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Nuclear oscillations and nuclear filament formation accompany single-strand annealing repair of a dicentric chromosome in *Saccharomyces cerevisiae*

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Summary

Dicentric chromosomes undergo breakage during mitosis as a result of the attachment of two centromeres on one sister chromatid to opposite spindle poles. Studies utilizing a conditional dicentric chromosome III in *Saccharomyces cerevisiae* have shown that dicentric chromosome repair occurs primarily by deletion of one centromere via a *RAD52*-dependent recombination pathway. We report that dicentric chromosome resolution requires *RAD1*, a gene involved in the single-strand annealing DNA repair pathway. We additionally show that single-strand annealing repair of a dicentric chromosome can occur in the absence of *RAD52*. *RAD52*-independent repair requires the adaptation-defective *cdc5-ad* allele of the yeast polo kinase and the DNA damage checkpoint gene *RAD9*. Dicentric chromosome breakage in *cdc5-ad rad52* mutant cells is associated with a prolonged mitotic arrest, during which nuclei undergo microtubule-dependent oscillations, accompanied by dynamic changes in nuclear morphology. We further demonstrate that the frequency of spontaneous direct repeat recombination is suppressed in yeast cells treated with benomyl, a drug that perturbs microtubules. Our findings indicate that microtubule-dependent processes facilitate recombination.

Key words: Dicentric chromosome, Microtubules, Homology search, Recombination, Single-strand annealing

Introduction

The study of DNA repair mechanisms has been aided by the development of experimental systems that generate DNA double-strand breaks (DSBs) at specific chromosomal locations and during defined periods of the cell cycle. One such system is the budding yeast conditional dicentric chromosome (Hill and Bloom, 1989). This inducible dicentric chromosome was constructed by inserting a second copy of centromere 3 (*CEN3)* into chromosome III, approximately 45 kb upstream of the endogenous centromere 3 (Fig. 1A). The inserted centromere is under control of the *GAL1* promoter, allowing for the reversible inactivation of this centromere when cells are grown in the presence of galactose. Studies utilizing dicentric chromosomes are unique in that DSBs are generated by mitotic pulling forces when centromeres on the same sister chromatid are aligned to opposite spindle poles during mitosis. Previous work showed that efficient healing of a broken dicentric chromosome in *Saccharomyces cerevisiae* requires *RAD52*, indicating that repair occurs primarily through homologous recombination (Brock and Bloom, 1994). In cells lacking *RAD52*, infrequent dicentric chromosome repair events occur through non-homologous end joining (Kramer et al., 1994).

Resolution of a conditional dicentric chromosome is accompanied by the deletion of one centromere and the intervening DNA. These events were initially thought to result from gene conversion (GC) accompanied by crossover (Jager and Philippsen, 1989). However, other studies of direct repeat recombination have shown that deletions commonly occur without generating the circular product predicted to form through a crossover event (Fishman-Lobell et al., 1992). Moreover, many deletion events are dependent on *RAD1* and *RAD10* (Klein, 1988; Schiestl and Prakash, 1990; Ivanov and Haber, 1995). The Rad1 and Rad10 proteins form a structure-specific endonuclease that generates 5′ single-stranded ends (Siede et al., 1993). These genes function in a recombination pathway known as single-strand annealing (SSA) (Sugawara and Haber, 1992; Sung et al., 1993; Ivanov and Haber, 1995). The recognition of SSA as a major pathway for direct repeat recombination suggests that this mechanism plays a role in the repair of a dicentric chromosome.

Previous studies have shown that recombination frequency is primarily governed by the degree of sequence homology rather than the location of the recombining sequences within the genome (Haber and Leung, 1996). This implies the existence of a homology search mechanism as part of the recombination process. The integration of *E. coli* lacO and tetO sequences into chromosomes of yeast strains encoding lac repressor-GFP and tet repressor-GFP fusion proteins has recently provided visual evidence of long-distance interactions between like sequences (Aragon-Alcaide and Strunnikov, 2000). Such interactions may represent a constitutive homology search process. The mechanisms that bring homologous sequences into close association are unknown. Efficient meiotic recombination in the fission yeast *Schizosaccharomyces pombe* requires microtubule-dependent oscillatory nuclear movements (Chickashige et al., 1994; Ding

et al., 1998; Yamamoto et al., 1999). It has been suggested that such movements promote the alignment of homologous chromosomes. Microtubule-driven nuclear movements could similarly promote the association and recombination of homologous sequences during the vegetative cycle.

In this study we show a requirement for *RAD1* in the repair of a dicentric chromosome, which is consistent with recombination via SSA. Dicentric chromosome repair also exhibits a requirement for *RAD52*; however we show that this process can occur via *RAD52*-independent SSA in cells containing the adaptation-defective *cdc5-ad* allele of the yeast polo kinase and the DNA damage checkpoint gene *RAD9*. Dicentric *cdc5-ad rad52* mutant cells additionally experience an extended arrest that is characterized by microtubule and ATP-dependent nuclear oscillations. Finally, recombinational repair of a dicentric chromosome containing direct and inverted centromere repeats, as well as spontaneous recombination events between *his3* heteroalleles, also show a dependency on microtubule function. These latter findings suggest that microtubule-dependent processes play a general role in recombination. We discuss ways in which nuclear movements might contribute to chromosome repair.

Materials and Methods

Strain construction, yeast culture and Southern blotting

Growth conditions and media used to evaluate the kinetics of dicentric chromosome repair were as previously described (Brock and Bloom, 1994). Genotypes and construction of the monocentric (J178-1D), dicentric inverted (J178#24) and dicentric direct repeat (J178#27) strains used for benomyl sensitivity assay are likewise detailed elsewhere (Brock and Bloom, 1994). The LS20 and *cdc5-ad* strains were a gift of Lee Hartwell (Fred Hutchison Cancer Center, Seattle, WA). Strain HUWT (Qiu et al., 1999) was derived from progeny of W1021-7c and W1089-6c (Thomas and Rothstein, 1989). Strains developed for this work include KBY3009, a dicentric direct repeat strain created in the LS20 genetic background (*MAT*Δ *ade2 ade3*::*GalHO lys5 trp1 leu2 his3 ura3 can1 cyh2 his4*Δ::*URA3GALCEN3*); KBY3002 (KBY3009 with *rad1*::KAN^r); KBY3010 (KBY3009 with *rad52*::*LEU2*); KBY3006 (KBY3002 with *rad52*::*LEU2*); KBY3003 (KBY3009 with *rad9*::*TRP1*); KBY3011, identical to KBY3009 except that it contains the *cdc5-ad* point mutation (L251T); KBY3012 (KBY3011 with *rad1*::KAN^r); KBY3034 (KBY3011 with *rad52*::*LEU2*); KBY3014 (KBY3012 with *rad52*::*LEU2*); KBY3015 (KBY3034 with *rad52*::*LEU2*); KBY3031 (KBY3039 with *dhc1*::*LEU2* and *rad52*::*TRP1*); KBY3039 (KBY3011 with *HTB1-GFP-KAN^r*); KBY3037 (KBY3039 with *rad52*::*LEU2*); KBY3038 (KBY3009 with *HTB1-GFP-KAN^r*); KBY3040 (KBY3038 with *rad52*::*LEU2*); and KBY5004 (J178-1D MAT a, *ade1, met 14, ura3-52, leu2-3, 112, his3-11,15, H2B-GFP-KAN^r*). KBY5004 was transformed with a *GAL-MPS1* construct on a 2 μm plasmid to generate a cell population arrested in mitosis by *MPS1* overexpression. Strains were constructed by one step gene replacement (Rothstein, 1991) using plasmids or PCR fragments. Analysis of *CEN3* recombination following activation of the dicentric chromosome was as previously described (Brock and Bloom, 1994) except that digoxigenin-labeled probes labeled with the DIG PCR synthesis kit (Roche, Indianapolis, Indiana) were used in Southern blotting. Probes were hybridized to genomic DNA bound to nylon membranes using the DIG Easy Hyb system (Roche). Southern blots were developed with the AttoPhos Substrate Kit (Roche) and imaged with a Storm gel analyzer (Molecular Diagnosics). A minimum of two clones of each strain were used for physical and microscopic analyses.

Histone 2B-GFP fusion constructs and image acquisition

Yeast strains were transformed with an integrating PCR fragment generated with oligos HTB2#1: gggctgttaccaaatactcctcctctactcaag-ccgcagcagcagtcagcagcagcaatgagtaaaggagaagaa, HT2BN#2: tcatatc-tggacaagtaacagaaccctaatgttactggcggcgttagtatcg and the plasmid pKanMX2-GFP (K.B., unpublished), which contains the green fluorescence protein coding region and the G418 resistance marker. Cells were added to the surface of a gelatin slab formed on the surface of a microscope slide. Slabs contained 25% gelatin, 2% glucose, 0.5% casamino acids, 50 μg/ml tryptophan, 50 μg/ml adenine and 16.5 μg/ml uracil (YCAUT). Coverslips were sealed to slides with Valap (1:1:1 vaseline:lanolin:paraffin). A flow chamber was constructed by attaching a coverslip to a microscope slide with petroleum jelly to observe the effects of microtubule and ATP inhibitors on nuclear dynamics. The coverslip was precoated with a 1 mg/ml solution of poly-L-lysine to facilitate cell attachment. Filling and rinsing of the chamber was accomplished by adding liquid to one end and simultaneously withdrawing liquid from the opposite end with a filter paper wick. Microscopic observations were made at room temperature (22°C). The microscopy system and GFP fluorescence imaging have previously described (Shaw et al., 1997a), with the exception that observations in the current study were made using a 100×/1.4 NA Plan Apo objective. Metamorph software (Universal Imaging) was used to control shutters, focusing and image acquisition. Five z-axis fluorescence images separated by 0.75 μm were collected for each time point and converted to a single 3D image using Metamorph software. A single differential interference contrast image was collected at the medial z-step.

Quantification of nuclear oscillations

Hydroxyurea (HU)-arrested cells were obtained by growing KBY5004 to log phase and transferring cells to YPD supplemented with 40 μg/ml adenine and 200 mM HU. A *MPS1*-arrested population was obtained by growing KBY5004 cells containing a *GAL-MPS1* expression plasmid overnight in adenine-supplemented sucrose media lacking uracil to maintain the plasmid (Lauze et al., 1995). Cells were grown to log phase and switched to galactose media lacking uracil for 6 hours to promote overexpression of Mps1. Large budded cells were filmed on YCAUT glucose + 25% gelatin slabs for 2 hours. Images were acquired every 2 minutes with an exposure time of 250 milliseconds. Images of dicentric *cdc5-ad rad52*, dicentric *cdc5-ad* and dicentric *Rad52* cells were obtained with the same exposure and time intervals described above following 6 hours of exposure to YCAUT glucose media. A cell was scored as oscillation + if during the time course it underwent at least one cycle of nuclear movement from mother to bud to mother, such that at each stage >50% of the nuclear material was in either the mother or bud.

Image analysis and calculation of dynamic parameters

Measurements of nuclear filaments were taken with Metamorph software. Filaments were measured from the filament tip to the geometric center of the nuclear mass. Length measurements were made in triplicate using appropriate calibrations from a stage micrometer and corrected for specimen tilt angle with a Microsoft Excel algorithm containing the formula c^2=square root ($a^2 + b^2$), where a is the measured distance taken from the 3D image and b is the distance between the uppermost and lowest focal planes in which the filament was visible. Growing and shortening rates were calculated from a least squares fit of four or more data points. Criteria for dynamic parameters were based on those used to quantify microtubule dynamics (Tirnauer et al., 1999). Growth and shortening events were defined as a change in length sustained over four time points having a correlation coefficient ≥0.85 and yielding a total length change >0.6 μm. The proportion of time spent in growing, shortening or paused phases was determined by measuring the amount

of time spent in a given phase for all filaments and dividing by total time. Dynamicity (Toso et al., 1993) was calculated by dividing the sum of all filament length changes measured by total observation time.

Recombination assay

The spontaneous *his3* heteroallele recombination frequency of strain HUWT was determined by tabulating the formation of *HIS+* colonies on synthetic media lacking histidine (Qiu et al., 1999). Cells were plated in triplicate on media with and without added benomyl for each experiment. Cells were plated on media lacking uracil both in the presence and absence of benomyl to correct for differences in the cell growth/viability resulting from drug treatments.

Results

Dicentric chromosome repair requires *RAD1*

Previous studies have shown that the resolution of a conditional dicentric chromosome III in *S. cerevisiae* occurs primarily through homologous recombination between centromere (*CEN*) repeats, resulting in the elimination of one copy of *CEN3* and 45 kb of intervening DNA (Fig. 1A,B) (Brock and Bloom, 1994). Deletion of *RAD52* prevents this recombination event and results in a significant decrease in viability (Fig. 1C, Table 1) (Brock and Bloom, 1994). The requirement for *RAD52* is consistent with either GC accompanied by crossover or SSA. We deleted *RAD1* in the dicentric chromosome strain to distinguish between these possibilities. The viability of the *rad1* deletion strain was 26-fold lower than an isogenic strain containing a functional copy of *RAD1*. This was similar to the loss in viability that accompanied the deletion of *RAD52* (Table 1). Physical analysis of *CEN3* recombination showed that *RAD1*, in addition to *RAD52*, is required for the formation of the *CEN3-GALCEN3* recombination product (Fig. 1D). The viability of the *rad1 rad52* double deletion mutant was the same as either of the single mutants (Table 1), suggesting that both genes function in the same pathway. This result is in contrast to several genetic studies of spontaneous recombination events that have revealed a synergistic effect of the deletion of both *RAD1* and *RAD52*, suggesting that these genes operate in different pathways (Klein, 1988; Schiestl and Prakash, 1988; Thomas and Rothstein, 1989; Liefshitz et al., 1995). However, other studies utilizing endonucleases to create DSBs have shown that loss of *RAD52* reduces the efficiency of SSA when homologous repeats are 5 kb or less in length (Fishman-Lobell et al., 1992; Plessis et al., 1992; Sugawara and Haber, 1992). It is possible that the structure of the DSB determines the requirement for *RAD52*. DNA ends produced during dicentric chromosome breakage may be more similar to those generated by endonucleases than they are to DNA ends formed during spontaneous recombination events. Physical studies of recombination events have revealed that *rad52* mutant strains show an increased rate of DNA strand excision (White and Haber, 1990; Sugawara and Haber, 1992). This suggests that *RAD52* may protect DNA ends from excessive resection by nucleases as opposed to playing a direct role in the SSA process. It is also possible that some dicentric chromosome repair events occur through a one-end strand invasion pathway (Sugawara and Haber, 1992; Prado and Aguilera, 1995).

The DNA damage checkpoint gene *RAD9* is required for efficient utilization of some repair pathways (Fasullo et al., 1998). A *RAD9*-dependent midanaphase delay has been observed in yeast cells containing a dicentric chromosome, but loss of this gene does not adversely affect strain viability (Table 1) (Yang et al., 1997). We examined the kinetics of *CEN3* recombination in a *rad9* deletion strain and found them

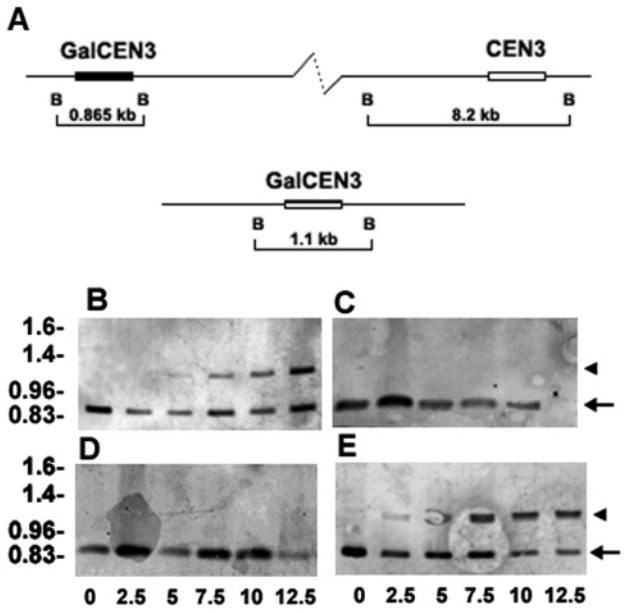

Fig. 1. Homologous repair of a dicentric chromosome in budding yeast requires *RAD52* and *RAD1* but not *RAD9*. (A) The diagram shows locations of *Bam*HI restriction sites (labeled 'B') flanking the endogenous *CEN3*, *GALCEN3* (upper diagram) and *CEN3-GALCEN3* recombination products (lower diagram). (B) Southern analysis of *Bam*HI-digested genomic DNA hybridized to a labeled *CEN3* fragment reveals the loss of a 0.865 kb *GALCEN3* fragment (arrow) and the formation of a 1.1 kb fragment (arrowhead) following a shift from galactose to glucose to activate the dicentric chromosome. No recombination product is observed in a *rad52* deletion strain (C) nor in a *rad1* deletion strain (D). The kinetics of *CEN3-GALCEN3* recombination are unaffected by deletion of *RAD9* (E). The numbers below lanes refer to the time of sample collection, in hours, following transfer of cells to glucose. The positions of molecular weight markers (kb) are indicated.

The DNA damage checkpoint gene *RAD9* is required for efficient utilization of some repair pathways (Fasullo et al., 1998). A *RAD9*-dependent midanaphase delay has been observed in yeast cells containing a dicentric chromosome, but loss of this gene does not adversely affect strain viability (Table 1) (Yang et al., 1997). We examined the kinetics of *CEN3* recombination in a *rad9* deletion strain and found them

Table 1. Effect of *rad1*, *rad52* and *rad9* deletion on cell viability

Strain	%Viability*	s.d.
Dicentric	60	9.0
Dicentric *rad1*	2.3	1.4
Dicentric *rad52*	2.0	0.9
Dicentric *rad1 rad52*	1.9	0.8
Dicentric *rad9*	77	12
Dicentric *cdc5ad*	63	8.9
Dicentric *cdc5ad rad1*	2.3	0.7
Dicentric *cdc5ad rad52*	1.7	0.7
Dicentric *cdc5ad rad1 rad52*	2.0	0.7
Dicentric *cdc5ad rad9*	78	9.2
Dicentric *cdc5ad rad9 rad52*	0.4	0.5
Dicentric *cdc5ad dhc1*	30	6.4
Dicentric *cdc5ad dhc1 rad52*	0.9	0.3

*Viabilities of dicentric strains were determined by the number of colony forming units (CFU) on plates containing glucose relative to those that grew on galactose. Values are means of four to seven determinations.

identical to the corresponding wild-type strain (Fig. 1E, compare with Fig. 1B). Our findings suggest that dicentric chromosome repair events are completed rapidly and do not require the additional time provided by the *RAD9*-dependent cell cycle delay.

The *cdc5-ad* allele promotes *RAD52*-independent SSA

Cells containing the *cdc5-ad* allele of the yeast polo-like kinase experience an extended mitotic delay in response to an unrepaired DSB (Toczyski et al., 1997). We considered that such a delay might allow us to detect *RAD52*-independent SSA events by allowing additional time for completion of repair prior to the end of mitosis. *CEN3* recombination induced by dicentric chromosome breakage occurred with the same kinetics regardless of whether cells contained the *cdc5-ad* or *CDC5* wild-type allele (Fig. 2A, compare with Fig. 1B). However, in contrast to the dicentric *CDC5 rad52* strain (Fig. 1C), *CEN3* recombination was observed in the dicentric *cdc5-ad rad52* strain (Fig. 2B). No *CEN3* recombination product was detected in a *cdc5-ad rad1 rad52* deletion strain (Fig. 2C), indicating that this event occurs through SSA. We additionally showed that *RAD52*-independent repair in a *cdc5ad* strain requires *RAD9* (Fig. 2D). Analysis of populations derived from individual dicentric *cdc5-ad rad52* cells revealed that recombination occurred through a *RAD1*-dependent mechanism in 19 of 20 clones (data not shown).

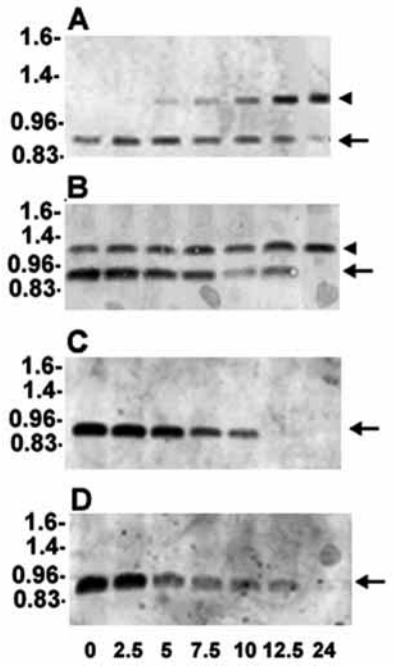

Fig. 2. *RAD52*-independent homologous repair of a dicentric chromosome in cells containing the *cdc5-ad* allele requires *RAD1* and *RAD9*. (A) Southern analysis of *CEN3-GALCEN* recombination in a *cdc5-ad* strain following activation of a dicentric chromosome. (B) *CEN3-GALCEN* recombination also occurs in a *cdc5-ad rad52* strain following activation of a dicentric chromosome but this recombination product is not detected in a *cdc5-ad rad1 rad52* double deletion strain (C) or in a *cdc5-ad rad9 rad52* strain (D). The arrow indicates the position of the original band and the arrowhead indicates the recombinant band. The numbers below the lanes refer to time of sample collection, in hours, following transfer of strains from galactose to glucose. The positions of molecular weight markers (kb) are indicated.

Dicentric *cdc5ad rad52* cells exhibit nuclear oscillations and dynamic alterations in nuclear morphology

Over the course of 24 hours following activation of the dicentric chromosome in the *cdc5-ad rad52* strain, 89% of the population arrested as abnormally large budded cells, similar to the phenotype previously reported for *cdc5-ad* cells containing unrepaired DNA damage (Toczyski et al., 1997). This extended mitotic arrest was not observed in dicentric *CDC5* cells, dicentric *cdc5-ad* cells, dicentric *rad52* cells, *cdc5-ad RAD52* cells or in *cdc5-ad rad9 rad52* cells (Table 2). These findings indicate that the requirements for arrest include a source of DSBs (the activated dicentric chromosome), the *cdc5-ad* allele, the presence of *RAD9* and deletion of *RAD52*, consistent with the original characterization of the *cdc5-ad* allele (Toczyski et al., 1997). It was previously shown that that the cell cycle arrest was generated by the *cdc13-1* allele (Toczyski et al., 1997). By contrast, cells containing a conditional dicentric chromosome experience a midanaphase delay (Yang et al., 1997). To determine if dicentric *cdc5-ad rad52* cells also pause in midanaphase, we replaced the endogenous *HTB2* (histone 2B) gene with an *HTB2-GFP* fusion construct. Images of 25 histone-GFP expressing *cdc5-ad rad52* cells were collected 2 hours after activation of the dicentric chromosome. Nineteen of the 25 large-budded cells contained a bilobed nucleus that straddled the bud neck (data not shown), which is indicative of a midanaphase arrest (Yang et al., 1997).

Real-time observations of dicentric *cdc5ad rad52* cells expressing *HTB2*-GFP revealed an extended period of oscillatory nuclear movements (Fig. 3). This behavior initiated with the retraction of the bud-proximal lobe of a bilobed nucleus, resulting in the formation of a single nuclear mass. In 52% of arrested cells, the nucleus oscillated between the mother cell and bud. This behavior was not observed in dicentric *cdc5-ad RAD52* cells or in *CDC5 rad52* cells (Table 3). Time-lapse images of cells collected at 15 minute intervals over a 24 hour period revealed that more than 90% of cells exhibited nuclear oscillations for at least 14 hours after activation of the dicentric chromosome. Nuclear oscillations were also accompanied by the extension and collapse of filamentous nuclear protrusions (Fig. 3). Approximately 1% of

Table 2. Accumulation of very large-budded cells following dicentric chromosome breakage in a *cdc5-ad rad52* mutant population

Strain	Percentage of very large budded cells
Dicentric	<1%
Dicentric *cdc5-ad*	<1%
Dicentric *rad52*	2%
Dicentric *cdc5-ad rad52*	89%
Dicentric *cdc5-ad rad9 rad52*	<1%

Numbers are means of three determinations made 24 hours after activation of the dicentric chromosome (approximately 100 cells/determination). Very large-budded cells are categorized by a long axial diameter (mother + bud) >12 μm.

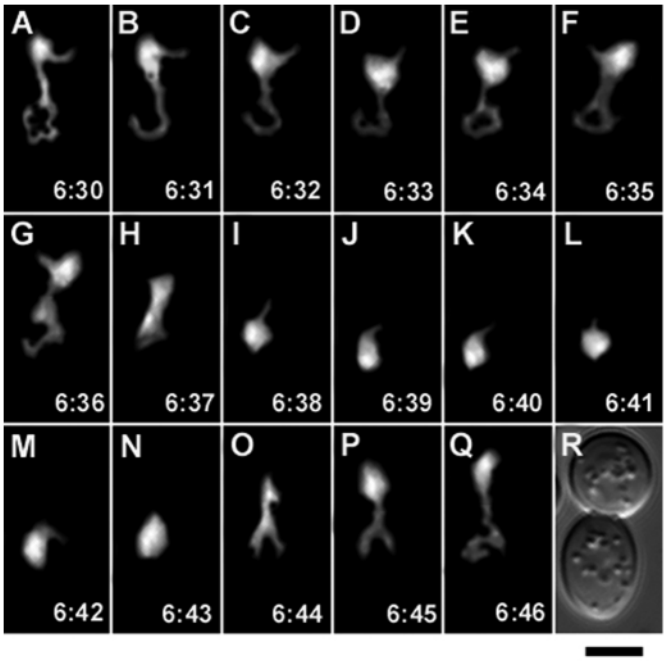

Fig. 3. Changes in nuclear morphology and repeated nuclear oscillations result from activation of a dicentric chromosome in *cdc5-ad rad52* cells. (A-Q) Sequential fluorescence images of histone2B-GFP-labeled nuclei in a dicentric *cdc5-ad rad52* cell. (R) DIC image of cell in A-Q. The time after activation of dicentric chromosome is indicated (hours, minutes) in the lower right corner of each panel. Bar, 5 μm.

cells that remained arrested longer than 6 hours succeeding in completing nuclear division and cytokinesis (data not shown). The divided cells rebudded and proceeded through the next cell cycle with normal kinetics.

To determine if nuclear oscillations are a general feature of cells that have experienced a mitotic arrest, we characterized nuclear movements following exposure to hydroxyurea (HU) or elevated expression of *MPS1*. Treatment with HU causes a DNA-damage-induced mitotic block. Overexpression of *MPS1*, a protein involved in the spindle checkpoint and spindle pole body duplication, also results in a mitotic arrest (reviewed in Winey and Huneycutt, 2002). Nuclear oscillations were not observed in HU-arrested cells (Table 3). Cells overexpressing *MPS1* did undergo nuclear oscillations, albeit less frequently than dicentric *cdc5ad rad52* cells (Table 3). Our results indicate that the sequestration of cells in a mitotic arrest state is not of itself sufficient to promote nuclear oscillations.

Table 3. Nuclear oscillations occur in dicentric *cdc5-ad rad52* cells and following *MPS1* overexpression but not in HU-treated cells

Strain	Proportion of large-budded cells exhibiting nuclear oscillations
Dicentric *cdc5-ad*	0/20
Dicentric *rad52*	0/20
Dicentric *cdc5-ad rad52*	11/20
Monocentric, HU treated	0/20
Monocentric, GAL-MPS1 overexpression	5/20

Results are based on time-lapse images collected at 2 minute intervals over a period of 2 hours.

Table 4. Growth and shortening of nuclear filaments in dicentric *cdc5ad rad52* cells treated with sodium azide and deoxyglucose

Parameter	Pre-treatment	Azide + DOG	Post-washout
Growth rate	0.82±0.41	0.20±0.03	0.71±0.17
Shortening rate	0.77±0.53	0.66±0.42	0.87±0.30
% time in growth	33.6	2.8	28.5
% time in shortening	17.5	4.3	21.7
% time in pause	48.9	92.9	48.9
Dynamicity	0.40	0.03	0.49

Rates and dynamicity are reported in μm/minute. Values are means±1 s.d. Data was taken from measurements of filaments in 16 nuclei.

Nuclear oscillations in dicentric *cdc5ad rad52* cells are microtubule- and ATP-dependent but do not require dynein

Microtubules are required for the oscillatory nuclear movements that occur during meiotic prophase *S. pombe* (Ding et al., 1998). To determine if nuclear oscillations in dicentric *cdc5-ad rad52* cells also depend on microtubules, we treated cells with media containing 100 μg/ml of the microtubule-destabilizing drug benomyl. Real-time observation of 10 benomyl-treated cells over a period of 30 minutes to 1 hour revealed no evidence of the nuclear movements seen in the untreated cells (Fig. 4A-D). Some of the benomyl-treated cells contained nuclear filaments, but no growth or shortening of the filaments was observed and no new filaments formed during the period when cells were exposed to the drug. This result demonstrates that nuclear oscillations in dicentric *cdc5-ad rad52* cells require microtubules.

In addition to microtubules, meiotic nuclear oscillations in fission yeast require the microtubule motor dynein (Yamamoto

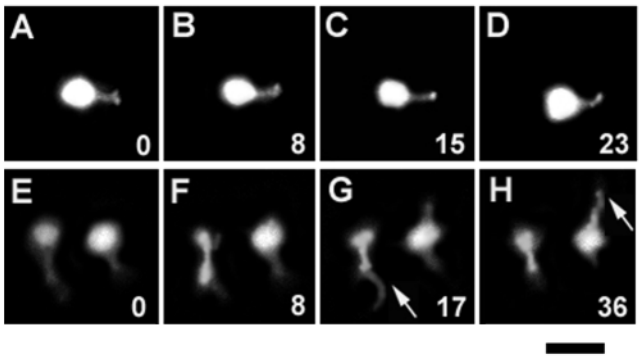

Fig. 4. Requirement for microtubules but not dynein for nuclear dynamics in dicentric *cdc5-ad rad52* cells. (A-D) Sequential fluorescence images of a dicentic *cdc5-ad rad52* cell expressing histone2B-GFP following treatment with benomyl. (E-H) Sequential fluorescence images of two dicentric *cdc5-ad dhc1 rad52* cells expressing histone2B-GFP. Note the change in nuclear morphology of the right-hand cell from round with a tail to 'dumbbell' (Fig. 4E,F). Also note the growth of filaments from both nuclei, indicated by arrows in Fig. 4G,H. Elapsed time is indicated in seconds. Bar, 5 μm.

et al., 1999). In budding yeast, dynein provides the major pulling force during anaphase and is responsible for the nuclear oscillations observed prior to anaphase onset in wild-type yeast cells (Yeh et al., 2000). Deletion of the gene encoding dynein heavy chain (*DHC1)* reduces the viability of cells containing a dicentric chromosome (Table 1). We deleted *DHC1* to determine if dynein is required for nuclear oscillations in *cdc5-ad rad52* cells. The nuclear dynamics of *dhc1* mutant cells were indistinguishable from cells containing a functional copy of *DHC1* (Fig. 4E-H). Thus, although we cannot rule out a role for dynein in anaphase nuclear oscillations in dicentric *cdc5-ad rad52* cells, it is clearly not the only source of motility.

Because microtubule motors use ATP hydrolysis to generate movement, we tested the ATP requirement for nuclear oscillations by treating dicentric *cdc5-ad rad52* cells with a solution containing 0.02% sodium azide and 1% deoxyglucose. To quantify the nuclear dynamics, length changes for individual nuclear filaments were measured over time. A plot of length changes for one nuclear filament in a cell treated with azide and deoxyglucose is shown in Fig. 5. The azide/deoxyglucose treatment resulted in the immediate cessation of both nuclear oscillations and changes in nuclear morphology. Nuclear dynamics resumed almost immediately upon removal of the azide and deoxyglucose. Growing and shortening rates were calculated, and the percentage of total time that filaments spent in growing, shortening or paused phases was determined (see Materials and Methods). The dynamicity parameter, derived from studies of microtubule dynamics, was also calculated as an overall measure of filament growth and shortening (Toso et al., 1993). The results are shown in Table 4. Depletion of ATP resulted in a significant suppression of filament dynamics. The most notable changes were the increase in pause time from 50% to over 90% of the total time, and the decrease in dynamicity, with post-treatment values 13-fold lower than the pre-treatment values. Interestingly, the rates of nuclear filament formation and collapse in untreated cells (0.8 μm/minute) are similar to rates of yeast cytoplasmic microtubule dynamics during anaphase (Tirnauer et al., 1999). Our findings demonstrate that nuclear oscillations in dicentric *cdc5-ad rad52* cells require ATP, which is consistent with a possible role of microtubule motors in this process.

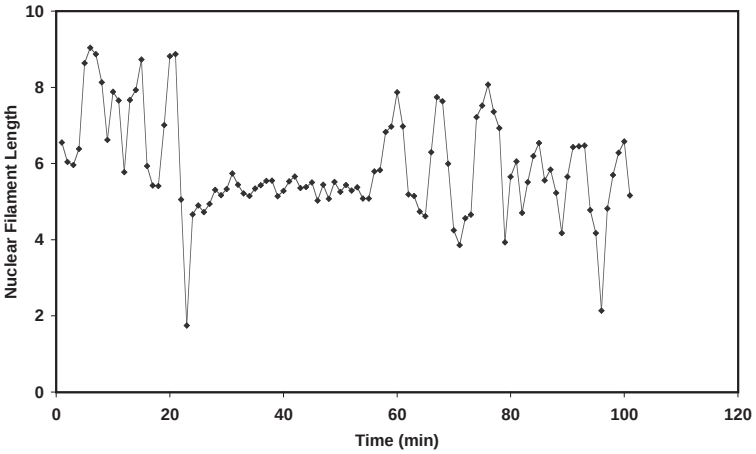

Fig. 5. Suppression of nuclear filament dynamics in dicentric *cdc5-ad rad52* cells by ATP depletion. Life history plot of a nuclear filament before, during and after removal of sodium azide and deoxyglucose. Media containing azide and deoxyglucose was added after 23 minutes and replaced by with drug-free media after 58 minutes.

Perturbation of microtubule function suppresses recombination between non-centromere sequences

The association between nuclear movements and enhanced *CEN* repeat recombination suggested that microtubules play a role in other recombination events. We tested this by determining if the survival of cells containing an inverted repeat dicentric chromosome was compromised by exposure to benomyl. Inverted repeat dicentric chromosomes undergo *RAD52*-dependent recombination between repeated sequences that flank the centromere region, resulting in deletion of one of the *CEN* repeats (Brock and Bloom, 1994). A reduction in the viability of an inverted dicentric strain following treatment with benomyl would thus indicate a microtubule dependence for recombination between non-centromeric sequences. A range of benomyl concentrations was chosen that did not significantly inhibit cell cycle progression nor result in detectible microtubule depolymerization (data not shown). As shown in Fig. 6, a monocentric strain exhibited only minor suppression of colony forming ability when plated on the highest benomyl concentration (15 μg/ml). By contrast, the viability of both inverted and direct repeat dicentric strains was significantly compromised on plates containing 10-15 μg/ml of benomyl. These results indicate that efficient recombinational repair of a dicentric chromosome requires microtubules whether occurring through *CEN* repeat recombination or by means of recombination between other repeated sequences.

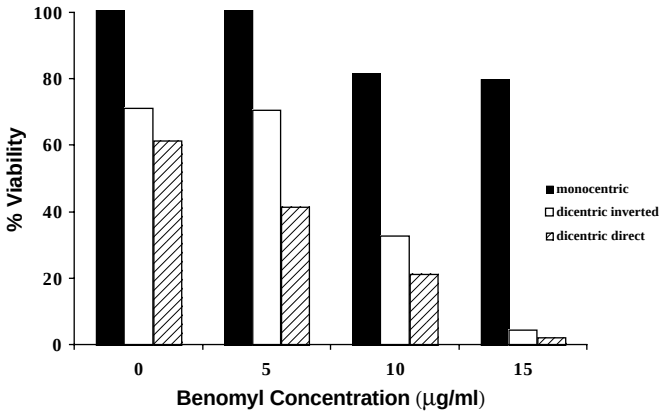

Fig. 6. Viability of cells containing either an inverted or a direct repeat dicentric chromosome is inhibited by low concentrations of benomyl. Strains containing either inverted or direct repeat dicentric constructs were grown in galactose media, washed and plated on solid media containing either galactose or glucose and the indicated concentrations of benomyl. The proportion of viable cells was determined by dividing the number of colonies that grew on glucose media by those on galactose. Shown are mean values for the results of three independent experiments, normalized to a control viability of 100%.

Table 5. *HIS3* recombination frequency as a function of benomyl concentration

Benomyl concentration (μg/ml)	Recombination frequency	Relative recombination frequency
0	4.05±1.06	1
10	3.11±1.37	0.76
15	1.77±0.33	0.43

Recombination frequencies are means±1 s.d. obtained from four independent experiments.

We further examined the potential role of microtubules in recombination by determining the effect of benomyl on the frequency of spontaneous recombination between two *his3* heteroalleles flanking a copy of the *URA3* gene (Table 5). Cells were plated on media containing concentrations of benomyl in the range of those used in the dicentric chromosome assay, and the *his3* recombination frequency was compared with cells that had not been exposed to the drug. Recombination was suppressed 2.3-fold by 15 μg/ml benomyl. These findings indicate that microtubule-dependent processes enhance spontaneous recombination events in budding yeast, in addition to promoting recombination events associated with dicentric chromosome resolution and meiotic recombination in fission yeast.

Discussion

We have utilized a conditional dicentric chromosome in budding yeast to study the repair of DSBs that occur during mitosis. We found that *RAD1* is required for dicentric chromosome repair both in the presence and absence of *RAD52* (Figs 1 and 2). The requirement for *RAD1* in recombinational deletion events is characteristic of the SSA pathway (Ivanov and Haber, 1995). The role of *cdc5-ad* in *RAD52*-independent repair of the dicentric chromosome is currently unclear. The presence of the *cdc5-ad* allele alone does not alter the frequency of SSA events resulting from a DSB caused by HO endonuclease in cells that contain Rad52 (Galgoczy and Toczyski, 2001). In addition, we did not observe any difference in dicentric chromosome recombination kinetics when *CDC5 RAD52* and *cdc5-ad RAD52* strains were compared (data not shown). However, it is a reasonable assumption that *CEN* recombination in the absence of *RAD52* is a consequence of the *cdc5-ad*-dependent mitotic block, given that both require *RAD9*.

Centromere recombination was consistently detected prior to activation of the dicentric chromosome in *cdc5-ad rad52* cells (Fig. 2B, lane 1). By contrast, this was not observed with dicentric *CDC5 RAD52* or *cdc5-ad RAD52* strains (Fig. 1B, lane 1; Fig. 2A, lane 1). In cells grown on galactose, transcriptional readthrough at the *GALCEN* prevents the activation of the dicentric chromosome. However, it has been shown that the conditional dicentric chromosome III is lost more frequently than its monocentric counterpart on galactose (Neff and Burke, 1992). Thus a functional kinetochore can form, albeit infrequently, even in the presence of galactose. We suggest that the nuclear oscillations associated with the *cdc5-ad rad52* strain result in an increase in dicentric chromosome breakage, leading to more frequent recombination on galactose. The reduced functional capability of the conditional kinetochore on galactose does not promote efficient dicentric chromosome breakage but may induce sufficient tension on the chromosome to trigger the DNA damage response. The DNA-damage-induced arrest in dicentric *cdc5-ad rad52* cells results in a prolonged mitotic arrest characterized by a sustained period of nuclear oscillations. These oscillations could promote more frequent dicentric chromosome breakage events than would normally occur in the presence of galactose and as a consequence, more frequent *CEN3* recombination. Consistent with this, a small portion (<10%) of very large budded cells with nuclear morphology identical to that in Fig. 3R were found in populations of dicentric *cdc5-ad rad52* cells grown in galactose media (data not shown). It is unlikely that this mechanism accounts for a significant proportion of the recombination events that occur when dicentric cells are grown on glucose, given the high efficiency of chromosome breakage promoted by an activated *GALCEN*.

Live cell imaging of GFP-labeled nuclei following dicentric chromosome activation in *cdc5-ad rad52* cells allowed us to observe an extended period of nuclear oscillations accompanied by the formation and collapse of nuclear filaments (Figs 3-5). The association of these nuclear movements with *RAD52*-independent dicentric chromosome recombination suggests that this behavior might be responsible for the *CEN* recombination events observed in these cells. Microtubule-dependent oscillatory nuclear movements are also observed during meiosis in fission yeast and are required for efficient meiotic recombination (Chickashige et al., 1994; Ding et al., 1998; Yamamoto et al., 1999). It has been suggested that nuclear oscillations observed during meiosis in *S. pombe* facilitate recombination by promoting the alignment of homologous chromosomes (Yamamoto et al., 1999). Although this exact mechanism is unlikely to explain enhanced recombination in dicentric *cdc5-ad rad52* cells, the contortions that the nucleus undergoes during this process (Fig. 3) could increase the frequency of collisions between *CEN* repeats located in the two halves of a broken dicentric chromosome. A previous study revealed that recombination events promoted by the HO endonuclease occur with similar frequency regardless of the location of the recombining sequences within the genome, consistent with the idea that a homology search mechanism may facilitate such events (Haber and Leung, 1996). It has also been demonstrated that increasing the copy number of a sequence can increase the likelihood a sequence will undergo recombination, suggesting that recombination is stimulated by increasing the frequency with which sequences interact (Melamed and Kupiec, 1992). Collision frequency may play a greater role in promoting recombinational repair of a broken dicentric chromosome, where each half of the broken chromosome is actively separated from the other, than it does in repair of other types of DSBs.

Nuclear oscillations are not unique to dicentric *cdc5-ad* cells. We have observed cells exhibiting this behavior as a consequence of *MPS1* overexpression (Table 3), and nuclear transits between the yeast mother cell and bud have previously been reported in *cdc13, cdc16,* and *cdc23* mutant cells following their release from a mitotic arrest (Palmer et al., 1989). The proteins encoded by *CDC16* and *CDC23* are part of the anaphase-promoting complex (APC) that facilitates entry into and exit from anaphase. The *cdc13* arrest occurs

through a *RAD9*-dependent DNA damage response pathway that prevents progression into anaphase by inhibiting the APC-dependent degradation of Pds1/Securin. Recent studies have shown that a reduction in Pds1 levels at anaphase onset is required for anaphase spindle stability (Jensen et al., 2001; Severin et al., 2001). Spindle instability could result in alterations in spindle length and could account for the conversion of elongated/bilobed nuclei to spherical structures observed in dicentric *cdc5-ad rad52* cells (see Fig. 3H-I). Interestingly, APC complexes immunoprecipitated from *cdc5-ad* mutant cells exhibit a reduced degree of ubiquitination activity (Charles et al., 1998). Ubiquitination of Pds1 is a prerequisite for its degradation, and the persistence of elevated levels of Pds1 in *cdc5-ad* mutant cells could lead to spindle instability and contribute to nuclear oscillations. Future experiments will be required to determine whether nuclear oscillations result from spindle microtubule dynamics, cytoplasmic microtubule dynamics or a combination of the two.

The association of microtubule-dependent nuclear oscillations with *CEN* recombination raised the question of whether microtubules play a role in recombination events involving other homologous repeats. To test this hypothesis we examined the effect of low concentrations of benomyl on a dicentric chromosome repair process that involves recombination between non-centromere sequences (Fig. 6) and the effect of this drug on spontaneous recombination between of a pair of *his3* heteroalleles (Table 5). We have observed that concentrations of benomyl in the range used in our experiments suppress the dynamic instability behavior of cytoplasmic microtubules and yeast kinetochore movements during mitosis (K.B. and Chad Pearson, unpublished). The growth and shortening of microtubules is responsible, at least in part, for nuclear movements that occur throughout the cell cycle in budding yeast (Shaw et al., 1997b) and for chromosome movements during mitosis (Pearson et al., 2001). Thus it is likely that the significant reduction in viability of cells containing a dicentric chromosome in response to exposure to benomyl is due to decreased chromosome breakage resulting from suppressed microtubule dynamics. The suppression of recombination revealed by the *his3* assay system indicates that microtubules may play a more general role in facilitating efficient recombination. It is unlikely that this effect was the result of the moderate decrease in growth rate that accompanied the benomyl treatments given that previous studies have shown that cell cycle delays have either a positive effect or no significant impact on DNA repair (Weinert and Hartwell, 1988; Brock and Bloom, 1994). Previous studies have shown that spontaneous intrachromosomal recombination occurs at a higher rate than spontaneous recombination between sequences located on different chromosomes (Lichten and Haber, 1989; Jinks-Robertson et al., 1993). This indicates that a collision-based homology search may not play a significant role in this type of recombination. It is assumed from the similar genetic requirements of recombination induced by agents that produce DSBs and those required for spontaneous recombination that the latter events are likewise promoted by DSBs. The source of these cryptic DSBs has not been definitively identified. On the basis of our finding that low concentrations of benomyl suppress recombination, we suggest that a portion of the DSBs involved in these recombination events result from microtubule-generated nuclear movements. Further, the modest effect of the drug treatments on this process indicates that both dependent and microtubule-independent sources of DSBs give rise to spontaneous recombination events.

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References

Aragon-Alcaide, L. and Strunnikov, A. V. (2000). Functional dissection of in vivo interchromosome association in *Saccharomyces cerevisiae*. *Nat. Cell. Biol.* **2**, 812-818.

Brock, J. A. and Bloom, K. (1994). A chromosome breakage assay to monitor mitotic forces in budding yeast. *J. Cell Sci.* **107**, 891-902.

Charles, J. F., Jaspersen, S. L., Tinker-Kulberg, R. L., Hwang, L., Szidon, A. and Morgan, D. O. (1998). The Polo-related kinase Cdc5 activates and is destroyed by the mitotic cyclin destruction machinery in *S. cerevisiae*. *Curr. Biol.* **8**, 497-507.

Chikashige, Y., Ding, D. Q., Funabiki, H., Haraguchi, T., Mashiko, S., Yanagida, M. and Hiraoka, Y. (1994). Telomere-led premeiotic chromosome movement in fission yeast. *Science* **264**, 270-273.

Ding, D. Q., Chikashige, Y., Haraguchi, T. and Hiraoka, Y. (1998). Oscillatory nuclear movement in fission yeast meiotic prophase is driven by astral microtubules, as revealed by continuous observation of chromosomes and microtubules in living cells. *J. Cell Sci.* **111**, 701-712.

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

Fishman-Lobell, J., Rudin, N. and Haber, J. E. (1992). Two alternative pathways of double-strand break repair that are kinetically separable and independently modulated. *Mol. Cell. Biol.* **12**, 1292-1303.

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

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

Hill, A. and Bloom, K. (1989). Acquisition and processing of a conditional dicentric chromosome in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **9**, 1368-1370.

Ivanov, E. L. and Haber, J. E. (1995). *RAD1* and *RAD10*, but not other excision repair genes, are required for double-strand break-induced recombination in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **15**, 2245-2251.

Jager, D. and Philippsen, P. (1989). Stabilization of dicentric chromosomes in *Saccharomyces cerevisiae* by telomere addition to broken ends or by centromere deletion. *EMBO J.* **8**, 247-254.

Jensen, S., Segal, M., Clarke, D. J. and Reed, S. I. (2001). A novel role of the budding yeast separin Esp1 in anaphase spindle elongation: evidence that proper spindle association of Esp1 is regulated by Pds1. *J. Cell Biol.* **152**, 27-40.

Jinks-Robertson, S., Michelitch, M. and Ramcharan, S. (1993). Substrate length requirements for efficient mitotic recombination in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **13**, 3937-3950.

Klein, H. (1988). Different types of recombination events are controlled by the *RAD1* and *RAD52* genes of *Saccharomyces cerevisiae*. *Genetics* **120**, 367-377.

Kramer, K. M., Brock, J. A., Bloom, K., Moore, J. K. and Haber, J. E. (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.

Lauze, E., Stoelcker, B., Luca, F. C., Weiss, E., Schutz, A. R. and Winey, M. (1995). Yeast spindle pole body duplication gene MPS1 encodes an essential dual specificity protein kinase. *EMBO J.* **14**, 1655-1663.

Lichten, M. and Haber, J. E. (1989). Position effects in ectopic and allelic

mitotic recombination in *Saccharomyces cerevisiae*. *Genetics* **123**, 261-268.

Liefshitz, B., Parket, A., Maya, R. and Kupiec, M. (1995). The role of DNA repair genes in recombination between repeated sequences in yeast. *Genetics* **140**, 1199-1211.

Melamed, C. and Kupiec, M. (1992). Effect of donor copy number on the rate of gene conversion in the yeast *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* **235**, 97-103.

Neff, M. W. and Burke, D. J. (1992). A delay in the *Saccharomyces cerevisiae* cell cycle that is induced by a dicentric chromosome and dependent upon mitotic checkpoints. *Mol. Cell. Biol.* **12**, 3857-3864.

Palmer, R. E., Koval, M. and Koshland, D. (1989). The dynamics of chromosome movement in the budding yeast *Saccharomyces cerevisiae*. *J. Cell. Biol.* **109**, 3355-3366.

Pearson, C. G., Maddox, P. S., Salmon, E. D. and Bloom, K. (2001). Budding yeast chromosome structure and dynamics during mitosis. *J. Cell Biol.* **152**, 1255-1266.

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

Prado, F. and Aguilera, A. (1995). Role of reciprocal exchange, one-ended invasion crossover and single-strand annealing on inverted and direct repeat recombination in yeast: different requirements for the *RAD1*, *RAD10*, and *RAD52* genes. *Genetics* **139**, 109-123.

Qiu, J., Qian, Y., Chen, V., Guan, M. X. and Shen, B. (1999). Human exonuclease 1 functionally complements its yeast homologues in DNA recombination, RNA primer removal, and mutation avoidance. *J. Biol. Chem.* **274**, 17893-17900.

Rothstein, R. (1991). Targeting, disruption, replacement, and allele rescue: integrative DNA transformation in yeast. *Meth. Enzymol.* **194**, 281-301.

Schiestl, R. H. and Prakash, S. (1990). RAD10, an excision repair gene of *Saccharomyces cerevisiae*, is involved in the RAD1 pathway of mitotic recombination. *Mol. Cell. Biol.* **10**, 2485-2491.

Severin, F., Hyman, A. A. and Piatti, S. (2001). Correct spindle elongation at the metaphase/anaphase transition is an APC-dependent event in budding yeast. *J. Cell Biol.* **155**, 711-717.

Shaw, S. L., Yeh, E., Bloom, K. and Salmon, E. D. (1997a). Imaging green fluorescence protein fusion proteins in *Saccharomyces cerevisiae*. *Curr. Biol.* **7**, 701-704.

Shaw, S. L., Yeh, E., Maddox, P., Salmon, E. D. and Bloom, K. (1997b). Astral microtubule dynamics in yeast: a microtubule-based searching mechanism for spindle orientation and nuclear migration into the bud. *J. Cell Biol.* **139**, 985-994.

Siede, W., Friedberg, A. S. and Friedberg, E. C. (1993). Evidence that the Rad1 and Rad10 proteins of *Saccharomyces cerevisiae* participate as a complex in nucleotide excision repair of UV radiation damage. *J. Bacteriol.* **175**, 6345-6347.

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

Sung, P., Reynolds, P., Prakash, L. and Prakash, S. (1993). Purification and characterization of the *Saccharomyces cerevisiae* RAD1/RAD10 endonuclease. *J. Biol. Chem.* **268**, 26391-26399.

Thomas, B. J. and Rothstein, R. (1989). Elevated recombination rates in transcriptionally active DNA. *Cell* **56**, 619-630.

Tirnauer, J. S, O'Toole, E., Berrueta, L., Bierer, B. E. and Pellman, D. (1999). Yeast Bim1p promotes the G1-specific dynamics of microtubules. *J. Cell Biol.* **145**, 993-1007.

Toczyski, D. P., Galgoczy, D. J. and Hartwell, L. H. (1997). CDC5 and CKII control adaptation to the yeast DNA damage checkpoint. *Cell* **90**, 1097-1106.

Toso, R. J., Jordan, M. A., Farrell, K. W., Matsumoto, B. and Wilson, L. (1993). Kinetic stabilization of microtubule dynamic instability in vitro by vinblastine. *Biochemistry* **32**, 1285-1293.

Weinert, T. A. and Hartwell, L. H. (1988). The RAD9 gene controls the cell cycle response to DNA damage in *Saccharomyces cerevisiae*. *Science* **241**, 317-322.

White, C. I. and Haber, J. E. (1990). Intermediates of recombination during mating type switching in *Saccharomyces cerevisiae*. *EMBO J.* **9**, 663-673.

Winey, M. and Huneycutt, B. J. (2002). Centrosomes and checkpoints: the MPS1 family of kinases. *Oncogene* **21**, 6161-6169.

Yamamoto, A., West, R. R., McIntosh, J. R. and Hiraoka, Y. (1999). A cytoplasmic dynein heavy chain is required for oscillatory nuclear movement of meiotic prophase and efficient meiotic recombination in fission yeast. *J. Cell Biol.* **145**, 1233-1249.

Yang, S. S., Yeh, E., Salmon, E. D. and Bloom, K. (1997). Identification of a mid-anaphase checkpoint in budding yeast. *J. Cell Biol.* **136**, 345-354.

Yeh, E., Yang, C., Chin, E., Maddox, P., Salmon, E. D., Lew, D. J. and Bloom, K. (2000). Dynamic positioning of mitotic spindles in yeast: role of microtubule motors and cortical determinants. *Mol. Biol. Cell* **11**, 3949-3961.