (stock preparation containing 1 unit/ μ l). The spheroplasts were washed twice in 1 M sorbitol, resuspended in STC buffer (1 M sorbitol, 10 mM Tris, pH 7.5, 10 mM CaCl₂) and then the described protocol was followed. Spheroplast preparations were used that had less than 2% lysed spheroplasts in 1 M sorbitol and exhibited 100% lysis after water was added (spheroplasts were examined using a phase-contrast microscope). An agarose plug (about 100 μ l) containing chromosomal size DNA of donor strains was equilibrated three times for 1 h by soaking in 1 ml of 25 mM NaCl. The dialyzed plug was melted for 3–5 min at 65°C, transferred to 42°C for 10 min before addition to spheroplasts. After equilibrating, the agarase, 10-fold diluted in 25 mM NaCl (1 unit/ μ l), was carefully added (1 unit/100 mg agarose). The sample was incubated for 15 min at 42°C. Each transformation mixture contained 400 μ l of spheroplasts which were added by gentle mixing to the treated melted plug. All other steps were performed as described (53). Plates were incubated 6–8 days at 30°C for formation of the individual transformants selected for the *Leu*⁺ phenotype.

The yield of Leu⁺ transformants with the YVL6 recipient strain varied from 50 to 200 colonies for 100 μ l agarose plugs containing about 10⁸ molecules of 360 kb *S.cerevisiae* chromosome III. The same yield of transformants was observed in retransformation experiments of the host strain with a *S.carlsbergensis* chromosome III. The frequency of Leu⁺ transformants was about 5–10 times lower with the *rad52* deficient mutant strain YVL6- Δ 1.

Preparation of agarose plugs containing yeast chromosomal size DNAs

For retransformation experiments, yeast cells containing a chromosome III of interest were grown to stationary stage in YPD medium. Low-melting-point agarose plugs were prepared at a final density of 10⁹ cells/ml as described by Carle and Olson (54). After purification of the chromosomes and agarase treatment, 100 μ l of the sample was used for spheroplast transformation. Chromosome size yeast DNA for electrophoresis analysis was prepared in a similar manner. Chromosomal size DNAs were stored in 0.05 M EDTA, 0.01 M Tris, pH 7.5, at 4°C.

Transverse alternating field electrophoresis (TAFE)

Transverse alternating field electrophoresis (TAFE) was used to analyze DNA size. The DNA was run in 1% agarose gels with recirculated (at 5°C) 0.25 \times TBE buffer (1 \times TBE = 0.1 M Tris–borate, 0.002 M EDTA, pH 8.3) using 350 mA and a pulse time of 31 s for 18 h. After electrophoresis, the gels were stained with ethidium bromide. The positions of chromosome III bands were visualized by Southern blotting with labeled *LEU2* and *MAT* probes. Chromosome III sizes in transformants were determined by comparison to concatemers of λ phage, assuming 48,502 base pairs for the λ DNA.

Analysis of mutations in chromosome III arising during yeast transformation

The integrity of a transformed chromosome III was determined by assessing the ability of the transformed chromosome to substitute for the resident chromosome. Loss of the resident chromosome was induced by transferring the Leu⁺ Ura⁺ transformants to complete YPG medium containing 2% galactose and incubating plates overnight. The cultures were streaked out on complete YPD medium containing 2% glucose to obtain individual colonies. The colonies were replica-plated to minimal medium lacking uracil to identify loss of the resident chromosome III (the loss of the centromere-linked *URA3* marker signals the loss of the complete chromosome). This procedure typically resulted in 50% of the cells having lost the resident chromosome if the transformed chromosome III was functional. Transformants having lost the resident chromosome III, as well as the original

Leu⁺ Ura⁺ clones, were analyzed by TAFE. In order to estimate the frequency of mutations in non-essential genes of chromosome III during transformation, the Ura⁻ segregants of transformants (lacking the resident chromosome III) were checked for the presence of an altered phenotype, i.e. slow growth, cold (12°C) and heat (37°C) sensitivity, ability to mate, and threonine or histidine auxotrophy (resulting from mutations in the two genes located on chromosome III, *HIS4* and *THR4*). Since the recipient strains carry a mutant allele of *HIS3*, mutations in the *HIS4* locus of the incoming chromosome III were checked after crossing the Ura⁻ segregants with the *MAT α his4* tester strain SDP113 containing a functional *HIS3* gene.

Rates of spontaneous mutations during mitotic growth

Fluctuation analysis was used to determine the rates of spontaneous mutation at the *CAN1^s*, *MET14*, *LYS2* and *URA3* loci during mitotic propagation. Spontaneous mutation frequencies were determined for reversion of the Lys⁻ and Met⁻ mutations and for forward mutation in the *CAN1^s* locus resulting in resistance to canavanine. Spontaneous forward mutation frequencies of the *URA3* gene linked to the centromere of chromosome III were measured using selection for 5-fluoroorotic acid resistance. The mutation rates (mutations/cell/division) were estimated using the method of the median (55) for five different cultures.

RESULTS

Mutations in homologous chromosome III during transformation into a *rad52* mutant

Previously it was shown that the absence of *RAD52* function results in increased rates of spontaneous frameshift and missense mutations (40, 41). For the *rad52* deletion mutant strain (YVL6- Δ 1) used in this study, the rates of spontaneous reversion (*met14* and *lys2*) and forward mutation (*URA3* and *CAN1^s*) are at least five times higher than in the isogenic RAD⁺ strain YVL6 (Table 1). Since the increased mutation rates could compromise the use of a *rad52* mutant for studying large genomic fragments, especially if their genetic function is to be examined when returned to the original organism, we have investigated whether this mutator activity also affected chromosomal DNA transformed into a *rad52* mutant.

For these studies we used the well-characterized chromosome III that contains 182 open reading frames for proteins longer than 100 amino acids (56). Based on experiments involving random disruption of genes in the genome and disruption of coding reading frames in chromosome III (56, 57), ~30% of the genes in this chromosome when mutated are expected to result in altered growth and loss of viability. Thus, there is a large genetic target for detecting mutations.

Table 1. Rates of spontaneous mutation in recombination-deficient and wild-type strains

Strain	Spontaneous mutation rate ^a ($\times 10^{-8}$)			
	<i>MET14</i>	<i>CAN1^s</i>	<i>URA3</i>	<i>LYS2</i>
RAD ⁺	0.22	1.5	2.3	0.11
<i>rad52</i>	1.23	28.0	20	5.10

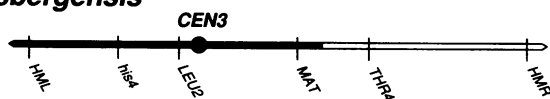
^aSpontaneous mutation was measured as reversion of Met⁻ and Lys⁻ auxotrophs or in the case of *CAN1* and *URA3*, as forward mutation to canavanine and 5-fluoroorotic acid resistance, respectively. Rates were determined according to Lea and Coulson (55) as described in Materials and Methods.

In order to identify mutations that might arise during transformation, we developed a system in which such mutations could be genetically detected by including in the recipient strain a chromosome III with a conditional centromere (Fig. 1). Adjacent to the centromere is a *GAL1-10* promoter so that transfer of cells from glucose to galactose results in inactivation of the centromere resulting in chromosome III loss due to malsegregation (50). Cells that receive a functional chromosome III are viable when the resident conditional chromosome III is lost. Mutations of essential genetic material in the transformed chromosome III are indicated by inability to recover cells on galactose that lacked the host chromosome III (i.e. they could not lose the *URA3* centromere-linked marker). They could be due to large chromosomal deletions, detectable using gel electrophoresis, or small localized changes in essential genes. Other types of mutations, such as those resulting in slow growth and requirement for specific nutrients, could also be identified in the transformed chromosomes. Since the retransformation was done with chromosomal DNA from haploids, the mutations could not have accumulated during mitotic growth. The mitotic mutation rate (see Table 1) is too low to account for the results described below.

The consequences of transformation on genetic integrity of homologous chromosome III were determined using techniques common for isolation of large genomic DNA. Haploid cells containing a *LEU2*-marked *S.cerevisiae* chromosome III were lysed in agarose plugs and the DNA was used to transform the *leu2 rad52* recipient strain YVL6-Δ1. 190 of 206 *Leu⁺ Ura⁺*

transformants of the donor chromosome III could substitute for the resident chromosome (Table 2). Forty five among 190 transformants were examined further; all had the two chromosomal bands corresponding to the incoming and resident chromosomes III as determined by TAFE analysis (Fig. 2). Most transformants capable of losing a chromosome III were *MATα/MATa*. ~7% of the *Leu⁺ Ura⁺* transformants (13/190) were *MATα*. The change in mating type in the transforming chromosome III was not due to intrachromosomal events such as *MAT* switching because all such events were accompanied by reciprocal exchange between the incoming and the resident chromosomes (the 340 kb resident chromosome with the *URA3*-marked centromere became 360 kb in size and the 360 kb incoming chromosome marked by *LEU2* became 340 kb). A possible change of phenotype (including cold and heat sensitivity, slow growth, inability to mate and inability to grow on minimal growth media) was examined in 156 *Ura⁻* segregants having lost the resident chromosome III. Mutations were observed in ~5% of the transformed chromosomes (Table 3). Of the 16 chromosomes (estimated to represent 8% of the original *Leu⁺* isolates) that could not substitute for the resident chromosome III, 10 were normal size and are likely to have contained small mutations inactivating one of the essential genes in this chromosome. The remaining 6 chromosomes, that had deletions ranging from 50 to 80 kb (data not shown), might have resulted from intramolecular recombination during transformation of the incoming chromosome [similar to that observed in YAC retransformation experiments (35)] or from interaction of fragments of the transforming chromosome with the resident chromosome III.

S. carlsbergensis



S. cerevisiae

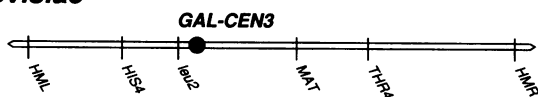


Figure 1. Schematic representation of chromosome III of *S.cerevisiae* and *S.carlsbergensis*. While the two chromosomes are functionally equivalent and exhibit the same gene order, they are diverged over the 'left' two-thirds (indicated by filled-in portion of *S.carlsbergensis* chromosome). The recipient strain contains a *S.cerevisiae* chromosome III with a *GAL1-10* promoter next to the centromere. Induction of the promoter by placing cells on galactose-containing medium inactivated the adjacent centromere resulting in chromosome loss.

Mutations in diverged chromosome III during transformation into a *rad52* mutant

Since a *rad52* mutant is not completely lacking in recombination and since recombination appears to play a role in producing large deletions in YACs containing mammalian DNA (35), the appearance of transformation-associated mutations was examined with diverged chromosomal DNA. The opportunity for recombination is expected to be reduced as homology is decreased. We used chromosome III from *S.carlsbergensis*, which is functionally homologous to chromosome III of *S.cerevisiae*. Genetic and molecular analyses indicate that the chromosome is composed of two sections (Fig. 1) where the right one-third is homologous to the *S.cerevisiae* chromosome and the remainder is diverged by ~15% (58), as confirmed by sequencing of four loci (*HML*, *HIS4*, *LEU2* and *MAT*) (59, 60). The divergence is a barrier for meiotic recombination and greatly decreases mitotic recombination in yeast (44–46) as well as recombination during transformation (33, 34, 47). Among 97%

Table 2. Integrity of homeologous and homologous chromosomes III during transformation^a into recombination deficient and wild-type strains of *S.cerevisiae*

Recipient strain	Transformed chromosome	Number of <i>Leu⁺</i> transformants analyzed	Number (%) of transformants capable of losing the resident chromosome III
<i>RAD⁺</i>	Homeologous	604	546 (90%)
<i>rad52</i>	Homeologous	201	196 (97%)
<i>RAD⁺</i>	Homologous	482	251 (52%)
<i>rad52</i>	Homologous	206	190 (92%)

^aEach result corresponds to chromosome isolates from 3–8 independent transformation experiments.

of the transformants (196/201) containing the diverged chromosome, the transformed chromosome could substitute for the resident chromosome (Table 2). Among 45 isolates examined by TAFE, 41 contained bands corresponding to the resident and incoming chromosome III. It is interesting that the remaining 4 isolates (among the examined 45) had chromosomes that were 20 kb larger than the incoming chromosome. A similar category of chromosomes with increased size is described below for the RAD⁺ strain. The reason for the increase remains to be determined. In all the segregants lacking the resident chromosome III, a *S. carlsbergensis* size chromosome III was observed. Figure 3 shows the chromosomal bands in the original Leu⁺ Ura⁺ transformants of the *rad52* strain and resulting Ura⁻ segregants. Thus, most of the *rad52* transformants receiving a diverged chromosome contained a complete set of essential genes.

Among 159 chromosomes that could substitute for the resident chromosome, only 1% resulted in altered phenotypes (Table 3), similar to results described above for transformation with the *S. cerevisiae* chromosome III. In 4 of the 5 Leu⁺ transformants

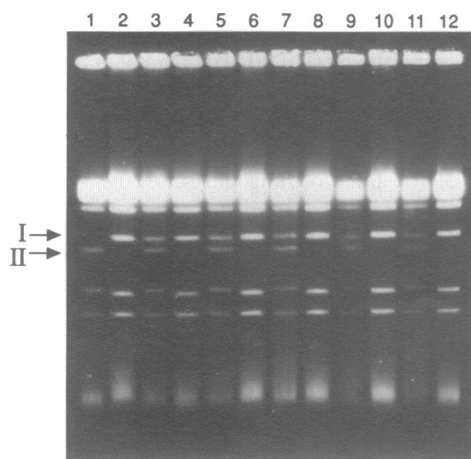


Figure 2. Physical characterization of chromosomes III in a *rad52* strain transformed with a homologous chromosome III where the transformed chromosome can substitute for the resident chromosome. Lane 1: a *rad52* recipient strain (YVL6-Δ1). Lane 2: a donor strain (YVL6). Lanes 3, 5, 7, 9 and 11: Leu⁺ Ura⁺ transformants of the *rad52* strain containing a functional homologous chromosome III (from YVL49). Lanes 4, 6, 8, 10 and 12: Ura⁻ segregants of the transformants lacking the resident chromosome III. Arrows indicate positions of a 340 kb and 360 kb resident and donor chromosome III, respectively.

(Table 2) in which the incoming chromosome could not substitute for the resident chromosome, the *S. carlsbergensis* chromosome was not altered in size. The mutations in these chromosomes were presumably small intragenic mutations. The other chromosome had a 90 kb deletion (data not shown).

We conclude that in a *rad52* mutant there is a relatively low rate of transformation-associated mutation that is reduced even further when homology is decreased. As described below, this contrasts with the high rates of transformation-associated mutation obtained in a wild-type host.

Homologous chromosome III transformed into a RAD⁺ strain is frequently altered

The integrity of a homologous chromosome III transformed into a RAD⁺ strain was greatly reduced in comparison to transformation into a *rad52* mutant or if the chromosome was diverged. Only 52% of the Leu⁺ transformants contained a homologous chromosome III capable of substituting for the resident chromosome (Table 2). Among 19 of 23 isolates examined containing a functional chromosome, two chromosome III bands corresponding to the resident (340 kb) and incoming chromosomes (360 kb) were detected (data not shown). The other 4 isolates contained the resident chromosome III and the transformed chromosome that was 30 kb larger than expected. There were additional genetically detectable changes in the transformants capable of losing a chromosome III. For approximately one-third of the transformants, there was an exchange of mating type loci between the two chromosomes, i.e. the transformed chromosome became *MATα* and the resident chromosome was *MATa*. The change in mating type in the transforming chromosome III is due to interchromosomal events because all such events were accompanied by a reciprocal exchange between the incoming and the resident chromosomes. Since the *CEN3-MAT* interval covers about 80 kb of chromosome III (56) and one-third of all transformants experienced an exchange in this interval, the incoming chromosome can be considered to be recombinationally active in a RAD⁺ strain.

For the rest of the Leu⁺ transformants, the resident chromosome was not dispensable. Physical analysis of the transformants showed that most of them (25 from 40 analyzed by TAFE) contained only one chromosome III band corresponding to that in the recipient strain. These transformants are likely to have arisen by gene conversion between the *LEU2* region of the incoming DNA and the resident chromosome. In 38% of the transformants (15 from 40), there were new bands capable of hybridizing with *LEU2* and *MAT* probes, indicating

Table 3. Transformation-associated small mutations in homeologous and homologous chromosomes III

Recipient strain	Incoming/resident chromosome	Mutant phenotypes after loss of a resident chromosome ^a			Clones analyzed ^c
		Slow growth	Cold sensitivity	Auxotrophic	
RAD ⁺	<i>carl/cere</i> ^b	8	0	1	152 (6%)
<i>rad52</i>	<i>carl/cere</i>	1	1	0	159 (1%)
RAD ⁺	<i>cere/cere</i>	5	6	0	103 (10%)
<i>rad52</i>	<i>cere/cere</i>	3	3	1	156 (5%)

^aTransformants were selected on medium lacking leucine. Loss of the resident chromosome III containing a conditional centromere was induced in the transformants as described in Materials and Methods. The appearance of new phenotypes was determined in the segregants (Ura⁻) lacking the resident chromosome.

^bThe '*cere*' and '*carl*' refer to chromosome III originating from either *S. cerevisiae* or *S. carlsbergensis*.

^cValue in parentheses corresponds to percent of chromosomes with detectable mutations.

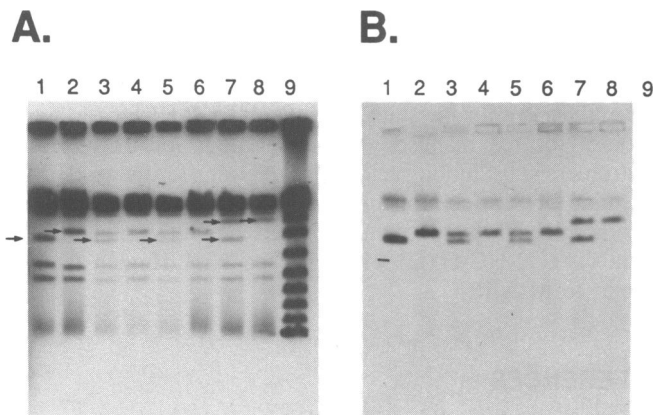


Figure 3. Physical characterization of chromosomes III in a *rad52* strain transformed with a diverged chromosome III where the transformed chromosome can substitute for the resident chromosome. In (A) the chromosomes are ethidium bromide-stained (the picture is a negative rather than a positive as shown in Fig. 2). In (B) the chromosomes are identified with a labelled *LEU2* probe. Lane 1: a *rad52* recipient strain (YVL6-Δ1). Lane 2: a donor strain (SDP86-4A) containing a *S.carlsbergensis* chromosome III. Lanes 3, 5 and 7: three randomly chosen transformants containing a functional *S.carlsbergensis* chromosome III. Lanes 4, 6 and 8: *Ura*⁻ segregants of the transformants lacking the *S.cerevisiae* resident chromosome III (derived from strains identified in lanes 3, 5 and 7, respectively). Lane 9: λ multimers ladder. In some isolates, the transformed chromosome III is about 20 kb bigger than the original chromosome (lanes 7, 8). Arrows indicate positions of 340 kb and 360 kb chromosome III of the resident and donor strains, respectively.

that they contained transformed chromosome III DNA. The sizes of the new bands were 50–120 kb shorter than the original incoming chromosome in 12 from 15 *Leu*⁺ transformants (Fig. 4). Approximately half of these transformants were *MATα/MATa*. Of the remaining 3, there was a chromosome III band that was 25 kb (for two isolates) and 75 kb larger than the original donor chromosome III (Fig. 4). The chromosomes that are larger are likely to have arisen by interchromosomal recombination. Note that this category of events was not detected when the *RAD*⁺ strain was transformed with a diverged chromosome (described below) or if the host strain was a *rad52* mutant.

Integrity of the diverged chromosome III transformed into a *RAD*⁺ strain

Unlike the situation with homologous chromosome transfer, most (90%) of the *Leu*⁺ isolates following transformation with the diverged chromosome were able to lose the resident chromosome III (Table 2). This is somewhat less (although significantly different) from that observed for the *rad52*. Similar to results with the *rad52* mutant, almost all the isolates (44/45) with functional transformed chromosomes III had two bands corresponding in size to the resident and incoming chromosomes. (The remaining one contained a 20 kb shorter *S.carlsbergensis* chromosome.) Also, transformed chromosomes that could substitute for the resident chromosome exhibited relatively few detectable genetic changes (Table 3). Among 152 chromosomes examined genetically, only 9 resulted in slow growth or auxotrophy for threonine (Table 3). None of these were altered in size (data not shown).

For the remaining *Leu*⁺ transformants unable to tolerate loss of the resident chromosome III, 8 among 16 analyzed by TAFE contained a normal size transformed chromosome III. Two others

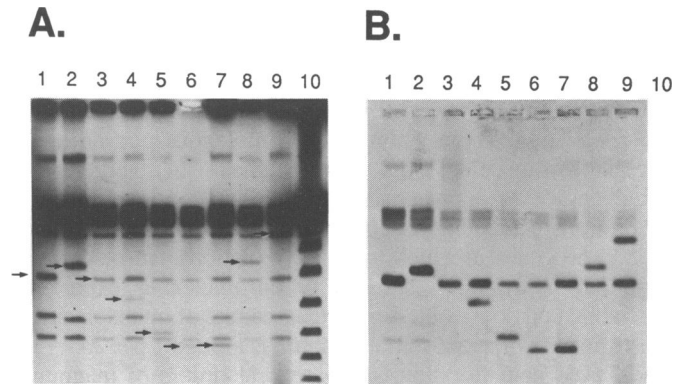


Figure 4. Physical characterization of chromosomes III in *RAD*⁺ strain transformed with a homologous chromosome III where the transformed chromosome cannot substitute for the resident chromosome. In (A) the chromosomes are ethidium bromide-stained (the picture is a negative rather than a positive as shown in Fig. 2). In (B) the chromosomes are identified with a labelled *LEU2* probe. Lane 1: a *rad52* recipient strain (YVL6). Lane 2: a donor strain (YVL49) containing a homologous chromosome III. Lanes 3–9: randomly chosen *Leu*⁺ *Ura*⁺ transformants that are unable to lose chromosome III. Lane 10: λ multimers ladder. Arrows indicate positions of chromosome III bands in the transformants.

had *S.carlsbergensis* chromosomes that were 30 and 60 kb shorter than the original. The remaining 6 *Leu*⁺ transformants did not contain a second chromosomal band that could hybridize with the *LEU2* probe. These transformants (estimated to represent 4% of the original *Leu*⁺ isolates) were likely to have arisen by recombination as is proposed to occur during transformation with homologous chromosomes. There were no examples of isolates where recombination had taken place between the centromere and *MAT* as described above for homologous chromosomes. Thus, the genetic activity of the transforming chromosomes resulting in mutations depends largely on opportunities for recombination.

DISCUSSION

Transformation and transfection are prominent tools for genetic analysis and genome manipulation in many organisms. A general requirement is that the integrity of the transformed DNA in the recipient be comparable to that in the host organism. The system we described has proven useful for obtaining a minimum estimate of the consequences of transformation on the integrity of chromosomal size DNA in yeast as well as determining the contribution of recombination and the *RAD52* gene. The chromosome III used in transformation experiments is largely comprised of unique DNA (56). When opportunities for recombination are reduced by transforming a *rad52* deletion mutant with a diverged chromosome, the integrity of the resulting transformed chromosome is high based on the relatively few mutations detected, suggesting that large molecules of primarily unique DNA can be cloned accurately into yeast.

In order to estimate the mutation frequency in the transforming DNA, it is necessary to know the number of mutant genes that could be identified in our assays. Chromosome III contains 182 open reading frames for proteins longer than 100 amino acids (56). Approximately 30% of genes in yeast when mutated yield a detectable phenotype including inability to grow (56, 57). Thus, mutations in ~50–60 genes might be picked up in our assay.

For the *rad52* strain the frequency of transformed diverged chromosomes having lost an essential cellular function or giving rise to altered growth was ~4% (3% large and 1% small mutations). Although a small amount of recombination can occur in a *rad52* mutant (possibly in the homologous portion of the diverged chromosomes), this value obtained with diverged DNA would appear to approximate the minimum rate of mutation during transformation. Assuming that on chromosome III there are 50–60 genes in which localized mutations can be detected using our assays and that 4% of chromosomes experience detectable mutations, we estimate that the rate of mutation during transformation of a *rad52* mutant strain is around 1 per 200–300 genes. (We note that this is still several orders of magnitude higher than mitotic rates of mutation; cf. Table 1). The rate for the RAD⁺ strain is estimated to be at least 4-fold higher, which may reflect greater opportunities for recombination. Therefore, while *rad52* mutants are known to be mutators for frameshift and base pair mutations during mitotic growth, the incidence of small mutations appears to be comparable or reduced in a *rad52* mutant for incoming chromosomal size DNA.

Our results with the RAD⁺ strain using homologous and diverged chromosome III DNAs are helpful for understanding how chromosomal DNA undergoes changes during transformation and the role of recombination. Clearly, homologous transforming DNA has a great potential for undergoing change. The potential is directly related to the opportunity for recombination since the rearrangements are greatly reduced when the chromosomes are diverged and/or the strain is a *rad52* mutant.

These observations are important for understanding the often low accuracy during the cloning of human DNAs into yeast. As much as half the human YACs cloned into RAD⁺ strains have rearrangements, deletions and/or are chimeras (see Introduction). Presumably this is due to the recombinational potential within the large molecules as suggested from the present experiments as well as experiments with small plasmids and model YACs (32, 34–36). Since the recombinational potential appears large, changes internal to cloned human DNAs could arise by recombination between commonly occurring repeats such as Alu's similarly to recombination between homologous chromosomes. The present experiments suggest that small mutations are infrequent in transforming DNA.

Thus, while linear chromosomal size DNA transformed into yeast has considerable recombinational potential, reducing the opportunity for interactions with another molecule by reducing homology and/or transforming into a *rad52* mutant nearly abolishes changes generated by recombination. The *rad52* host is particularly useful since mutations as we have shown are not increased. The source(s) of the relatively low level of changes observed in human DNAs when cloned into *rad52* remains to be determined. Since recombination can still be detected in a *rad52* mutant, there may be other pathways or unique categories of substrates within YACs that are responsible for the observed changes.

Our results also suggest an alternative method for genetic manipulation of yeast. Previously, chromosome transfer between yeast strains could be accomplished by cytoduction using a *kar1* mutant (61) resulting in disomy for one, and frequently more than one, chromosome. The present experiments demonstrate an alternative means for producing genetically identifiable disomic strains using standard spheroplast transformation procedures.

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