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A Point Mutation in the *Aspergillus nidulans sonB*^{Nup98} Nuclear Pore Complex Gene Causes Conditional DNA Damage Sensitivity

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ABSTRACT

The nuclear pore complex (NPC) is embedded in the nuclear envelope where it mediates transport between the cytoplasm and nucleus and helps to organize nuclear architecture. We previously isolated *sonB*1, a mutation encoding a single amino acid substitution within the *Aspergillus nidulans* SONBn^{Nup98} NPC protein (nucleoporin). Here we demonstrate that this mutation causes marked DNA damage sensitivity at 42°. Although SONBn^{Nup98} has roles in the G_2 transition, we demonstrate that the G_2 DNA damage checkpoint is functional in the *sonB*1 mutant at 42°. The MRN complex is composed of MRE11, RAD50, and NBS1 and functions in checkpoint signaling, DNA repair, and telomere maintenance. At 42° we find that the DNA damage response defect of *sonB*1 mutants causes synthetic lethality when combined with mutations in *scaA*^{NBS1}, the *A. nidulans* homolog of NBS1. We provide evidence that this synthetic lethality is independent of MRN cell cycle checkpoint functions or MREA^{MRE11}-mediated DNA repair functions. We also demonstrate that the single *A. nidulans* histone *H2A* gene contains the C-terminal SQE motif of histone H2AX isoforms and that this motif is required for the DNA damage response. We propose that the *sonB*1 nucleoporin mutation causes a defect in a novel part of the DNA damage response.

THE nuclear pore complex (NPC) is an evolutionarily conserved structure made up of multiple copies of ~30 different NPC proteins (nucleoporins) embedded in the nuclear envelope (for review see Hetzer *et al.* 2005; Tran and Wente 2006). The NPC restricts diffusion of proteins and nucleic acids between the nucleus and cytoplasm and facilitates active nucleocytoplasmic transport through the nuclear envelope. Other roles for the NPC are only just beginning to be understood. For example, in *Saccharomyces cerevisiae* the NPC has been demonstrated to play roles in tethering telomeres to the nuclear periphery, which helps to facilitate transcriptional silencing of subtelomeric genes (Galy *et al.* 2000; Feuerbach *et al.* 2002; Therizols *et al.* 2006). Somewhat paradoxically, certain nucleoporins have been demonstrated to preferentially associate with transcriptionally active genes (Ishii *et al.* 2002; Casolari *et al.* 2004, 2005; Dilworth *et al.* 2005; Menon *et al.* 2005; Schmid *et al.* 2006). Interestingly, budding yeast nucleoporin null alleles that display sensitivity to DNA-damaging agents have been identified (Galy *et al.* 2000; Bennett *et al.* 2001; Chang *et al.* 2002; Loeillet *et al.* 2005; Therizols *et al.* 2006). Although the mechanism leading to DNA damage sensitivity of these nucleoporin nulls is currently not known, it is likely that NPC function is required for the normal DNA damage response.

In response to DNA damage, cells both activate DNA repair pathways and enforce checkpoints to arrest cell cycle progression until DNA has been repaired (for review see Zhou and Elledge 2000; McGowan and Russell 2004). In the presence of DNA damage, the G_2 DNA damage checkpoint prevents mitotic entry via tyrosine phosphorylation of the cyclin-dependent kinase Cdc2 (Cdk1 or NIMX^{Cdc2} in *Aspergillus nidulans*). Tyrosine-phosphorylated Cdc2 must be dephosphorylated for mitosis to occur and mutation of tyrosine to nonphosphorylatable phenylalanine (cdc2F mutants) results in premature mitotic entry in the presence of DNA damage (Ye *et al.* 1997; for review see Zhou and Elledge 2000). The evolutionarily conserved phosphatidyl inositol 3-kinase-like kinases (PIKK) ATR and ATM function to signal both repair and checkpoint pathways in response to DNA damage. The central importance of ATR and ATM is underscored by human diseases such as ataxia telangiectasia, which result from mutation of these genes. More recently the *M*RE11, *R*AD50, *N*BS1 (MRN) complex has been demonstrated to function early in the DNA damage response together with the ATM and ATR kinases, and mutations in MRN genes have been linked to the diseases Nijmegen breakage syndrome (NBS) and ataxia telangiectasia-like disorder (Uziel *et al.* 2003; Difilippantonio *et al.* 2005; Lee and Paull 2005; Stiff *et al.* 2005; You *et al.* 2005; Jazayeri *et al.* 2006; for review see Abraham and Tibbetts 2005; Stavridi and Halazonetis 2005; Zhang *et al.* 2006).

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The MRN complex has roles in cell cycle checkpoint signaling as well as in DNA repair and telomere maintenance (for review see D'Amours and Jackson 2002; Zhang *et al.* 2006). Mre11p has DNA nuclease, strand dissociation, and strand annealing activities, while RAD50 has similarity to structural maintenance of chromosome proteins and is thought to form a dimer that bridges DNA strands at a double-strand break (for review see D'Amours and Jackson 2002; Stavridi and Halazonetis 2005; Zhang *et al.* 2006). The precise function of NBS1 is less clear although it contains a forkhead-associated (FHA) and breast cancer C terminus (BRCT) domain, suggesting that it binds phosphorylated proteins (Becker *et al.* 2006; for review see D'Amours and Jackson 2002; Stavridi and Halazonetis 2005; Zhang *et al.* 2006). Indeed, NBS1 has been demonstrated to bind the γ-H2AX phosphoserine epitope, which is phosphorylated early in the DNA damage response by the ATM/ATR kinases in nucleosomes surrounding DNA damage (Downs *et al.* 2000; Kobayashi *et al.* 2002; Celeste *et al.* 2003; Nakamura *et al.* 2004; Unal *et al.* 2004; for review see Vidanes *et al.* 2005).

A. nidulans has long been utilized as a model genetic system and the cell cycle and DNA damage response in this organism is well characterized (for review see Osmani and Ye 1996; Goldman *et al.* 2002; Goldman and Kafer 2004; Osmani and Mirabito 2004). Temperature-sensitive mutants of the *A. nidulans nimA* kinase reversibly arrest in G_2 at the nonpermissive temperature of 42° even though the Cdc2/cyclinB kinase is fully activated (Osmani *et al.* 1987). This is likely because the Cdc2/cyclinB kinase is cytoplasmic at a *nimA*1 G_2 arrest and cannot enter the nucleus (Wu *et al.* 1998). We have previously isolated mutations in two nucleoporins, $SONA^{Gle2}$ and $SONBn^{Nup98}$, which suppress a *nimA*1 G_2 arrest and allow entry into mitosis (Wu *et al.* 1998; De Souza *et al.* 2003). Both $SONA^{Gle2}$ and $SONBn^{Nup98}$ disperse from the NPC during the partial disassembly of the NPC in *A. nidulans* (De Souza *et al.* 2004). It is likely that these NPC mutants suppress the *nimA*1 G_2 arrest by allowing sufficient Cdc2/cyclinB and tubulin into the nucleus to allow mitotic entry (Wu *et al.* 1998; De Souza *et al.* 2004). Here we show that the *sonB*1 mutation displays a high degree of sensitivity to DNA-damaging agents at 42° but that this DNA damage sensitivity is independent of the G_2 DNA damage checkpoint. Epistasis analysis indicates that $SONBn^{Nup98}$ functions on a different pathway of the DNA damage response from those involving $UVSC^{Rad51}$, $UVSH^{Rad18}$, γ-H2AX phosphorylation, and the G_2 DNA damage checkpoint. The defect in *sonB*1 mutants that causes DNA damage sensitivity also results in synthetic lethality at 42° when combined with mutations in $scaA^{NBS1}$, which encodes the *A. nidulans* homolog of NBS1 (Bruschi *et al.* 2001; Semighini *et al.* 2003). Similar synthetic lethality was not observed between *sonB*1 and $mreA^{MRE11}$ mutants, suggesting that *sonB*1 synthetic lethality with $scaA^{NBS1}$ mutants is independent of the DNA repair activities of the MRN complex. We provide evidence that the synthetic lethality between *sonB*1 and $scaA^{NBS1}$ mutants is also independent of the cell cycle checkpoint functions of $SCAA^{NBS1}$. Our data suggest that the $SONBn^{Nup98}$ nucleoporin may have a novel role in the DNA damage response.

MATERIALS AND METHODS

General techniques: Media and general techniques for *A. nidulans* culture, transformation, and DAPI staining for chromosome mitotic index were as previously described (Osmani *et al.* 1987, 1991, 1994; Oakley and Osmani 1993; Ye *et al.* 1995; Wu *et al.* 1998). Western analysis was carried out preparing lysates in sample buffer containing 6 M urea as described previously (De Souza *et al.* 2000). The phospho-Cdc2 (Tyr15) antibody was purchased from Cell Signaling Technology.

DNA damage sensitivity assays: Quiescent conidiospores and germlings were tested for sensitivity to UV irradiation as previously described (Ye *et al.* 1997) using a microprocessor-controlled UV crosslinker (FBUVXL-1000; Fischer Biotech; 254 nm). Hydroxyurea, 1,2,7,8 diepoxyoctane (DEO), methyl methanesulfonate (MMS), and camptothecin were all purchased from Sigma (St. Louis) and added to media at the appropriate concentrations immediately prior to pouring plates. All plates were prewarmed to 32° or 42° as appropriate prior to inoculation. Entry into mitosis after MMS treatment of either conidiospores or germlings arrested at the G_2 *nimA*5 arrest point was as described previously (Ye *et al.* 1997).

Plasmid constructs: Serine 129 of the *A. nidulans* histone *H2A* gene (May and Morris 1987) in plasmid pRG3-H2A-H2B (De Souza *et al.* 2003) was mutated to alanine using the Quickchange site-directed mutagenesis kit (Stratagene, La Jolla, CA) to generate plasmid pRG3 H2A S129A-H2B. Introduction of the appropriate mutation was confirmed by sequencing.

***A. nidulans* strains:** Genotypes of strains used in this study are listed in supplemental Table 1 at http://www.genetics.org/supplemental/. Although the $mreA^{MRE11}$ disruption strain (TMRE) was previously reported as sterile (Semighini *et al.* 2003), we were able obtain viable progeny in sexual crosses. The *H2A S129A* mutant was constructed by a two-step gene replacement (Ye *et al.* 1996). GR5 (*pyrG*89, *wA*3, *pyroA*4) and CDS40 (*pyrG*89, *wA*2, *pyroA*4, *sonB*1) were transformed with plasmid pRG3 H2A S129A-H2B and single-site integration at the histone *H2A/H2B* locus confirmed by Southern blot analysis and PCR using primers external to the region of duplication (Yang *et al.* 2004). Plasmid loss was selected for with 5-fluoroorotic acid (Osmani *et al.* 1994) and evictants maintaining the *H2A S129A* mutation were selected for by screening for DNA damage sensitivity. Introduction of the *H2A S129A* mutation was confirmed by PCR amplification and sequencing of the histone *H2A* locus. Double-mutant strains generated between nucleoporin mutants and DNA damage response mutants were confirmed by crossing strains back to a wild-type strain to recover the single-mutant phenotypes. Strains IM69, MKF11, SCA299-16, and TMRE were kind gifts from Gustavo Goldman (Universidade de São Paulo, São Paulo, Brazil).

RESULTS

The *sonB*1 NPC mutant is highly DNA damage sensitive at 42°: The *nimA*1 temperature-sensitive mutation causes cells to arrest in G_2 of the cell cycle at the restrictive temperature of 42°. Intriguingly, we previously

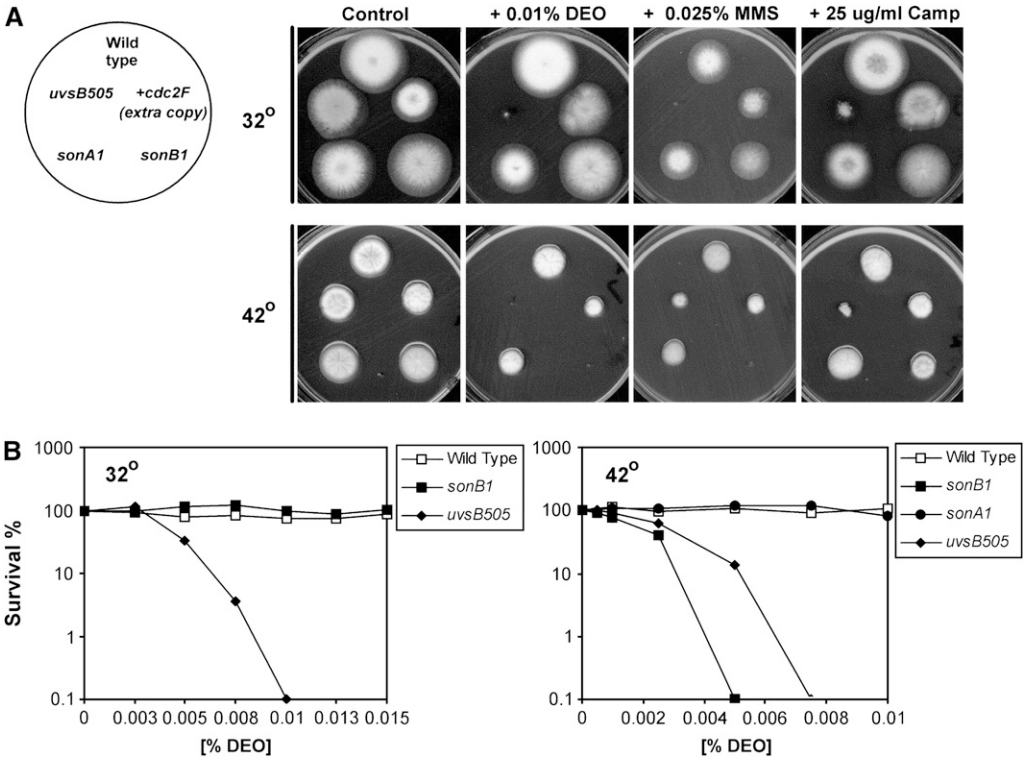

FIGURE 1.—The *sonB*1 mutant is highly sensitive to DNA-damaging agents at 42°. (A) Colony formation of wild-type (R153), *uvsB*505^{ATR} (CDS314), *cdc2*F (FRY24), *sonA*1 (CDS365), and *sonB*1 (CDS364) strains under the indicated conditions. Plates were incubated for 3 days. Note that the sectoring of the *cdc2*F mutant at 32° in the presence of DEO or camptothecin is due to loss of the *cdc2*F allele by plasmid eviction, leaving the wild-type allele. (B) Viability of the indicated strains (250 conidiospores spread/plate, two plates/strain) at 32° and 42° in the presence of different concentrations of the DNA alkylating agent DEO. Viability was assessed after 3 days incubation.

isolated single point mutations in two essential NPC proteins, SONA^{Gle2} and SONBn^{Nup98}, which suppress the *nimA*1 G_2 arrest and allow mitotic entry at 42° (WU *et al.* 1998; DE SOUZA *et al.* 2003). This suggests that the *sonA*1 and *sonB*1 NPC mutants are defective in some aspect of G_2 regulation at 42°. As loss of G_2 checkpoint functions over mitotic entry can cause DNA damage sensitivity, we tested the ability of *sonA*1 and *sonB*1 mutants to form a colony in the presence of DNA-damaging agents. Wild-type and the G_2 checkpoint-deficient *uvsB*505^{ATR} and *cdc2*F mutant strains were used as controls (YE *et al.* 1997; DE SOUZA *et al.* 1999). Strikingly, the *sonB*1 mutant displayed marked sensitivity to the DNA alkylating agents MMS and DEO at 42° but behaved similarly to wild type at 32° (Figure 1A). Similar results were obtained using survival assays that indicated that the *sonB*1 mutant was more sensitive than the *uvsB*505^{ATR} mutant to DEO at 42° (Figure 1B). Moreover, this DNA damage sensitivity was specific to the *sonB*1 mutant as the *sonA*1 nucleoporin mutant remained viable at 42° in the presence of DEO or MMS (Figure 1, A and B). Importantly, the DNA damage sensitivity of *sonB*1 mutants at 42° was not due to general cellular stress as *sonB*1 mutants were not sensitive to nocodazole, or camptothecin at 42° (Figure 1A; data not shown). Thus the *sonB*1 mutation causes a defect in the DNA damage response specifically at the temperature at which it suppresses the *nimA*1 G_2 arrest. It is particularly intriguing that a single amino acid substitution within an essential NPC protein (DE SOUZA *et al.* 2003) would cause sensitivity to DNA-damaging agents.

In *A. nidulans*, mutants with defective G_2 checkpoint functions are sensitive to DNA damage only if damage is elicited after cells have entered the cell cycle but not if damage is elicited to quiescent conidiospores (YE *et al.* 1997; DE SOUZA *et al.* 1999). This is because after DNA damage has occurred, quiescent conidiospores take several hours to enter the cell cycle, allowing time for DNA repair before DNA replication and mitotic entry. We therefore compared the UV irradiation sensitivity of *sonB*1 germlings and quiescent conidiospores at 42°. The *cdc2*AF mutant, which is defective in the G_2 checkpoint over mitotic entry (YE *et al.* 1997), was also tested as a control. The *sonB*1 mutant behaved similarly to the *cdc2*AF mutant with germlings, but not conidiospores, displaying sensitivity to UV irradiation at 42° (Figure 2). In contrast, both germlings and conidiospores of the *uvsH*304^{Rad18} DNA-repair-deficient mutant (KAFER and MAYOR 1986; YOON *et al.* 1995) were sensitive to UV irradiation elicited at 42°. Notably, the UV irradiation sensitivity of *sonB*1 germlings was restricted to 42° (Figure 2), similar to the case for *sonB*1 sensitivity to MMS and DEO (Figure 1). These data are consistent with the *sonB*1 mutant potentially having a defective G_2 DNA damage checkpoint at 42°, the temperature at which this mutation suppresses the *nimA*1 G_2 arrest.

In *sonB*1 mutants, Cdc2 undergoes tyrosine phosphorylation in response to DNA damage: Similar to humans, the G_2 DNA damage checkpoint prevents mitotic entry through a pathway leading to tyrosine phosphorylation of Cdc2 in *A. nidulans* (YE *et al.* 1997). We therefore examined the ability of *sonB*1 mutants

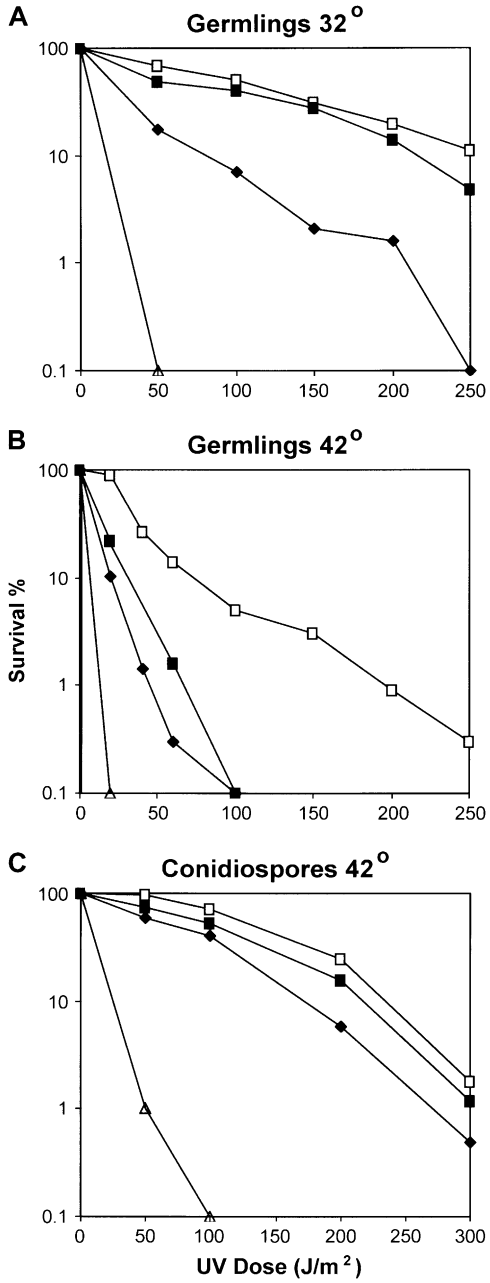

FIGURE 2.—Differential UV sensitivity of *sonB*1 quiescent conidiospores and germlings at 42°. Conidiospores (250/plate, two plates/strain) of (□) wild type (GR5), (♦) *cdc*2AF (FRY20-1), (■) *sonB*1 (CDS40), and (△) *uvsH*304^{Rad18} (A329) were spread onto plates and either were allowed to germinate at (A) 32° or (B) 42° for 6 hr prior to UV irradiation or (C) were immediately UV irradiated. After irradiation, plates were incubated at the indicated temperatures for 2 days to allow colony formation. The percentage survival after UV irradiation is expressed as the percentage of colonies produced in the absence of treatment. This experiment was performed twice with similar results.

to tyrosine phosphorylate Cdc2 in response to DNA damage at 42°. Asynchronous wild-type or *sonB*1 log-phase cultures were shifted to 42° for 3 hr and then either treated or not treated with MMS for an additional 2 hr at 42°. Lysates were prepared at each stage and immunoblotted with an antibody specific for tyrosine-phosphorylated Cdc2. Cdc2 tyrosine phosphorylation increased similarly in either wild-type or the *sonB*1 mutant in response to DNA damage (Figure 3A). To ensure that cells were in G_2 prior to the addition of MMS, we also performed this experiment in strains that contained the *nimA*5 temperature-sensitive mutation, which arrests cells in G_2 at the restrictive temperature of 42° (OSMANI *et al.* 1987). At *nimA*5 arrest, Cdc2 was not tyrosine phosphorylated in the absence of DNA damage, but became tyrosine phosphorylated following the addition of MMS (Figure 3B), consistent with our previous results (YE *et al.* 1997). As shown in Figure 3B, *sonB*1 mutants were able to tyrosine phosphorylate Cdc2 in response to DNA damage elicited at the G_2 *nimA*5 arrest point. This provides strong evidence that the G_2 DNA damage checkpoint pathway leading to Cdc2 tyrosine phosphorylation is functional in *sonB*1 mutants at 42°. Interestingly, *sonB*1 mutants displayed a higher level of tyrosine-phosphorylated cdc2 compared to wild-type strains (Figure 3, A and B). As this was observed at 32° as well as at 42°, this effect is likely not related to the DNA damage sensitivity of *sonB*1 mutants at 42° but may reflect the demonstrated defect of *sonB*1 mutants in G_2/M regulation (DE SOUZA *et al.* 2003).

While the above data demonstrate that *sonB*1 mutants are able to tyrosine phosphorylate Cdc2 in response to DNA damage, it is still possible that just enough Cdc2/cyclinB is able to enter the nucleus, be activated, and allow inappropriate mitotic entry. To determine if this is the case, we next examined if *sonB*1 mutants enter mitosis prematurely if DNA is damaged. To do this, we first synchronized cells at the *nimA*5 G_2 arrest point (YE *et al.* 1997). These G_2-arrested cells were either treated or not treated with MMS to elicit DNA damage. Cells were then released to the *nimA*5 permissive temperature of 32° by media exchange and mitotic entry followed by examining cells for condensed DNA at time points after release. As expected, the *nimA5* control delayed mitotic entry in the presence of DNA damage while cells also containing the *cdc*2AF mutation entered mitosis similarly in the presence or absence of DNA damage (Figure 3C). Cells containing the *sonB*1 mutation delayed mitotic entry in the presence of DNA damage (Figure 3C), consistent with these cells having an intact G_2 DNA damage checkpoint. However, as *sonB*1 mutants are not DNA damage sensitive at 32° (Figure 1), it is possible that the delay in mitotic entry of the *nimA*5 *sonB*1 mutant in these experiments was due to a rapid reactivation of *sonB*1 upon shifting cultures to 32°. To determine if *sonB*1 mutants delay mitotic entry if cells are maintained at 42°, we germinated wild-type or *sonB*1 conidiospores at 42° in the presence or absence of MMS and followed entry into the first mitosis. Under these conditions, *sonB*1 mutants delayed entry into mitosis in

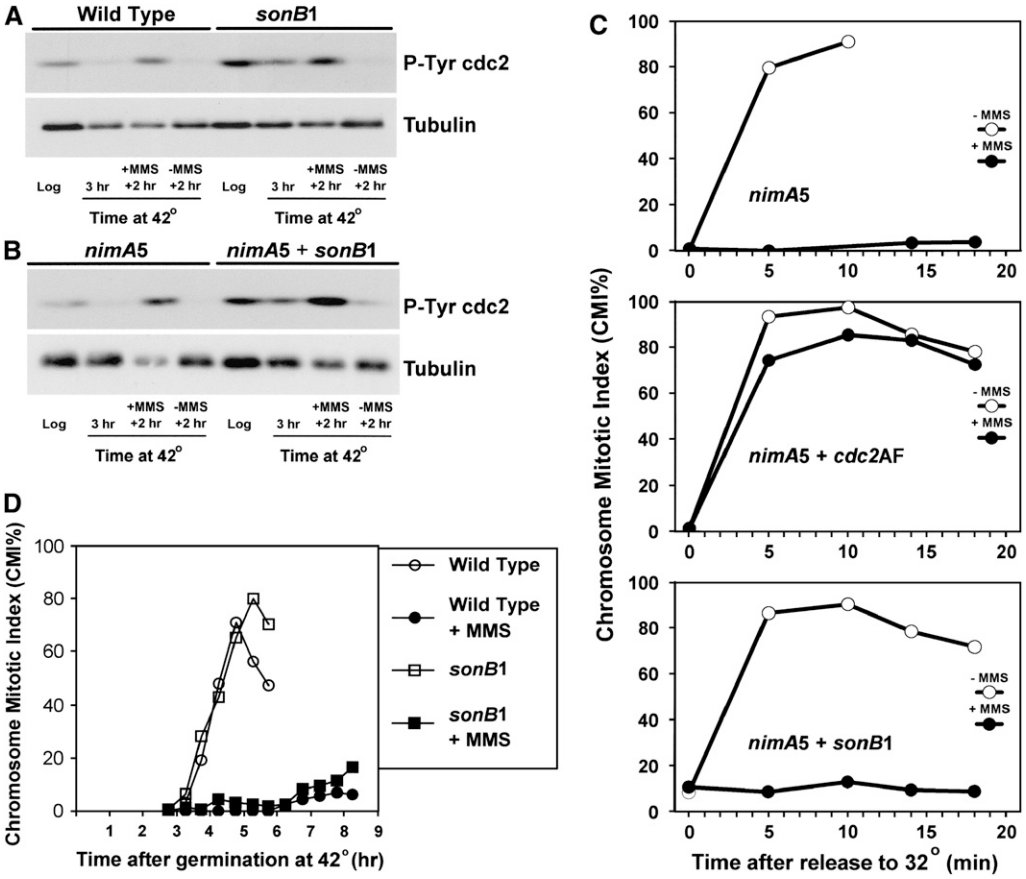

FIGURE 3.—*sonB*1 mutants undergo tyrosine phosphorylation of Cdc2 and arrest in G_2 in response to DNA damage. (A and B) Log-phase wild-type (GR5), *sonB*1 (CDS40), *nimA*5 (SO6), and *nimA*5 *sonB*1 (CDS119) cultures were shifted to 42° for 3 hr. Cultures were then divided into two and grown for another 2 hr in the presence or absence of 0.04% MMS. The relative levels of tyrosine 15-phosphorylated Cdc2 were determined by immunoblotting with an antibody specific for this epitope at the indicated time points. Levels of tubulin are shown as a loading control. (C) Chromosome mitotic index (CMI) of *nimA*5 (SO54), *nimA*5 *cdc2*AF (AT27), and *nimA*5 *sonB*1 (CDS119) germlings treated or not with 0.025% MMS during a *nimA*5 G_2 arrest (42°) prior to release to *nimA*5 permissive temperature (32°) in the absence of MMS. Note that *nimA*5 and *nimA*5 + *sonB*1 cells did not display an increase in CMI percentage even 30 min following release from *nimA*5 arrest in the presence of MMS. (D) Wild-type (GR5) and *sonB*1 (CDS40) conidiospores were germinated in the presence or absence of 0.01% MMS and the CMI was determined by DAPI staining. Nocodazole (5 μg/ml) was included to prevent mitotic exit once cells entered mitosis.

the presence of DNA damage similarly to a wild-type strain (Figure 3D). Together, these results indicate that *sonB*1 mutants have a functional G_2 DNA damage checkpoint, even though *sonB*1 germlings are more sensitive to UV irradiation than are *sonB*1 quiescent conidiospores.

***sonB*1 mutants display synthetic lethality with *scaA*^{NBS1} mutants at 42° without DNA damage:** The above indicates that the *sonB*1 mutant is defective in some part of the DNA damage response other than the G_2 DNA damage checkpoint. To further investigate this, we determined if *sonB*1 mutants genetically interacted with mutants defective in different aspects of the DNA damage response in *A. nidulans* (KAFER and MAYOR 1986; YOON *et al.* 1995; KAFER and MAY 1997; VAN HEEMST *et al.* 1997; YE *et al.* 1997; DE SOUZA *et al.* 1999; HOFMANN and HARRIS 2000; BRUSCHI *et al.* 2001; SEMIGHINI *et al.* 2003; MALAVAZI *et al.* 2006; NAYAK *et al.* 2006). Most striking were the genetic interactions between *sonB*1 and mutants of the *A. nidulans* NBS1 ortholog *scaA*^{NBS1} (BRUSCHI *et al.* 2001). Notably, *sonB*1 mutants were not viable at 42° when combined with the *scaA*1^{NBS1} mutation, even without addition of any genotoxic agent (Figure 4A). Although *sonB*1 *scaA*1^{NBS1} double mutants formed smaller colonies at 32° and 37° than either single mutant, the synthetic lethality was restricted to 42°, the same temperature at which *sonB*1 mutants display DNA damage sensitivity (Figure 4A). The *scaA*1 mutation is predicted to result in a truncated SCAA^{NBS1} protein due to a single base transversion introducing a stop codon in the *scaA*1 reading frame (BRUSCHI *et al.* 2001). To determine if the temperature-dependent synthetic lethality between *sonB*1 and *scaA*1^{NBS1} was due to complete loss of SCAA^{NBS1} function, we constructed a double mutant between *sonB*1 and a null allele of *scaA* (SEMIGHINI *et al.* 2003). The *sonB*1 Δ*scaA*^{NBS1} and *sonB*1 *scaA*1^{NBS1} double mutants behaved identically, confirming that SCAA^{NBS1} function is required for *sonB*1 survival at 42°. SCAA^{Nbs1} is a component of the MRN complex, which consists of MRE11, RAD50, and NBS1 (SEMIGHINI *et al.* 2003; for review see D'AMOURS and JACKSON 2002; D'ADDA DI FAGAGNA *et al.* 2004; STAVRIDI and HALAZONETIS 2005; ZHANG *et al.* 2006). We next determined if *sonB*1 displayed a similar synthetic lethal interaction with a disrupted allele of *mreA*^{MRE11}, the *A. nidulans* ortholog of MRE11 (SEMIGHINI *et al.* 2003). In contrast to the synthetic lethality observed with *scaA*^{NBS1} mutants, *sonB*1 *mreA*^{MRE11} double mutants were able to

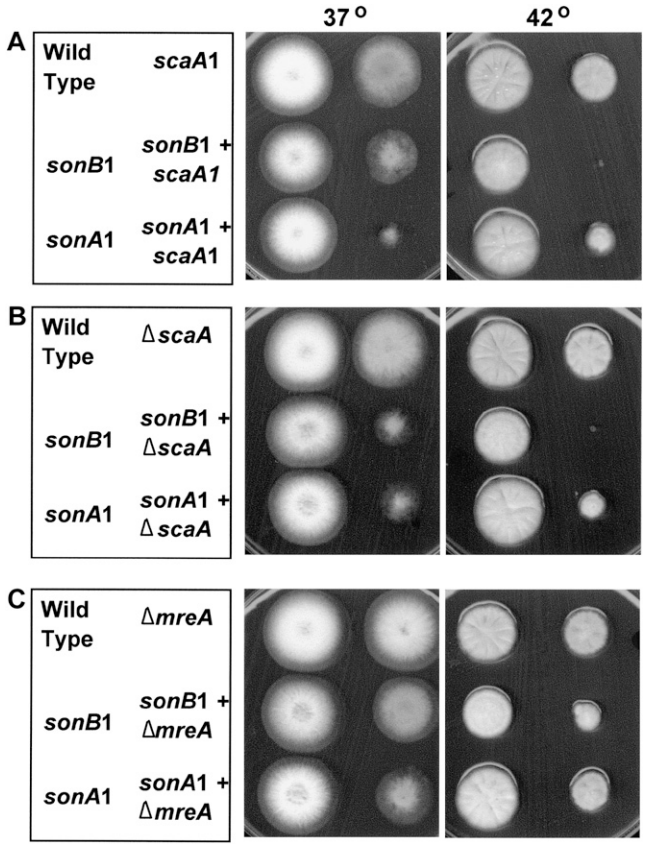

FIGURE 4.—The *sonB*1 mutant is synthetically lethal with *scaA*^{NBS1} mutants at 42°. Wild type and the indicated single or double mutants were inoculated on plates and grown at either 37° (2 days) or 42° (3 days). Note that *sonB*1 displays synthetic lethality with either (A) *scaA*1^{NBS1} or (B) the *scaA*^{NBS1} null at 42° but not with the (C) *mreA*^{MRE11} mutant. Strains used were CDS323, CDS324, CDS326, CDS350, CDS351, CDS364, CDS365, CDS373, CDS374, CDS375, MKF11, and R153 and are listed in supplemental Table S1 at http://www.genetics.org/supplemental/.

form a colony at 42° (Figure 4C). The fact that MRE11 encodes the DNA nuclease, strand annealing, and strand dissociation activity of the MRN complex (SEMIGHINI *et al.* 2003; for review see D'AMOURS and JACKSON 2002) suggests that the synthetic lethality between *sonB*1 and the *scaA*^{NBS1} mutants at 42° is independent of these MRN complex activities.

Both the *sonA*1 and *sonB*1 NPC mutants suppress the *nimA*1 G_2 arrest at 42°. SONA^{Gle2} and SONBn^{Nup98} directly bind each other and the mutations in these genes that suppress *nimA*1 likely do so by similar mechanisms (DE SOUZA *et al.* 2003). However, these NPC mutants differ in that only *sonB*1, and not *sonA*1, is sensitive to DNA-damaging agents at 42° (Figure 1). We therefore determined whether the *sonA*1 mutation genetically interacted with MRN complex mutants in a similar manner to *sonB*1 at 42°. As with *sonB*1 mutants, *sonA*1 mutants did not display synthetic lethality with the *mreA*^{MRE11} disruption at 42° (Figure 4C). Additionally, and in contrast to the synthetic lethality between *sonB*1 and *scaA*^{NBS1} mutants at 42°, both the *sonA*1 *scaA*^{NBS1} null and *sonA*1 *scaA*1^{NBS1} double mutants were viable at 42° (Figure 4, A and B). Together, these data suggest that *sonB*1 synthetic lethality with the *scaA*^{Nbs1} mutants at 42° is likely independent of the *sonB*1 mutant defect that suppresses a *nimA*1 G_2 arrest. Rather, *sonB*1 *scaA*^{NBS1} mutant synthetic lethality at 42° may result from combining the DNA damage response defect of *sonB*1 with lack of SCAA^{NBS1} function. However, this interpretation should be viewed with caution as the *sonA*1 mutant displayed synthetic sickness with *scaA*^{NBS1} and *mreA*^{MRE11} mutants at 37° (Figure 4), perhaps suggesting a more general genetic interaction between the MRN complex and the SONA^{Gle2}/SONBn^{Nup98} NPC subcomplex.

Synthetic lethality between *sonB*1 and *scaA*^{NBS1} mutants at 42° is independent of the *scaA*^{NBS1} checkpoint functions: One of the functions of NBS1 is to regulate checkpoint pathways in response to DNA damage and this function is conserved for *A. nidulans* SCAA^{NBS1} (SEMIGHINI *et al.* 2003). The role of NBS1 in checkpoint regulation involves the ATR/ATRIP complex and/or ATM and ultimately prevents mitotic entry by tyrosine 15 phosphorylation of Cdc2 (for review see ZHOU and ELLEDGE 2000). We rationalized that if loss of checkpoint function in *scaA*^{NBS1} mutants was causing synthetic lethality with *sonB*1 mutants, *sonB*1 mutants should also show synthetic lethality with other checkpoint-deficient mutants. However, in contrast to *sonB*1 synthetic lethality with *scaA*^{NBS1} mutants at 42°, *sonB*1 was viable at 42° when combined with the *uvsB*505^{ATR}, *uvsD*308^{ATRIP}, *cdc2*F, or Δ*atmA*^{ATM} checkpoint-deficient mutants (Figure 5). Therefore, it is unlikely that loss of checkpoint function in *scaA*^{NBS1} mutants causes synthetic lethality with *sonB*1 at 42°.

Although the *sonB*1 mutant was viable in combination with the above checkpoint-deficient mutants, *sonB*1 did significantly increase the DNA damage sensitivity of *uvsB*505^{ATR}, *uvsD*308^{ATRIP}, *cdc2*F, and Δ*atmA*^{ATM} at 42° (Figure 5). This is consistent with the *sonB*1 mutation affecting a different part of the DNA damage response from the G_2 DNA damage checkpoint, supporting our earlier conclusion (Figure 2).

The *sonA*1 nucleoporin mutant was viable at 42° in combination with all checkpoint mutants tested (Figure 5). However, interestingly, the *sonA*1 mutation increased the DNA damage sensitivity of the *cdc2*F mutant (Figure 5D). Given that the *sonA*1 nucleoporin mutation allows Cdc2/cyclinB into the nucleus at 42° during a *nimA*1 arrest (WU *et al.* 1998), it is likely that increased nuclear access of active Cdc2F/cyclinB in the *sonA*1 *cdc2*F double mutant leads to increased DNA damage sensitivity. Somewhat surprisingly, the *sonA*1 mutation did not increase the DNA damage sensitivity of *uvsB*505^{ATR}, *uvsD*308^{ATRIP}, or Δ*atmA*^{ATM} mutants, which are defective in the pathway leading to tyrosine phosphorylation of Cdc2. This may reflect the relative levels

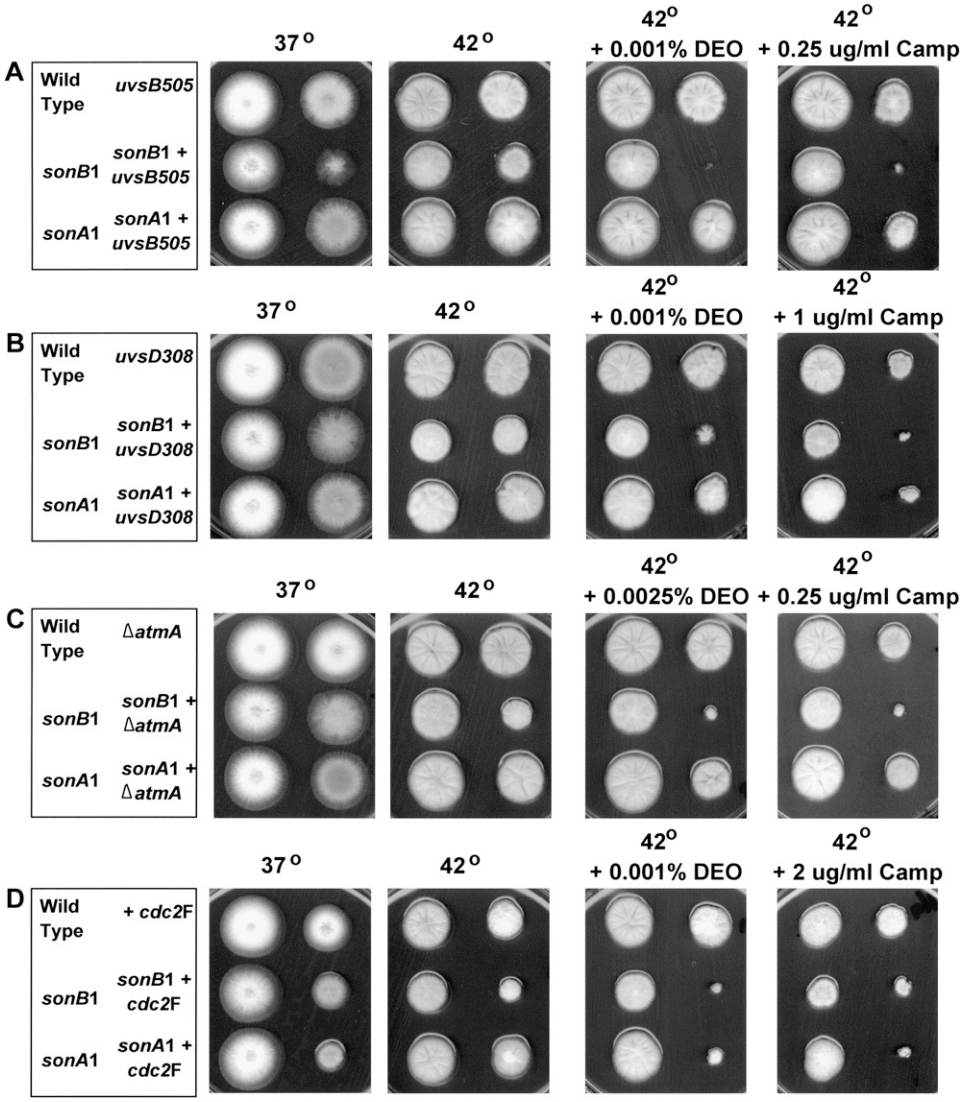

FIGURE 5.—The *sonB*1 mutant is not synthetically lethal with checkpoint-deficient mutants at 42°. (A–D) Wild type and the indicated single or double mutants were inoculated on plates and grown at either 37° (2 days) or 42° (3 days). Colony formation of strains in the presence of the DNA-damaging agents DEO or camptothecin was also evaluated as indicated. Strains used were A574, CDS204, CDS207, CDS293, CDS314, CDS319, CDS320, CDS353, CDS364, CDS365, CDS366, CDS367, CDS369, FRY24, and R153 and are listed in supplemental Table S1 at http://www.genetics.org/supplemental/.

of nontyrosine-phosphorylated Cdc2 in *uvsB*505^{ATR}, *uvsD*308^{ATRIP}, or Δ*atmA*^{ATM} mutants compared with that of the *cdc2*F mutant under these conditions.

The *sonB*1 mutation does not cause DNA damage: Another possibility to explain the synthetic lethal interaction between *sonB*1 and *scaA*^{NBS1} mutants is that the *sonB*1 mutation itself leads to DNA double-strand breaks, which are not repaired in the absence of SCAA^{NBS1} function. If this were the case, *sonB*1 mutants should also display synthetic lethality at 42° with mutants deficient in the repair of DNA double-strand breaks. DNA double-strand breaks are repaired by either homologous recombination or nonhomologous end joining (NHEJ). In *A. nidulans*, the *uvsC*^{Rad51} gene encodes a Rad51 ortholog and *uvsC*^{Rad51} mutants are sensitive to DNA double-strand breaks and display defects in homologous recombination (CHAE and KAFER 1997; SEONG *et al.* 1997; VAN HEEMST *et al.* 1997; ICHIOKA *et al.* 2001). We generated the *sonB*1 *uvsC*114^{Rad51} double mutant, which was viable at 42° (Figure 6A), arguing that the *sonB*1 mutation does not lead to DNA double-strand breaks. Similarly, deletion of *nkuA*^{Ku70}, the *A. nidulans* ortholog of the *Ku*70 gene that functions in NHEJ in other systems (for review see HOPFNER *et al.* 2002), had no effect on *sonB*1 viability at 42° (Figure 6B). However, surprisingly, the *nkuA*^{Ku70} null displays no sensitivities to DNA-damaging agents, indicating that Ku70-mediated NHEJ may play only a minor role in the DNA damage response in *A. nidulans* or that there is a second NHEJ pathway functioning without *nkuA*^{Ku70} (NAYAK *et al.* 2006). We next tested the viability of the *sonB*1 mutation when combined with the *A. nidulans uvsH*77^{Rad18} postreplication repair-deficient mutant (YOON *et al.* 1995). The *sonB*1 *uvsH*77^{Rad18} double mutant was viable at 42°, providing further evidence that the *sonB*1 mutation does not cause DNA damage (Figure 6C).

Interestingly, *sonB*1 *uvsC*114^{Rad51} and *sonB*1 *uvsH*77^{Rad18} double mutants were not viable at 42° in the presence of concentrations of DEO at which the respective single mutants formed viable colonies (Figure 6, A and C).

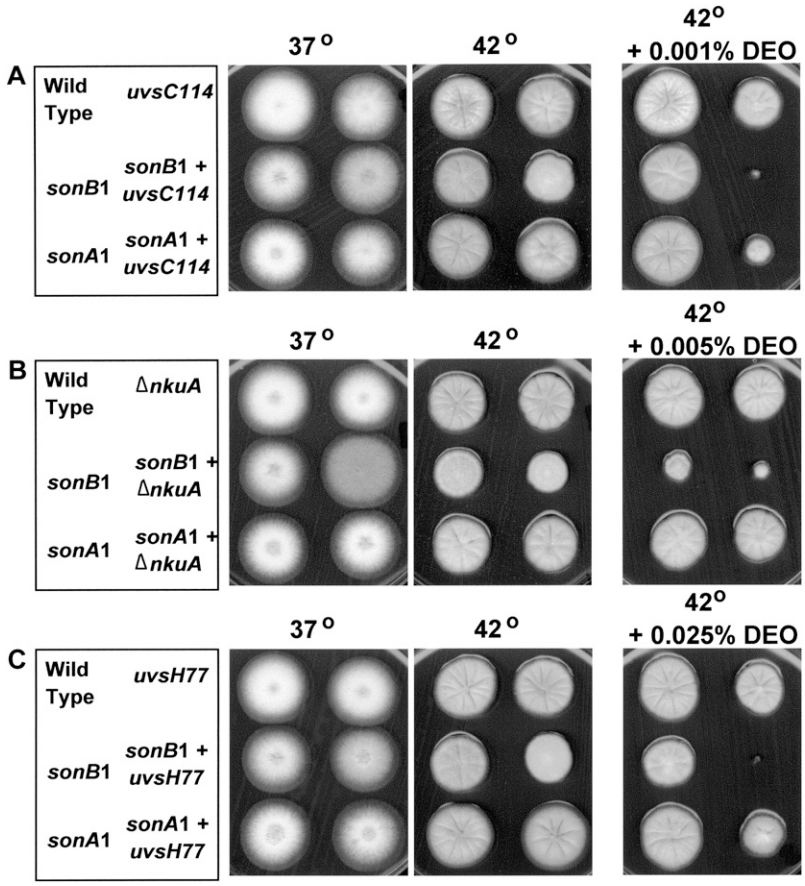

FIGURE 6.—The *sonB*1 mutant is not synthetically lethal with DNA-repair-deficient mutants at 42°. (A–C) Wild type and the indicated single or double mutants were inoculated on plates and grown at either 37° (2 days) or 42° (3 days). Colony formation of strains in the presence of the DNA-damaging agent DEO was also evaluated as indicated. Strains used were CDS260, CDS261, CDS311, CDS315, CDS330, CDS352, CDS368, CDS370, CDS364, CDS365, R153, and TN02 are listed in supplemental Table S1 at http://www.genetics.org/supplemental/.

Moreover, this effect was specific to the *sonB*1 mutant and was not observed with the *sonA*1 mutant (Figure 6). The *sonB*1 *nku*A null double mutant did not display any significant increase in sensitivity to DEO or camptothecin (Figure 6; data not shown) in comparison to the single mutants. These data provide further evidence that SONBn^{Nup98} has a role in the DNA damage response and indicate that this function is likely on a different pathway than either UVSC^{Rad51} or UVSH^{Rad18}.

γ-H2AX phosphorylation has a role in the DNA damage response independent of SONBn^{Nup98}: We have previously shown that the histone *H2A/H2B* gene pair acts as a copy-number suppressor of *sonB*1 cold sensitivity and sensitivity to hydroxyurea at 32°, but has no effect on the *sonA*1 mutant (DE SOUZA *et al.* 2003). Phosphorylation of a conserved serine in the histone H2AX variant (to generate γ-H2AX) in nucleosomes located near sites of DNA double-strand breaks has important roles in the DNA damage response, including regulating MRN complex localization to sites of DNA damage (KOBAYASHI *et al.* 2002; CELESTE *et al.* 2003; for review see VIDANES *et al.* 2005). This conserved serine of histone H2AX isoforms is present near the C terminus of the single *A. nidulans* histone *H2A* gene (Figure 7A) (MAY and MORRIS 1987), which we term *H2AX*. Given the genetic interaction between *sonB*1 and histone *H2AX/H2B*, and the role of γ-H2AX phosphorylation for MRN complex function, we determined the effect of preventing γ-H2AX phosphorylation on *sonB*1 mutants at 42°. We endogenously replaced the conserved serine in the C-terminal of H2AX with alanine to generate a *H2AX-S129A* mutant that was viable. The DNA damage sensitivity of the *H2AX-S129A* mutant was compared to mutants in the *uvsB*^{ATR} and *atmA*^{ATM} PIKK kinases, orthologs of which phosphorylate H2AX in other systems (DOWNS *et al.* 2000; CELESTE *et al.* 2003; NAKAMURA *et al.* 2004). The *H2AX-S129A* mutant was sensitive to DEO and camptothecin (Figure 7B), demonstrating for the first time in *A. nidulans* that serine 129 is required for the DNA damage response and is likely phosphorylated in response to DNA damage. The *H2AX-S129A* mutant displayed no sensitivity to hydroxyurea (Figure 7B), indicating that serine 129 is not essential for the response to a slow S-phase in *A. nidulans*. The *H2AX-S129A* mutant was less sensitive than the *uvsB505*^{ATR} mutant to DNA-damaging agents, consistent with UVSB^{ATR} having roles in the DNA damage response in addition to γ-H2AX phosphorylation. In contrast to *uvsB*505^{ATR}, the *atmA*^{ATM} null was more sensitive than the *H2AX-S129A* mutant only in the presence of camptothecin, which leads to the formation of DNA double-strand breaks (Figure 7B). This is consistent with ATM function being more specific in response to DNA double-strand breaks and with ATM being required for events in addition to

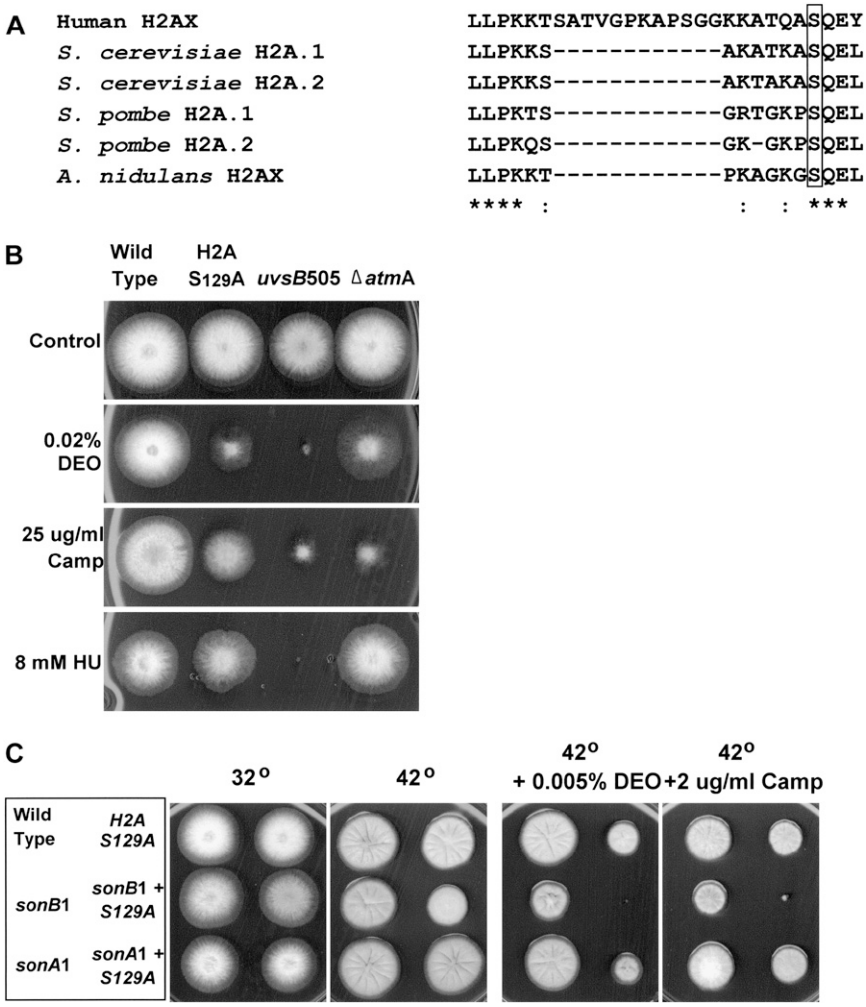

FIGURE 7.—γ-H2AX phosphorylation is required for the DNA damage response in *A. nidulans* but not for viability of the *sonB*1 mutant at 42°. (A) Alignment (ClustalW, Biology Workbench at http://workbench.sdcs.edu/) of the C-terminal tail of H2AX from the indicated organisms. The serine in the conserved SQE motif is boxed. Identical (*) and conserved groups (:) are indicated. (B) DNA damage sensitivities of wild-type (R153), *H2AX S129A* (CDS198), *uvsB*505^{ATR} (CDS314), and *atmA*^{ATM} null (CDS320) strains at 32°. (C) Colony formation of wild type and the indicated single or double mutants at 32° and 42° in the presence or absence of 0.005% DEO or 2 μg/ml camptothecin. Strains used were CDS198, CDS200, CDS201, CDS364, CDS365, and R153 and are listed in supplemental Table S1 at http://www.genetics.org/supplemental/.

H2AX-S129 phosphorylation during double-strand break repair (MALAVAZI *et al.* 2006).

We next assessed whether preventing H2AX-S129A phosphorylation had any effect on the *sonB*1 mutant. The *sonB*1 *H2AX-S129A* double mutant was not synthetically lethal at 42° and therefore H2AX phosphorylation is not essential for viability of *sonB*1 mutants at 42°. Notably, *sonB*1 *H2AX-S129A* double mutants displayed a much greater sensitivity to both DEO and camptothecin at 42° compared to either single mutant (Figure 7C). This effect was specific for *sonB*1 as *sonA*1 *H2AX-S129A* double mutants did not display any such additive effects (Figure 7C). These data are consistent with *sonB*1 functioning on a different pathway of the DNA damage response from that leading to H2AX-S129 phosphorylation.

DISCUSSION

Here we report that the *sonB*1 nucleoporin mutant is highly sensitive to DNA-damaging agents specifically at 42°, indicating that this mutation causes a defect in the DNA damage response at 42°. As the *sonB*1 mutation also suppresses a *nimA*1 G_2 arrest at 42° (DE SOUZA *et al.* 2003), we hypothesized that *sonB*1 mutants may have a defective G_2 DNA damage checkpoint resulting in cells entering mitosis without repair of DNA damage. Supporting this, we found that *sonB*1 germlings that had entered the cell cycle were sensitive to UV irradiation but *sonB*1 quiescent conidiospores were not. This differential in the UV sensitivity of germlings and conidiospores is similar to that seen for *A. nidulans* mutants, which are unable to tyrosine phosphorylate Cdc2, causing a defective G_2 DNA damage checkpoint (YE *et al.* 1997; DE SOUZA *et al.* 1999). However, we surprisingly found that in *sonB*1 mutants Cdc2 is tyrosine phosphorylated and cells arrest in G_2 normally in response to DNA damage, indicating that the G_2 DNA damage checkpoint is functional. Further, *sonB*1 *cdc2*F double mutants were more sensitive than the respective single mutants to DNA-damaging agents, providing genetic evidence that SONBn^{Nup98} functions on a different pathway in the DNA damage response from that leading to Cdc2 tyrosine phosphorylation. Therefore, the DNA damage sensitivity of the *sonB*1 mutant at 42° is independent of the G_2 DNA damage checkpoint.

It has become clear that the MRN complex is a key player in the DNA damage response (for review see D'Amours and Jackson 2002; Stavridi and Halazonetis 2005; Zhang *et al.* 2006). The MRN complex initially functions early in the DNA damage response, together with the ATM and ATR kinases, to regulate signaling and checkpoint pathways and then later in the DNA damage response to help facilitate homologous recombination and NHEJ (Uziel *et al.* 2003; Difilippantonio *et al.* 2005; Lee and Paull 2005; Stiff *et al.* 2005; You *et al.* 2005; Jazayeri *et al.* 2006; for review see Abraham and Tibbetts 2005; Stavridi and Halazonetis 2005; Zhang *et al.* 2006). The most striking finding of this study is the temperature-dependent, synthetic lethal interaction between *sonB*1 and either the *scaA*1^{NBS1} mutant or the *scaA*^{NBS1} null. This synthetic lethality was restricted to 42°, the same temperature at which *sonB*1 displays high DNA damage sensitivity. Although *sonB*1 mutants are defective in some aspect of G_2 regulation at 42°, we do not believe that this is the defect causing synthetic lethality with *scaA*^{NBS1} mutants. This is because, while both the *sonA*1 and *sonB*1 nucleoporin mutants suppress a *nimA*1 G_2 arrest at 42° (Wu *et al.* 1998; De Souza *et al.* 2003), only *sonB*1, and not *sonA*1, displays DNA damage sensitivity and is completely dead in combination with *scaA*^{NBS1} mutants at 42°. This argues that *sonB*1 synthetic lethality with *scaA*^{NBS1} mutants is likely not due to *sonB*1 G_2 regulation defects at 42°, but rather due to the defect of the *sonB*1 mutant in the DNA damage response. Our data therefore suggest that it is a combination of the *sonB*1 DNA damage response defect and the lack of SCAA^{NBS1} function that causes synthetic lethality.

One potential link between *sonB*1 and the MRN complex is the histone *H2AX/H2B* gene pair. We previously isolated histone *H2AX/H2B* as a copy-number suppressor of *sonB*1 cold sensitivity and hydroxyurea sensitivity at 32° (De Souza *et al.* 2003). However, the high level of DNA damage sensitivity of *sonB*1 mutants is not suppressed by extra-copy histone *H2AX/H2B* (data not shown). Phosphorylation of H2AX by the ATM/ATR kinases to generate γ-H2AX is important for the localization of the NBS1 to sites of DNA damage (Kobayashi *et al.* 2002; Celeste *et al.* 2003). This function is likely conserved in *A. nidulans* as we found that the *H2AX-S129A* mutant was sensitive to DNA-damaging agents and it has been shown that SCAA^{NBS1} accumulates in the nucleus in an UVSB^{ATR}-dependent manner in response to DNA damage (Fagundes *et al.* 2005). However, we found that *sonB*1 *H2AX-S129A* double mutants were viable at 42°, suggesting that it is not a defect in NBS1 localization to γ-H2AX that causes synthetic lethality between *sonB*1 and the *scaA*^{NBS1} mutants. Further, the marked increase in DNA damage sensitivity of *sonB*1 *H2AX-S129A* double mutants relative to the respective single mutants suggests that SONBn^{Nup98} and γ-H2AX phosphorylation are on different pathways of the DNA damage response.

Intriguingly, the *sonB*1 mutant was viable at 42° when combined with a disrupted allele of *mreA*^{MRE11}, the *A. nidulans* ortholog of *mre*11 that encodes the DNA nuclease, strand annealing, and strand dissociation activities of the MRN complex (Semighini *et al.* 2003; for review see D'Amours and Jackson 2002; Zhang *et al.* 2006). These MRE11 DNA-modifying activities are likely important for the DNA repair functions of the MRN complex. Therefore, *sonB*1 synthetic lethality is likely independent of the DNA repair functions of the MRN complex, which is further supported by our finding that the *sonB*1 mutant was not synthetically lethal with other DNA repair mutants at 42°. One NBS1 function that is independent of MRE11 is NBS1 binding to the γ-H2AX phosphoserine epitope via the FHA/BRCT domain located in the N-terminal of NBS1 (Kobayashi *et al.* 2002; for review see Zhang *et al.* 2006). While we have shown that γ-H2AX phosphorylation is not required for survival of *sonB*1 mutants at 42°, it is likely that the NBS1 FHA/BRCT domain also binds other as yet unidentified phosphoserine epitopes. Given our data, it is tempting to speculate that SCAA^{NBS1} binding to phosphoserine epitopes may be required for survival of *sonB*1 mutants at 42°. Although the FHA/BRCT domain of NBS1 orthologs is not well conserved, a recent bioinformatics study suggests that SCAA^{NBS1} contains the conserved residues required for binding to phosphoserine epitopes (Becker *et al.* 2006).

Our data suggest that the loss of cell cycle checkpoint functions that occurs in *scaA*^{NBS1} mutants (Semighini *et al.* 2003) is not the defect in these mutants causing synthetic lethality in combination with *sonB*1 mutants at 42°. This is because the *sonB*1 mutation is not synthetically lethal in combination with mutations in other cell cycle checkpoint regulators, including the *A. nidulans* orthologs of *ATR*, *ATRIP*, *ATM*, or the *cdc2*F mutant (Ye *et al.* 1997; De Souza *et al.* 1999; Hofmann and Harris 2000; Malavazi *et al.* 2006). In addition, given that the *sonB*1 mutant was viable when combined with these cell cycle checkpoint mutants or the *uvsC*^{Rad51} or *uvsH*^{Rad18} DNA repair mutants, it is unlikely that *sonB*1 in itself causes DNA damage at 42°. Notably, however, as double mutants between *sonB*1 and *uvsC*^{Rad51} or *uvsH*^{Rad18} mutants were more sensitive to DEO than were the respective single mutants, it is likely that SONBn^{Nup98} functions on a different pathway in the DNA damage response from that with UVSC^{Rad51} or UVSH^{Rad18}.

Interestingly, the NPC has been demonstrated to have roles in tethering telomeres to the nuclear periphery in budding yeast (Galy *et al.* 2000; Feuerbach *et al.* 2002; Therizols *et al.* 2006). Further, a recent study has shown that anchoring of telomeres to the nuclear periphery is required for efficient DNA double-strand break repair (Therizols *et al.* 2006). These authors found that nucleoporin mutants that failed to properly tether telomeres to the nuclear periphery display a decreased efficiency in the repair of DNA double-strand breaks

induced proximal to telomeres. It will therefore be interesting to determine if telomeric localization and/or function is disrupted in *sonB*1 mutants and whether this contributes to the DNA damage sensitivity of *sonB*1 mutants. Further, given that the MRN complex functions in telomere regulation (Verdun *et al.* 2005; for review see d'Adda di Fagagna *et al.* 2004), it is possible that the synthetic lethality between *sonB*1 and *scaA*^{NBS1} mutants may be due to combining different defects in telomere biology caused by these mutations. Another explanation for the DNA-damage-sensitive phenotype of *sonB*1 is that some aspect of nucleocytoplasmic transport required for the damage response does not function in this mutant at 42°. We consider this unlikely, given that nuclear transport of a nuclear localization sequence reporter construct is normal in *sonB*1 mutants at 42° (C. De Souza and S. A. Osmani, unpublished observations) and that the *sonA*1 nucleoporin mutant does not display similar DNA damage sensitivities. However, given that the recruitment of the MRN complex to sites of DNA damage has been reported to require relocation of MRE11 and RAD50 from the cytoplasm to the nucleus (Tauchi *et al.* 2001; Kobayashi *et al.* 2002), we cannot rule out the involvement of SONBn^{Nup98} in regulating specific nuclear transport pathways during the DNA damage response.

Null alleles of certain budding yeast nucleoporins display sensitivity to DNA-damaging agents (Galy *et al.* 2000; Bennett *et al.* 2001; Chang *et al.* 2002; Loeillet *et al.* 2005; Therizols *et al.* 2006); however, we know of no single amino acid substitutions in yeast nucleoporins that cause sensitivity to DNA-damaging agents. Similar to the case for *sonB*1, the DNA damage sensitivity of budding yeast nucleoporin nulls does not appear to be caused by general defects in DNA repair (Loeillet *et al.* 2005; Therizols *et al.* 2006). Interestingly, genomewide screens have revealed that null alleles of the budding yeast *nup*120 and *nup*133 nucleoporins, which are sensitive to DNA-damaging agents, display synthetic lethality with null alleles of MRN (MRX in budding yeast) coding genes (Loeillet *et al.* 2005). While the mechanism of this interaction has not been established, it suggests that the genetic interaction between the NPC and the MRN complex is likely conserved.

Notably, of the 13 nucleoporins that are nonessential in *A. nidulans*, none display obvious sensitivities to DNA-damaging agents (Osmani *et al.* 2006). This makes it even more intriguing that a single amino acid substitution in the essential *sonB*^{Nup98} nucleoporin gene causes conditional DNA damage sensitivity at 42°. Most DNA-damage-sensitive mutations identified to date in simple organisms occur in nonessential genes. This is because the genetic screens used to identify them require mutations to be viable but display sensitivity to DNA-damaging agents. This fact has selected for DNA-damage-sensitive mutations in nonessential genes. Screens for conditional DNA-damage-sensitive mutations, similar to the heat-dependent DNA damage sensitivity of the *sonB*1 mutation, may therefore identify essential genes that function in novel aspects of the DNA damage response.

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