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Deletion of *MAG1* and *MRE11* enhances the sensitivity of the *Saccharomyces cerevisiae HUG1*P-GFP promoter-reporter construct to genotoxicity

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ABSTRACT

Eukaryotic yeast-based DNA damage cellular sensors offer many advantages to traditional prokaryotic-based mutagenicity assays. The *HUG1*P-GFP promoter-reporter construct has proven to be an effective method to selectively screen for multiple types of DNA damage. To enhance the sensitivity and selectivity of the system to different types of DNA damage, two genes involved in distinct DNA damage responses were deleted. Deletion of *MAG1*, a gene encoding a DNA glycosylase and member of the base excision repair (BER) pathway, increased the biosensor's sensitivity to the alkylating agents methyl methanesulfonate (MMS) (lowering the sensitivity threshold to 0.0001% (v/v)) and ethyl methanesulfonate (EMS). Deletion of *MRE11*, part of the highly conserved RMX complex that aids in sensing and repairing double strand breaks in budding yeasts, enhanced sensitivity to gamma radiation (γ-ray) (detection threshold of 50 Gy) and camptothecin. The *mre11*Δ phenotype dominated in *mag1*Δ *mre11*Δ strains. Through the deletions, we were able to engineer increased selectivity to alkylating agents, γ-ray, and camptothecin, since increased sensitivity to one type of damage did not alter the quantitative response to other genotoxins. The enhancements to the *HUG1*P-GFP system did not affect its ability to detect several other DNA damaging agents, including 1,2-dimethyl hydrazine (SDMH), phleomycin, and hydroxyurea (HU), or affect its lack of response to the potentially non-genotoxic carcinogen formaldehyde.

1. Introduction

Because of the high correlation between a compound's ability to damage DNA and its ability to induce cancer, determining the genotoxicity of environmental contaminants and newly synthesized chemicals is critical to protect human health. Traditional cell-based mutagenic assays, such as the Ames test (Ames et al., 1973), *umu*-test (Oda et al., 1985), and SOS-chromotest (Quillardet et al., 1982), rely on prokaryotic subjects, so their results may not always be directly applicable to higher eukaryotic organisms, including humans. Yeast-based genotoxicity assays have emerged as alternative mutagenic assays, often showing better results than traditional prokaryotic tests (Averbeck and Averbeck, 1994; Cole et al., 1987; Elledge and Davis, 1989; Ichikawa and Eki, 2006; Jia et al., 2002; Lichtenberg-Frate et al., 2003; Walmsley et al., 1997). These cell-based assays typically contain a DNA cassette encoding a readily detectable reporter gene under the control of a promoter sensitive to DNA damage. The cassette is either transformed into a yeast cell via a self-replicating plasmid or incorporated into the host genome at a targeted locus. Because yeasts are eukaryotic, the gene regulation and biochemical pathways involved in the DNA damage response are similar to, although simpler than, those in humans, potentially making the results of these yeast-based assays more relevant to human cancer susceptibility than those of the prokaryotic-based methods.

We recently reported the creation of *HUG1*P-GFP promoter-reporter construct, which proved to be effective in detecting multiple types of genotoxicity (Benton et al., 2007). While the exact biological function of *HUG1* remains a mystery, it is known to be involved in the Mec1p kinase pathway (Basrai et al., 1999), a crucial pathway for signal transduction after DNA damage in yeast (Gasch et al., 2001). *HUG1* is induced in response to multiple types of DNA damage (Basrai et al., 1999), and exhibits a dose dependent transcriptional response to DNA alkylation and to gamma radiation (γ-ray) (Benton et al., 2006). This induction pattern makes the *HUG1* promoter region an excellent choice for regulating reporter gene expression in response to DNA damage; however, optimal genotoxicity detection requires enhancing the strain's sensitivity and selectivity to specific types of DNA damage.

Due to the robustness of yeasts, the minimum detection threshold of the *HUG1*P-GFP biosensor strains to some mutagens is above concentrations that may be harmful to humans over the

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long term. The sensitivity of yeast-based biosensors to genotoxicity has been improved by using host strains with compromised cell wall integrity (Walsh et al., 2005), disabled efflux transporters (Lichtenberg-Frate et al., 2003), and defective DNA damage repair pathways (Jia and Xiao, 2003).

In this study, the gene encoding the DNA glycosylase Mag1p (Chen et al., 1989), a crucial member of the base excision repair pathway (BER), was deleted to enhance sensitivity of the *HUG1*P-GFP biosensor strain to DNA alkylating agents. Previous studies have shown *mag1*Δ strains to be more sensitive than wild type cells to killing by DNA alkylating agents such as methyl methanesulfonate (MMS), dimethyl sulfate (DMS), *N*-methyl-*N*-nitrosourea (MNU), and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) (Chen et al., 1989; Chen et al., 1990). Other genotoxins such as UV radiation, 4-nitroquinoline-1-oxide, and γ-ray produced similar death rates in wt and *mag1*Δ cells (Chen et al., 1989, 1990).

*mag1*Δ cells accumulate spontaneous mutations at a rate similar to that of wild type cells (Maclean et al., 2003; Xiao et al., 2001). Interestingly, when exposed to alkylating agents such as MMS, *mag1*Δ strains often exhibit lower mutation frequency than wt strains (Chen et al., 1990; Xiao et al., 2001). One possible explanation is that Mag1p activity increases mutagenicity by removing alkylated bases and creating AP sites, leading to base misincorporation by DNA polymerases (Chen et al., 1990; Xiao et al., 2001). *mag1*Δ strains treated with MMS accumulate methylated bases at a greater rate than wt strains treated with MMS. This presumably leads to increased replication fork stalling and cell cycle arrest, inducing the Mec1p pathway at lower alkylating agent doses than in wt cells. This induction should lead to increased transcription of *HUG1* and other members of the Mec1p pathway at lower doses of MMS. This hypothesis is consistent with the observation that *RNR3* is induced at lower doses in *mag1*Δ cells than wt cells (Jia and Xiao, 2003). Therefore, we tested the hypothesis that deleting *MAG1* from a *HUG1*P-GFP biosensor strain will enhance sensitivity to DNA alkylating agents such as MMS.

MRE11 was deleted in an attempt to enhance *HUG1*P-GFP biosensor sensitivity to double strand breaks (Ajimura et al., 1993). Mre11p is a member of the RMX complex (Rad50p, Mre11p, and Xrs2p), a highly conserved region that functions in sensing DNA damage and repairing double strand breaks by homologous recombination and non-homologous end joining. *mre11*Δ strains are extremely sensitive to ionizing radiation, but not to UV radiation (Bressan et al., 1998; Nisson and Lawrence, 1986). *mre11*Δ cells are deficient in double strand break repair and grow more slowly than wt cells. The lack of sensitivity to UV radiation and deficiency in double strand break repair suggest an *mre11*Δ mutant will specifically increase the sensitivity of the *HUG1*P-GFP construct to double strand breaks such as those caused by γ-ray.

In this work, we have engineered novel yeast strains with enhanced sensitivity to multiple types of DNA damage. Specifically, we have improved the detection threshold of the *HUG1*P-GFP biosensor construct to alkylation and γ-ray through selective deletion of genes involved in repair of these types of DNA damage. By carefully selecting target genes for deletion, we have created a novel genotoxin detection strain which outperforms our previous versions. Furthermore, the methods utilized in this study will be used to engineer further improvement in subsequent sensor organisms.

2. Materials and methods

2.1. Yeast strains

Table 1 lists the yeast strains used in this study. Supplemental Table 1 lists all primers used. SPY1102, SPY1103, and SPY1104 were created as previously described (Benton et al., 2007).

Table 1
Yeast strains used in this study

Name	Genotype	Source
SPY810	W303 *MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15*	Collection
SPY1101	SPY810 with *hug1*Δ*::GFP-HIS3*	Benton et al. (2007)
SPY1102	SPY1101 with *mag1*Δ*:: kan*^{r}	This study
SPY1103	SPY1101 with *mre11*Δ*:: kan*^{r}	This study
SPY823	W303 *MAT α ura3-1 his3::hisG*	Collection
SPY1104	SPY823 with *mre11*Δ*::kan*^{r}	This study
SPY1105	SPY1101 with *mag1*Δ*::kan*^{r} *mre11*Δ*::kan*^{r}	This study
SKY2164	*MAT a ura4*	Collection
SKY2165	*MAT α ura4*	Collection

SPY1105 was derived from a cross of SPY1102 and SPY1104. SPY1102 and SPY1104 were mated on GNA medium (5% glucose, 3% Difco nutrient broth, 1% yeast extract, 2% agar). Successfully mated cells were selected on synthetic complete (SC) medium lacking histidine and leucine, grown again on GNA medium, and then sporulated on NGS medium (1% potassium acetate, amino acids, 2% agar) for 7 days to induce them back to the haploid state. Individual haploid cells were isolated by random spore analysis. The resulting strains were screened on SC medium lacking histidine and on YPD medium (1% yeast extract, 2% bactopeptone, 2% dextrose) containing G418 antibiotic. Strains that passed both screenings were analyzed by PCR for *MAG1* and *MRE11* deletions as described above. A *LEU2* deletion was verified by observing no growth on SC medium lacking leucine. Mating type and ploidy were verified by a mating cross with SKY2164 (*MAT a ura4-52*) and SKY2165 (*MAT α ura4-52*) on SC medium lacking uracil.

2.2. DNA damage exposure

DNA damage was induced as previously described (Benton et al., 2007). Two hours after exposure began, 1 mL of each culture was removed and the OD_{600} was recorded in order to monitor cell proliferation. The cells were then washed in PBS, resuspended in fresh PBS to an OD_{600} of 0.2, and placed in the dark on ice until flow cytometry analysis. This process was repeated after 4 h, 6 h, and 8 h had passed since the initial DNA damage exposure.

2.3. Colony forming unit assays

All colony forming unit assays (CFU) consisted of a minimum of three independent trials. Cells were grown in liquid YPD at 30 °C and 250 rpm to mid log phase and then diluted to an OD_{600} of 0.1. Cultures were then treated with either MMS or γ-ray and returned to 30 °C incubation. After 4 h of continuous MMS exposure or 4 h after γ-ray exposure, cells were diluted and sonicated. The cells were divided into 3 groups of approximately 100 cells each and each group of 100 was plated on a different YPD plate. The procedure was repeated 4 h later, after 8 h of continuous MMS exposure or 8 h after γ-ray exposure. The plates were incubated at 30 °C for 72 h, and then colonies were counted. The number of colonies on the three plates from the same culture was averaged, and the average of each of the three cultures was averaged to determine the number of viable colonies for a given condition. The number of colonies on each plate was normalized with the number of colonies on control plates not treated with any DNA damaging agent.

2.4. Growth rate analysis

To determine the effect of DNA damaging agent exposure on cell proliferation and doubling time, culture OD_{600} was monitored

as a function of exposure time. Two hours after exposure began, 1 mL of each culture was removed and the OD_{600} was recorded. The OD_{600} was converted to cell titer using a standard curve constructed by measuring the OD_{600} of samples whose cell concentration was determined by counting cells on a hemocytometer. Culture doubling time was calculated using the following equation:

$$N = 2^{t/t_d} N_0$$

where N is the number of cells at time t, N_0 is the initial number of cells, and t_d is the average doubling time.

2.5. Flow cytometry

For each replicate, at least 10,000 cells were analyzed for fluorescence following protocols previously described (Benton et al., 2007).

3. Results and discussion

3.1. mag1Δ strains show enhanced sensitivity to genotoxicity caused by MMS

We have previously described a yeast-based biosensor which selectively senses and reports DNA damage through GFP expression (Benton et al., 2007). This strain contains a GFP encoding region regulated by a HUG1 promoter, leading to GFP expression in response to many types of genotoxicity. In the current work, we sought to increase the sensitivity of our original strain (SPY1101) to lower doses of DNA damage through the deletion of genes involved in the cellular DNA damage response, thereby enhancing the utility of the sensor.

Strain SPY1102 was created by deleting *MAG1* from the original *HUG1*P-GFP strain, SPY1101. Mag1p is a DNA glycosylase that removes alkylated bases from the DNA backbone, creating AP sites which are later cleaved by endonucleases. The deletion of *MAG1* created cells which were more susceptible to death than the wild type and SPY1101 strains upon exposure to the alkylating agent MMS. SPY1102 was no more susceptible to death after exposure to γ-ray than wild type and SPY1101 cells (data not shown).

SPY1102 (*mag1*Δ) was exposed to multiple doses of MMS and γ-ray (Fig. 1). Fig. 1A shows the difference in relative fluorescence levels caused by the *MAG1* deletion after 8 h of exposure (8 h is the time point at which GFP fluorescence was the maximum). The MMS dose-dependent responses of SPY1101 and SPY1102 (*mag1*Δ) show the enhanced sensitivity acquired through the *MAG1* deletion. The *mag1*Δ strain had a peak relative fluorescence of 154-fold at 0.005% MMS, whereas the optimum relative fluorescence of SPY1101 peak was 148-fold at 0.01%. At 0.005% MMS, SPY 1101 fluorescence was up-regulated 97-fold, indicating the *MAG1* deletion caused a 1.5-fold increase in relative GFP fluorescence at that dose. The *MAG1* deletion had a more profound effect at the low MMS doses. SPY1101 had a relative fluorescence of 4 at 0.0005% MMS, whereas SPY1102 (*mag1*Δ) had a relative fluorescence of 34, an improvement of more than 8-fold. The lowest detectable dose of MMS by the *HUG1*P-GFP system was lowered through the *MAG1* deletion, while the kinetics of the response was basically unchanged (see Supplemental Information).

The increase in *HUG1*P-GFP sensitivity upon *MAG1* deletion probably results from the accumulation of methyl groups on the DNA backbone. These alkylations cause the replication fork to stall during DNA synthesis, activating the Mec1p kinase pathway (Pasero et al., 2003), of which Hug1p is a member. The lack of functional Mag1p presumably causes higher levels of alkylation at lower doses of MMS, inducing *HUG1* at lower doses than in SPY1101 cells. This notion is consistent with the dose-responses for both strains, SPY1101 (*MAG1*) and SPY1102 (*mag1*Δ), having the same response pattern but with the optimum fluorescence shifted toward lower MMS doses for SPY1102 (*mag1*Δ). *MAG1* deletion therefore reduces the detection threshold of the *HUG1*P-GFP strain for MMS.

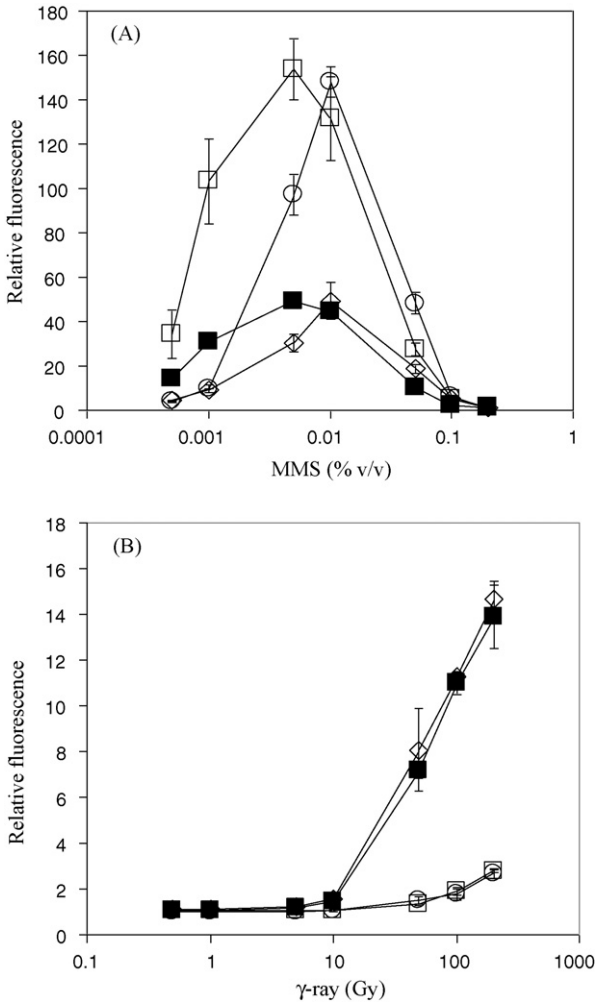

Fig. 1. GFP induction in SPY1101, SPY1102 (*mag1*Δ), SPY1103 (*mre11*Δ), and SPY1105 (*mag1*Δ *mre11*Δ) by (A) MMS and (B) γ-ray. Cells were grown to log phase in rich medium, and then treated with the indicated genotoxin. After 8 h of continuous exposure to MMS or 8 h after exposure to γ-ray, GFP expression relative to untreated control cells of the same strain was quantified by flow cytometry. Results are the average of at least three independent experiments, and error bars represent standard deviation. (◯) SPY1101, (□) SPY1102, (◊) SPY1103, and (■) SPY1105.

To ensure that *MAG1* deletion selectively enhanced sensitivity to MMS, SPY1102 (*mag1*Δ) was exposed to multiple doses of γ-ray, whose most lethal effects are the result of double strand breaks (Friedl et al., 1993). The responses of SPY1101 (*MAG1*) and SPY1102 (*mag1*Δ) were indistinguishable at every dose tested (Fig. 1B). This result shows that the sensitivity to specific types of DNA damage can be increased, without changing the response to other types of damage, by disabling or weakening a specific DNA repair pathway.

3.2. mre11Δ strains show enhanced sensitivity to genotoxicity induced by γ-ray

Strain SPY1103 was created by deleting *MRE11* from the original *HUG1*P-GFP strain, SPY1101. Mre11p is crucial for sensing double

strand breaks and recruits other proteins to aid in their repair. To assess whether SPY1103 (*mre11*Δ) was selectively sensitized to γ-ray, cells were exposed to multiple doses of γ-ray and MMS (Fig. 1). Fig. 1B shows the sensitivity of SPY1103 (*mre11*Δ) was greater than that of SPY1101 (*MRE11*) in response to γ-ray 8 h after exposure, the time point that resulted in the highest fluorescence induction (data not shown). The minimum detection threshold of SPY1101 (*MRE11*) was 200 Gy, with a relative fluorescence of 2.7. The same dose of γ-ray induced a relative fluorescence of 14.7 in SPY1103 (*mre11*Δ), more than a 5-fold increase in signal over SPY1101. Meanwhile, SPY1103 (*mre11*Δ) was able to detect 50 Gy, with an 8-fold induction of fluorescence.

Fig. 1A shows a decrease in relative fluorescence of SPY1103 (*mre11*Δ) cells exposed to MMS for 8 h, the time point that yielded maximum GFP induction (data not shown). 0.01% MMS produced the maximum fluorescence for both strains: a 148-fold increase over control cells for SPY1101 (*MRE11*) and a 49-fold increase for SPY1103 (*mre11*Δ), about a 3-fold decrease in relative fluorescence as a result of the deletion. The dose dependent response of SPY1103 (*mre11*Δ) did not show an increase or decrease in sensitivity to MMS over SPY1101; the fluorescent response was simply attenuated. The decrease in relative fluorescence in the *mre11*Δ strain is likely due to a slight increase in basal GFP expression level. *MRE11* null mutants accumulate DNA damage in the absence of exogenous genotoxins (Grenon et al., 2001). Basal GFP fluorescence in SPY1103 (*mre11*Δ) is about 2.7-fold higher than that of SPY1101 (*MRE11*) cells when measured after culturing cells for 6 h with no genotoxin present (data not shown). The lowest detectable dose of γ-ray by the *HUG1*P-GFP system did not change significantly through the *MRE11* deletion, while the time necessary for a positive response increased slightly (see Supplemental Information).

These results show that *MRE11* deletion increases peak fluorescence and sensitivity to γ-ray in a *HUG1*P-GFP biosensor, without affecting the sensitivity to MMS. This increase is likely due to an accumulation of double strand breaks in the *mre11*Δ strain, which then induces *HUG1* promoter activity. *MRE11* mutants have been shown to be sensitive to MMS (Ajimura et al., 1993). It is interesting to note that although *mre11*Δ strains exhibited reduced viability in response to MMS (data not shown), the *HUG1*P-GFP did not show an enhanced sensitivity for MMS upon *MRE11* deletion. This seems to indicate that *HUG1* and *MRE11* are not regulated by the same cellular checkpoints. Both are involved in the response to MMS, but they do not appear to have an epistatic relationship.

In budding yeast, the RMX complex, consisting of Rad50p, Mre11p, and Xrs2p, has multiple functions including roles in DNA damage repair through homologous recombination and non-homologous end joining, meiotic recombination, and telomere maintenance (reviewed in D'Amours and Jackson (2002)). Additionally, the RMX complex activates the Mec1p signaling pathway in response to double strand breaks (Nakada et al., 2004). It is possible that the deletion of *MRE11* actually lowered sensitivity to low doses of γ-ray by limiting the cells' ability to initiate key portions of the DNA damage response.

3.3. *mre11*Δ phenotype dominates in *mag1*Δ *mre11*Δ cells

A *mag1*Δ *mre11*Δ *HUG1*P-GFP biosensor strain, SPY1105, was created to determine the synergistic or antagonistic effect of *MAG1* and *MRE11* deletion on biosensor detection of MMS and γ-ray. When exposed to MMS, strain SPY1105 (*mag1*Δ *mre11*Δ) displayed an *mre11*Δ phenotype (Fig. 1A). The sensitivity to MMS was similar to that of SPY1102 (*mag1*Δ) with the peak fluorescence induction at 0.005%, but the relative fluorescence at each dose was lower than that in SPY1102 (*mag1*Δ). The relative fluorescence induction at 0.005% MMS was 49-fold for SPY 1105, identical to the fluorescence induction of SPY1103 (*mre11*Δ) at its peak fluorescence. This induction was about 3-fold lower than the relative fluorescence induction of SPY1102 (*mag1*Δ), likely due to the elevated background GFP expression resulting from *mre11*Δ deletion. Basal GFP expression in SPY1105 (*mag1*Δ *mre11*Δ) was about 2.6-fold higher than that of SPY1101 cells when measured after culturing cells for 6 h with no genotoxin present (data not shown).

When SPY1105 (*mag1*Δ *mre11*Δ) cells were exposed to γ-ray, the *MRE11* deletion dominated (Fig. 1B). Peak fluorescence for SPY1103 (*mre11*Δ) and SPY1105 (*mag1*Δ *mre11*Δ) occurred at 200 Gy, and the values were not statistically different. In SPY1102 (*mag1*Δ) the *MAG1* deletion had no effect on sensitivity to γ-ray, and the same appeared to be true for SPY1105 (*mag1*Δ *mre11*Δ).

MAG1 and *MRE11* deletion both affected GFP expression in the *HUG1*P-GFP biosensor performance. This is a promising result from the perspective of biosensor design as it suggests multiple genes can be deleted, yielding viable cells that have increased sensitivity for specific types of damage without compromising the responses to other types of damage. With proper deletion choices, it should be possible to increase the sensitivity of *HUG1*P-GFP biosensor to other types of DNA damage besides alkylation and double strand breaks.

3.4. *MAG1* deletion enhanced *HUG1*P-GFP sensitivity to genotoxins other than MMS

All four *HUG1*-GFP strains were exposed to multiple types of DNA damaging agents, to assess how the deletions of *MAG1* and *MRE11* affect the strain's sensitivity and selectivity in detecting different genotoxins. SPY1102 (*mag1*Δ) exhibited enhanced sensitivity to ethyl methanesulfonate (EMS) (Fig. 2A), a DNA alkylating agent, as would be expected given the response to MMS (Fig. 1A). This decrease in the dose that elicits the peak GFP response peak dose in a *mag1*Δ strain is consistent with previous observations (Jia and Xiao, 2003).

More interestingly, SPY1102 (*mag1*Δ) showed increased sensitivity over SPY1101 to 4-nitroquinoline-1-oxide (4-NQO), a UV mimetic agent that causes single strand adducts (Fig. 2B). The lowest detectable dose of 4-NQO was 0.05 μg/mL for SPY1101, while SPY1102 (*mag1*Δ) was able to detect 0.01 μg/mL. Furthermore, at all doses of 4-NQO above 0.05 μg/mL, SPY1102 (*mag1*Δ) exhibited a 2-fold or more increase in GFP fluorescence compared to SPY1101. Mag1p is a member of the BER, which removes individual bases in response to damage such as alkylation. Damage caused by 4-NQO induces the nucleotide excision repair (NER) pathway, which removes multiple nucleotides around a damaged site (Friedberg et al., 1995). The observed increase in sensitivity suggests some synergy between the NER and BER in a *hug1*Δ *mag1*Δ null mutant. Some models suggest that the RMX complex could play a role in the response to DNA damage that triggers the NER (Nakada et al., 2004).

3.5. *MRE11* deletion enhanced *HUG1*P-GFP sensitivity to camptothecin

Camptothecin is a topoisomerase I inhibitor, which allows topoisomerase I to cleave a strand of DNA, but prohibits its religation, effectively creating single strand breaks in the DNA backbone. These single-strand breaks are often converted to double strand breaks by the cell during DNA replication (Ferrara and Kmiec, 2004). SPY1103 (*mre11*Δ) and SPY1105 (*mag1*Δ *mre11*Δ) cells were more sensitive to DNA damage caused by camptothecin than SPY1101 cells (Fig. 2C). Both *mre11*Δ strains were able to detect

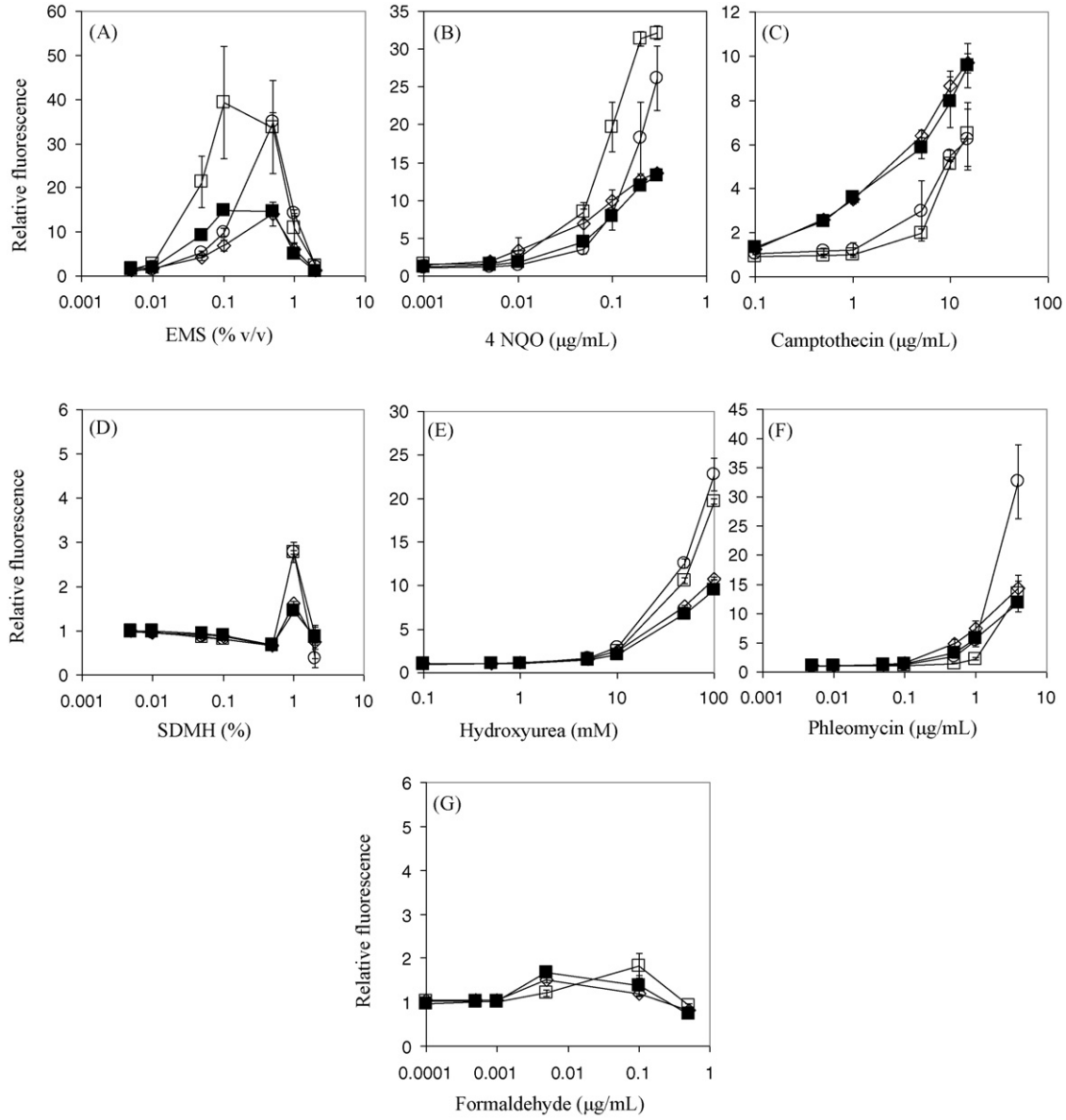

Fig. 2. GFP induction in SPY1101, SPY1102 (*mag1*Δ), SPY1103 (*mre11*Δ), and SPY1105 (*mag1*Δ *mre11*Δ) by multiple DNA damaging agents. Cells were grown to log phase in rich medium, and then treated with the indicated genotoxin for 6 h (A, B, D, and E) or 8 h (C, F, and G). GFP expression relative to untreated control cells of the same strain was measured by flow cytometry. Results are the average of at least three independent experiments, and error bars represent standard deviation. (○) SPY1101, (□) SPY1102, (◊) SPY1103, and (■) SPY1105.

camptothecin doses of 0.5 μg/mL, whereas SPY1101 had a detection limit of 5 μg/mL. GFP fluorescence increased in the *mre11*Δ strains for all doses of camptothecin tested above 0.5 μg/mL. Camptothecin induces both homologous recombination and non-homologous end joining repair pathways. As seen earlier with γ-ray, the improved sensitivity to camptothecin is likely due to an accumulation of double strand breaks in the *mre11*Δ strain, which then induces *HUG1* promoter activity.

3.6. *MAG1 and MRE11 deletions increase selectivity of HUG1P-GFP for specific genotoxins*

The *HUG1*P-GFP strains were exposed to four additional genotoxins to determine if selectivity for specific genotoxins was conferred by *MAG1* and *MRE11* deletions (Fig. 2D–G). None of the strains were able to improve upon SPY1101's ability to detect 1,2-dimethyl hydrazine (SDMH), a carcinogenic DNA alkylating agent (Fig. 2D). The lack of improvement by SPY1102 (*mag1*Δ) was surprising given its ability to detect lower doses of the other alkylating agents tested, MMS and EMS.

SPY1101 outperformed the *mag1*Δ and *mre11*Δ deletion strains in the detection of hydroxyurea (HU) and phleomycin (Fig. 2E and F). Hydroxyurea is a ribonucleotide reductase inhibitor, which causes stalled replication forks that can eventually lead to double strand break formation (Diffley et al., 2000; Shechter et al., 2004). Phleomycin is an antibiotic that induces double strand breaks in yeast (Moore, 1988). One might have expected *HUG1*P-GFP *mre11*Δ null mutants to show enhanced sensitivity to hydroxyurea and phleomcycin as they did for γ-ray and camptothecin. The reduction in signal may be due to decreased ability of the *mre11*Δ cells to recognize double strand breaks, as previously discussed. Finally, no variations of the *HUG1*P-GFP strains were able to detect the presence of formaldehyde, a putative carcinogen which many consider to be "non-genotoxic" (Fig. 2G).

4. Conclusions

The *HUG1*P-GFP promoter-reporter construct has demonstrated the ability to detect a wide variety of DNA damaging agents without responding to general cell stresses (Benton et al., 2007). In this study we enhanced the utility of the cellular biosensor through the systematic deletion of genes involved in the DNA damage response. By deleting *MAG1*, a DNA glycosylase and member of the BER pathway, the *HUG1*P-GFP sensitivity to the alkylating agents MMS and EMS was greatly enhanced. Deletion of *MRE11*, part of the highly conserved RMX complex that aids in sensing and repairing double strand breaks in budding yeasts, enhanced sensitivity to γ-ray and camptothecin. The effects of a double deletion of *MAG1* and *MRE11* were additive.

We were able to engineer increased selectivity through deletion of components of the DNA damage repair pathway, since increased sensitivity to one type of damage did not alter sensitivity to other genotoxins. The enhancements to the *HUG1*P-GFP system did not improve its ability to detect several other DNA damaging agents, including SDMH, phleomycin, and HU, or its ability to detect the carcinogen formaldehyde. Based on the results of this study, it seems likely that the directed deletion of genes in DNA damage repair pathways can enhance sensitivity to those and other genotoxins.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2008.06.033.

References

Ajimura, M., Leem, S.H., Ogawa, H., 1993. Genetics 133 (1), 51–66.
Ames, B.N., Lee, F.D., Durston, W.E., 1973. Proc. Natl. Acad. Sci. U.S.A. 70 (3), 782–786.
Averbeck, D., Averbeck, S., 1994. Mutat. Res. 315 (2), 123–138.
Basrai, M.A., Velculescu, V.E., Kinzler, K.W., Hieter, P., 1999. Mol. Cell. Biol. 19 (10), 7041–7049.
Benton, M.G., Glasser, N.R., Palecek, S.P., 2007. Mutat. Res. 633, 21–34.
Benton, M.G., Somasundaram, S., Glasner, J.D., Palecek, S.P., 2006. BMC Genomics 7, 305.
Bressan, D.A., Olivares, H.A., Nelms, B.E., Petrini, J.H., 1998. Genetics 150 (2), 591–600.
Chen, J., Derfler, B., Maskati, A., Samson, L., 1989. Proc. Natl. Acad. Sci. U.S.A. 86 (20), 7961–7965.
Chen, J., Derfler, B., Samson, L., 1990. EMBO J. 9 (13), 4569–4575.
Cole, G.M., Schild, D., Lovett, S.T., Mortimer, R.K., 1987. Mol. Cell. Biol. 7 (3), 1078–1084.
D'Amours, D., Jackson, S.P., 2002. Nat. Rev. Mol. Cell. Biol. 3 (5), 317–327.
Diffley, J.F., Bousset, K., Labib, K., Noton, E.A., Santocanale, C., Tercero, J.A., 2000. Cold Spring Harb. Symp. Quant. Biol. 65, 333–342.
Elledge, S.J., Davis, R.W., 1989. Mol. Cell. Biol. 9 (11), 4932–4940.
Ferrara, L., Kmiec, E.B., 2004. Nucleic Acids Res. 32 (17), 5239–5248.
Friedberg, E.C., Walker, G.C., Siede, W., 1995. DNA Repair and Mutagenesis. ASM Press, Washington, DC.
Friedl, A.A., Beisker, W., Hahn, K., Eckardt-Schupp, F., Kellerer, A.M., 1993. Int. J. Radiat. Biol. 63 (2), 173–181.
Gasch, A.P., Huang, M., Metzner, S., Botstein, D., Elledge, S.J., Brown, P.O., 2001. Mol. Biol. Cell 12 (10), 2987–3003.
Grenon, M., Gilbert, C., Lowndes, N.F., 2001. Nat. Cell Biol. 3 (9), 844–847.
Ichikawa, K., Eki, T., 2006. J. Biochem. (Tokyo) 139 (1), 105–112.
Jia, X., Xiao, W., 2003. Toxicol. Sci. 75 (1), 82–88.
Jia, X., Zhu, Y., Xiao, W., 2002. Mutat. Res. 519 (1/2), 83–92.
Lichtenberg-Frate, H., Schmitt, M., Gellert, G., Ludwig, J., 2003. Toxicol. In Vitro 17 (5/6), 709–716.
Maclean, M.J., Aamodt, R., Harris, N., Alseth, I., Seeberg, E., Bjoras, M., Piper, P.W., 2003. Aging Cell 2, 93–104.
Moore, C.W., 1988. Cancer Res. 48 (23), 6837–6843.
Nakada, D., Hirano, Y., Sugimoto, K., 2004. Mol. Cell. Biol. 24 (22), 10016–10025.
Nisson, P.E., Lawrence, C.W., 1986. Mutat. Res. 165 (3), 129–137.
Oda, Y., Nakamura, S., Oki, I., Kato, T., Shinagawa, H., 1985. Mutat. Res. 147 (5), 219–229.
Pasero, P., Shimada, K., Duncker, B.P., 2003. Cell Cycle 2 (6), 568–572.
Quillardet, P., Huisman, O., D'Ari, R., Hofnung, M., 1982. Proc. Natl. Acad. Sci. U.S.A. 79 (19), 5971–5975.
Shechter, D., Costanzo, V., Gautier, J., 2004. DNA Repair (Amst.) 3 (8/9), 901–908.
Walmsley, R.M., Billinton, N., Heyer, W.D., 1997. Yeast 13 (16), 1535–1545.
Walsh, L., Hastwell, P.W., Keenan, P.O., Knight, A.W., Billinton, N., Walmsley, R.M., 2005. Mutagenesis 20 (5), 317–327.
Xiao, W., Chow, B.L., Hanna, M., Doetsch, P.W., 2001. Mutat. Res. 487 (3/4), 137–147.