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Regulation of Hyphal Morphogenesis and the DNA Damage Response by the *Aspergillus nidulans* ATM Homolog *AtmA*

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**ABSTRACT**

Ataxia telangiectasia (A-T) is an inherited disorder characterized by progressive loss of motor function and susceptibility to cancer. The most prominent clinical feature observed in A-T patients is the degeneration of Purkinje motor neurons. Numerous studies have emphasized the role of the affected gene product, ATM, in the regulation of the DNA damage response. However, in Purkinje cells, the bulk of ATM localizes to the cytoplasm and may play a role in vesicle trafficking. The nature of this function, and its involvement in the pathology underlying A-T, remain unknown. Here we characterize the homolog of ATM (*AtmA*) in the filamentous fungus *Aspergillus nidulans*. In addition to its expected role in the DNA damage response, we find that *AtmA* is also required for polarized hyphal growth. We demonstrate that an *atmA* mutant fails to generate a stable axis of hyphal polarity. Notably, cytoplasmic microtubules display aberrant cortical interactions at the hyphal tip. Our results suggest that *AtmA* regulates the function and/or localization of landmark proteins required for the formation of a polarity axis. We propose that a similar function may contribute to the establishment of neuronal polarity.

**ATM** is a phosphatidyl-3-kinase-related protein kinase (PIKK) that functions as a central regulator of the DNA damage response in eukaryotic cells (Shiloh 2001; McKinnon 2004). Like its homolog ATR, ATM responds to damage such as double-strand breaks (DSBs) by phosphorylating multiple targets that collectively act to maintain genome integrity. Key targets include proteins that direct chromatin modification (histone H2AX), promote DNA repair (BRCA1, BLM), activate cell cycle checkpoints (Chk1, Chk2, Nbs1, FancD2, Mdm2), and trigger apoptosis (p53) (summarized by McKinnon 2004). Many of these proteins contain consensus ATM phosphorylation sites whose importance has been demonstrated in functional studies (O’Neill et al. 2000). Notably, studies in yeast have shown that the ATM homolog Tel1 is one of the first proteins recruited to DSBs, where it appears to mediate formation of protein complexes involved in checkpoint activation and repair (Lisby et al. 2004). Accordingly, it is generally thought that the primary function of ATM is to choreograph the response to DNA damage.

In humans, mutations in ATM cause the devastating neurodegenerative disease ataxia telangiectasia (A-T; summarized by Gatti 1998; Chun and Gatti 2004). The most prominent neurological feature of A-T is progressive cerebellar ataxia, which is triggered by the loss of Purkinje cells within the cerebellum. Although the number of Purkinje cells initially appears normal at birth, the cells begin to degenerate shortly thereafter. ATM is predominantly a nuclear protein (Lakin et al. 1996), but it surprisingly displays prominent cytoplasmic localization in mouse Purkinje cells and cerebellar tissue (Kuljis et al. 1999; Barlow et al. 2000). Additional biochemical studies show that ATM associates with cytoplasmic vesicles (Watters et al. 1997). Both in vitro and in vivo assays demonstrate that ATM interacts with β-adaptins (Lim et al. 1998), which are components of the adaptor complexes involved in clathrin-mediated vesicle transport (Boehm and Bonifacino 2001). Consistent with these observations, A-T cells and mouse Atm−/− mutants display aberrant accumulation of lysosomes (Barlow et al. 2000). Additional cytoplasmic abnormalities observed in A-T cells include the presence of unusually thick actin stress fibers (McKinnon and Burgoyne 1985) and possible defects in exocytosis (O’Connor and Linticum 1980). Given the large size of ATM, these studies collectively support the notion that ATM has a cytoplasmic function independent of its role in the DNA damage response.

The filamentous fungus *Aspergillus nidulans* possesses a sophisticated DNA damage response that ensures the maintenance of genome integrity (Goldman et al. 2002;
Goldman and Kafer 2004). The ATR homolog UvsB<sup>ATR</sup> is a central component of this response, in which it controls the activation of multiple checkpoints, regulates damage-induced gene expression, and also promotes DNA repair (De Souza et al. 1999; Hofmann and Harris 2000). UvsB<sup>ATR</sup> displays an extensive web of genetic interactions with other proteins involved in the DNA damage response, including the Mre11 complex (SacA<sup>BstI</sup>, MreA<sup>Mre11</sup>, and Sld1<sup>Rad5</sup>), the cdc2-related kinase NpKα, and SepB<sup>ETR</sup> (Fagundes et al. 2004, 2005; Gygax et al. 2005; Malavazi et al. 2005). However, the function of UvsB<sup>ATR</sup> relative to its paralog ATM remains unclear for the filamentous fungi. In particular, ATM and ATR homologs possess both shared and unique functions in the DNA damage responses of the model yeast <i>Saccharomyces cerevisiae</i> (Rouse and Jackson 2002). To determine if a similar relationship exists in <i>A. nidulans</i>, we cloned and initiated the functional characterization of the A. nidulans ATM homolog, AtmA<sub>ATM</sub>. As expected, AtmA<sub>ATM</sub> is intimately involved in the DNA damage response. However, to our surprise, it is also required for the formation of a stable axis of hyphal polarity. On the basis of our observations, we propose that AtmA<sub>ATM</sub> performs a conserved cytoplasmic function that is important for the growth of highly polarized cells.

**Materials and Methods**

**Strains, media, and growth conditions**: The strains used in this study are A28 (<i>pabaA6</i>; <i>biA1</i>), A781 (<i>waA2</i>; <i>nimA5</i>), GR5 (<i>pyrG89</i>; <i>waA3</i>; <i>pyroA4</i>), R21 (<i>pabaA1</i>; <i>yA2</i>), IM69 (<i>pyrG89</i>; <i>waA3</i>; <i>pyroA1</i>; <i>AtmA:<i>pyrG</i></i>), and IM69-221 (<i>pyrG89</i>; <i>waA3</i>; <i>nimA5</i>; <i>AtmA:<i>pyrG</i></i>). Rich media (YG: 2% glucose, 0.5% yeast extract, trace elements) and minimal glucose media (MM: 1% glucose, original high nitrate salts, trace elements, pH 6.5) were prepared using standard approaches (Hill and Kafer 2001; Fagundes et al. 2005). For glycerol media, 1.2% glycerol was substituted for glucose in MM. In all media, uridine (5 mM) and uracil (10 mM) were added when necessary for complementation of the pyrG89 marker. Routine genetic approaches were used for strain construction (Kafar 1977, Appendix; available upon request). For the UV-light viability assays, conidiospores were suspended in 0.2% Tween-20 and plated out on YAG plates (YG plus 2% agar; ~100 conidia/plate). The plates were then irradiated immediately with UV using a UV Stratalinker 1800 (Stratagene, La Jolla, CA) and incubated at 30° for 48 hr to determine UV sensitivity of nondividing cells. To determine UV survival of dividing cells, conidiospores on YAG plates were first allowed to germinate for 4.5 hr at 30°. By this time the germinated spores had entered the cell cycle and were about to undergo the first mitosis. These germ cells were UV irradiated on the plates and then similarly incubated at 30° for 48 hr. Viability was determined as the percentage of colonies on treated plates compared to untreated controls.

**DNA manipulations and construction of the ΔatmA strain**: DNA manipulations were according to Sambrook and Russell (2001). DNA fragment probes for Southern blots were labeled with [α<sup>-32P</sup>]dCTP using the RTS Rad prime DNA labeling system kit (GIBCO-BRL, Gaithersburg, MD). PCR primers were designed for amplifying each DNA fragment necessary for PCR-mediated technique by using Primer Express Version 1.0 (Applied Biosystems, Foster City, CA) design software. In the deletion construction, the <i>A. fumigatus pyrG</i> gene was amplified from the pCDA21 plasmid (Chaverroche et al. 2000) and was referred to as the zeopyrG cassette because the amplified fragment also contains the zeocin-resistance gene. The PCR-mediated construction for the atmA gene consisted of three initial amplifications that generated a 5'- and 3'-flanking region of atmA gene and a final fusion PCR. For the DNA fragments containing the flanking regions, genomic DNA was used as a template. The 5'-flanking fragment, which encompasses 2000 bp upstream from the nucleotide +5214 of the atmA genomic sequence, was amplified with the primers ATM1 5'-ATTCTTAAGCATGATATGTC-3' and ATM2 5'-CTGAAATTCGGCGTTGAGGAC-3' (underlined bases indicate the region of homology to the zeo-pyrG cassette). The 3'-flanking region, which encompasses 750 bp downstream of nucleotide +7632 of the atmA genomic sequence, was amplified with the primers ATM3 5'-TGAAGCGAATTCGTCGAG-3' and ATM4 5'-TCATGCAATAACG GCC-3' (underlined bases indicate the region of homology to the zeo-pyrG cassette). The zeopyrG cassette was amplified using plasmid pCDA21 as a template and the following primers: ATM5’ZEO 5'-GGTAGCTTCTCCAGGCGAATTC-3' and ATM3’PRY 5'-AGGCGGCTGGCCCTCAT GAATTCGCCCTAACACATT-3' (where underlined bases indicate the region of homology to the 5'- and 3'-atmA flanking regions, respectively). The final 5167-bp fusion PCR fragment was amplified by using the three previous DNA fragments as templates and ATM1 and ATM4 as primers. The 50-μl amplification mixture included 1× Platinum Taq DNA Polymerase high-fidelity buffer (Invitrogen, San Diego), 20 pmol of each primer, 0.4 mM deoxynucleotide triphosphate mix, 1.0 unit of Platinum Taq high-fidelity DNA polymerase (Invitrogen), and 500 ng of genomic DNA or 100 ng of plasmid DNA. PCR amplification was carried out in a PTC100 96-well thermal cycler (MJ Research, Watertown, MA), at 94° for 2 min, and 30 times at 94° for 1 min, 52°–60° (depending on the fragment) for 1 min, and 68° for 2–3 min, followed by an extension step at 68° for 10 min. After the reaction, the PCR products were gel purified with a QIAGEN (Chatsworth, CA) PCR clean-up kit following the manufacturer’s instructions. Transformation of <i>A. nidulans</i> strain GR5 was according to the procedure of Osmani et al. (1987) using 5 μg of linear DNA fragments. Transformants were scored for their ability to grow on YG medium. Southern analysis demonstrated that the deletion cassette had integrated at the atmA locus.

**Protein expression, purification, and phosphorylation assays**: Protein assays were performed by initially growing conidia from the wild type (GR5) and IM69 (ΔatmA) strains in a reciprocal shaker at 37° for 16 hr in liquid YG medium (plus uridine and uracil if necessary). Mycelia were aseptically transferred to fresh YG medium plus 25 μM camptothecin (CPT) for different periods of time at 37°. Each sample was harvested by filtration through a Whatman filter number 1, washed thoroughly with sterile water, quickly frozen in liquid nitrogen, and disrupted by grinding. Total protein was extracted at 4° with extraction buffer (15 mM β-nitrophenylphosphate, 25 mM Tris pH 7.5, 15 mM EGTA, pH 7.5, 15 mM MgCl2) plus protease inhibitors (1 μg/ml leupeptin, 10 μg/ml aprotinin, 5 mM benzamidine, 15 mM PMSF). Protein concentration was determined using a modified Bradford assay (Bio-Rad, Hercules, CA). Twenty-five μg of ScaA<sup>RNA</sup>::His<sub>6</sub> fusion protein (purified as described in Fagundes et al. 2005) was incubated with 25 μg of GR5 or IM69 total extract in a final volume of 100 μl and incubated for 5 min at 30°. Phosphorylation reaction buffer (0.2 mM ATP, 2.5 mM MgCl2, 2.5 mM MnCl2, 0.5 μl [γ<sup>32P</sup>]dATP) was added to this protein mixture and incubated
for 1 hr at 30°C. The Ni-NTA super flow (QIAGEN) resin was prepared by washing in PBS buffer and equilibrated with ligation buffer (20 mM sodium phosphate, pH 7.6, 500 mM NaCl, 20 mM imidazole, 1 mM PMSF, 1 mM benzamidine). The resin (90 µl) was subsequently combined with 100 µl of the protein mixture and incubated for 1 hr at room temperature. The resin was recovered by centrifugation at 3000 rpm for 1 min, washed three times with the ligation buffer, and finally resuspended in 30 µl of SDS–PAGE sample buffer (62.5 mM Tris–HCL, pH 6.8, 2% SDS, 10% glycerol, 5% β-mercaptoethanol, and 5% bromophenol blue). The proteins were eluted from the resin by heating at 100°C for 3 min and separated by SDS–PAGE gels. Gels were stained with Coomassie solution (Coomassie 0.25%, methanol 50%, and acetic acid 30%), dried, and exposed to Kodak (MXG/Plus 35 cm) films.

Microscopy: Conidiospores from wild-type and ΔatmA mutant strains were germinated at 37°C and 5% bromofenol blue). The proteins were eluted from the resin by heating at 100°C for 3 min and separated by SDS–PAGE gels. Gels were stained with Coomassie solution (Coomassie 0.25%, methanol 50%, and acetic acid 30%), dried, and exposed to Kodak (MXG/Plus 35 × 43 cm) films.

RESULTS

Cloning and disruption of AtmAATM: BLAST searches of the recently completed A. nidulans genome sequence (Galagan et al. 2005; http://www.genome.wi.mit.edu/annotation/fungi/aspergillus/) were used to identify a homolog of ATM. These searches resulted in the identification of AN0038.2 (renamed here as AtmAATM; note that the previously characterized UvsB ATR is annotated as AN0675.2). The atmA gene possesses nine introns (nucleotide positions 83–133, 177–231, 1594–1648, 1960–2244, 2530–2643, 6503–6548, 6940–6982, 8051–8097, and 9059–9123, respectively) and encodes a predicted 2793-amino-acid protein with 30% identity and 48% similarity to ATM (4e-137).

To characterize the function of AtmAATM, an internal deletion was generated using a fusion PCR-based approach (see MATERIALS AND METHODS). This 2418-bp deletion removes 806 amino acids that encompass the entire FAT domain and a considerable portion of the predicted catalytic kinase domain (Figure 1A). Moreover, RT–PCR experiments verified that no atmA transcripts were generated from the region upstream of the deletion (data not shown). Protoplasts of A. nidulans strain GR5 were transformed using the fusion PCR product, and several transformants were obtained by their ability to grow in YAG. Allelic replacement of atmA was verified in at least one transformant as confirmed by the analysis of Southern blots (Figure 1B), thereby generating the ΔatmA strain IM69.

When tested on rich media (i.e., YG), the extent of radial colony growth and the production of asexual spores were not dramatically affected by the loss of AtmA (Figure 2A). However, no viable meiotic ascospores were recovered when IM69 was self-fertilized (data not shown).
Role of AtmA in the DNA damage response: As an initial test of AtmA function in the DNA damage response, the effects of several different DNA-damaging agents on the growth of IM69 were assessed. The ΔatmA strain was more sensitive to several agents, including CPT, bleomycin (BLEO), and methyl methanesulfonate (MMS), whereas it was no more sensitive than wild type to other agents such as hydroxyurea (HU) and 4-nitroquinoline-1-oxide (4-NQO) (Figure 2A). In addition, germinating ΔatmA conidiospores (i.e., mitotically active cells) exhibited a slight increase in sensitivity to UV irradiation, while dormant spores (i.e., mitotically quiescent cells) displayed wild-type levels of resistance (Figure 2B), suggesting that AtmA is important for UV-light-induced DNA damage only during cell cycle progression. To understand the basis for the sensitivity of the ΔatmA mutant to DNA damage, we examined its ability to undergo checkpoint-mediated delay of mitotic entry. The G2/M DNA damage checkpoint prevents entry into mitosis in the presence of damaged DNA (for reviews, see Hartwell and Weinert 1989; Carr 1995). To address the role of AtmA in this checkpoint, we first constructed a nimA5 ΔatmA double-mutant strain (IM69-221). The nimA5 mutation causes a late G2 arrest at the restrictive temperature of 44°C, thereby making it possible to synchronize cells in G2 before causing DNA damage. Preliminary tests verified that the nimA5 mutation did not affect the growth of the ΔatmA mutant. MMS (0.0025%) was added to either single-mutant nimA5 ΔatmA+ or double-mutant nimA5 ΔatmA G2-arrested cells for 75 min to cause transient DNA damage and then removed from the culture by replacement with fresh medium as cells were downshifted to release the G2 block. The fresh medium contained 5 µg/ml of nocodazole to cause cell block in mitosis and to allow chromosomal mitotic index (CMI) determination. Addition of MMS to nimA5 ΔatmA+ cells markedly delayed entry into mitosis after return to 30°C (Figure 3, top). In contrast, no delay was observed when MMS was added to nimA5 ΔatmA (Figure 3, bottom). These observations highlight the role of AtmA in the G2/M checkpoint that operates to prevent mitosis in the presence of DNA damage.

As a second approach to testing the role of AtmA in the DNA damage checkpoint response, we determined the ability of the ΔatmA mutant to undergo radiation-resistant DNA synthesis (RDS). The failure of A-T cells to undergo transient RDS after irradiation is a characteristic feature of the disease (for review, see D’Amours and Jackson 2002). Wild-type hyphae exposed to the radiomimetic drug BLEO display a transient delay in the kinetics of nuclear division (Figure 4A, left), whereas no such delay is observed in the ΔatmA mutant (Figure 4A, right). This observation implies that exposure to BLEO does not delay DNA synthesis when AtmA function is compromised.
Finally, we tested the capacity of AtmA to phosphorylate a downstream target in response to DNA damage. A general function of ATM homologs is the ability to phosphorylate p95/Nbs1 on serine 343 \textit{in vitro} and \textit{in vivo} after ionizing radiation (IR; Gatei et al. 2000; Lim et al. 2000; Wu et al. 2000; Zhao et al. 2000). Considering that DSBs are the main effect of IR, and that CPT induces DSBs, we used the antitopoisomerase I drug in experiments to verify whether the \textit{DatmA} strain would be unable to phosphorylate ScaA NBS1. Accordingly, protein extracts prepared from the \textit{DatmA} mutant and a wild-type strain were tested for the ability to phosphorylate the apparent \textit{A. nidulans} Nbs1 homolog ScaA NBS1 upon treatment with CPT (Figure 4B). For the wild-type strains, the intensity ratio (radioactivity signal/amount ScaA) increased with incubation time in the presence of CPT (from 0.8 at 5 min to 7.6 at 120 min). In contrast, extracts from the \textit{DatmA} mutant were unable to phosphorylate ScaA NBS1 to the same levels observed in the wild-type strain (Figure 4C). A heat-inactivated extract (KD), \textit{i.e.}, a protein extract from the wild-type grown for 30 min and heated at 100° for 10 min, was used as a negative control for the ScaA NBS1 phosphorylation reaction (Figure 4C, bottom).

Taken together, these data strongly suggest that AtmA shares with other ATM homologs a central and conserved role in the DNA damage response.

\textbf{Role of AtmA\textsuperscript{ATM} in the septation checkpoint:} In \textit{A. nidulans}, the DNA damage checkpoint inhibits septum formation by modulating the activity of NimX\textsuperscript{CDKI} (Harris and Kraus 1998; De Souza et al. 1999). In particular, chronic exposure of predivisional hyphae to low levels of DNA damage causes increased AnkA\textsuperscript{WEI1} mediated Tyr-15 phosphorylation of NimX\textsuperscript{CDKI} (Kraus and Harris 2001), which prevents septation even though nuclear division can occur (Harris and Kraus 1998). Notably, the ability of DNA damage to block septum formation requires UvsB\textsuperscript{ATR} function (Harris and Kraus 1998). We evaluated the role of AtmA in the septation checkpoint by determining whether the \textit{DatmA} mutant could undergo septum formation in the presence of a DNA-damaging agent. As expected, prolonged exposure to 0.0025% MMS reduced the level of septation in wild-type hyphae (42% of MMS-treated hyphae possessed septa \textit{vs.} 82% of untreated controls). However, unlike uvsB\textsuperscript{ATR} mutants, septum formation was not restored in the \textit{DatmA} mutant. Indeed, the septation block was much more reduced than in the wild type (2% of MMS-treated \textit{DatmA} hyphae possessed septa \textit{vs.} 92% of untreated controls). This observation suggests that AtmA does not play a direct role in blocking septum formation upon activation of the DNA damage response.

\textbf{AtmA\textsuperscript{ATM} is required for the formation of a stable polarity axis:} During the analysis of \textit{DatmA} phenotypes, it was noted that the mutant displayed pronounced morphological defects even when grown under normal conditions (\textit{i.e.}, in the absence of DNA damage). The morphogenetic program associated with the germination of \textit{A. nidulans} conidiospores includes \textit{(i)} a brief period of isotropic spore swelling, \textit{(ii)} the establishment
of a stable polarity axis, and (iii) the emergence of a polarized germ tube (Momany 2002). This program leads to the formation of a multinucleate hyphal cell that grows solely by apical extension. Landmark proteins that act as positional cues to specify sites of polarized growth have not yet been identified in A. nidulans (Harris and Momany 2004).

On a standard rich glucose medium (YG), ΔatmA conidiospores produce polarized germ tubes with kinetics similar to wild type. However, the resulting hyphae exhibit zig-zag or curled growth patterns (Figure 5) suggestive of a defect in the microtubule-dependent regulation of hyphal growth. Consistent with this view, the normal bipolar pattern of germ-tube emergence is abolished in the ΔatmA mutant. Whereas >90% of wild-type conidiospores produce a second germ tube opposite from the first, <40% of ΔatmA conidiospores display the same pattern (Figure 5). Previous observations suggest that the bipolar pattern requires an intact cytoskeleton (Harris et al. 1999), which presumably transports a landmark protein to the incipient polarization site. The wild-type growth pattern leads to the formation of mycelial colonies whose edge consists of well-spaced hyphae that grow outward in a linear manner (Figure 5). Because of the defects in growth pattern, ΔatmA colony edges are extremely disorganized and contain hyphae whose growth appears randomly oriented with respect to each other and the colony center (Figure 5). On a poorer minimal glycerol medium (M-glycerol), ΔatmA mutants display a pronounced defect in polarity establishment. Instead of forming a germ tube, they continue to grow in an isotropic manner and produce large round multinucleate spheres (Figure 5E). The aberrant growth patterns on rich media and the failure to establish polarity on poorer media occur despite apparently normal nuclear division and cell growth. Indeed, swollen ΔatmA conidiospores actually contain a greater number of nuclei than wild-type hyphae grown for the same length of time. These results suggest that AtmA might play a role in recruiting the morphogenetic machinery to polarization sites.

We established an assay that would allow us to quantify the ability of a mutant to retain a stable axis of polarized hyphal growth. This assay is based on the ability of the microfilament-depolymerizing agent cytochalasin A (CA) to cause depolarization of growing hyphal tips. When incubated in the presence of 2 μg/ml of CA for 1 hr, wild-type hyphal tips swell in an isotropic manner (Figure 6A). Upon removal of CA and return to untreated media, polarized growth resumes. Most importantly, in 94% of wild-type hyphae tested (n = 300; two independent experiments), growth resumes along the original polarity axis (Figure 6A). This observation suggests that, despite the loss of detectable actin filaments, the morphogenetic machinery is retained at polarization sites for at least 1 hr. When subjected to the same CA treatment regimen, ΔatmA mutants grown in MM also form swollen hyphal tips (Figure 6B). However,
upon washout, growth did not resume using the original polarity axis. Instead, in 79% of tested hyphae (n = 300; two independent experiments), a new axis was established in the subapical region of the hyphal tip cell, thereby producing a new lateral branch (Figure 6B). Moreover, 16% of the tested ΔatmA hyphae failed to resume growth, which was never observed in CA-treated wild-type hyphae. These results imply that the morphogenetic machinery is not stably localized at the hyphal tip of ΔatmA mutants. We propose that this reflects the failure to properly utilize positional cues at the hyphal tip or the loss of these cues altogether.

**AtmA** regulates microtubule organization at hyphal tips: In *A. nidulans*, the FITC-conjugated lectin WGA can be used to detect sites of cell-wall deposition (Harris et al. 1999). At a concentration of 5 μg/ml, FITC–WGA staining is solely confined to the tips of wild-type hyphae (Figure 7A). In ΔatmA mutants, FITC–WGA stained hyphal tips as well, but prominent spore body staining was also observed (Figure 7B). This observation

**Figure 5.**—Morphological defects caused by the ΔatmA mutation. Wild-type hyphae grown on YG (A) or M-glycerol (B) media. ΔatmA mutant grown on YG (C and D) or M-glycerol (E) media. Note the zig-zag or curled hyphae in C and D and the failure to establish polarity in E. Wild-type (F) and ΔatmA (G) hyphae growing on the surface of a YG plate. Note that wild-type hyphae radiate outward in a linear fashion, whereas mutant hyphae do not and frequently curl backward. (H) Germination pattern of wild-type and ΔatmA conidiospores. Spores were allowed to germinate on YG media for 11 hr. Spores possessing two germ tubes were classified as displaying (left to right) bipolar, quarterpolar, or random germination patterns. N = 200. Bar, 10 μm.

**Figure 6.**—ΔatmA mutants fail to reestablish a lost polarity axis. Hyphae were incubated with cytochalasin A to disrupt polarized growth and cause tip swelling (arrows). Upon return to drug-free medium, wild-type hyphae resumed polarized growth along the original axis (A, arrow). By contrast, ΔatmA mutants established new axes that became lateral branches (B, arrow). Bar, 10 μm.
suggests that a pool of vesicles carrying precursors for cell-wall biosynthesis was inappropriately retained within the spore body. Wild-type hyphae grown in the presence of the microtubule-depolymerizing agent benomyl exhibit a similar intense localization of FITC–WGA within the spore body. Wild-type hyphae grown in the presence of 5 μg/ml benomyl, growth is largely confined to the spore body (C). Bars, 10 μm.

**Figure 7.**—Growth is not confined to the hyphal tip in ΔatmA mutants. Hyphae were stained with FITC-conjugated wheat germ agglutinin to localize sites of active cell-wall deposition. Growth occurs at the tips of wild-type hyphae (A, arrow). In ΔatmA mutants (B), growth is also observed in the spore body (arrow). In wild-type hyphae that were germinated in the presence of 5 μg/ml benomyl, growth is largely confined to the spore body (C). Bars, 10 μm.

**DISCUSSION**

ATM and ATR are paralogous PIKKs that orchestrate the DNA damage response in eukaryotic cells (Shiloh 2001; McKinnon 2004). These kinases possess both overlapping and distinct roles in the regulation of this response. In *A. nidulans*, we have previously shown that UvsB<sup>ATR</sup> regulates the DNA damage checkpoint response, inhibits septation in predivisional hyphae that have experienced DNA damage, and controls both the expression and the localization of proteins involved in the processing and repair of DNA damage (Harris and Kraus 1998; Hofmann and Harris 2000; Fagundes et al. 2005). In this study, we present the first analysis of ATM in a filamentous fungus. Like other ATM kinases, we show that AtmA<sup>ATM</sup> modulates DNA damage checkpoint responses and phosphorylates the downstream target ScaA<sup>NBS1</sup>. However, we also demonstrate that AtmA<sup>ATM</sup> has an additional role in the formation of stable axes of polarized hyphal growth. In this respect, AtmA may share a pivotal function with its homologs in other highly polarized cell types such as neurons.

**Role of AtmA<sup>ATM</sup> in the DNA damage response:** Our results demonstrate that AtmA<sup>ATM</sup> prevents mitotic entry when DNA is damaged during S or G2 phases of the cell cycle. To some extent, this may account for the DNA damage sensitivity of the ΔatmA mutant, because dormant conidiospores, in which the checkpoint is dispensable, display wild-type levels of resistance to some forms of DNA damage. On the other hand, AtmA<sup>ATM</sup> likely has additional roles in promoting DNA repair. Like other eukaryotes, one such function may be to regulate the activity of the MRN complex (D’Amours and Jackson 2002). Homologs of each component of this complex have been characterized in *A. nidulans* (*i.e.*, MreA = Mre11, Sld1 = Rad50, and ScaA = Nbs1) and shown to be involved in multiple facets of the DNA damage response (Bruschi et al. 2000; Semighini et al. 2003; Malavazi et al. 2005). Notably, UvsB<sup>ATR</sup> regulates both the expression and the nuclear localization of ScaA<sup>NBS1</sup> (Fagundes et al. 2005), whereas AtmA<sup>ATM</sup> controls the level of ScaA<sup>NBS1</sup> phosphorylation. These distinct regulatory inputs could potentially confer ATR- and ATM-specific functions on the *A. nidulans* MRN complex. Alternatively, they could act in concert to ensure that the complex is fully activated in response to the presence of damaged DNA. A more detailed analysis of the interactions between the MRN complex and both UvsB<sup>ATR</sup> and AtmA<sup>ATM</sup> is needed to address these possibilities.

UvsB<sup>ATR</sup> inhibits septum formation in predivisional hyphae that have been exposed to low levels of DNA damage (Harris and Kraus 1998). Although this effect is mediated by Ank<sup>WEE-1</sup>-dependent Tyr-15 inhibitory phosphorylation of NimX<sup>Cdk1</sup> (Kraus and Harris 2001), the mechanism by which UvsB<sup>ATR</sup> may influence the Tyr-15...
phosphorylation state of NimX$^{CDK1}$ has not been determined. By contrast, AtmA$_{ATM}$ does not appear to be involved in the inhibition of septum formation. Instead, the ΔatmA mutation exacerbates the septation block. This could simply reflect an increase in the strength of the DNA damage signal caused by loss of AtmA$_{ATM}$. However, an intriguing possibility is that AtmA$_{ATM}$ may directly promote septum formation during recovery from the DNA damage response. We have previously shown that this function requires the A. nidulans RecQ/BLM helicase homolog MusNBLM (Hofmann and Harris 2001). Because extensive interactions between ATM and BLM have been characterized in animal cells (Beamish et al. 2002), it is tempting to speculate that similar interactions could trigger recovery from the DNA response in predivisional hyphae.

**Role of AtmA$_{ATM}$ in polarized hyphal growth:** The observation that AtmA$_{ATM}$ is required for the formation of a stable axis of hyphal polarity was unexpected. In yeast cells, ATM function has never been linked to the establishment or maintenance of cell polarity. Moreover, only in human and mouse Purkinje cells has ATM been implicated in functions related to cellular morphogenesis (Barlow et al. 2000). Accordingly, our results raise the interesting possibility that ATM possesses a unique and previously uncharacterized role in the formation of stable polarity axes that is specific to highly polarized cells such as hyphae and neurons. Furthermore, we propose that failure to complete this function causes morphological defects that disrupt neuronal differentiation and thus contribute to the disease pathology of A-T.

Our observations suggest that AtmA is required to specify the growth zone at hyphal tips. In the filamentous fungi, an apical cluster of vesicles and cytoskeletal elements termed the Spitzenkorper plays a critical role in defining the growth zone (summarized by Harris et al. 2005). Notably, interactions between cytoplasmic microtubules and the cell cortex appear to play an important role in maintaining the position of the Spitzenkorper within the middle of the growth zone (Konzack et al. 2005). We propose that AtmA-dependent phosphorylation may influence these interactions, thereby helping to define a discrete zone for the cortical recruitment of cytoplasmic microtubules. The absence of this zone in the ΔatmA mutant would presumably lead to random growth.}

**Figure 8.—Microtubules fail to converge at the hyphal tip in ΔatmA mutants.** Wild-type (A–D) and ΔatmA (E–H) hyphae were examined by immunofluorescence microscopy. (A and E) Nuclei visualized using Hoechst 33258. (B and F) Microtubules visualized with an anti-β-tubulin antibody. (C and G) merged image. (D and H) Brightfield images (single section, whereas all other images are maximum projection of Z stacks). (I) The microtubule organization at the hyphal tip is enlarged to highlight the defect. Bars, 5 μm.
interactions between microtubules and the cortex at hyphal tips, which would in turn affect Spitzenkorper position and the maintenance of a stable polarity axis. Consistent with this hypothesis, Horio and Oakley (2005) demonstrate that although microtubules are not strictly required for polarized growth, they are rate limiting for the growth of hyphal-tip cells. What proteins at the hyphal tip could be the targets of ATM-dependent regulation? Potential candidates include proteins that associate with the plus ends of cytoplasmic microtubules, such as dynactin, which has been suggested as a possible ATM target (O’Neill et al. 2000), or plus-end-directed kinesins involved in the transport of these proteins. One such kinesin, KipA, has already been shown to regulate Spitzenkorper position (Konzack et al. 2005). Alternatively, homologs of known cortical microtubule-anchoring proteins from budding or fission yeast (i.e., Kar9, Tea1, Tea4) may be ATM targets. Finally, fungal β-adaptins, whose animal homologs are known substrates of ATM, may be AtmA targets that control the trafficking of a landmark protein that specifies a cortical zone for microtubule interactions. Proteomic approaches designed to identify AtmA-interacting partners should ultimately reveal the relevant target.

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