MOLECULAR AND CELLULAR BIOLOGY, June 1997, p. 3356–3363 Vol. 17, No. 6
0270-7306/97/$04.00+0

Discrete Roles of the Spc1 Kinase and the Atf1 Transcription Factor in the UV Response of *Schizosaccharomyces pombe*

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Received 22 November 1996/Returned for modification 23 January 1997/Accepted 18 March 1997

Exposure of mammalian cells to UV irradiation or alkylating agents leads to the activation of the c-Jun N-terminal kinase and p38 stress-activated protein kinase cascades, phosphorylation of c-Jun and ATF-2 bZIP transcription factors, and finally to selective induction of gene expression. This UV response is believed to be crucially important for cell survival, although conclusive evidence is lacking. Here, we address this issue by investigating a homologous UV response pathway in the fission yeast *Schizosaccharomyces pombe*. In fission yeast cells, UV irradiation induces activation of Spc1 stress-activated protein kinase, which in turn phosphorylates the Atf1 bZIP transcription factor. *spc1* mutants are hypersensitive to killing by UV at a level equivalent to some checkpoint *rad* mutants. Whereas checkpoint *rad* mutants fail to arrest division in response to DNA damage, *spc1* mutants are defective at resuming cell division after UV exposure. Levels of basal and UV-induced transcription of *ctt1*$^+$, which encodes a catalase believed important for combating oxidative stress caused by UV, are extremely low in *spc1* mutants. Atf1 is required for UV-induced transcription of *ctt1*$^+$, but *atf1* mutants are not hypersensitive to killing by UV. This surprising finding is explained by the observation that *ctt1*$^+$ basal expression is unaffected in *atf1* single mutant and *spc1 atf1* double mutant cells, suggesting that unphosphorylated Atf1 represses *ctt1*$^+$ expression in *spc1* cells. In fact, the level of UV sensitivity of *spc1 atf1* double mutant cells is intermediate between those of the wild type and *spc1* mutants. These findings suggest the following. (i) Key properties of UV response mechanisms are remarkably similar in mammals and *S. pombe*. (ii) Activation of Spc1 kinase greatly enhances survival of UV-irradiated cells. (iii) Induction of gene expression by activation of Atf1 may not be the most important mechanism by which stress-activated kinases function in the UV response.

Mammalian cells exposed to shortwave UV irradiation or alkylating agents such as methyl methanesulfonate (MMS) respond by inducing the transcription of a specific set of genes by a process that is generically known as the UV response (8, 10, 11, 13). Most of the details of the mammalian UV response remain to be determined, but the emerging picture is that of two stress-activated protein kinase cascades activating multiple transcription factors that typically function in heterodimeric complexes. One pathway involves a related family of c-Jun N-terminal kinases (JNKs) that are activated by a variety of forms of stress and hence are also referred to as stress-activated protein kinases (SAPKs) (8, 20). JNKs are related to mitogen-activated protein kinases (MAPKs) and thus follow the pattern of being activated by MAPK kinases (MAPKKs or MEKs) that are themselves activated by MAPKK kinases (MAPKKKs or MKKs) (39). A second pathway involved in the UV response leads to activation of a class of related protein kinases that are most well known as p38/RK kinases. p38/RK kinases are also regulated by upstream protein kinase cascades that follow the MAPK format (9, 16, 22, 28, 30). Like JNKs, p38 kinases are activated in response to a variety of stresses, including UV and MMS.

The best-defined transcription factor targets of JNK and p38 kinases belong to the family of bZIP transcription factors, so called because they contain a region of basic amino acids followed by leucine zipper sequences (42). Notable members of the bZIP transcription factor family include Jun, Fos, and ATF-2. A well-established target of JNKs is Jun, which together with Fos forms the AP-1 transcription factor complex (8, 17, 20). JNKs and p38 kinases are also capable of activating ATF-2 by direct phosphorylation (15, 37).

UV and MMS are well-known DNA-damaging agents, but it is believed that the mammalian UV response is not triggered by DNA damage (10). Indeed, in enucleated HeLa cells, JNK becomes highly activated in response to UV exposure, thereby ruling out the possibility that damage of nuclear chromosomal DNA is required for the UV response (11). Instead, it is believed that the UV response may be primarily induced at the cell membrane, possibly through the peroxidation of lipids. Likewise, alkylating agents may activate the UV response through reactions with free SH groups. These reactions cause a depletion of the intracellular pool of reduced glutathione (GSH), resulting in oxidative stress (21). A key finding in support of this notion is that the AP-1-induced transcription of c-*jun* is prevented by incubation of HeLa cells in medium containing *N*-acetylcysteine (NAC) (10). NAC is rapidly converted to GSH, which in turn acts as an effective intracellular scavenger of free radicals.

The functional significance of the mammalian UV response is not clearly understood. None of the AP-1-dependent-inducible genes are involved in DNA repair, whereas there are at least two UV-inducible genes regulated by AP-1 for which a role in counteracting the adverse effects of free radicals might be inferred: genes encoding glutathione *S*-transferase and metallothioneins (2). Thus, it appears that the UV response is likely to be involved in combating oxidative damage, although it remains to be established whether the induction of oxidative stress genes actually has a protective effect against UV irradiation and exposure to alkylating agents. It is possible that in addition to activating transcription factors, stress-activated ki-

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nases may have other functions that are important for protecting cells from UV damage.

Recent studies of the fission yeast *Schizosaccharomyces pombe* have resulted in the identification of a protein kinase, Spc1, that closely resembles the stress-activated kinases of the JNK-p38 families in mammalian cells (19, 24, 34) and budding yeast Hog1p kinase (4). Spc1, also known as Sty1 and Phh1, is activated by a variety of stresses, including nitrogen limitation, high osmolarity, heat stress, and hydrogen peroxide treatment (7). The transmission of all of these stress signals requires Wis1, a MAPKK homolog (38). Spc1 activity is negatively regulated by Pyp1 and Pyp2 tyrosine-specific phosphatases via direct dephosphorylation of the tyrosine residue phosphorylated by Wis1. Interestingly, the Wis1-Spc1 kinase cascade is linked to the G_2-M cell cycle control mechanism (24, 34). Under optimum growth conditions, *spc1* mutants exhibit a moderate delay of the onset of mitosis that is greatly exacerbated under stressful conditions. Recent studies have provided strong evidence that Spc1 directly regulates Atf1, a bZIP transcription factor (18, 35, 36, 41). Atf1 phosphorylation is directly catalyzed by Spc1 in vivo. Moreover, osmotic stress-induced transcription of genes such as *gpd1*$^+$, which encodes an enzyme required for glycerol synthesis, is greatly reduced in *spc1* and *atf1* mutants. The discovery that Spc1 and Atf1 have close structural similarities to p38 and ATF-2, respectively, and that Spc1 phosphorylates Atf1 in response to a range of stress stimuli suggests a remarkable degree of conservation between stress-activated signal transduction pathways in fission yeasts and mammals.

The discovery of similar stress response mechanisms in fission yeasts and mammals presents an opportunity to investigate these signal transduction systems by means that were not previously possible, taking advantage of genetic methods to answer questions that delve into causality and physiological significance. Accordingly, in this paper, we report experiments that have addressed a number of issues relevant to the response of fission yeast cells to genotoxic agents such as UV and MMS. We have found that the Wis1-Spc1 pathway is essential for a UV response that is triggered by oxidative stress. Moreover, failure to activate Spc1 leads to a profound sensitivity to killing by UV and MMS. We report that Atf1 is required for UV-induced elevation of stress response genes and explain the paradoxical finding that *atf1* mutants are not abnormally sensitive to UV and MMS. These studies reveal discrete roles for a UV-responsive MAPK and its transcription factor substrate in mediating the cellular response to UV irradiation.

MATERIALS AND METHODS

Yeast strains, media, and general methods. The *S. pombe* strains used in this study are described in Table 1. YES and synthetic EMM2 media were used for growth media. Fission yeast experimental methods and media have been described previously (1, 25).

Gene expression analysis. Northern hybridization analyses were performed as described previously (31). A 1-kb *Eco*RV fragment from pJK148 was used for the control *leu1*$^+$ probe. A 2,461-bp fragment of *rad8*$^+$ was amplified by PCR with the 5′ oligonucleotide 5′-TCGGATGCTTTGGTGTGGAGG-3′ and the 3′ oligonucleotide 5′-GTCTCTAAAGCCGTCGACCTC-3′. A 1,021-bp fragment of *ctt1*$^+$ was amplified by PCR with the 5′ oligonucleotide 5′-TCCTGAACGTGT CGTCCATGCAAAGG-3′ and the 3′ oligonucleotide 5′-GACGGATTGAGG ATGGATAGTTTG-3′. A 2.3-kb *Bam*HI fragment from the pREP3-pyp2 plasmid was used to probe for *pyp2*$^+$ mRNA.

Detection of the Spc1Ha6H and Atf1Ha6H proteins. Immunoblot analysis of Spc1 protein was performed with a strain having a chromosomal copy of *spc1-Ha6H* (34). This gene encodes Spc1 protein having a C-terminal tag containing two copies of the influenza virus hemagglutinin (HA) epitope followed by six consecutive histidine residues. Atf1 was also tagged at its C terminal end with two copies of HA and six histidine residues, and this construct was expressed from the *atf1*$^+$ promoter (35). Spc1 and Atf1 proteins were purified by Ni^{2+}-nitrilotriacetic acid (NTA)-agarose chromatography, resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), electroblotted to nitrocellulose, and then detected with either anti-HA (12CA5) or anti-phosphotyrosine (4G10; Upstate Biotechnology) antibodies as described previously (34). Immunoreactive bands were detected with horseradish peroxidase-conjugated secondary antibodies and the ECL (enhanced chemiluminescence) Western blotting detection system (Amersham). For the UV irradiation studies, cells were collected by filtration on 25-mm-diameter Metricel membrane filters (Gelman), irradiated with a Bio-Rad Genelinker, and then resuspended in liquid YES medium.

TABLE 1. *S. pombe* strains used in this study

Strain[a]	Genotype	Source or reference
PR109	*h*$^-$	Laboratory stock
KS1147	*h*$^+$ *spc1-M13*	34
KS1366	*h*$^-$ *spc1::ura4*$^+$	34
KS1376	*h*$^-$ *spc1HA6H(ura4*$^+$*)*	34
KS1455	*h*$^-$ *rad24::ura4*$^+$	T. Carr
KS1479	*h*$^-$ *atf1HA6H(ura4*$^+$*)*	35
KS1497	*h*$^-$ *atf1::ura4*$^+$	35
KS1533	*h*$^-$ *atf1::ura4*$^+$ *spc1::ura4*$^+$	35
KS1572	*h*$^-$ *spc1::ura4*$^+$ *atf1HA6H(ura4*$^+$*)*	35
JL1188	*h*$^+$ *rad1-1*	S. Subramani
JM544	*h*$^-$ *wis1::ura4*$^+$	Laboratory stock
GD1520	*h*$^+$ *cdc17-K42 spc1HA6H(ura4*$^+$*)*	This study
GD1683	*h*$^+$ *wis1::ura4*$^+$ *spc1HA6H(ura4*$^+$*)*	This study

[a] All strains are *leu1-32 ura4-D18*.

UV and MMS survival studies. For the UV sensitivity experiments, 2,000 cells of a log-phase culture were plated in duplicate for each dose and irradiated at 254 nm with a Bio-Rad Genelinker. Survival curves represent the mean value of three experiments. For the MMS sensitivity experiments, exponentially growing cells were incubated for 1 h with the indicated concentrations of MMS. The drug was inactivated with 5% sodium thiosulfate, and appropriate dilutions of cells were plated on YES medium. Survival curves represent the mean value of three experiments.

RESULTS

UV irradiation and MMS treatment stimulate Spc1 tyrosine phosphorylation. The initial aim of this study was to determine whether *S. pombe* has a UV response mechanism that is similar to the JNK and p38 kinase pathways in mammalian cells. MAPKKs activate MAPKs by phosphorylating threonine and tyrosine residues in the sequence Thr-X-Tyr; thus, a hallmark of the mammalian UV response is the detection of tyrosine-phosphorylated JNK and p38 by the use of antiphosphotyrosine antibodies. Therefore, the potential involvement of Spc1 in a UV response in *S. pombe* was carried out by analysis of Spc1 tyrosine phosphorylation. These studies used a strain that has a single chromosomal copy of *spc1*$^+$ tagged with a sequence encoding two copies of the HA epitope and six consecutive histidine residues (34). This allowed us to purify Spc1 by using Ni^{2+}-NTA beads and to detect it by using anti-HA antibodies.

Spc1 underwent rapid tyrosine phosphorylation in response to UV irradiation (Fig. 1A). The response was quite transient, nearing peak levels within 10 min of irradiation and decreasing to preirradiation levels within 60 min posttreatment. To further confirm the activation of Spc1 by UV irradiation, we have examined the phosphorylation state of the Atf1 transcription factor. Atf1 is a downstream component of the Wis1-Spc1 pathway that becomes phosphorylated by Spc1 kinase during stress (35). For this analysis, we used a strain carrying a chromosomal *atf1*$^+$ gene tagged with a sequence encoding the HA antigen and six consecutive histidine residues (35). Atf1 protein purified from unstressed cells migrated with an apparent molecular mass of ~85 kDa in SDS-PAGE (Fig. 1B). Fifteen minutes after UV irradiation, Atf1 migrated as a somewhat diffuse band with a reduced mobility of ~90 kDa. This mobility shift was previously shown to be caused by phosphorylation

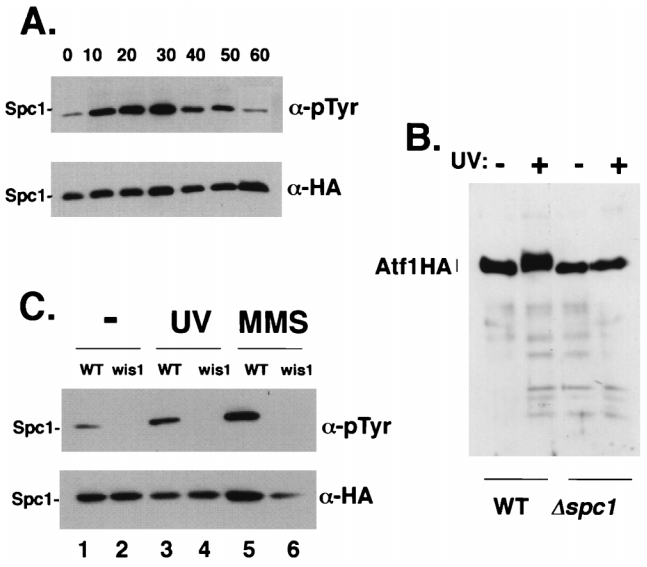

FIG. 1. Wis1-dependent activation of Spc1 in response to UV and MMS. (A) Wild-type (WT) cells (KS1376) were UV irradiated at 200 J/m^2 (as described in Materials and Methods) and incubated for the indicated time (minutes) in YES medium. Epitope-tagged Spc1 was isolated by Ni^{2+}-NTA affinity precipitation and immunoblotted to detect phosphotyrosine (α-pTyr) or HA epitope tag (α-HA). (B) Spc1-dependent phosphorylation of Atf1 after UV irradiation. Wild-type (KS1479) and Δ*spc1* (KS1572) strains were grown in YES medium at 30°C, and aliquots were harvested before or 15 min after UV irradiation (200 J/m^2). Epitope-tagged Atf1 was isolated by Ni^{2+}-NTA affinity precipitation and analyzed by SDS-PAGE followed by immunoblotting with anti-HA antibodies. (C) UV and MMS activation of Spc1 is dependent on Wis1. Wild-type and Δ*wis1* mutant cells were incubated under unstressful conditions (lanes 1 and 2), UV irradiated at 200 J/m^2 followed by 15 min of incubation in YES medium (lanes 3 and 4), or exposed to 0.05% MMS for 20 min (lanes 5 and 6). Spc1 was analyzed as described above.

carried out by Spc1 kinase (35). In contrast, Atf1 protein purified from a Δ*spc1* strain migrated with an apparent molecular mass of ~85 kDa even after UV irradiation. These data show that Atf1 is phosphorylated in vivo in response to UV irradiation in an *spc1*$^+$-dependent manner.

Wis1 MAPKK is required for the transmission of high-temperature, oxidative stress and osmotic stress signals to Spc1 (7, 24, 34). We therefore examined whether Wis1 was also required for the UV-induced activation of Spc1 by comparing Spc1 tyrosine phosphorylation in wild-type and Δ*wis1* backgrounds. This analysis showed that UV-induced tyrosine phosphorylation of Spc1 was abolished in Δ*wis1* cells (Fig. 1C). Similar experiments were carried out with cells treated with MMS. As shown in Fig. 1C, MMS treatment induced a high level of Spc1 tyrosine phosphorylation that was abolished in a Δ*wis1* background. These studies establish that the Wis1-Spc1 kinase cascade is activated in response to UV irradiation and an alkylating agent, findings that are highly reminiscent of the mammalian UV response.

Mechanism of Spc1 activation by UV irradiation. The mammalian UV response appears to be activated by damage to components in the cell membrane, as opposed to damage of nuclear DNA. Moreover, it is believed that the mammalian UV response occurs mainly as a reaction to oxidative stress, probably by the formation of free radicals in a process involving lipid peroxidation (3, 10). To explore the situation in *S. pombe*, we examined the effect of incubating cells in NAC, which when taken up by cells is readily converted to GSH, a scavenger of free radicals (23). By itself, NAC had no effect on the Spc1 tyrosine phosphorylation in unstressed cells (Fig. 2).

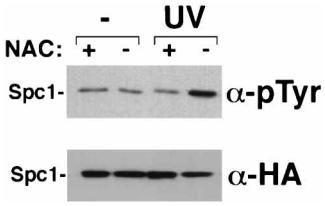

FIG. 2. NAC prevents Spc1 tyrosine phosphorylation in response to UV irradiation. Wild-type cells were incubated in the presence or in the absence of NAC (40 mM) for 1 h prior to exposure to UV irradiation (100 J/m^2). Samples were collected 15 min later. Spc1 was isolated by Ni^{2+}-NTA affinity precipitation and probed for the presence of phosphotyrosine (α-pTyr) or HA epitope tag (α-HA).

However, the UV-induced increase in Spc1 tyrosine phosphorylation was completely abrogated by preincubation of cells with NAC (Fig. 2). Similarly, MMS activation of Spc1 was prevented by incubation in medium containing NAC (data not shown).

To more directly address the possibility that DNA damage by itself leads to activation of Spc1, an experiment was carried out to introduce DNA lesions into the genome without causing environmental stress. This experiment employed a strain carrying the *cdc17-K42* mutation, which encodes a temperature-sensitive version of DNA ligase (27). Incubation of *cdc17-K42* cells at the restrictive temperature of 35°C results in the accumulation of unligated DNA. As shown in Fig. 3A, incubation of *cdc17-K42* cells at the restrictive temperature also leads to the induction of expression of *rad8*$^+$, a gene believed to encode a DNA helicase that is involved in DNA repair (12). However, during the time course of this experiment, there was no change in the level of Spc1 tyrosine phosphorylation (Fig. 3B), even after 5 h of incubation at the restrictive temperature (data not shown). (Note that in this experiment the culture temperature

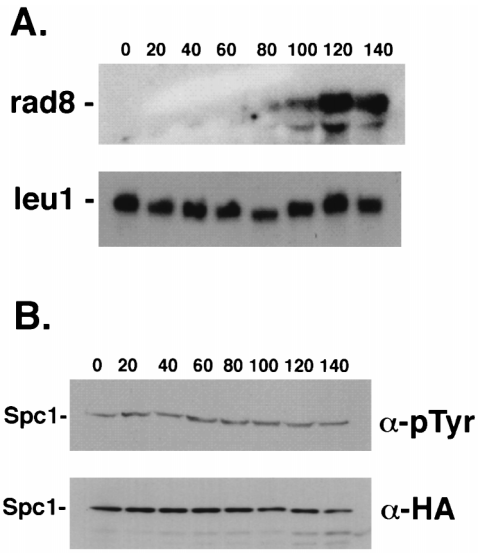

FIG. 3. Spc1 tyrosine phosphorylation is not responsive to unligated DNA. Strain GD1514, having a temperature-sensitive mutation of the DNA ligase gene (*cdc17-K42*), was grown to mid-log phase at the permissive temperature of 30°C. The culture temperature was then gradually shifted to the restrictive temperature of 35°C over a period of 30 min. Samples were collected at the indicated times after the temperature had been shifted. (A) Northern analysis showed elevated expression of the *rad8*$^+$ gene, believed to encode a DNA helicase involved in DNA repair. (B) Spc1 tyrosine phosphorylation was unchanged during the time course of the experiment.

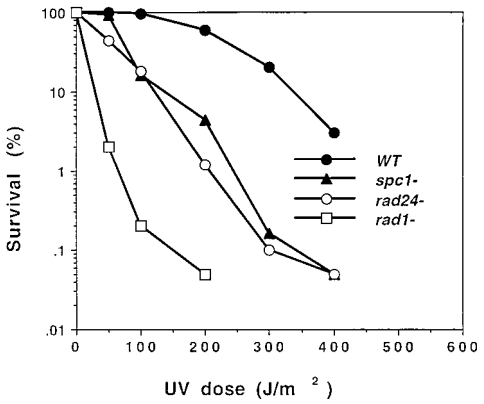

FIG. 4. Spc1 is important for survival of UV irradiation. Wild-type (WT) (PR109), *spc1-M13* (KS1147), *rad24::ura4*$^+$ (KS1455), and *rad1-1* (JL1188) strains were exposed to the indicated doses of UV irradiation, and survival was measured as described in Materials and Methods. The *spc1-M13* cells were hypersensitive to killing by UV at a level equivalent to that of the Δ*rad24* cells, whereas *rad1-1* cells were several orders of magnitude more sensitive to UV.

was gradually raised from 30°C to 35°C to avoid causing heat stress.)

These findings strongly suggest that treatments such as UV irradiation and exposure to alkylating agents activate Spc1 kinase by causing oxidative stress. DNA lesions do not appear to be involved in activating the Spc1 pathway.

Survival of *spc1-M13* mutants after exposure to UV and MMS. Although the existence of a UV response in mammalian cells has been established for a number of years, a clear understanding of the physiological importance of the UV response has been lacking, mainly due to the inability to specifically ablate the UV response. Therefore, the existence of *spc1* mutants of fission yeast presented a valuable opportunity to address the question of whether the UV response is important for combating the cytotoxic effects of UV irradiation. Wild-type and *spc1-M13* mutant cells were grown to mid-log phase, exposed to increasing doses of UV, and assayed for viability by colony-forming ability. This analysis revealed that *spc1-M13* or Δ*spc1* cells were highly sensitive to UV irradiation (Fig. 4 and data not shown). For example, at a dose of 300 J/m^2, at which ~30% of the wild-type cells survived, only ~0.2% of the *spc1-M13* cells were able to form colonies. Δ*wis1* cells were also profoundly sensitive to UV irradiation, at a level equivalent to that of *spc1-M13* cells (data not shown), in agreement with the observation that Wis1 is required for the tyrosine phosphorylation of Spc1 that is induced by UV.

An experiment was carried out to gain a sense of the importance of the Spc1-regulated UV response relative to the DNA damage repair and checkpoint mechanisms. Fission yeast *rad* mutants can be broadly divided into two groups: those that are defective both for DNA repair and the G_2-M checkpoint, such as *rad1* mutants, and those that are defective only in the checkpoint itself, such as *rad24* mutants (5). We therefore compared the UV sensitivities of *spc1-M13*, *rad1-1*, and *rad24::ura4*$^+$ mutants (Fig. 4). This analysis showed that the UV sensitivity of *spc1-M13* cells was approximately the same as that of *rad24::ura4*$^+$ cells. In contrast, *rad1-1* cells were much more UV sensitive than either *spc1-M13* or *rad24::ura4*$^+$ cells.

The ability of *spc1-M13* cells to survive exposure to MMS was also investigated. Consistent with the studies of UV sensitivity, we found that the ability of *spc1-M13* cells to survive MMS treatment was dramatically decreased relative to that of wild-type cells (data not shown and see Fig. 6B). As was the case for UV treatment, the MMS survival properties of *spc1-M13* cells were equivalent to those of Δ*wis1* and *rad24* strains (data not shown and Fig. 6B). These findings show that activation of Spc1 is very important for survival of UV and MMS treatment.

Cell cycle defect of *spc1* mutants exposed to UV irradiation. In *S. pombe*, a number of UV-sensitive *rad* mutants have been classified as checkpoint-defective mutants because they fail to arrest cell cycle progression in G_2 in response to DNA-damaging agents (33). In this sense, checkpoint proteins such as Rad24 are involved in inhibiting cell cycle progression. In contrast, Spc1 kinase has a positive role in promoting the onset of mitosis (24, 34). The discovery that Spc1 is involved in controlling the response to agents that have both cytotoxic and genotoxic consequences prompted an examination of the effect of UV irradiation on the cell division properties of *spc1* mutant cells. As was the case for wild-type cells, UV exposure (200 J/m^2) caused the cell division (septation) index of *spc1-M13* cells to drop to 0 within 90 min after irradiation. However, UV-irradiated *spc1-M13* cells were highly defective in resuming cell division. At 6 h postirradiation, *spc1-M13* cells were elongated and very few cells had division septa, whereas wild-type cells had resumed cell division at this time (Fig. 5). Δ*wis1* cells behaved exactly like *spc1-M13* mutants (data not shown). These findings show that activation of Spc1 kinase as part of the UV response is required to resume cell division after exposure to UV irradiation.

Δ*atf1* cells are not UV sensitive. A hallmark of the mammalian UV response is the transcriptional induction of stress response genes. In *S. pombe*, Spc1 kinase promotes the induction of expression of stress response genes such as *gpd1*$^+$ and *pyp2*$^+$ by a mechanism that requires Atf1, a bZIP transcription factor (35, 41). In fact, both basal transcription and stress-induced transcription of *gpd1*$^+$ and *pyp2*$^+$ are extremely low in Δ*atf1* mutants. Consistent with this finding, Δ*atf1* mutants grow very poorly on medium supplemented with 1 M KCl, although the defect is less severe than that of *spc1* mutants. As shown in Fig. 1B, Atf1 is phosphorylated after UV irradiation in an Spc1-dependent manner. To further explore the role of Atf1 in the UV response, we examined the UV sensitivity of Δ*atf1* mutants. Much to our surprise, we found that the UV sensitivity of Δ*atf1* mutants was not enhanced relative to that of wild-type cells (Fig. 6A). This pattern was replicated when

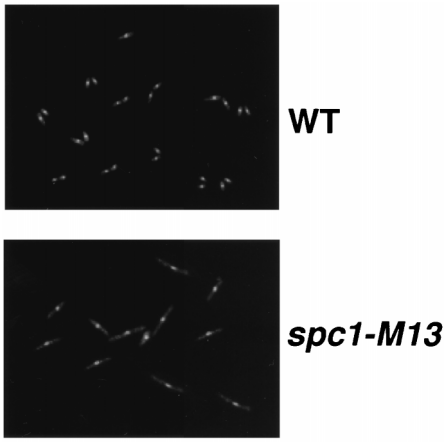

FIG. 5. *spc1-M13* cells exhibit a cell cycle arrest phenotype when exposed to UV irradiation. Exponentially growing wild-type (WT) and *spc1-M13* cells were irradiated at 200 J/m^2. Cells were then incubated in YES medium at 30°C for 6 h, fixed with ethanol, and stained with the DNA dye 4′,6-diamidino-2-phenylindole.

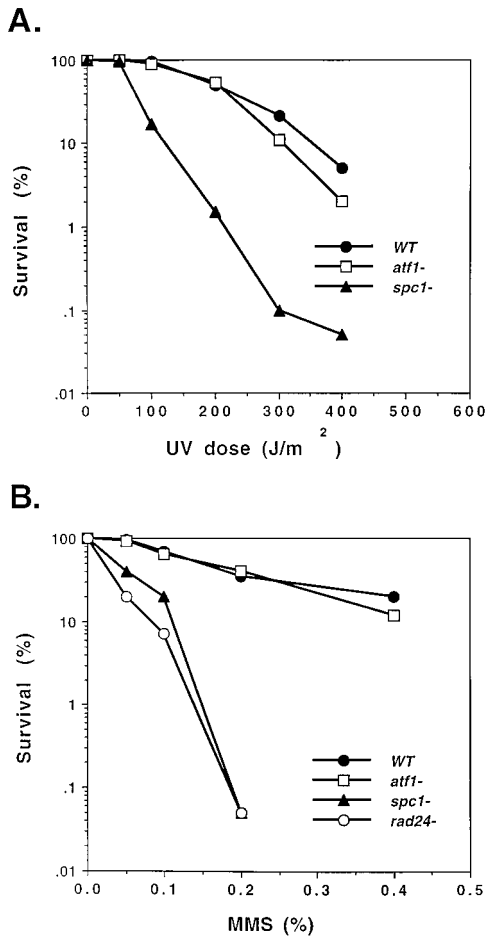

FIG. 6. Δ*atf1* cells are not hypersensitive to killing by UV irradiation or MMS treatment. (A) Cell survival assays were performed with wild-type (WT) (PR109), *atf1::ura4*$^+$ (KS1497), and *spc1-M13* (KS1147) cells exposed to the indicated doses of UV irradiation. (B) Cell survival assays were performed with wild-type (PR109), Δ*atf1* (KS1497), *spc1-M13* (KS1147), and *rad24::ura4*$^+$ (KS1455) cells exposed to the indicated doses of MMS.

Δ*atf1* cells were tested for MMS sensitivity: Δ*atf1* and wild-type cells survived equally well following exposure to MMS, whereas *spc1-M13* cells were profoundly sensitive to MMS treatment (Fig. 6B).

These results might be explained if Atf1 is not required for transmitting the Spc1-dependent transcription induction signal that is activated following exposure to UV irradiation. Perhaps there is a transcription factor target other than Atf1 that is important for the UV response. We addressed this question by measuring *pyp2*$^+$ expression in Δ*atf1* cells following UV exposure. UV-induced expression of *pyp2*$^+$ was abolished in Δ*atf1* cells and Δ*spc1* cells (Fig. 7). These findings show that an intact Spc1-Atf1 signaling pathway is required for the UV-induced expression of *pyp2*$^+$.

Atf1 is required for UV-induced transcription of *ctt1*$^+$, a gene encoding catalase. These results called into question the notion that Spc1-dependent induction of stress response genes is important for survival of UV and MMS treatment. It is likely that Spc1 has important functions that do not involve Atf1; indeed the cell cycle defects of *spc1-M13* mutants are not replicated in Δ*atf1* strains (35, 41). Therefore, it is possible that Spc1 carries out functions required for UV survival that do not involve transcriptional induction. However, a deficiency in this argument was that genes such as *pyp2*$^+$ have no obvious roles in combating oxidative stress; thus, a defect in expressing *pyp2*$^+$ is in itself not incompatible with the fact that Δ*atf1* cells are not abnormally sensitive to UV and MMS. Therefore, it became important to identify an Atf1-regulated gene that was likely to be directly involved in protecting cells from oxidative stress. One such protein is catalase, which rapidly catalyzes the decomposition of hydrogen peroxide to water and oxygen. Quite recently, the gene encoding cytosolic catalase, *ctt1*$^+$, was identified in *S. pombe* (26). Both oxidative stress and UV irradiation cause a large increase in the level of *ctt1*$^+$ mRNA, indicating that induction of *ctt1*$^+$ transcription may be an important part of the UV response in *S. pombe*. In *Saccharomyces cerevisiae*, catalase T is important for survival of oxidative stress, heat shock, and osmotic stress (40). Moreover, the HOG1 pathway regulates the osmotic induction of the *CTT1* gene (32).

We found that transcription of *ctt1*$^+$ was regulated by Spc1. UV irradiation caused a large increase in the level of *ctt1*$^+$ mRNA by a mechanism that was abolished in a Δ*spc1* or *spc1-M13* background (Fig. 8A and data not shown). Moreover, the basal level of *ctt1*$^+$ expression was also reduced in Δ*spc1* or *spc1-M13* cells. These observations suggested that the reduced level of *ctt1*$^+$ basal expression in Δ*spc1* cells may, in part, account for their high sensitivity to killing by UV and MMS.

Interestingly, there was a very different pattern of *ctt1*$^+$ expression in Δ*atf1* mutants relative to Δ*spc1* cells (Fig. 8A). The basal amount of *ctt1*$^+$ mRNA was not decreased in Δ*atf1* mutants relative to that in the wild type, but there was no increase in the level of *ctt1*$^+$ mRNA after UV irradiation. Thus, Atf1 was not required for the basal level of *ctt1*$^+$ expression but was necessary for the increased level of *ctt1*$^+$ expression that is part of the UV response. These differences between Δ*spc1* and Δ*atf1* mutants may explain why Δ*spc1* cells are sensitive to UV and MMS, whereas Δ*atf1* cells behave like the wild type.

Atf1 represses *ctt1*$^+$ mRNA expression and enhances UV sensitivity in Δ*spc1* cells. Two explanations came to mind to explain how *ctt1*$^+$ expression could be highly dependent on Spc1 for both basal and UV-induced expression but require Atf1 only for the increased level of expression which occurs in response to UV stress. Perhaps the simplest possibility was that Spc1 regulated two transcription factors: one was sufficient for the basal level of *ctt1*$^+$ expression as observed in Δ*atf1* mutants, whereas the second factor, Atf1, was required for expression that is induced by UV irradiation. In this scheme, the first transcription factor would not be sufficient for UV-in-

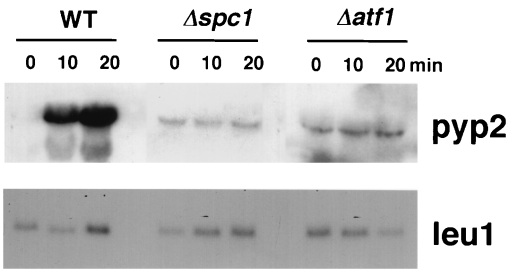

FIG. 7. Induction of *pyp2*$^+$ mRNA transcription in response to UV is dependent on Atf1 and Spc1. RNA samples were collected at the indicated times (minutes) from wild-type (WT), Δ*spc1*, and Δ*atf1* cells after the cells had received a 200-J/m^2 dose of UV irradiation. Northern analysis was performed with *pyp2*$^+$ and *leu1*$^+$ probes.

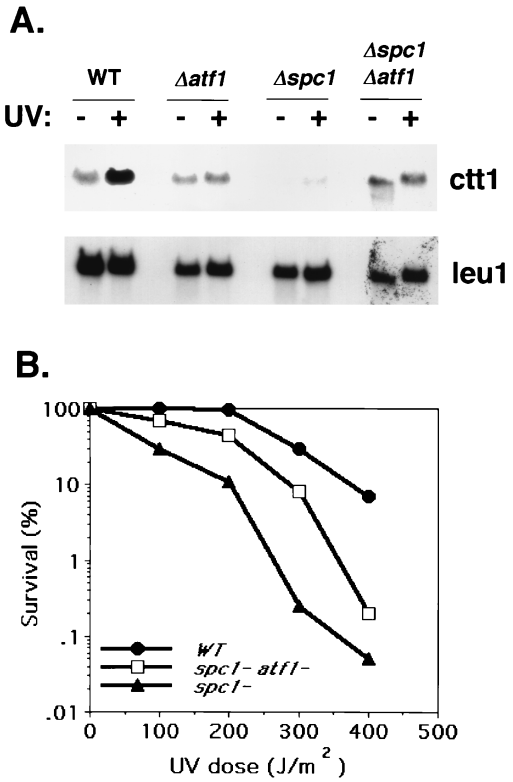

FIG. 8. Induction of expression of the *ctt1*$^+$ catalase gene in response to UV is regulated by Spc1 and Atf1. (A) RNA was isolated from wild-type (WT) (PR109), Δ*spc1* (KS1366), Δ*atf1* (KS1497), and Δ*spc1* Δ*atf1* (KS1533) cells after the cells had received a 200-J/m^2 dose of UV irradiation. Northern analysis was performed with *ctt1*$^+$ and *leu1*$^+$ probes. In wild-type cells, there was a large increase of *ctt1*$^+$ mRNA in response to UV. This response was absent in the three mutant strains. However, basal transcription of *ctt1*$^+$ was reduced only in the Δ*spc1* strain, suggesting that Atf1 may act as a repressor of *ctt1*$^+$ expression in Δ*spc1* cells. (B) UV irradiation survival properties of wild-type, Δ*spc1*, and Δ*spc1* Δ*atf1* cells. The Δ*spc1* Δ*atf1* double mutant exhibits a UV survival phenotype intermediate between that of wild-type and Δ*spc1* mutant cells.

duced transcription. The second model proposed that Atf1 has both transcriptional activator and repressor properties. In the absence of Spc1, Atf1 acts as a transcriptional repressor. On the other hand, in the absence of Atf1 a transcriptional mechanism that is independent of both Spc1 and Atf1 provides the basal level of *ctt1*$^+$ expression. In this model, Spc1 converts Atf1 from a transcriptional repressor to a transcriptional activator.

The two possibilities, which we named the two-factor and Atf1 activator-repressor models, made contrary predictions about the pattern of *ctt1*$^+$ expression in Δ*spc1* Δ*atf1* double mutants. The two-factor model proposed that *ctt1*$^+$ mRNA expression should be very low in the double mutant, equivalent to that observed in Δ*spc1* single mutant cells. In contrast, the Atf1 activator-repressor model made the opposite prediction, namely that there should be significant basal expression of *ctt1*$^+$ in the double mutant, equivalent to that seen in Δ*atf1* single mutant. Analysis of the Δ*spc1* Δ*atf1* double mutant strongly supported the activator-repressor model: the pattern of *ctt1*$^+$ expression in the double mutant was indistinguishable from that of the Δ*atf1* single mutant (Fig. 8A). This observation strongly suggests that Spc1 activity converts Atf1 from a transcriptional repressor to a transcriptional activator.

The question arose as to whether the basal expression of genes such as *ctt1*$^+$ contributed to cell survival following exposure to UV. This question was addressed by comparing the UV survival properties of wild-type, Δ*spc1* single mutant, and Δ*spc1* Δ*atf1* double mutant cells. Interestingly, the UV survival phenotype of the Δ*spc1* Δ*atf1* cells was intermediate between those of the wild-type and Δ*spc1* cells (Fig. 8B). Thus, the transcriptional repressor activity of Atf1 enhances UV killing in cells that lack Spc1 activity, although defects that cannot be ascribed to the repressor activity of Atf1 also contribute to the UV sensitivity of Δ*spc1* cells.

DISCUSSION

The major aim of our studies was to use fission yeast to investigate how eukaryotic cells respond to cytotoxic damage caused by agents such as UV and MMS. The first goal of the studies was to determine whether *S. pombe* has a UV response mechanism that is broadly analogous to the mammalian system. As described above, the mammalian UV response is believed to involve the activation of two classes of MAPK homologs, JNKs and p38 kinases. Thus, we initially asked whether Spc1 kinase, which is most closely related to p38 kinase, is activated in response to UV and MMS treatment. This analysis showed that Spc1 undergoes a large increase in activating tyrosine phosphorylation in response to both types of genotoxic agents. Importantly, the increase in Spc1 tyrosine phosphorylation was quite rapid and transient, typically approaching maximal levels within 10 min of exposure to UV and decreasing to basal levels within 60 min. Wis1 kinase was required for the increase of Spc1 tyrosine phosphorylation in response to UV and MMS. We also found that UV and MMS exposure resulted in the induced expression of the *pyp2*$^+$ and *ctt1*$^+$ genes, and this response was dependent on Atf1 transcription factor. Moreover, we observed that Atf1 was phosphorylated in vivo after UV irradiation in an Spc1-dependent manner. Thus, our findings show that fission yeast has a UV response mechanism that is transmitted through the Wis1-Spc1 kinase cascade via Atf1 transcription factor.

A second aim of our studies was to identify the nature of the stress or damage that is sensed by the Spc1 UV response pathway. As noted above, UV and MMS are well-known mutagens; thus, in theory these genotoxic agents could trigger the Spc1 UV response by damaging DNA. However, our findings strongly suggest that the Spc1 pathway is not responsive to DNA damage, or at least not to the presence of unligated DNA as occurs in *cdc17-K42* mutant cells. More importantly, our studies have shown that incubation of cells in NAC abolishes the UV-induced activation of Spc1. Intracellular NAC is converted to GSH, which in turn reacts with oxygen free radicals. Thus, the ability of NAC to suppress UV-induced activation of Spc1 kinase strongly suggests that Spc1 kinase is responding to oxidative stress. Indeed, previous studies have shown that the Spc1 kinase pathway becomes highly activated when cells are exposed to hydrogen peroxide, which causes acute oxidative stress (7).

The third important aim of our studies was to evaluate whether the UV response is important for survival of UV exposure. This is a disarmingly straightforward question that does not have a simple answer. First, it is clear that activation of Spc1 via Wis1 kinase is quite important for survival of UV irradiation and MMS treatment. For example, at a UV dose of 300 J/m^2, the survival of *spc1-M13* or Δ*spc1* cells is decreased ~100-fold relative to wild-type cells. The UV sensitivity of *spc1-M13* cells is comparable to that of Δ*rad24* mutants, which are defective in the G_2-M checkpoint (14). Likewise, *spc1-M13* cells exhibit a hypersensitivity to MMS treatment that is comparable to that exhibited by Δ*rad24* cells.

Questions relating to the physiological importance of the UV response become complicated when one considers the role of Atf1 transcription factor. Surprisingly, Δ*atf1* mutants are not hypersensitive to killing by UV or MMS; in fact, Δ*atf1* mutants and wild-type cells cannot be distinguished in this assay. This result could be easily understood if Atf1 was not involved in the UV response or if there were other Atf1-like transcription factors that provided redundant activities, but our studies show that Atf1 is required for the transcriptional induction of several UV-responsive genes. Of particular significance is the *ctt1*$^+$ gene, which encodes catalase, because the role of catalase in combating oxidative stress is well established. Expression of *ctt1*$^+$ mRNA is highly elevated in response to UV; this response is abolished in a Δ*atf1* mutant. Why then are Δ*atf1* and Δ*spc1* mutants not similarly sensitive to killing by exposure to UV or MMS? Our studies suggest the difference between Δ*atf1* and Δ*spc1* mutants in UV and MMS sensitivity may be partly explained by differences in basal expression of important stress response genes. In Δ*spc1* cells, the basal expression of *ctt1*$^+$ is very low, much less than is observed in wild-type cells grown under unstressful conditions. In contrast, basal *ctt1*$^+$ expression in Δ*atf1* mutants is not decreased relative to that of the wild type. Basal expression of *ctt1*$^+$ is also approximately normal in Δ*spc1* Δ*atf1* double mutant cells, indicating that Atf1 acts as a repressor of *ctt1*$^+$ expression in Δ*spc1* cells. The basal expression of *ctt1*$^+$ and perhaps other genes appears to be physiologically important, because UV survival is enhanced in the Δ*spc1* Δ*atf1* double mutant cells relative to that in the Δ*spc1* single mutant.

These observations lead to the following additional conclusions. (i) The increased expression of *ctt1*$^+$ that occurs following exposure is not important for survival of exposure to UV or MMS, at least not as assayed by the methods described here. The same can be said for other stress response genes regulated by Atf1. (ii) The fact that Δ*spc1* Δ*atf1* double mutant cells are more sensitive to UV killing than Δ*atf1* single mutant cells, even though both strains exhibit the same patterns and levels of *ctt1*$^+$ expression, shows that Spc1 contributes to UV survival by a mechanism that does not involve Atf1. In theory this could occur by a transcriptional induction mechanism that does not require Atf1 or regulate *ctt1*$^+$ expression, or it may be that Spc1 has important substrates that are not involved in transcriptional regulation. Resolution of this question will come with the identification of additional in vivo substrates of Spc1.

An ancillary discovery arising from our studies is that Atf1 transcription factor appears to act as a repressor of *ctt1*$^+$ expression in Δ*spc1* cells. As mentioned above, this conclusion is based on the observation that basal expression of *ctt1*$^+$ is enhanced in a Δ*spc1* Δ*atf1* double mutant relative to that in a Δ*spc1* single mutant. The simplest interpretation of this observation is that Atf1 is negatively influencing *ctt1*$^+$ expression in the absence of Spc1 activity. To our knowledge, this is the first indication that a bZIP transcription factor is converted from a transcriptional repressor to activator by the action of a MAPK homolog. We do not yet know exactly how Spc1 regulates Atf1 activity, other than that it involves direct phosphorylation of Atf1 by Spc1 in vivo (35).

The mammalian UV response, first identified as a characteristic pattern of altered gene expression induced by brief exposure to UV, has been recognized for more than a decade. Recent investigations have resulted in the identification of proteins that are believed to have important signal transduction functions in the UV response, such as p38/RK kinase and ATF-2 transcription factor. Other recent advances have also led to the availability of ATF-2-deficient mice and compounds that specifically inhibit p38/RK kinases (6, 22, 29), but as of yet, the effect of these mutations and kinase inhibitors on the survival of cells following UV exposure has not been described. Thus, the actual physiological significance of the mammalian UV response is uncertain. Our findings may be instructive in several regards. First, our studies of Spc1 kinase in fission yeast cells provide direct evidence that UV-activated kinases are important for survival of UV irradiation; thus, we may expect that JNK and p38 kinase will have similarly important functions in mammalian cells. Second, although our studies have clearly shown that Spc1 kinase is required for the transcriptional induction of a gene encoding a protein that has a well known role in combating oxidative stress, namely catalase, it cannot be assumed that transcriptional induction of stress response genes is important for survival of UV stress. Δ*atf1* mutants fail to induce *ctt1*$^+$ expression in response to UV and yet exhibit no defect in surviving UV irradiation. There has been an implicit assumption that the increased expression of AP-1-regulated genes that is the hallmark of the mammalian UV response is critically important for survival of UV damage, but our findings clearly indicate that for fission yeast cells, this assumption may be incorrect. Our findings suggest that stress-activated kinases such as p38 and JNK may have substrates other than transcription factors that have primary importance in promoting survival of cells in the face of UV irradiation. A challenge of future studies will be to identify these substrates; we anticipate that fission yeast cells will prove useful for accomplishing these goals.

ACKNOWLEDGMENTS

We thank the TSRI Cell Cycle Groups for their support, with particular thanks going to Kazuhiro Shiozaki, Frederique Gaits, and Junko Kanoh. Suresh Subramani, Tony Carr, and Susan Forsburg provided *rad* strains and the *cdc17-K42* strain.

G.D. was supported by an American Cancer Society International Cancer Research Fellowship. This work was funded by an NIH grant awarded to P.R.

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