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Stimulation of Sister Chromatid Exchanges and Mutation by Aflatoxin B_1-DNA Adducts in *Saccharomyces cerevisiae* Requires *MEC1* (ATR), *RAD53*, and *DUN1*

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Abstract

The hepatocarcinogen aflatoxin B_1 (AFB_1) is a potent recombinagen but weak mutagen in the yeast Saccharomyces cerevisiae. AFB_1 exposure induces DNA damage-inducible genes, such as *RAD51* and those encoding ribonucleotide reductase (RNR), through a *MEC1* (ATR homolog)-dependent pathway. Previous studies have indicated that *MEC1* is required for both AFB_1-associated recombination and mutation, and suggested that AFB_1-DNA adducts are common substrates for recombination and mutagenesis. However, little is known about the downstream effectors of *MEC1* required for genotoxic events associated with AFB_1 exposure. Here we show that AFB_1 exposure increases frequencies of *RAD51*-dependent unequal sister chromatid exchange (SCE) and activates Rad53 (CHK2). We found that *MEC1*, *RAD53*, and *DUN1* are required for both AFB_1-associated mutation and SCE. Deletion of *SML1*, which encodes an inhibitor of *RNR*, did not suppress the *DUN1*-dependent requirement for AFB_1-associated genetic events, indicating that higher dNTP levels could not suppress the dun1 phenotype. We identified AFB_1-DNA adducts and show that approximately the same number of adducts are obtained in both wild type and *rad53* mutants. Since *DUN1* is not required for UV-associated mutation and recombination, these studies define a distinct role for *DUN1* in AFB_1-associated mutagenesis and recombination. We speculate that AFB_1-associated DNA adducts stall DNA replication, a consequence of which can either be mutation or recombination.

Keywords

aflatoxin B_1; DNA adducts; mutation recombination; cell-cycle checkpoint; *Saccharomyces cerevisiae*

INTRODUCTION

The incidence of hepatocellular carcinoma (HCC) strongly correlates with exposure to aflatoxin B_1 (AFB_1) and to hepatitis B and C virus [1]. The correlation of the p53 Ser249 mutation in HCC associated with AFB_1 exposure is consistent with the idea that AFB_1 is a

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strong liver carcinogen because it is a mutagen [2-4]. A current hypothesis is that recurrent regeneration after injury renders liver cells more susceptible to carcinogen-associated DNA damage [5]. Thus, active DNA replication may enhance AFB_1-associated genotoxic effects.

AFB_1-associated mutagenesis is correlated with the number of AFB_1-associated DNA adducts. The highly unstable 8,9-dihydro-8-(N^7-guanyl)-9-hydroxyaflatoxin B1 (AFB^1-N^7-Gua) adduct converts to both a formamidopyrimidine (FAPY) and an apurinic site [6]. The FAPY structures are highly stable, stall replication, and are poor substrates for the *Escherichia coli* polV translesion polymerase [6]. However, introduction of oligonucleotides containing AFB_1-associated FAPY structures in *E. coli* generates mutations, similar to those observed in liver carcinomas, at or near the sties of the AFB_1-DNA adducts [6]. These studies suggest that AFB_1-associated FAPY structures are indeed mutagenic adducts.

Considering that DNA repair mechanisms are similar in yeast *Saccharomyces cerevisiae* and higher eukaryotes, yeast is a useful organism to study how genetic factors can increase AFB_1-associated genotoxic effects, including mutations and homologous recombination events, such as gene conversion events, and translocations. Observations that *rad51* mutants, defective in recombinational repair, exhibit enhanced AFB_1-associated mutagenesis implies that a common DNA adduct stimulates both recombination and mutagenesis [7]. These observations suggest that DNA damage tolerance pathways are important in triggering AFB_1-associated recombination and mutation; however, how recombinational or mutagenic pathways are selected is unclear.

These AFB_1-associated genotoxic responses occur in the context of a global genome response to AFB_1-associated DNA damage. The global transcription response to AFB_1 includes an induction of recombinational repair, mismatch repair, and nucleotide excision repair genes in nongrowing cells [8], while genes involved in nucleotide metabolism and recombination, such as *RNR* and *RAD51*, are induced in growing cells [9]. Cells exposed to AFB_1 exhibit an extended S phase and a fivefold decline in histone transcripts, suggesting that AFB_1-associated DNA adducts trigger a replication block and a checkpoint response [9]. This is supported by the observation that *MEC1* (ataxia telangiectasia and Rad3 related, ATR) is required for both AFB_1-associated recombination [8] and mutation [9].

Genes that are involved in the S phase checkpoint include *MEC1* (ATR), *RAD53*, and *DUN1*. *RAD53* is required for replication delay [10]. *DUN1* induces *RNR* (ribonucleotide reductase) transcription by inactivating the Crt1 repressor [11] and activates Rnr enzymatic activities by phosphorylating and thereby inactivating the Sml1 inhibitor [12]. While *RAD53* is required for DNA damage-associated homologous recombination [13], *DUN1* is neither required for UV or X-ray associated homologous recombination [14].

We determined whether *MEC1*, *RAD53*, and *DUN1* were involved in either AFB_1-associated mutation or sister chromatid exchange (SCE). Based on the identification of AFB_1-associated DNA adducts in yeast, we speculate that these DNA adducts initiate the checkpoint activation. We observed that *MEC1*, *RAD53*, and *DUN1* were required for both AFB_1-associated mutation and SCE. However, we observed that *DUN1* was not required for UV-associated SCE or mutation. We therefore suggest that distinct genetic requirements for AFB_1-associated and UV-associated genotoxic effects.

MATERIALS AND METHODS

Strains and Media

The genotypes of yeast strains used in this study are listed in Table 1. Strains for measuring SCE and mutation are derived from the canavanine sensitive strain YB163, which contains

his3 recombination substrates in tandem at *TRP1* [15]. Mutants containing *sml1*:: *KanMX mec1*-Δ::*TRP1, sml1*:: *KanMX, rad53*-Δ::*LEU2,* and *dun1*::*KanMX* were generated by one-step gene disruption [16] using PCR amplified gene fragments and selecting for Kan^R or the appropriate auxotrophic selection. The primers used for amplifying these gene fragments are listed in the "Yeast Deletion Database" (http://www-deletion.-stanford.edu). The *dun1*::*kanMX sml1*::*URA3* double mutant was made by genetic cross. Ura^- derivatives of the *rad51* mutant YB205 [17] and *dun1*::*kanMX sml1*::*URA3* strains were made by selecting for 5-fluorouracil (FOA) resistance. Plasmid pCS316 [18], containing cytochrome P450 1A2 (CYP1A2) and hOR, was introduced into wild type and checkpoint mutants by selecting for Ura^+. pJW730, containing *DUN1,* was introduced into *dun1* mutants by selecting for Trp^+ transformants.

Standard media were used for the culture of yeast cells. YP (yeast extract, peptone), YPD (YP, dextrose), SC (synthetic complete, dextrose), SC-LEU (SC lacking leucine), SC-TRP (SC lacking tryptophan), SC-URA (SC lacking uracil), and FOA media are described by Burke et al. [19]. Media to select for canavanine resistance contain SC-ARG (synthetic complete lacking arginine) and 60 μg/mL canavanine (CAN) sulfate.

Measuring DNA Damage-Associated Recombination and Mutation Events

To measure AFB_1-associated genotoxic events, log phase yeast cells (A_{600}=0.5-1) were exposed to indicated doses of AFB_1, previously dissolved in DMSO. Cells were maintained in nutrient media (SC-URA) during the carcinogen exposure. To measure UV-associated events, log phase cells (A_{600}=0.5-1) were washed and resuspended in sterile H_2O and then exposed to indicated doses of UV (260 nM, $2J/m^2/s$). After the exposure, cells were washed twice in H_2O, and then plated on SC-HIS or SC-ARG CAN to measure SCE or mutation frequency, respectively. An appropriate dilution was inoculated on YPD to measure viability.

Detection and Quantification of DNA Adducts

To measure the AFB_1-associated DNA adducts in yeast, we used liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI/MS/MS) [20]. Log phase cultures of yeast expressing human CYP1A2 (pCS316) were exposed to 50 μM AFB_1 for 4 h. Because standard protocols for isolating yeast DNA involve alkaline buffers, rendering the highly unstable AFB_1 N^7-Gua DNA adducts labile, we have modified the "smash-and-grab" protocol [21] so that we are using a neutral buffer containing 10 mM Tris-HCl, 1 mM EDTA, 100 mM NaCl, 2% Triton X-100, 1% SDS, pH 7. DNA was then isolated from two independent samples of yeast cells. The DNA adducts were identified and measured by high performance liquid chromatography and tandem mass spectroscopy (LC-ESI/MS/MS) after acid hydrolysis [20].

Determining Rad53 (Chk2) Activation

Activation of Rad53 was determined by Western blots. Cells were inoculated in SC-URA medium. Log phase cells (A_{600}=0.5-1) were concentrated threefold in SC-URA and exposed to 50 μM AFB_1 for 4 h. After washing cells twice in H_2O, aliquots were plated directly on SC-HIS to measure recombination, appropriately diluted and plated on YPD to measure viability. Protein extracts were prepared as previously described by Foiani et al. [22], separated on 10% acrylamide/0.266% bis-acrylamide gels for Rad53 detection, and transferred to nitrocellulose membranes. Rad53 was detected by Western blotting using goat anti-Rad53 (yC-19, Santa Cruz, Biotechnology, Santa Cruz, CA). The secondary antibody used was anti-goat IgG-HRP (Santa Cruz).

RESULTS

AFB_1-Associated SCE Requires *RAD51*

AFB_1 efficiently stimulates homologous recombination in diploid yeast expressing human CYP1A2 and CYP1A1 [8]. Since, compared to wild type, AFB_1-associated mutation frequencies increase in *rad51* haploid mutants [7], which are defective in homologous recombination, we investigated whether there was a common genetic control for AFB_1-associated SCE and mutagenesis in yeast.

We measured mutation and unequal SCE in yeast strains (Table 1) expressing human CYP1A2 by selecting for Can^R or His^+, respectively (Figure 1). The spontaneous frequency of SCE in wild type and the *rad51* mutant was 2×10^{-5}, consistent with previous results [23], while the spontaneous mutation frequency was 10-fold higher ($5\times10^{-5}/5\times10^{-6}$) in the *rad51* mutant, compared to wild type. Previous experiments indicated 100-fold and 20-fold increase in translocation and gene conversion frequencies, respectively, in wild-type log-phase cells that were maintained in phosphate buffer while exposed to AFB_1 [18]. We detected no AFB_1-associated SCE in wild-type log-phase cells that were maintained in phosphate buffer while exposed to AFB_1 (data not shown). We exposed log phase wild-type and *rad51* cells to AFB_1 for 4 h in growth (SC-URA) medium, as performed by Guo et al. [7]. While we detected a significant threefold and eightfold stimulation of SCE in wild-type cells after exposure to 15 and 50 μM AFB_1, respectively, we detected no significant stimulation of SCE in *rad51* cells. However, we did detect a higher net frequency of Can^R mutants after exposure to 50 μM AFB_1 (35×10^{-6}, n=4) in *rad51* cells, compared to wild type (17×10^{-6}, n=4), consistent with a previous study [7]. Thus, AFB_1 exposure stimulates *RAD51*-dependent SCE recombination in haploid cells that are maintained in growth medium at concentrations previously observed to stimulate both gene conversion events and directed translocations in diploid cells.

MEC1-Dependent Rad53 Activation Correlates With AFB_1-Associated SCE

Considering that *RNR2* and *DUN1* expression is induced in actively growing yeast cells exposed to AFB_1 [9], we next determined whether the *RAD53*-dependent signaling pathway that activates Dun1 was *MEC1*-dependent by following Rad53 phosphorylation after AFB_1 exposure (Figure 2). To obtain sufficient protein and minimize AFB_1 amounts, concentrated log phase cells (~5×10^7 cells/mL) were exposed to AFB_1. Similar to previous experiments, we detected greater than fourfold increase in recombination in wild-type cells. By western blots, we measured Rad53 (Chk2) phosphorylation in *mec1*, *dun1*, and wild-type cells after 4 h of AFB_1 exposure. Rad53 activation occurred in the *dun1* mutant, but was reduced or abolished in the *mec1-21* (missense) or *mec1*-Δ (null) mutants, respectively, confirming the requirement for the *MEC1* pathway as a response to DNA damaging agents [24]. These studies thus indicate that AFB_1 exposure coincidentally stimulates recombination and checkpoint activation.

AFB_1-Associated SCE and Mutation Require *MEC1*, *RAD53*, and *DUN1*

We then determined whether *MEC1*, *RAD53*, and *DUN1* gene functions were required for both AFB_1-associated SCE and mutation. We found no increase in AFB_1-associated SCE in *mec1*, *rad53*, and *dun1* mutants (Figure 3, left). The *DUN1* requirement was surprising, considering that UV-associated SCE is *DUN1*-independent [14]. Interestingly, while *rad53* and *mec1* mutants exhibit similar viability after AFB_1 exposure, we observed that *dun1* mutants were the most sensitive to AFB_1 (Figure 3, right). These studies indicate that AFB_1-associated SCE requires *RAD53*, *DUN1*, and *MEC1*.

We then determined whether, similar to SCE recombination, AFB_1-associated mutation also required *MEC1*, *RAD53*, and *DUN1* by measuring Can^R mutants. The spontaneous frequency of Can^R in wild type was 5×10^{-6}, similar to Guo et al. [7]. After exposure to 100 μM AFB_1,

we observed approximately sixfold increase in mutation frequency in wild type. However, in *dun1*, *mec1*, and *rad53* mutants, we observed at most a twofold increase in the frequencies of Can^R mutants (Figure 3, center). We observed a 30-fold increase in the frequencies of UV-associated Can^R in wild type and the *dun1* mutant after cells were exposed to 120 J/m^2 (data not shown, *n*=3). Thus, similar to SCE, we observed that AFB_1-associated mutation requires *MEC1*, *RAD53*, and *DUN1*.

To determine that *dun1* phenotypes are recessive we introduced the *DUN1*-containing plasmid (pJW730) in the *dun1* strain by selecting for Trp^+ transformants. We then measured unequal SCE, mutation, and survival after the Trp^+ transformants were exposed to 100 μM AFB_1. Compared to spontaneous frequencies, we observed fourfold ($22\times10^{-6}/5\times10^{-6}$, *n*=3) and fivefold ($9\times10^{-5}/1.9\times10^{-5}$, *n*=3) increase in mutation and recombination frequencies, respectively. Compared to the *dun1* mutant, we observed an approximately twofold increase in percent survival (51%/28%). These data indicate that extrachromosomal expression of *DUN1* suppresses *dun1* phenotypes.

DUN1 has multiple functions including induction of RNR, regulation of the G2 checkpoint, and stabilization of stalled replication forks [25]. Since the *dun1* DNA damage sensitivity can be suppressed by mutation in the negative regulator of RNR, *SML1* [12], we determined whether the *dun1* mutation and recombination phenotypes could also be suppressed in a *dun1 sml1* mutant. We introduced CYP1A2 (pCS316) into a *dun1 sml1* strain and then measured survival, mutation, and recombination frequencies after exposure to 100 μMAFB_1. The *dun1 sml1* mutant was as resistant (76% survival, *n*=5) as wild type. Compared to the spontaneous frequencies, we observed less than a twofold increase in the AFB_1-associated frequencies of recombination [$(8.3\pm5.4)/(6.5\pm5.4)\times10^{-5}$, *n*=4] and mutation [$[(13.7\pm4.5)/(8.7\pm4.2)\times10^{-6}$], *n*=5], respectively. We conclude that increasing deoxynucleotide triphosphate (dNTP) levels per se is insufficient to suppress the *dun1* defect in stimulating AFB_1-associated recombination and mutation. However, considering that *sml1* mutations do suppress AFB_1-associated lethality in *dun1*, higher dNTP levels increase survival after AFB_1 exposure.

AFB_1-N^7-Gua and AFB_1-FAPY DNA Adducts Can be Detected and Measured

We speculate that the AFB_1-DNA adducts, such as the N^7-Gua and FAPY-AFB_1 adducts, stall DNA replication. We therefore set out to detect AFB_1-DNA adducts in both wild type and checkpoint mutants [20]. Log phase cultures of yeast expressing human CYP1A2 were exposed to 50 μM AFB_1 for 4 h. DNA was then isolated from two independent samples of yeast cells, and subjected to acid hydrolysis. Both the AFB_1-N^7-Gua and AFB_1-FAPY adducts were identified and measured by LC-ESI/MS/MS (Figure 4). The data indicate that the level of AFB_1 adducts are essentially the same for both the wild type and the *rad53* mutant (Table 2). We deduced the number of adducts to be ~1-2 for every 1000 kb. Thus, for each yeast genome equivalent, ~15-30 AFB_1 adducts are generated under these conditions.

DISCUSSION

AFB_1 is a strong recombinagen but weak mutagen in yeast [8,18]. Higher AFB_1-associated mutation frequencies in a haploid *rad51* mutant, compared to wild type [7], suggest that a common DNA lesion could stimulate either SCE or mutation. AFB_1 exposure stimulates SCE in lymphocytes and rat hepatoma lines (for review, see [26]); however, no similar studies have been done in yeast. Since AFB_1-associated mutation requires *MEC1* [7] and *RNR* induction results from AFB_1 exposure [6], we determined whether AFB_1-associated genotoxicity requires the *MEC1* (ATR), *RAD53*, *DUN1*-mediated pathway involved in increasing dNTP levels after DNA damage exposure. The major conclusion of this article is that both recombination and mutation require *MEC1*, *RAD53*, and *DUN1*; however, higher dNTP levels per se do not circumvent the *DUN1* requirement, indicating that other checkpoint pathway

functions, such as stabilizing replication forks, may be required for AFB_1-associated genotoxic effects. We speculate that AFB_1-associated mutation and SCE occur when the DNA replication apparatus encounters AFB_1-DNA adducts.

The studies thus extended previous work indicating that *MEC1* and *RAD53* are required for mutagenesis [7] by showing that SCE and mutagenesis are jointly regulated. We observed that AFB_1-associated Rad53 activation correlated with higher frequencies of SCE, and detected AFB_1-N^7-Gua and FAPY DNA adducts that have been previously suggested to impede DNA replication [6]. Indeed although AFB_1-N^7-guanine adducts are unstable, they were more abundant than FAPY DNA adducts after 4 h AFB_1 exposure, indicating that the former is present in growing cells. The protracted presence of these adducts, which are poor templates for DNA polymerases [6] may explain why cells exposed to AFB_1 exhibit elongated S phases [9]. The AFB_1-associated Rad53 activation could also result from stalled replication forks [27]. We do not know, however, whether recombination precedes mutation, as suggested by observations that Rev1 (error-prone polymerase) accumulates during late S and G2 phases of the cell-cycle [28,29]. Further studies are necessary to determine whether defects in translesion synthesis lead to more AFB_1-associated SCE events.

The *DUN1*-mediated checkpoint pathway for both AFB_1 mutation and recombination is not required for UV-associated mutation and SCE [14], but is required for mutagenesis in DNA replication mutants [30]. One possibility is that translesion polymerases bypass UV lesions, such as thymidine dimers [31], more easily compared to AFB_1-associated DNA lesions. We speculate that the *DUN1* requirement for AFB_1-associated events result from *DUN1* function in stabilizing DNA replication forks at replication blocks [25]. *DUN1* may be involved in repressing translation of *RAD5* RNA transcripts, and *RAD5* overexpression sensitizes cells to replication blocks [32]. *RAD5* is also required for template switching events, indicating a possible function at stalled replication forks [33]. Further experiments are necessary to determine whether AFB_1 specific adducts stall DNA polymerase.

The genetic requirements of AFB_1-associated SCE are distinct from those for X-ray associated SCE, which is *DUN1*-independent [14], suggesting that the initiating lesion is not a double-strand break. Standard protocols to measure X-ray associated SCE use log-phase cells incubated in H_2O (nutrient-depleted media), not nutrient medium, during the radiation exposure. These observations strengthen the idea that AFB_1-associated SCE occurs as a consequence of the DNA replication fork encountering the AFB_1-associated DNA lesion.

Although other types of AFB_1-associated genotoxic events, such as translocations, require *MEC1* [8], we do not know whether the AFB_1-DNA adducts that initiate SCE are the same as those that initiate translocations and gene conversion events [8,18]. Fifteen DNA repair genes were induced in cells exposed to AFB_1 in phosphate buffer [8], while only *RAD51* and *RAD54* were induced in cells exposed in nutrient media [9]. It will be important to determine whether different genotoxic events following AFB_1 exposure are the consequence of the induction of different DNA repair genes or of different DNA adducts. Further experiments are also necessary to determine whether other AFB_1-associated recombination events require *RAD53* and *DUN1*.

In conclusion, we have found that both AFB_1-associated recombination and mutation require *MEC1, RAD53,* and *DUN1* and coincide with Rad53 activation. Although AFB_1-DNA adducts have been measured in yeast [34], this is the first study to positively identify specific AFB_1-DNA adducts in yeast, and indicate that the unstable AFB_1-N^7-Gua can be detected after a 4 h exposure time. Future studies may reveal how individual adducts are repaired and contribute to AFB_1-associated genotoxic effects in yeast. Our results may have some interesting implications for AFB_1-associated genotoxicity in humans. For example, liver injury, resulting

in increased liver cell proliferation, correlates with a higher incidence of HCC. We speculate that the DNA replication in the presence of AFB_1-DNA adducts may correspond to a higher level of AFB_1-associated genotoxicity in mammalian cells.

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Abbreviations

HCC, hepatocellular carcinoma; AFB_1, aflatoxin B1; AFB_1-N^7-Gua, 8,9-dihydro-8-(N^7-guanyl)-9-hydroxyaflatoxin B_1; FAPY, formamidopyrimidine; RNR, ribonucleotide reductase; ATR, ataxia telangiectasia and Rad3 related; SCE, sister chromatid exchange; CYP1A2, cytochrome P450 1A2; LC-ESI/MS/MS, liquid chromatography-electrospray ionization tandem mass spectrometry; dNTP, deoxynucleotide triphosphate.

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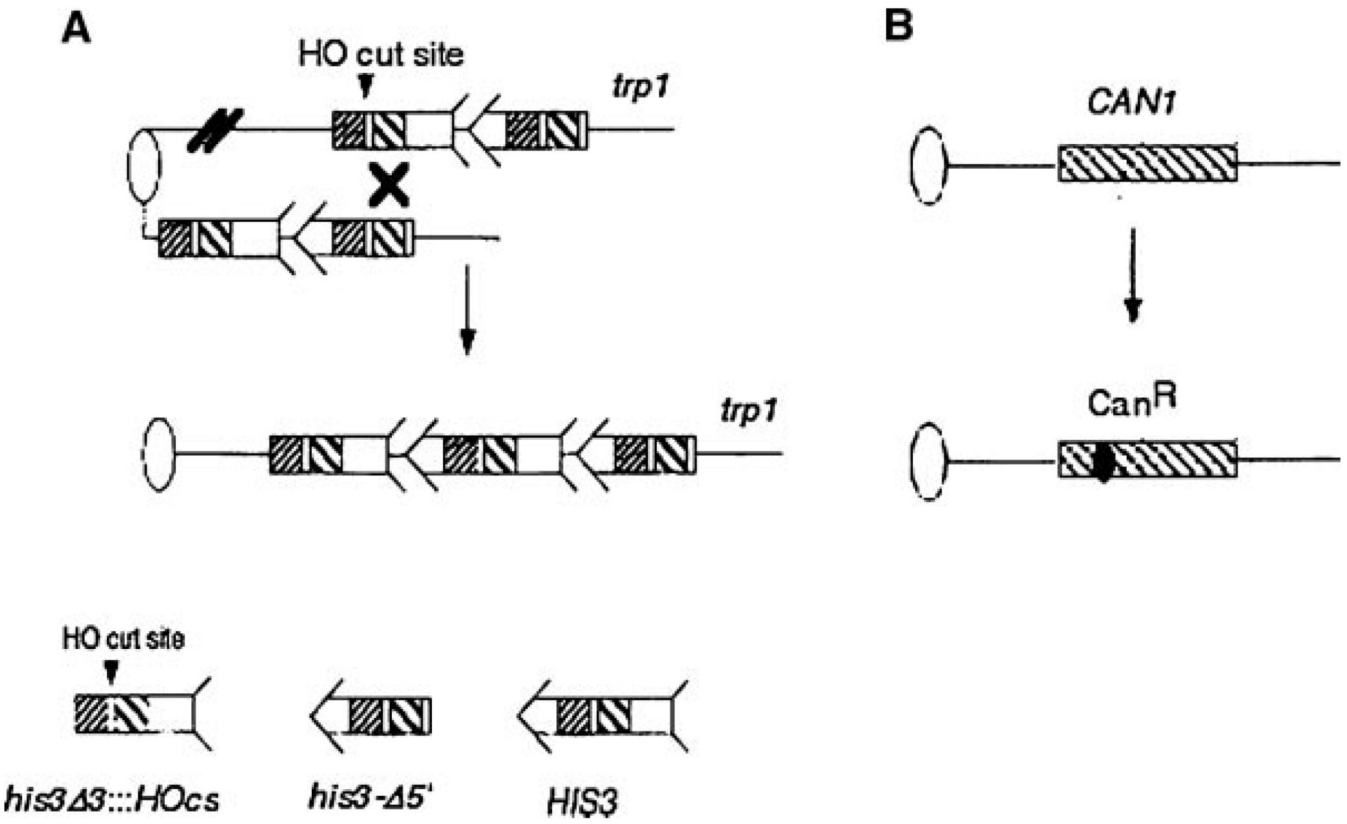

Figure 1.

Recombination and mutation assay used in this study. The oval represents the centromere and the single line represents duplex DNA. For simplicity, the left arm of chromosome IV is not shown. (A) Unequal sister chromatid recombination is monitored by selecting for His^+ prototrophs that result from recombination between the juxtaposed, truncated *his3* fragments. The *his3-Δ3′* lacks the 3' sequences (arrow head), while the *his3-Δ5′* lacks to promoter sequences (feathers). Both *his3* fragments are located with the amino acid reading frames oriented to the centromere. The *his3* fragments share a total of 450 bp sequence homology. (B) The mutation assay is a forward mutation assay by selecting for canavanine resistance.

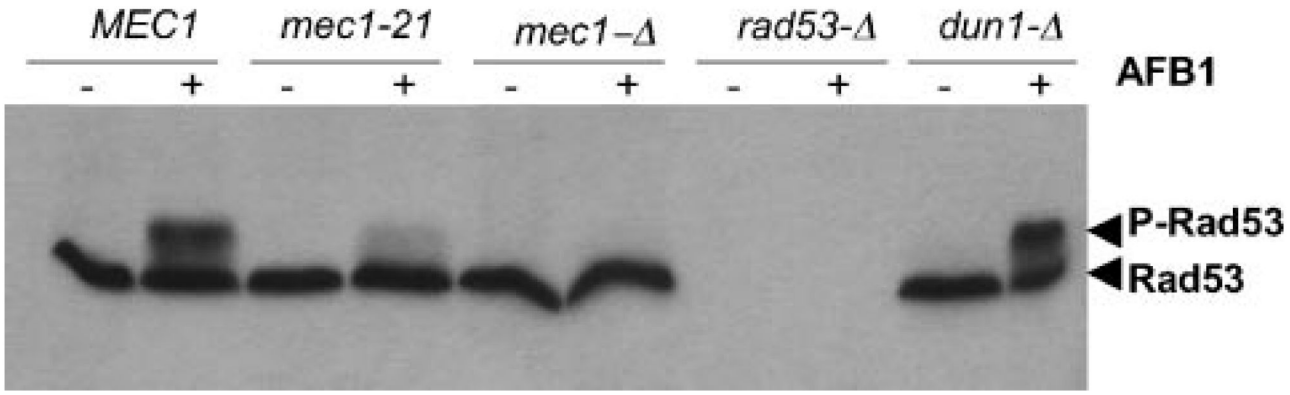

Figure 2.

AFB_1-associated Rad53 activation occurs in wild-type and *dun1* cells but not in *mec1* null mutants. Cells were exposed to 50 μM AFB_1 for 4 h and Rad53 activation was measured in the wild type (YB163), *dun1* (YB387), *mec1-21* (YB385), and *mec1* null (YB386) mutants. The arrow points to phosphorylated Rad53.

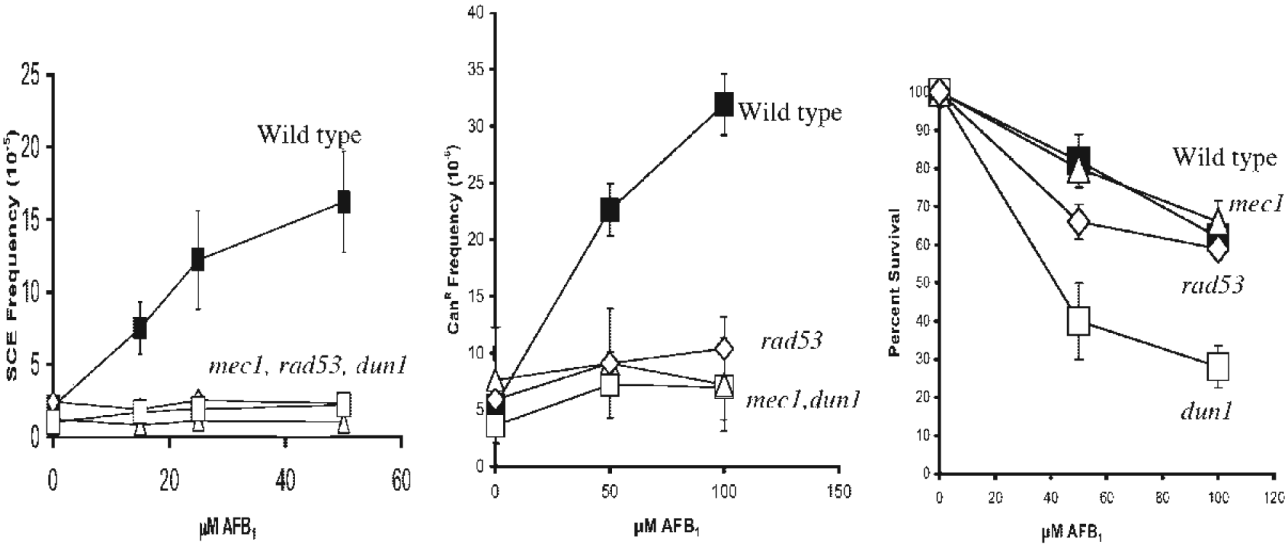

Figure 3.

Genetic requirements for AFB_1-associated mutagenesis and recombination. Mutation frequency is plotted against AFB_1 concentration. The total exposure time is 4 h. Survival is plotted against AFB_1 concentration. The solid black square is wild type (YB163), the open black square is *dun1*(YB387), the open triangle is *mec1*(YB86), and the open diamond is *rad53* (YB353). Genotypes are given next to the symbols.

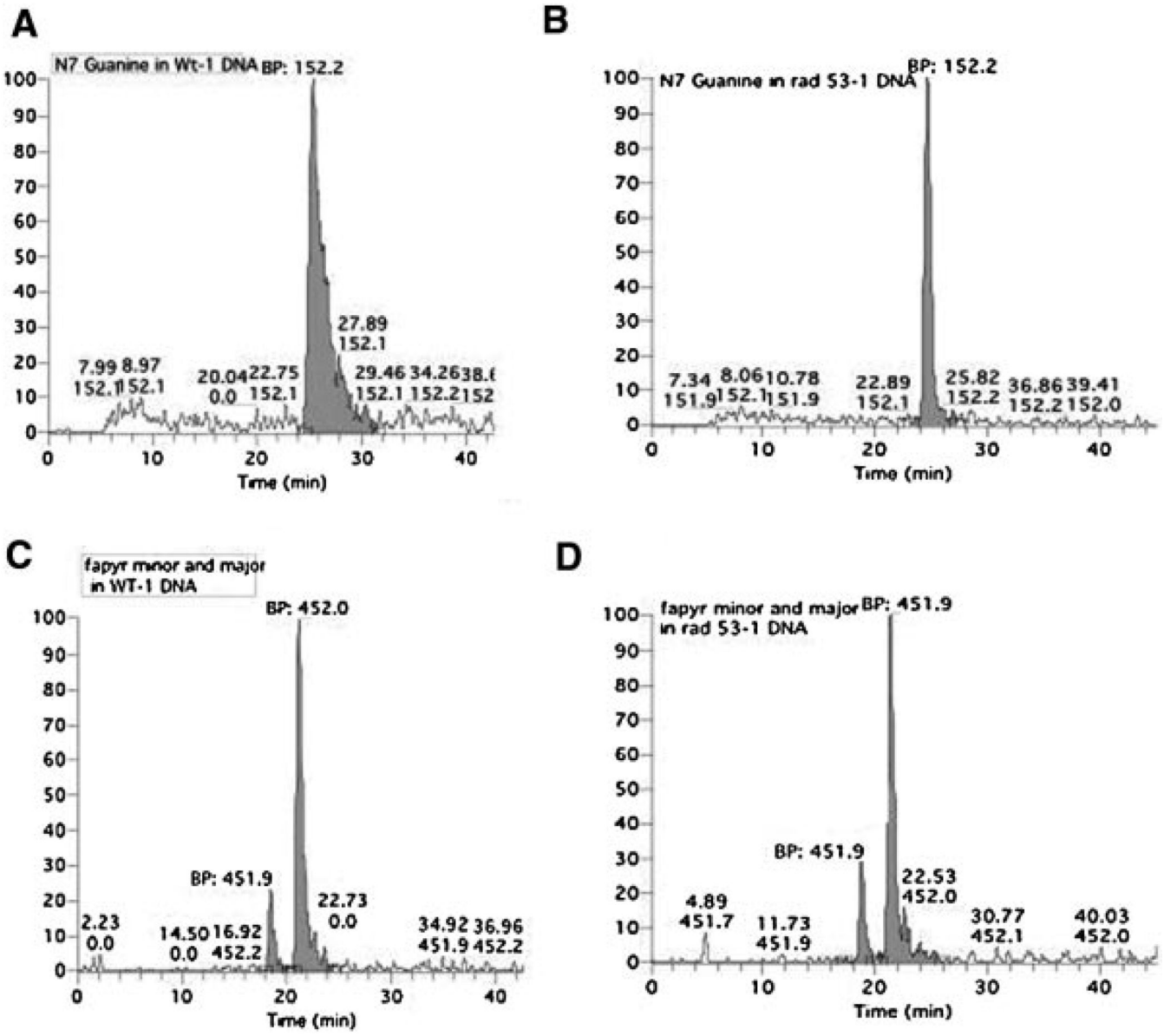

Figure 4.

N^7-Gua AFB_1 and FAPY AFB_1 adducts present in wild type (YB163) and *rad53* (YB230) strains. AFB_1 adducts were identified by LC-ESI/MS/MS. Relative abundance is plotted against time of detection; adduct quantities were computed from shaded areas under base peaks. DNA from both wild type (A,C) and *rad53* (B,D) strains were obtained after cells were exposed to 50 μM AFB_1 for 4 h. Base peak (BP) m/z 152.2 (A,B) corresponds with AFB_1, while m/z 452 (C,D) corresponds with major and minor FAPY-AFB_1 adducts.

Table 1

Yeast Strains*

Strain	Genotype	Plasmid	Source
YB353	MATα ura3-52 his3-Δ200 ade2-n trp1-Δ 1 gal3$^-$ leu2-3, 112 GAL1::his3-Δ 5′ trp1::his3-Δ 3′::HOcs Iys2$^-$ (leaky) sml1::KanMX rad53::LEU2	pCS316	This laboratory
YB163	*MAT***a**-inc ura3-52his3-Δ200 ade2-101 lys-801 trp1-Δ1 gal3- trp1 ::[his3-Δ3′::HOcs, his3-Δ5′]		This laboratory
YB384	*MAT***a** *rad51 leu2-Δ1*	pCS316	This laboratory
YB368	*MAT***a**-inc *mec1-21 leu2*		This laboratory
YB385	*MAT***a**-inc *mec1-21 leu2*	pCS316	pCS316 introduced in YB368
YB327	*MAT***a**-inc *mec1-Δ::TRP1 sml1::KanMX*		This laboratory
YB386	*MAT***a**-inc *mec1-Δ::TRP1 sml1::KanMX*	pCS316	pCS316 introduced in YB327
YB230	*MAT***a**-inc *leu2-Δ1 mec2-1(rad53)::LEU2*		This laboratory
YB387	*MAT***a**-inc *dun1::KanMX*	pCS316	
YB388	*MAT***a**-inc *dun1::KanMX*	pCS316 + pJW730	pJW730 i n YB387
YB389	*MAT***a**-inc *dun 1::KanMX sml1::URA3*		
YB390	*MAT***a**-inc *dun 1::KanMX sml1::ura3*	pCS316	pCS316 introduced in a FOA^R derivative of YB389

* Strains listed below YB163 have the same genotype as YB163 unless indicated.

Table 2

Concentrations of AFB_1-DNA Adducts in Yeast DNA

Yeast genotype (strain)[a]	AFB_1-N^7-Gua adducts/ mg DNA1[b] (nmol)	FAPY adducts/mg DNA[c] (nmol)	Ratio[d]
Wild type (YB163)	4×10^{-3}	1.1×10^{-3}	4:1
rad53 (YB320)	4.5×10^{-3}	2.3×10^{-3}	2:1

[a] Relevant genotype respect to *RAD53*, both strains derived from S288c. See Table 1 for complete genotype.

[b] AFB1-N^7 Gua, 8,9-dihydro-8-(N^7-guanyl)-9-hydroxyaflatoxin B1; $n = 2$.

[c] FAPY, formamidopyrimidine; FAPY determined for one sample of each genotype.

[d] Ratio = major AFB1-N^7-Gua adduct: FAPY adduct.