15. J. D. Mackenzie, *J. Am. Ceram. Soc.* **46**, 461 (1963); J. Arndt and D. Stöffler, *Phys. Chem. Glasses* **10**, 117 (1969).
16. P. W. Bridgman, *Proc. Am. Acad. Arts Sci.* **71**, 387 (1937).
17. In the recompression experiments, samples were initially loaded to 50 GPa, decompressed to 0 GPa, and then recompressed to high pressures.
18. In contrast, work hardening can increase the yield strength by more than a factor of 2 in crystalline materials with a limited number of slip systems [H. C. Heard, in *Flow and Fracture of Rocks*, H. C. Heard *et al.*, Eds. (American Geophysical Union, Washington, DC, 1972), pp. 191–209].
19. B. A. Proctor, I. Whitney, J. W. Johnson, *Proc. R. Soc. London Ser. A* **297**, 534 (1967).
20. At 0 GPa, silica glass fractures because the critical stress for nucleating and extending cracks is less than the critical stress for moving defects and dislocations. Thus the ductile creep strength must be greater than 4 GPa at room pressure and temperature. Extrapolations of experimental data suggest that the viscosity of silica glass is greater than 10^{30} Pa·s at 0 GPa and 300 K [W. Primak, *J. Appl. Phys.* **55**, 852 (1984); see also (*21*)]. These extrapolations provide an upper bound on the change in creep strength at room temperature; they imply that the time scale for viscous deformation decreases by 18 orders of magnitude between 0 and 10 GPa.
21. G. Urbain, Y. Bottinga, P. Richet, *Geochim. Cosmochim. Acta* **46**, 1061 (1982).
22. R. Hemley *et al.*, *Phys. Rev. Lett.* **57**, 547 (1986).
23. In previous experiments (*13*), we measured plastic flow in individual glass fragments under shear stresses of ~0.1 GPa at 10 GPa confining pressure. This value compares well with the results in Fig. 1, and it shows that the low strength at ~10 GPa is not due to grain boundary sliding in our samples.
24. The shape of a diamond cell sample resembles a thin disk. If the pressure gradient is measured outward from the center over a distance, r, the average pressure ($\overline{P}$) over that interval occurs at the point $r/\sqrt{2} = 0.707r$ from the center.
25. The observations of (*5*) have been interpreted as a range of Si coordinations distributed between four and six at 39 GPa, the highest pressure of their study. The strength data suggest that tetrahedral coordination may persist up to 60 GPa at 300 K.
26. This value is the average increase in Si–O distance in crystalline silicates between tetrahedral and octahedral structures. We compared bond lengths between α-quartz [L. Levien, C. T. Prewitt, D. J. Weidner, *Am. Mineral* **65**, 920 (1980)] and stishovite [W. Sinclair and A. E. Ringwood, *Nature* **272**, 714 (1978)] and between enstatite [N. Morimoto and K. Koto, *Z. Kristallogr.* **129**, 65 (1969)] and $MgSiO_3$ perovskite [E. Ito and Y. Matsui, *Earth Planet. Sci. Lett.* **38**, 443 (1978); T. Yagi, H. K. Mao, P. M. Bell, *Phys. Chem. Minerals* **3**, 97 (1978)].
27. M. A. Spackman, R. J. Hill, G. V. Gibbs, *Phys. Chem. Minerals* **14**, 139 (1987).
28. T. Murase, A. R. McBirney, *Geol. Soc. Am. Bull.* **84**, 3563 (1973).
29. Whereas eruptions of silica-rich magmas produce steep cinder cones, silica-poor melts form broad shield volcanoes and continental flood basalts [*Basaltic Volcanism Study Project* (Pergamon, New York, 1981)].
30. We thank H. K. Mao and Q. Williams for helpful discussions and two anonymous reviewers for helpful comments on an earlier manuscript. This work was supported by the National Science Foundation. R.J. thanks the Fairchild scholar program at the California Institute of Technology for partial support.

4 May 1988; accepted 18 July 1988

Mitotic Recombination Within the Centromere of a Yeast Chromosome

SUSAN W. LIEBMAN,* LORRAINE S. SYMINGTON,† THOMAS D. PETES‡

Centromeres are the structural elements of eukaryotic chromosomes that hold sister chromatids together and to which spindle tubules connect during cell division. Centromeres have been shown to suppress meiotic recombination in some systems. In this study yeast strains genetically marked within and flanking a centromere, were used to demonstrate that gene conversion (nonreciprocal recombination) tracts in mitosis can enter into and extend through the centromere.

CENTROMERIC DNA FORMS A TIGHT complex with proteins that is very resistant in vitro to nuclease digestion (*1*). One might expect that recombination could not occur within such a protein-DNA complex. Indeed, in *Drosophila*, meiotic recombination near the centromere is greatly suppressed (*2*). However, in yeast, we found that meiotic conversion tracts frequently span the centromere (*3*). We also found that meiotic conversion occurs at approximately normal rates near the centromere. In contrast, Lambie and Roeder (*4*) suggested that the yeast centromere represses meiotic conversion about fivefold. These different findings on the effect of the centromere on the rates of meiotic conversion may reflect differences in genetic background or the types of constructions used.

Mitotic gene mapping in yeast is based on the frequency of crossovers between genes and their centromeres. The original presumption (*5*) was that in mitosis, duplicated sister chromatids were held together by their centromeres while they recombined with their nonsister homologs. Consequently, the further a gene was from its centromere, the greater the chance of a crossover uncoupling sister alleles from the same centromere. Thus, about half the time, depending on the segregation of the homologous chromosomes, mitotic crossing-over will lead to heterozygous markers distal to the crossover becoming homozygous.

Recent evidence (*6–8*) suggests that a considerable portion of mitotic recombination occurs prior to chromosome duplication. The two-strand–stage model of recombination (*6*) suggests that crossovers occur in G_1 between homologous DNA single strands of the same polarity and are resolved

Table 1. Numbers and types of presumptive convertants derived from SLP-6 and S-5 parent strains. Standard genetic procedures for yeast were used to score for markers and to determine mating types (*22*). Trichodermin (TCM) resistance was scored on nonselective media containing 0.20 mg of the drug per milliliter. Trichodermin was stored as a 20 mg of solution in 50% ethanol at −20°C. The pluses and minuses indicate the growth of the strains on media lacking Leu, Ura, His, or Thr or the presence or absence of the Xho I or Nru I restriction sites. NA means not applicable. Representative presumptive α/α and **a/a** strains were shown to be diploid because the viability of meiotic progeny after crossing to diploid mating-type homozygous testers was good, while bad spore viability (characteristic of a triploid) was obtained when they were crossed to haploid testers. As expected genetic tests indicated that all heterozygous markers not on chromosome III remained heterozygous in the SLP-6 convertants. Numbers in heading state the number of convertants obtained.

Marker	SLP-6						S-5					
	ura3⁻						*ura3*⁻			Tcm resistance		
	21	1	2	1	1	2	2	2	2	5	10	4
Leu	+	−	+	+	−	+	+	+	−	+	+	−
Ura	−	−	−	−	−	−	−	−	−	+	−	−
Xho I	+/−	+/−	−/−	−/−	−/−	−/−	+/−	+/+	+/+	+/+	+/+	+/+
Nru I	+/−	+/−	+/−	+/+	+/+	+/+	NA	NA	NA	NA	NA	NA
His	NA	NA	NA	NA	NA	NA	+	−	−	−	−	−
Thr	NA	NA	NA	NA	NA	NA	+	+	+	+	+	+
MAT	α/**a**	α/**a**	α/**a**	α/**a**	α/**a**	α/α	α/**a**	α/**a**	**a/a**	α/**a**	α/**a**	**a/a**

Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637.

*On sabbatical leave from the Laboratory for Cellular Molecular and Developmental Biology, Department of Biological Sciences, University of Illinois at Chicago, Box 4348, Chicago, IL 60680.
†Present address: Institute of Cancer Research, College of Physicians and Surgeons of Columbia University, New York, NY 10032.
‡Present address: Department of Biology, University of North Carolina, Chapel Hill, NC 27599.

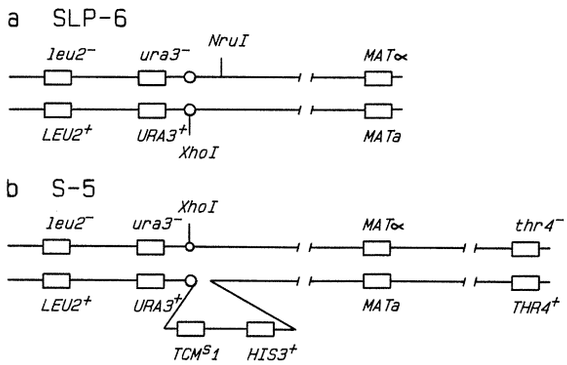

Fig. 1. Arrangement of heterozygous markers used to detect mitotic gene conversion in the centromere. (**a**) Strain SLP-6. SLP-6 was obtained by mating haploid segregants LS61-153A (*MAT***a**) and LS61-386B (*MAT*α) from a previously described (*3*) diploid, LS-61. In addition to the chromosome III markers shown, SLP-6 was heterozygous for *can*^{R}*1* (conferring recessive resistance to canavanine), *ade6*, and *trp1-1*. It was homozygous for *aro7*, and for *ura3* located at the normal chromosome V position. (**b**) Strain S-5. S-3, the *MAT*α parent of S-5 was obtained by transforming (*23, 24*) a *leu2*$^-$, *ura3*$^-$, *MAT*α, *thr4*$^-$, *his3*$^-$, *tcm*^{R}*1* haploid with the Eco RI fragment of the centromere substitution vector pJC3-13 (*25*) containing the 300-bp *CEN3* Alu I fragment with the Xho I site. This Eco RI fragment also contains the *URA3*$^+$ gene and sequences that normally flank *CEN3* on chromosome III. The Ura$^+$ transformants were isolated and subjected to DNA blot analysis (*26*) to confirm the transplacement (*24*) event on chromosome III. A Ura$^-$ derivative, called S-3, was then isolated by growth on medium containing 5-FOA. S-4, the *MAT***a** parent of S-5 was obtained by transforming (*23*) a *LEU2*$^+$, *ura3*$^-$, *MAT***a**, *THR4*$^+$, *his3*$^-$, *tcm*^{R}*1* haploid with the Eco RI fragment of the same centromere substitution vector pJC3-13 used above that contained the 630-bp wild-type *CEN3* and two Bam HI fragments. The Bam HI fragments contained the *TCM*^{S}*1* gene (3.2 kb) (*27*) and the *HIS3*$^+$ gene (1.7 kb) (*20*) and were inserted at the unique Bam HI site to the right of the centromere. The Ura$^+$ transformants were isolated and the transplacement event confirmed by DNA blot analysis (*26*). The distances shown in the figure are not to scale.

in G_2 upon DNA replication. After duplication the centromere would define the sister chromatids, and as above, all genes distal to the crossover would be uncoupled from their centromeres. According to this model, there is no reason to presume any special role for the centromere during mitotic recombination, since the recombination could occur before centromeres serve as attachment sites either for sister chromatids or for microtubules. Indeed, Malone *et al.* (*9*) presented evidence that recombination near the centromere is either repressed in meiosis or enhanced in mitosis. We decided to determine whether the centromeres themselves could undergo recombination in mitosis by observing whether mitotic gene conversion tracts can include centromeres.

Gene conversion is the nonreciprocal transfer of DNA sequence information, where donor sequence replaces recipient sequence. The mechanism involved is not yet clear. It has been suggested that conversion may involve heteroduplexes or double-strand gaps (or both) (*10–13*). Gene conversion events are frequently associated with a reciprocal exchange of flanking sequence in both meiosis and mitosis (*14*). Mitotic conversion can be detected in a diploid by selecting for rare homozygous progeny that arise from a heterozygous parent. Homozygous progeny resulting from gene conversion can be distinguished from those caused by mitotic crossing-over by requiring that distal markers remain heterozygous (*15*).

To look for gene conversion in a centromere, we used the X78 mutation (*16*), which contains a new Xho I site in the centromere of chromosome III (CEN3). This mutation does not affect *CEN3* function during mitosis or meiosis (*17*). Since we could not directly select for conversion of this mutation, we took advantage of the observation that gene conversion tracts often cover several kilobases of adjacent DNA (*18*). We constructed two diploids, SLP-6 and S-5 (see Fig. 1), that were heterozygous for X78 and an auxotrophic marker, *URA3*, placed within 1 kb of the centromere. By selecting for conversion of *URA3*, we hoped to detect conversion at the adjacent centromere. To determine whether the conversion tracts could extend through the centromere into the opposite chromosome arm, in strain SLP-6 we put a heterozygous Nru I restriction site marker on the other side of the centromere about 1 kb away. In strain S-5, instead of the Nru I site, we inserted dominant trichodermin-sensitive (*TCM*^{S}*1*) and *HIS3*$^+$ genes immediately adjacent to the centromere in one of the chromosome III homologs. Since S-5 was homozygous for the recessive resistant *tcm*^{R}*1* and *his3*$^-$ markers at their normal (non–chromosome III) locations, loss of the heterozygous chromosome III *TCM*^{S}*1–HIS3*$^+$ insert by extension of the *ura3* conversion tract would be detected as trichodermin-resistant histidine auxotrophs. In addition, we used the heterozygous insert to select directly for convertants that cause the loss of the *TCM*^{S}*1* gene. Finally, to detect crossing-over, we also included heterozygous distal chromosome III markers in the strains.

To select for conversion of the *URA3*$^+$ allele to *ura3*$^-$, we plated the diploids on 5-fluoro-orotic acid media (5-FOA), which selects against *URA3*$^+$ (*19*). To select for loss of the dominant *TCM*^{S}*1* allele, we plated the diploid S-5 on nutritional media (YPD) containing 0.20 mg of the drug trichodermin per milliliter. Only one resistant uracil auxotroph or trichodermin-resistant derivative was picked from each subclone plated on the selective media. The most common causes of uracil auxotrophy or trichodermin resistance should either be the desired gene conversion, a centromere-proximal reciprocal exchange, or loss of the entire chromosome III homolog that bears the *URA3*$^+$ allele. We distinguished among these possibilities by examining the fates of the heterozygous distal chromosome III markers: *LEU2*$^+$*/leu2*$^-$, which is centromere-distal to *URA3*$^+$, and *MAT*α*/MAT***a** and *THR4*$^+$*/thr4*$^-$ located on the opposite arm (Fig. 1). The Xho I and Nru I sites of selected strains were also examined by DNA hybridization analysis (Table 1 and Fig. 2).

Among the 112 and 58 independent uracil auxotrophs isolated from SLP-6 and S-5, respectively, 84 and 52 had also become able to mate with **a** cells and were auxotrophic for all markers on the α chromosome III homolog. These strains were presumed to have arisen by loss of the chromosome III homolog that carries the *URA3*$^+$ allele. In support of this hypothesis, the *CEN3* Xho I site and the flanking region lacking the Nru-I site that are found on this homolog were also missing in all nine representatives of these SLP-6 revertants examined with DNA hybridization analysis. These presumed chromosome loss mutants were not studied further. Likewise, among 43 independent revertants selected from S-5 for trichodermin resistance, 24 were clearly not the result of the desired gene conversion. Among these 24 strains, 21 were prototrophic for histidine and therefore could not involve loss of the chromosome III insert. These may have arisen by mutation of the chromosome III *TCM*^{S}*1* gene or by conversion of this gene with the normal non–chromosome III *tcm*^{R}*1* allele as a template. The three others were chromosome loss mutants. The remaining uracil auxotrophs and trichodermin-resistant mutants were scored for all chromosome III markers. The data are presented in Table 1.

Twenty-two SLP-6 *ura3*$^-$ derivatives remained heterozygous at the *MAT*, Xho I, and Nru I sites, and all but one of these remained prototrophic for leucine. These strains probably resulted from conversions of the *URA3*$^+$ locus that did not extend as far as the Xho I site on the right, nor as far as *LEU2* on the left. Such strains may result from conversion between the chromosome III homologs or from ectopic pairing (*20,*

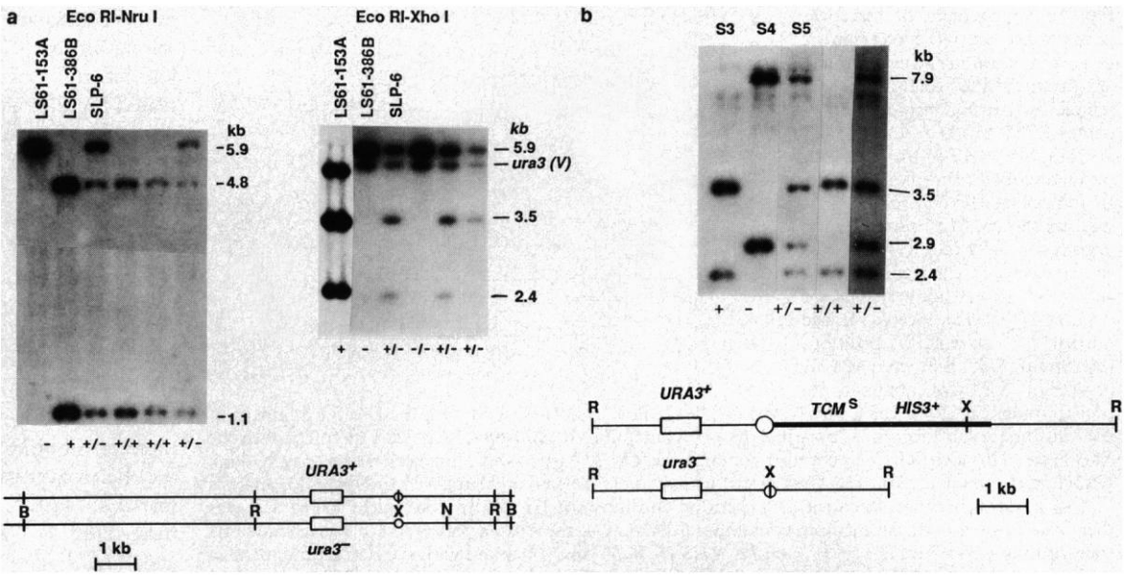

Fig. 2. Physical analysis of DNAs from spontaneous *ura3*⁻ derivatives of SLP-6 and S-5. DNAs isolated from 5-ml stationary phase cultures (*22*) were doubly digested with Eco RI and Xho I, with Eco RI and Nru I, or with Bgl II and Nru I (not shown). Digests were analyzed on 0.8% agarose gels, transferred to Hybond-N (Amersham) and hybridized with ^{32}P-labeled JC303-4 (*25*) DNA. This probe contains the 5.9-kb Eco RI piece including *CEN3* and the inserted *URA3*. The genomic copy of *ura3* on chromosome V hybridizes to probe in all lanes. (**a**) The SLP-6 diploid and its haploid parents, LS61-153A (Xho I⁺, Nru I⁻), and LS61-386B (Xho I⁻, Nru I⁺ are controls. Unmarked lanes are representative *ura3*⁻ derivatives of SLP-6. The 5.9-kb band indicates the absence of the Xho I or Nru I site in at least one of the chromosome III copies. The diagnostic smaller bands indicate that the site is present in at least one of the chromosome copies. If both the 5.9-kb and the smaller bands are present, the strain is heterozygous. The presence or absence of the Nru I or Xho I sites, respectively, is indicated at the bottom of each lane. The Eco RI–Xho I lane of LS61-153A is from another gel. The bottom of the Eco RI–Nru I figure is from an autoradiogram that was exposed longer than that used for the top. The restriction map is of the SLP-6 diploid. (**b**) The S-5 diploid and its haploid parents, S-4 (insert +), and S-3 (insert −) are controls. Unmarked lanes are representative *ura3*⁻ derivatives of S-5. The presence of the 7.9-kb and 2.9-kb bands indicate the absence of the Xho I site in *CEN3* and the presence of the *TCM*^{S} and *HIS3*⁺ insert in at least one of the chromosome III copies. The 7.9-kb and 2.9-kb bands were present or absent together in all strains. The absence of these bands indicated that both chromosome III copies contained the *CEN3* Xho I site and lacked the insert. The presence or absence of the *CEN3* Xho I is indicated at the bottom of each lane. The restriction map is of the S-5 diploid. The thick line indicates the *TCM*^{S} *HIS3*⁺ insert. R, Eco RI; X, Xho I; N, Nru I; B, Bgl II.

21) of the chromosome III *URA3*⁺ allele with the chromosome V *ura3*⁻ allele. Conversion events resulting from homologous chromosome pairing may or may not have been associated with a crossover. In other studies, about 10 to 55% of mitotic conversion events are associated with crossing-over (*14*). In our strains, associated crossovers would lead to leucine auxotrophy half the time. The finding of only a single leucine auxotroph suggests that many of the conversion events are not associated with crossovers. The strain auxotrophic for leucine could be the result of a crossover between *URA3* and the centromere. Alternative possibilities are conversion of *URA3*⁺ that does not extend into the Xho I site but that extends leftward covering *LEU2* or a conversion event associated with a crossover between *LEU2* and the centromere.

Two other uracil auxotrophs derived from SLP-6 had become homozygous for the loss of the Xho I site but remained heterozygous at Nru I, *LEU2*, and *MAT*. These auxotrophs probably arose as a result of a conversion tract that covered both the *URA3*⁺ gene and the Xho I site within the centromere, but that ended before the flanking Nru I site.

Four other uracil auxotrophs derived from SLP-6 became homozygous for the loss of the Xho I site and the presence of the Nru I site. In these cases, the conversion tracts cover the *URA3*⁺ gene, extend through the centromere and include the Nru I site on the opposite chromosome arm. In one case, the tract did not include the *LEU2* or *MAT* loci, since these sites remained heterozygous. The other three convertants became homozygous either for *leu2*⁻ or *MAT*α. These strains resulted either from crossovers associated with the conversions or from extensive conversion tracts that covered *LEU2*⁺ (around 22 kb away) or *MAT***a** (about 100 kb away).

Similar results were obtained from the S-5 strain. Of the six *ura3*⁻ conversions obtained on 5-FOA media, two did not extend as far as the Xho I site within the centromere, whereas four more included the Xho I site and the *TCM*^{S}*1–HIS3*⁺ insert on the opposite chromosome arm. The two **a**/**a** convertants cannot be explained as the result of continuous conversion tracts derived from one chromosome since such tracts would produce *ura3*⁻/*ura3*⁻ α/α cells. The *ura3*⁻/*ura3*⁻ **a**/**a** recombinants could be produced in a variety of ways: (i) patchy repair of a long symmetric heteroduplex formed in either G_1 or G_2, (ii) repair of a double-strand break in G_1 with resolution of the crossed-strand, Holliday intermediate by DNA replication, or (iii) two independent conversion events.

All of the trichodermin-resistant, histidine auxotrophs selected from S-5 were homozygous for the presence of the Xho I site. Thus, in all cases, conversion tracts that included the insert extended into the centromere. In five strains, the conversion tract did not extend into the flanking *ura3* gene on the other side of the centromere, whereas in ten strains this gene was included in the tract. The four remaining **a**/**a** convertants can be explained several ways as described above.

In summary, we selected for gene conversion events involving loci within 1 kb of the centromere. Out of 53 independent such conversion events, at least 29 extended into the centromere, and 22 of these involved a coconversion of a mutation of the opposite side of the centromere. These convertants demonstrate that mitotic gene conversion tracts can span centromeres. Since the present study did not examine frequencies of mitotic recombination, we cannot comment on the effect of the centromere on the rate of mitotic exchange. Our observations, however, clearly indicate that mitotic recombination events can occur within and propagate through the centromere.

REFERENCES AND NOTES

1. K. S. Bloom and J. Carbon, *Cell* **29**, 305 (1982).
2. Th. Dobzhansky, *Genetics* **15**, 347 (1930).
3. L. S. Symington and T. D. Petes, *Cell* **52**, 237 (1988).
4. E. J. Lambie and S. Roeder, *ibid.*, p. 863.
5. R. K. Mortimer and D. C. Hawthorne, in *The*

Yeasts, A. H. Rose and J. S. Harrison, Eds. (Academic Press, New York, 1969), vol. 1, pp. 385–460.
6. M. S. Esposito, *Proc. Nat. Acad. Sci. U.S.A.* **75**, 4436 (1978).
7. H. Roman, *Carlsberg Res. Commun.* **45**, 211 (1980).
8. F. Fabre, *Nature* **272**, 795 (1978).
9. R. E. Malone, J. E. Golin, M. S. Esposito, *Curr. Gen.* **1**, 241 (1980).
10. M. Meselson and C. Radding, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 358 (1975).
11. C. Bruschi and M. Esposito, *ibid.* **80**, 7566 (1983).
12. R. Holliday, *Genet. Res.* **5**, 282 (1964).
13. J. W. Szostak, T. L. Orr-Weaver, R. J. Rothstein, *Cell* **33**, 25 (1983).
14. M. Esposito and J. Wagstaff, in *The Molecular Biology of the Yeast Saccharomyces*, J. N. Strathern, E. W. Jones, J. R. Broach, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982), vol. 1, pp. 341–370.
15. T. L. Orr-Weaver and J. W. Szostak, *Microbiol. Rev.* **49**, 33 (1985).
16. A. Gaudet and M. Fitzgerald-Hayes, *Mol. Cell. Biol.* **7**, 68 (1987).
17. D. Koshland, L. Rutledge, M. Fitzgerald-Hayes, L. H. Hartwell, *Cell* **48**, 801 (1987).
18. S. Fogel, R. K. Mortimer, K. Lusnak, in *The Molecular Biology of the Yeast Saccharomyces*, J. N. Strathern, E. W. Jones, J. R. Broach, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982), vol. 1, pp. 289–339.
19. J. D. Boeke, F. Lacroute, G. R. Fink, *Mol. Gen. Genet.* **197**, 345 (1984).
20. S. Jinks-Robertson and T. D. Petes, *Genetics* **114**, 731 (1986).
21. M. Lichten, R. H. Borts, J. E. Haber, *ibid.* **115**, 233 (1987).
22. F. Sherman, G. R. Fink, J. B. Hicks, *Methods in Yeast Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1983).
23. H. Ito, Y. Fukuda, K. Murata, A. Kimura, *J. Bacteriol.* **153**, 163 (1983).
24. R. J. Rothstein, *Methods Enzymol.* **101**, 202 (1893).
25. L. Clarke and J. Carbon, *Nature* **305**, 23 (1983).
26. E. M. Southern *J. Mol. Biol.* **98**, 503 (1975).
27. P. A. Brown and J. W. Szostak, *Methods Enzymol.* **101**, 278 (1983).
28. We thank M. Fitzgerald-Hayes for providing the *CEN3* probes, J. Szostak for providing for the *TCM*^{S}*1* gene on plasmid pSZ414 and S. Jinks-Robertson for providing plasmid pSR7; Nina Brown for technical assistance; S. Picologlou, and D. Morrison for helpful comments about the manuscript; and W. O. Godtfredsen and Leo Pharmaceutical Products for the gift of trichodermin. Supported by NIH grant GM24110 from the National Institute of General Medical Sciences.

25 March 1988; accepted 13 June 1988

A Ligase-Mediated Gene Detection Technique

ULF LANDEGREN, ROBERT KAISER, JANE SANDERS, LEROY HOOD

An assay for the presence of given DNA sequences has been developed, based on the ability of two oligonucleotides to anneal immediately adjacent to each other on a complementary target DNA molecule. The two oligonucleotides are then joined covalently by the action of a DNA ligase, provided that the nucleotides at the junction are correctly base-paired. Thus single nucleotide substitutions can be distinguished. This strategy permits the rapid and standardized identification of single-copy gene sequences in genomic DNA.

DNA ANALYSIS IS ATTAINING increasing importance for the diagnosis of disease caused by single-gene defects as well as for the detection of infectious organisms (*1*). Moreover, a number of genes, predominantly those encoded in the major histocompatibility complex, have been found to be associated with an increased susceptibility to a variety of disease states (*2*). Of a total of approximately 2000 defined human genetic loci (*3*), approximately 100 have currently been studied at the DNA level for their role in genetic disease (*4*). A number of genetic diseases are caused by alleles present in the population at relatively high frequencies, perhaps because of selective advantages to the heterozygous carriers (*5*). The ongoing characterization of disease-causing or disease-associated gene sequences makes large-scale screening for carrier status and genetic counseling a possibility. It may also sharpen the diagnostic accuracy for diseases such as autoimmune conditions where the susceptibility may be influenced by defined alleles. Such prospects are currently limited by the cumbersome nature of the available DNA detection methods.

A majority of polymorphisms in the human genome are caused by point mutations that involve one or a few nucleotides. Current DNA analysis procedures capable of detecting the substitution of a single nucleotide are based on differential denaturation of mismatched probes as in allele-specific oligonucleotide hybridization (*6*) or denaturing gradient gel electrophoresis (*7*). Alternatively, the sequence of interest can be investigated for polymorphisms that affect the recognition by a restriction enzyme (*8*) or that will allow ribonuclease A (RNase A) to cleave at mismatched nucleotides of an RNA probe hybridized to a target DNA molecule (*9*). Although denaturing gradient gel or RNase A can survey long stretches of DNA for mismatched nucleotides, they are estimated to detect only about half of all mutations that involve single nucleotides (*7, 9*). Similarly, less than half of all point mutations give rise to gain or loss of a restriction enzyme cleavage site (*10*). The only existing technique capable of identifying any single nucleotide difference, short of DNA sequence analysis, is allele-specific oligonucleotide hybridization. This technique involves immobilizing separated (*6*) or enzymatically amplified (*11*) fragments of target DNA, hybridizing with oligonucleotide probes, and washing under carefully controlled conditions to discriminate single nucleotide mismatches.

We have devised a strategy that permits the facile distinction of known sequence variants differing by as little as a single nucleotide. The approach combines the ability of oligonucleotides to hybridize to the sequence of interest and the potential of a DNA-specific enzyme, T4 DNA ligase, to distinguish mismatched nucleotides in a DNA double helix (Fig. 1). Two oligonucleotide probes are permitted to hybridize to the denatured target DNA such that the 3′ end of one oligonucleotide is immediately adjacent to the 5′ end of the other. The ligase can then join the two juxtaposed oligonucleotides by the formation of a phosphodiester bond, provided that the nucleotides at the junction are correctly base-paired with the target strand. The ligation event thus positively identifies sequences complementary to the two oligonucleotides. A heterozygous sample is therefore scored as positive for both alleles. The joining of the oligonucleotides may be conveniently demonstrated, for instance, by labeling one of the oligonucleotides with biotin and the other one with ^{32}P. After the ligation reaction, the biotinylated oligonucleotides are allowed to bind to streptavidin immobilized on a solid support. Radioactive oligonucleotides that have become ligated to biotinylated oligonucleotides remain on the support after washing and are detected by autoradiography.

The gene encoding human β globin was selected as a model system to test the technique. There are two relatively frequent alleles, β^S and β^C, each differing from the normal allele, β^A, by a single nucleotide substitution in positions 2 and 1, respectively, of codon six (Figs. 2 and 3) (*12*). Subjects homozygous for the β^S allele suffer from sickle cell anemia. Moreover an increased risk of sudden death during exertion has been observed among individuals heterozygous for β^S (*13*).

The ligase-mediated gene detection procedure was used to distinguish β^A and β^S genes in equivalent amounts of DNA present in cells, in cloned DNA, and in genomic DNA (Fig. 2). One of two synthetic oligonucleotides (B131 or B132), specific for each of the alleles, was used in conjunction

Division of Biology, California Institute of Technology, Pasadena, CA 91125.