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Differential regulation of two closely clustered yeast genes, *MAG1* and *DDI1*, by cell-cycle checkpoints

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ABSTRACT

Eukaryotic DNA-damage checkpoint genes have been shown to not only arrest cells at certain stages, but are also involved in the transcriptional response to DNA damage. However, while the signal transduction for cell-cycle checkpoint is well characterized, it is not clear whether the same signal transduction pathway is responsible for the regulation of all DNA damage-inducible genes. In order to understand how different checkpoint genes are involved in gene regulation, the effects of various checkpoint mutations on the expression of a unique yeast *MAG1-DDI1* dual promoter were examined in this study. *MAG1* and *DDI1* are transcribed from a common promoter region and co-induced by a variety of DNA damaging agents. However, gene-specific *cis*-acting elements were also identified, and the two genes are indeed differentially expressed under certain conditions. We found that *DDI1* induction was not affected in any of the checkpoint mutants. In contrast, *MAG1* induction was completely abolished in the *pol2* and *rad53* mutants. However, in the *mec1-1* or any of the G_1/S and G_2/M checkpoint mutants, including *rad9*, *rad17* and *rad24*, DNA damage-induced *MAG1* expression was not significantly affected, and a *rad9 rad17* double mutation only slightly reduced *MAG1* induction. Based on this and previous studies, we present two models for the role of checkpoint genes in transcriptional regulation in response to DNA damage.

INTRODUCTION

Cells respond to DNA damage by delaying cell cycle progression and by increasing the expression of genes involved in the repair and tolerance of DNA damage (1). Eukaryotic cells have developed surveillance mechanisms that monitor and regulate the key events of the cell cycle so that its progression is dependent on the completion of previous events. These surveillance mechanisms are known as cell-cycle checkpoints (2,3). The checkpoint is considered to be a signal transduction pathway, which is activated by a signal and results in an inhibition of cell cycle progression (3,4). The DNA-damage checkpoint is the mechanism that detects damaged DNA and generates a signal that arrests cells in the G_1 or G_2 phases of the cell cycle, and slows down S phase (DNA synthesis) progression (4). This mechanism is thought to prevent the replication of damaged templates and the segregation of broken chromosomes. At least seven genes, namely *RAD9*, *RAD17*, *RAD24*, *MEC3*, *POL2*, *MEC1* and *RAD53*, have been identified in the budding yeast *Saccharomyces cerevisiae* that, when mutated, inactivate certain checkpoint controls (4–6). While *RAD9*, *17*, *24* and *MEC3* are required to activate the DNA damage checkpoint when cells are in G_1 or G_2 (2,7–9), and *POL2* is required to sense UV damage and replication blocks when cells are in S-phase (10), *MEC1* (11) and *RAD53* (12) appear to form a downstream signal transduction cascade required for all three checkpoints (5,6)

In addition to their functions in cell cycle progression, some checkpoint genes also play roles in the control of DNA damage-induced gene expression of DNA repair and synthesis genes (13–15), suggesting that the cellular response to DNA damage is co-ordinated. This regulatory function is thought to be achieved through phosphorylation of a nuclear protein kinase Dun1 (16) by the Rad53 protein kinase (11). Interestingly, a similar signal transduction cascade also exists in mammalian cells. For example, activation of the human *GADD45* gene by ionizing radiation is dependent on the tumor suppressor and transcription factor p53; however, this activation is not observed in *ATM*-deficient cells (17). A single signal transduction pathway activating a large number of genes in response to DNA damage is reminiscent of the *Escherichia coli* SOS response, where RecA-LexA controls expression of >30 genes (1,18). However, previous reports appear to be inconclusive regarding the ability of checkpoint genes to exert such a blanket response. One study (14) indicates that the *rad9* mutation affects DNA damage-induction of essentially all the genes examined, whereas another study (13) suggests that *checkpoint* mutations differentially affect target gene induction by DNA damage. Similarly, DNA damage-inducible genes under the above study (13) can also be divided into several groups according to their response to various *checkpoint* mutations. Thus, it appears that a unified SOS-type response to DNA damage may not operate in eukaryotic cells.

In order to address how different checkpoint genes are involved in transcriptional regulation, and if the signal transduction cascade for gene regulation parallels that for cell-cycle checkpoint control, we undertook the present study by analyzing the dual *MAG1-DDI1* gene expression in various checkpoint mutants. *MAG1* encodes a 3-methyladenine (3MeA) DNA glycosylase (19), the first enzyme in a multistep base-excision-repair pathway

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for the removal of lethal lesions such as 3MeA, and protects yeast cells from killing by DNA alkylating agents such as methyl methanesulfonate (MMS) (20). *MAG1* is inducible by various DNA damaging agents, regardless of whether or not Mag1 is required for the repair of these types of damage (21–23). *DDI1* is located immediately upstream of *MAG1*, transcribed in an opposite direction, and is co-regulated with *MAG1* (24). *DDI1* encodes a protein that is highly conserved in eukaryotes; however, deletion of *DDI1* did not result in any noticeable phenotypic alterations (W.Xiao, unpublished results). Deletion analyses in the *DDI1-MAG1* promoter region (23–25) have identified several *cis*-acting regulatory elements either shared by both genes, or specific for only one of the genes. In addition, *MAG1* and *DDI1* are differentially regulated in the presence of the protein synthesis inhibitor cycloheximide (25). Hence, studying how cell-cycle checkpoint genes regulate *MAG1-DDI1* expression may help to establish the signal transduction pathway from sensing the damage to controlling DNA damage-inducible gene expression. In this study, we demonstrate that *MAG1* and *DDI1* expression are differentially affected by checkpoint mutations, that only the S-phase checkpoint sensor transmits damage signals to the *MAG1* regulatory pathway, and that, more importantly, the cell-cycle checkpoint signal transduction cascade may differ from the gene regulation pathway.

MATERIALS AND METHODS

Yeast strains, cell culture and transformation

Haploid yeast strains used in this study are listed in Table 1. THY107 was created by transforming DBY747 with *Eco*RI+*Sal*I-cleaved pRR330 containing the *rad9Δ::hisG-URA3-hisG* cassette (26). The Ura$^+$ transformants with *rad9Δ* deletion were confirmed by Southern hybridization and other *rad9* phenotypes.

Yeast cells were grown at 30°C in either complete YPD medium or SD medium supplemented with the appropriate nutrients (27). Plasmids were transformed into yeast cells by a modified lithium acetate protocol (28). The transformants were streaked onto a fresh selective plate before being utilized for further analysis.

Plasmids

Plasmid pZZ2 (YCp, *URA3*, *RNR3-lacZ*; 29) was obtained from Dr S. Elledge (Baylor College of Medicine, Houston, TX) and utilized to determine *RNR3* expression by a β-galactosidase (β-gal) assay. Plasmids YEpMAG1-lacZ (YEp, *LEU2*, *MAG1-lacZ*; 23) and YEpDDI1-lacZ (YEp, *URA3*, *DDI1-lacZ*; 24) have been described previously. In order to transform checkpoint mutant strains with different selectable markers, two additional *lacZ* fusion plasmids were made. Plasmid pWX1254 (YEp, *URA3*, *LEU2*, *MAG1-lacZ*) was constructed by inserting a 1.2 kb *Hin*dIII fragment from YEp24 (30) containing the *URA3* gene into the unique *Hin*dIII site of YEpMAG1-lacZ. Plasmid pWX1813 (YEp, *LEU2*, *DDI1-lacZ*) was created by cloning a 0.53 kb *Xba*I fragment from pWX1807 (24) into YEp367R (31) in the orientation of *DDI1-lacZ* fusion. This fusion construct contains a 368 bp *DDI1* promoter region and a 158 bp coding sequence, which is in-frame with the *lacZ* gene in YEp367R. The basal and damage-induced expression of pWX1254 and pWX1813 in the same host strain DBY747 was determined and found to be indistinguishable from that of YEp-MAG1-lacZ and YEp-DDI1-lacZ, respectively.

Table 1. *Saccharomyces cerevisiae* strains

Strain[a]	Genotype	Source/reference
DBY747	*MAT**a** leu2-3,112 ura3-52 his3-Δ1 trp1-289*	D. Botstein
THY107	*MAT**a** leu2-3,112 ura3-52 his3-Δ1 trp1-289 rad9Δ::hisG-URA3-hisG*	This study
Y203	*MAT**a** ade2-1 his3 leu2-3,112 lys2 trp1 ura3-Δ100 rnr3::RNR3-URA3-TRP1*	29
Y400	*MAT**a** leu2-3,112 his3 his7-2 pol2-12*	10
Y300	*MAT**a** can1-100 ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1*	12
Y301	*MAT**a** can1-100 ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 sad1-1*	12
TWY394	*MAT**a**, ura3 his7 leu2 trp1*	13
TWY177	*MAT**a**, ura3 his3 leu2 trp1 mec1-1*	13
TWY178	*MATα trp1 ura3 mec2-1*	13
TWY102	*MAT**a**, ura3 his3 leu2 trp1 rad9Δ::LEU2*	T. Weinert
WXY9619	TWY394 with *rad17Δ::HIS3*	This study
WXY9620	TWY102 with *rad17Δ::HIS3*	This study
TWY297	*MAT**a**, ura3 his3 trp1 rad24-1*	T. Weinert

[a]All the Y strains were obtained from Dr S. Elledge. Y203 was used as the wild type control of Y400, and Y300 was used as the wild type control of Y301. All TWY strains were from Dr T. Weinert (University of Arizona) and are congenic with TWY394.

To make a *rad17Δ::HIS3* disruption cassette, plasmid pUC-RAD17 obtained from Dr W. Siede (Emory University, GA, USA) was first subcloned to contain a 1.9 kb *Bam*HI–*Xba*I fragment with entire *RAD17* coding region, a 1.0 kb *Mlu*I–*Sty*I fragment containing 893 bp *RAD17* 5′ coding region and 94 bp promoter was removed and replaced by a *Bgl*II linker to form pUC-rad17ΔBg. A 1.2 kb *Bam*HI fragment containing the *HIS3* gene isolated from plasmid YDp-H (32) was inserted at the *Bgl*II site of pUC-rad17ΔBg to create prad17Δ::HIS3. The *rad17Δ::HIS3* cassette was released by *Bam*HI–*Xba*I double digestion prior to yeast cell transformation, and the transformants with chromosomal *rad17* deletion was confirmed by Southern hybridization before further characterization.

DNA-damage treatment and β-gal assay

The β-gal assay was performed as described previously (23). Briefly, 0.5 ml of overnight yeast culture was used to inoculate 2.5 ml of fresh SD selective medium and incubation was continued for another 2 h. At this point, chemicals were added at the concentration indicated and cells were incubated for another 4 h. For UV treatment, cells were spread on YPD agar in a petri dish, exposed to 254 nm UV light at different intensities in a UV crosslinker (Fisher model FB-UVXL-1000 at ~2400 μW/cm^2), collected into 3 ml of SD selective medium, and incubated in the dark to prevent photoactivation. One ml of the above unsynchronized log-phase cell suspension was used for determining cell titer at OD_{600nm}, and the remaining cells were used for the β-gal assay. The β-gal activity is expressed in Miller units (33). Several measures were taken to maintain consistency of quantitative analysis. First, only freshly streaked yeast transformants were used for the β-gal assay. Second, the β-gal assay was performed with several independent transformants from a single experiment to avoid transformation 'jackpots'. Third, a sample of recipient cells with the vector plasmid was included in every experiment as a background control, with a typical β-gal activity of ~0.03 Miller unit. Fourth, results from various transformants/treatments presented for comparison were always from the same experiment to avoid interexperimental variations. Last, all the results presented were the average of at least three independent experiments with standard deviations as shown, or within 30%.

RNA isolation and northern hybridization

Overnight culture (1 ml) was used to inoculate 3 ml of fresh medium and cells were incubated for another 2 h. For MMS treatment, MMS was added to the indicated final concentrations and the incubation was continued for another 30 min. RNA was isolated by a glass-bead method (34), separated by gel electrophoresis, blotted on a *GeneScreen Plus* membrane (DuPont) and hybridized with α-^{32}P-labeled DNA probe as instructed. After an ~24 h hybridization, the membrane was washed and exposed to an X-ray film. The mRNA band intensity was measured by a Bio-Rad (Hercules, CA) model 620 densitometer equipped with 1-D Analyst software.

After agarose gel electrophoresis, DNA fragments were isolated by a Sephadex G10 spin column method (35) and labeled with [α-^{32}P]dCTP using a Random Primer Labeling kit from Gibco-BRL (Gaithersburg, MD). The 0.76 kb *Eco*RI–*Bgl*II *MAG1* probe contains nucleotides –138 to +630 relative to the *MAG1* gene. The 0.93 kb *Eco*RI *DDI1* probe contains nucleotides –149 to +786 relative to the *DDI1* gene. The *ACT1* probe was isolated as a 1.6 kb *Bam*HI–*Hin*dIII fragment from pAA93 (a gift from Dr F. Sherman, Rochester University).

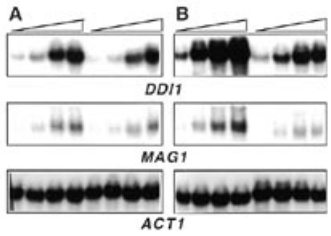

Figure 1. Northern hybridization of *DDI1* and *MAG1* expression in G_1/S and G_2/M checkpoint mutants induced by MMS. Total RNA was isolated from (**A**) DBY747 (wt, left half) and THY107 (*rad9*Δ, right half); and (**B**) TWY394 (wt, left half) and TWY297 (*rad24*, right half). Cells were treated with increasing concentrations (0, 0.01, 0.05 and 0.1%, indicated on top of the figure) of MMS for 30 min before RNA isolation. Each lane contains 15 μg of total RNA. The blots were sequentially hybridized and stripped with *MAG1*, *DDI1* and *ACT1* probes as described in Materials and Methods.

RESULTS

G_1/S and G_2/M checkpoint single mutations do not affect *MAG1* and *DDI1* DNA damage induction

Mutations in *RAD9*, *17*, *24* and *MEC3* are defective in G_2/M as well as G_1/S checkpoints in response to DNA damage (4,5). We examined the *MAG1* and *DDI1* expression in *rad9*, *rad17* and *rad24* mutants by both northern hybridization and β-gal assay of *lacZ* fusion constructs. Northern hybridization shows that although overall levels of *DDI1* and *MAG1* expression decreased slightly in the *rad9* and *rad24* mutants, the relative induction of *DDI1* and *MAG1* was not affected in these *rad* mutants (Fig. 1). Likewise, β-gal activity of the *MAG1-lacZ* and *DDI1-lacZ* transformants was not significantly affected in all three *rad* mutants examined (Fig. 2A and B). One may argue that MMS at the given doses specifically induces retarded S-phase progression, whereas the above *rad* mutants are specific for the G_1/S and G_2/M checkpoints. In order to determine whether or not the above *rad* mutations affect *MAG1* and *DDI1* induction by DNA damage that triggers G_1/S and G_2/M checkpoints, we also used UV as a damage-inducing agent and found that *rad9* mutation did not alter UV induction of *MAG1-lacZ* (Fig. 2C) and *DDI1-lacZ* (data not shown).

Recently, it was reported that while each *rad9*, *rad17* or *rad24* single mutation influences gene expression to a certain extent, *rad9* acts additively with *rad17* or *rad24* mutation to reduce gene expression in response to DNA damage (36). To examine if such double mutants are defective in *MAG1* and *DDI1* expression, we created a *rad9*Δ *rad17*Δ double mutant and compared the *DDI1-lacZ* and *MAG1-lacZ* expression with its isogenic single mutants. As shown in Figure 2, the *rad9 rad17* double mutation does not appear to affect *DDI1-lacZ* expression; however, it reduced the *MAG1-lacZ* induction by ~1/3 compared with the corresponding single mutations, although *MAG1* was still DNA-damage inducible to a certain extent (Fig. 2B). The *rad9* mutation is known to affect S-phase checkpoint, and this effect is additive to *rad17/rad24* mutations (37). However, loss of S-phase checkpoint arrest *per se* does not appear to account for the observed *MAG1* and *DDI1* induction, since *MAG1* expression is not cell-cycle regulated (22), and other S-phase checkpoint

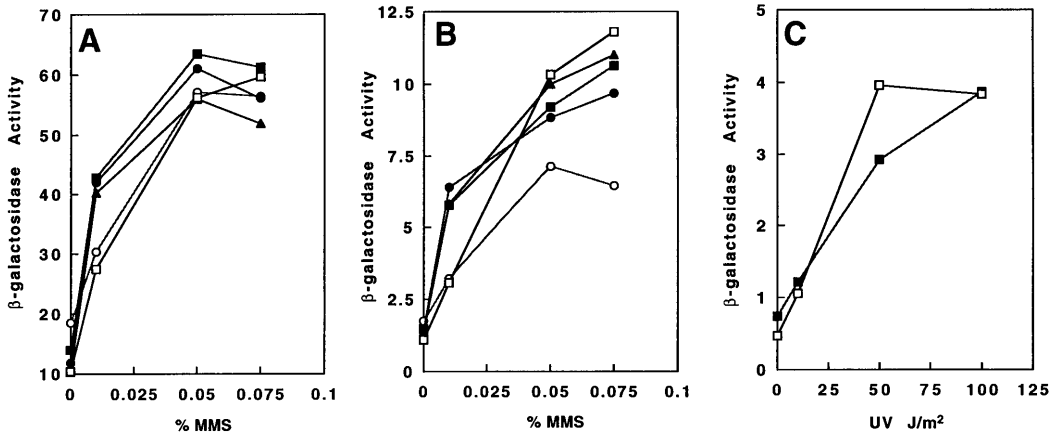

Figure 2. DNA damage-induced *DDI1-lacZ* and *MAG1-lacZ* expression in G_1/S and G_2/M checkpoint mutants. (**A**) MMS-induced *DDI1-lacZ* expression; (**B**) MMS-induced *MAG1-lacZ* expression; (**C**) UV-induced *MAG1-lacZ* expression. (□) TWY394 (wt); (■) TWY102 (*rad9*Δ); (▲) WXY9619 (*rad17*Δ); (●) TWY297 (*rad24*); and (○) WXY9620 (*rad9*Δ *rad17*Δ). The results are the average of three to six independent experiments. β-gal activity is given in Miller units.

mutations may or may not affect *MAG1* induction by MMS (see later). Hence, our observations suggest that either G_1/S and G_2/M checkpoint genes are not involved in the DNA damage induction of *MAG1* and *DDI1* genes, or there is an alternative pathway which bypasses the requirement for these *RAD* genes.

The S-phase checkpoint mutation abolishes DNA damage induction of *MAG1*, but not *DDI1*

POL2 encodes the catalytic subunit of an essential DNA polymerase ε (Polε). Mutation of *POL2* exclusively affects S-phase progression in the presence of DNA damage (10), the only known example of such a phenomenon. Like G_1/S and G_2/M checkpoint mutations, the *pol2-12* mutation also slightly reduced basal-level *MAG1* and *DDI1* expression (Fig. 3A). However, *pol2-12* differentially affects *MAG1* and *DDI1* induction after MMS treatment; while *DDI1* expression was not significantly affected, *MAG1* induction was completely abolished (Fig. 3A). To confirm this result, quantitative β-gal assay was performed with *MAG1-lacZ* and *DDI1-lacZ* transformants. The results, as presented in Figure 3B and C, are consistent with northern hybridization results (Fig. 3A). Furthermore, the *pol2-12* mutant is also defective in UV-induced *MAG1-lacZ* expression (data not shown). Thus, *POL2* appears to be involved in the signal transduction of DNA damage to control *RNR3* (10) as well as *MAG1* expression.

Rad53 and *mec1* mutations behave differently with respect to the DNA damage induction of *MAG1* gene expression

Current yeast cell-cycle checkpoint models indicate that Mec1 protein kinase phosphorylates the Rad53/Mec2/Sad1/Spk1 protein and controls its activity (4,5,12,38). The observation that overexpression of the *RAD53* gene suppresses the *mec1* mutant phenotype further supports the notion that *RAD53* functions downstream of *MEC1* (39). We examined the effects of *rad53* and *mec1* mutations on MMS-induced *MAG1* and *DDI1* expression. Since both *MEC1* and *RAD53* are essential genes, specific mutant alleles known to affect cell-cycle checkpoint functions were employed in this study. Strains bearing the *mec1-1* mutation are defective in both S-phase and G_2/M DNA damage checkpoints

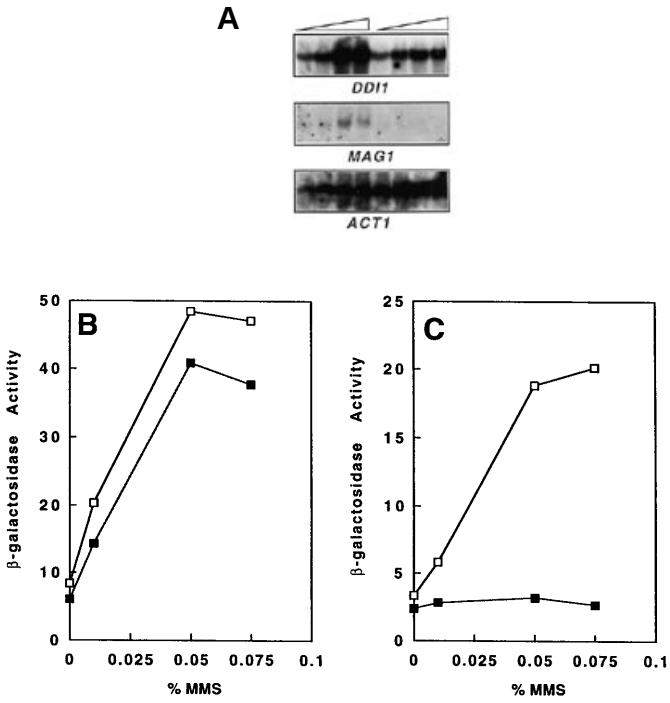

Figure 3. MMS induction of *DDI1* and *MAG1* expression in the S-phase checkpoint mutant. (**A**) Northern analysis. Total RNA was isolated from Y203 (wt, left half) and Y400 (*pol2-12*) after 0, 0.01, 0.05 and 0.1% MMS treatments (indicated on top of figure) for 30 min. Each lane contains 15 µg of total RNA. The blot was sequentially hybridized and stripped with *MAG1*, *DDI1* and *ACT1* probes. (**B**) *DDI1-lacZ* and (**C**) *MAG1-lacZ* induction by MMS in the wild type and *pol2* mutant. (□) Y203 (wt) and (■) Y400 (*pol2-12*). The results in (B) and (C) are the average of three independent experiments.

(40,41). To our surprise, neither *DDI1* nor *MAG1* expression was affected by the *mec1-1* mutation (Fig. 4A and B). We also confirmed that, as expected, TWY177 (*mec1-1*) was sensitive to DNA damaging agents such as UV and MMS (data not shown) and was largely defective in *RNR3* induction by MMS (Fig. 4C and 13). Furthermore, UV-induced expression of all three *lacZ* fusion

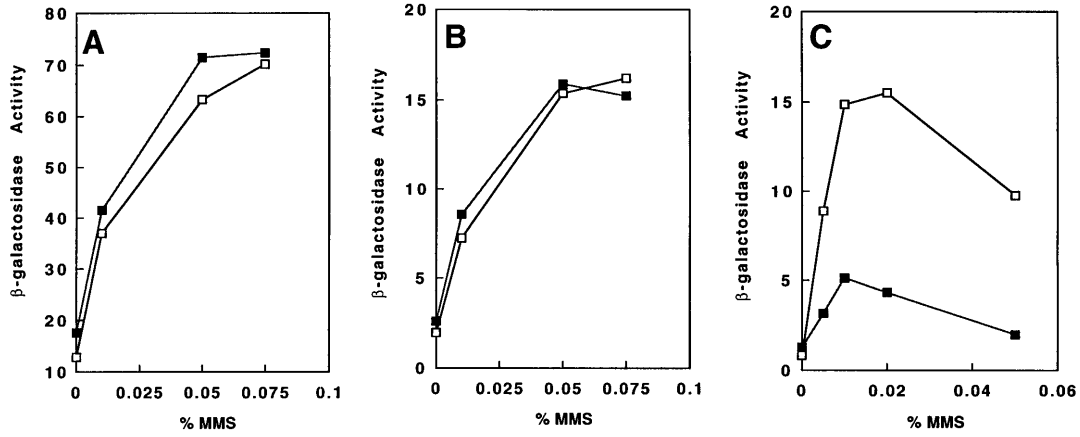

Figure 4. MMS-induced expression of *lacZ*-fusion genes in the *mec1-1* mutant. (**A**) *DDI1-lacZ* transformants, (**B**) *MAG1-lacZ* transformants and (**C**) *RNR3*-lacZ transformants. (□) TWY394 (wt); and (■) TWY177 (*mec1-1*). All results are the average of three independent experiments.

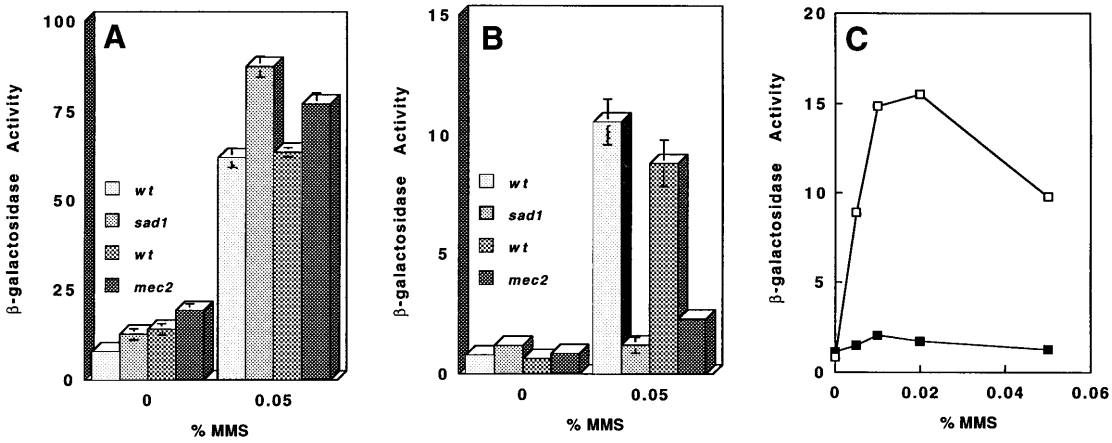

Figure 5. MMS-induced expression of *lacZ*-fusion genes in *rad53* mutants. (**A**) *DDI1-lacZ* transformants, (**B**) *MAG1-lacZ* transformants and (**C**) *RNR3-lacZ* transformants. Comparison was made between Y300 (wt) and Y301 (*sad1-1*), and between TWY394 (wt) and TWY178 (*mec2-1*), as indicated in the figures. (□) TWY394 (wt); and (■) TWY178 (*mec2-1*). All results are the average of at least three independent experiments with standard deviations shown in (A) and (B) by a bar.

constructs in the *mec1-1* mutant parallels that of MMS-induction (data not shown). This result suggests that either the cell-cycle checkpoint function of *MEC1* is distinct from its transcriptional regulation function for certain transcripts, or some DNA-damage inducible genes are not under the control of *MEC1*.

We were even more surprised to find that *rad53* mutation did not affect *DDI1* induction (Fig. 5A); however, it completely abolished the *MAG1* induction by MMS (Fig. 5B). This effect was displayed by both *rad53* mutant alleles available to us, namely *sad1-1* (12) and *mec2-1* (9,13). Furthermore, the *mec2-1* mutant was also completely defective in *RNR3* induction (Fig. 5C and 13). Thus, with regard to *MAG1* gene regulation in response to DNA damage, cell-cycle checkpoint genes can be divided into two groups: *RAD53* and *POL2* are absolutely required for the *MAG1* induction, whereas each of *RAD9*, *RAD17*, *RAD24* and *MEC1* is dispensable for *MAG1* induction.

DISCUSSION

In this study, we examined whether or not yeast cell-cycle checkpoint genes have a universal effect on a dual *MAG1-DDI1* gene induction in response to DNA damage. Apparently, DNA damage-induced transcriptional regulation is far more complicated than one has anticipated. First of all, the *DDI1* induction by MMS and UV is not affected by mutations in any of the checkpoint genes. Thus, the *DDI1* expression pattern appears to be different from that of *MAG1*, but similar to that of *UBI4*, which is also unaffected by all checkpoint mutations examined (13). Since *cis*-acting regulatory elements unique to *MAG1*, namely UAS_{MAG1} and URS_{MAG1}, have been identified (23–25), we infer that checkpoint genes may control *MAG1* induction through these elements. Secondly, *MAG1* induction is differentially affected by various checkpoint mutations. While *rad9*, *rad17*, *rad24* and

mec1-1 mutations have little effect on *MAG1* induction, *pol2-12*, *sad1-1* and *mec2-1* completely abolish MMS- and UV-induced expression of *MAG1*. The effect of *rad9*Δ on *MAG1* induction by UV was previously examined in G_1-arrested cells, and the maximum induction ratio decreased from 2.1 in the wild type cells to 1.3 in the *rad9*Δ cells (14). We did not observe such an effect in either asynchronous or G_1-arrested cell populations (data not shown). The discrepancy could derive from different strain backgrounds, since in TWY wild type cells, we were able to obtain an 8-fold *MAG1* induction after 50 J/m^2 UV treatment. Thirdly, the signal transduction cascade administrated by checkpoint genes does not appear to discriminate among lesions produced by different DNA-damaging agents. Both UV and MMS are well-characterized DNA-damaging agents and are used extensively in the study of cell-cycle checkpoint functions; however, UV mainly causes G_1/S (7) and G_2/M arrest (8,42), whereas MMS treatment delays S-phase progression (9,37) and causes G_1 pulse (43). Although checkpoint mutants respond differently to UV- and MMS-induced cell cycle arrest, UV and MMS induction of *MAG1* expression is indistinguishable in various checkpoint mutants. This phenomenon was also observed with some other DNA damage inducible genes examined to date (13).

At least two models could explain the results obtained from this study for the checkpoint regulation of *MAG1* induction by UV and MMS. A simple model would suggest a signal transduction pathway with Polε as a sensor to detect lesions and/or replication blocks in DNA and initiating the cascade, transmitting a signal either directly or indirectly to Rad53. This transmission does not require Mec1 protein kinase; however, if Rad53 phosphorylation is necessary for its activity in transcriptional regulation, a different ATM-like protein kinase, such as Tel1 (40,41), may be required. Indeed, both *MEC1* and *TEL1* are involved in the control of Rad53 phosphorylation (39). A second model argues that the lack of altered *MAG1* DNA damage induction in *rad9*, *rad17*, *rad24* and *mec1* mutants is probably due to the existence of alternative pathways parallel to these genes. Previous studies (44,45) have suggested that the gene regulation by checkpoint genes is not necessarily a linear sequence from Mec1 to Rad53 to Dun1. Recently, it was shown that *RAD9* and *RAD24/RAD17/MEC3* may define two additive, interacting branches of a DNA damage checkpoint pathway and that the double mutants defective in both branches have more severe effects with regard to cell cycle progression, DNA damage-induced gene regulation and Rad53 phosphorylation (36). This model is also consistent with the observations that *RAD9* is in a different epistasis group from *RAD17* and *RAD24* with respect to S-phase checkpoint and MMS sensitivity (37), and that *RAD9* has a different role than *RAD17*, *RAD24* and *MEC3* in the *in vivo* processing of DNA damage (46). However, our results with the *rad9*Δ *rad17*Δ double mutant indicate that genes in these two branches play a minor, if any, role in the control of DNA damage-induced expression of *DDI1-MAG1*. Alternatively, a third branch may exist parallel to *RAD9* and *RAD17/RAD24/MEC3*. In the case of *MEC1*, an alternative pathway for the gene regulation function is likely to be *TEL1*. *TEL1* is not only a sequence homolog of *MEC1*, but overexpression of *TEL1* is able to rescue the essential cellular function in a *mec1* disruption mutant, as well as the sensitivity to DNA-damaging agents of the *mec1-1* mutant (41). It is interesting to note that *TEL1* is required for telomere maintenance (40), but has no distinguishable role in checkpoint arrest. However, it may be able to replace *MEC1* in the transcriptional response to DNA damage (39). This second model would also be consistent with the observation (13 and this study) that *mec1-1* only partially reduces MMS-induced *RNR3* expression, in contrast to the *mec2-1* mutation that completely abolishes *RNR3* induction. In this regard, recent observations (47) that phosphorylated Rad9 specifically binds to Rad53 and that Mec1 is probably involved in Rad9 phosphorylation in response to DNA damage but not replication inhibition, may shed light on the understanding of alternative regulatory pathways. This, however, does not rule out the possibility that the *mec1-1* is a partial loss-of-function mutation with regard to transcriptional activation.

The *RNR3* gene, and possibly some other DNA damage inducible genes, responds to checkpoint mutations differently than *MAG1*, in that the *RNR3* induction by DNA damaging agents is affected by all checkpoint mutations, including *rad9*, *17*, *24*, *mec3* and *mec1-1* (13–15,46). This differential response is not yet clear at the mechanistic level. However, it is noticed in this study that the optimal induction of *RNR3* by MMS is at 0.01%, whereas that of *MAG1* is at 0.05–0.075%; the different thresholds for DNA damage induction together with the different response to checkpoint mutations would be better explained by the second proposed model.

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