A Novel Selection System for Chromosome Translocations in *Saccharomyces cerevisiae*

Rachel B. Tennyson, Nathalie Ebran, Anissa E. Herrera and Janet E. Lindsley[1]

Department of Biochemistry, University of Utah, Salt Lake City, Utah 84132-3201

Manuscript received September 17, 2001
Accepted for publication January 16, 2002

ABSTRACT

Chromosomal translocations are common genetic abnormalities found in both leukemias and solid tumors. While much has been learned about the effects of specific translocations on cell proliferation, much less is known about what causes these chromosome rearrangements. This article describes the development and use of a system that genetically selects for rare translocation events using the yeast *Saccharomyces cerevisiae*. A translocation YAC was created that contains the breakpoint cluster region from the human *MLL* gene, a gene frequently involved in translocations in leukemia patients, flanked by positive and negative selection markers. A translocation between the YAC and a yeast chromosome, whose breakpoint falls within the *MLL* DNA, physically separates the markers and forms the basis for the selection. When *RAD52* is deleted, essentially all of the selected and screened cells contain simple translocations. The detectable translocation rates are the same in haploids and diploids, although the mechanisms involved and true translocation rates may be distinct. A unique double-strand break induced within the *MLL* sequences increases the number of detectable translocation events 100- to 1000-fold. This novel system provides a tractable assay for answering basic mechanistic questions about the development of chromosomal translocations.

CHROMOSOME translocations are a type of gross genetic rearrangement found in many kinds of cancer. The genetic consequences of chromosome translocations include deregulated gene expression, expression of novel fusion proteins, and loss of heterozygosity if the translocation is unbalanced (Rabbitts 1994; Rowley 1998). While much is understood or currently being studied about how particular translocations are involved in malignant processes, the fundamental events that lead to chromosomal translocations are poorly understood (Nowell 1997). In a subset of lymphoid leukemias and lymphomas the RAG recombination system, normally involved in immunoglobulin and T-cell receptor gene rearrangements, is implicated in an aberrant recombination or transposition-like reaction at the affected loci (Hiom *et al.* 1998). However, in the majority of cases there is no evidence that RAG proteins are involved in generating the translocations. So how do all the other translocations occur?

One of the very few methods presently available to study this problem is the analysis of the DNA sequences that occur at the breakpoints of the translocations. The results of such studies have suggested that individual translocation events may involve recombination between repeated ALU sequences, the interactions of the translin protein, illegitimate recombination, or some combination of these and other pathways (Aoki *et al.* 1995; Strout *et al.* 1998; Zucman-Rossi *et al.* 1998; Gillert *et al.* 1999). One class of translocations of particular interest is that involving the *MLL* gene (*ALL-1*, *HRX*) at chromosome band 11q23 (Canaani *et al.* 1995). The *MLL* gene is involved in translocations in >80% of infant acute lymphoblastic leukemia (ALL) cases and ~85% of therapy-related acute myeloid leukemia (t-AML) cases when topoisomerase II drugs were used in a prior cancer treatment protocol (Rowley 1998). Translocations involving *MLL* are unusual in that they are known to involve at least 38 different partner genes on a large number of different chromosomes (Rowley 2000). The breakpoints for the majority of the translocations cluster in an 8.3-kb region between exons 5 and 11 (the breakpoint cluster region). Strong topoisomerase II cleavage sites have been found within this 8.3-kb region (Felix *et al.* 1995; Aplan *et al.* 1996; Strissel *et al.* 1998; Lovett *et al.* 2001), but there is so far no direct evidence for the involvement of this enzyme or any other in causing the translocations. Therefore, while knowing sequences of the breakpoints provides important clues to the potential mechanisms, it is very difficult to accurately determine the pathway of events leading to a translocation solely by examining the final products.

One reason that so much less is known about the mechanism of chromosome translocations compared to other types of genome alterations is the lack of a genetic selection system specific for nonhomology-based translocations. Our present understanding of mismatch repair, nucleotide excision repair, and aneuploidy comes

[1]*Corresponding author:* Department of Biochemistry, University of Utah, 20 N. 1900 East, Salt Lake City, UT 84132-3201.
E-mail: janet.lindsley@hsc.utah.edu

largely from genetic and eventually biochemical studies using model organisms (Lengauer *et al.* 1998). The goal of experiments described in this article is to show that chromosome translocations between heterologous sequences of the type seen in human tumors can be studied in a model genetic organism, the yeast *Saccharomyces cerevisiae*. Yeast has been used for many years to study DNA recombination, although not in this context. Ectopic recombination between homologous markers integrated on two different chromosomes generates translocations (Sugawara and Szostak 1983; Jinks-Robertson and Petes 1986; Lichten and Haber 1989; Haber and Leung 1996; Fasullo *et al.* 1998). Forward mutation assays have led to the identification of rare translocations among other gross chromosome rearrangements (Chen *et al.* 1998; Chen and Kolodner 1999; Myung *et al.* 2001), but not specifically for their selection. Nonreciprocal products of break-induced replication (BIR) have been described as translocations (Morrow *et al.* 1997; Bosco and Haber 1998; Galgoczy and Toczyski 2001). However, neither ectopic recombination nor a strictly nonreciprocal BIR mechanism could explain many of the translocations seen in humans. Therefore, a system for the direct selection of cells containing untargeted chromosome translocations that do not occur primarily by BIR was developed and is presently described.

MATERIALS AND METHODS

Plasmids, yeast artificial chromosomes, and strains: The plasmids pRT9+ and pJEL261 were the circular versions of the small linear yeast artificial chromosomes (YACs), 1a and 3a, used in these studies. They were prepared using standard cloning techniques by incorporating the following elements. The *URA3* gene, as well as bacterial sequences telomeric of the *HIS3* gene, was derived from YIplac211 (Gietz and Sugino 1988). The *HIS3* gene (base pairs −179 to +835 relative to the start codon) and the *LEU2/CYH2* gene cassette were PCR amplified from the plasmids FAT-RS 303′b′ (Conrad *et al.* 1990) and pRS318 (Sikorski and Hieter 1989), respectively. The *CAN1* gene was amplified from plasmid M3093 (a kind gift of David Stillman, University of Utah), with removal of the *Sal*I site. The *ARS1* and the *GAL1/10* promoter sequences adjacent to *CEN3* were amplified from plasmids GARS1(U) (Snyder *et al.* 1988) and pGALCEN[2] (Hill and Bloom 1987), respectively. The 8.3-kb *Bam*HI fragment containing the *MLL* breakpoint cluster region was amplified from λmg11.1 DNA (Gu *et al.* 1992). The two final plasmids, pRT9+ and pJEL261, were identical except that the *Xho*I site near the center of the *MLL* DNA was replaced by an I-*Sce*I site in pJEL261 (Plessis *et al.* 1992). The two translocation YACs were created by linearizing these plasmids with *Sal*I and ligating telomere seed sequences, obtained from pYAC55 (Burke and Olson 1991), onto the ends of the linear ~21-kb plasmids. The relative orientation of each of these elements on the final YACs is shown in Figure 1B. The final orientation of the *MLL* sequence is the same as in the human genome. I-*Sce*I expression was induced from a *GAL1* promoter on the high copy, *TRP1*-marked plasmid pJX14, a derivative of pPEX7 (Plessis *et al.* 1992) and pRS424 (Sikorski and Hieter 1989). Oligonucleotides used for cloning, PCR, sequencing, and labeling reactions were made at the DNA Peptide Synthesis Facility, Huntsman Cancer Institute, University of Utah (National Institutes of Health grant CA 42014).

The yeast strains used in this study were all derived from S288C via FY250 (Winston *et al.* 1995). The *URA3, CAN1*, and in some cases, *RAD52* genes were completely deleted using the pMPY-ZAP plasmid system (Schneider *et al.* 1996), which leaves behind a small portion of the bacterial *hisG* gene. Cycloheximide resistance was selected as previously described (Sikorski and Boeke 1991). Isogenic haploids of opposite mating-type and diploids of the strains were made as previously described (Herskowitz and Jensen 1991), complementing Δ*rad52* with a centromeric plasmid containing the entire *RAD52* gene where necessary. All strain phenotypes were verified by plating on synthetic complete (SC) media lacking appropriate amino acids or uracil and containing drugs. Gene deletions were verified by PCR. Ploidy of all strains was verified by FACS analysis. The strains used in these studies were RTY20 (*MAT*α *his3*Δ*200 leu2*Δ*1 trp1*Δ*63 ura3*Δ*::hisG cyh2 can1*Δ*::hisG*), RTY22 (the isogenic *MAT***a**/α diploid of RTY20), RTY29 (*MAT*α *his3*Δ*200 leu2*Δ*1 trp1*Δ*63 ura3*Δ*::hisG cyh2 can1*Δ*::hisG rad52*Δ*::hisG*), and RTY31 (the isogenic *MAT***a**/α diploid of RTY29).

Yeast were grown in YPAD, prepared by adding adenine hemisulfate, 40 mg/liter, to YPD (Sherman 1991). Transformed strains were grown in SC medium lacking appropriate amino acids or uracil (Rose 1987). In some cases the drugs canavanine, cycloheximide, or 5-fluoroorotic acid (5-FOA) were added to final concentrations of 60 μg/ml, 10 μg/ml, or 1 mg/ml, respectively. Strains were grown at 30°.

Translocation assay: Cultures of the YAC-transformed strains were grown for 48 hr in 25 ml of SC-ura-leu + glucose medium. The cultures were washed twice in sterile distilled water and once in SC-ura-his + 2% glucose medium. They were then diluted into five or seven cultures each to an OD_{600} of 0.05 in 50 ml of the same medium and allowed to grow for 24 hr with shaking. The cells were again collected, washed twice in sterile distilled water, and diluted to an OD_{600} of 0.4 in SC-ura-his + 2% galactose medium. A small aliquot of each culture was diluted to 1×10^3 cells/ml and 100 μl of cells were plated on YEPD to establish a cell count. The cells were grown for another 16–24 hr, collected, and resuspended in sterile distilled water at a concentration of $\leq 2.5 \times 10^8$ cells/ml. For each culture 200 μl of cells were plated on each of 5–20 selective plates (SC-ura-his-arg + canavanine + cycloheximide + glucose). One 50-μl and two 100-μl aliquots of cells diluted to 1×10^3 cells/ml were plated on YEPD to establish a final cell count for each culture. Colony counts were made 3 days after plating for the YEPD plates and 5–8 days after plating for the selective plates. A secondary screen that verified the loss of the left arm of the YAC was performed by restreaking colonies from selective plates to SC-leu and YEPD plates. Colonies that failed to grow on SC-leu were subsequently plated on SC + 5-FOA and YEPD to test the stability of the resultant translocant, as described by Shero *et al.* (1991). Colonies that grew on YEPD, but failed to grow on SC + 5-FOA, were scored as stable. Translocation rates were calculated using Luria-Delbrück fluctuation analysis and the method of the median as indicated (Luria and Delbruck 1943; Lea and Coulson 1948). The translocation experiments testing the effect of a double-strand break in the YAC were done in an identical manner except that the media lacked tryptophan to ensure maintenance of the I-*Sce*I expression or control plasmids, pJX14 and pRS424, respectively. Translocation rates could not be properly determined in the I-*Sce*I experiments by fluctuation analysis. Instead, the total number of translocation events, divided by the number of cells plated, was compared to the results of control experiments.

Pulsed-field gel electrophoresis: Pulsed-field gel plugs were

made as previously described (Gerring *et al.* 1991) and 1-mm slices were embedded into the 1% agarose gels, made, and run in 1× transverse alternating field electrophoresis (TAFE) II buffer (22.5 mm Tris-borate/0.5 mm EDTA) at 15°. Pulsed-field gel electrophoresis was done using the TAFE GeneLine II system (Beckman, Fullerton, CA) and the following program: stage 1, 12 hr at 350 mA with a 1-min switching time; stage 2, 12 hr at 370 mA with a 2-min switching time; and stage 3, 12 hr at 390 mA with a 3-min switching time. Gels were stained in ethidium bromide, destained in water, and photographed over a UV transilluminator.

DNA analysis: Genomic DNA preparation was based on the protocol of Hoffman and Winston (1987). Southern blots were performed as previously described (Southern 1975) using alkaline transfer (Reed and Mann 1985) and probes radiolabeled by random priming (Feinberg and Vogelstein 1983). The approximate positions of the translocation breakpoints within the YACs were determined by PCR using a set of eight primer pairs that spanned the YAC from *CAN1* to *URA3*. Since all of the translocation samples contained the *URA3* gene, this reaction was used as a positive control. The remaining seven regions were designated by the letters A through G starting at the *CAN1* gene. The translocation breakpoint was designated to occur in the first region (G → A) in which there was no PCR product. For example, a sample with a breakpoint in region D gave PCR products for the *URA3* gene and regions G, F, and E. Translocation breakpoints were isolated using one of three methods: plasmid rescue following *Sph*I digestion of the genomic DNA and ligation, thermal asymmetric interlaced (TAIL) PCR (Liu *et al.* 1995), or inverse PCR (Ochman *et al.* 1989). Sequencing was performed by the DNA Sequencing Facility at the University of Utah. Sequences were analyzed using an unfiltered, gapped BLAST search at the Saccharomyces Genome Database web site (http://genome-www2.stanford.edu/cgi-bin/SGD/nph-blast2sgd).

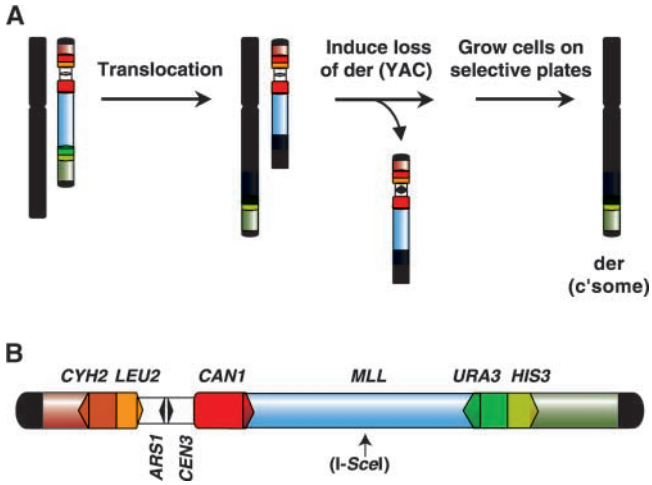


Figure 1.—Schematic of translocation assay and YAC. (A) The black chromosome represents the native chromosomes in the yeast genome and the multicolored chromosome is the translocation YAC; for purposes of clarity they are not drawn to scale. The red and green regions of the YAC represent the regions containing negative and positive selection markers, respectively. The arrowheads within the white region represent the bidirectional *GAL1-10* promoter. Cells containing translocations within the *MLL* sequences (blue) or the *CAN1* gene can be selected in this assay as described in the text. While the translocation event shown is reciprocal, nonreciprocal events may also be selected by this assay. (B) The translocation YAC with its labeled features is shown to scale (23 kb total length). An I-*Sce*I cleavage site is present near the center of the *MLL* region in the YAC used in some experiments.

RESULTS

Design of a selection system for untargeted translocations: One consistent feature of chromosome translocations is the division of genetic material originally present on one parental chromosome onto separate derivative chromosomes. This is the underlying principle used in the present design, as illustrated in Figure 1A. A small yeast artificial chromosome, the translocation YAC, was designed to have positive selection markers at one end and negative selection markers at the opposite end. A translocation breakpoint that falls between the markers will place the positive selection markers on a separate derivative chromosome [der(c'some)] from the negative selection markers. The derivative YAC [der(YAC)], or remaining original YAC in the case of a nonreciprocal translocation, can be destabilized by inactivating its conditional centromere. Therefore, even very rare translocation events can be detected by selecting for the stable presence of the positive selection markers and the loss of negative selection markers. While the target size is essentially the entire genome in diploids, only about one-half of the translocation events will give rise to monocentric (as opposed to acentric or dicentric) derivative chromosomes and thereby be selectable. Since there are very few haplo-insufficient genes in *S. cerevisiae* (Chial *et al.* 1999; Stevens and Davis 1998), loss of an arm of a chromosome on the resulting derivative YAC due to a reciprocal translocation or fragment loss should be well tolerated (see Figure 1A). In haploid cells, the breakpoints of selectable reciprocal translocations may be limited to the ends of chromosomes, telomeric of all essential genes. While this experimental design forces the loss of one of the two derivative chromosomes in a reciprocal translocation, it uniquely allows for the specific selection of untargeted chromosome translocations.

The details of the translocation YAC are shown in Figure 1B. The positive selection markers are the *URA3* and *HIS3* genes, and the negative selection markers are the *CAN1* and *CYH2* genes. The translocation breakpoint region used in the present study is the 8.3-kb breakpoint cluster region of the human *MLL* gene. It was chosen because of its frequent involvement in human translocation events (see Introduction) and its lack of homology with the yeast genome. However, we presently have no evidence to suggest that the *MLL* DNA behaves uniquely in the translocation assay. The placement of the divergent *GAL1/10* promoter between the centromere (*CEN3*) and origin of replication (*ARS1*) makes these genetic elements conditional; addition of galactose induces this promoter strongly and inactivates both the centromere and origin function (Hill and

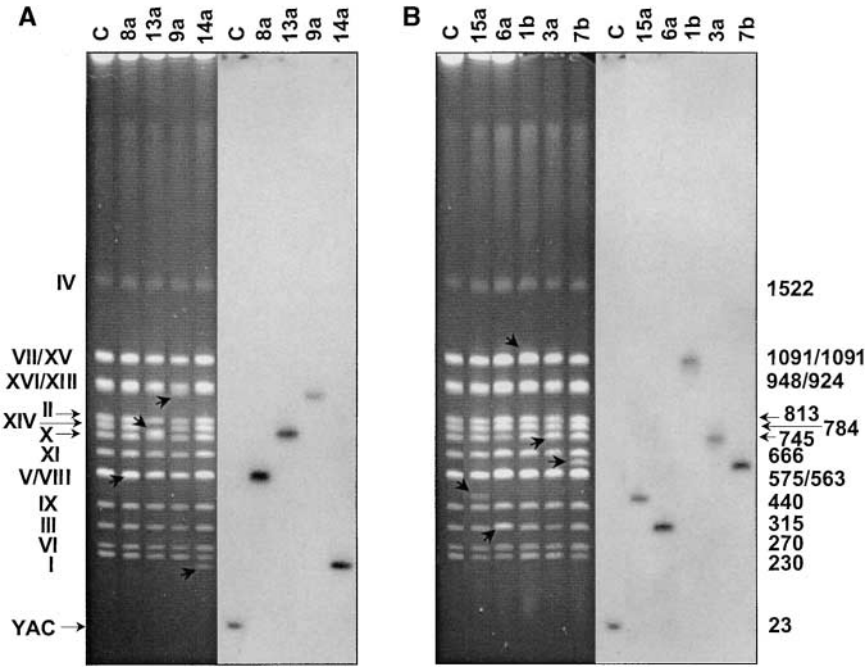


FIGURE 2.—Pulsed-field gel analysis of chromosomes isolated from cells selected and screened for translocations. Selected results from haploid (A) and diploid (B) cells are shown. The left halves of A and B show the ethidium bromide-stained gels, while the right halves show the Southern blot analysis (probing for the *URA3* and *HIS3* genes) to identify the position of the derivative chromosome. Arrowheads indicate the positions of derivative chromosomes on the ethidium-stained gels. The sample names are given above the lanes and correspond to the names used in Table 1 and Figure 5. "C" designates control lanes containing chromosomes isolated from the starting haploid strain RTY29-1a; while the small YAC is not visible in the ethidium-stained gel, it is visible in the Southern blot. The chromosomes are identified to the left of the gels; chromosome *XII* is not visible as a discrete band on these gels and is therefore not labeled. The lengths (in kilobases) of the labeled chromosomes are given to the right.

BLOOM 1987). While there is no evidence that addition of galactose is critical to the function of this assay, it was added to all of the cultures for the experiments described in this manuscript. The *LEU2* gene is an additional auxotrophic marker to screen for loss of the left arm of the YAC.

The *URA3, HIS3*, and *CAN1* genes were completely deleted from the yeast strains used in these studies, leaving no extensive homology between the right arm of the YAC and the yeast genome. The strains also contained deletions in the *LEU2* and *TRP1* genes and a point mutation in the *CYH2* gene. The translocation assays were performed by growing yeast in medium that selected for the presence of the right arm of the YAC and repressed the *GAL1/10* promoter. The cells were then shifted to galactose-containing medium to induce loss of any derivative YACs. In some experiments, this shift to galactose-containing medium also induced expression of an endonuclease (I-*Sce*I) that could uniquely cleave within the *MLL* region of one of the YACs (YAC 3a). The rare cells that contained a translocation were selected on plates lacking uracil and histidine and containing the drugs canavanine and cycloheximide, to simultaneously select for the presence of the *URA3* and *HIS3* genes and select against the presence of the wild-type *CAN1* and *CYH2* genes, respectively. Colonies arising from surviving cells were then screened for loss of the *LEU2* gene and for stability of the *URA3* gene. Cells that had stably maintained the *URA3* gene, but had lost the *CAN1*, *CYH2*, and *LEU2* genes, were considered candidates for having a chromosome translocation and were further analyzed.

This selection system was most effective in cells with a crippled homologous recombination system due to deletion of the *RAD52* gene (PAQUES and HABER 1999). In *RAD52* diploid strains a very large number of colonies appeared on the initial selection plates that failed to pass the subsequent screens. Unlike *rad52*Δ cells, many of the selected and screened *RAD52* cells did not contain obvious chromosome translocations. This made it very difficult to obtain accurate translocation rates. Therefore, the following analyses of the translocation products were all done using *rad52*Δ strains. We do not presently understand why the selection is effective in *rad52*Δ strains only.

Chromosome analysis from selected cells: The vast majority of the *rad52*Δ cells passing the selection and screens contained chromosome translocations, as defined by the criteria presented below. Analysis of the chromosomes from these cells by pulsed-field gel electrophoresis and Southern blotting indicated the movement of the right arm of the YAC to a larger, chromosome-sized DNA (Figure 2). In some cases the derivative chromosomes could be seen as new bands on the ethidium bromide-stained pulsed-field gel. In other cases the derivative chromosome was not apparent until the Southern blot revealed the right arm of the YAC had moved to a position overlapping an intact yeast chromosome. Independent isolates from different cultures gave rise to many different-sized bands, suggesting that translocations were occurring at many locations. Approximately 200 samples from 22 separate selection and screening experiments were analyzed in this way and 98% showed the right arm of the YAC translocated to a larger derivative chromosome.

To ensure that the movement of the *URA3* and *HIS3* genes represented a simple chromosome translocation (similar to that illustrated in Figure 1A) as opposed to

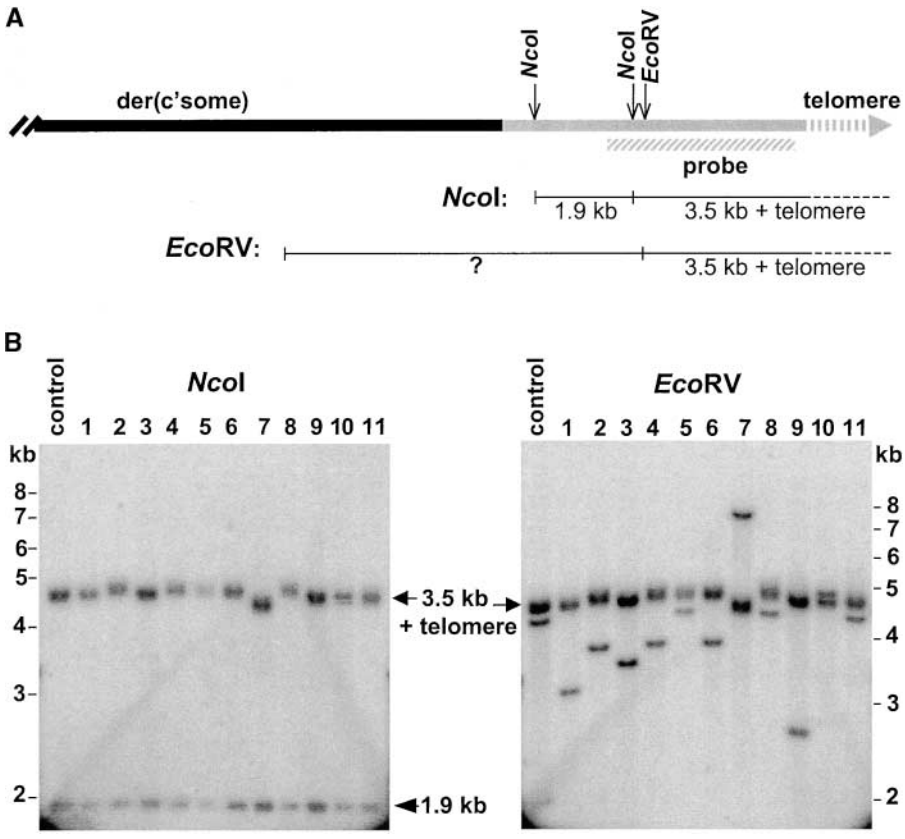


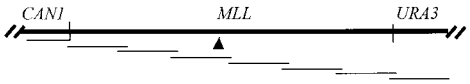
FIGURE 3.—The selected translocation events place the right arm of the YAC at the telomere of the derivative chromosomes. (A) This schematic shows a derivative chromosome resulting from a simple translocation. The sequences originating from the right arm of the YAC are shaded. The positions of *Nco*I and *Eco*RV sites, as well as the probe used for Southern analysis, are indicated. The fragment sizes expected from digestion with either *Nco*I or *Eco*RV for a simple translocation are indicated. One of the two fragments expected from the *Eco*RV digestion is expected to vary depending on position of the breakpoint (as indicated by ?). The length of the telomeres is variable, ranging from ~1 to 1.3 kb. (B) Southern blot analysis of genomic DNA isolated from selected yeast cells, digested with either *Nco*I or *Eco*RV, and separated by agarose gel electrophoresis is shown. The samples in the control lanes are from the starting YAC-containing strain, RTY31-1a. The 11 remaining samples are from six separate selection and screening experiments, each taken from a separate culture. Note that the bands migrating at ~4.5 kb (labeled 3.5 + telomere) are somewhat variable and smeary, as expected for telomere-containing fragments. Only one-half of the samples analyzed by this method are shown.

a more complex type of genetic rearrangement, the derivative chromosomes were further analyzed. First, in a simple translocation the right arm of the YAC should be the most telomeric DNA on one arm of the derivative chromosome. Genomic DNA from the selected cells was separately digested with either *Nco*I or *Eco*RV and analyzed by agarose gel electrophoresis and Southern blotting, again probing for the *URA3* and *HIS3* genes (Figure 3). In all cases tested (22 of 22) the results indicated that these genes were telomeric on the derivative chromosomes.

The translocation breakpoints of derivative chromosomes were analyzed next. The locations of breakpoints in a large number of samples were narrowed to ~1.5-kb regions on the right arm of the YAC between the *CAN1* and *URA3* genes by PCR analysis of genomic DNA (Figure 4). The region containing the breakpoint was the first region (analyzing from right to left) in which no PCR product was detected. In the haploid *rad52*Δ cells the breakpoints were distributed throughout the 8.3-kb *MLL* translocation breakpoint region and the *CAN1* gene. By contrast, in the diploid cells the breakpoints tended to cluster at each end of the *MLL* region. Long runs of adenosines (15–30 bp) occur at each end of the *MLL* DNA (in regions B and G, Figure 4), and as shown below, many of the breakpoints were at these A runs. In haploid cells where I-*Sce*I was induced to create a double-strand break near the middle of the *MLL* region, the majority of the breakpoints clustered around the break. In diploids, I-*Sce*I cleavage shifted the clustering of breakpoints to being adjacent and telomeric to the cut site; while no breakpoints were now detected in region B, a large number still fell within region G.

Eighteen translocation breakpoints, all from experiments using *rad52*Δ cells, were sequenced. Ten of these were at runs of A's within regions B and G from diploid cells. One representative example of this class is shown in Table 1 (sample 1b). The length of homologous overlap between the two parental chromosomes that exists in the derivative chromosomes ranged from 3 to 22 bp, almost all of which were A's in this class. Four different partner chromosomes were involved in this translocation class. Six of the events involved chromosome *XII*; five of the six were within the rDNA repeats (see Figure 5). Three of these five breakpoints, selected in completely separate experiments, were at essentially identical positions within the repeat sequence, differing only by a single A in the overlap region. A run of 22 consecutive A's occurs at this location both in the rDNA repeats and within region G of the *MLL* breakpoint cluster, suggesting why this may be a "hotspot."

This preference for breakpoints containing runs of



Experiment:	Number of breakpoints per region: A	B	C	D	E	F	G	total:
Haploid *rad52Δ*	4	3	4	4	4	2	2	23
Diploid *rad52Δ*	3	10	3	0	1	2	16	35
Haploid *rad52Δ* + I-*Sce*I induced break	0	1	0	14	2	1	1	19
Diploid *rad52Δ* + I-*Sce*I induced break	1	0	0	6	3	4	8	22

FIGURE 4.—Positions of breakpoints within the translocation YAC as defined by PCR. The diagram shows an ~11-kb segment of the translocation YAC; the position of the I-*Sce*I cleavage site present in some experiments is indicated by the arrowhead. Eight sets of PCR primers were designed to amplify regions within this segment, and the resulting amplicons, designated A–G and *URA3*, are illustrated by the eight thin lines.

A's was not seen in haploid strains, although limited homology between the YAC and the partner chromosome was seen (Table 1, samples 8a, 9a, 11a, and 12a). In four of five breakpoints sequenced from I-*Sce*I-induced events the breakpoints fell at or immediately adjacent to the cleavage site (samples 6a, 7a, 11a, and 12a). In one case from a diploid cell, the breakpoint fell ~750 bp telomeric to the cleavage site, within a run of A's (sample 7b).

The partner chromosome for the translocations selected was quite variable (Figure 5); 9 of the 16 yeast chromosomes were represented in the 18 breakpoints sequenced. The 4 haploid breakpoints fell at the very ends of chromosomes such that there were no essential genes telomeric of these breakpoints. It makes sense that only haploids with translocations that do not cause the loss of essential genes would survive. By contrast, none of the 14 breakpoints in the partner chromosomes from diploid cells was within 35 kb of the native telomere.

In all cases (18 of 18) the length of the derivative chromosome predicted from the breakpoint sequence, assuming a simple translocation, matched the length determined by pulsed-field gel electrophoresis (within the resolution of the technique, ~30 kb). Four examples (6a, 7b, 8a, and 9a) can be seen by comparing the results in Figures 2 and 5. It is not possible to predict a unique length for the five derivative chromosomes with breakpoints within the rDNA repeats and the two within the subtelomeric repeated sequences (11a and 12a), but in each of these cases the measured length falls within the expected range. Together this evidence indicates that simple, untargeted chromosome translocations between a YAC and the yeast genome can occur and be selected using the system presently described.

The translocation events resulted in some cells with an apparently normal chromosome number, in which a derivative chromosome replaced a normal chromosome, and other cells that contained the derivative chromosome in addition to the normal chromosome complement. For haploids the chromosome number could sometimes be clearly determined by analysis of the pulsed-field gel; samples 14a and 13a are examples of cells with and without extra chromosomes, respectively (Figure 2A). Of 36 haploid genomes analyzed by pulsed-field gel, 7 clearly had and 10 clearly did not have extra chromosomes by visual inspection. For the remaining 19 it was not possible to discern the ploidy from gels. In sample 9a the breakpoint occurs within the *DIA1* gene near the right end of chromosome *XIII* (Figure 5). While the pulsed-field gel does not show a conclusive shortening of chromosome *XIII* (Figure 2A), the *FET4* gene, which is normally telomeric to *DIA1*, was shown to be missing from this strain by PCR analysis (not shown). Sample 9a is therefore an example of a translocation strain with a normal chromosome number that could not be discerned by visual inspection of the pulsed-field gel.

It was more difficult to analyze ploidy of the diploid translocation strains. Sample 6a is an example in which the relative band intensity of chromosome *X* is diminished (Figure 2B), suggesting that this strain had only one full-length copy of chromosome *X* in addition to the half-length der(*X*) chromosome (Figure 5). To test this hypothesis, the strain was sporulated and 11 tetrads were dissected. The resulting spores segregated 2:2 for viability and the surviving two spores lacked the der(*X*) chromosome as evidenced by their inability to grow on plates missing uracil and histidine. This result confirmed that sample 6a had only two copies of chromosome *X*, one full length and one half length. Seven diploid strains were analyzed by tetrad dissection and only this one segregated 2:2. Therefore, it appears that a smaller percentage of diploids gave rise to translocations with detectable loss of a full-length chromosome than haploids. This is surprising given the fact that most such events would give rise to viable colonies in a diploid but not a haploid.

Translocation rates: Translocation rates were calculated using Luria-Delbrück fluctuation analysis (LURIA and DELBRUCK 1943), assuming that all of the colonies that passed the selection and both screens contained translocations (Table 2). The rates for the *RAD52* strains were ~5- to 10-fold higher (although considerably less reliable, as described above) than those for the *rad52Δ* strains. The measured translocation rates for both haploid and diploid *rad52Δ* cells were the same, 2×10^{-9} translocations per cell per generation, even though the mechanisms involved in generating the translocations may have been distinct. Untargeted and uninduced translocations are thought to be rare events, agreeing with the low rates presently seen.

Translocation rates could not be properly determined when I-*Sce*I was induced to create a double-strand break. Instead, the total number of translocation events, relative to the total number of cells, was compared between experiments with cells containing both an I-*Sce*I site in the *MLL* region of the YAC and an I-*Sce*I expression plasmid and cells lacking either the cleavage site or the

TABLE 1

Translocation breakpoint sequences

Name[a]	Breakpoint sequence[b]	
1b	*XII*:	CCATTTACTTAAAAAAAAAAAAAAAAAAAAAATGTCCCCAC
(G, d)	der (*XII*):	CCATTTACTTAAAAAAAAAAAAAAAAAAAAAACTATATGGG
	YAC:	ACTCCATCTCAAAAAAAAAAAAAAAAAAAAAACTATATGGG
10a	*XIV*:	AGGGTGACGCTGCTCTGCAGCCCTTGAAAGAACGGTACGAG
(E, d)	der (*XIV*):	TCACTATGGTGCTCAGGCTGGTCTTGAAAGAACGGTACGAG
	YAC:	TCACTATGGTGCTCAGGCTGGTCTTGAACTCCTGAGCTCAA
8a	*VIII*:	AGCGAAACAATCTTAGTGATTGCTGGCATGTCATTAGCGA
(E, h)	der (*VIII*):	AGCGAAACAATCTTAGTGAGCAAATATTCTCTTAGTCCCT
	YAC:	CCTACTATTCATTTACTAAGCAAATATTCTCTTAGTCCCT
9a	*XIII*:	TCCATTATTCCAAATTATCAATTGCACTCTACCGTTT
(E, h)	der (*XIII*):	TCCATTATTCCAAATTATCAATCCCATCTTAGTTTGG
	YAC:	AAGATAATATATAAAGCACAATCCCATCTTAGTTTGG
6a	*X*:	TGGTACTAGTAGATGAAGACGCGTAAGGAGGCTTTGATAGGAG
(D, d I-*Sce*I)	der (*X*):	TGGTACTAGTAGATGAAGACGCGTCGAGAGGCTGAGGCAGGAG
	YAC:	TAGGGATAACAGGGTAATACGCGTCGAGAGGCTGAGGCAGGAG
7a	*XIII*:	GGAAACTGTCGTAGTAAATTAACAGGGGAATCCGATTGA
(D, d I-*Sce*I)	der (*XIII*):	GGAAACTGTCGTAGTAAATTAACAGGGTAATACGCGTCAA
	YAC:	CGAGCGCGCACGCTAGGGATAACAGGGTAATACGCGTCAA
7b	*VII*:	TTTACTAAAAAAAAAAAAAAGTAAAAAAAAATTCATTACT
(E, d I-*Sce*I)	der (*VII*):	TTTCCTAAAAAAAAAAAAAAATACCAGGCATGGTAGCATG
	YAC:	CTACTAAAAAAAAAATAAAATACCAGGCATGGTAGCATG
11a	*IV*:	TCGGTCAGAAAGCCGGGTAAGGAGTGACAGCGAGAGTAA
(D, h I-*Sce*I)	der (*IV*):	TCGGTCAGAAAGCCGGGTAACAGGGTAATACGCGTCGAG
	YAC:	AGCGCGCACGCTAGGGATAACAGGGTAATACGCGTCGAG
12a	*VI*:	TCAGAAAGCCGGGTAAGGTATGACAGCGAGAGTAGAGGTA
(D, h I-*Sce*I)	der (*VI*):	TCAGAAAGCCGGGTAAGGTATAACAGGGTAATACGCGTCG
	YAC:	TCGAGCGCGCACGCTAGGGATAACAGGGTAATACGCGTCG

[a] The name of the derivative chromosome is designated by the experiment number and a lowercase letter indicating the culture. Following the name, in parentheses, an uppercase letter indicates the region within the YAC where the translocation occurred (see Figure 4), and "d" or "h" indicates that the strain was diploid or haploid, respectively. Translocations induced by cleavage of the YAC with I-*Sce*I are indicated.

[b] Sequences in boldface type indicate positions of identity among the native chromosomes (designated by roman numerals), YACs, and derivative chromosomes. Underlined sequences indicate identity between either the YACs or the native chromosomes and the derivative chromosomes. The I-*Sce*I cleavage sites are indicated by arrows.

I-*Sce*I gene (Table 3). In haploids, the relative number of translocation events increased 1200-fold upon I-*Sce*I cleavage. In diploids, the increase was ~140-fold. Since there are no I-*Sce*I sites in the yeast genome (Thierry *et al.* 1991), the large increase in number of translocation events seen indicates that either the creation of a double-strand break in the YAC is a rate-limiting step in the translocation process or the break induces a new mechanism for translocation.

DISCUSSION

We have designed a new system in yeast that specifically selects for cells containing untargeted, nonhomology-based chromosome translocations. This translocation selection system is based on the separation of positive and negative selection markers originally present on a small YAC (Figure 1). Essentially all of the *rad52*Δ cells passing the selection and screening steps had simple chromosome translocations. Since these events occur in the absence of DNA sequence homology and Rad52p, they are not simply the result of ectopic recombination or BIR (Malkova *et al.* 1996; Bosco and Haber 1998). The translocations occurred in both haploid and diploid yeast and involved many different sites on at least 9 of the 16 native chromosomes. Induction of a double-strand break on the YAC increased the number of translocation events 100- to 1000-fold. This

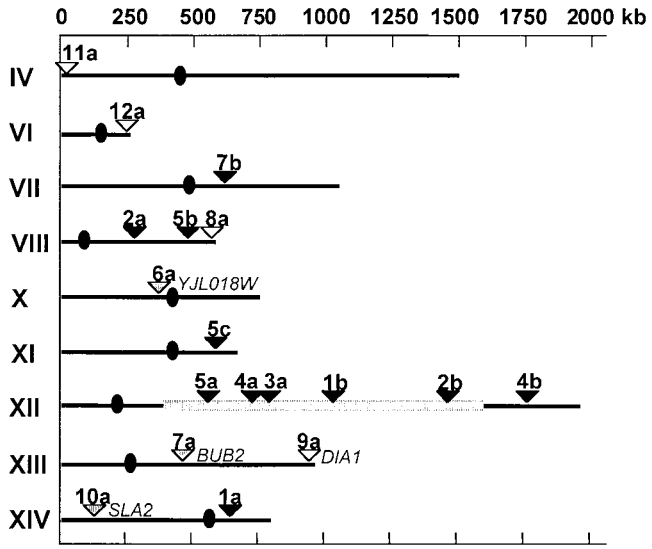


FIGURE 5.—Chromosomal map positions of the sequenced breakpoints. Open, shaded, and solid arrowheads represent the positions of translocation breakpoints isolated from haploids, diploids, and diploids where the breakpoint fell at a run of A's, respectively. Ovals indicate centromeres. The sample names are given above the arrowheads. When the breakpoint fell within an open reading frame (ORF), the ORF or gene name is given to the right of the breakpoint. The rDNA repeats are shown as a shaded bar on chromosome *XII*. Nine of the diploid breakpoints occurring at runs of A's that are not shown in Table 1 are included in this map.

is the first system described that specifically selects for cells containing untargeted chromosome translocations.

While the detectable translocation rates for haploids and diploids were equivalent, the mechanisms involved in generating these events were most likely distinct. First, the amount of sequence identity between the YAC and the native chromosome that was found at the breakpoint of the derivative chromosome was consistently longer in diploids (Table 1). The majority of the diploid breakpoints occurred at runs of adenosines, which represent the longest stretches of homology between the right arm of the YAC and the yeast genome. This bias for A runs was not seen in haploids, where the sequence identity at the breakpoints ranged from only 1 to 4 bp. This suggests that the translocations may involve a nonhomologous end-joining (NHEJ) pathway more frequently in haploids than in diploids. While an I-*Sce*I-generated double-strand break increased the number of translocation events in both haploids and diploids, the positions of the breakpoints differed between them. In the majority of haploid cells, the breakpoint fell close to the initial cut site, while for diploids the breakpoints were often telomeric to the initial cut, probably at runs of A's (Figure 4 and Table 1). This indicates that involvement of both exonuclease activity and homology may be more important in diploids than in haploids. Second, each of the events analyzed from haploids occurred close to the telomere of a native chromosome, such that no essential genes fell telomeric to the breakpoints. This proximity to the native telomere was not seen for any of the diploid translocation events. While we cannot presently rule out a preference for translocations at subtelomeric sequences specifically in haploids, the most likely explanation for these results is that translocations actually occur at a higher rate in haploid cells, but the majority are lethal due to loss of essential genes. Approximately 10% of the yeast genome is telomeric of all essential genes, suggesting that the haploid translocation target size for selectable events is one-twentieth of that in diploids. Therefore, the true translocation rate in haploids may well be 20-fold higher than those in diploids. Last, a crude analysis of the ploidy of the selected haploid *vs.* diploid cells suggested that fewer haploid cells contained extra chromosomes than diploid cells.

One potential mechanistic explanation for all of these results involves the differential efficiency of NHEJ and homologous recombination in yeast, depending on mating-type status. NHEJ is downregulated in nonmating *MAT***a**/*MAT*α cells compared to either *MAT***a** or *MAT*α cells (ASTROM *et al.* 1999; LEE *et al.* 1999), while homologous recombination is more efficient in **a**/α cells compared to **a**/**a** cells (CLIKEMAN *et al.* 2001). Recently it was shown that one of the genes required for efficient NHEJ, LIF2/NEJ1, is repressed in **a**/α diploids (FRANK-VAILLANT and MARCAND 2001; KEGEL *et al.* 2001). Therefore, even though the central component of homologous recombination, *RAD52*, has been deleted in the present studies, **a**/α diploid cells may still have a preference to repair DNA breaks by homologous pathways. While BIR has been described as completely *RAD52* dependent (MALKOVA *et al.* 1996; BOSCO and HABER

TABLE 2

Translocation rates

Experimental description	Strain used	Translocation rate (translocations/cell/division)	No. of experiments
RAD52; haploid	RTY20-1a	$<1.3 \times 10^{-8}$	2
RAD52; diploid	RTY22-1a	$<1.7 \times 10^{-8}$	2
*rad52*Δ; haploid	RTY29-1a	$2.1 (\pm 0.3) \times 10^{-9}$	3
*rad52*Δ; diploid	RTY31-1a	$2.2 (\pm 0.8) \times 10^{-9}$	4

TABLE 3

I-*Sce*I cleavage increases the number of translocation events seen

Experimental description	Strain:plasmid used	No. of selected and screened colonies/total no. of colonies plated	No. of experiments
*rad52*Δ; haploid; I-*Sce*I cleavage	RTY29-3a:pJX14	4.1 (± 2.3) × 10^{-6}	5
*rad52*Δ; diploid; I-*Sce*I cleavage	RTY31-3a:pJX14	1.8 (± 0.7) × 10^{-6}	3
*rad52*Δ; haploid; I-*Sce*I control[a]	RTY29-3a:pRS424[a]	3.3 (± 0.8) × 10^{-9}	3
*rad52*Δ; haploid; I-*Sce*I control[b]	RTY29-1a:pJX14[b]	3.4 (± 3.9) × 10^{-9}	3
*rad52*Δ; diploid; I-*Sce*I control[a]	RTY31-3a:pRS424[a]	1.6 (± 1) × 10^{-8}	3
*rad52*Δ; diploid; I-*Sce*I control[b]	RTY31-1a:pJX14[b]	1.1 (± 0.7) × 10^{-9}	2

[a] There is no I-*Sce*I gene in the expression vector for these controls.
[b] There is no I-*Sce*I site in the YAC for these experiments.

1998), it may occur at a sufficient rate in *rad52*Δ diploids to account for the higher frequency of extra chromosomes seen in these cells. In the haploids, the combination of the very short microhomologies seen at the breakpoints and the majority of cells with a normal ploidy is consistent with the translocations occurring primarily by a NHEJ pathway (Kramer *et al.* 1994). The ~20% of selected haploids that contain an extra chromosome may result from the combination of a more frequent translocation event at a nontelomeric location in G2 cells followed by a nondisjunction event. The translocations seen in haploids, particularly those induced by I-*Sce*I cleavage of the YAC, cannot easily be explained by BIR. For a double-strand break within the *MLL* region of the YAC to cause a selectable translocation by BIR, the broken YAC would have to invade on one end of the native chromosome, and have replication proceed through the native centromere, and continue off the far arm. This would result in the derivative chromosome being an extra copy of that chromosome and would not explain why the breakpoints tend to fall near telomeres. It has been reported that BIR does not proceed through centromeres (Morrow *et al.* 1997) and requires homology at the breakpoint (Sugawara and Haber 1992). For these reasons it seems unlikely that BIR is a major pathway in generating the translocations selected in haploids using this system. Additionally, Myung *et al.* (2001) recently reported that the translocation rate dropped 5- to 10-fold in a haploid *rad52* strain if *LIG4* was also deleted. Since the only known function of Lig4p is in NHEJ, their results also suggest the involvement of end joining in chromosome translocations in haploid yeast.

While further studies will be required to verify this hypothesis, these initial results suggest that the translocation assay done in haploid yeast may be a more appropriate model for human translocations than the assay done in diploids. In fact, of 25 published breakpoint sequences in the *MLL* gene from leukemia patients, 12 show microhomologies of 1–4 bp, while none have longer homologies or occur at runs of A's (Domer *et al.* 1995; Felix *et al.* 1995; Malkova *et al.* 1996; Gill Super *et al.* 1997; Sobulo *et al.* 1997; Atlas *et al.* 1998; Bosco and Haber 1998; Megonigal *et al.* 1998; Gillert *et al.* 1999). The breakpoints in humans are distributed throughout the 8.3-kb region of *MLL*, similar to what was found in the haploid yeast. However, as mentioned above, there is presently no evidence that the human *MLL* breakpoint cluster sequence confers any unique properties to this yeast translocation assay.

The results presented here indicate that our new translocation assay selects for recombination events that are quite distinct from most of those previously described in yeast. Very recently, Myung *et al.* (2001) reported an increase in the rate of gross chromosomal rearrangements (GCRs), analyzed by the loss of function of two genes near the end of chromosome *V*, in *rad52*Δ yeast. While they did not specifically select for translocations, ~70% of the GCRs in cells lacking Rad52p were translocations. Further analysis will be required to determine if the translocations are occurring by similar mechanisms in these two different selection schemes. One advantage of the system described herein is that it allows for the inclusion of essentially any nonyeast DNA sequence in place of the *MLL* sequence in the translocation YAC. This system will be useful in determining what gene products are involved in causing or preventing translocations in general and whether DNA topoisomerase II is specifically involved in translocations at the *MLL* locus.

The authors thank Tim Formosa and David Stillman for helpful discussions, Jing Xu for technical help, and Diana Lim for figure preparation. This work was funded by a pilot project grant from the Huntsman Cancer Institute and by National Institutes of Health grant GM60420. The oligonucleotide synthesis and DNA sequencing core facilities were supported by the Cancer Center Support grant 2P30CA42014.

LITERATURE CITED

Aoki, K., K. Suzuki, T. Sugano, T. Tasaka, K. Nakahara *et al.*, 1995 A novel gene, Translin, encodes a recombination hotspot binding protein associated with chromosomal translocations. Nat. Genet. **10:** 167–174.

Aplan, P. D., D. S. Chervinsky, M. Stanulla and W. C. Burhans, 1996 Site-specific DNA cleavage within the *MLL* breakpoint cluster region induced by topoisomerase II inhibitors. Blood **87:** 2649–2658.

Astrom, S. U., S. M. Okamura and J. Rine, 1999 Yeast cell-type regulation of DNA repair. Nature **397:** 310.

Atlas, M., D. Head, F. Behm, E. Schmidt, N. J. Zeleznik-Le *et al.*, 1998 Cloning and sequence analysis of four t(9;11) therapy-related leukemia breakpoints. Leukemia **12:** 1895–1902.

Bosco, G., and J. E. Haber, 1998 Chromosome break-induced DNA replication leads to nonreciprocal translocations and telomere capture. Genetics **150:** 1037–1047.

Burke, D. T., and M. V. Olson, 1991 Preparation of clone libraries in yeast artificial chromosome vectors. Methods Enzymol. **194:** 251–270.

Canaani, E., P. C. Nowell and C. M. Croce, 1995 Molecular genetics of 11q23 chromosome translocations. Adv. Cancer Res. **66:** 213–234.

Chen, C., and R. D. Kolodner, 1999 Gross chromosomal rearrangements in *Saccharomyces cerevisiae* replication and recombination defective mutants. Nat. Genet. **23:** 81–85.

Chen, C., K. Umezu and R. D. Kolodner, 1998 Chromosomal rearrangements occur in *S. cerevisiae rfa1* mutator mutants due to mutagenic lesions processed by double-strand-break repair. Mol. Cell **2:** 9–22.

Chial, H. J., T. H. Giddings, Jr., E. A. Siewert, M. A. Hoyt and M. Winey, 1999 Altered dosage of the *Saccharomyces cerevisiae* spindle pole body duplication gene, *NDC1*, leads to aneuploidy and polyploidy. Proc. Natl. Acad. Sci. USA **96:** 10200–10205.

Clikeman, J., G. Khalsa, S. Barton and J. Nickoloff, 2001 Homologous recombinational repair of double-strand breaks in yeast is enhanced by *MAT* heterozygosity through yKU-dependent and -independent mechanisms. Genetics **157:** 579–589.

Conrad, M., J. Wright, A. Wolf and V. Zakian, 1990 RAP1 protein interacts with yeast telomeres in vivo: overproduction alters telomere structure and decreases chromosome stability. Cell **63:** 739–750.

Domer, P. H., D. R. Head, N. Renganathan, S. C. Raimondi, E. Yang *et al.*, 1995 Molecular analysis of 13 cases of MLL/11q23 secondary acute leukemia and identification of topoisomerase II consensus-binding sequences near the chromosomal breakpoint of a secondary leukemia with the t(4;11). Leukemia **9:** 1305–1312.

Fasullo, M., T. Bennett, P. AhChing and J. Koudelik, 1998 The *Saccharomyces cerevisiae RAD9* checkpoint reduces the DNA damage-associated stimulation of directed translocations. Mol. Cell. Biol. **18:** 1190–1200.

Feinberg, A. P., and B. Vogelstein, 1983 A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132:** 6–13.

Felix, C. A., B. J. Lange, M. R. Hosler, J. Fertala and M. Bjornsti, 1995 Chromosome band 11q23 translocation breakpoints are DNA topoisomerase II cleavage sites. Cancer Res. **55:** 4287–4292.

Frank-Vaillant, M., and S. Marcand, 2001 NHEJ regulation by mating type is exercised through a novel protein, Lif2p, essential to the Ligase IV pathway. Genes Dev. **15:** 3005–3012.

Galgoczy, D. J., and D. P. Toczyski, 2001 Checkpoint adaptation precedes spontaneous and damage-induced genomic instability in yeast. Mol. Cell. Biol. **21:** 1710–1718.

Gerring, S. L., C. Connelly and P. Hieter, 1991 Positional mapping of genes by chromosome blotting and chromosome fragmentation. Methods Enzymol. **194:** 57–77.

Gietz, R. D., and A. Sugino, 1988 New yeast-*Escherichia coli* shuttle vectors constructed with *in vitro* mutagenized yeast genes lacking six-base pair restriction sites. Gene **74:** 527–534.

Gill Super, H., P. L. Strissel, O. M. Sobulo, D. Burian, S. C. Reshmi *et al.*, 1997 Identification of complex genomic breakpoint junctions in the t(9;11) *MLL-AF9* fusion gene in acute leukemia. Genes Chromosomes Cancer **20:** 185–195.

Gillert, E., T. Leis, R. Repp, M. Reichel, A. Hosch *et al.*, 1999 A DNA damage repair mechanism is involved in the origin of chromosomal translocations t(4;11) in primary leukemia cells. Oncogene **18:** 4663–4671.

Gu, Y., T. Nakamura, H. Alder, R. Prasad, O. Canaani *et al.*, 1992 The t(4;11) chromosome translocation of human acute leukemias fuses the ALL-1 gene, related to drosophila trithorax, to the AF-4 gene. Cell **71:** 701–708.

Haber, J. E., and W. Y. Leung, 1996 Lack of chromosome territoriality in yeast: promiscuous rejoining of broken chromosome ends. Proc. Natl. Acad. Sci. USA **93:** 13949–13954.

Herskowitz, I., and R. E. Jensen, 1991 Putting the HO gene to work: practical uses for mating-type switching. Methods Enzymol. **194:** 132–146.

Hill, A., and K. Bloom, 1987 Genetic manipulation of centromere function. Mol. Cell. Biol. **7:** 2397–2405.

Hiom, K., M. Melek and M. Gellert, 1998 DNA transposition by the RAG1 and RAG2 proteins: a possible source of oncogenic translocations. Cell **94:** 463–470.

Hoffman, C., and F. Winston, 1987 A ten-minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of *Escherichia coli*. Gene **57:** 267–272.

Jinks-Robertson, S., and T. Petes, 1986 Chromosomal translocations generated by high-frequency meiotic recombination between repeated yeast genes. Genetics **114:** 731–752.

Kegel, A., J. O. O. Sjostrand and S. U. Astrom, 2001 Nej1p, a cell type-specific regulator of nonhomologous end joining in yeast. Curr. Biol. **11:** 1611–1617.

Kramer, K. M., J. A. Brock, K. Bloom, J. K. Moore and J. E. Haber, 1994 Two different types of double-strand breaks in *Saccharomyces cerevisiae* are repaired by similar *RAD52*-independent, nonhomologous recombination events. Mol. Cell. Biol. **14:** 1293–1301.

Lea, D. E., and C. A. Coulson, 1948 The distribution of the numbers of mutants in bacterial populations. J. Genet. **49:** 264–284.

Lee, S. E., F. Paques, J. Sylvan and J. E. Haber, 1999 Role of yeast *SIR* genes and mating type in directing DNA double-strand breaks to homologous and non-homologous repair paths. Curr. Biol. **9:** 767–770.

Lengauer, C., K. W. Kinzler and B. Vogelstein, 1998 Genetic instabilities in human cancers. Nature **396:** 643–649.

Lichten, M., and J. E. Haber, 1989 Position effects in ectopic and allelic mitotic recombination in *Saccharomyces cerevisiae*. Genetics **123:** 261–268.

Liu, Y. G., N. Mitsukawa, T. Oosumi and R. F. Whittier, 1995 Efficient isolation and mapping of *Arabidopsis thaliana* T-DNA insert junctions by thermal asymmetric interlaced PCR. Plant J. **8:** 457–463.

Lovett, B. D., D. Strumberg, I. A. Blair, S. Pang, D. A. Burden *et al.*, 2001 Etoposide metabolites enhance DNA topoisomerase II cleavage near leukemia-associated MLL translocation breakpoints. Biochemistry **40:** 1159–1170.

Luria, S. E., and M. Delbruck, 1943 Mutations of bacteria from virus sensitivity to virus resistance. Genetics **28:** 491–511.

Malkova, A., E. L. Ivanov and J. E. Haber, 1996 Double-strand break repair in the absence of *RAD51* in yeast: a possible role for break-induced DNA replication. Proc. Natl. Acad. Sci. USA **93:** 7131–7136.

Megonigal, M. D., E. F. Rappaport, D. H. Jones, T. M. Williams, B. D. Lovett *et al.*, 1998 t(11;22) (q23;q11.2) in acute myeloid leukemia of infant twins fuses *MLL* with hCDC*rel*, a cell division cycle gene in the genomic region of deletion in DiGeorge and velocardiofacial syndromes. Proc. Natl. Acad. Sci. USA **95:** 6413–6418.

Morrow, D. M., C. Connelly and P. Hieter, 1997 "Break-copy" duplication: a model for chromosome fragment formation in *Saccharomyces cerevisiae*. Genetics **147:** 371–382.

Myung, K., C. Chen and R. D. Kolodner, 2001 Multiple pathways cooperate in the suppression of genome instability in *Saccharomyces cerevisiae*. Nature **411:** 1073–1076.

Nowell, P. C., 1997 Genetic alterations in leukemias and lymphomas: impressive progress and continuing complexity. Cancer Genet. Cytogenet. **94:** 13–19.

Ochman, H., J. A. Ajioka, D. Garza and D. L. Hartl, 1989 Inverse polymerase chain reaction, pp. 105–111 in *PCR Technology: Principles and Applications for DNA Amplification*, edited by H. A. Erlich. Stockton Press, New York.

Paques, F., and J. E. Haber, 1999 Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. Microbiol. Mol. Biol. Rev. **63:** 349–404.

Plessis, A., A. Perrin, J. E. Haber and B. Dujon, 1992 Site-specific recombination determined by I-*Sce*I, a mitochondrial group I intron-encoded endonuclease expressed in the yeast nucleus. Genetics **130:** 451–460.

Rabbitts, T. H., 1994 Chromosomal translocations in human cancer. Nature **372:** 143–149.

Reed, K. C., and D. A. Mann, 1985 Rapid transfer of DNA from agarose gels to nylon membranes. Nucleic Acids Res. **13:** 7207–7221.

Rose, M. D., 1987 Isolation of genes by complementation in yeast. Methods Enzymol. **152:** 481–504.

Rowley, J. D., 1998 The critical role of chromosome translocations in human leukemias. Annu. Rev. Genet. **32:** 495–519.

Rowley, J. D., 2000 Molecular genetics in acute leukemia. Leukemia **14:** 513–517.

Schneider, B. L., B. Steiner, W. Seufert and A. B. Futcher, 1996 pMPY-ZAP: a reusable polymerase chain reaction-directed gene disruption cassette for *Saccharomyces cerevisiae*. Yeast **12:** 129–134.

Sherman, F., 1991 Getting started with yeast. Methods Enzymol. **194:** 3–21.

Shero, J. H., M. Koval, F. Spencer, R. E. Palmer, P. Hieter *et al.*, 1991 Analysis of chromosome segregation in *Saccharomyces cerevisiae*. Methods Enzymol. **194:** 749–773.

Sikorski, R. S., and J. D. Boeke, 1991 *In vitro* mutagenesis and plasmid shuffling: from cloned gene to mutant yeast. Methods Enzymol. **194:** 302–318.

Sikorski, R. S., and P. Hieter, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:** 19–27.

Snyder, M., R. J. Sapolsky and R. W. Davis, 1988 Transcription interferes with the elements important for chromosome maintenance in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8:** 2184–2194.

Sobulo, O. M., J. Borrow, R. Tomek, S. Reshmi, A. Harden *et al.*, 1997 MLL is fused to CBP, a histone acetyltransferase, in therapy-related acute myeloid leukemia with a t(11;16)(q23;p13.3). Proc. Natl. Acad. Sci. USA **94:** 8732–8737.

Southern, E. M., 1975 Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:** 503–517.

Stevens, R. C., and T. N. Davis, 1998 Mlc1p is a light chain for the unconventional myosin Myo2p in Saccharomyces cerevisiae. J. Cell Biol. **142:** 711–722.

Strissel, P. L., R. Strick, J. D. Rowley and N. J. Zeleznik-Le, 1998 An in vivo topoisomerase II cleavage site and a DNase I hypersensitive site colocalize near exon 9 in the MLL breakpoint cluster region. Blood **92:** 3793–3803.

Strout, M. P., G. Marcucci, C. D. Bloomfield and M. A. Caligiuri, 1998 The partial tandem duplication of *ALL1* (*MLL*) is consistently generated by *Alu*-mediated homologous recombination in acute myeloid leukemia. Proc. Natl. Acad. Sci. USA **95:** 2390–2395.

Sugawara, N., and J. E. Haber, 1992 Characterization of double-strand break-induced recombination: homology requirements and single-stranded DNA formation. Mol. Cell. Biol. **12:** 563–575.

Sugawara, N., and J. W. Szostak, 1983 Recombination between sequences in nonhomologous positions. Proc. Natl. Acad. Sci. USA **80:** 5675–5679.

Thierry, A., A. Perrin, J. Boyer, C. Fairhead, B. Dujon *et al.*, 1991 Cleavage of yeast and bacteriophage T7 genomes at a single site using the rare cutter endonuclease I-*Sce*I. Nucleic Acids Res. **19:** 189–190.

Winston, F., C. Dollard and S. L. Ricupero-Hovasse, 1995 Construction of a set of convenient *Saccharomyces cerevisiae* strains that are isogenic to S288C. Yeast **11:** 53–55.

Zucman-Rossi, J., P. Legoix, J.-M. Victor, B. Lopez and G. Thomas, 1998 Chromosome translocation based on illegitimate recombination in human tumors. Proc. Natl. Acad. Sci. USA **95:** 11786–11791.

Communicating editor: L. S. Symington