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Nature and Distribution of Chromosomal Intertwinings in *Saccharomyces cerevisiae*

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To elucidate yeast chromosome structure and behavior, we examined the breakage of entangled chromosomes in DNA topoisomerase II mutants by hybridization to chromosomal DNA resolved by pulsed-field gel electrophoresis. Our study reveals that large and small chromosomes differ in the nature and distribution of their intertwinings. Probes to large chromosomes (450 kb or larger) detect chromosome breakage, but probes to small chromosomes (380 kb or smaller) reveal no breakage products. Examination of chromosomes with one small arm and one large arm suggests that the two arms behave independently. The acrocentric chromosome XIV breaks only on the long arm, and its preferred region of breakage is approximately 200 kb from the centromere. When the centromere of chromosome XIV is relocated, the preferred region of breakage shifts accordingly. These results suggest that large chromosomes break because they have long arms and small chromosomes do not break because they have small arms. Indeed, a small metacentric chromosome can be made to break if it is rearranged to form a telocentric chromosome with one long arm or a ring with an "infinitely" long arm. These results suggest a model of chromosomal intertwining in which the length of the chromosome arm prevents intertwinings from passively resolving off the end of the arm during chromosome segregation.

Sister chromatids are held together from the time of replication until the end of metaphase. As spindle fibers from opposite poles pull on the connected sisters, the tension generated appears to be essential to maintain stable microtubule attachment (37, 38). Therefore, the connection between sister chromatids contributes directly to ensuring that the two sister centromeres are oriented towards opposite poles. As anaphase begins, the connection between the sisters is dissolved, and because they are properly oriented, the chromosomes segregate correctly to the poles. Thus, the connection between sister chromatids is essential for ensuring that sister chromatids disjoin correctly. Several models have suggested that this connection is provided by topological intertwining between the replicative sisters (24, 33, 34, 50).

Several observations suggest that replicative sister DNA molecules are wound around one another prior to anaphase. Intertwinings of sister molecules appear to result from steric exclusion of swivelases from the termination regions between some replication forks, leading to DNA replication without concomitant unwinding (9, 50, 61). The twists of the double helix in the termination region are thus translated into intertwinings between the daughter DNA molecules. Because it makes double-strand breaks in the DNA, DNA topoisomerase II is the only topoisomerase that can disentangle the replicated sisters (1, 30). When topoisomerase II is inhibited by hypertonic conditions, the genome of simian virus 40 becomes intertwined following DNA replication (61). The intertwinings that are present at the termination region then become distributed over the entire simian virus 40 genome (59). Intertwining of circular DNA molecules has also been observed in topoisomerase II (*top2*) mutants of *Saccharomyces cerevisiae* (6, 9, 27).

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As with circular plasmids, eukaryotic chromosomes appear to be intertwined following DNA replication. The presence of intertwinings in natural chromosomes is inferred from the phenotypes of temperature-sensitive *top2* mutants in yeasts. In the fission yeast *Schizosaccharomyces pombe*, the tangled nuclei seen in *top2* mutants appear to result from the inability of the cells to resolve intertwinings of sister chromatids (58). Indeed, in situ hybridization has allowed the visualization of the spindle pulling at the centromeres, stretching the chromatin fibers while the tangled chromatin mass remains behind (10). In the budding yeast *S. cerevisiae*, chromosome separation gels reveal chromosomes that have broken when intertwined molecules were segregated in the absence of topoisomerase II activity (26).

Although it has been proposed that chromosomal intertwinings play an important role in chromosome segregation (24, 33, 34, 50), little is known about their nature and distribution. One reason for this lack of information is that the transient nature of intertwinings makes them difficult to study in wild-type cells. The existence of *top2* mutants in yeast, however, provides a cell type in which intertwinings are relatively stable. In addition, because of their small size, budding yeast chromosomes lend themselves well to analysis by pulsed-field gel electrophoresis (2, 46). Furthermore, elements of chromosomal substructure, such as the centromere, are well characterized in budding yeast cells, and these elements can be easily relocated by homologous recombination. These advantages allow the manipulation of genotype and chromosomal conformation to address specific questions about chromosome structure.

We have undertaken a study of chromosome intertwining in *S. cerevisiae* in order to answer two questions. (i) What is the distribution of intertwinings in mitotic yeast chromosomes? (ii) What is the functional significance of chromosomal intertwining in holding sister chromatids together until anaphase? Using hybridization to pulsed-field gels of chromosomal DNA from topoisomerase II mutants, we find that

TABLE 1. *S. cerevisiae* strains used in this study

Strain	Genotype	Source or reference
CH322	*MATa his4-539am lys2-801am ura3-52 top2-2*	25
CH325	*MATa his4-539am lys2-801am ura3-52 top2-4*	25
CH332	*MATa his4-539am lys2-801am ura3-52 TOP2*	25
CH335	*MATa his4-539am lys2-801am ura3-52 TOP2*	25
CH561	*MATa his4-539am lys2-801am ura3-52 TOP2*	25
CH732	*MATa his4-539am lys2-801am ura3-52 top2-13*	55
CH734	*MATa his4-539am lys2-801am ura3-52 TOP2*	55
CH897	*MATa leu2-3,112 lys2-801am ura3-52 top2-4*	56
CH898	*MATa leu2-3,112 lys2-801am ura3-52 TOP2*	56
CH1565	*MATa his4-539am lys2-801am ura3-52 rad50Δ::URA3 top2-4*	This study
CH1567	*MATa his4-539am lys2-801am ura3-52 rad50Δ::URA3 TOP2*	This study
CH1634	*MATa his4-539am lys2-801am ura3-52 rad52Δ::URA3 TOP2*	This study
CH1640	*MATa his4-539am lys2-801am ura3-52 rad52Δ::URA3 top2-2*	This study
CH1869	*MATa lys2-801am met2Δ*::CEN7.*LEU2*[a] cen14Δ::*URA3 top2-4*	This study
CH1870	*MATa lys2-801am met2Δ*::CEN7.*LEU2*[a] cen14Δ::*URA3 TOP2*	This study
CH1998	*MATα ade1 leu2 lys2-801am ura3-52 top2-4* ring chromosome III	This study
CH1999	*MATα top2-4* telocentric chromosome III	This study
CH2000	*MATα leu2 lys2-801am ura3-52 TOP2* ring chromosome III	This study
CH2001	*MATα TOP2* telocentric chromosome III	This study

[a] The *LEU2* gene is inserted adjacent to CEN7.

intertwinings lead to breakage preferentially located in a region centering around 200 kb from the centromere. The absence of breakage of small chromosomes in *top2* mutants suggests that intertwinings are not sufficient to prevent sister chromatid separation.

MATERIALS AND METHODS

Strains and media. *S. cerevisiae* strains used in this study are listed in Table 1. Standard genetic techniques and media were used (47).

Chromosome breakage assay. Yeast strains were grown to early log phase at 25°C. For temperature shift experiments, the culture was split. One-half was shifted to 35°C for one and one-half generation times, and the other half was returned to 25°C for one and one-half generation times. For cold-sensitive alleles, the permissive and restrictive temperatures were 30°C and 14°C, respectively. The morphology and viability of the cultures were tested in each experiment to confirm the expected lethality and accumulation of doublets in *top2* mutants at restrictive temperature (data not shown) (25).

Chromosomal DNA was isolated by using a modification of the protocol described by Carle and Olson (3). Cells were spun and washed with 0.05 M EDTA, pH 8.0. Cells were then resuspended in 0.05 M EDTA at 5×10^9 cells per ml and kept at 35°C. Zymolyase (2 mg/ml) in 0.01 M $NaPO_4$–50% glycerol was added to cells to a final concentration of 0.1 mg/ml. Next, two volumes of 1% low-melt agarose in 0.125 M EDTA at 50°C were added to the resuspended cells, mixed by pipetting up and down, poured into a plug mold, and cooled on ice. The plugs were incubated in 0.05 M EDTA–0.01 M Tris-HCl (pH 7.6)–7.5% β-mercaptoethanol at 37°C overnight and then incubated in 0.05 M EDTA–0.01 M Tris-HCl–1% Sarkosyl–1 mg/ml-proteinase K at 50°C overnight. Plugs were stored in 0.05 M EDTA at 4°C.

The plugs were cut to the size of a well and run on a 1% pulsed-field agarose gel in 0.5× Tris-borate-EDTA (TBE) in a CHEF-DR pulsed-field gel apparatus (Bio-Rad) (4). Electrophoresis was performed in a 4°C constant-temperature room with circulation of the buffer through several feet of tubing running through a heat sink. The running conditions were generally 60-s switch time for 18 to 20 h and then 90-s switch time for 12 to 14 h. The gel was stained in 0.5-mg/ml ethidium bromide for 1/2 h. To avoid any problems from incomplete transfer to membranes, the gels were probed directly (39, 57). All washes were done with gentle shaking. To prepare for hybridization, the gel was denatured at room temperature for 1 h in 0.5 N NaOH–0.15 M NaCl and then renatured in 0.15 M NaCl–0.5 M Tris-HCl (pH 7.6) for 1 h at room temperature. The gel was then dried onto Whatman 3MM filter paper on a Bio-Rad gel dryer at 60°C for 1.5 to 2 h. The dried gel affixed to the paper was stored at room temperature until further use. Prior to hybridization, the dried gel was rehydrated off the paper in distilled water and restained in ethidium bromide.

Probes were made by random-primer labeling (see Table 2 for the origin of the DNA fragments used to make the probes). Gels were prehybridized for 1 h and hybridized overnight in BLOTTO (31) in sealable bags at 65°C. They were washed in 1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate (SDS) at 65°C for 2 h and then in 0.1× SSC–5% SDS at 65°C for 2 h. The gels were stored wrapped at −20°C and exposed to preflashed X-ray film at −70°C. Densitometry was done on the exposures with a PDI densitometer.

Assessing sensitivity. To estimate the sensitivity of our assay, we determined the level of breakage that could go undetected by comparing two exposures of the hybridization of a probe to a large chromosome (*SPO13*, chromosome VIII) with similar exposures of the hybridization of a probe to a small chromosome (*ADE1*, chromosome I) (data not shown). The bands in these exposures that were analyzed by densitometry were all in linear range of the film. Although the breakage products of the large chromosome were clearly visible on the longer exposure, they could not be visualized on the shorter exposure. No breakage products from the small chromosome were visible on the longer exposure, even though the intact chromosome I band was overexposed. Taking into account the different specific activities of the probes and the different lengths of the exposures, comparison of the bands of chromosome VIII and chromosome I

allowed us to estimate that 2% breakage of chromosome I could go undetected.

***rad50* and *rad52* experiments.** Strains for the *top2 rad50* experiment were produced by transforming a *rad50* deletion plasmid into strains CH325 (*top2-4*) and CH335 (*TOP2*), as described elsewhere (43). The correct construction was verified by UV sensitivity assay and by hybridization to Southern blots (data not shown) (49). Strains for the *top2 rad52* experiment were created similarly with the plasmid pCH1068, which carries a deletion of *RAD52* containing an insertion of *URA3* (56). The *rad52* genotype of the transformants was confirmed by hybridization to Southern blots (data not shown) (49).

Gamma-ray irradiation. To detect randomly generated chromosome breakage, we employed a ^{137}Cs source with a fixed dose of 64.4 rads and a dose rate of 16.2 rads/s. Aliquots of a culture of strain CH332 (*TOP2*) in early log phase were irradiated with 0, 15, 34, or 62 kilorads, and chromosomal DNA was isolated as usual.

Centromere relocation. The centromere of chromosome XIV was relocated by simultaneous single step gene transplacements (44). We replaced the centromere on chromosome XIV with a *URA3* marker and inserted a fragment containing CEN7 and *LEU2* into a deletion of the *MET2* locus on the left arm of chromosome XIV. The plasmids containing CEN7 and CEN14 (p202 and p203, respectively) were kindly provided by P. Hieter (20). The plasmid containing *MET2*, pPL4-2-1, was kindly provided by G. Faugeron (28). These plasmids were used to construct plasmids appropriate for centromere relocation, as described below.

To construct the cen14 disruption plasmid, plasmid pCH1369 was created by subcloning the 2.3-kb *Hin*dIII fragment containing CEN14 from plasmid p203 into *Hin*dIII-digested plasmid pCH1050, a plasmid created by disrupting the *Eco*RV site in the plasmid pBSKS (Stratagene). All of the consensus centromere sequence (125 bp) in plasmid pCH1369 was deleted by digesting with double *Xmn*I and *Eco*RV partials and ligating in the 1.6-kb *Nru*I-*Sma*I *URA3*-containing fragment from the plasmid YCp50, creating plasmid pCH1398. The lack of centromere activity in this plasmid was confirmed by plasmid stability assay (data not shown) (33).

To create a plasmid carrying a CEN7 insertion at *MET2*, plasmid pCH1399 was constructed by ligating the 1.0-kb *Hin*dIII-*Bgl*II fragment containing CEN7 from plasmid p202 into plasmid YEp351 (21), which was digested with *Hin*dIII and *Bam*HI. The 2.1-kb *Bam*HI-*Sal*I fragment containing the *MET2* gene from plasmid pPL4-2-1 was subcloned into plasmid pBR322 digested with *Bam*HI and *Sal*I, creating plasmid pCH1367. Most of the *MET2* open reading frame in plasmid pCH1367 was deleted by digesting with *Bgl*II, filling in with T4 DNA polymerase, digesting with *Sst*I, and ligating in the 3.0-kb *Sst*I-*Hpa*I fragment containing CEN7 and *LEU2* from plasmid pCH1399, creating plasmid pCH1422. The centromere activity on this plasmid was confirmed by plasmid stability assay (data not shown).

We used these plasmids to construct *top2-4* and *TOP2* strains in which the centromere of chromosome XIV was relocated to the *MET2* locus. The 3.8-kb *Hin*dIII fragment of plasmid pCH1398 containing cen14Δ::*URA3* and the 5.3-kb *Bam*HI-*Sal*I fragment of plasmid pCH1422 containing *met2*Δ::CEN7.*LEU2* (i.e., *MET2* disrupted by CEN7 with an adjacent *LEU2* gene) were simultaneously transformed into strains CH897 (*top2-4*) and CH898 (*TOP2*) by a modified lithium acetate procedure (48). Transformants were selected on complete medium plates lacking uracil and/or leucine. Prototrophic colonies were then tested for methionine auxotrophy on complete medium plates lacking methionine. Ura$^+$ Leu$^+$ Met$^-$ transformants were tested by Southern blotting for the correct constructions at both insertion/deletion loci (data not shown) (49). Transformants were also tested in genetic crosses to strains containing the wild-type chromosome XIV structure. As expected, diploids heterozygous for the wild-type and the relocated chromosome XIV had low spore viability, and most tetrads were 2:2 for viability (data not shown). This result is expected because an odd number of crossovers between the insertion/deletion loci of the rearranged chromosome XIV and the wild-type chromosome XIV leads to the production of acentric and dicentric versions of chromosome XIV.

Telocentric and ring chromosome III strains. The 320-kb circular and telocentric chromosome III strains TA1145 (*MAT***a** *ade1 leu2 ura3 can1* ring chromosome III) and TA1145-I1 (*MAT***a** *ade1 leu2 ura3 can1* telocentric chromosome III::*URA3.LEU2*) were generously provided by A. Murray (36). The strains used to compare the breakage of these chromosomes in a *top2-4* background were made by backcrossing strain TA1145 and strain TA1145-I1 to strain CH325 twice. *TOP2* and *top2-4* spores with the ring, telocentric, or wild-type chromosome III were identified by their genetic markers. The circular configuration of the ring chromosome III was confirmed by pulsed-field gel analysis. The ring chromosome III does not normally run into pulsed-field gels (12), but random breakage induces the ring to run as a linear 320-kb band, as expected (data not shown). The construction of the telocentric chromosome III strains was tested by pulsed-field gel analysis and by hybridization to Southern blots (data not shown) (36, 49).

RESULTS

Unlike large chromosomes, small chromosomes do not break at detectable levels in *top2* strains at restrictive temperature. To study yeast chromosomal intertwinings and their functional significance, we examined the consequences of chromosome segregation in cells lacking the DNA-untangling activity of DNA topoisomerase II. In the absence of topoisomerase II activity, chromosome segregation leads to nondisjunction and chromosome breakage (26, 58). Although topoisomerase II has been proposed to play a structural role in the chromosome scaffold (14, 19), the damage in *top2* mutants is probably not caused by perturbation of a possible structural function of topoisomerase II, because removal or inactivation of topoisomerase II does not affect chromosome structure in vitro or in vivo (22, 53). Therefore, chromosome damage is most likely caused by the inability to resolve topological intertwinings between sister chromatids. In order to determine the nature and distribution of the topological intertwinings, we examined the effect of *top2* mutations on breakage of several chromosomes.

Examination of two chromosomes suggests that they differ in their response to a lack of topoisomerase II activity. Wild-type cells (*TOP2*) and temperature-sensitive *top2* mutants (*top2-4*) were grown to early log phase at permissive temperature. Each culture was split and incubated at permissive or restrictive temperature for one and one-half generation times. Intact chromosomal DNA was then isolated from cells (3) and resolved on a contour-clamped homogeneous electric field (CHEF) pulsed-field gel (Fig. 1A) (4). As with many strains, our strains show karyotypic differences from the strains used in physical mapping of the

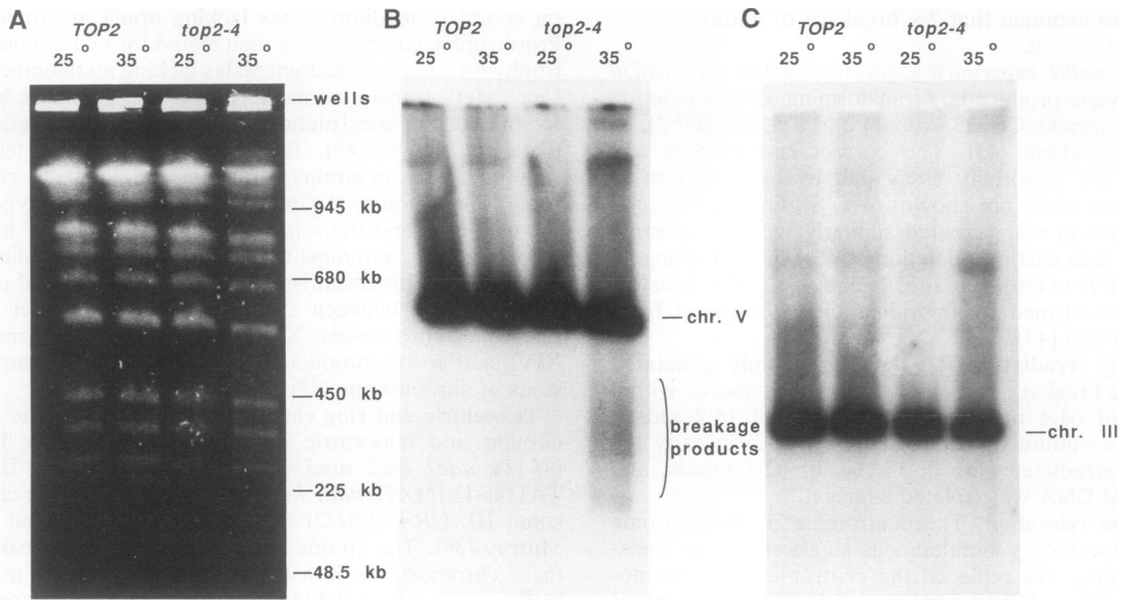

FIG. 1. Unlike chromosome V, chromosome III does not break in *top2* strains at restrictive temperature. Logarithmically growing cultures of strains CH335 (*TOP2*) and CH325 (*top2-4*) were split, and aliquots were incubated at 25 or 35°C for one and one-half generation times (3 1/2 or 2 1/2 h, respectively). Chromosomal DNA was isolated from these cultures and run on a CHEF pulsed-field gel. (A) Ethidium bromide-stained agarose gel. Size standards from phage lambda DNA and yeast chromosomes (Bio-Rad) are indicated on the right. (B) Autoradiograph of dried agarose gel hybridized with *URA3* probe from chromosome V (600 kb). The DNA in the wells is not always maintained throughout the hybridization process because of the fragile nature of the plugs. Note the smear of breakage products (180 to 450 kb) only in DNA from the restrictive-temperature *top2* culture. (C) Autoradiograph of HMRP probe to chromosome III (380 kb) hybridized to a sample of the same DNA shown in panel A run on an adjacent section of the gel. Note the lack of visible breakage products. Breakage products are not visible even on longer exposures (data not shown).

yeast genome (32, 41). Therefore, chromosome sizes in our strains were estimated by using DNA standards (Bio-Rad). To assay chromosome breakage, we hybridized chromosome-specific probes to the dried agarose gel (Table 2) (39, 57). A probe to chromosome V detects the intact 600-kb chromosome V band in DNA from *TOP2* and *top2-4* cells at both permissive and restrictive temperatures. However, a smear of chromosome breakage products appears only in the DNA from the mutant culture grown at restrictive temperature (Fig. 1B). Surprisingly, when samples of the same DNA are run on an adjacent section of the gel and hybridized to a probe to chromosome III, no breakage products of the 380-kb chromosome III are detected (Fig. 1C); breakage cannot be detected even in much longer exposures (data not shown). Similar results were obtained with DNA prepared from strains carrying other *top2* alleles (*top2-2* and *top2-13*; data not shown). Thus, it appears that unlike chromosome V, chromosome III does not have detectable levels of breakage in *top2* strains at restrictive temperature.

TABLE 2. Plasmid origin of probes used in this study

Probe	Plasmid	Fragment	Source or reference
3LT	p78-4.4	1.0-kb *Eco*RI-*Sal*I	40
14R	pCH1343	0.9-kb *Hin*dIII-*Bam*HI	This study
14M	pCH1381	1.2-kb *Hin*dIII-*Eco*RI	This study
ADE1	pUC19-*ADE1*	0.7-kb *Eco*RI	J. Haber
CEN14	pCH1369	0.5-kb *Eco*RV	This study
CDC14	pCH1103	1.0-kb *Eco*RI-*Kpn*I	This study
HMRP	pJH340	1.8-kb *Eco*RI	J. Haber
HOP1	pNH35-1	2.2-kb *Hin*dIII	23
KAR1	pMR24	2.3-kb *Bam*HI-*Bgl*II	G. Fink
MET2	pCH1367	1.1-kb *Bgl*II-*Sal*I	This study
PET494	pMC203	0.9-kb *Xho*I-*Kpn*I	5
RAD52	pSM22	1.4-kb *Bam*HI-*Cla*I	N. Kleckner
SPO13	p(spo13)16	0.4-kb *Pst*I-*Hin*dIII	60
TOP2	pCH1050	0.3-kb *Hin*dIII-*Eco*RI	55
URA3	p(spo13)16	1.1-kb *Hin*dIII	60

Examining the breakage of additional chromosomes revealed that the size of a chromosome determines whether or not it breaks. We found that probes to chromosomes 450 kb or larger detect chromosome breakage, but probes to chromosomes 380 kb or smaller detect no breakage products (the data are summarized in Table 3). To confirm that the difference in the levels of chromosome breakage between small and large chromosomes is significant, we quantitated the level of breakage of large chromosomes using densitometry. Such analysis is important because the breakage products are distributed over a wide range of sizes, making their signal appear relatively weak. Quantitation was done with autoradiographs in which the exposures of both the chromosome band and the breakage products were in the linear range of the film. This analysis was possible for three large chromosomes: chromosome XIII (970 kb) exhibited 42% breakage, chromosome XIV (820 kb) exhibited 35% breakage, and chromosome VIII (600 kb) exhibited 38% breakage. Because only one sister chromatid of each intertwined pair may break during segregation, the proportion of chromosomes that are intertwined may be twofold higher (see Discussion). Using densitometry, we also estimated the amount of breakage that could go undetected (see Materials and Methods), which is approximately 2%. Therefore, although approximately one-third of the large chromosomes

TABLE 3. Chromosome breakage depends on chromosome size[a]

Chromosome	Size (kb)[b]	Probe[c]	Breakage
VII	1,120	*HXK2*	Yes[d]
XIII	970	*RAD52*	Yes
XIV	820	*MET2*	Yes
XIV	820	*KAR1*	Yes
XIV	820	*TOP2*	Yes
XIV	820	14M	Yes
XIV	820	CEN14	Yes
XIV	820	*PET494*	Yes
XIV	820	14R	Yes
V	600	*URA3*	Yes
VIII	600	*SPO13*	Yes
IX	450	*HOP1*	Yes
III	380	3LT	No
III	380	HMRP	No
VI	290	*CDC14*	No
I	260	*ADE1*	No

[a] In several experiments, chromosomal DNA was isolated from *TOP2* and *top2* strains grown at permissive temperature and then shifted to permissive or restrictive temperature for one and one-half generation times. The samples were run on a CHEF gel and prepared for hybridization. The gels were probed with DNA specific for particular chromosomes, producing autoradiographs similar to those in Fig. 1. This table presents a summary of the results.

[b] Because of karyotypic differences between our strains and strains used in physical mapping studies (32, 41, 42), chromosome sizes are estimated from a phage lambda ladder and yeast chromosome size standards (Bio-Rad).

[c] See Table 2 for the plasmid origin of these probes.

[d] Information for chromosome VII was derived from a previous study (26).

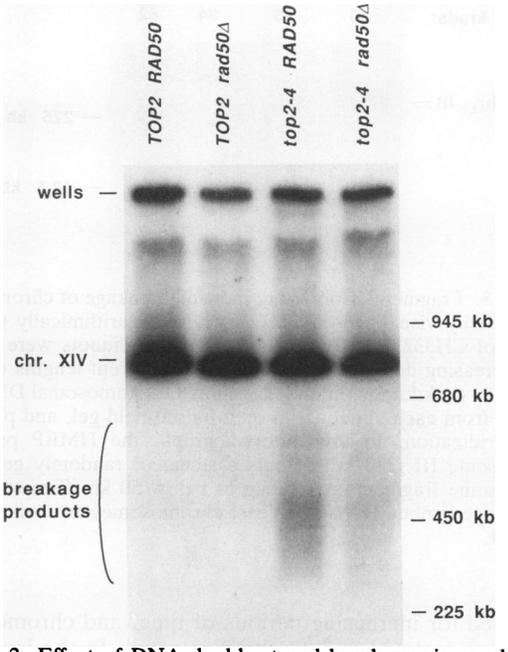

FIG. 2. Effect of DNA double-strand break repair on chromosome breakage in *top2* strains. Chromosomal DNA was isolated from strains CH335 (*TOP2 RAD50*), CH1567 (*TOP2 rad50Δ*), CH325 (*top2-4 RAD50*), and CH1565 (*top2-4 rad50Δ*), run on a pulsed-field gel, and prepared for hybridization, as for Fig. 1. Size standards from yeast chromosomes are indicated on the right. This autoradiograph shows the hybridization of a *KAR1* probe to the DNA from the restrictive-temperature cultures only. The probe detects DNA in the wells and the intact chromosome XIV (820 kb) in all of the lanes. A smear of breakage products is produced only in the DNA from *top2* cultures. Note that the amount of breakage products in the *top2-4 rad50Δ* strain is not greater than the amount in the *top2-4 RAD50* strain. Increased hybridization might have been expected if most breakage products were repaired in the *top2-4 RAD50* strain before DNA isolation.

break in *top2* mutants at restrictive temperature, small chromosomes break less than 2% of the time.

This unexpected result suggests that small and large chromosomes behave differently, perhaps because of differences in the number or stability of their intertwinings. For example, the numbers of intertwinings between sister chromatids may vary greatly according to the size of the chromosome. Alternatively, the size of a chromosome could affect the ability of intertwinings to be resolved "passively", by simply falling off the end of the chromosome when the sister chromatids separate. In order to draw either conclusion, however, it is necessary to eliminate the trivial possibility that breakage products from small chromosomes (small DNA fragments) are lost before detection by hybridization.

No class of breakage products is underrepresented because of repair or loss. Differences in the observed levels of chromosome breakage of small and large chromosomes could be due to the relative ability of the cell to repair large versus small chromosomes. Specifically, small breakage products (i.e., small fragments of small chromosomes) might be more efficiently repaired than large chromosome fragments. The role of DNA repair could be significant because our assay necessarily detects only those breaks that are not repaired. To rule out the possibility that small chromosomes are broken but that their fragments are repaired in the cell before DNA isolation, we examined the effect of DNA repair on the level of detectable breakage.

To determine whether double-strand DNA repair affects the number or size distribution of observed chromosome breakage products, we examined breakage in repair-deficient *rad50* and *rad52* strains. If most breaks are normally repaired before DNA isolation in *top2 RAD50* strains, then the level of breakage should be greater in a *top2 rad50* double mutant than in the *top2 RAD50* single mutant. We prepared DNA from *TOP2 RAD50*, *TOP2 rad50Δ*, *top2-4 RAD50*, and *top2-4 rad50Δ* strains at permissive and restrictive temperature, and a pulsed-field gel of the DNA was hybridized with a probe to chromosome XIV (Fig. 2). As expected, chromosome breakage is visible only in the *top2* cultures grown at restrictive temperature; no breakage products are visible in the permissive-temperature cultures of any strains (data not shown). Strikingly, there is no increase in the observed level of breakage in the *top2-4 rad50Δ* double mutant compared with the level in the *top2-4 RAD50* single mutant. (Similar results were observed with a *top2-2 rad52Δ* strain [data not shown].) Indeed, for reasons that remain unclear, the level of breakage is actually lower in the double mutants. Thus, it appears that DNA repair has little effect on our ability to visualize the products of broken chromosomes. Furthermore, it appears unlikely that efficient repair of breaks in small chromosomes causes the distinction between the levels of breakage observed for small and large chromosomes.

An alternative trivial explanation for the distinction between small and large chromosomes is that degradation of breakage products could cause preferential loss of small fragments, such as those produced by breakage of small chromosomes. To examine this possibility, we tested our ability to detect small chromosome fragments after random chromosome breakage was induced by gamma rays. A culture of *TOP2* cells growing in early log phase was

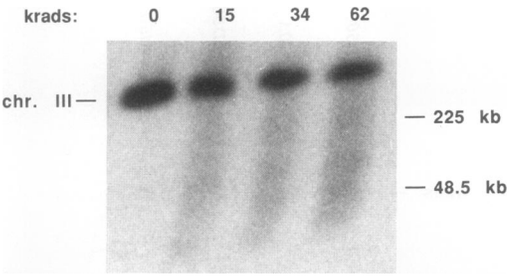

FIG. 3. Fragments from γ-ray-induced breakage of chromosome III are easily visualized on CHEF gels. A logarithmically growing culture of CH332 (*TOP2*) was split, and the aliquots were treated with increasing doses of γ-irradiation for different lengths of time; the results (in kilorads [krads]) are shown. Chromosomal DNA was isolated from each aliquot, run on a pulsed-field gel, and prepared for hybridization. In this autoradiograph, the HMRP probe to chromosome III (380 kb) detects a smear of randomly generated chromosome fragments extending to below 50 kb. Size standards from phage lambda DNA and yeast chromosomes are indicated on the right.

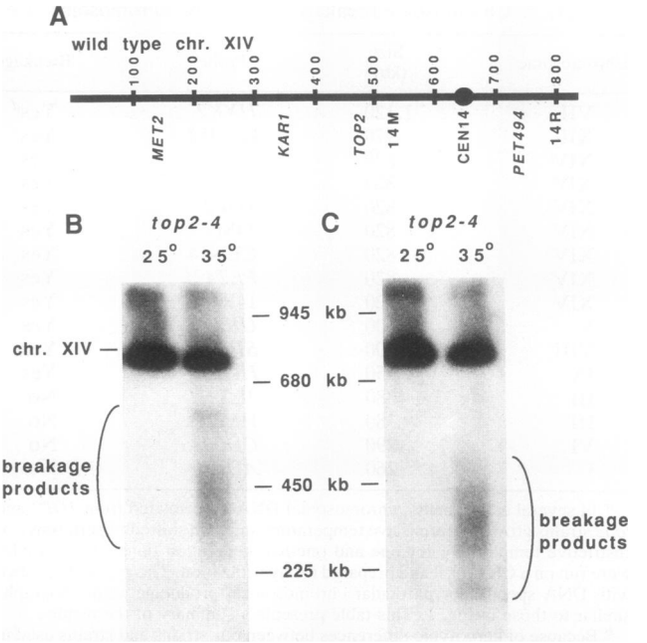

FIG. 4. Mapping breakage sites on the arms of chromosome XIV. Chromosomal DNA was isolated from strains CH335 (*TOP2*) and CH325 (*top2-4*), run on a pulsed-field gel, and prepared for hybridization. (A) Map of chromosome XIV. Distance (in kilobases) is indicated above the chromosome. Probes used to localize the region of breakage are indicated below the chromosome. (B) Autoradiograph revealing hybridization of *MET2* probe from the left arm of chromosome XIV to DNA from *top2-4* cultures at permissive and restrictive temperatures. Size standards from yeast chromosomes are indicated at the right. The location of hybridization to chromosome XIV (820 kb) and the smear of breakage products (300 to 600 kb) are indicated on the left. (C) Autoradiograph of 14R probe from the right arm of chromosome XIV hybridized to a sample of the same DNA as shown in panel B, run on an adjacent section of the gel. The smear of breakage products (180 to 540 kb) is indicated on the right; size standards are at the left. The lack of breakage products smaller than 180 kb suggests that breakage does not occur on the short arm of chromosome XIV.

irradiated for increasing periods of time, and chromosomal DNA was isolated and resolved as usual. In the irradiated samples, a probe to chromosome III (HMRP [Table 2]) detects a smear of fragments, including molecules smaller than 50 kb (Fig. 3). Since densitometry shows that the total signal is constant in all lanes, it is clear that breakage products of small chromosomes are not lost or degraded in our gel system. Thus, it appears that the difference in the level of breakage we detect reflects a real difference in the behavior or structure of large versus small chromosomes.

The level of breakage is determined independently for each chromosome arm. Large chromosomes may break because of their overall size or because of the size of their arms. If the latter hypothesis is true, each arm of a chromosome might behave independently on the basis of the size of that arm. For example, arms 250 kb or smaller (the size of the largest arm of a chromosome that does not show detectable breakage [the right arm of chromosome III]) should not break, even when they are components of a large chromosome (>500 kb).

To test this hypothesis, we determined the preferred region of breakage on chromosome XIV, which has one long arm (660 kb) and one short arm (160 kb) (32, 42). If breaks occur only on arms longer than 250 kb, then probes to chromosome XIV should detect only fragments that result from breaks on the long arm. The location of chromosome breakage on chromosome XIV can be determined by plotting the size of the breakage products along the chromosome. The results from using telomeric probes are the most straightforward to analyze (11), but probes to several more-proximal sites give consistent results (data not shown). The most telomeric probe from the left arm of chromosome XIV (*MET2*) detects breakage products of 300 to 600 kb (Fig. 4B); the most telomeric probe to the right arm (14R) detects breakage products of 180 to 540 kb (Fig. 4C). Assuming one break per molecule, these size ranges suggest that breakage occurs in the region between 300 and 600 kb from the left telomere; there is a preferred region of breakage between 300 and 540 kb and a minor region of breakage approximately 600 kb from the left telomere. The sizes of the breakage products suggest that most breaks occur on the long arm of chromosome XIV. Thus, it appears that the small arm of chromosome XIV does not break even though it is part of a large (820-kb) chromosome. These results are consistent with the results from the examination of two other chromosomes, chromosome V (150- and 450-kb arms) and chromosome VIII (130- and 470-kb arms) (32, 41). The sizes of the breakage products (chromosome V, 180 to 450 kb; chromosome VIII, 210 to 530 kb) detected by probes to the short arm of each chromosome suggest that breakage occurs on the long arms but not on the short arms (Fig. 1B and data not shown). Thus, the determination of whether a chromosome arm will break appears to be made independently for each chromosome arm, without regard to the total size of the chromosome.

Changing the size of the chromosome arm alters its behavior. To distinguish whether the region of breakage is determined by the size of a chromosome arm rather than by some inherent weak point in its structure, we altered the length of two chromosome arms by moving a centromere. The centromere of chromosome XIV was relocated by deleting the wild-type centromere and simultaneously inserting a new centromere at the *MET2* locus on the left arm (see Materials and Methods for details). The wild-type chromosome XIV has a large left arm and a small right arm (660 and 160 kb, respectively). The altered chromosome XIV has a small left

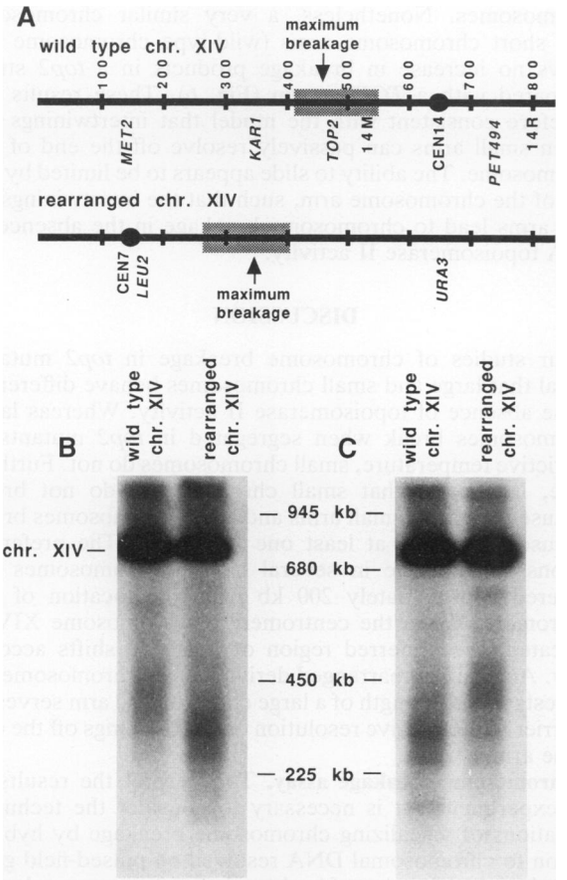

FIG. 5. The effect of centromere relocation on breakage of chromosome XIV. Chromosomal DNA was isolated from strains CH897 (*top2-4* wild-type chromosome XIV) and CH1869 (*top2-4* rearranged chromosome XIV), run on a pulsed-field gel, and prepared for hybridization. (A) The structures of the wild-type chromosome XIV and the rearranged chromosome XIV. The centromere of chromosome XIV was relocated by simultaneous deletion of the centromere at its wild-type locus and insertion of a new centromere at the *MET2* locus. The preferred region of breakage on each chromosome is indicated by the grey box. Distance (in kilobases) is indicated above the chromosome. Probes used to localize the region of breakage are indicated below the chromosome. (B) Autoradiograph revealing the hybridization by a *MET2* probe from the left arm of chromosome XIV. Size standards from yeast chromosomes are indicated on the right. (C) Autoradiograph of 14R probe from the right arm of chromosome XIV hybridized to a sample of the same DNA as shown in panel B, run on an adjacent section of the gel. The range of sizes of breakage products in the smears is altered in the centromere relocation strain; size standards are at the left.

arm and a large right arm (110 and 710 kb, respectively) (Fig. 5A).

If the length of a chromosome arm and not some inherent weak point in chromosome structure determines the region of chromosome breakage, then changing the location of the centromere should alter the region of chromosome breakage. We probed the DNA from *top2-4* strains containing the wild-type chromosome XIV or the rearranged chromosome XIV with the two most telomeric probes, *MET2* and 14R (Fig. 5B and C). (The results from more internal probes [Fig. 5A] are consistent with the results from the telomeric probes [data not shown].) As predicted, the relocation of the centromere caused a shift in the size of the breakage products detected by both probes. The shift indicates that the breakage points are different in the wild-type chromosome XIV and the rearranged chromosome XIV. For both the wild-type and the rearranged chromosome, the distances from the preferred region of breakage to the appropriate centromere are similar, centering around 200 kb. This distance between the preferred region of breakage and the centromere is similar to what we have observed for other natural chromosomes (chromosome V and chromosome VIII). Thus, it appears that the preferred region of breakage for each chromosome is determined not by weak points in the chromosome structure but by its distance from the centromere.

A small chromosome can be made to break if it has one long arm. If chromosome arms are truly autonomous, then rearranging a small chromosome so that it has long arms should transform it from a nonbreaking chromosome to a breaking chromosome. We tested a derivative of chromosome III in which the two short arms were rearranged to create one large arm (36). The natural chromosome III in our strains is a metacentric chromosome with arms of approximately 100 and 250 kb. In the rearranged chromosome, most of the left arm of chromosome III was relocated to the right arm, creating a telocentric chromosome III with one long arm of 320 kb. Thus, the two chromosomes are virtually identical in sequence, but they differ in the arrangement of their arms.

To determine whether a small chromosome with one long arm would break, we examined DNA from strains carrying either the natural chromosome III or the telocentric chromosome III. Breakage was assessed by using a probe to sequences proximal to *HMR* (Fig. 6B, lanes 1, 2, 4, and 5). As expected, hybridization to the DNA from strains containing the natural chromosome III reveals that there is no significant difference between *TOP2* and *top2-4* strains. In contrast, a smear of breakage products is clearly visible below the intact chromosome band in the *top2-4* strain containing the telocentric derivative. Therefore, although the rearranged chromosome is only 320 kb long, the size of its arm is sufficient to cause breakage. Thus, small chromosomes break if they have long arms.

The length of chromosome arms acts as a barrier to the passive resolution of intertwinings. Why does the length of a chromosome arm determine whether it will suffer chromosome breakage? One explanation is that the probability of intertwinings is related to the length of the chromosome arm, and longer arms are intertwined whereas shorter arms are not. This explanation would be surprising, in light of numerous observations of small circular plasmids being intertwined in *top2* strains at restrictive temperature (6, 9, 27). Alternatively, all arms may possess intertwinings, but in short chromosome arms the intertwinings may be able to resolve passively (i.e., without requiring DNA topoisomerase II activity) by sliding off the end of the chromosome.

To test the possibility that intertwinings can fall off the end of short chromosome arms, we examined the behavior of a small chromosome in a circular conformation. Circular DNAs are unique in that any intertwinings between them result in catenation that cannot be resolved in the absence of topoisomerase II activity. Thus, the behavior of the circular chromosome is expected to reveal the consequences of all intertwinings, not just those that fail to resolve passively on a similar linear molecule.

We compared the behavior of a circular derivative of chromosome III with both the natural metacentric chromosome III and a telocentric derivative in *TOP2* and *top2-4* strains. The ring chromosome III was created by recombination between the silent mating type cassettes (17). Be-

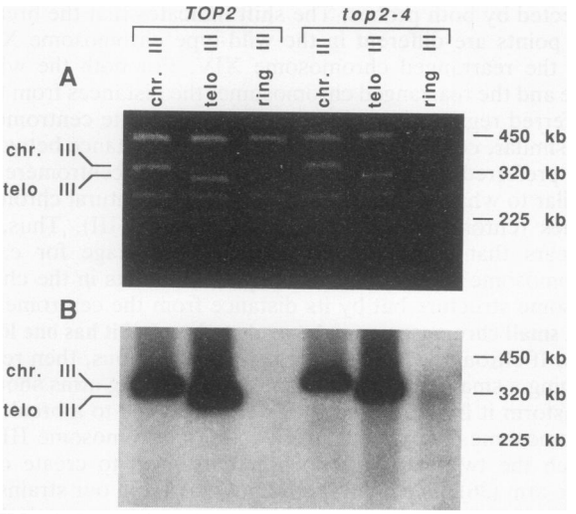

FIG. 6. Increasing the length of an arm of chromosome III causes it to break. Chromosomal DNA was isolated from strains CH335 (*TOP2* wild-type chromosome III), CH2001 (*TOP2* telocentric chromosome III), CH2000 (*TOP2* ring chromosome III), CH325 (*top2-4* wild-type chromosome III), CH1989 (*top2-4* telocentric chromosome III) and CH1988 (*top2-4* ring chromosome III). The samples were run on a pulsed-field gel and prepared for hybridization. (A) Ethidium bromide-stained agarose gel of DNA from the restrictive-temperature culture of each strain. Size standards from yeast chromosomes are indicated at the right. Chromosomes IX, III, VI and I are visible in this gel (top to bottom). The wild-type and the telocentric chromosome III bands are marked on the left. Note that the telocentric chromosome III (320 kb) is somewhat smaller than the wild-type chromosome III (380 kb). Although the ring chromosome does not enter the gel (data not shown), the similar intensity of the bands in each lane demonstrates that similar amounts of chromosomal DNA were run in each lane. (B) Autoradiograph revealing the hybridization of the HMRP probe to chromosome III sequences. The localization of the hybridization to the wild-type and the telocentric chromosome III is marked on the left. The localization of the hybridization to the linearized ring chromosome III is indicated on the right (320 kb), together with other size standards. Note the similarity of the hybridization to the wild-type chromosome III in the *TOP2* strain and in the *top2-4* strain. However, note the smear of breakage products only in the *top2-4* strains containing the telocentric chromosome III (150 to 320 kb) and the ring chromosome III (170 to 320 kb).

cause of its topology, this circular chromosome does not enter a pulsed-field gel (12), so we confirmed that the expected amount of material is present in the wells by hybridization to a Southern blot (data not shown) (49). One break in the ring chromosome III will create a linear molecule that runs at 320 kb. In the *TOP2* ring chromosome III strain, no molecules running at 320 kb are detected (Fig. 6B, lane 3). In contrast, DNA from the *top2* ring chromosome III strain exhibits a light band of broken linearized rings at 320 kb as well as a lighter smear of smaller breakage products (Fig. 6B, lane 6). This result suggests that small chromosomes are indeed intertwined in *top2* mutants.

Surprisingly, the level of breakage of the ring chromosome is very similar to the level of breakage in the telocentric chromosome III. This similarity is apparent by eye when the telocentric chromosome band is covered and the smear of breakage products from the telocentric lane is compared with all of the hybridization in the ring chromosome lane. This result suggests that intertwinings cannot be passively resolved on long arms any more than they can on ring chromosomes. Nonetheless, a very similar chromosome with short chromosome arms (wild-type chromosome III) shows no increase in breakage products in a *top2* strain compared with a *TOP2* strain (Fig. 6). These results are therefore consistent with the model that intertwinings between small arms can passively resolve off the end of the chromosome. The ability to slide appears to be limited by the size of the chromosome arm, such that the intertwinings on long arms lead to chromosome breakage in the absence of DNA topoisomerase II activity.

DISCUSSION

Our studies of chromosome breakage in *top2* mutants reveal that large and small chromosomes behave differently in the absence of topoisomerase II activity. Whereas large chromosomes break when segregated in *top2* mutants at restrictive temperature, small chromosomes do not. Furthermore, it appears that small chromosomes do not break because they have small arms and large chromosomes break because they have at least one large arm. The preferred regions of breakage in several natural chromosomes are centered approximately 200 kb from the location of the centromere. When the centromere of chromosome XIV is relocated, the preferred region of breakage shifts accordingly. Analysis of rearranged derivatives of chromosome III suggests that the length of a large chromosome arm serves as a barrier to the passive resolution of intertwinings off the end of the arm.

Chromosome breakage assay. To interpret the results of our experiments, it is necessary to consider the technical limitations of visualizing chromosome breakage by hybridization to chromosomal DNA resolved on pulsed-field gels. If a substantial fraction of broken chromosomes were lost at any step, then we might fail to see breakage products simply for technical reasons. This possibility seems unlikely for a variety of reasons. First, hybridization was done directly to the dried agarose gel (39, 57). This method avoids any error from uneven DNA transfer, and it is more sensitive than conventional hybridization to Southern blots (54). Second, long exposures were made for each hybridization to ensure that we were not simply missing a smear of breakage products. Finally, small chromosome fragments were easily detected in a control experiment in which we induced chromosome breakage by irradiation. Thus, small chromosome fragments, when present, are easily detectable in this system.

The ability of the cell to repair breaks in the DNA could also have had dramatic effects on the results of these experiments. For example, if small chromosomes were repaired more efficiently than large chromosomes, our results would have a trivial explanation. Experiments with *rad50* and *rad52* strains, however, reveal that double-strand break repair has little effect on the type of breakage products observed in the use of this assay. This result is not surprising, because DNA breakage probably occurs as the sister chromatids are being segregated (26). Since the broken fragments are segregated to different daughter haploid cells, there is no template from which double-strand break repair could occur. In summary, the difference in the observed levels of breakage of large versus small chromosomes seems to reflect real differences in the structure or behavior of the chromosomes.

What proportion of chromosomes are intertwined? Because natural eukaryotic chromosomes are linear, it has been difficult to examine the extent to which they are intertwined.

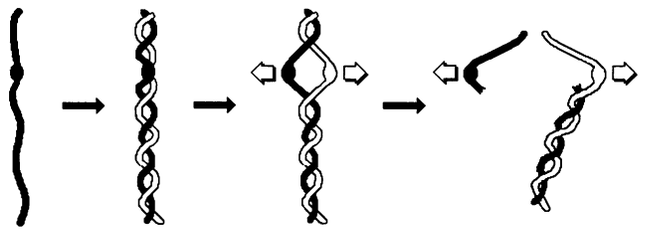

FIG. 7. Representation of the segregation of an acrocentric chromosome in the absence of DNA topoisomerase II activity. This illustration shows the DNA replication, chromosome segregation, and subsequent breakage of a chromosome with one short arm and one long arm in a *top2* mutant at restrictive temperature. After replication, an unknown number of intertwinings remain between the sister chromatids. In the absence of topoisomerase II activity, the intertwinings cannot be resolved except by falling off the end of the chromosome (passive resolution). As the spindle (white arrows) pulls at the centromeres of the tangled chromatids during chromosome segregation, intertwinings are able to migrate off the end of the short chromosome arms, but the length of the long arms prevents the passive resolution of all of its intertwinings. The breakage of just one of the chromatids should allow the broken chromosome fragments to disjoin. Thus, the remaining unresolved intertwinings do not contribute to the breakage products visualized by our assay.

Our studies suggest that intertwining of sister chromatids is prevalent in *top2* mutants at restrictive temperature. For chromosome XIV, for example, densitometry reveals that approximately one-third of the molecules undergo breakage in *top2* mutants at restrictive temperature. Because only one sister chromatid must break to remove the connection between the two sister centromeres of chromosome XIV (Fig. 7), this result suggests that approximately two-thirds of the sister chromatids may be intertwined such that they require topoisomerase II for resolution. The suggestion that most chromatids are intertwined is consistent with earlier observations of catenated plasmids in *top2* mutants (6, 26, 27). In contrast, this estimate of the level of intertwining is much higher than what might have been expected on the basis of the rates of mitotic recombination in *top2* strains at restrictive temperature (26). It is important to note, however, that the recombination studies were limited to measuring DNA damage in the 5 to 10% of *top2* cells that survived an incubation at restrictive temperature. It has been suggested that these survivors may not be representative of the population as a whole (26). The assay used in the present study has the important advantage of allowing the visualization of all of the products of chromosome segregation, even those present in inviable cells. Thus, our analysis demonstrates that when measuring chromosome damage or loss, experiments which can look only at the survivors may underrepresent the absolute level of damage.

Sister chromatids that remain intact in *top2* mutants may not have been intertwined, or they may have nondisjoined rather than broken. It is difficult to estimate what percentage of intertwined sisters nondisjoin. In an earlier study of nondisjunction among *top2* cells surviving an incubation at restrictive temperature, the observed rate of nondisjunction was 0.3%, approximately 15-fold higher than that seen at permissive temperature (26). Although these studies demonstrated that the rates of nondisjunction increase in *top2* mutants at restrictive temperature, estimating the overall rates of nondisjunction is impossible because of the limitation of examining only viable cells. Accurate measurement of the absolute level of nondisjunction in all *top2* cells will await methods that do not require cell viability, such as in situ hybridization to nuclei (45). Until this information is available, our best estimate is that at least two-thirds of the sister chromatids are intertwined.

The nature and distribution of intertwinings. Our results indicate that chromosome breakage is localized to general regions but not to specific sites. The distributed nature of chromosome breakage in *top2* mutants probably derives from two sources. First, the termination of DNA replication occurs over a broad region in a non-sequence-specific manner in *S. cerevisiae* (16). Since it appears that intertwinings are generated at the points of termination of DNA replication (9, 61), the initial localization of intertwinings on any one chromosome probably varies from molecule to molecule. Second, our results suggest that intertwinings can move after they are generated. The normally metacentric chromosome III exhibits no detectable breakage in a *top2* mutant, but when it is rearranged into a ring chromosome, it exhibits detectable levels of breakage. Thus, it appears that although intertwinings are trapped on circular molecules in the absence of topoisomerase II activity, they can migrate off the ends of short linear molecules. The suggestion that intertwinings are mobile is consistent with work using simian virus 40, which shows that intertwinings generated at a specific termination site become distributed over the entire genome (59). Prior to the present work, however, it was not clear whether chromosomal structure would prevent the movement of intertwinings along a eukaryotic chromosome. It was possible, for example, that the chromosome scaffold might obstruct the movement of intertwinings (13). The absence of discrete bands of breakage products suggests that the barrier to passive resolution is unlikely to be a precisely positioned, stable chromosomal structure. Furthermore, it appears that intertwinings, once generated, can move along the chromosome.

Although intertwinings appear to be mobile in *S. cerevisiae* and other organisms, our results suggest that there is a general limit to the distance that intertwinings can travel. The most economical interpretation of our results with natural and rearranged chromosomes is that increasing the length of a chromosome arm prevents intertwinings from being able to fall off its end. The topology of a very long chromosome arm approximates that of a circular DNA molecule, in that the length of the arm prevents the easy rotation of one sister chromatid about the other and therefore the resolution of intertwinings off the end of the molecule. In addition to explaining why only long chromosome arms break, this hypothesis is consistent with the regions of breakage we observe in long chromosome arms. Intertwinings appear to be able to fall off chromosome arms that are shorter than 250 kb, and arms that are longer than 250 kb have regions of breakage centered approximately 200 kb away from the centromere. It is straightforward to imagine that intertwinings can slide only so far, and when they can slide no more, the chromosome breaks. Visualizing this process is especially easy in light of recent work in which the segregation of intertwined chromosomes is visualized in *top2* cells fixed as they were dividing at restrictive temperature (10). While the mass of the chromosome remains behind on the metaphase plate, a thin thread of DNA can be seen being pulled away from the chromosomal mass by the mitotic spindle.

Beyond a certain size, it appears that increasing the length of a chromosome arm does not lead to an increase in breakage. For example, the "infinitely" long arm of the ring chromosome III exhibits levels of breakage that are similar

to those of a telocentric chromosome of the same size, even though a ring chromosome must have all intertwinings resolved by a type II topoisomerase. It is theoretically possible that a ring chromosome has many fewer intertwinings than a similarly sized linear chromosome, or it could have similar levels of intertwinings but undergo nondisjunction much more frequently. Either way, the breakage levels of the ring and telocentric chromosomes would have to be very similar solely by chance. It is more economical to propose that lengthening a long chromosome arm has little effect on the level of breakage. This hypothesis not only explains the results with chromosome III; it also explains our results with other natural and rearranged chromosomes. When we quantitated the level of breakage in three large natural chromosomes, we did not see a significant increase in the level of breakage as chromosome size increased. This result makes sense in light of the mechanics of chromosome separation. If sister chromatids of an acrocentric chromosome are intertwined, one break in one chromatid should be sufficient to allow the sister centromeres to separate (Fig. 7). Once a particular chromatid has broken, the existence of additional intertwinings between the sisters is irrelevant.

Implications of the nature and distribution of chromosomal intertwinings for chromosome behavior. Although there are examples of size-dependent chromosome behavior in yeast, the effects that have been observed are probably distinct from those we report here. For example, studies with synthetic chromosomes (20, 35) or chromosome III deletions (34, 51) show that the mitotic stability of these artificial chromosomes increases with their size. However, this effect is observed only with constructs that are much smaller than natural yeast chromosomes. For molecules over 100 kb, an increase in chromosome size produces no concomitant increase in stability (34, 51). Size-dependent behavior has also been reported for natural chromosomes in mutant backgrounds. For example, the frequency of chromosome transfer in *kar1/KAR1* heterokaryons was reported to be inversely proportional to the genetic size of the chromosomes tested (7). Now that the genetic and physical sizes of yeast chromosomes are better characterized, however, a reevaluation of the data shows that the transfer rate in heterokaryons is not strictly size dependent. Finally, a study of *chl1* mutants showed that the smallest and the third-smallest chromosomes were lost more frequently than six other chromosomes (29). The second smallest chromosome was never lost, and it has been suggested that the difference in the measured loss rates may be determined by lethality caused by chromosome aneuploidy (15). Therefore, although the stability of unnaturally small molecules may depend on their size, there is no clear correlation between chromosome stability and the size of natural yeast chromosomes.

Although our analysis suggests that the level of nonresolvable intertwinings is lower in small chromosomes, large and small chromosomes appear to segregate equally well. It is apparent from many experiments that the error rates are very low for chromosome segregation of all natural yeast chromosomes. Estimates of rates of loss vary according to genetic background and experimental design, but most estimates fall into the range of $\sim 10^{-4}$ to 10^{-5} chromosome loss events per cell division (8, 18, 52). Since loss rates for large and small chromosomes are similar, it seems unlikely that structural features in which they differ are essential for proper chromosome segregation.

The differences we observe between large and small chromosomes have implications for models of chromosome segregation. It has been suggested that chromosomal intertwinings play a functional role in holding sister chromatids together until they segregate at anaphase (24, 33, 34, 50). This attachment would guarantee proper attachment of the spindle fibers and therefore proper segregation of the chromosomes. One model suggests that the regulation of topoisomerase II activity prevents the resolution of the intertwinings until anaphase. In this model, it is only the release of the intertwinings by topoisomerase II that triggers the segregation of the sisters at anaphase (24, 33, 34). Work with plasmids in *S. cerevisiae* casts some doubt on this model, because it shows that plasmid DNA molecules are untangled while the cells are still in metaphase, and yet the plasmids segregate well (27). Because this work was done with artificial plasmids, one could argue that it bears little relationship to the behavior of natural chromosomes. However, our results with authentic yeast chromosomes also cast doubt on this model. We find that even when topoisomerase II is absent, small chromosomes can segregate without breaking, whether because of the complete absence of intertwinings or the ability of the intertwinings to resolve passively. Since small chromosomes segregate with a high degree of fidelity, it appears unlikely that intertwinings are necessary to ensure their proper segregation.

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