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Enhanced stimulation of chromosomal translocations by radiomimetic DNA damaging agents and camptothecin in *Saccharomyces cerevisiae rad9* checkpoint mutants

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Abstract

Saccharomyces cerevisiae rad9 checkpoint mutants exhibit pleiotropic phenotypes, including higher frequencies of chromosome loss, radiation sensitivity, and decreased induction of DNA damage-inducible genes. We had previously shown that *rad9* mutants exhibit higher frequencies of DNA damage-associated translocations but lower frequencies of DNA damage-associated sister chromatid exchange (SCE), compared to wild type. Herein, we have shown that differences between the frequencies of DNA damage-associated recombination in the *rad9* mutant and wild type depend on the identity and the concentration of the DNA damaging agent. Translocation and SCE frequencies were measured in strains containing truncated *his3* fragments, located either on chromosomes II and IV, or located in tandem on chromosome IV, respectively. DNA damage-associated frequencies of translocations after exposure to hydrogen peroxide (H_2O_2), bleomycin, phleomycin, cisplatin, and camptothecin are higher in the *rad9* diploid than in wild type. However, translocation frequencies after exposure to 4-nitroquinoline 1-oxide (4-NQO) and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) are similar in *rad9* and wild-type strains. We suggest that the deficiency in triggering G_2 arrest after exposure to specific DNA damaging agents results in the higher levels of DNA damage-associated translocations in *rad9* mutants.

Keywords

Recombination; DNA damaging agent; Yeast; G_2 checkpoint

1. Introduction

Cell cycle checkpoints may reduce chromosomal instability by allowing time for DNA repair before replication or segregation of chromatids. In *Saccharomyces cerevisiae* (yeast), four distinct cell cycle delays have been described: one G_1/S phase, two S phase, and one G_2/M phase [1]. Both single stranded DNA and stalled replication forks have been postulated to signal cell cycle checkpoints (for review, see [2]). Activation of one or more

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cell cycle checkpoints may occur after cells are exposed to specific DNA damaging agents. For example, alkylating agents that methylate in the DNA minor groove may block DNA polymerase progression, while methylation in the major groove may be repaired by enzymes which indirectly produce DNA strand breaks [3]. Thus, how DNA damage is processed and whether the damage is sufficient to block DNA replication may be key factors in triggering particular checkpoints.

RAD9 is required for cell cycle arrest in G_2 after exposure to DNA damaging agents [4]. DNA damaging agents that trigger cell cycle arrest in G_2 include UV and X rays [4], and chemical compounds that directly or indirectly cause double-strand breaks, such as hydrogen peroxide (H_2O_2) [5] and methyl methanesulfonate (MMS) [6]. Chemical compounds that cause intrastrand and interstrand cross-links, such as cisplatin [7], and topoisomerase I inhibitors, such as camptothecin [8], also trigger G_2 arrest. However, other DNA damaging agents which cause bulky adducts, such as the UV-mimetic agent 4-Nitroquinoline 1-oxide (4-NQO), do not trigger G_2 arrest in eukaryotic cells [9]. Thus, *RAD9*-mediated G_2 arrest can be triggered both by radiomimetic chemical agents, as well as specific chemical compounds which indirectly cause DNA single or double-strand breaks.

RAD9-mediated G_2 cell-cycle arrest allows more time for recombinational repair between sister chromatids, which are preferred substrates for X-ray-associated repair in yeast [10,11]. Consistent with this idea, *rad9* mutants exhibit lower frequencies of X-ray-associated SCEs but higher frequencies of X-ray-associated translocations [12]. When *rad9* cells are exposed to UV and MMS, there is only a minor decrease in frequencies of DNA damage-associated SCEs, compared to wild type [12]. These DNA damaging agents may generate a greater number of DNA lesions that trigger the *MEC1*-dependent S phase checkpoint to prevent replication fork collapse [13]. Thus, checkpoint mutants exposed to only a subset of DNA damaging agents exhibit higher frequencies of DNA damage-associated translocations and lower frequencies of DNA damage-associated SCE [12].

Besides its function to trigger cell cycle arrest in G_2, *RAD9* is required to prevent mitosis when DNA replication is incomplete [14] and delaying entry into S phase after exposure either to ionizing radiation, UV, or 4-NQO [15]. *RAD9* is required to repair chromosome breaks that occur during S phase as a consequence of defective topoisomerase action [16] and in DNA damage tolerance mechanisms in excision repair mutants [17]. *rad9* mutants are defective in maintaining large yeast artificial chromosomes (YACs) when such YACs contain only one replication origin [18]. Thus, *RAD9* maintains genetic stability by triggering delays at other stages in the cell cycle.

In this manuscript, we have exposed cells to DNA damaging agents to identify compounds that stimulate more translocations but fewer SCEs in *rad9* mutants, compared to wild type. In addition to DNA damaging agents that directly cause strand breaks, we also chose cross-linking and alkylating agents and agents that cause bulky adducts. Compared to wild type, we observed more DNA damage-associated translocations when *rad9* cells were exposed to particular X-ray-mimetic agents, cisplatin or camptothecin but not to 4-NQO and MNNG.

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2. Materials and methods

2.1. Strains and media

Standard media, including YPD (yeast extract, peptone, dextrose), synthetic complete lacking histidine (SC-HIS) have been previously described [19]. The strains used to measure homology-directed translocations and sister chromatid exchange (SCE) are listed in Table 1, and their construction was previously described [12]. These strains contain two truncated fragments of *his3, his3- 3´* and *his3- 5´* [12], as illustrated (Fig. 1). Directed translocations were measured in diploids, which were derived from one haploid containing the *his3* fragments and one haploid lacking the truncated fragments.

2.2. DNA damaging agents

Compounds used in this study included methyl methanesulfonate (MMS), *N*-methyl-*N´*-nitro-*N*-nitrosoguanidine (MNNG), cisplatin, 4-nitroquinoline 1-oxide (4-NQO), bleomycin, phleomycin, H_2O_2, camptothecin. H_2O_2 (30% w/w) was purchased from Sigma. Chemicals were purchased from Sigma or Aldrich Chemicals. Chemicals were dissolved in either dimethyl sulfoxide (DMSO), dimethyl formamide (DMF) or water, depending on the specifications provided by the vendor.

2.3. Plate test assay for induced recombination in rad9 diploids

A single colony from the appropriate strain was inoculated in YPD medium and incubated at 30 °C with agitation. Approximately 10^7 cells were then plated on SC-HIS plates and the indicated amounts of the chemical were added in the center of the plate. The zone of toxicity is indicative of the lethality of the chemical, while a halo of induced His^+ colonies is indicative of translocation events. Concentrations of solutions added to plates included H_2O_2 (30% w/w), 10.5 M MMS, 100 mM MNNG, 140 mM 4-NQO, 133 mM cisplatin, 10 mg/ml camptothecin, 3 units/ml bleomycin, 10 mM phleomycin.

2.4. Culturing and treatment of yeast

Protocols used to test the recombinogenicity of chemical DNA damaging agents have been described [20]. For measuring stimulation of SCE and translocations, cells were pre-incubated for 30 min in YPD after treatment with the DNA damaging agent, washed twice with sterile H_2O, and then plated on selective medium (SC-HIS). We exposed cells to concentrations of chemicals that exhibit equivalent levels of lethality in both wild-type and *rad9* strains (Table 2). Because spontaneous frequencies of translocations are significantly higher in *rad9* mutants, we also measured net frequencies of DNA damage-associated recombination by subtracting the spontaneous frequency from the frequency obtained after chemical exposure, as done previously [12,21,22]. At least three independent experiments were done for each DNA damaging agent. The significance of the differences between *rad9* mutants and *RAD9*$^+$ strains was determined using the two-tailed paired sample *t*-test [23].

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2.5. CHEF gels

Electrophoretic karyotypes of His+ recombinants were determined by pulse field electrophoresis using contour clamped gel electrophoresis (CHEF) to resolve chromosomal DNA. The running time was 26 h at 250 V (6 V/cm) and the pulse time was 90 s [22].

3. Results

3.1. Recombination assays

Our goal was to identify chemical DNA damaging agents that stimulate more translocations in *rad9* mutants, compared to wild type. Considering that *rad9* mutants are defective in X-ray-associated SCE [12], we then asked whether the frequencies of DNA damage-associated SCE were lower in *rad9* mutants after exposure to these chemical agents. To measure frequencies of DNA damage-associated translocation and SCE, we select His+ prototrophs that result from recombination between the *his3* fragments located on chromosomes II and IV or located in tandem at the *trp1* locus, respectively. The rate of spontaneous translocations in the *rad9* diploid YB134 is 2×10^{-7}, which is fourfold higher than the rate in the wild-type diploid YB110 [12]. The rates of spontaneous SCE in *rad9* haploid and diploid mutants and in wild-type haploid and diploid strains are the same, ~2×10^{-6} [12].

We employed a simple plate assay to detect recombinants that result from exposure to DNA damaging agents [20]. Because spontaneous rates of translocations are low [12,20], DNA damage-associated His+ recombinants containing translocations appear as halos after a chemical compound is diffused from the center of a SC-HIS plate inoculated with either wild-type or *rad9* cells (Fig. 2). This assay indicates when there are more stimulated His+ recombinants per total number of inoculated cells after exposure to the DNA damaging agent. We observed a thicker halo of stimulated His+ recombinants and more spontaneous His+ recombinants after *rad9* cells were exposed to MMS and H_2O_2, compared to wild type (Fig. 2). Other DNA damaging agents, including bleomycin, phleomycin, camptothecin and cisplatin, did not stimulate more His+ recombinants using this plate assay (data not shown). However, as shown in previous studies [20], particular DNA damaging agents stimulate more recombination in log phase cell cultures.

3.2. Agents that directly cause DNA strand breaks stimulate more recombination in rad9 mutants

We had previously shown that *rad9* cells exhibit higher frequencies of translocations after exposure to MMS [12], an agent known to promote DNA breaks. Actively growing log phase cultures of both wild-type and *rad9* diploid strains were then exposed to other DNA damaging agents that create single and double-strand breaks. Exposure to 2 mM H_2O_2 increased translocation frequencies in both *RAD9* and *rad9* diploid strains by 31- and 22-fold, respectively; however, compared to wild type, there was a three-fold difference in the net frequency of DNA damage-associated translocations in the *rad9* diploid compared to the *RAD9* strain. Bleomycin and phleomycin exposure increased translocation frequencies 11- and 33-fold in the *rad9* diploid, and we observed an eight and fivefold difference in the net translocation frequencies, respectively, in *rad9* diploid compared to wild type. The higher frequencies of DNA damage-associated translocations obtained after exposure

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of *rad9* cells to bleomycin, compared to the related compound phleomycin, may result from the higher number of double-strand breaks that are caused by bleomycin [24]. Thus, exposure to chemical agents that cause DNA strand breaks, including H_2O_2, bleomycin, and phleomycin, results in higher translocation frequencies in *rad9* than in wild-type (Table 2).

3.3. Camptothecin and cross-linking agents that trigger G_2 arrest stimulate more homology-directed translocations in rad9 mutants

DNA damaging agents, including cross-linking agents and topoisomerase inhibitors, do not directly induce single and double-strand breaks but can trigger G_2 arrest in budding yeast. Exposure to 1.3 mM cisplatin increased translocation frequencies 81- and 22-fold in wild-type and *rad9* strains, respectively; net frequencies were threefold higher in *rad9* cells (Table 2). Net frequencies of camptothecin-associated translocations were fivefold higher in *rad9* cells, compared to wild type, although we observed more modest increases in DNA damage-associated frequencies of translocations after camptothecin exposure compared to other compounds. These results indicate that frequencies of DNA damage-associated translocations may be higher in checkpoint mutants after exposure to compounds that do not directly induce strand breaks.

3.4. DNA damage-associated SCE is decreased in rad9 mutants after exposure to a subset of DNA damaging agents

To determine whether higher frequencies of DNA damage-associated translocations correlated with lower frequencies of DNA damage-associated SCE, we measured SCE frequencies in both wild-type (YB163 and YB146) and *rad9* (YB147) strains after exposure to H_2O_2, camptothecin, phleomycin, cisplatin and 4-NQO. After exposure to phleomycin and camptothecin, we observed significant twofold increases in SCE frequencies in wild type ($P < 0.05$) but not in the *rad9* strain ($P > 0.05$) (Table 3). SCE frequencies after exposure to bleomycin and cisplatin were 4.7×10^{-5} (average, $n = 3$) and 3.2×10^{-5} (average, $n = 3$) but not higher than wild type (YB147), 2.4×10^{-5} ($P > 0.05$). We observed a fourfold stimulation of recombination in both wild-type and *rad9* cells after exposure to H_2O_2 (Table 3) and 4-NQO (data not shown). Thus, differences in SCE recombination frequencies in wild type and *rad9* mutants after exposure to DNA damaging agents was only observed for a subset of DNA damaging agents.

3.5. RAD9 checkpoint is less efficient in suppressing 4-NQO and MMNG-associated recombination

Several DNA damaging agents create particular DNA adducts that require replication to initiate recombination, while other DNA damaging agents more frequently form DNA adducts in replicating regions. For example, recombinogenicity of 4-NQO is enhanced when cells undergo replication [25] and DNA adducts formed after MNNG exposure occur most frequently in active replicating regions [26]. We observed a 43- and 23-fold increase in translocation frequencies after wild-type and *rad9* cells were exposed to 4-NQO, respectively (Table 2). We observed a 52- and 21-fold increase in translocation frequencies after wild-type and *rad9* cells were exposed to MNNG, respectively (Table 2). Net DNA damage-associated frequencies after exposure to either MNNG or 4-NQO were less than

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twofold higher in *rad9*. This data suggest that *RAD9* poorly suppresses recombination that is either stimulated by MMNG or 4-NQO.

3.6. Electrophoretic karyotypes of DNA damage-associated His$^+$ recombinants

We previously observed that most spontaneous and radiation-associated His$^+$ recombinants from wild type contain reciprocal translocations, *CEN2* :: IV and *CEN4* :: II, while most His$^+$ recombinants from *rad9* contain one non-reciprocal translocation, *CEN2* :: IV [12]. We therefore characterized the electrophoretic karyotypes of DNA damage-associated translocations that occurred after exposure to H_2O_2 and camptothecin, DNA damaging agents that do not directly stimulate double-strand breaks (Fig. 3). We observed that four out of four H_2O_2-associated His$^+$ recombinants from the *rad9* strain contain only one non-reciprocal translocation, *CEN2* :: IV After exposure to camptothecin, we characterized the electrophoretic karyotype of six His$^+$ recombinants from the *rad9* strain and three from wild type. Of the His$^+$ recombinants from the *rad9* strain, we observed that four contain non-reciprocal translocations and two contain reciprocal translocations. We observed that one His$^+$ recombinant contained reciprocal translocations and two contained non-reciprocal translocations from the wild-type strain. This data suggest that DNA damage caused by camptothecin stimulates both reciprocal and non-reciprocal translocations.

4. Discussion

S. cerevisiae rad9 mutants exhibit complex phenotypes, including radiation sensitivity, enhanced frequencies of chromosome loss, and failure to arrest the cell cycle in G_2 [15,17,18,27]. Here we report that, depending on the chemical compound, *RAD9* can partially suppress the frequencies of DNA damage-associated translocations and increase the frequencies of DNA damage-associated SCE. We observed higher frequencies of translocations in *rad9* mutants after exposure to DNA damaging agents that are known to specifically trigger cell cycle arrest in the G_2 phase, and these agents include bleomycin, phleomycin, H_2O_2, cisplatin and camptothecin. Since frequencies of DNA damage-associated SCE were the same in wild type and *rad9* after exposure to specific DNA damaging agents, we speculate that S phase checkpoints are important in enhancing recombinational repair. Because *rad9* and wild-type strains did not exhibit different recombination phenotypes after exposure to MNNG and 4-NQO but *rad9* mutants are hypersensitive to both MNNG and 4-NQO, we speculate that *RAD9* participates in additional DNA repair pathways to confer resistance both to alkylating compounds and compounds that cause bulky adducts.

The data supporting these conclusions was obtained using both a simple plate assay and a quantitative liquid assay. The plate assay provides a quick method to identify a subset of chemical compounds that stimulate more translocations in *rad9* mutants. However, the liquid assay provides a more accurate measurement of the translocation frequencies after exposure to particular DNA damaging agents, which are active in log phase cells or in the presence of oxygen. Although a subset of DNA damage-associated translocation frequencies are higher in *rad9* mutants than in wild type, the fold induction (ratio of the DNA damage-associated translocation frequency to the spontaneous frequencies) may be higher in wild type than in

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the *rad9* mutant because the spontaneous translocation frequencies are lower in the wild-type strain.

We previously documented that frequencies of X-ray-associated translocations are increased but frequencies of X-ray-associated SCE are decreased in *rad9* mutants [12]. Particular DNA damaging agents that mimic the effects of ionizing radiation, such as phleomycin, thus stimulate more translocations but fewer SCEs in *rad9* mutants. We suggest that *RAD9* may confer resistance to several radiomimetic compounds by simply triggering a G_2 arrest and allowing more time for sister chromatid gap repair.

Higher frequencies of DNA damage-associated translocations in *rad9* cells after camptothecin and cisplatin exposure support ideas that both camptothecin [28] and cisplatin [7] create DNA adducts that indirectly generate recombinogenic double-strand breaks. Cisplatin-induced interstrand crosslinks creates replication-dependent double-strand breaks that trigger G2 arrest [29]. Modest stimulation of SCE after cisplatin exposure could result from the low percentage of interstrand crosslinks among total number of DNA adducts [29] and the relatively high frequencies of spontaneous SCE.

Double-strand breaks resulting from camptothecin exposure occur when the DNA replication apparatus encounters trapped topoisomerase I intermediates, leading to replication fork collapse [16]. His^+ recombinants resulting from either unequal SCE or translocations after camptothecin exposure could result from break-induced replication (BIR) mechanisms [30,31], and *RAD9*-mediated G_2 arrest may provide time for unequal SCE. Non-reciprocal translocations are postulated to occur by BIR [22]. However, we detected both reciprocal and non-reciprocal translocations among camptothecin-associated His^+ recombinants. Considering that we exposed asynchronous log phase cell cultures to camptothecin, it is possible that camptothecin-associated DNA damage occurred after DNA replication was completed and gap repair mechanisms initiated recombination resulting in reciprocal translocations.

We observed that in *rad9* mutants, higher frequencies of DNA damage-associated translocations and lower frequencies of DNA damage-associated SCE do not correlate after exposure to particular DNA damaging agents, such as H_2O_2. Frequencies of MMS and UV-associated SCE are also similar in wild type and *rad9* mutants [12]. Both UV and MMS create DNA adducts that impede DNA replication fork progression and trigger S phase checkpoints [32], although longer MMS-induced S phase has been also attributed to the inhibition of replication initiation at late replication origins [13]. Thus, it would be interesting to determine whether DNA damage-associated SCE was deficient in mutants defective in S phase checkpoints.

We found that *RAD9* does not function to suppress DNA damage-associated translocations after exposure to either 4-NQO or MNNG. Consistent with previous literature [26,33], we speculate that these compounds are recombinogenic in S phase, and *RAD9*-independent S phase checkpoints regulate DNA damage-associated recombination. The *RAD9* requirement for efficient UV excision repair [34] and for the G_1 cell cycle delay [15] after exposure to

DNA damaging agents may explain the sensitivity of *rad9* mutants to the UV-mimetic agent 4-NQO and to particular alkylating agents.

Our results are interesting to compare with those from a related study concerning the types of DNA damaging agents that stimulate gross chromosomal rearrangements [35]. The DNA damage-associated frequencies of translocations are higher than frequencies of gross chromosomal rearrangements, possibly reflecting the greater efficiency of homologous recombination, compared to non-homologous recombination. DNA damage resulting from X-rays and bleomycin exposure, generate more gross chromosomal rearrangements than DNA damaging agents that do not directly cause double-strand breaks. However, our results indicate that a subset of alkylating agents and bulky adducts are potent stimulators of chromosomal rearrangements directed by homologous recombination. Although there are several reasons that may account for these differences, it is interesting to note that the *RAD9* checkpoint suppresses directed translocations; whereas, the S phase checkpoint is important in suppressing gross chromosomal rearrangements [36]. Thus, it will be interesting to extend these studies and determine whether *RAD9*-independent S phase checkpoints also suppress homology-directed translocations.

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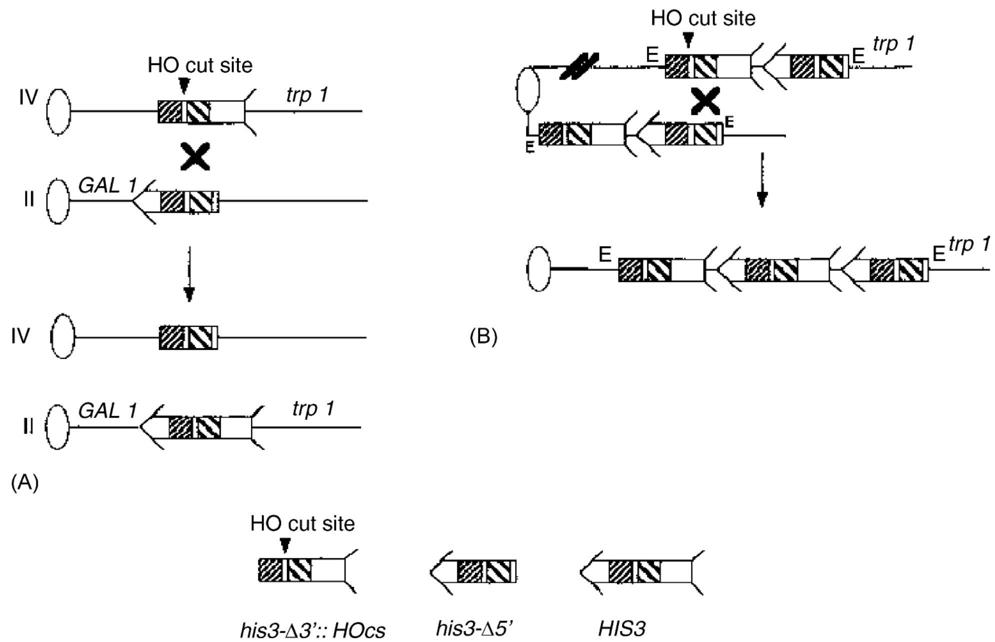

Fig. 1.

Recombination assays used in this study. Ovals represent centromeres and lines represent chromosomes. For simplicity, the left arms of chromosomes are not included. The position and orientation of the *his3* recombinational substrates are shown as present in strains used to measure (A) reciprocal translocations and (B) unequal SCE. An "X" designates potential sites of crossovers, and the resulting chromosomal rearrangement is presented. An arrow and feathers denote *HIS3*. As indicated on the bottom of the figure, the 5′ deletion lacks the feathers and the 3′ deletion lacks the arrow. The two regions of sequence identity shared by the *his3* fragments are indicated by decorated boxes; broadly spaced diagonal lines indicate a region of ~300 bp, and tightly spaced diagonal lines indicate a region of 167 bp.

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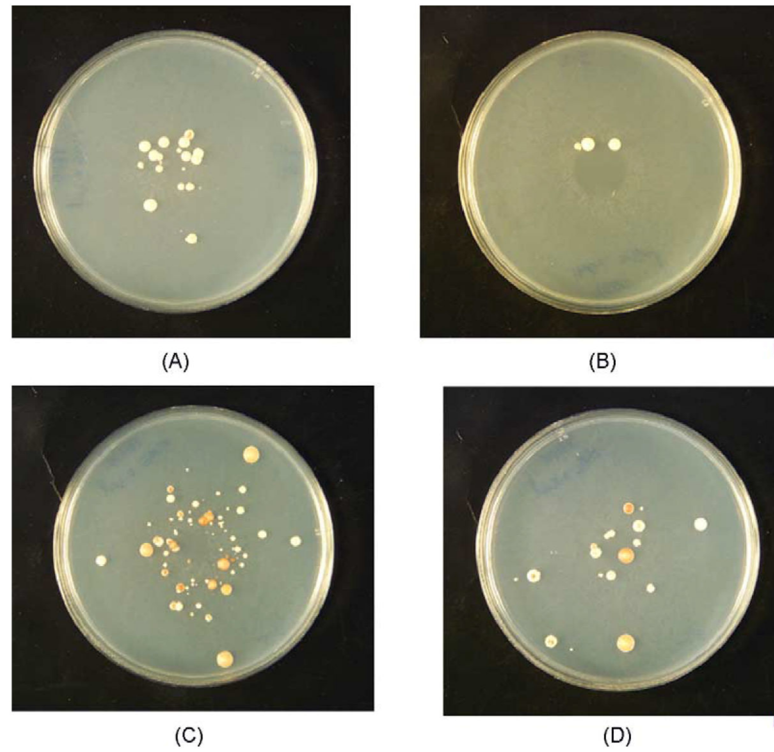

Fig. 2.

Plate assay demonstrating higher levels of DNA damage-associated translocations in a *rad9* diploid compared to a wild-type diploid. (A) YB110 (wild-typediploid) + 0.2 μl of MMS. (B) YB110 (wild-typediploid) + 1 μl of H_2O_2. (C) YB134 (*rad9*mutant) + 0.2 μl of MMS. (D) YB134 (*rad9*mutant) + μl of H_2O_2.

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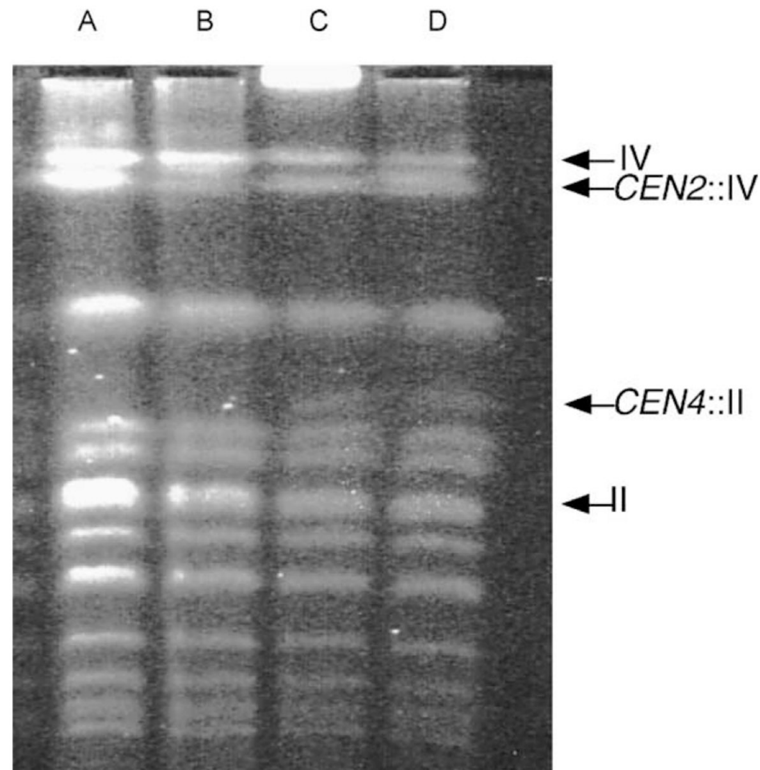

Fig. 3.
Electrophoretic karyotype of camptothecin-associated His$^+$ recombinants generated in the *rad9* diploid YB134. (A) YB134 His$^-$-parent. (B) His+ recombinant containing a non-reciprocal translocations. (C) His$^+$ recombinant containing a reciprocal translocation. (D) His$^+$ recombinant containing a reciprocal translocation. The positions of chromosomes II, IV, and translocations *CEN2* :: IV and *CEN4* :: V are indicated by the arrow.

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Table 1

Yeast strains

Laboratory name	Genotype[a]	Source
YA102	*MAT***a***ura3-52, his3– 200, ade2-101, 1ys2-801, trp1– 1, gal3*⁻	M. Carlson
YB109	*MATα leu2-3,112,GAL1 :: his3- 5′,trp1 :: his3– 3' :: HOcs, lys2*⁻ (leaky)	This laboratory
YB130	*MAT***a***rad9 :: URA3*	This laboratory
YB132	*MATα leu2-3,112, GAL1 :: his3- 5′,trp1 :: his3- 3' :: HOcs, lys2*⁻*(leaky), rad9 :: URA3*	This laboratory
YB110	YB109 × YA102	This laboratory
YB134	YB130 × YB132	This laboratory
YB163	*MAT***a-inc***trp1 :: [his3- 3′ :: HOcs, his3- 5′]*	This laboratory
YB146	*MAT***a***trp1 :: [his3- 3′ :: HOcs, his3- 5′]*	This laboratory
YB147	*MAT***a***trp1 :: [his3- 3′ :: HOcs, his3- 5′] rad9 :: URA3*	This laboratory

[a]Genotype the same as YA102 unless indicated.

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Table 2

Stimulation of translocations by DNA damaging agents in *RAD9* (YB110) and the *rad9* :: *URA3* diploid (YB134)

Agent (concentration or dose)	Stimulation in *RAD9* (YB110)[a]		Stimulation in *rad9*$^-$ (YB134)[a]		Ratio[c]
	His$^+$/CFU × 10^7 (survival%)	Net[b]	His$^+$/CFU × 10^7 (survival%)	Net[b]	
Spontaneous	6 ± 2	0	23 ± 14	0	NA
Alkylating agents[d]					
MMS 0.1% (10.5 (mM))	62 ± 16(94)	59 ± 13	248 ± 24(81)	246 ± 21	4.2
MNNG 50 (μM)	100 ± 9 (88)	92 ± 1	173 ± 46 (90)	153 ± 38	1.7
MNNG 100 (μM)	250 ± 10 (85)	248 ± 11	347 ± 40 (76)	326 ± 37	1.3
MNNG 200 (μM)	345 ± 38 (53)	338 ± 21	306 ± 62 (16)	253 ± 43	0.7
Bulky adducts					
4-NQO 1 (μM)	28 ± 9 (100)	27 ± 14	43 ± 6 (100)	33 ± 10	1.2
4-NQO 10 (μM)	494 ± 163(67)	484 ± 160	666 ± 91 (47)	636 ± 96	1.3
Cisplatin 1.3 (μM)	122 ± 33 (100)	120 ± 25	347 ± 71(87)	330 ± 70	2.8
Free radical agents					
Bleomycin 0.015 units	60 ± 52 (94)	54 ± 52	446 ± 130(86)	403 ± 136	7.5
Phleomycin 10 (μM)	67 ± 31(93)	59 ± 31	295 ± 117 (60)	286 ± 116	4.8
H_2O_2 2 (μM)	219 ± 92 (76)	212 ± 92	585 ± 259 (76)	558 ± 260	2.6
H_2O_2 4 (μM)	346 ± 138(71)	329 ± 192	533 ± 167 (54)	523 ± 165	1.6
TopoI inhibitor					
Camptothecin 50 (μg/ml)	5 ± 5 (95)	<1	38 ± 14 (99)	26 ± 14	5.2
Camptothecin 100 (μg/ml)	34 ± 7 (95)	33 ± 7	163 ± 50 (85)	153 ± 54	4.6

[a] For complete genotype, see Table 1.

[b] Net stimulation = (His$^+$ frequency w/agent – His$^+$ spontaneous frequency) × 10^7; $N > 3$.

[c] (Net His$^+$ per 10^7 CFU for *rad9*$^-$)/(Net His$^+$ per 10^7 CFU for *RAD9*$^+$); NA, not applicable.

[d] DNA damage-associated frequencies for MMS were previously published, see [12].

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Table 3

Stimulation of SCE by H_2O_2, camptothecin, and phleomycin in *RAD9* and *rad9* :: *URA3* strains

Chemical agent	Stimulation in *RAD9* (YB163)[a]		Stimulation in *rad9* :: *URA3* (YB147)	
	His$^+$/CFU × 10^6 (survival %)	Fold increase[b]	His$^+$/CFU × 10^6 (survival %)	Fold increase[b]
Spontaneous	16 ± 2 (100)	1	15 ± 2 (100)	1
0.04% H_2O_2	59 ± 12 (40)	3.7	60 ± 20 (35)	4
50 μg/ml camptothecin	32 ± 7 (88)	2	20 ± 3 (91)	1.3
10 μM phleomycin	33 ± 6 (41)	2.1	17 ± 3 (45)	1.1

[a] For complete genotype, see Table 1.

[b] His$^+$ frequency with agent/spontaneous His$^+$ frequency.