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High-frequency transformation of yeast: Autonomous replication of hybrid DNA molecules

(recombinant DNA/cloning vector/yeast plasmid/eukaryotic genetics/minichromosome)

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ABSTRACT **A set of vector DNAs (Y vectors) useful for the cloning of DNA fragments in *Saccharomyces cerevisiae* (yeast) and in *Escherichia coli* are characterized. With these vectors, three modes of yeast transformation are defined. (*i*) Vectors containing yeast chromosomal DNA sequences (YIp1, YIp5) transform yeast cells at low frequency (1–10 colonies per μg) and integrate into the genome by homologous recombination; this recombination is reversible. (*ii*) Hybrids containing endogenous yeast plasmid DNA sequences (YEp2, YEp6) transform yeast cells at much higher frequency (5000–20,000 colonies per μg). Such molecules replicate autonomously with an average copy number of 5–10 covalently closed circles per yeast cell and also replicate as a chromosomally integrated structure. This DNA may be physically isolated in intact form from either yeast or *E. coli* and used to transform either organism at high frequency. (*iii*) Vectors containing a 1.4-kilobase yeast DNA fragment that includes the centromere linked *trp1* gene (YRp7) transform yeast with an efficiency of 500–5000 colonies per μg; such molecules behave as minichromosomes because they replicate autonomously but do not integrate into the genome. The uses of Y vectors for the following genetic manipulations in yeast are discussed: isolation of genes; construction of haploid strains that are merodiploid for a particular DNA sequence; and directed alterations of the yeast genome. General methods for the selection and the analysis of these events are presented.**

The molecular analysis of gene structure, function, and regulation depends upon the ability to correlate physiological, genetic, and structural data relating to a specific gene or set of genes. Many mutants of the yeast *Saccharomyces cerevisiae* have been isolated, and techniques for the manipulation and mapping of the associated genetic characteristics are routinely performed (1). Genetically defined yeast DNA sequences have been isolated in the form of viable molecular hybrids with bacteriophage λ or *Escherichia coli* plasmids (2–5). Derivatives of the cloned *his3* gene that delete DNA sequences near or in the structural gene have been isolated and physically defined (unpublished data). The demonstration by Hinnen *et al.* (5) that recombinant DNA containing cloned yeast genes can be used to transform yeast cells clearly expands the potential of molecular analysis considerably. These workers showed that yeast transformation occurred at low frequency and that it was usually accompanied by homologous recombination between the transforming DNA and the host chromosomal DNA.

The present paper reports two additional modes of yeast transformation. In both, the transformation event occurs at high frequency and is associated with autonomous replication of the transforming DNA. Yeast vectors useful for a wide variety of genetic manipulations have been constructed by combining the three mechanistically different modes of transformation with structural information of the endogenous yeast plasmid (6), the *his3* gene, the *trp1* gene, and the *ura3* gene (D. Botstein, personal communication). In particular, physically defined, cloned alterations of wild-type yeast DNA sequences may be introduced back into yeast cells in order to examine their *in vivo* phenotypic effects.

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MATERIALS AND METHODS

Organisms, DNAs, and Enzymes. The following strains were used: yeast—A3617C (a *his3*-532 *gal2*) (3) and D13-1A (a *his3*-532 *trp1 gal2*); *E. coli*—*trpC* 9830 (7), *hisB* 463, SF8, C600 (rK^-mK^+) (2), and MB1000 ($rK^-mK^+lac^-trp^-pyrF^-$) (D. Botstein, personal communication); phage—λgt-Sc2601 (2), λ590 (8), and λgt-Sc4104; plasmid DNAs—pMB9-Sc2601 (3), Scp1 (6), pBR322 (9), pGT2-Sc2605, pBR322-Sc2676 (unpublished data), and pMB1068 (D. Botstein, personal communication). Propagation of strains and preparation of DNAs have been described (2, 3, 6).

*Eco*RI, *E. coli* DNA ligase, and deoxynucleotidyl terminal transferase were the gifts of Marj Thomas, Robert Alazard, and Tom St. John, respectively. Other restriction endonucleases were purchased from New England BioLabs and Bethesda Research Laboratories (Rockville, MD) and used as directed. Cloning procedures have been described (2, 10).

Where appropriate p2,EK1 conditions, as described by the National Institutes of Health Guidelines fo Recombinant DNA Research, were used.

Rapid Yeast DNA Preparations. Total yeast DNA was prepared from 5-ml cultures of cells grown to the stationary phase. Yeast cells were harvested and resuspended in 0.4 ml of 0.9 M sorbitol/50 mM potassium phosphate, pH 7.5/14 mM 2-mercaptoethanol. Lyticase (25 units) (a gift from R. Schekman) was added and spheroplast formation was allowed to proceed for 30 min at 30°C. At this stage, the procedure for rapid phage DNA preparations (6) was used with two changes: the ethanol precipitation was done at room temperature, and the resulting pellet was resuspended in 50–100 μl of 10 mM Tris, pH 7.4/1 mM EDTA containing 0.5 μg of pancreatic RNase. These preparations yielded approximately 1 μg of DNA per ml of original culture. The DNA is of high molecular weight [95% is greater than 25 kilobases (kb)], is relatively un-nicked (the yeast plasmid is isolated predominantly in the closed circular form), and is cleavable by all restriction enzymes tested.

Transformation of Yeast Cells. The procedure of Hinnen *et al.* (5) was followed with some modifications. Spheroplasts were prepared by treating 100 ml of an exponentially growing culture with 300 units of lyticase for 30 min at 30°C. After treatment with polyethylene glycol, the cells were immediately plated in the regeneration agar (10^7–10^8 viable spheroplasts per plate). The relative efficiency of transformation with the different vector DNAs was simultaneously determined on individual preparations of spheroplasts.

Abbreviation: kb, kilobase(s).

Nucleic Acid Hybridization. Preparations of ^{32}P-labeled probes and hybridization to nitrocellulose filters have been described (3). Hybridizations were usually performed at 65°C in 1 M Na^+. More stringent conditions were achieved in 50% formamide/1 M Na^+ at 42°C.

RESULTS AND DISCUSSION

Yeast Transformation Vectors. All yeast transformation (Y) vectors used in this study have the following properties: the ability to replicate in yeast and *E. coli* cells; genetic characteristics selectable in yeast or *E. coli* after DNA transformation into either of these organisms; sites of cleavage into which essentially any fragment of DNA can be inserted; and capability to isolate hybrid DNAs as covalently closed circles in at least one of these organisms. These vectors combine *E. coli* and yeast genetics into a single system, thus making it possible for cloning technology to be applied directly to yeast cells. Table 1 describes the properties of the Y vectors. Fig. 1 diagrams the physical structures of these vectors. Hybrid DNAs were enzymatically constructed *in vitro* and propagated in *E. coli* cells. Structural analysis of these DNAs was performed on material isolated from *E. coli* cells.

DNA Structural Analysis of the Yeast Transformation Event. The transformation event is operationally defined as the selected, genotypic change of a yeast cell dependent upon a particular DNA molecule. Hinnen *et al.* (5) analyzed the transformation of a *leu2*$^-$ yeast strain to Leu$^+$ by a combination of genetic and molecular techniques. However, it is possible to analyze the structure of the transforming DNA in greater detail by using well-marked restriction endonuclease cleavage maps

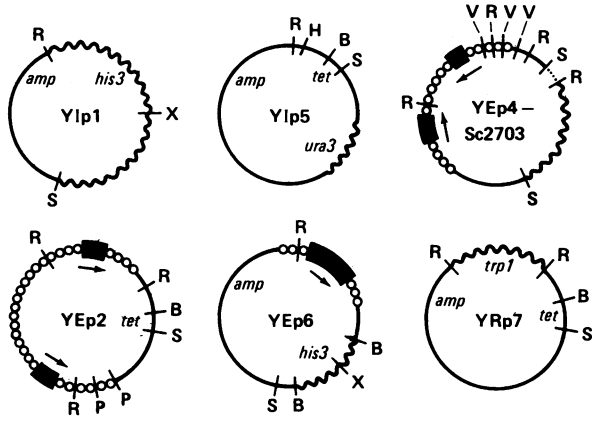


FIG. 1. Structure of Y vectors. DNA sequences diagrammed as follows: solid line, *E. coli* plasmid pBR322; wavy line, yeast chromosomal; circles, yeast plasmid Scpl; dashed line, λ; solid bar, Scpl sequence which is repeated in an inverted orientation (arrows). Restriction endonuclease sites: R, *Eco*RI; B, *Bam*HI; S, *Sal* I; X, *Xho* I; P, *Pst* I; V, *Pvu* I; H, *Hin*dIII. The vectors were constructed as follows: YIp1, ligation of *Eco*RI- and *Sal* I-cleaved pBR322 and pGT2-Sc2605 DNAs; YEp2 and YEp4, joining of dG-tailed *Pst* I-cleaved pBR322 DNA and dC-tailed *Hpa* I-cleaved Scp1 DNA; YIp5, joining of dC-tailed *Ava* I-cleaved pBR322 DNA and dG-tailed *Hin*dIII-cleaved λ590-Sc2904 DNA (Sc2904 is the 1.1-kb *ura3* *Hin*dIII fragment of pMB1068); YEp6, joining of dG-tailed *Eco*RI-cleaved pBR322-Sc2676 DNA and dC-tailed *Hpa* I-cleaved Scp1 DNA; YRp7, ligation of *Eco*RI-cleaved pBR322 and λgt-Sc4104 DNAs. YEp2 and YEp4 have the XY form of Scp1 (6) but differ by the number of *Pst* I sites regenerated by the cloning procedure (one for YEp2; two for YEp4) and by the orientation of Scp1 DNA with respect to pBR322 DNA. The structure of YEp6 DNA is most probably explained by a single deletion that removes approximately 4 kb of Scp1 DNA sequences and 0.2 kb of pBR322 sequences. The Scp1 sequences in YEp6 DNA correspond to coordinates 3700–5800 of the XY form.

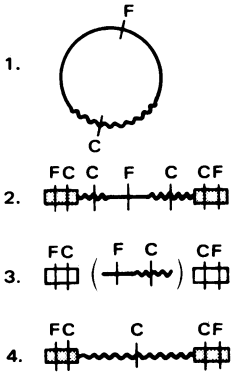
Table 1. Properties of Y vectors

Vector*	Size, kb	Markers	Cloning sites
YIp1	9.8	*amp, his3*	*Eco*RI, *Sal* I, *Xho* I
YEp2	10.4	*tet*	*Pst* I
YIp5	5.4	*amp, tet, ura3*	*Eco*RI, *Bam*HI, *Sal* I, *Hin*dIII
YEp6	7.9	*amp, his3*	*Eco*RI, *Xho* I, *Sal* I
YRp7	5.7	*tet, amp, trpl*	*Bam*HI, *Sal* I

* Abbreviations: I, chromosomal integrator; E, episomal replicon; R, chromosomal replicon; p, *E. coli* plasmid.

of the relevant DNAs. Fig. 2 presents four hypothetical mechanisms to explain a given transformation event: (*i*) autonomous replication of the transforming DNA, (*ii*) integration of the transforming DNA into the yeast genome by homologous recombination, (*iii*) integration via illegitimate (nonhomologous) recombination, and (*iv*) transformation not associated with autonomous replication or stable chromosomal integration of foreign DNA. To facilitate description of the DNA sequence organization of the transformants, we define three types of DNA sequences. *Common sequences* are present in both the transforming DNA and in the chromosomal DNA of the transformed strain. *Foreign sequences* are present in the transforming DNA but not in the chromosomal DNA; because the yeast plasmid (Scp1) does not normally integrate into the chromosome (6), it is defined as a foreign sequence. *Flanking sequences* are located immediately adjacent to either side of the common sequence in the chromosome of the transformed strain.

The diagrams in Fig. 2 show that the four postulated mechanisms of transformation predict different physical organizations of the transforming DNA. The autonomous replication mechanism predicts a low molecular weight form of the transforming DNA in the yeast cell which is identical to (or a linear permutation of) the covalently closed circular form found in *E. coli*. Both integration models predict that the transforming DNA will exist in the yeast cells as part of a high molecular weight species. However, the homologous recombination model predicts a perfectly aligned, nontandem duplication of the entire common sequence. The duplication is separated by one copy of the foreign sequence. This specific organization results from the insertion of a linear permutation of the transforming DNA into any site of the chromosomal copy of the common sequence. The illegitimate recombination model predicts in-



	Not cleaved	Endo F cleaved	Endo C cleaved
1.	Low M_r species	Linear	Linear
2.	High M_r species	2 linears (known length)	Linear (same as model 1)
3.	High M_r species	2 linears (unknown length)	Linear (unknown length)
4.	None	None	None

FIG. 2. Structural predictions for four transformation models. F and C, restriction endonuclease sites. Enzyme F cleaves foreign but not common sequences; enzyme C cleaves common but not foreign sequences. Predictions listed are based on hybridization of ^{32}P-labeled foreign sequence-specific probe across a gel of electrophoretically separated samples. See text for details.

tegration of the transforming DNA at an unknown genomic site. The structural organization will not be precisely predictable, and it will differ from that predicted for homologous recombination. The fourth mechanism predicts a structure indistinguishable from a nontransformed cell.

The analysis of any yeast transformant with respect to these four basic mechanisms proceeds in the following manner (see Fig. 2). DNA from a transformant is treated in three ways. One sample is uncleaved by restriction endonucleases; another sample is treated with an enzyme that cleaves at an internal cleavage site in the foreign sequence but does not cleave the common sequence (endo F); the third sample is treated with an enzyme that cleaves at least once in the common sequence or at the joint between the foreign and common sequences (endo C). DNA molecules in each sample are electrophoretically separated, transferred to a nitrocellulose filter, and challenged for hybridization with a ^{32}P-labeled probe specific for foreign DNA sequences or total transforming DNA sequences. This method is sufficiently rapid and general to be useful for the analysis of all yeast transformants.

Low-Frequency Transformation of Yeast Is Accompanied by Homologous Recombination. *his3* hybrids (such as YIp1 and pBM9-Sc2601) transform *his3*$^-$ yeast cells to His$^+$ at a frequency of 1–10 colonies per μg of DNA. Hybridization results such as those shown in Fig. 3 indicate that all 30 His$^+$ transformants examined contain one copy of the entire transforming DNA integrated at the *his3* locus. None of the low-frequency transformants contain any autonomously replicating DNA. The clearest example of the predicted integrated structure is that found in KY114, a cell transformed to His$^+$ by pMB9-Sc2683. Sc2683 contains an internal 1.4-kb deletion of the original *Eco*RI DNA fragment Sc2601 (M. Brennan and K. Struhl: unpublished data). Because the *Eco*RI fragments containing the common sequences of pMB9-Sc2683 and the host chromosome are distinguishable, the nontandem duplication is easily seen by *Eco*RI cleavage of KY114 DNA (Fig. 3, lane 7).

The nontandem duplicated structure generated upon transformation is not stable (Fig. 4). After 15 generations of growth in nonselective medium, approximately 1% of the colonies are His$^-$. This segregation is accompanied by the complete loss of the transforming DNA (Fig. 3, lane 6) and almost certainly results from a reversal of the original transformation event—i.e., excision by homologous recombination.

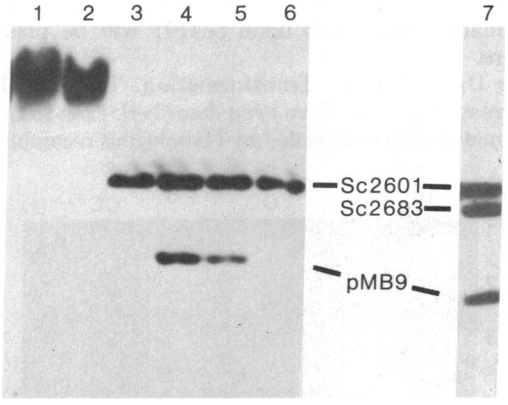


FIG. 3. Separation in 0.7% agarose; 1 μg of DNA per lane. Lanes: 1, 4, and 5, A3617C (pMB9-Sc2601); 2, A3617C (YIp1); 3, A3617C (untransformed); 6, his$^-$ segregant of A3617C (pMB9-Sc2601); 7, A3617 (pMB9-Sc2683). Samples in lanes 3–7 were cleaved with *Eco*RI. Samples in lanes 1 and 2 were uncleaved. The hybridization probe was ^{32}P-labeled pMB9-Sc2601 DNA. Appropriate size standards were present in each gel. Lane 7 is not from the same gel as lanes 1–6.

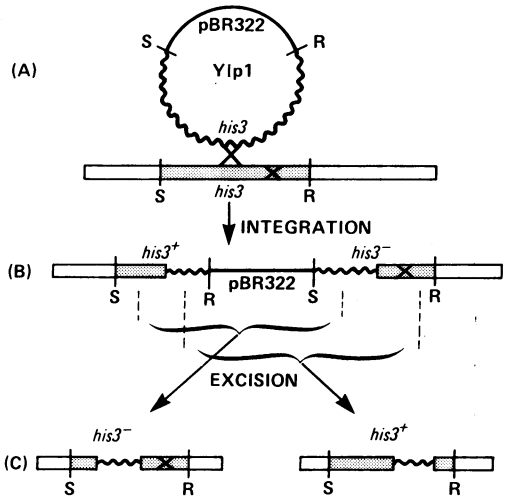


FIG. 4. (*A*) Homologous recombination at *his3* sequences between YIp1 and yeast chromosomal DNAs. (*B*) Integrated structure of a low-frequency transformant. (*C*) Possible structures after excision of transforming DNA. The *his3*-532 lesion maps in the middle of all cloned His$^+$ DNA fragments used in this paper (unpublished data).

The results indicate that low-frequency yeast transformation by *his3* hybrid DNAs is always associated with homologous recombination at the *his3* locus. Hinnen *et al.* (5) reported that most transformation events by a hybrid DNA molecule containing the yeast *leu2* gene could be accounted for by homologous recombination at the *leu2* locus. They also found transformants in which the *leu2*$^+$ character was unlinked to *leu2*$^-$ (as in model 3) and transformants that did not contain any foreign DNA sequences (as in model 4). However, the transforming DNA used in their experiments contains a sequence of yeast DNA that is present at many sites in the yeast genome (11). Therefore, *leu2*$^+$ transformants that have the transforming DNA integrated at loci other than *leu2* may be explained by homologous recombination at one of these repeated DNA sequences.

High-Frequency Transformation by Hybrid DNAs Containing Yeast Plasmid Sequences Is Accompanied by Autonomous Replication and Chromosomal Integration. Naturally occurring deletions across the unique *Hpa* I cleavage site of the endogenous yeast plasmid DNA (Scp1) do not affect replication of the plasmid in yeast cells (6). Vectors YEp2 and YEp4 contain the entire yeast plasmid sequences in functional form. A 7.1-kb *Sal* I DNA fragment (Sc2703) was inserted into YEp4. YEp4-Sc2703 DNA transforms *his3*$^-$ yeast to His$^+$ at a frequency of 5000–20,000 colonies per μg. The structural analysis of 42 of these His$^+$ transformants (3 of which are shown in Fig. 5) indicates that all 42 contain autonomously replicating, covalently closed, circular molecules of identical size to that of the transforming DNA. Based on ethidium bromide staining of the agarose gel (Fig. 5), we estimate that each cell has an average of 5–10 hybrid DNA molecules. In addition to replicating autonomously, the transforming DNA (in all cases) is integrated into the chromosome by homologous recombination at the *his3* locus; thus, these molecules are yeast episomes.

High-frequency transformants dependent upon endogenous yeast plasmid (Scp1) DNA sequences are extremely unstable with respect to the His$^+$ character. After 15 generations of growth in nonselective liquid medium, 97% of the cells are His$^-$. Fifteen of 15 His$^-$ segregants lost both the autonomously replicating and the chromosomally integrated structures. Seven of seven colonies that remained His$^+$ after this nonselective passaging contained both forms of the transforming DNA (data

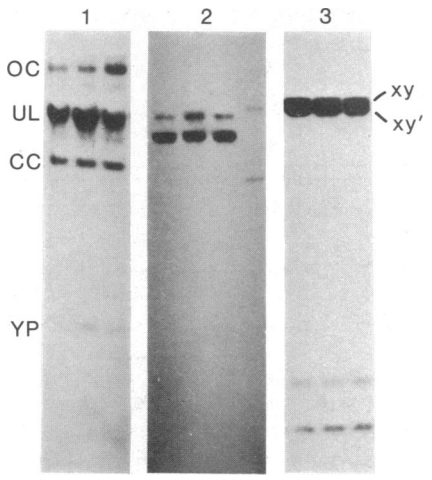


FIG. 5. Three isolates of A3617 (YEp4-Sc2703). Samples: 1, uncleaved DNA separated in 0.4% agarose; 2, *Pvu* I-cleaved DNA separated in 0.4% agarose [includes one lane of A3617C (YIp1) DNA]; 3, *Eco*RI-cleaved DNA separated in 0.7% agarose. The hybridization probe was ^{32}P-labeled pBR322 DNA. Abbreviations: CC, closed circles of transforming DNA; OC, open circles of transforming DNA; UL, uncleaved linear chromosomal DNA of high molecular weight; xy,xy', linear fragments containing either the XY or the XY' form of Scp1 DNA; YP, closed circular Scp1 molecules. The mobilities of these species were determined with appropriate standards present in the same gel. Scp1 DNA contains a sequence that is nontandemly repeated in an inverted orientation (6) (Fig. 1). Recombination in *E. coli* across these inverted repeat sequences has not been detected; i.e., YEp4-Sc2703 DNA is isolated from *E. coli* in only one form. This figure indicates that high-frequency transformants contain two forms of these molecules; therefore, recombination in yeast across these inverted repeat sequences does occur. Analogously, this suggests that the XY and XY' forms of Scp1 DNA are normally interchangeable in yeast. Because the transforming DNA (in one form) rapidly becomes equilibrated to roughly equimolar quantities of both forms, it is likely that the recombination across the inverted repeat sequence occurs by site-specific recombination.

not shown). Comparison of the genetic instability of transformants containing hybrids with or without Scp1 DNA sequences suggests that integration of the yeast plasmid into the chromosome may be detrimental to growth of the yeast cell. This is consistent with the observation that Scp1 does not normally integrate into the chromosome (6).

The entire sequence of Scp1 is not essential for the autonomous replication of hybrid DNA molecules in yeast. Naturally occurring deletions of Scp1 DNA replicate autonomously in yeast cells (6). YEp6 contains only 2 kb of Scp1 DNA, yet its transformation properties are indistinguishable from those of YEp2. Other fragments of Scp1 DNA, which are sufficient for autonomous replication of hybrid DNA molecules, have no sequences in common except for one copy of the inverted repeat sequence (data not shown). It is possible that the inverted repeat sequences are essential for Scp1 DNA replication.

Although high-frequency transformation is almost certainly dependent upon autonomous replication of the transforming DNA, it is striking that all 42 His$^+$ transformants tested also contain integrated structures. Because the His$^+$ character has not been separated from the presence of both the autonomous and integrated forms, it is not clear which (or both) form expresses *his3*. It is also possible that some cells of a culture of a high-frequency transformant lack either of the two forms; i.e., the transforming DNA may equilibrate rapidly between autonomous and integrated copies. In relation to *his3* expression, the intracellular location of the autonomously replicating form of the transforming DNA is significant. Although it is believed that Scp1 DNA is predominantly found in the cytoplasm (12), the observation that hybrid DNA molecules can integrate into the chromosome strongly suggests their presence in the nucleus. The possibility of nuclear copies of Scp1 DNA is also suggested by the fact that yeast cells transcribe discrete species of poly(A)-containing RNA from Scp1 DNA (J. Broach, personal communication).

In the course of these experiments, we observed that there is DNA sequence homology between the *E. coli* plasmids pBR322 and pMB9 and the yeast plasmid Scp1. This is best illustrated in Fig. 5. The degree of homology is fairly weak because hybridization was not detected under more stringent renaturation conditions.

Autonomous Replication and High-Frequency Transformation Dependent upon a Yeast Chromosomal Sequence. In other work we had isolated a 1.4-kb yeast DNA fragment (Sc4101) containing the *trp1* gene by complementation of *E. coli* mutants lacking *N*-(5'-phosphoribosyl) anthranilate isomerase. In initial transformation experiments, hybrid DNA molecules containing Sc4104 (such as YRp7) transformed *trp1*$^-$ yeast cells to Trp$^+$ at the surprisingly high frequency of 500–2000 colonies per μg. Furthermore, YRp7-Sc2605 hybrids (containing both *trp1* and *his3* genes) cotransformed *trp1*$^-$ *his3*$^-$ strains to His$^+$ and Trp$^+$ in all 175 transformants examined, demonstrating that the high transformation efficiency is dependent upon the presence of the 1.4-kb chromosomal sequence and is not due to an aberration of the *trp1* transformation. When 30 of the resulting transformants were examined (4 are shown in Fig. 6), the transforming sequences were always detected as closed circular DNA molecules. None of the hybrids was found integrated into the yeast chromosomal DNA.

The behavior of hybrid DNAs containing Sc4101 suggests that these molecules act like yeast minichromosomes. Thus, a yeast chromosomal sequence permits hybrid DNA molecules to replicate autonomously and to express structural genes (both *trp1* and *his3*) without recombining with host chromosomal sequences. This observation is striking because other modes of transformation are always accompanied by homologous recombination even when the integrated structures are highly unstable (as in Scp1 hybrid molecules). Because Sc4101 hybrids containing other yeast sequences (e.g., YRp7-Sc2605) exhibit this behavior, the failure to integrate is not explained by suppression of homologous recombination at the *trp1* locus. These transformation properties of Sc4101 hybrids are of special interest in light of the close proximity of *trp1* to the centromere of chromosome IV (1). Further studies on high-frequency transformation dependent upon Sc4101 will be presented elsewhere.

Some Uses of Yeast Transformation. Three modes of transformation of yeast have been described. Low-frequency transformation is accompanied by homologous recombination

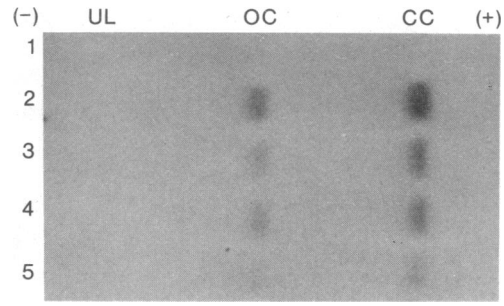


FIG. 6. Uncleaved DNA from four isolates of D13-1A (YRp7-Sc2605) (lanes 2–5) and one isolate of D13-1A (lane 1) were separated in 0.5% agarose and probed with ^{32}P-labeled pBR322 DNA. Abbreviations as in Fig. 5.

between transforming DNA sequences and yeast chromosomal DNA. High-frequency transformation dependent upon yeast plasmid sequences is accompanied by autonomous replication and chromosomal integration of the transforming DNA. The high-frequency transformation dependent upon a yeast chromosomal sequence (Sc4101) is also accompanied by autonomous replication of the transforming DNA, but the hybrid molecules fail to integrate into the host chromosomal DNA. Although the mechanisms for the two modes of high-frequency transformation are unclear, the properties of these modes are sufficiently defined so that they may be applied to a wide range of genetic manipulations.

By using yeast transformation, a hybrid containing a desired yeast gene can be directly identified by complementation of a yeast mutation. In particular, high-frequency transformation should prove invaluable as a general method for the cloning of any yeast gene. Because all high-frequency transformation vectors are autonomous replicons in yeast and in *E. coli*, the hybrid DNA molecules are readily interchanged between these two organisms. For example, a pool of hybrid DNA molecules of YEp2 containing unselected fragments of yeast DNA could be isolated from *E. coli* cells and transformed into an appropriate yeast strain selecting for the gene of interest. The autonomously replicating DNA isolated from such a yeast transformant could then be introduced back into *E. coli*. Plasmid DNA isolated from these *E. coli* cells should contain the desired yeast gene. As a test of this system, we have cloned *Pst* I DNA fragments of yeast into YEp2. From such a hybrid pool, we have selected a hybrid that contains the predicted 2.8-kb *Pst* I DNA fragment containing the *his3* gene. In some cases, the yeast mutation to be complemented may not be directly selectable. However, because YEp6 contains the *his3* gene, transformation of a YEp6 hybrid pool into a strain that contains the mutation of interest and is also *his3*$^-$ allows one to select His$^+$ transformants before screening for the desired marker.

An important application of yeast transformation is that of introducing physically defined alterations of a given DNA fragment back into yeast cells in order to examine the phenotypic effect of the alteration. For example, the introduction of a cloned sequence into yeast cells without radically altering the existing genome would permit detailed complementation analysis. Because yeast DNA sequences cloned in YRp7 are autonomously replicated and functionally expressed in the absence of significant recombinations with the yeast genome, this vector is ideal for such manipulations. Thus, haploid strains containing YRp7 hybrid DNA molecules are merodiploid for the cloned yeast sequences in a manner analogous to F′-containing *E. coli* strains. Such complementation analyses have been performed to study the expression of the yeast *his3* gene.

In addition, it would be useful to replace completely a functional genomic yeast sequence with a physically or genetically altered derivative. Low-frequency transformation could be used to perform these manipulations. It is essential that common DNA sequences flank both sides of the cloned alteration (e.g., deletion, insertion, altered restriction endonuclease site, genetic lesion). Integration and excision of transforming DNA containing an altered common DNA sequence generates two classes of segregants (Fig. 4). Some contain one copy of the altered common sequence at the normal chromosomal location; the others are indistinguishable from the original untransformed strain. The inclusion of a separate, functional yeast gene in the hybrid DNA makes it possible to select independently for the transformation and segregation events. Alterations of a given sequence are possible even if the sequence itself has no selectable phenotype.

The ability to isolate defined yeast genes and to introduce them back into yeast is essential for detailed studies of gene structure and function in this eukaryote. In addition, it is now possible to study the expression of other eukaryotic DNAs in yeast. Methods such as those described in this paper make it possible to genetically manipulate *S. cerevisiae* as easily as *E. coli*.

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