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 (Aboushehra et al. 1996; Kiser and Weiner 1996). Furthermore, in *Saccharomyces cerevisiae* 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 cdks (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...
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 adeE20 biA1; uvbs505 ssba1; s3; chaA1; chaA1), AAH1 (uvbs110; nimA5), AAH2 (uvbs110; musN227; nimA5), AAH4 (uvds115; musN227; wA2), AAH11 (uvbs505; musN227; chaA1), AAS280 (muS3227; nimX1248; wA2), AA5315 (uvbs110; musN227; pabaA1 acrA1 actA1 riboB2 chaA1), AAS381 (sepB3; uvbs110; musN227; chaA1), AAS1 (uvbs110; musN227; chaA1), ASH206 (sepB3; uvbs110; chaA1), ASH204 (uvbs110; argB2; wA2), ASH206 (uvds115; wA2), ASH208 (sepB3; pabaA6; wA2), ASH286 (nimX1248; wA2), AAS380 (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, 2% malt extract, 0.2% peptone, 0.1% yeast extract, 0.1% casamino acids, trace elements, and vitamins; pH 6.5), and CM (1% dextrose, 2% malt extract, 0.2% peptone, trace elements, and vitamins; pH 6.5). The MMS was subsequently neutralized with sodium bisulfite. Spores were diluted and plated onto MAG at a density of ~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°C and the number of survivors on each plate counted. For each treatment, duplicate plates 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: Conidiospores from the appropriate strain 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°C and the number of survivors on each plate counted. For each treatment, duplicate plates were performed and the average number of survivors calculated. Viability was determined as the percentage of colonies on treated plates compared to untreated controls.

Isolation of total RNA and Northern analysis: Isolation of total RNA was performed as described (Dobinson et al. 1994; Harris and Kraus 1998). The extent of mitotic index was determined by measuring the chromosome mitotic index (Harris and Hame 1995; n = 100).

Isolation of total RNA and Northern analysis: Strains were grown in liquid YGV for 12 hr at 28°C with constant shaking. MMS was added to a concentration of 0.025% and the incubation continued for another 3 hr before harvesting. For sepB3 strains, cultures were grown for 8 hr at 28°C before shifting to 42°C 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-
UVSB is required for the DNA damage checkpoint: Mutations in uvsB cause sensitivity to UV irradiation,
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,
germlings had entered mitosis compared to only 8% of triggers, significant changes in gene expression through uvsB110 shown that the double mutants undergo nuclear division with uvsB110 used (sepB3 uvsB110) (Harris 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 non-disjunction (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.

**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 nimXcdc2AF 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 activity. 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; Abouszekhra et al. 1996; Kiser and Weinert 1996) In S. cerevisiae, the transcriptional response to DNA damage has been shown to be dependent on Meclp function (Kiser and Weinert 1996). Similarly, in humans, ATM likely triggers significant changes in gene expression through

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**TABLE 1**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Control 5-FOA-resistant colonies/10⁶ survivors</th>
<th>+MMS 5-FOA-resistant colonies/10⁶ survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td>A28</td>
<td>Wild type</td>
<td>0.7</td>
<td>6.9</td>
</tr>
<tr>
<td>ASH 201</td>
<td>uvsB110</td>
<td>0</td>
<td>1.3</td>
</tr>
<tr>
<td>ASH 383</td>
<td>musN227</td>
<td>0.2</td>
<td>9.8</td>
</tr>
<tr>
<td>AAS315</td>
<td>uvsB110 musN227</td>
<td>0</td>
<td>8.3</td>
</tr>
</tbody>
</table>

A total of 10⁶ 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 10⁶ 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.
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 Helden 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 due to artificial cell cycle synchronization caused by MMS treatment, since it still occurred in hyphae 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 was 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.

### TABLE 2

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>uvsC induction ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>A28</td>
<td>Wild type</td>
<td>3.3 ± 0.4</td>
</tr>
<tr>
<td>ASH 201</td>
<td>uvsB110</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>ASH 383</td>
<td>musN227</td>
<td>2.7 ± 0.4</td>
</tr>
<tr>
<td>AAS315</td>
<td>uvsB110 musN227</td>
<td>2.8 ± 0.1</td>
</tr>
</tbody>
</table>

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.

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

### TABLE 3

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>uvsC induction ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASH 208</td>
<td>sepB3</td>
<td>2.3 ± 0.6</td>
</tr>
<tr>
<td>ASH 202</td>
<td>sepB3 uvsB110</td>
<td>1.1 ± 0.1</td>
</tr>
</tbody>
</table>

Strains were grown at either permissive (28°C) or restrictive temperature (42°C). 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°C compared to 42°C. The data shown represent the mean average of two independent experiments.
TABLE 4  
Effects of the uvsB110 and musN227 mutations on septum formation

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Septation index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A28</td>
<td>Wild type</td>
<td>91</td>
</tr>
<tr>
<td>ASH208</td>
<td>sepB3</td>
<td>1</td>
</tr>
<tr>
<td>ASH202</td>
<td>sepB3 uvsB110</td>
<td>86</td>
</tr>
<tr>
<td>ASH380</td>
<td>sepB3 musN227</td>
<td>2</td>
</tr>
<tr>
<td>AAS381</td>
<td>sepB3 uvsB110 musN227</td>
<td>24</td>
</tr>
</tbody>
</table>

Conidia were germinated on coverslips for 12 hr at 42°C. 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 septum (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 NIMXcdc2AF. In A. nidulans, the presence of DNA damage triggers inhibitory Tyr-15 phosphorylation of the cdk NIMXcdc2AF (Ye et al. 1997). This phosphorylation event leaves NIMXcdc2AF in an inactive state, resulting in inhibition of mitosis and septum formation (Ye et al. 1997; Harris and Kraus 1998). The nimXcdc2AF mutation makes NIMXcdc2AF refractory to Tyr-15 phosphorylation (Ye et al. 1996). As a result, nimXcdc2AF 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, then the musN227 nimXcdc2AF double mutant should have no effect on septation in nimXcdc2AF mutants. To test this possibility, we compared the MMS sensitivity of the musN227 nimXcdc2AF double mutant to the parental nimXcdc2AF mutant (Table 1). This observation suggests that MUSN regulates septation via a separate pathway from that which regulates NIMXcdc2AF tyrosine phosphorylation. To further explore this possibility, we compared the MMS sensitivity of the musN227 nimXcdc2AF double mutant to the parental single mutants. The double mutant showed enhanced sensitivity to MMS (Figure 5), indicating that musN227 and nimXcdc2AF belong to different epistasis groups of DNA repair mutants.


**TABLE 5**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Septation index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A28</td>
<td>Wild type</td>
<td>11</td>
</tr>
<tr>
<td>ASH 286</td>
<td>nimX&lt;sup&gt;802AF&lt;/sup&gt;</td>
<td>36</td>
</tr>
<tr>
<td>ASH 383</td>
<td>musN227</td>
<td>0</td>
</tr>
<tr>
<td>AAS280</td>
<td>musN227 nimX&lt;sup&gt;802AF&lt;/sup&gt;</td>
<td>15</td>
</tr>
</tbody>
</table>

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.

**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 Mayor 1986), 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<sup>802AF</sup>, 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 musN277 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 musN277 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 musN277 modifies several, but not all, of the phenotypes caused by uvsB mutations. In particular, the inability of uvsB110 mutants to restrain septation formation and induce mutagenesis in the presence of DNA damage was negated by the musN277 mutation. In contrast, musN277 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 musN277 uvsB110 double mutant may account, in part, for the partial nature of the suppression. Collectively, our observations suggest that the musN277 mutation does not cause a general increase in UVSB activity. Instead, we propose that musN277 modifies a specific UVSB function that has gone awry in uvsB mutants. Furthermore, we suggest 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 musN277 mutation does not reflect a direct physical interaction between MUSN and UVSB. First, musN277 can suppress defects caused by different alleles of uvsB (Figure 4). Second, the musN277 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 musN277, suppresses uvsB defects to the same apparent extent as the musN277 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<sup>cdk1</sup> 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<sup>cdk1</sup> 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 *sepB* uvsB double mutants is consistently higher than that seen in *sepB* nimX<sup>cdk1</sup> mutants (Harris and Kraus 1998). If the sole effect of DNA damage on septation was mediated by inhibitory tyrosine phosphorylation of NIMX<sup>cdk1</sup>, the level of septation in the double mutants should be similar. Second, the observation that the mus227 mutation affects septation independent of NIMX<sup>cdk1</sup> 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<sup>cdk1</sup> is required for septum formation (Harris and Kraus 1998), we suggest that this pathway affects septation by negatively regulating NIMX<sup>cdk1</sup> 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<sup>cdk1</sup> 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; Sidorenko 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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**LITERATURE CITED**


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