Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 177–181, January 1994
Genetics

The frequency of gene targeting in yeast depends on the number of target copies

JOHN H. WILSON*, WAI-YING LEUNG†, GIOVANNI BOSCO†, DE DIEU*, AND JAMES E. HABER†‡

*Department of Biochemistry, Baylor College of Medicine, Houston, TX 77030; and †Rosenstiel Center and Department of Biology, Brandeis University, Waltham, MA 02254

Communicated by Salih J. Wakil, September 13, 1993 (received for review February 5, 1993)

ABSTRACT **We have compared the efficiency of transformation by linear DNA fragments in yeast strains carrying different numbers of homologous targets for recombination. In strains carrying dispersed copies of a target and in strains carrying tandem arrays, the frequency of transformation is proportional to the number of targets. This result is in contrast to previous studies of transformation in mammalian cells, where targeted integration was insensitive to the number of targets. We conclude that, in yeast, the search for a homologous partner is a rate-limiting step in the successful recombination of linearized DNA fragments. Furthermore, the fact that we obtain the same results with both dispersed and clustered targets argues against models of homology searching in which DNA becomes nonspecifically associated with a chromosome and then slides along the DNA until homology is encountered.**

Double-strand breaks (DSBs) in DNA promote homologous recombination in eukaryotes (reviewed in refs. 1–4). Thus, when DSBs are created at the boundaries of a DNA segment that shares homology with a target site, one observes the efficient replacement of the target site region with the "ends-out" DNA, both in *Saccharomyces cerevisiae* (2) and in mammalian cells (3, 5). Although some of the molecular steps in recombination have been directly observed in synchronized yeast cells undergoing DSB-mediated recombination (6–8), many questions remain about how the ends of DNA locate their homologous partners. One unresolved issue is: what are the rate-limiting steps in recombination?

Recently, Zheng and Wilson (9) investigated the rate-limiting steps in the transformation of mammalian cells by linearized DNA. They used two cell lines, one of which contained 2 copies of the dihydrofolate reductase (DHFR) gene. The second line contained ≈800 copies of the DHFR gene, located in three principal clusters of amplified copies found in different chromosomes. When these two lines were transformed with a segment of the DHFR gene into which a selectable gene had been introduced, a few percent of the total transformants arose by homologous recombination and the rest by nonhomologous insertions into various chromosomes. Surprisingly, the proportion of events which occurred by targeted integration into a DHFR gene was the same whether there were 2 copies or 800. These results suggested that successful homologous recombination was not rate-limited by the search for a homologous partner.

It was therefore of interest to know whether the same rules would apply in *S. cerevisiae*, where nearly all integration of linearized DNA containing a selectable marker occurs by homologous recombination (3, 10). We have examined this question by measuring transformation efficiencies in strains with a single copy of a target sequence and in isogenic strains with multiple copies of the identical target, located at several dispersed sites or tandemly arrayed at one site.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Strains. Strains WYL161, WYL162, and WYL198 are isogenic strains containing varying numbers of the *leu2-A,R* target gene, in which both the *Asp*718 (A) and *Eco*RI (R) sites have been ablated. These strains have the basic genotype: *MATa/MATα leu2-A,R/leu2-A,R met13-2/met13-4 lys2/lys2 ade1/ade1 trp5/TRP5 TRP1/trp1 can1/CAN1 cyh2/CYH2*. Strain WYL161 (Fig. 1*A*) carries a single copy of the *leu2-A,R* allele at its normal location; the gene on the other homologue was deleted by transformation with a *Pst* I fragment in which the *Xho* I–*Eco*RV segment including most of the *LEU2* gene was replaced by a *Hin*dIII *URA3* fragment (E. J. Louis and J.E.H., unpublished work). Strain WYL198 (Fig. 1*B*) is homozygous for the deletion at *LEU2* but has a single copy of the *leu2-A,R* gene inserted by homologous recombination at *HIS4*, creating a disruption of the *HIS4* gene (11, 12). Strain WYL162 (Fig. 1*C*) carries eight copies of the *leu2-A,R* target, including the normal *leu2-A,R* alleles and homozygous insertions of the *leu2-A,R* gene at *URA3*, *HIS4*, and *MAT*, as described (11, 12). This strain was constructed by a series of crosses between isogenic derivatives carrying single insertions of *leu2-A,R* at one of the three ectopic locations.

Strains with varying numbers of tandemly arranged targets for transformation were provided by C. Denis (University of New Hampshire). Strain 500-16 (*MATa ura1 his4 trp1 adh3 adr1-1*) (13) was transformed with the pBR322-derived plasmid ADR1-262 (13) to provide a series of isogenic derivatives that contained 1–20 tandem copies of the plasmid integrated at the *trp1* locus.

Transformation Conditions. Cells were transformed by the lithium acetate method of Schiestl and Gietz (14). Purified DNA fragments of the *LEU2* gene were obtained by gel electrophoretic separation of a 1.97-kb *Hpa* I–*Sal* I fragment from a pGEM (Promega) plasmid, pJH353, that contains the *Pst* I fragment surrounding *LEU2* (15). The fragment was purified by Prep-a-Gene (Bio-Rad) according to the manufacturer's instructions. Similarly, a 4.57-kb *Eco*RI–*Pvu* II fragment containing the *LYS2* gene (16) was purified from plasmid pJH146. Approximately 4 μg of purified *LEU2* fragment and 2.5 μg of the *LYS2* fragment were used in each transformation experiment.

Plasmid pJH835 was constructed for these experiments. The 2.2-kb *Sma* I–*Hae* III fragment of the *URA1* gene (17) was inserted in the *Sac* I site of pBR322. A *Pst* I–*Aat* II fragment of this plasmid, thus containing the *URA1* gene flanked by pBR322 sequences (Fig. 1*D*), was purified as described above. A 7.5-kb *Pst* I restriction fragment containing the *HIS4* gene was isolated from plasmid R82 (18).

RESULTS

To compare targeting at multiple dispersed sites, we constructed three diploid strains: two with a single copy of a

Abbreviations: DHFR, dihydrofolate reductase; DSB, double-strand break.

‡To whom reprint requests should be addressed.

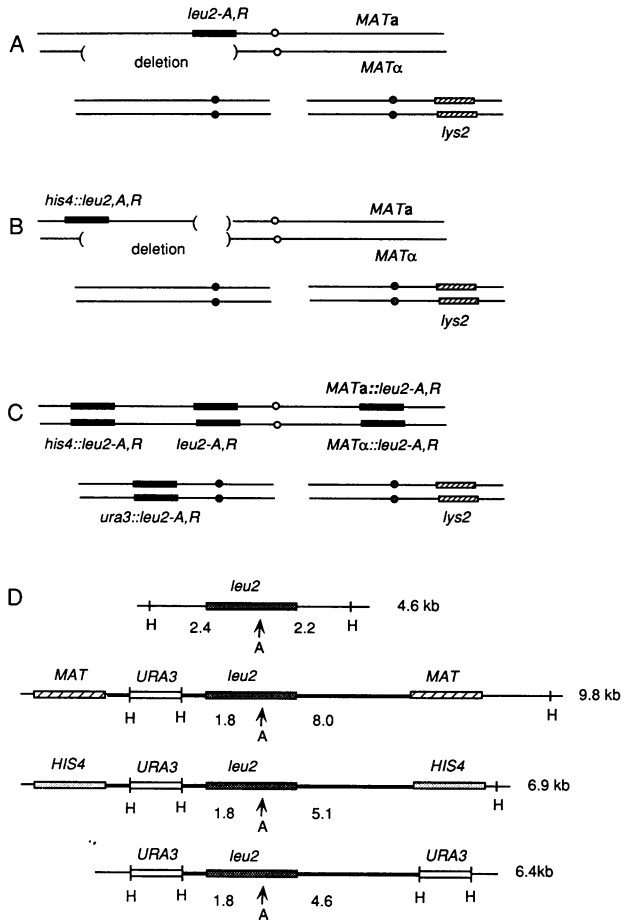

FIG. 1. An isogenic set of strains containing varying numbers of the *leu2-A,R* target gene. (*A*) Strain WYL161 carries a single copy of the *leu2-A,R* allele at its normal location. (*B*) Strain WYL198 is homozygous for a deletion at *LEU2* but has a single copy of the *leu2-A,R* gene inserted by homologous recombination at *HIS4*. (*C*) Strain WYL162 carries eight copies of the *leu2-A,R* target, with insertions at *URA3*, *HIS4*, and *MAT*. (*D*) A restriction map of the *leu2-A,R* locus and of the *URA3–leu2-A,R*–pBR322 construct inserted at the ectopic locations *his4*, *MAT*, and *ura3*. Transformation of *leu2-A,R* to Leu$^+$ results in the creation of two *Hin*dIII–*Asp*718 restriction fragments (A, *Asp*718; H, *Hin*dIII). In the case of the inserts at the three ectopic locations, the sizes of the smaller *Hin*dIII–*Asp*718 restriction fragment are identical, whereas the larger fragments are different for each location. The sizes of the *Hin*dIII fragments homologous to a *LEU2* probe are given to the right of each locus. The sizes of the *Hin*dIII/*Asp*718-cut fragments are given below each locus.

leu2-A,R (Leu$^-$) target site and one with eight copies (Fig. 1). Strain WYL161 contains a single copy of the *leu2-A,R* double mutant at its normal chromosomal location (the copy on the other chromosome has been deleted). WYL198 also contains only one copy of *leu2-A,R*, but in this instance it is integrated at the *HIS4* locus (the DNA at the normal *LEU2* locus is deleted from both homologues). WYL162 contains eight copies of the *leu2-A,R* DNA, two at the normal location and two each integrated at *HIS4*, *URA3*, and *MAT* (11). All three strains are also homozygous for a *lys2* mutation. These strains were transformed with a mixture of purified linear fragments of *LEU2* and *LYS2* DNA, as further described in Table 1. The numbers of Leu$^+$ and Lys$^+$ transformants were determined independently and their ratio was then calculated to normalize for any differences in DNA concentrations or transformation efficiency from experiment to experiment. The results in Table 1 show that the strain with eight targets for transformation to Leu$^+$ yielded 9.6 times as many transformants as the single-target strain WYL161. Compared with the single-target strain WYL198, the eight-target strain had 3.8 times as many transformants. The difference between the two single-target strains appears to reflect an inherent difference in efficiency of recombination at the two target sites, though this was not evident when the sites of gene conversion were analyzed in WYL162 (see below). An average of the two results gives a 6.7-fold advantage to the eight-target strain compared with single-target strains.

These results suggest that, in yeast, the number of targets alters the efficiency of transformation. However, it was also possible that one of the sites where *leu2-A,R* was inserted was unusually "hot" for recombination. We have ruled out this possibility by analyzing the sites of integration of the wild-type *LEU2* sequences by Southern blotting. DNA from individual Leu$^+$ transformants was cleaved with *Hin*dIII, which cuts outside of the *leu2-A,R* or *LEU2* sequences, and with *Asp*718, which cleaves *LEU2* but not *leu2-A,R*. A typical analysis is shown in Fig. 2, with the sources of restriction fragments containing the *leu2-A,R* targets indicated on the left and the sources of the *LEU2* fragments (the sites of targeting) shown on the right. Among 58 Leu$^+$ transformants, we found nearly equal numbers of conversions at each *leu2-A,R* site, with 12 at the original *leu2* locus, 11 at *HIS4*, 13 at *MAT*, and 14 at *URA3*. Thus, despite the difference in the efficiencies of transforming a single-target strain at *leu2* (WYL161) or at *HIS4* (WYL198), there was no significant difference in the frequencies with which one or the other site was used as a target in WYL162. Eight of the transformants (14%) were not simple gene convertants. Three of these had two conversion events each, and 2 others were complex, with unexpected additional *LEU2*-homologous bands that may reflect nonhomologous insertions of LEU2 (10). The remaining 3 showed the parental pattern (see lanes 7 and 14 in Fig. 2); we have not analyzed these three exceptions further.

One difference between these results and those of Zheng and Wilson (9) using mammalian cells is that the *leu2-A,R* targets were all dispersed, while the DHFR genes were highly clustered. If the ends of linearized DNA sought their partners by "tracking" along DNA until a homologous sequence was found, a cluster of copies might not differ significantly from a single copy as a target for transformation. To investigate this question further in yeast, we took advantage of a set of isogenic strains that contained multiple, tandemly integrated copies of a pBR322 plasmid integrated at the *TRP1* locus (13) (Fig. 3). We chose four of these strains, carrying 1 to ≈20 copies of the plasmid (Table 2). These strains also carry *his4* and *ura1* mutations. We inserted a *URA1* gene into the *Sca* I site of pBR322 so that this marker could be integrated into the pBR322 sequences in the various strains. To control for differences in transformation efficiency, we mixed linear, purified *HIS4* DNA (for which there is one target in all strains) and a segment of pBR322 carrying the *URA1* gene. The results of several transformation experiments are shown in Table 2. As we found with multiple, dispersed copies of a target sequence, the efficiency of transformation to Ura$^+$, relative to His$^+$, increased linearly with the number of possible targets, so that there were about 7.2 times as many Ura$^+$ transformants for a strain carrying approximately 19 tandem copies of the target as there were for a strain with only 1 copy.

Because even a strain with no integrated copies of the pBR322 plasmid also could be transformed to wild type by the *URA1* sequences by conversion of the resident *ura1* mutation, we also examined a strain with no inserts. A comparison of the transformation of this strain and of a strain with one insert shows that the resident locus was used only about one-ninth as often as the plasmid insert (Table 2). We attribute this difference to the fact that the linearized trans-

Table 1. Transformation to Leu$^+$ of strains containing multiple dispersed copies of the *leu2-A,R* target

Strain	No. of target copies	No. of Leu$^+$ transformants	No. of Lys$^+$ transformants	Ratio of Leu$^+$/Lys$^+$	Normalized ratio
		Experiment 1			
WYL161	1	19	11	1.7	1.0
WYL198R	1	124	30	4.1	2.4
WYL162	8	1249	76	16.4	9.5
		Experiment 2			
WYL161	1	62	78	0.8	1.0
WYL198R	1	115	55	2.1	2.7
WYL162	8	1456	189	7.7	9.8

Strains were transformed, as described in *Materials and Methods*, with a mixture of two DNA fragments: a 1.98-kb *Hpa* I–*Sal* I fragment containing the *LEU2* gene and a 4.57-kb *Eco*RI–*Pvu* II *LYS2* fragment. The *LEU2* and *LYS2* DNAs were combined in approximately equal amounts before transformation. The same mixture of the two fragments was used in all of the transformations of one experiment. For the most part, Lys$^+$ transformants were not Leu$^+$ or vice versa.

forming DNA has ends which can recombine directly with pBR322 sequences inserted at *TRP1* but which would have to be removed before the *URA1* gene could recombine with the resident *ura1* locus (see Fig. 3*D*). The conclusion that nearly all of the Ura1$^+$ transformations involve integrations into one of the possible copies of pBR322 was confirmed by Southern blot analysis of 20 transformants, all of which showed integrations into the tandem array of plasmid copies at *trp1*. Thus, the frequency of targeted integration increases proportionally to the number of target copies.

DISCUSSION

Our experiments argue that the efficiency of targeted recombination in yeast is approximately linearly dependent on the number of chromosomal targets, whether the targets are dispersed in the genome or tandemly arranged. The equal frequencies of targeting to each of the dispersed copies of *leu2-A,R* indicate that targeting efficiency is not strongly influenced by the surrounding chromosomal context at the four sites we have tested. We cannot easily explain the 2-fold difference in transformation efficiency when a *leu2-A,R* target was located at *leu2* or adjacent to *HIS4* (Table 1), especially as this difference was not reflected in the frequency with which the two sites were used as targets in the eight-target diploid. The general lack of an effect of chromosomal context was also found in a previous study of spontaneous mitotic ectopic recombination between *leu2* alleles at these same four sites (12). Previous studies of integrative transformation can also be interpreted in this way, but in those experiments a plasmid with unique and repeated (ribosomal DNA) sequences was found to integrate preferentially into the ribosomal DNA tandem repeats (19); however, in such studies the differences could also be attributed to inherent differences in recombination of the two different homologous targets.

We interpret our data to say that finding a homologous partner is the rate-limiting step for targeting of linear DNA to

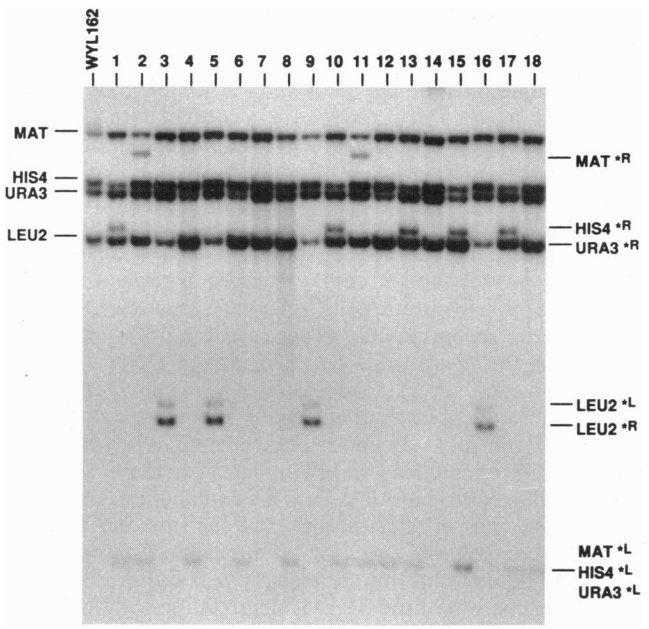

FIG. 2. Analysis of Leu$^+$ transformants of strain WYL162, a diploid strain containing *leu2-A,R* genes at *leu2* and inserted at *his4*, *MAT*, and *ura3*. As described in Fig. 1, a *Hin*dIII/*Asp*718 restriction endonuclease digestion produces unique fragments diagnostic of the location of the *leu2-A,R* segment that is transformed to Leu$^+$. The positions of the *leu2-A,R Hin*dIII restriction fragments are marked on the left; the positions of the *Asp*718–*Hin*dIII restriction fragments of the Leu$^+$ transformants, to the left (*L) and right (*R) of the *Asp*718 site, are indicated on the right.

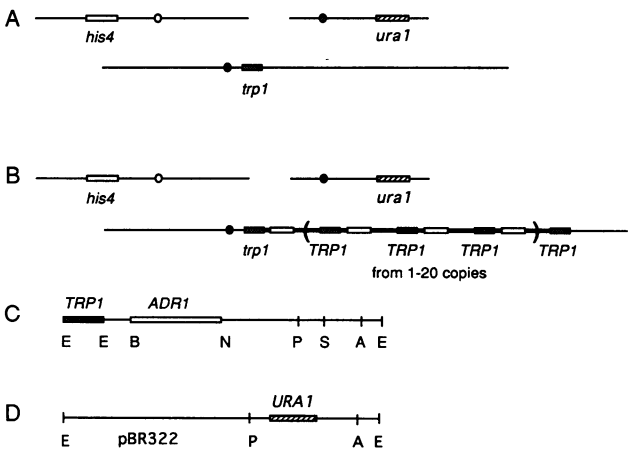

FIG. 3. Strains with varying numbers of tandemly arranged targets for transformation. Strain 500-16 (*A*) was transformed with the pBR322-derived plasmid ADR1-262 (*C*) to produce a series of isogenic derivatives that contained between 1 and 20 copies of the plasmid integrated at the *trp1* locus (*B*). These strains were transformed with *Pst* I–*Aat* II fragment of plasmid pJH835 (*D*) that contains the 2.2-kb *Sma* I–*Hae* III fragment of the *URA1* gene inserted at the *Sca* I site. The 2.8-kb *Pst* I–*Aat* II fragment can integrate directly into the homologous pBR322 sequences inserted at the *TRP1* locus on chromosome IV. Ura$^+$ transformants can also arise by gene conversion of the *ura1* mutation on chromosome XI. These strains also contain a mutation in the *his4* gene that can be transformed to His$^+$ with a 7.5-kb *Pst* I restriction fragment containing the *HIS4* gene. E, *Eco*RI; B, *Bam*HI; N, *Nru* I; P, *Pst* I; S, *Sca* I; A, *Aat* II.

Table 2. Transformation of strains with different numbers of tandemly arranged targets for *URA1*

Strain	No. of pBR322 target copies*†	No. of His+ transformants	No. of Ura+ transformants	Ratio of Ura+/His+	Normalized ratio
		Experiment 1			
12	1.0	31	13	0.42	1.0
35	18.7	29	122	4.2	10.0
		Experiment 2			
12	1.0	76	38	0.5	1.0
2-11	6.4	49	46	0.94	1.9
2-18	9.1	55	81	1.47	2.9
35	18.7	124	281	2.27	4.5
		Experiment 3			
500-16	0	75	12	0.16	0.12
12	1.0	31	43	1.39	1.0
2-11	6.4	56	219	3.91	2.8
2-18	9.1	47	385	8.19	5.9
35	18.7	93	911	9.80	7.1

An isogenic set of strains derived from strain 500-16 (*ura1 his4*) and containing 0–20 copies of a *TRP1–ADR1*–pBR322 plasmid integrated into the *trp1* locus (see *Materials and Methods*) were compared for their transformation efficiency. The cells were transformed with a mixture of linearized DNA for the *HIS4* gene (the 7.5-kb *Pst* I fragment) and for the *URA1* gene (a 2.8-kb *Pst* I–*Aat* II fragment; see Fig. 3*D*) inserted into pBR322. The transformants for each marker were counted separately and the ratios of transformation efficiency were calculated. The results of three experiments are shown.

*The average number of pBR322-containing plasmids integrated in each strain was determined by densitometric analysis of a Southern blot of *Aat* II-digested DNA, probed with labeled pBR322. A single integration of plasmid pJH835 produces a pBR322-homologous restriction fragment of 5.7 kb, while tandem duplications of the plasmid yields both a single copy of the 5.7-kb band and one or more copies of a 9.5-kb band. The total number of copies integrated at *trp1* is therefore the sum of the radioactivity in these two bands, normalized to the intensity of the single-copy band. The non-integral numbers most likely reflect the fact that copy number within repeated arrays may vary among some cells of the population.

†All strains also have a copy of the *ura1* gene on chromosome XI.

homologous sequences in yeast. Copy-number dependence of gene targeting in yeast stands in sharp contrast to the apparent lack of such dependence in mammalian cells. For mammalian cells the conclusion that the search for homology is not rate-limiting is based on two observations: similar frequencies of targeting to DHFR sequences present at 2 or 800 copies (9) and similar frequencies of targeting to chromosomally integrated plasmid sequences present as 1 copy, 4 dispersed copies, or 5 tandem copies (20). This conclusion is also supported by microinjection studies which show that the frequency of gene targeting does not change over the range of a few copies to a few thousand copies of injected DNA (20, 21). [In yeast, the efficiency of recombination does seem to be dependent on donor copy number (11, 22).]

Given numerous observations in yeast (1, 2) and mammalian cells (23–26) that gene targeting is consistent with DSB repair models of recombination, it seems unlikely that the pathways for gene targeting are fundamentally different; however, the molecular details of these events are not known. Even if the mechanisms are fundamentally similar, the rate-limiting steps that govern the efficiency of homologous recombination might be very different. Such a difference in the rate-limiting step might reflect different levels or efficiencies of individual recombination proteins. Alternatively, the difference in rate-limiting step may result from a difference in the activity of some interfering process. For example, it is likely that most input DNA is ultimately destroyed by exo- and/or endonucleases in both yeast and mammalian cells. A slow rate of destruction in mammalian cells could give the input DNA adequate time to search the entire genome, with the result that homology searching would not show up as the rate-limiting step. If destruction were faster in yeast, the window of opportunity might be sufficiently restricted so that only a fraction of the genome could be searched before the input DNA was destroyed, with the result that homology searching would show up as the rate-limiting step. Further experiments in yeast and mammalian cells will be required to decide among these alternatives. The fact that the increase in transformation efficiency was less than 1:1 with the increase of target copies may mean that more than one step influences the final outcome, though the principal one must be the location of a homologous partner.

Our results also might seem to be in conflict with a previous study we carried out on the effects of copy number on meiotic gene conversion in yeast (11). In that study, the likelihood of converting *LEU2* to *leu2-A,R* was approximately the same when there was one or six *leu2-A,R* donor copies at various ectopic locations. There are several possible reasons why this result is, in fact, not in conflict with our present study of transformation in mitotic cells. A trivial explanation would be that meiotic recombination proceeds by a different mechanism than the transformation of linearized DNA in mitotic cells. However, several recent studies have shown that DSBs are a major type of lesion that initiates meiotic recombination (27, 28). Even so, DSBs in meiotic cells might be acted upon by different exonucleases that might allow the DSBs to be more stable than in mitotic cells. This explanation, too, seems unlikely in view of observations that the rate and extent of 5′-to-3′ exonuclease digestion of breaks on chromosomal DNA in meiotic and mitotic cells appear quite comparable [there seems to be little 3′-to-5′ degradation (6, 29, 30)]. In more general terms, one might argue that meiotic cells must engage in a much higher level of homology searching than mitotic cells, as reflected in much higher rates of recombination in meiosis. Possibly, then, the homology-searching machinery is not rate-limiting during meiosis.

The explanation we favor is that, in the transformation experiments, where the input DNA is short-lived, the cut DNA is in the form of extrachromosomal fragments. In the meiotic experiments the DSBs are created in chromosomes.

We suggest that a DSB in a chromosome may signal a cellular "checkpoint" mechanism (31) to prevent the cell from progressing into mitosis until the lesion is repaired. Moreover, even if there is extensive 5′-to-3′ degradation of the chromosomal DNA, the 3′ ends would still be able to locate homologous regions and complete recombination (6, 8). We imagine that this would be true not only for meiotic cells, but for mitotic cells as well. In contrast, the presence of linearized transforming DNA may not signal the checkpoint, and extensive 5′-to-3′ degradation may destroy the relatively short DNA fragments.

These data also provide information about the way in which a transforming piece of DNA locates its homologous target. Various studies of the way genome-regulatory proteins locate their binding sites suggest that they first associate nonspecifically with DNA and then "slide" along the DNA until they reach their high-affinity binding site (32, 33). This sort of "one-dimensional" searching has also been suggested for the way DNA finds its homologous partners (34). Our experiments do not support this notion for yeast transformation. If transforming DNA first associated nonspecifically with chromosomal DNA and then moved along the chromosome until homology were found, we would expect that a cluster of target sites would not differ significantly from a single site in the efficiency with which transforming DNA was integrated. The similar dependence on copy number for both dispersed and tandem targets instead suggests that the search for homologous sequences is more likely to result from a series of random collisions ("three-dimensional" searches) rather than from a processive search of long segments of chromosomal DNA.

We are indebted to Clyde Denis, who provided the series of strains with tandem duplications of the pBR322 target sequence, and to A. Roy, who provided plasmid pFL392 containing the *URA1* gene. G.B. especially thanks Neal Sugawara for his role as a patient mentor. Neal Sugawara, Genya Ivanov, Susan Lovett, and Michael Lichten all made insightful suggestions to improve the manuscript. This work was supported by National Institutes of Health Grants GM29736 (to J.E.H.) and GM38219 (to J.H.W.).

1. Szostak, J. W., Orr-Weaver, T., Rothstein, R. J. & Stahl, F. W. (1983) *Cell* **33,** 25–35.
2. Petes, T. D., Malone, R. E. & Symington, L. S. (1991) in *Molecular and Cellular Biology of the Yeast Saccharomyces*, eds. Broach, J. R., Pringle, J. R. & Jones, E. W. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 407–521.
3. Capecci, M. (1989) *Science* **244,** 1288–1292.
4. Zimmer, A. (1992) *Annu. Rev. Neurosci.* **15,** 115–137.
5. Rothstein, R. (1991) *Methods Enzymol.* **194,** 281–301.
6. White, C. I. & Haber, J. E. (1990) *EMBO J.* **9,** 663–673.
7. Sugawara, N. & Haber, J. E. (1992) *Mol. Cell. Biol.* **12,** 563–575.
8. Fishman-Lobell, J., Rudin, N. & Haber, J. E. (1992) *Mol. Cell. Biol.* **12,** 1293–1303.
9. Zheng, H. & Wilson, J. H. (1990) *Nature (London)* **344,** 170–173.
10. Schiestl, R. & Petes, T. D. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 7585–7589.
11. Haber, J. E., Leung, W. Y., Borts, R. H. & Lichten, M. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 1120–1124.
12. Lichten, M. & Haber, J. E. (1989) *Genetics* **123,** 261–268.
13. Bemis, L. T. & Denis, C. L. (1988) *Mol. Cell. Biol.* **8,** 2125–2131.
14. Schiestl, R. & Gietz, R. D. (1989) *Curr. Genet.* **16,** 339–346.
15. Andreadis, A., Hsu, Y.-P., Kohlhaw, G. B. & Schimmel, P. R. (1982) *Cell* **31,** 319–325.
16. Morris, M. E. & Jinks-Robertson, S. (1991) *Gene* **98,** 141–145.
17. Roy, A. (1992) *Gene* **118,** 149–150.
18. Donahue, T. F., Farabaugh, P. J. & Fink, G. R. (1982) *Gene* **18,** 47–59.
19. Szostak, J. W. & Wu, R. (1980) *Nature (London)* **284,** 426–430.
20. Thomas, K. R., Folger, K. R. & Cappecci, M. R. (1986) *Cell* **44,** 419–428.
21. Rommerskirch, W., Graeber, I., Grässmann, M. & Grässmann, A. (1988) *Nucleic Acids Res.* **16,** 941–952.
22. Melamed, C. & Kupiec, M. (1992) *Mol. Gen. Genet.* **235,** 97–103.
23. Jasin, M. & Berg, P. (1988) *Genes Dev.* **2,** 1353–1363.
24. Valancius, V. & Smithies, O. (1991) *Mol. Cell. Biol.* **11,** 1402–1408.
25. Pennington, S. & Wilson, J. H. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 9498–9502.
26. Hasty, P., Rivera-Perez, J. & Bradley, A. (1992) *Mol. Cell. Biol.* **12,** 2464–2474.
27. Sun, H., Treco, D., Schultes, N. P. & Szostak, J. W. (1989) *Nature (London)* **338,** 87–90.
28. Cao, L., Alani, E. & Kleckner, N. (1990) *Cell* **61,** 1089–1101.
29. Sun, H., Treco, D. & Szostak, J. W. (1991) *Cell* **64,** 1155–1161.
30. Ray, B. L., White, C. I. & Haber, J. E. (1991) *Mol. Cell. Biol.* **11,** 5372–5380.
31. Hartwell, L. H. & Weinert, T. A. (1989) *Science* **246,** 629–634.
32. Berg, O. G., Winter, R. B. & von Hipple, P. H. (1982) *Trends Biochem. Sci.* **7,** 52–55.
33. Fried, M. G. & Coruthers, D. M. (1984) *J. Mol. Biol.* **172,** 263–282.
34. Gonda, D. K. & Radding, C. M. (1983) *Cell* **34,** 647–654.