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The *Aspergillus nidulans uvsB* Gene Encodes an *ATM*-Related Kinase Required for Multiple Facets of the DNA Damage Response

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ABSTRACT

In *Aspergillus nidulans*, *uvsB* and *uvsD* belong to the same epistasis group of DNA repair mutants. Recent observations suggest that these genes are likely to control cell cycle checkpoint responses to DNA damage and incomplete replication. Consistent with this notion, we show here that UVSB is a member of the conserved family of ATM-related kinases. Phenotypic characterization of *uvsB* mutants shows that they possess defects in additional aspects of the DNA damage response besides checkpoint control, including inhibition of septum formation, regulation of gene expression, and induced mutagenesis. The *musN227* mutation partially suppresses the poor growth and DNA damage sensitivity of *uvsB* mutants. Although *musN227* partially suppresses several *uvsB* defects, it does not restore checkpoint function to *uvsB* mutants. Notably, the failure of *uvsB* mutants to restrain septum formation in the presence of DNA damage is suppressed by the *musN227* mutation. We propose that UVSB functions as the central regulator of the *A. nidulans* DNA damage response, whereas MUSN promotes recovery by modulating a subset of the response.

SUCCESSFUL cellular proliferation is absolutely dependent on replication and segregation of the genome with the highest fidelity. The DNA damage response (DDR) is a protective mechanism that ensures the maintenance of genomic integrity during cellular reproduction. The DDR consists of extensive repair systems that deal directly with DNA damage, as well as surveillance systems, known as checkpoints, which arrest the cell cycle and provide a period of time for repair before the critical events of replication and segregation occur (Friedberg *et al.* 1995). Activation of the DNA damage checkpoint can halt the cell cycle at the G1-S transition (Siede *et al.* 1993, 1994), slow progression through S phase (Paulovich and Hartwell 1995), or cause arrest at the G2-M transition (Weinert and Hartwell 1988). Extensive molecular characterization of DNA damage checkpoint pathways in numerous eukaryotic organisms has revealed that regardless of the specific effect, checkpoint activation is controlled by the conserved family of ataxia telangiectasia mutated (ATM) kinases (Zakian 1995; Rotman and Shiloh 1998). In response to the presence of DNA damage, these PI-3 related kinases modulate cell cycle progression by phosphorylating multiple substrates, including p53 (Banin *et al.* 1998; Canman *et al.* 1998), and the Rad53/Cds1/Chk2 protein kinases (Sanchez *et al.* 1996; Matsuoka *et al.* 1998; Brown *et al.* 1999). In addition to their checkpoint function, several observations suggest that the ATM kinases regulate additional facets of the eukaryotic DDR. For example, in *Saccharomyces cerevisiae*, the ATM kinase Mec1p is required for the transcriptional induction of genes involved in the metabolism and repair of DNA damage (Aboussekhra *et al.* 1996; Kiser and Weinert 1996). Furthermore, in *Schizosaccharomyces pombe* and human cells, ATM kinases have been implicated in the control of DNA damage-induced mutagenesis (Gentner *et al.* 1978; Bennett and Rainbow 1988; Hilgers *et al.* 1989). The functional importance of the ATM kinases in the DDR is exemplified by the observation that mutations in the human ATM gene cause the cancer-prone syndrome ataxia telangiectasia (Savitsky *et al.* 1995).

Extensive genetic analyses have resulted in the characterization of a large number of DNA repair mutants in the filamentous fungus *Aspergillus nidulans* (reviewed by Kafer and May 1998). Furthermore, a subset of these mutants has been sorted into four distinct epistasis groups (Kafer and Mayor 1986; Chae and Kafer 1993), and representative members from three of these groups have been cloned (Yoon *et al.* 1995; Kafer and May 1997; Han *et al.* 1998). Despite this progress, the *A. nidulans* DDR remains poorly characterized. Analysis of cell cycle responses to DNA damage has shown that *A. nidulans* possesses a G2/M checkpoint that depends on inhibitory tyrosine phosphorylation of the cyclin-dependent kinase $NIMX^{cdk1}$ (Ye *et al.* 1997). We have shown that this pathway also mediates DNA damage-induced inhibition of cell division (septum formation; Harris and Kraus 1998). We have also demonstrated that *uvsB* and *uvsD* mutants, which define the remaining uncharacterized epistasis group of repair mutants, are unable to restrain septum formation in the presence of

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DNA damage (Harris and Kraus 1998). This result, together with the observation that *uvsB* and *uvsD* mutants are sensitive to a diverse array of DNA damaging agents (Kafer and Mayor 1986; Harris and Kraus 1998), suggests that these genes encode proteins involved in DNA damage checkpoint function. Here, we demonstrate that UVSB is a member of the family of ATM kinases that have been implicated in the cellular response to DNA damage. We show that UVSB is required for multiple facets of the *A. nidulans* DDR, including nuclear division arrest, induced mutagenesis, and possibly, DNA damage-regulated gene expression. Furthermore, we report that the *musN227* mutation partially suppresses specific DDR defects caused by *uvsB* mutations. We suggest that UVSB functions as the central regulator of the *A. nidulans* DDR, whereas MUSN is required for recovery and the resumption of normal cellular proliferation.

MATERIALS AND METHODS

Strains, media, and growth conditions: The following strains were used in this study: A28 (*pabaA6 biA1*), A781 (*nimA5; wA2*), A826 (*suA1adE20 adE20 biA1; uvsB505 ssbA1; sB3; choA1; chaA1*), AAH1 (*uvsB110; nimA5*), AAH2 (*uvsB110; musN227; nimA5*), AAH4 (*uvsD153; musN227; wA2*), AAH11 (*uvsB505; musN227; chaA1*), AAS280 (*musN227*; *nimX*^{cdc2AF};*wA2*), AAS315 (*uvsB110; musN227 pabaA1 acrA1 actA1 riboB2 chaA1*), AAS381 (*sepB3; uvsB110; musN227; chaA1*), ASH201 (*uvsB110; chaA1*), ASH202 (*sepB3; uvsB110; chaA1*), ASH204 (*uvsB110; argB2; wA2*), ASH206 (*uvsD153*; *wA2*), ASH208 (*sepB3; pabaA6; wA2*), ASH286 (*nimX*^{cdc2AF}; *wA2*), ASH380 (*sepB3; musN227; wA2*), and ASH383 (*musN227; chaA1*).

Media used were MAG (2% dextrose, 2% malt extract, 0.2% peptone, trace elements, and vitamins; pH 6.5), CM (1% dextrose, 0.2% peptone, 0.1% yeast extract, 0.1% casamino acids, nitrate salts, trace elements, and vitamins; pH 6.5), MNV (1% dextrose, nitrate salts, trace elements, and vitamins; pH 6.5), or YGV (2% dextrose, 0.5% yeast extract, and vitamins). Trace elements, nitrate salts, and vitamins were as described previously (Kafer 1977). Uridine (250 mm) and uracil (500 mm) were added as necessary to support the growth of pyrimidine requiring strains. Triton X-100 (0.01%) was added to solid media to promote colonial growth for viability assays. Hydroxyurea (HU; Sigma Chemical Co., St. Louis), diepoxyoctane (DEO; Aldrich Chemical Co., Milwaukee) and methyl methane sulfonate (MMS; Aldrich Chemical Co.) were added to the appropriate concentration after autoclaving. 5-Fluoroorotic acid (5-FOA; Angus Buffers and Biochemicals, Niagara Falls, NY) was added to media at a concentration of 1 mg/ml after autoclaving. All genetic manipulations performed in this study have been described previously (Harris *et al.* 1994). *sepB3; uvsB110; musN227* triple mutants were identified as temperature-sensitive segregants that were sensitive to 5 mm HU but exhibited slightly better growth on 0.005 and 0.01% MMS than the *sepB3*;*uvsB110* double mutant. The *musN227*; *nimX*^{cdc2AF} double mutants were identified as segregants that were sensitive to 5 mm HU and exhibited enhanced sensitivity to MMS as compared to the *musN227* mutant. Double and triple mutant genotypes were confirmed by a backcross to wild type.

Cloning of the *uvsB* gene: Strain ASH204 was cotransformed with the *argB*-based autonomously replicating plasmid pDHG25 (Gems *et al.* 1991) and pools of cosmids from a chromosome IV specific cosmid library (Brody *et al.* 1991). Transformants that could grow on MNV media containing 5 mm HU were recovered at 32°. Pools were subdivided until a single cosmid (W24C04) that could complement the HU hypersensitivity of *uvsB110* mutants was identified. Subsequent transformations with fragments derived from this cosmid localized the complementing activity to a 10-kb *Eco*RI-*Not*I fragment. Sequencing of this fragment and primer synthesis were performed by the Molecular Core Facility at the University of Connecticut Health Center. The BLAST algorithm at the NCBI (Altschul *et al.* 1997) was used to search the available DNA sequence databases. Several approaches were used to locate introns in the *uvsB* sequence, including (i) alignment of the amino acid sequence with that of its closest orthologue Rad3p, (ii) examination of the sequence for the presence of conserved splicing sequences (Gurr *et al.* 1987), and (iii) reverse transcriptase (RT)-PCR. RT-PCR was performed using the RETROscript First Strand Synthesis kit (Ambion, Austin, TX).

To replace the *uvsB* gene with the *argB* marker, plasmid pAS1 was constructed. First, plasmid pASUB10, which contains the entire *uvsB* open reading frame, was digested to completion with *Bam*HI. The resulting 6.5-kb *Bam*HI fragment containing the vector backbone plus flanking *uvsB* sequences was then ligated to a 1.8-kb *Bam*HI fragment containing *argB*.

Viability assays: Conidiospores from the appropriate strains were diluted and plated at ~100 conidia per plate on CM + Triton X-100 media containing the appropriate concentration of HU or MMS. For UV viability assays, conidiospores were plated and allowed to germinate for 4.5 hr before UV irradiation. Plates were incubated for 3 days at 32° and the number of survivors on each plate counted. For each treatment, duplicate platings were performed and the average number of survivors calculated. Viability was determined as the percentage of colonies on treated plates compared to untreated controls.

Mutagenesis assays: For each strain tested, 10^7 conidiospores were treated for 30 min at 30° in 1 ml YGV containing 0.05% MMS. The MMS was subsequently neutralized with sodium thiosulfate and the conidiospores were washed with sterile distilled water. Spores were diluted and plated onto MNV + 5-FOA (to select pyrimidine requiring mutants) and CM + Triton X-100 (to measure viability). Plates were incubated at 32° for 3 days. All platings were performed in quadruplicate, and the mean number of survivors and 5-FOA-resistant colonies determined. The level of mutagenesis was expressed as the number of FOA-resistant colonies per 10^6 viable conidia.

Microscopy: Cells were grown, fixed, and stained as previously described (Harris *et al.* 1994; Harris and Kraus 1998). Hoechst 33258 and Calcofluor were used to visualize nuclei and septa, respectively. Nuclear division kinetics and septation indices were determined as previously described (Harris *et al.* 1994; Harris and Kraus 1998). To assess the effect of the *uvsB110* and *musN227* mutations on mitotic entry in synchronized germlings, strains containing the temperature-sensitive *nimA5* mutation were exposed to 0.025% MMS following a 6-hr incubation at 43.5°. The extent of mitotic entry was determined by measuring the chromosome mitotic index (Harris and Hamer 1995; $n = 100$).

Isolation of total RNA and Northern analysis: Strains were grown in liquid YGV for 12 hr at 28° with constant shaking. MMS was added to a concentration of 0.025% and the incubation continued for a further 3 hr before harvesting. For *sepB3* strains, cultures were grown for 8 hr at 28° before shifting to 42° and incubating for an additional 8 hr before harvesting. Isolation of total RNA was performed as described (Dobinson *et al.* 1993).

RNA gels were run, blotted, and probed according to standard protocols (Ausubel *et al.* 1995). The *uvsC* probe con-

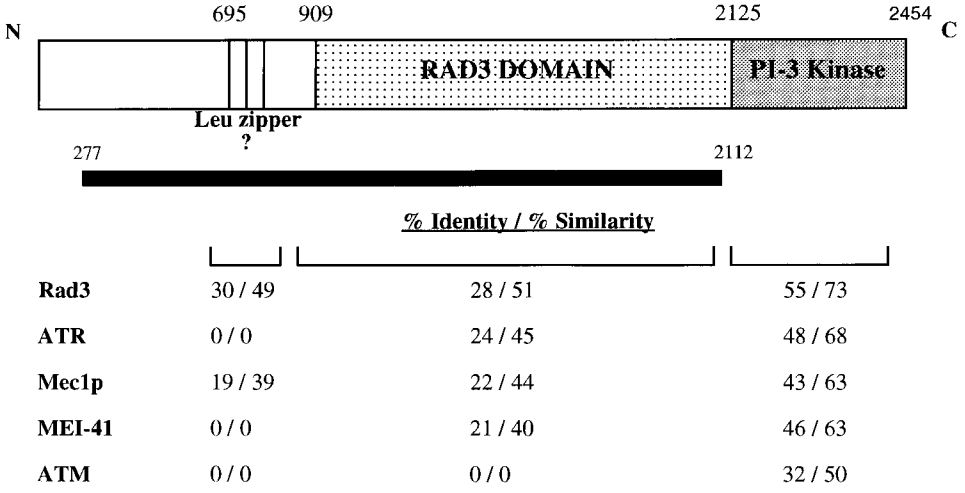

Figure 1.—Molecular characterization of UVSB. A schematic representation of UVSB is shown, illustrating the locations of the PI-3 kinase domain, the RAD3 domain, and the putative leucine zipper. Levels of sequence identity between UVSB and other ATM-related kinases within each domain are also provided. Percentage identity was calculated by the BLAST algorithm at NCBI. The aminoterminal 647 amino acids show no significant similarity to any sequence in the database. The GenBank accession number for the *uvsB* sequence is AF176575. The solid black line depicts the region of UVSB missing in the deletion plasmid pAS1.

sisted of a 0.6-kb *Sph*I cDNA fragment derived from plasmid pGW1525 (van Heemst *et al.* 1997). The actin probe used as a loading control was a 5.1-kb *Hin*dIII genomic fragment from plasmid pSH16. Blots were exposed to Kodak X-OMAT AR film and an intensifying screen for 24–48 hr at −70°. Band intensity was quantitated by phosphoimager analysis.

RESULTS

Cloning of the *uvsB* gene: The *uvsB* gene had previously been mapped to chromosome IV (Jansen 1970). By transforming pools of cosmids from chromosome IV into a *uvsB110* mutant, we identified a single cosmid, W24C04, that was capable of complementing the HU sensitivity caused by the *uvsB110* mutation. Additional transformation experiments using fragments derived from W24C04 identified a 10-kb *Eco*RI-*Not*I fragment that possessed complementing activity. The complete DNA sequence of both strands of this fragment was determined. Analysis of the sequence revealed that *uvsB* encodes a predicted protein of 2454 amino acids, with an estimated molecular weight of 277 kD. Searches of available databases revealed that UVSB belongs to the ATM family of PI-3 related kinases (Zakian 1995). In particular, UVSB is most similar to *S. pombe* Rad3p, *S. cerevisiae* Mec1p, *Drosophila melanogaster* MEI-41, and human ATM and RAD3-related protein (ATR) (Jeggo *et al.* 1998; Figure 1). The identity with its closest orthologue, Rad3p, is primarily confined to three regions (Figure 1): the C-terminal PI-3 kinase domain (55%), the Rad3 domain (28%), and the putative leucine zipper region (30%).

To determine if *uvsB* is an essential gene, a plasmid was constructed in which 75% of the *uvsB* open reading frame was replaced with the selectable *argB* marker (Figure 1). When transformed into a haploid strain, we failed to recover Arg^+ spores displaying phenotypes consistent with loss of UVSB function (*i.e.*, HU or MMS sensitivity, slow growth; A. Hofmann and S. Harris, unpublished results). Furthermore, DNA blot analysis of a set of Arg^+ transformants displaying wild-type phenotypes revealed that the plasmid had integrated ectopically in all cases. These observations imply that the *uvsB* gene is essential. Moreover, they also suggest that the existing *uvsB* alleles are hypomorphic.

UVSB is required for the DNA damage checkpoint: Mutations in *uvsB* cause sensitivity to UV irradiation,

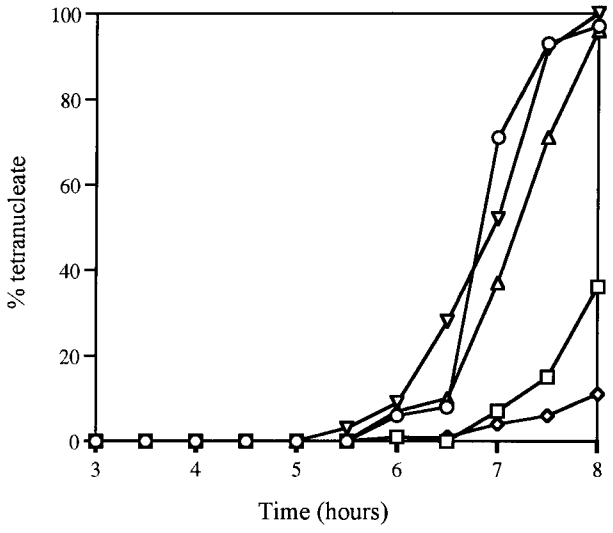

Figure 2.—UVSB is required for the nuclear division delay triggered by the *sepB3* mutation. Conidia from strains A28 (wild type, circles), ASH208 (*sepB3*, squares), ASH202 (*sepB3 uvsB110*, triangles), ASH380 (*sepB3musN227*, diamonds), and AAS381 (*sepB3uvsB110musN227*, inverted triangles) were germinated on coverslips for 3 hr at 42°. Thereafter, one coverslip was removed every 30 min over a 5-hr period and processed for microscopy. Coverslips were stained with Hoechst 33258 and Calcofluor to visualize nuclei and septa, respectively. For each strain and time point, 200 randomly selected germlings were scored for the number of nuclei present. Data points represent the percentage of germlings possessing four or more nuclei.

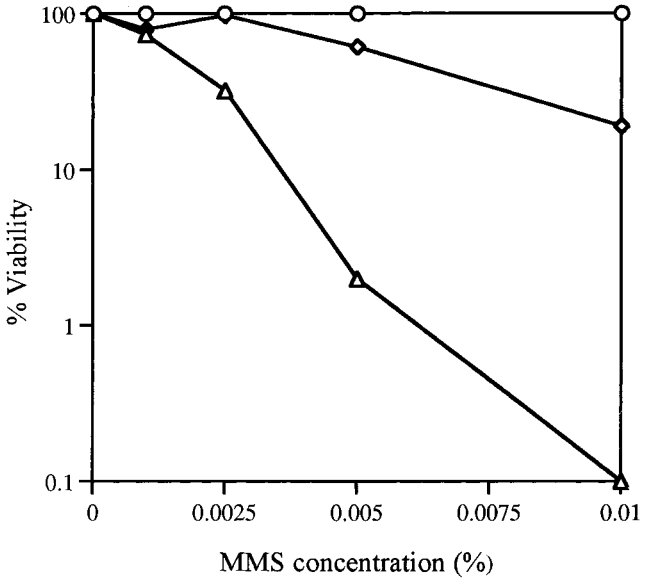

Figure 3.—*uvsB110* mutants are more sensitive to MMS than *nimX*^{cdc2AF} mutants. Conidia from strains A28 (wild type, circles), ASH201 (*uvsB110*, triangles), and ASH286 (*nimX*^{cdc2AF}, diamonds) were diluted and plated at ~100 conidia per plate on CM + Triton X-100 media containing the indicated concentration of MMS. The number of survivors on each plate was determined after 3 days incubation at 32°. The percentage of viable colonies represents the percentage of colonies remaining on the treated plates as compared to the untreated control plates. The data plotted represent the average of four plates tested per dose of MMS.

DNA alkylating agents, and the replication inhibitor HU (Jansen 1970; Harris and Kraus 1998). Recently, it became apparent that UVSB was likely to function as a component of the DNA damage checkpoint, as mutations in *uvsB* abolish the cell cycle delay and inhibition of septum formation triggered by the exposure of predivisional hyphae to DNA damaging agents (Harris and Kraus 1998).

To confirm the role of UVSB in the DNA damage checkpoint, we examined the kinetics of nuclear division in a *sepB3 uvsB110* double mutant. SEPB is likely to function in chromosomal DNA metabolism, since temperature-sensitive *sepB* mutations cause increased levels of mitotic recombination and chromosome nondisjunction (Harris and Hamer 1995). Consistent with previous observations, *sepB3* mutants displayed a pronounced nuclear division delay at restrictive temperature (Harris and Hamer 1995; Figure 2). This delay is completely abolished by the *uvsB110* mutation, as *sepB3 uvsB110* double mutants undergo nuclear division with kinetics similar to wild type (Figure 2). We have also shown that the *uvsB110* mutation can abrogate the mitotic delay observed when cells synchronized at the G2-M transition using the *nimA5* mutation are released into media containing 0.025% MMS. In particular, following 20-min exposure to MMS at 42°, 49% of *nimA5 uvsB110* germlings had entered mitosis compared to only 8% of *nimA5* germlings. The fraction of untreated germlings that had entered mitosis was 58 and 36% for *nimA5 uvsB110* and *nimA5* germlings, respectively. These observations demonstrate that UVSB is required for the damage-induced inhibition of cell cycle progression.

TABLE 1

Effects of *uvsB110* and *musN227* mutations on induced mutagenesis

		5-FOA-resistant colonies / 10^6 survivors	
Strain	Genotype	Control	+MMS
A28	Wild type	0.7	6.9
ASH201	*uvsB110*	0	1.3
ASH383	*musN227*	0.2	9.8
AAS315	*uvsB110 musN227*	0	8.3

A total of 10^7 conidia were treated in 1 ml YGV containing 0.025% MMS for 30 min at 30°. Treated conidia were diluted and plated onto MNVUU + 5-FOA (to select for pyrimidine requiring mutants) and CM + Triton X-100 (to measure viability). After 3 days at 32°, the number of colonies on each plate was determined. The level of mutagenesis is expressed as the number of 5-FOA-resistant colonies / 10^6 viable conidia plated. The mean number of surviving colonies on the CM + Triton X-100 plates ranged from 49 to 120. The data presented here are from one representative experiment. Three independent experiments were performed, and although there was variation in the specific values, the overall trends between the mutants were identical.

UVSB is required for additional aspects of the DDR: Phenotypic characterization of *uvsB* mutants suggested that UVSB may have additional roles in the DDR other than simply controlling the checkpoint response. For example, *uvsB110* mutants are much more sensitive to MMS treatment than *nimX*^{cdc2AF} mutants, which appear to be solely defective in checkpoint function (Figure 3; Ye *et al.* 1997). In addition, *uvsB* mutations cause mitotically quiescent conidia, in which checkpoint function is presumably dispensable, to be sensitive to both UV irradiation and MMS (Kafer and Mayor 1986).

These observations led us to examine other aspects of the DDR that may depend upon UVSB function. One critical element of the DDR is the induction of mutagenesis (Friedberg *et al.* 1995). Using 5-FOA resistance as an assay, the *uvsB110* mutation virtually abolished induced mutagenesis (Table 1). Notably, a similar phenotype has also been observed in AT cells (Bennett and Rainbow 1988; Hilgers *et al.* 1989)

Another critical element of the DDR is the transcriptional induction of genes involved in the metabolism and repair of DNA damage (Witkin 1991; Aboussekhra *et al.* 1996; Kiser and Weinert 1996) In *S. cerevisiae*, the transcriptional response to DNA damage has been shown to be dependent on Mec1p function (Kiser and Weinert 1996). Similarly, in humans, ATM likely triggers significant changes in gene expression through

TABLE 2

Effects of the *uvsB110* and *musN227* mutations on DNA damage-induced transcription

Strain	Genotype	*uvsC* induction ratio
A28	Wild type	3.3 ± 0.4
ASH201	*uvsB110*	2.1 ± 0.1
ASH383	*musN227*	2.7 ± 0.4
AAS315	*uvsB110 musN227*	2.8 ± 0.1

Transcript levels were determined by Northern analysis and quantification of band intensities using a phosphoimager. Levels were normalized to the loading control (actin) by dividing the counts per minute in the *uvsC* bands by the counts per minute in the actin bands. Induction ratios represent the ratio of treated levels to untreated levels. The data shown represent the mean average of two independent experiments.

TABLE 3

***uvsC* induction in *sepB3* and *sepB3 uvsB110* mutants**

Strain	Genotype	*uvsC* induction ratio
ASH208	*sepB3*	2.3 ± 0.6
ASH202	*sepB3 uvsB110*	1.1 ± 0.1

Strains were grown at either permissive (28°) or restrictive temperature (42°). *uvsC* transcript levels were determined by Northern analysis and quantification of band intensities using a phosphoimager. Transcript levels were normalized to the loading control (actin) by dividing the counts per minute in the *uvsC* bands by the counts per minute in the actin bands. Induction ratios represent the ratio of *uvsC* transcript levels at 28° compared to 42°. The data shown represent the mean average of two independent experiments.

its ability to phosphorylate p53 (Banin *et al.* 1998; Canman *et al.* 1998). Given the similarity between UVSB and both Mec1p and ATM, it seemed reasonable to test whether UVSB is similarly required for the transcriptional response to DNA damage in *A. nidulans.* One *A. nidulans* gene that has been shown to be induced in the presence of DNA damage is *uvsC* (van Heemst *et al.* 1997), which encodes the *A. nidulans* orthologue of *S. cerevisiae* Rad51p. Treatment of wild-type cells with MMS results in a 3.3-fold increase in *uvsC* transcript levels (Table 2). This increase is not due to artificial cell cycle synchronization caused by MMS treatment, since it still occurred in hyphae arrested at the G2/M transition by the *nimA5* mutation. For example, in one experiment, *nimA5* mutants arrested at 43.5° and treated with 0.025% MMS for 3 hr showed a 5.8-fold induction of *uvsC* transcript. The elevation of *uvsC* transcript levels caused by MMS treatment is reduced by ~35% in a *uvsB110* mutant (Table 2). As an independent means of testing the role of UVSB in the transcriptional response to DNA damage, we examined *uvsC* transcript levels in *sepB3* mutants that had been shifted to restrictive temperature. The observation that these conditions cause an increase in *uvsC* transcript levels suggests that the *sepB3* mutation generates a DNA damage signal (Table 3). However, as with MMS-treated wild-type cells, this response is only partially diminished (~50%) by the *uvsB110* mutation (Table 3). Thus, given the relatively weak effect of the *uvsB110* mutation on *uvsC* expression, we can only conclude at this time that UVSB plays a modest role at best in the transcriptional response to DNA damage in *A. nidulans.*

The *musN227* mutation suppresses specific DDR defects caused by *uvsB* mutations: The recessive *musN227* mutation was originally isolated in a screen for MMS-sensitive mutants (Kafer and Mayor 1986). Construction of double mutants for epistasis analysis revealed that the *musN227* mutation could partially suppress the poor growth and DNA damage sensitivity of several different *uvsB* mutant alleles (Kafer and Chae 1994). Consistent with this result, we found that the *musN227* mutation caused a 10-fold increase in the viability of *uvsB110* and *uvsB505* mutants exposed to low doses of MMS (Figure 4). A similar effect was noted at low doses of HU (A. Hofmann and S. Harris, unpublished results). The partial suppression conferred by the *musN227* mutation was not limited to *uvsB* mutations, since *musN227 uvsD153* double mutants also grew better at low doses of MMS (Figure 4).

Since UVSB appears to be required for multiple aspects of the DDR, we sought to determine which elements of the DDR are affected by the *musN227* mutation. We first assessed the ability of the *musN227* mutation to restore damage-induced nuclear division delay to *uvsB110* mutants. To do this, we constructed a *sepB3 uvsB110 musN227* triple mutant and examined the kinetics of nuclear division at the restrictive temperature. The triple mutant divided with kinetics similar to that of the *sepB3 uvsB110* double mutant (Figure 2), indicating that the *musN227* mutation did not restore the nuclear division delay. The observation that the mitotic delay is not restored in *nimA5 uvsB110 musN227* triple mutants that have been synchronized at G2-M and then released into MMS supports this notion. In particular, following 20-min exposure to 0.025% MMS at 42°, 35% of *nimA5 uvsB110 musN227* germlings had entered mitosis, compared to 49% of *nimA5 uvsB110* germlings and 8% of *nimA5* germlings. These results suggest that the *musN227* mutation must affect other UVSB-dependent aspects of the DDR.

As previously shown, the *uvsB110* mutation abolishes the damage-induced inhibition of septum formation (Harris and Kraus 1998; Table 4). To determine the effect of the *musN227* mutation on this aspect of the DDR, we assessed the ability of the *sepB3 uvsB110 musN227* triple mutant to undergo septation at the restrictive temperature. Remarkably, the level of septation

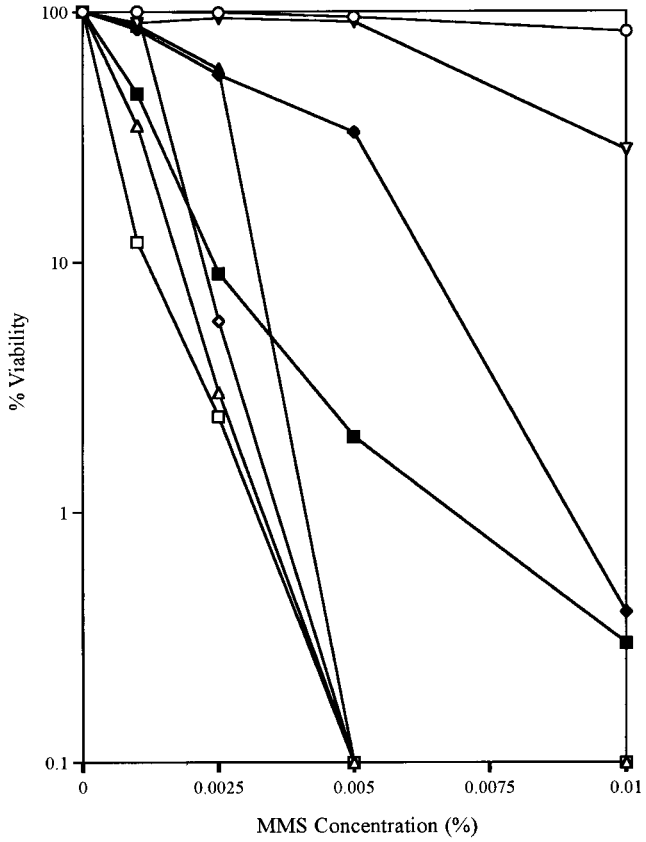

Figure 4.—The *musN227* mutation partially suppresses the MMS sensitivity of *uvsB* and *uvsD* mutants. Conidia from strains A28 (wild type, open circles), ASH201 (*uvsB110*, open triangles), A826 (*uvsB505*, open squares), ASH206 (*uvsD153*, open diamond), ASH383 (*musN227*, inverted open triangle), AAS315 (*uvsB110 musN227*, solid triangles), AAH11 (*uvsB505 musN227*, solid squares), and AAH4 (*uvsD153 musN227*, solid diamonds) were diluted and plated at ~100 conidia per plate on CM + Triton X-100 media containing the indicated concentration of MMS. The number of survivors on each plate was scored after 3 days incubation at 32°. The percentage of viable colonies represents the percentage of colonies remaining on the treated plates as compared to the untreated control plates. Data represent the average of four plates counted for each dose.

was severely reduced by the addition of the *musN227* mutation to the *sepB3uvsB110* background (Table 4). Thus, we conclude that *musN227* partially restores to *uvsB* mutants the ability to block septation in the presence of DNA damage.

The *uvsB110* mutation causes a defect in induced mutagenesis. To determine the effect of the *musN227* mutation on this aspect of the DDR, we tested the ability of *musN227 uvsB110* double mutants to form FOA-resistant colonies following treatment with MMS. The *musN227* mutation partially restored induced mutagenesis to the *uvsB110* mutant (Table 1). Indeed, the *musN227* mutation itself appears to cause a slight induced mutator phenotype (Table 1). Thus, we conclude that *musN227* partially suppresses the inability of *uvsB* mutants to induce mutagenesis when exposed to a DNA damaging agent.

TABLE 4

Effects of the *uvsB110* and *musN227* mutations on septum formation

Strain	Genotype	Septation index (%)
A28	Wild type	91
ASH208	*sepB3*	1
ASH202	*sepB3 uvsB110*	86
ASH380	*sepB3 musN227*	2
AAS381	*speB3 uvsB110 musN227*	24

Conidia were germinated on coverslips for 12 hr at 42°. Coverslips were stained with Hoechst 33258 and Calcofluor to visualize nuclei and septa, respectively. The septation index reflects the percentage of germlings possessing at least one septa (n = 200). The values presented represent the mean average of three independent experiments.

The *uvsB110* mutation causes a modest reduction of *uvsC* transcript levels in the presence of DNA damage. To assess the effect of the *musN227* mutation on this aspect of the *uvsB* phenotype, we examined transcript levels from *uvsC* in *musN227 uvsB110* double mutants treated with MMS. The observation that the double mutant only displayed a slight increase in *uvsC* transcript levels suggests that the *musN227* mutation does not significantly restore DNA damage-induced transcription in *uvsB* mutants (Table 2).

The effect of *musN227* on septation is independent of NIMX^{cdk1}: In *A. nidulans*, the presence of DNA damage triggers inhibitory Tyr-15 phosphorylation of the cdk NIMX^{cdk1} (Ye *et al.* 1997). This phosphorylation event leaves NIMX^{cdk1} in an inactive state, resulting in inhibition of mitosis and septum formation (Ye *et al.* 1997; Harris and Kraus 1998). The *nimX*^{cdc2AF} mutation makes NIMX^{cdk1} refractory to Tyr-15 phosphorylation (Ye *et al.* 1996). As a result, *nimX*^{cdc2AF} mutants septate at high levels despite the presence of DNA damage (Harris and Kraus 1998). If the *musN227* mutation affects the DNA damage-induced inhibition of septum formation by regulating Tyr-15 phosphorylation of NIMX^{cdk1}, it should have no effect on septation in *nimX*^{cdc2AF} mutants. To test this possibility, *musN227 nimX*^{cdc2AF} double mutants were constructed and tested for the ability to septate in the presence of the alkylating agent DEO. The *musN227* mutation decreased the level of septation observed in the *nimX*^{cdc2AF} mutant (Table 5). This observation suggests that MUSN regulates septation via a separate pathway from that which regulates NIMX^{cdk1} tyrosine phosphorylation. To further explore this possibility, we compared the MMS sensitivity of the *musN227 nimX*^{cdc2AF} double mutant to the parental single mutants. The double mutant showed enhanced sensitivity to MMS (Figure 5), indicating that *musN227* and *nimX*^{cdc2AF} belong to different epistasis groups of DNA repair mutants.

TABLE 5

Septum formation in *musN227 nimX*^{cdc2AF} double mutants

Strain	Genotype	Septation index (%)
A28	Wild type	11
ASH286	*nimX*^{cdc2AF}	36
ASH383	*musN227*	0
AAS280	*musN227 nimX*^{cdc2AF}	15

Conidia were germinated on coverslips in YGV containing 0.025% DEO for 12 hr at 37°. Hoechst 33258 and Calcofluor were used to stain nuclei and septa, respectively. The septation index represents the percentage of germlings possessing at least one septum (n = 200). The data presented represent the mean average of three independent experiments.

DISCUSSION

Mutations in the *uvsB* and *uvsD* genes define one of four epistatic groups of DNA repair mutants in *A. nidulans* (Kafer and Mayor 1986). Characterization of the phenotypes caused by these mutations suggested that *uvsB* and *uvsD* may encode proteins required for both the DNA damage and the DNA replication checkpoints (Ye *et al.* 1997; Harris and Kraus 1998). Here, the function of UVSB in the *A. nidulans* DNA damage response is described. In particular, we show that (i) UVSB is a member of the conserved family of ATM-related protein kinases that control checkpoint signal transduction, and (ii) UVSB is required for several aspects of the DDR, including nuclear division arrest, induced mutagenesis, and perhaps, DNA damage-induced gene expression. In addition, we demonstrate that a subset of *uvsB* defects can be partially suppressed by the *musN227* mutation. We propose that UVSB functions as the central regulator of the *A. nidulans* DDR, whereas MUSN promotes recovery and the resumption of normal proliferative growth.

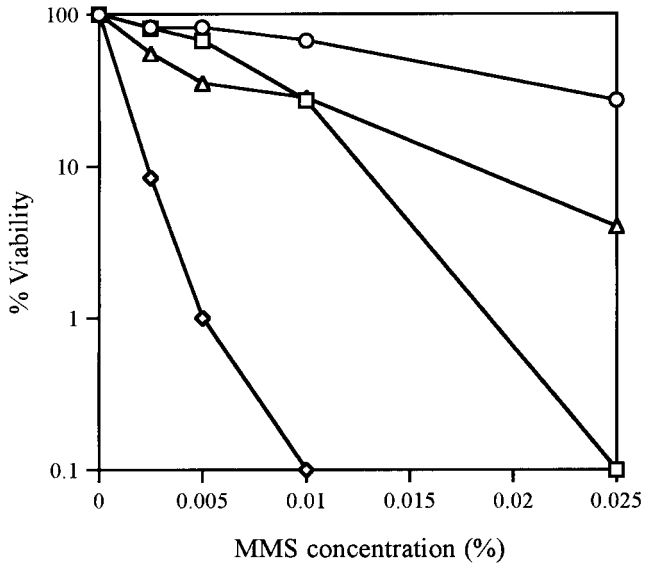

Figure 5.—The *musN227* and *nimX*^{cdc2AF} mutations belong to different epistasis groups. Conidia from strains A28 (wild type, circles), ASH383 (*musN227*, squares), ASH286 (*nimX*^{cdc2AF}, triangles), and AAS280 (*musN227 nimX*^{cdc2AF}, diamonds) were diluted and plated at ~100 conidia per plate on CM + Triton X-100 media containing the indicated concentration of MMS. The number of survivors on each plate was scored after 3 days incubation at 32°. The percentage of viable colonies represents the percentage of colonies remaining on the treated plates as compared to the untreated control plates. Data represent the average of six plates counted for each dose.

UVSB is a member of the ATM-related family of protein kinases: DNA sequence analysis shows that UVSB is a member of the ATM-related protein kinase family. In particular, UVSB shares greatest similarity with the ATM subfamily that includes human ATR, *S. cerevisiae* Mec1p, and *S. pombe* Rad3p. The similarity extends beyond the conserved carboxy-terminal PI-3 kinase domain and includes both the Rad3 domain and a putative leucine zipper (Jeggo *et al.* 1998). Despite the presence of the PI-3 kinase domain, members of the ATM family display protein kinase activity (Bentley *et al.* 1996; Banin *et al.* 1998; Canman *et al.* 1998; Cliby *et al.* 1998). Furthermore, this activity underlies the ability of the ATM kinases to trigger a cell cycle checkpoint response. For example, ATM, Mec1p, and Rad3 each appear to activate checkpoint responses by phosphorylating downstream protein kinases (*i.e.*, Rad53/Cds1/Chk2 and Chk1p; Sanchez *et al.* 1996; Martinho *et al.* 1998; Matsuoka *et al.* 1998; Brown *et al.* 1999). The presence of the conserved PI-3 kinase domain in UVSB suggests that it also exerts its checkpoint effect by phosphorylating downstream effector proteins. Three observations suggest that UVSD may be such a target. First, *uvsB* and *uvsD* mutations cause a similar spectrum of phenotypes (Harris and Kraus 1998; Kafer and May 1998), including failure to activate cell cycle checkpoints in the presence of DNA damage. Second, the epistatic relationship between *uvsB* and *uvsD* mutations demonstrates that both genes function in the same pathway (Kafer and Mayor 1986). Third, UVSD presumably functions downstream of UVSB, since preliminary experiments show that unlike *uvsB* mutants, the *uvsD153* mutation does not affect DNA damage-induced gene expression (A. Hofmann and S. Harris, unpublished results).

UVSB is required for multiple facets of the *A. nidulans* DDR: Since the DNA damage sensitivity caused by *uvsB* mutations greatly exceeds that caused by a typical DNA damage checkpoint mutant (*i.e.*, *nimX*^{cdc2AF}; Ye *et al.* 1997), we presumed that UVSB is required for other facets of the *A. nidulans* DDR in addition to the checkpoint response. In accordance with this hypothesis, we have previously shown that UVSB is required for the DNA damage-induced inhibition of septum formation (Harris and Kraus 1998). Results presented here suggest that UVSB could possibly play a role in DNA damage-induced gene expression. Transcriptional induction of at least one DNA damage-responsive gene was

found to be slightly affected by the *uvsB110* mutation. Although we cannot eliminate the possibility that DNA damage affects *uvsC* transcript stability, the presence of conserved MluI cell cycle box (MCB) elements (McIntosh 1993) in the *uvsC* promoter (van Heemst *et al.* 1997) suggests that, as in *S. cerevisiae* and *S. pombe* (Harris *et al.* 1996; Bachant and Elledge 1998), the effect is probably exerted at the level of transcriptional induction. Since the *uvsB110* mutation is likely to be hypomorphic, and its effect on *uvsC* transcript levels was rather small, analysis of DNA damage-induced expression of *uvsC* in a *uvsB* null background should provide further insight into the role of UVSB in this facet of the DDR. In addition, the expression of other genes showing comparable, if not greater, levels of DNA damage induction should be examined.

Another important aspect of the DDR is the induction of mutagenic DNA repair (Friedberg *et al.* 1995). Using a forward mutagenesis assay, we have found that a *uvsB* mutation virtually abolishes induced mutagenesis in *A. nidulans.* Other ATM family members have also been implicated in the control of mutagenesis. For example, *S. pombe rad3* mutants display pronounced defects in forward mutation (Gentner *et al.* 1978). In addition, examination of viral mutagenesis in infected AT cells demonstrated that they were defective for an inducible error-prone repair process (Bennett and Rainbow 1988; Hilgers *et al.* 1989). The mechanism by which ATM-related protein kinases influence DNA damage-induced mutagenesis is currently unknown. However, it is not inconceivable that the DNA damage-induced expression of genes required for mutagenic DNA repair is regulated by ATM family members.

Multiple defects observed in *uvsB* mutants are suppressed by the *musN277* mutation: The *musN227* mutation was originally isolated in a screen for mutants sensitive to MMS (Kafer and Mayor 1986). During the construction of double mutants for epistasis analysis, it was noted that *musN227* partially suppressed the slow growth and DNA damage sensitivity of *uvsB* mutants (Kafer and Chae 1994). We have confirmed this observation and, furthermore, shown that *musN227* modifies several, but not all, of the phenotypes caused by *uvsB* mutations. In particular, the inability of *uvsB110* mutants to restrain septum formation and induce mutagenesis in the presence of DNA damage was negated by the *musN227* mutation. In contrast, *musN227* does not appear to affect the loss of DNA damage-induced cell cycle delay caused by *uvsB* mutations, and its effect on *uvsC* transcription was not significant. The retention of the checkpoint defect in the *musN227 uvsB110* double mutant may account, in part, for the partial nature of the suppression. Collectively, our observations suggest that the *musN227* mutation does not cause a general increase in UVSB activity. Instead, we propose that *musN227* modifies a specific UVSB function that has gone awry in *uvsB* mutants. Furthermore, we suggest

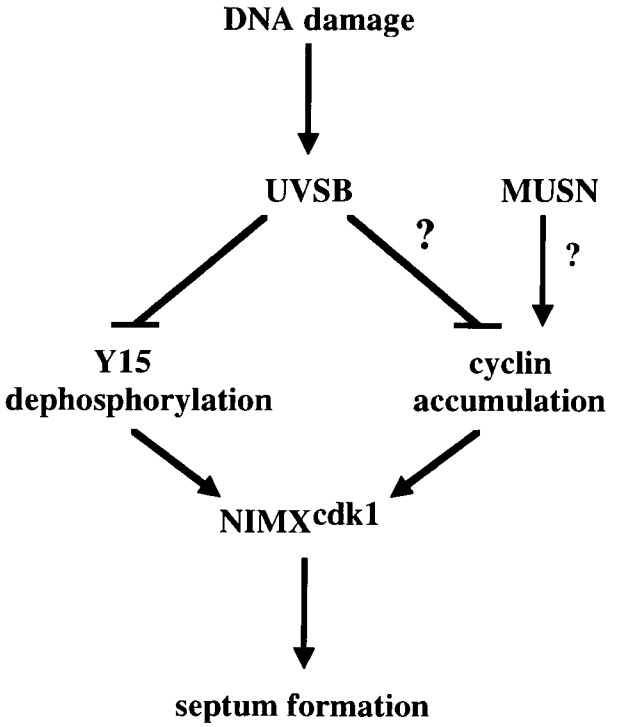

Figure 6.—A tentative model for the regulation of septum formation by DNA damage in *A. nidulans.* In the presence of DNA damage, activated UVSB is proposed to modulate NIMX^{cdk1} activity via two mechanisms: inhibition of Tyr-15 dephosphorylation and inhibition of cyclin accumulation. Septum formation is blocked by reduced NIMX^{cdk1} activity. See text for further details.

that this function may be the ability of UVSB to properly modulate the activity of gene products responsible for inhibiting septation and inducing mutagenesis in response to the presence of DNA damage.

Several observations suggest that the suppression of *uvsB* defects by the *musN227* mutation does not reflect a direct physical interaction between MUSN and UVSB. First, *musN227* can suppress defects caused by different alleles of *uvsB* (Figure 4). Second, the *musN227* mutation also suppresses DDR-related defects caused by the *uvsD153* mutation (Kafer and Chae 1994; Figure 4). Third, *musP234*, a mutant recovered from the same screen as *musN227*, suppresses *uvsB* defects to the same apparent extent as the *musN227* mutation (Kafer and Chae 1994; A. Hofmann and S. Harris, unpublished observations). We propose that the *musN* and *musP* mutations affect a regulatory pathway that normally antagonizes UVSB function. For example, MUSN and MUSP may be components of a pathway that downregulates the DNA damage-induced transcriptional response to promote recovery from the DDR and the resumption of normal proliferation. In the *mus uvsB* double mutants, the crippled DDR caused by the *uvsB* mutation may be sufficiently enhanced by the *mus* mutation so that some degree of resistance to DNA damage is restored. An important, but as yet untested, prediction of

this model is that the *mus* mutations may have no effect if the DDR is completely abolished (*i.e.*, in a *uvsB* null mutant).

Regulation of septum formation by DNA damage: We have previously shown that the presence of DNA damage inhibits septum formation in *A. nidulans* (Harris and Kraus 1998). Furthermore, we proposed that DNA damage triggers a regulatory response that leads to a reduction in the activity of the cyclin-dependent kinase $NIMX^{cdk1}$ below a threshold needed to trigger septation. Our genetic analyses indicated that the ultimate effect of this response is to increase the level of inhibitory $NIMX^{cdk1}$ tyrosine phosphorylation (Harris and Kraus 1998). However, two lines of evidence suggest that DNA damage may regulate septum formation via an additional mechanism. First, when incubated at restrictive temperature, the level of septation in *sepB3 uvsB* double mutants is consistently higher than that seen in *sepB3* $nimX^{cdc2AF}$ mutants (Harris and Kraus 1998). If the sole effect of DNA damage on septation was mediated by inhibitory tyrosine phosphorylation of $NIMX^{cdk1}$, the level of septation in the double mutants should be similar. Second, the observation that the *musN227* mutation affects septation independent of $NIMX^{cdk1}$ tyrosine phosphorylation implies that a second pathway for DNA damage regulation of septation exists. We propose that this pathway is controlled by UVSB. Moreover, since $NIMX^{cdk1}$ is required for septum formation (Harris and Kraus 1998), we suggest that this pathway affects septation by negatively regulating $NIMX^{cdk1}$ activity through a mechanism other than tyrosine phosphorylation. For example, UVSB, and potentially MUSN, could modulate the expression of a cyclin(s) that associates with $NIMX^{cdk1}$ to control septation (Figure 6). Although this cyclin could be NIME (O'Connell *et al.* 1992; Harris and Kraus 1998), it remains possible that *A. nidulans* possesses an additional cyclin whose accumulation is delayed by the presence of DNA damage (*i.e.*, similar to *S. cerevisiae* Cln1p and Cln2p; Sidorova and Breeden 1997). We note that the ability of DNA damage to specifically block cytokinesis in human cells may rely upon a similar ATM-dependent mechanism (Hermeking *et al.* 1997; Bunz *et al.* 1998).

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