DOI: 10.1534/genetics.105.041376

The *csnD/csnE* Signalosome Genes Are Involved in the *Aspergillus nidulans* DNA Damage Response

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Manuscript received February 1, 2005
Accepted for publication July 29, 2005

ABSTRACT

The signalosome (CSN) is a conserved multiprotein complex involved in regulation of eukaryotic development and is also required to activate ribonucleotide reductase for DNA synthesis. In *Aspergillus nidulans*, *csnD/csnE* are key regulators of sexual development. Here, we investigated whether the *csnD/csnE* genes are involved in the DNA damage response in this fungus. The growth of the *csnD/csnE* deletion mutants was reduced by subinhibitory concentrations of hydroxyurea, camptothecin, 4-nitroquinoline oxide, and methyl methanesulfonate. *A. nidulans* increases *csnD/csnE* mRNA levels when it is challenged by different DNA-damaging agents. There is no significant transcriptional induction of the *csnE* promoter fused with *lacZ* gene in the presence of DNA-damaging agents, suggesting that increased mRNA accumulation is due to increased mRNA stability. Septation was not inhibited in the *csnD/csnE* deletion mutants while Δ*uvsB* Δ*csnE* presented an increase in septation upon DNA damage caused by methyl methanesulfonate, suggesting that $uvsB^{ATR}$ and *csnE* genetically interact during checkpoint-dependent inhibition of septum formation. The double Δ*csnD/*Δ*csnE* Δ*npkA* mutants were more sensitive to DNA-damaging agents than were the respective single mutants. Our results suggest that *csnD/csnE* genes are involved in the DNA damage response and that NpkA and $UvsB^{ATR}$ genetically interact with the signalosome.

THE *co*nstitutive *p*hotomorphogenesis complex *9* (COP9), termed the *C*OP9 *sig**n*alosome (CSN), is a conserved nuclear-enriched multiprotein complex composed of eight subunits that are involved in regulation of eukaryotic development and the activation of ribonucleotide reductase for DNA synthesis (Schwechheimer and Deng 2001; Bech-Otschir *et al.* 2002; Cope and Deshaies 2003; Nielsen 2003; Schwechheimer 2004). It has been found in plants, mammals, *Drosophila melanogaster*, *Schizosaccharomyces pombe*, and *Aspergillus nidulans* (Schwechheimer 2004). The COP9 signalosome seemed to be absent from *Saccharomyces cerevisiae*; however, the verification of results from large-scale genomics and proteomics studies has recently resulted in the isolation of a CSN-related complex from this organism (Maytal-Kivity *et al.* 2002; Wee *et al.* 2002; Schwechheimer 2004). The COP9 signalosome was initially identified from *Arabidopsis thaliana* mutants with a light-brown seedling phenotype when grown in the dark (Chamovitz *et al.* 1996; Wei and Deng 1999). Later, most of the COP loci were subsequently found to be required for COP1 to go into the nucleus (Schwechheimer 2004). It was shown that COP1 negatively controls the levels of Hy5 and HyH, transcriptional regulators involved in photomorphogenesis, through subcellular localization and proteolysis (Osterlund *et al.* 2000).

The specific degradation of proteins in eukaryotes is mediated by the 26S proteasome, which is divided into the proteolytic core particle (CP) and two presumably identical 19S regulatory particles (RP) that are placed at either end of the 20S CP (Baumeister *et al.* 1998). The 19S RP consists of a base and a lid, and the eight subunits that form the lid are paralogs of the eight CSN subunits (Schwechheimer and Deng 2001). It has been suggested that the CSN and 19S RP lid have a common ancestor and possess similar biochemical properties (Schwechheimer and Deng 2001). They are characterized through the presence of two signature domains known as the *p*roteasome, *C*OP9 signalosome, *i*nitiation factor 3/*p*roteasome subunits, *I*nt-6, *N*ip-1, and *T*RIP-15 (PCI/PINT) and the *M*rp1p, *P*ad1 *N*-terminal family (MPN/MVO34) protein domains (Glickman *et al.* 1998; Kim *et al.* 2001). Protein degradation by the 26S proteasome is usually headed by protein ubiquitylation, a process mediated by an E1 ubiquitin-activating enzyme, an E2 ubiquitin-conjugating enzyme, and an E3 ubiquitin ligase (for a review, see Fang and Weissman

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2004). E3 ubiquitin ligases interact directly with protein substrates to mediate their ubiquitylation together with associated E2 ubiquitin-conjugating enzymes. It is noteworthy that most of the proteins that interact with the signalosome either are directly involved in protein degradation or are known to be regulated by protein degradation, such as transcription factors and cell cycle regulators (Schwechheimer and Deng 2001). The CSN directly interacts with E3 ubiquitin ligases (Scwechheimer 2004) and it is suggested that it regulates their activity toward its protein substrates by deneddylation of the E3 cullin subunit (Lyapina *et al.* 2001; Zhou *et al.* 2001; Cope *et al.* 2002; Yang *et al.* 2002) and by phosphorylation of the target proteins (Bech-Otschir *et al.* 2001; Sun *et al.* 2002).

In mammalian cells, CSN is involved in several processes such as the control of hormone signaling and tumor growth by regulation of c-Jun and p53 protein levels (Li *et al.* 2000; Pollmann *et al.* 2001) and inhibition of the mouse cyclin-dependent kinase inhibitor $p27^{KIP1}$ degradation and blockage of the G_1-S phase progression via deneddylation of SCF Cul1 (Tomoda *et al.* 1999; Yang *et al.* 2002). In insects and plants, the COP9 signalosome is an essential regulator of development and its malfunction results in postembryonic lethality (Wei *et al.* 1994; Freilich *et al.* 1999). Deletion mutants in *S. pombe csn1* and *csn2* mutants are delayed in S phase and are sensitive to UV light and ionizing radiation (Mundt *et al.* 2002). The *S. pombe* Csn1 and Csn2 subunits revealed their role in positively regulating the activity of the ribonucleotide reductase (RNR) through a proteolysis of the replication inhibitor Spd1 (Liu *et al.* 2003).

A. nidulans has been used as a model genetic system for the study of cell cycle control and DNA damage response (for reviews, see Kafer and May 1998; Aist and Morris 1999; Bruschi *et al.* 2001; Goldman *et al.* 2002; Fagundes *et al.* 2003, 2004; Semighini *et al.* 2003; for a review, see Goldman and Kafer 2004; Osmani and Mirabito 2004). *A. nidulans* forms asexual spores (conidia), which contain a single nucleus arrested in G_1 (Bergen and Morris 1983). During spore germination, conidia undergo an initial period of isotropic expansion before switching to polarized growth and forming an elongating germ tube. Two protein kinases, the $NimX^{cdc2}$ and NimA, are coordinately required to initiate mitosis in *A. nidulans* (Osmani and Ye 1996; Ye *et al.* 1997). Like other eukaryotic cells, the DNA damage checkpoint functions via Y15 phosphorylation of the $NimX^{cdc2}$ in *A. nidulans* (Ye *et al.* 1997). Loss of such checkpoint control regulation over mitosis can also cause defects in DNA rereplication after mitosis (De Souza *et al.* 1999). Although the Wee1 ortholog AnkA and the Cdc25 ortholog NimT control Y15 phosphorylation of NimX (Osmani *et al.* 1991; Kraus and Harris 2001), it is not clear how their activity and/or localization are influenced by DNA damage. De Souza *et al.* (1999) have shown that both *A. nidulans* $uvsB^{ATR}$ and $uvsD^{ATRIP}$ are involved in the G_2/M checkpoint in response to DNA damage. Ye *et al.* (1997) provided evidence that at least two S-phase checkpoint mechanisms control mitosis in *A. nidulans*. The first responds to the rate of DNA replication and inhibits mitosis via tyrosine phosphorylation of $NimX^{cdc2}$. If DNA replication is arrested, lack of tyrosine-phosphorylated $NimX^{cdc2}$ also cannot promote mitosis because of the presence of a second checkpoint mechanism over mitotic initiation, which involves the function of $BimE^{APC}$ (the homolog of the anaphase-promoting complex, APC). The DNA damage checkpoint also regulates septation in *A. nidulans* by modulating the activity of $NimX^{cdc2}$ and requiring functional AnkA (Harris and Kraus 1998; De Souza *et al.* 1999). Septum formation is triggered by high levels of $NimX^{cdc2}$ activity and is inhibited by DNA damage in a checkpoint-dependent manner (Harris and Kraus 1998). Harris and Kraus (1998) showed that mutations in $uvsB^{ATR}$ abolish the cell cycle delay and inhibition of septum formation triggered by the exposure of predivisional hyphae to DNA-damaging agents. Semighini *et al.* (2003) suggested that regulation of septation in *A. nidulans* is dependent not only on the $uvsB^{ATR}$ gene but also on the Mre11 complex.

Recently, Busch *et al.* (2003) identified two components of the COP9 signalosome, *csnD/csnE,* as novel regulators of sexual development in *A. nidulans*. The deletion of these two genes resulted in viable strains with mutant phenotypes, the most critical a block in maturation of cleistothecial primordia. Deneddylation of cullins is mediated by the fifth subunit of the signalosome and the characteristic *J*AB1/*M*PN/Mov34 *m*etalloenzyme (JAMM) motif, which is required for this metalloprotease activity, is present in *A. nidulans* CsnE, suggesting that this gene encodes the deneddylase activity of the fungal signalosome (Cope *et al.* 2002). Here, we investigated whether the *csnD/csnE* genes are involved in the DNA damage response in this fungus. We observed not only that these deletion mutant strains impaired growth in the presence of DNA-damaging agents, but also that septation is not inhibited in the *csnD/csnE* deletion mutants upon DNA damage caused by methyl methanesulfonate (MMS). Furthermore, we have also seen genetic interactions among *csnD/csnE* genes, the cdc2-related kinase NpkA, and $UvsB^{ATR}$ during *A. nidulans* DNA damage response.

MATERIALS AND METHODS

Strains, media, and methods of UV treatment: *A. nidulans* strains used are described in Table 1. Media were of two basic types: (1) a simple yeast extract complete medium with the three variants YAG (2% glucose, 0.5% yeast extract, 2% agar, trace elements), YUU (YAG supplemented with 1.2 g/liter each of uracil and uridine), and liquid YG medium (YAG but without 2% agar) and (2) a modified minimal medium (MM) of 1% glucose, original high nitrate salts, trace elements, 2% agar, pH 6.5 or a minimal medium without glucose (MC).

TABLE 1

***A. nidulans* strains**

Strains	Genotypes	References
GR5	*pyrG89; wA3; pyroA4*	FGSC A773
R21	*pabaA1 yA2*	FGSC A234
AGB152	*pyroA4; pyrG89*	Busch *et al.* (2003)
AGB195	*pyroA4; pyrG89; ΔcsnD::pyr4*	Busch *et al.* (2003)
AGB209	*pyroA4; pyrG89; ΔcsnE::pyr4*	Busch *et al.* (2003)
AAH14	*pabaA1 yA2; ΔuvsB; argB2*	Hofmann and Harris (2000)
MV3	*argB2; pyrG89; yA2; ΔnpkA::pyrG*	Kress-Fagundes *et al.* (2004)
JL195-3	*ΔcsnD::pyr4; ΔnpkA::pyrG*	This work
JL195-14	*ΔcsnD::pyr4; ΔuvsB::argB*	This work
JL209-3	*ΔcsnE::pyrG; ΔnpkA::pyrG*	This work
JL209-14	*ΔcsnE::pyrG; ΔuvsB::argB*	This work
APK35	*ankA; pabaA1*	Kraus and Harris (2001)
WG 355	*biA1, bgaO, argB2*	Van Gorcom *et al.* (1986)
AGB 246	*biA1; bgaO; argB2, lacZ <argB>*	This work
AGB 248	*biA1; bgaO; argB2, 5′csnE::lacZ <argB>*	This work
FGSC A4	Glasgow wild type	FGSC A4
A776	*PabaA1; acrA1; bimE7; riboB2; chaA1*	FGSC A776

Trace elements, vitamins, and nitrate salts are described by Kafer (1977, Appendix). Standard genetic techniques for *A. nidulans* were used for all strain constructions (Kafer 1977).

For the UV-light viability assays, conidiospores (dormant in a quiescent G_0 state) were suspended in 0.2% Tween-20 and plated out on YUU plates (~100 conidia/plate). The plates were then irradiated immediately with UV using a UV Stratalinker 1800 (Stratagene, La Jolla, CA) and incubated at 37° for 48 hr to determine UV sensitivity of nondividing cells. To determine UV survival of dividing cells, conidiospores on YUU plates were first allowed to germinate for 4.5 hr in a 37° incubator for colony formation. By this time the germinated spores had entered the cell cycle and were about to undergo the first mitosis. These germlings were UV irradiated on the plates and then similarly incubated at 37° for 48 hr. Viability was determined as the percentage of colonies on treated plates compared to untreated controls.

Construction of the 5′*csnE*::*lacZ* fusion plasmid: Primers ES59 (5′-TGA GGATCC GGC TTT CTC GTC AAC CAG-3′) and ES60 (5′-TAG GGA TCC CAT GAT GAT TGT CAG GTG-3′) containing *Bam*HI restriction sites were used for amplifying 1.0 kb of the 5′ regulatory region of the *A. nidulans csnE* gene from genomic DNA from the AGB152 strain. The 1.0-kb PCR fragment was fused to *lacZ* in the unique *Bam*HI site of plasmid pAN923-41B (Van Gorcom *et al.* 1986), resulting in pME 2817. The plasmid-borne *argB* wild-type gene was mutated by filling in the unique *Bgl*II site within the *argB* gene with PolIK, creating an insertion mutant *argB* allele (Punt *et al.* 1990). This mutation allows the selection of arginine prototrophy transformants generated by recovering the wild-type allele by crossing over with the *argB*2 mutation of WG355 strain.

A. nidulans transformation was performed as described (Eckert *et al.* 2000). The strain WG355 was transformed with plasmids pME2817 or pAN923-41B. Arginine prototrophy transformants were tested for single-copy integrations at the chromosomal *argB* gene locus by Southern blot analysis (Southern 1975) and by using the gene images random prime labeling and detection system (Amersham, Freiburg, Germany). The strain AGB 246 containing pAN923-41B serves as negative control. The AGB 248 strain contains the 5′*csnE::lacZ* fusion as single copy.

Specific β-galactosidase activity assay: *A. nidulans* strains were grown and harvested under the same conditions as for RNA isolation. Mycelia were harvested by filtration through no. 1 Whatman filter, washed thoroughly with sterile water, and quickly frozen in liquid nitrogen. About 300 μl of this ground mycelia was mixed with 500 μl B^+-buffer (100 mm Tris-HCl, pH 7.5, 200 mm NaCl, 20% glycerol, 5 mm EDTA, pH 8.0, and freshly added 1 μl/ml β-ME) and 5 μl/ml 200× 100 mm *p*-aminobenzamidin-HCL, 100 mm *Na-p*-tosyl-L-lysin-chlormethylketon, 100 mm *Na-p*-tosyl-L-phenylalanin-chlormethylketon, and 100 mm *o*-phenanthrolin and 100 mm phenylmethylsulfonyl fluoride (PIM) and vortexed four times for 15 sec. After centrifugation at 4° for 10 min, the supernatant was used directly for further analysis. Protein concentrations were determined according to Bradford (1976). β-Galactosidase assays were carried out at 28° as described by Miller (1972) using 0.5–5.0 μl of extracts.

Methods of replication checkpoint response: For the mitosis assay, conidiospores were inoculated onto coverslips in YUU medium with 0, 6, or 100 mm of hydroxyurea (HU). After 5–7 hr incubation at 30°, coverslips with adherent germlings were transferred to fixative solution (3.7% formaldehyde, 50 mm sodium phosphate buffer, pH 7.0, 0.2% Triton X-100) for 30 min at room temperature. Then they were briefly rinsed with PBS buffer (140 mm NaCl, 2 mm KCl, 10 mm $NaHPO_4$, 1.8 mm KH_2PO_4, pH 7.4) and incubated for 5 min in a solution with 100 ng/ml of 4′,6-diamidino-2-phenylindole (DAPI) (Sigma Chemical) and 100 ng/ml of calcofluor (fluorescent brightener, Sigma Chemical). After incubation with the dyes, they were washed with PBS buffer for 5 min at room temperature and then rinsed in distilled water and mounted on the slides. The material was photographed using a Zeiss epifluorescence microscope. The number of nuclei was assessed by DAPI staining. Germlings that had two or more nuclei after the HU incubation were scored as having a nonfunctional checkpoint response.

For the viability assay, 1.0×10^8 conidia were inoculated in YUU medium with 0, 6, or 100 mm of HU and incubated in a reciprocal shaker (250 rpm) at 30° for 7 hr. The conidiospores were washed with water, conveniently diluted and plated on YUU, and incubated at 30° for 48 hr. Viability was determined as the percentage of grown colonies on plates with drug-treated conidiospores compared to untreated controls.

RNA isolation: A total of 1.0×10^7 conidia/ml were used to inoculate 50 ml of liquid cultures that were incubated in a reciprocal shaker at 37° for 16 hr. Mycelia were aseptically transferred to fresh YG medium in the presence or absence of drugs for 30, 60, 90, 120, or 240 min. The following concentrations of

TABLE 2

Primers and fluorescent probes used in this work

Primers and probes	Sequences	Genes[a]
csnD_559RL	5′-CACTTCTTGGGAAAGCCGGAAG[FAM]G-3′	*csnD* AF236662
csnD_559RL_545FU	5′-CCGAGCAAGATCGAAGACCA-3′	*csnD* AF236662
csnE_342FL	5′-GACCATGACGAAGCAAACGAGTATATGG[FAM]C-3	*csnE* EAA64961
csnE_342FL_402RU	5′-CCCGTAGCCAGGGTGACTG-3′	*csnE* EAA64961
rib_reduct_820RL	5′-CACACCAGGCAGGCAAAGTCGGTG[FAM]G-3′	*rnrA* AAG40862
rib_reduct_820RL_768FU	5′-GGCTGAAGAAGCGAGGCTTG-3′	*rnrA* AAG40862
rns_2P_reduct_1560FL	5′-GACACCGCCCGATTGCTCTTGGTG[FAM]C-3′	*rnsA* XM_408517.1
rns_2P_reduct_1560RU	5′-GGCTTCAGCCGAATCGAAAG-3′	*rnsA* XM_408517.1
tubC_525FL	5′-CACTTTATGCCGTCGCCGAAAG[FAM]G-3′	*tubC* M17520
tubC_525FL_583RU	5′-GCAGAATGTCTCGTCCGAATG-3′	*tubC* M17520
uvsC_429FL	5′-GACGGTTGCCATACCCTTGCCG[FAM]C-3′	*uvsC* Z80341
uvsC_429FL_450RU	5′-CTTCGCCGCACCCAT-3′	*uvsC* Z80341

FAM, 6-carboxyfluorescein.
[a] NCBI access numbers follow gene name.

chemicals were used: 25 μM of camptothecin (CPT), 0.5 μg/ml of 4-nitroquinoline oxide (4-NQO), 0.003% of MMS, and 0.6 μg/ml of bleomycin (BLEO). Mycelia were harvested by filtration through no. 1 Whatman filter, washed thoroughly with sterile water, quickly frozen in liquid nitrogen, and disrupted by grinding, and total RNA was extracted with Trizol (Life Technologies). Ten micrograms of RNA from each treatment were then fractionated in 2.2 M formaldehyde and 1.2% agarose gel, stained with ethidium bromide, and then visualized with UV light. The presence of intact 28S and 18S ribosomal RNA bands was used as a criterion to assess the integrity of the RNA. RNAse-free DNAse treatment was done as previously described by Semighini *et al.* (2002).

Real-time PCR reactions: All the PCR and RT-PCR reactions were performed using an ABI Prism 7700 sequence detection system (Perkin-Elmer Applied Biosystems). Taq-Man EZ RT-PCR kits (Applied Biosystems, Foster City, CA) were used for RT-PCR reactions. The thermal cycling conditions composed an initial step at 50° for 2 min, followed by 30 min at 60° for reverse transcription, 95° for 5 min, and 40 cycles at 94° for 20 sec and 60° for 1 min. Taq-Man Universal PCR master mix kit was used for PCR reactions. The thermal cycling conditions composed an initial step at 50° for 2 min, followed by 10 min at 95°, and 40 cycles at 95° for 15 sec and 60° for 1 min. The reactions and calculations were performed according to Semighini *et al.* (2002). Table 2 describes the primers and Lux fluorescent probes (Invitrogen, San Diego) used in this work.

RESULTS

The *csnD/csnE* genes are involved in different aspects of the DNA damage response in *A. nidulans*: As a preliminary step to assess the involvement of the *csnD/csnE* genes in the DNA damage response, we verified their sensitivity to DNA-damaging agents. As can be seen in Figure 1, the Δ*csnD*/Δ*csnE* mutant strains are more

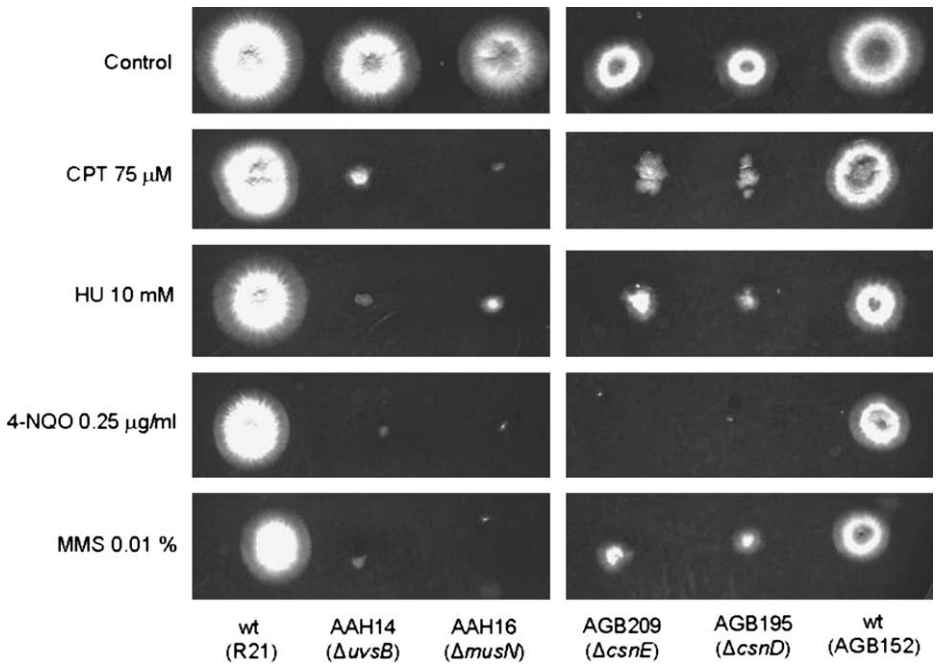

Figure 1.—Growth phenotypes of the Δ*csnD* and Δ*csnE* mutants. Strains R21 and AGB152 (wild type), AAH14 (Δ$uvsB^{ATR}$), AAH16 (Δ$musN^{RecQ}$), AGB195 (Δ*csnD*), and AGB209 (Δ*csnE*) were grown for 72 hr at 37° in YUU medium and YUU 75 μM CPT, YUU 10 mM HU, YUU 0.25 μg/ml 4-NQO, and YUU 0.01% MMS.

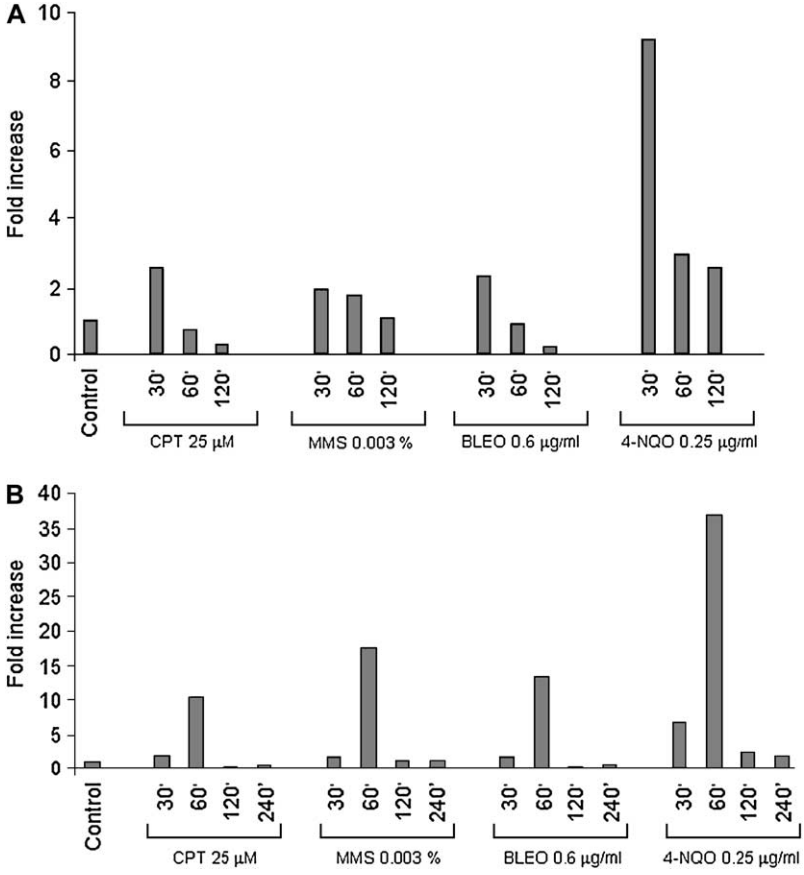

FIGURE 2.—Fold increase in *csnD/csnE* RNA levels in response to DNA-damaging agents. Mycelia were grown in the absence of any drug, 25 μM CPT, 0.003% MMS, 0.6 μg/ml BLEO, or 0.25 μg/ml 4-NQO for 30, 60, 120, and 240 min. Real-time RT-PCR was the method used to quantify the mRNA. The measured quantity of the *csnD/csnE* mRNA in each of the treated samples was normalized using the C_T values obtained for the *tubC* RNA amplifications run in the same plate. The relative quantitation of *csnD/csnE* and tubulin gene expression was determined by a standard curve (*i.e.*, C_T values plotted against the logarithm of the DNA copy number). Results of four sets of experiments were combined for each determination; means are shown. The values represent the number of times that the genes are expressed compared to the wild-type control grown without any drug (represented absolutely as 1.00).

sensitive to CPT, HU, 4-NQO, and MMS. We have also observed that germinating conidia of the *csnD/csnE* inactivation strains have increased sensitivity to UV light while there are no significant differences between the *csnD/csnE* inactivation and the wild-type strains in terms of sensitivity of quiescent conidia to UV light (data not shown).

We examined the mRNA expression of the *csnD/csnE* in the presence of DNA-damaging agents using real-time RT-PCR. *A. nidulans* wild type was grown in the absence of any drug and transferred to a specific concentration of a DNA-damaging agent for 30, 60, 120, and 240 min, and then RNA was isolated and analyzed for the expression of these genes (Figure 2). The *csnD* gene expression was increased after 30 min growth in the presence of MMS (~2 times), CPT and BLEO (~3 times), and 4-NQO (~9 times), and after 60 and 120 min growth in the presence of 4-NQO (~3 times). The *csnE* gene displays a comparable profile but with much higher expression than that of *csnD*: mRNA levels were increased after 60 min growth in the presence of CPT (~10 times), MMS (~17 times), and BLEO (~13 times), and after 30 and 60 min in the presence of 4-NQO (~7 and 37 times, respectively). These results indicate that *A. nidulans* has increased *csnD/csnE* mRNA levels when it is challenged by different DNA-damaging agents. To verify whether these increased mRNA levels were due to transcriptional induction or to increased mRNA stability, we fused the *csnE* promoter with the *lacZ* gene and evaluated its *in vivo* expression in the presence of DNA-damaging agents. As it can be seen in Figure 3, there is no significant induction at the promoter level in the presence of DNA-damaging agents. These results suggested that the observed increased *csnE* mRNA accumulation in the presence of DNA-damaging agents is due to increased mRNA stability.

In *A. nidulans*, septation is inhibited by DNA damage in a checkpoint-dependent manner (HARRIS and KRAUS 1998). We determined whether septation could be inhibited in Δ*csnD*/Δ*csnE* mutants by DNA damage caused by subinhibitory concentrations of MMS. Both mutants had normal septation levels in the absence of DNA damage when compared to the wild type (Table 3). The septation levels were dramatically reduced in wild-type mutant strain in the presence of DNA damage (Table 3). However, the septation levels were increased when the Δ*csnD*/Δ*csnE* mutants were grown in the presence of MMS (30.0 and 36.7%, respectively, against 7.5% from the wild-type strain; Table 3). These results suggest that the *csnD/csnE* signalosome genes are involved in the checkpoint-dependent inhibition of septum formation in *A. nidulans*.

One *A. nidulans* gene that has been shown to be induced in the presence of DNA damage is $uvsC^{RAD51}$ (VAN HEEMST *et al.* 1997). The $UvsC^{RAD51}$ is important for the initial steps of the homologous recombination by

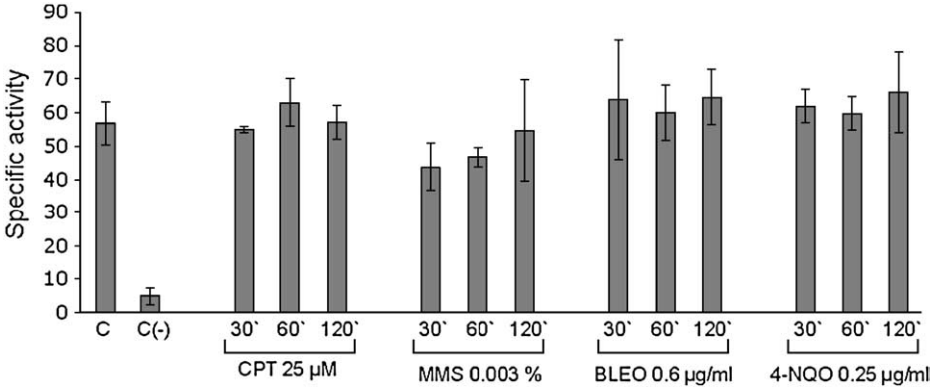

FIGURE 3.—Specific β-galactosidase activity of the *A. nidulans* 5′*csnE::lacZ* strain (AGB248) 30, 60, and 120 min after addition of DNA-damaging agents as indicated. Each value represents the mean of two independent measurements with standard deviations not exceeding 20%. The control shows the mean specific β-galactosidase activity of strain AGB248 grown without drug. The negative [C(−)] control strain AGB246 containing *lacZ* without any 5′ region did not show significant β-galactosidase activity. The applied drugs are CPT, MMS, BLEO, and 4-NQO.

binding the free ends of the DNA double strands (for a review, see NYBERG *et al.* 2002). In yeast cells, Rad51 homologs form irradiation-induced subnuclear foci (GASIOR *et al.* 2001; CASPARI *et al.* 2002). We examined the transcription of the *uvsC*^{RAD51} in the Δ*csnD*/Δ*csnE* mutants exposed to CPT. Thus, the mutants and the corresponding wild-type strain were grown in the absence of CPT and then transferred to complete medium containing 25 μM CPT for 30 to 120 min. After 30 min exposure to CPT, there is ~4.5-fold induction of the *uvsC*^{RAD51} gene in the wild-type and the mutant strains (Table 4). After 60 min exposure to CPT, the expression was still comparable among the strains and it had a variation of 6.84- to 10.9-fold induction; however, after 60 min induction, the expression of the *uvsC*^{RAD51} has decreased to 2.42- and 8.8-fold induction in the Δ*csnD*/Δ*csnE* mutants when compared to 14.46-fold induction in the wild-type strain. These results suggest that there is decreased *uvsC*^{RAD51} gene expression in the Δ*csnD*/Δ*csnE* mutants when they are exposed to an agent that causes double-strand breaks (DSBs), such as CPT. Moreover, since the activation of *uvsC*^{RAD51} mRNA expression is not affected but decreased, it is likely that the *csnD/csnE* inactivation mutants have a defect in sustaining the DNA damage response rather than a defect in activating it.

HU is an inhibitor of ribonucleoside diphosphate reductase, the rate-limiting step enzyme in deoxyribonucleotide (dNTP) biosynthesis. Depletion of dNTPs activates the DNA replication checkpoint, which slows progression through S phase (DESANY *et al.* 1998). Furthermore, initiation of DNA replication in the presence of high levels of HU causes DNA DSBs (MERRILL and HOLM 1999). Since HU is an effective inhibitor of DNA synthesis in *A. nidulans* (BERGEN and MORRIS 1983), we verified whether the *csnD/csnE* genes could play a role in the S-phase checkpoints by examining whether the Δ*csnD*/Δ*csnE* mutant strains could survive a transient period of growth in the presence of HU. Two different assays were used to verify if DNA replication checkpoint response is impaired in mutant strains (FAGUNDES *et al.* 2004). The first assay (*i.e.*, the mitosis assay) monitors mitosis in mutant and wild-type strains incubated in 6 or 100 mM of HU for 5–7 hr. The number of nuclei was assessed by DAPI staining, and a defect in

TABLE 3

Percentage of septation in *A. nidulans* germlings

Strains[a]	Control	+ MMS[b]
AGB152 (wild type)	100.0 ± 0.0	7.5 ± 2.1
AGB195 (Δ*csnD*)	98.0 ± 3.5	30.0 ± 5.0
AGB209 (Δ*csnE*)	96.7 ± 3.1	36.7 ± 6.7
AAH14 (Δ*uvsB*)	93.5 ± 6.4	22.5 ± 0.7
MV3 (Δ*npkA*)	100.0 ± 0.0	23.5 ± 0.7
JFL195-3(Δ*npkA* Δ*csnD*)	100.0 ± 0.0	11.7 ± 4.0
JFL195-14 (Δ*uvsB* Δ*csnD*)	100.0 ± 0.0	11.7 ± 6.2
JFL209-3 (Δ*npkA* Δ*csnE*)	100.0 ± 0.0	18.3 ± 7.0
JFL209-14 (Δ*uvsB* Δ*csnE*)	100.0 ± 0.0	60.0 ± 0.0

[a] All the experiments are the average of three independent experiments with 100 germlings evaluated in each of them.

[b] Conidia were germinated at 37° for 16 hr in YG medium in the absence or presence of 0.0015% MMS. Since these strains are MMS sensitive, a higher concentration of MMS was used for the wild type (0.005%). Septation was evaluated by calcofluor staining.

TABLE 4

Expression of the *uvsC*^{RAD51} gene in the *A. nidulans* wild-type and *csnD/csnE* inactivation mutants

Strains[a]	30 min	60 min	120 min
AGB152 (wild type)	4.60 ± 0.98	7.65 ± 0.17	14.46 ± 2.72
AGB195 (Δ*csnD*)	4.50 ± 0.86	6.84 ± 0.79	2.42 ± 0.06[b]
AGB209 (Δ*csnE*)	4.50 ± 0.27	10.9 ± 1.60	8.80 ± 0.40[b]

[a] The strains were grown 16 hr in YG medium and then mycelia was transferred to a fresh YG medium plus 25 μM CPT. The results (mean ± standard deviation) are expressed as the number of times the *uvsC*^{RAD51} gene is more expressed than the control without CPT. Statistical differences were determined by ANOVA followed, when significant, by the Newman-Keuls Multiple Comparison Test, using Sigma Stat statistical software (Jandel Scientifics). $P < 0.05$ was considered statistically significant.

[b] Significantly different from the wild type ($P < 0.002$).

TABLE 5

Mitosis assay from *A. nidulans* wild-type and mutant strains

HU[a]	0 mM	6 mM	100 mM
AGB152 (wild type)	61.3 ± 2.5	5.3 ± 3.2	0.0 ± 0.0
AGB195 (Δ*csnD*)	58.5 ± 5.5	8.3 ± 4.6	2.5 ± 1.3
AGB209 (Δ*csnE*)	62.0 ± 5.1	12.4 ± 1.7[b]	0.6 ± 0.5
MV3 (Δ*npkA*)	67.0 ± 3.0	27.3 ± 3.2[b]	2.7 ± 1.2
AAH14 (Δ*uvsB*)	61.5 ± 7.8	16.5 ± 2.1[b]	15.5 ± 0.7[b]
JFL195-3 (Δ*csnD* Δ*npkA*)	68.5 ± 1.3	11.3 ± 0.9	3.0 ± 1.4
JFL195-14 (Δ*csnD* Δ*uvsB*)	55.8 ± 9.8	5.5 ± 1.0	0.5 ± 0.5
JFL209-3 (Δ*csnE* Δ*npkA*)	51.2 ± 6.4	3.5 ± 0.6	0.0 ± 0.0
JFL209-14 (Δ*csnE* Δ*uvsB*)	55.7 ± 3.1	4.3 ± 0.9	0.0 ± 0.0

[a] Percentage of germlings that had two or more nuclei after the HU incubation were scored as germlings that did not have mitosis arrest. All the results are the average of determinations from three independent experiments with 100 germlings evaluated in each. Germlings that did not have mitosis arrest were scored. The results were expressed as mean ± standard deviation. Statistical differences were determined by ANOVA followed, when significant, by the Newman-Keuls Multiple Comparison Test, using Sigma Stat statistical software (Jandel Scientifics). $P < 0.05$ was considered statistically significant.

[b] Significantly different from the wild type ($P < 0.004$).

TABLE 6

Viability assay from *A. nidulans* wild-type and mutant strains

HU[a]	6 mM	100 mM
AGB152 (wild type)	63.0 ± 9.0	64.0 ± 10.0
AGB195 (Δ*csnD*)	48.0 ± 7.0[b]	46.0 ± 9.0[b]
AGB209 (Δ*csnE*)	53.0 ± 7.0	51.0 ± 8.0
MV3 (Δ*npkA*)	87.0 ± 7.0	98.0 ± 3.0
AAH14 (Δ*uvsB*)	75.0 ± 13.0	78.5 ± 11.7
JFL195-3 (Δ*csnD* Δ*npkA*)	56.0 ± 5.0	53.0 ± 5.0
JFL195-14 (Δ*csnD* Δ*uvsB*)	51.0 ± 6.0	30.0 ± 6.0[c]
JFL209-3 (Δ*csnE* Δ*npkA*)	65.0 ± 9.0	65.0 ± 13.0
JFL209-14 (Δ*csnE* Δ*uvsB*)	64.0 ± 3.0	62.0 ± 6.0

[a] Viability was determined as the percentage of colonies on HU-treated plates compared to untreated controls. All the results are the average of determinations from three independent experiments. The data are the average of three repetitions and means ± standard deviation are shown. Statistical differences were determined by ANOVA followed, when significant, by the Newman-Keuls Multiple Comparison Test, using Sigma Stat statistical software (Jandel Scientific). $P < 0.05$ was considered statistically significant.

[b] Significantly different from the wild type ($P < 0.004$).

[c] Significantly different from Δ*uvsB* and Δ*csnD* ($P < 0.001$).

the mitosis assay is defined as an increase in the number of germlings with two or more nuclei after the HU incubation (Table 5). The second assay (*i.e.*, the viability assay) assesses germling viability after incubation for 6 hr in the presence or absence of 6 or 100 mM of HU (Table 6). Both assays measure the state of the replication checkpoint response. The Δ*csnE* strain showed defects in the mitosis assay (6 mM) (Table 5). Taken together, all these evidences strongly indicate that *csnD/csnE* signalosome genes are involved in the *A. nidulans* DNA damage response.

Possible genetic interactions with *A. nidulans uvsB*^{ATR} and *npkA*: We investigated possible genetic interactions among *csnD/csnE* and *ankA*^{wee1}, *bimE*^{APC1}, *uvsB*^{ATR}, and the cdc2-related kinase *npkA* by constructing double mutants with Δ*csnD*/Δ*csnE* and the other mutants. We were unable to construct double mutants with the *bimE7* and *ankA* and Δ*csnD*/Δ*csnE* mutants. In both crosses, few cleistothecia of smaller size and without ascospores were produced. The double-mutant strains Δ*csnD* Δ*npkA* and Δ*csnE* Δ*npkA* were more sensitive to subinhibitory concentrations of 4-NQO, MMS, HU, and CPT than were the corresponding parental strains (Figure 4). The double-mutant strains Δ*csnD* Δ*uvsB* and Δ*csnE* Δ*uvsB* were inhibited to the same extent as the corresponding parental strain AAH14 (Δ*uvsB*) when grown in the presence of these DNA-damaging agents (Figure 4). The genetic interaction between *csnD/csnE* and *npkA* genes can also be observed when germinating conidia are exposed to UV light (Figure 5B, top). During septation in the presence of MMS, a synergism between *uvsB*^{ATR} and *csnE* can also be verified, since the double mutant for these genes (JFL209-14) presented an increase in the percentage of septation (60%) in comparison with the corresponding parental strains Δ*uvsB* and Δ*csnE* (22.5 and 36.7%, respectively). Our results suggest that both *csnD/csnE* and *npkA* genes genetically interact since there is a synergism in terms of sensitivity to DNA-damaging agents in the corresponding double-mutant strains. Furthermore, Δ*uvsB* and Δ*csnE* also genetically interact during checkpoint-dependent inhibition of septum formation in *A. nidulans*.

We also verified whether the S-phase checkpoints were intact in these double-mutant strains (Tables 5 and 6). As previously shown by Fagundes *et al.* (2004), the *uvsB* and *npkA* inactivation mutants have impaired S-phase checkpoints. During the mitosis assay at both 6 and 100 mM, no genetic interaction among the double-mutant strains was observed. However, the double-mutant Δ*csnD* Δ*uvsB* is impaired in the viability assay at 100 mM (Table 6). Interestingly, the *csnD/csnE* inactivation mutations are suppressing the absence of the DNA replication checkpoint response in the *uvsB* inactivation mutant (Table 5), providing additional evidence for genetic interaction between *csnD/csnE* and *uvsB* genes.

The ribonucleotide reductase genes have altered mRNA expression in the csnD/csnE inactivation strains: In fission yeast, the COP9 signalosome is required to activate ribonucleotide reductase for DNA synthesis (Nielsen 2003). As a preliminary step to characterize a possible dependence of the ribonucleotide

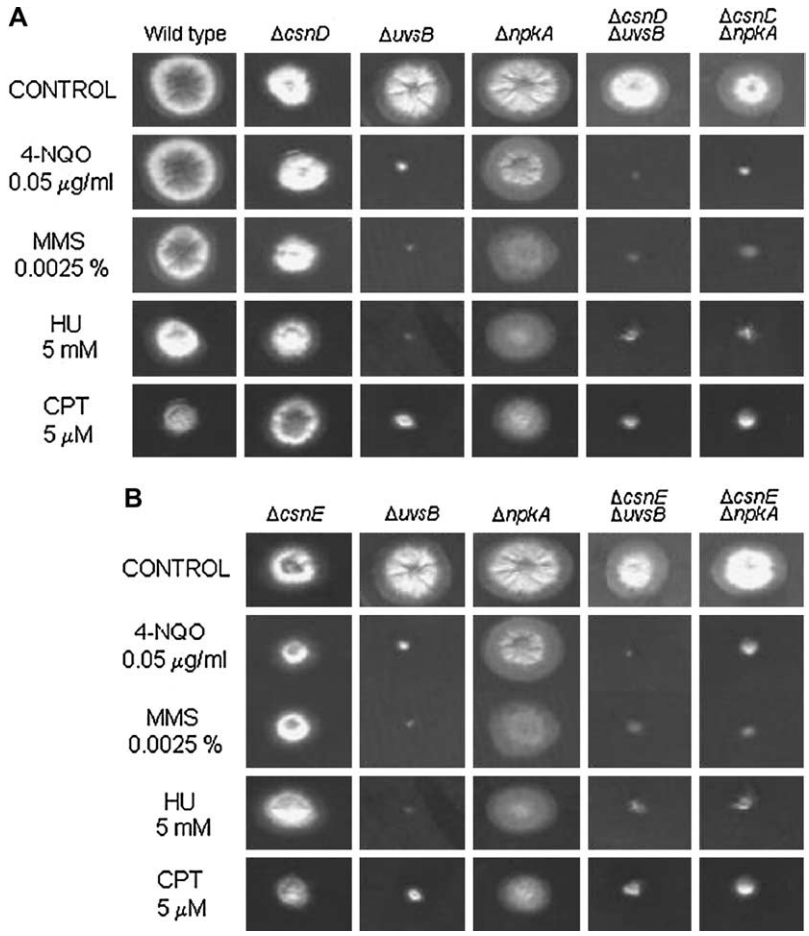

FIGURE 4.—The Δ*npkA* mutant shows synergistic interaction with the *csnD/csnE* inactivation mutants. (A) Strains AGB152 (wild type), AGB195 (Δ*csnD*), AAH14 (Δ*uvsB*^{ATR}), MV3 (Δ*npkA*), 195-14 (Δ*csnD* Δ*uvsB*), 195-3 (Δ*csnD* Δ*npkA*), and (B) AGB209 (Δ*csnE*), 209-14 (Δ*csnE* Δ*uvsB*), and 209-3 (Δ*csnE* Δ*npkA*) were grown for 72 hr at 37° in YUU medium and YUU 0.05 μg/ml 4-NQO, YUU 0.0025% MMS, YUU 5 mM HU, and YUU 5 μM CPT.

reductase activity on *csnD/csnE* genes, we identified the *A. nidulans* ribonucleotide reductase-encoding genes. Like other eukaryotes, *A. nidulans* has two genes that encode a small (*rnrA*, which was previously reported by KRAUS and HARRIS 2001; AAG40862) and a large (*rnsA*) subunit of ribonucleotide reductase (for a review, see JORDAN and REICHARD 1998). The predicted *rnsA* and *rnrA* protein products showed high identity with several ribonucleotide reductases from other eukaryotes (data not shown). We measured the mRNA expression of the *rnrA* and *rnsA* genes in the presence of DNA-damaging agents using real-time RT-PCR. As in Figure 2, *A. nidulans* wild-type GR5 was grown in the absence of any drug and transferred to a specific concentration of a DNA-damaging agent for 30, 60, and 120 min, and then RNA was isolated and analyzed for the expression of these genes (Figure 6). The *rnrA* gene expression was induced after 60 min growth in the presence of CPT and MMS (~4 times), BLEO (~3 times), and 4-NQO (~12 times) (Figure 6A). Accordingly, the *rnsA* gene expression was induced after 60 min in the presence of CPT (~10 times), MMS (~20 times), BLEO (~10 times), and 4-NQO (~45 times) (Figure 6B). These results indicate that *rnrA* and *rnsA* genes are induced at transcriptional level by different DNA-damaging agents.

As the next step, we verified *rnrA* and *rnsA* gene expression in the Δ*csnD*/Δ*csnE* mutant and wild-type strains when exposed to CPT. There is an average decrease (~50%) in *rnsA* and *rnrA* gene expression in the Δ*csnD* and Δ*csnE* mutants, respectively (Figures 7, A and B). We also observed the expression of *rnsA* and *rnrA* in the mutants Δ*npkA* (MV3), Δ*csnD* Δ*npkA* (JFL195-3), and Δ*csnE* Δ*npkA* (JFL209-3) when exposed to CPT (Figures 7, C and D). The Δ*npkA* mutant displayed lower levels of *rnrA* and *rnsA* gene expression than the wild-type strain did. However, the *rnrA* and *rnsA* mRNA expression levels are much more reduced in the double mutants Δ*csnD* Δ*npkA* and Δ*csnE* Δ*npkA* than in the corresponding parental strains. These results suggest that the ribonucleotide reductase gene expression upon DNA damage caused by CPT is dependent on the *csnD/csnE* and *npkA* genes.

DISCUSSION

This article investigates the possible involvement of the COP9 signalosome in the DNA damage response in *A. nidulans*. We examined this idea by assessing several features related to the DNA damage response in this fungus (for a review, see GOLDMAN and KAFER 2004). We observed that growth of the *csnD/csnE* inactivation

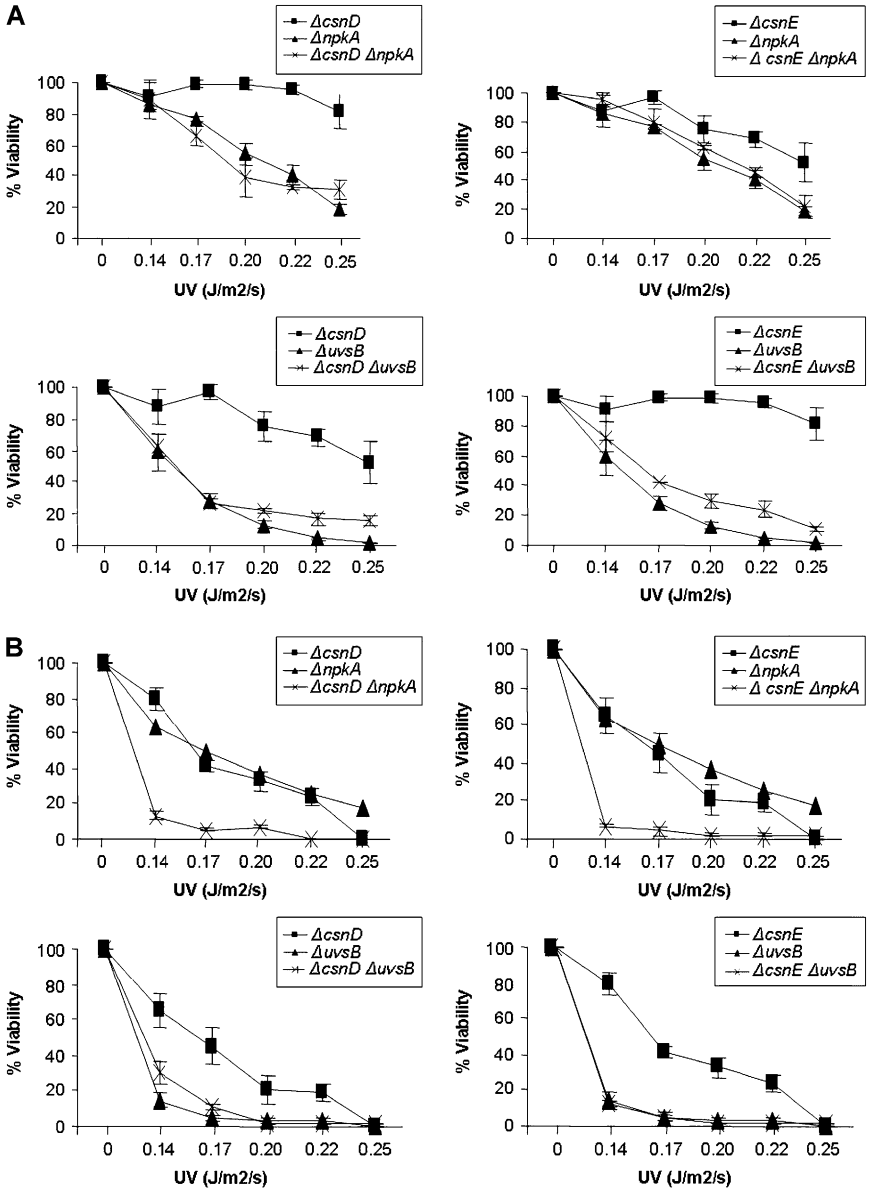

FIGURE 5.—The double mutants Δ*csnD* Δ*npkA* and Δ*csnE* Δ*npkA* showed increased sensitivity to UV light. Viability of germlings was scored after exposure to UV light of (A) quiescent and (B) germinating conidiospores. Viability was determined as the percentage of colonies on treated plates compared to untreated controls. The results were expressed by the average of three independent experiments and means are ± standard deviation. Statistical differences were determined by one-way analysis of variance (ANOVA) followed, when significant, by the Newman-Keuls Multiple Comparison Test, using Sigma Stat statistical software (Jandel Scientific, San Rafael, CA). The Δ*csnD* Δ*npkA* and Δ*csnE* Δ*npkA* strains were significantly different from Δ*csnD* and Δ*csnE* ($P < 0.01$).

mutants is sensitive to DNA-damaging agents and that *csnD/csnE* mRNA is increased in the presence of these genotoxins. Interestingly, in the *csnE* gene that encodes the deneddylase activity of the fungal signalosome, this mRNA accumulation is presumably due to increased mRNA stability. Furthermore, there is a decrease in *uvsC* mRNA expression and elimination of the checkpoint-dependent inhibition of septum formation in the presence of DNA damage in the Δ*csnD*/Δ*csnE* mutants.

Initially it was thought that in *A. thaliana* COP9 loss-of-function mutants, the loss of one subunit typically would result in the loss of the entire protein complex, and this could be used to explain the pleiotropic but identical phenotype of these mutants (SCHWECHHEIMER and DENG 2001). However, using weaker antisense and cosuppression lines, the reduction of individual CSN subunits brings about partially overlapping but also clearly distinct phenotypes; *e.g.*, while a partial reduction of CSN5 function results in plants with normal flowers, plants with reduced CSN1, CSN3, and CSN6 function have abnormal flower phenotypes (PENG *et al.* 2001; SCHWECHHEIMER *et al.* 2001; SCHWECHHEIMER 2004). Drosophila CSN4 and CSN5 losses of function have common but also distinct developmental phenotypes. CSN4 but not CSN5 mutants display a range of molting defects, while CSN5 but not CSN4 mutant larvae develop melanotic capsules (ORON *et al.* 2002). Deletion mutants in *S. pombe csn1* and *csn2* are delayed in S phase and are sensitive to UV light and ionizing radiation. However, mutants of *csn3*, *csn4*, and *csn5* display neither the phenotypes observed with *csn1* and *csn2* mutant strains nor any other obvious phenotypes, indicating that distinct CSN subunits may have distinct developmental roles in fission yeast (MUNDT *et al.* 2002).

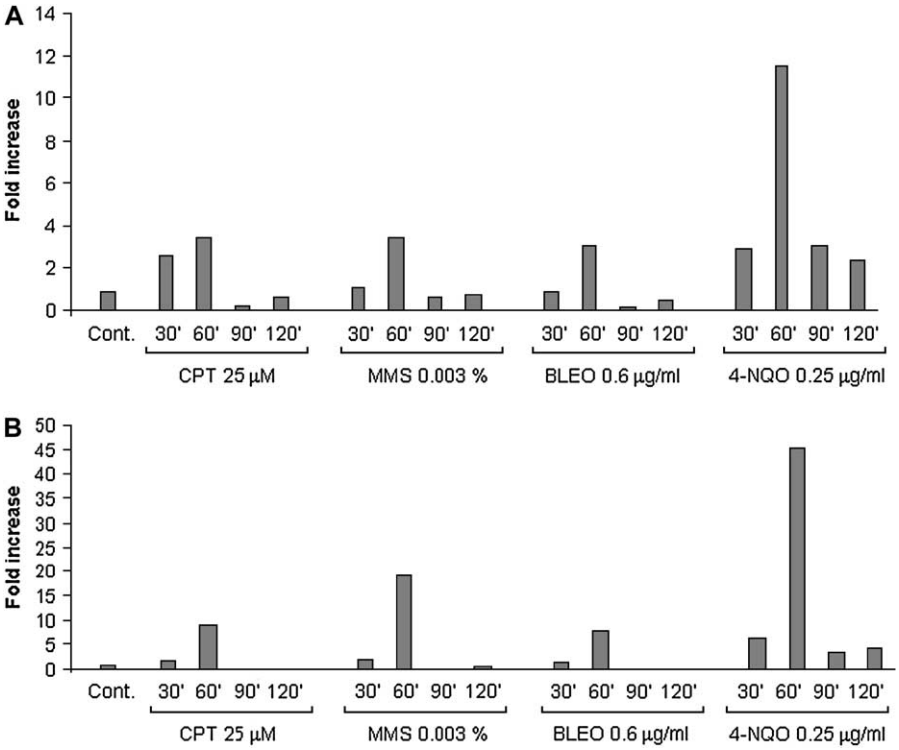

FIGURE 6.—Fold increase in *rnsA* and *rnrA* RNA levels in response to DNA-damaging agents. Mycelia were grown in the absence of any drug, 25 μM CPT, 0.003% MMS, 0.6 μg/ml BLEO, or 0.25 μg/ml 4-NQO for 30, 60, 90, and 120 min. Real-time RT-PCR was the method used to quantify the mRNA. The measured quantity of the (A) *rnsA* and (B) *rnrA* mRNA in each of the treated samples of the wild-type GR5 strain was normalized using the C_T values obtained for the *tubC* RNA amplifications run in the same plate. The relative quantitation of *rnsA* and *rnrA* and tubulin gene expression was determined by a standard curve (*i.e.*, C_T values plotted against the logarithm of the DNA copy number). Results of four sets of experiments were combined for each determination; means are shown. The values represent the number of times that the genes are expressed compared to the wild-type control grown without any drug (represented absolutely as 1.00).

We have observed that Δ*csnD*/Δ*csnE* mutations are epistatic for most of the phenotypes related to DNA damage that were investigated in this work. BUSCH *et al.* (2003) have established *csnD/csnE* genes as regulators of sexual development in *A. nidulans*. The deletion of each of these genes resulted in indistinguishable phenotypes, suggesting that they exhibit the expected epistatic behavior. However, there are circumstances in our work where they do not demonstrate the same phenotypes, for instance: (i) *csnD/csnE* mRNA expression is not synchronized since *csnE* expression seems to lag behind that of *csnD*; (ii) the Δ*csnE* inactivation mutant does not affect *rnsA* mRNA expression while the Δ*csnD* mutation does not really affect *rnrA* mRNA expression; (iii) the Δ*csnE* mutation enhances the septation of the Δ*uvsB* mutant during checkpoint-dependent inhibition of septum formation, whereas the Δ*csnD* mutation does not; and (iv) the Δ*csnD*/Δ*csnE* mutations have suppression effects on the Δ*uvsB* mutation in the viability and mitosis assays, respectively. Hence, despite its epistasis, specific biological functions can be attributed to individual CSN subunits. We are currently constructing a double-mutant Δ*csnD* Δ*csnE* strain aiming to study it for epistasis under some of the conditions where the single *csnD/csnE* mutants showed different phenotypes.

We also constructed double mutants with *cnsD-E* inactivation and deletion mutants of two genes, *uvsB*^{ATR} and *npkA*, which are involved in several aspects of the DNA damage response in *A. nidulans*. ATM/ATR are members of the family of phosphoinositide 3-kinase-related kinases and key regulators of the DNA damage response (CASPARI and CARR 1999; SHILOH 2001). The ATR pathway can respond to agents that interfere with the function of replication forks, such as HU, UV light, and DNA-alkylating agents such as MMS (NYBERG *et al.* 2002; OSBORN *et al.* 2002). The ATM/ATR kinases phosphorylate and activate signal transduction pathways that ultimately interface with the Cdk/cyclin machinery (ABRAHAM 2001). They trigger responses that promote the maintenance of genome integrity by phosphorylating multiple target proteins. *A. nidulans* UvsB^{ATR} is a member of the family of ATM/ATR kinases and functions as the central regulator of the *A. nidulans* DNA damage response (DE SOUZA *et al.* 1999; HOFMANN and HARRIS 2000; FAGUNDES *et al.* 2004). NpkA is a cdc2-related kinase that together with NimX^{Cdc2} and BimE^{APC1} monitors S-phase progression and/or recovery in response to DNA damage. We have observed that the double-mutant strains Δ*csnD* Δ*npkA* and Δ*csnE* Δ*npkA* were more sensitive to 4-NQO, MMS, HU, and CPT than the corresponding parental strains. Interestingly, the enhanced UV sensitivity of germinating conidia from the double mutants Δ*csnD*/Δ*csnE* Δ*npkA*, compared to the lack of interaction in quiescent conidia, suggests a possible role in checkpoint responses since there is no need to arrest the cell cycle for quiescent conidia. These data strongly support the notion that the signalosome may work in parallel with *npkA* to regulate DNA damage checkpoint responses. Another example of either gain or loss of CSN functions can perturb normal cell progression is the cell cycle inhibitor p27^{KIP1}, which is subject to regulation by CSN-mediated deneddylation through

SCF^{SKP2} via deneddylation and nuclear export (Tomoda *et al.* 1999; Yang *et al.* 2002; Wei and Deng 2003).

In *A. nidulans*, the DNA damage checkpoint regulates septation by modulating the activity of $NimX^{cdc2}$ (Harris and Kraus 1998; De Souza *et al.* 1999). Septum formation is triggered by high levels of $NimX^{cdc2}$ activity and is inhibited by DNA damage in a checkpoint-dependent manner (Harris and Kraus 1998). Harris and Kraus (1998) showed that mutations in $uvsB^{ATR}$ abolish the cell cycle delay and inhibition of septum formation triggered by the exposure of predivisional hyphae to DNA-damaging agents. We detected increased septation levels in the *csnD/csnE* inactivation mutants in the presence of DNA damage. Furthermore, Δ*uvsB* and Δ*csnE* also genetically interact during checkpoint-dependent inhibition of septum formation in *A. nidulans*. Our results suggest that regulation of checkpoint-dependent inhibition of septum formation in *A. nidulans* is dependent not only on the $uvsB^{ATR}$ gene but also on the CSN complex. Moreover, we have also observed that *csnD/csnE* inactivation mutations suppress the absence of DNA replication checkpoint response in the *uvsB* inactivation mutant. Thus, it is possible that the signalosome and UvsB function in parallel pathways to regulate the septation checkpoint, whereas they function in the same pathway to control mitotic checkpoints.

CSN is involved in multiple aspects of cell cycle and checkpoint control (for a review, see Wei and Deng 2003). Mutations in Drosophila CSN5 cause activation of mei-41-mediated meiotic DNA damage checkpoint during oogenesis (Doronkin *et al.* 2002). The fission yeast *csn1* and *csn2* mutants are synthetically lethal when combined with checkpoint pathway mutants such as *rad3*, *chk-1*, *cds-1*, and *cdc2.w* and more sensitive to gamma and UV irradiation (Mundt *et al.* 2002). In *S. pombe*, *csn1* and *csn2* are required for proper S-phase progression (Mundt *et al.* 2002). Further study has revealed that the S-phase delay observed in *S. pombe csn1* and *csn2* mutants is due at least in part to the misregulation of RNR, a key enzyme in the biosynthesis of deoxyribonucleotides (Liu *et al.* 2003). In *S. pombe*, RNR is composed of two small subunits (Suc22) and two large subunits (Cdc22). Activation of RNR involves nuclear export of Suc22, a process inhibited by a small cell cycle inhibitor, Spd1 (S phase delayed), whose level transiently declines in S phase and in response to DNA damage (Liu *et al.* 2003). Deletion of *csn1* leads to Spd1 accumulation owing to a defect in proteolysis. This prevents Suc22 nuclear export and ultimately leads to S-phase delay and DNA damage sensitivity. The Δ*csnE* strain showed defects in the mitosis assay (6 mM of HU). These results suggest a possible

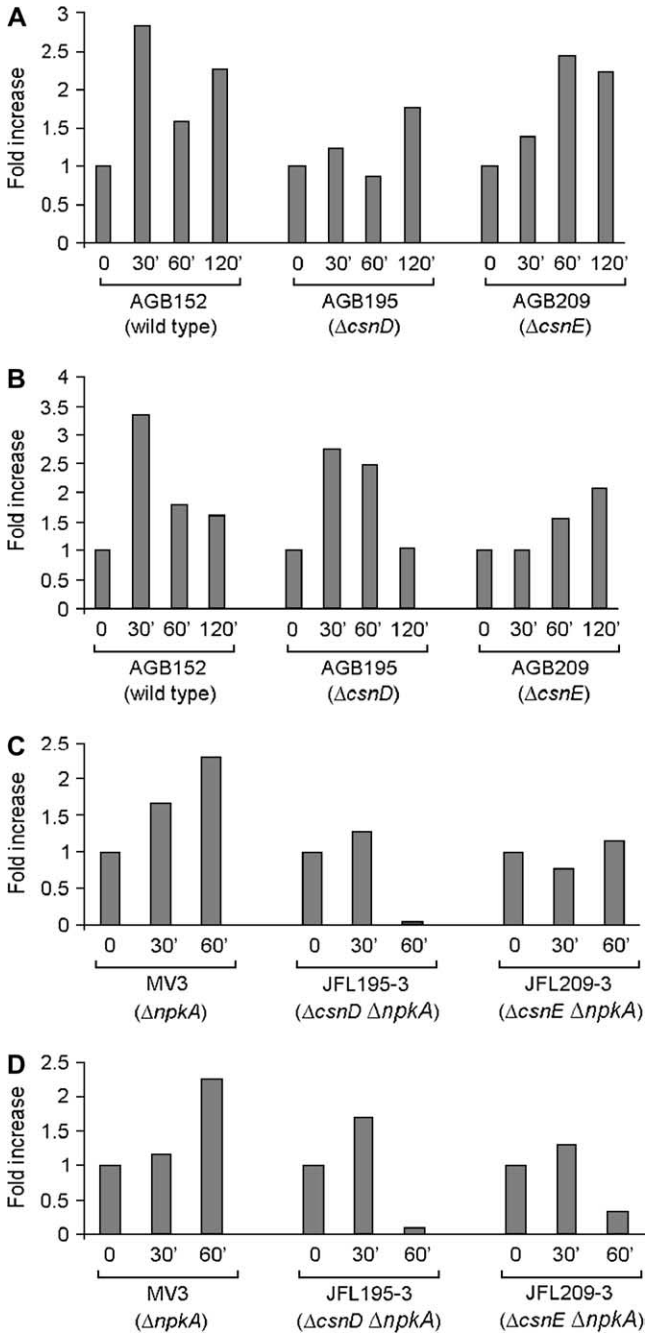

Figure 7.—The *rnsA* and *rnrA* genes showed decrease expression when Δ*csnD* and Δ*csnE* and the double mutants Δ*csnD* Δ*npka* and Δ*csnE* Δ*npkA* were grown in the presence of 25 μM camptothecin for 30, 60, and 120 min. Real-time RT-PCR was the method used to quantify the mRNA. (A and B) *rnsA* and *rnrA* mRNA expression from AGB152 (wild-type strain), AGB195 (Δ*csnD*), and AGB209 (Δ*csnE*), respectively. (C and D) *rnsA* and *rnrA* mRNA expression from MV3 (Δ*npkA*), JFL195-3 (Δ*csnD* Δ*npkA*), and JFL209-3 (Δ*csnE* Δ*npkA*), respectively. The measured quantity of the *rnsA* and *rnrA* mRNA in each of the treated samples was normalized using the C_T values obtained for the *tubC* RNA amplifications run in the same plate. The relative quantitation of *csnD/csnE* and tubulin gene expression was determined by a standard curve (*i.e.*, C_T values plotted against logarithm of the DNA copy number). Results of four sets of experiments were combined for each determination; means ± standard deviations are shown. The values represent the number of times that the genes are expressed compared to the wild-type control grown without any drug (represented absolutely as 1.00).

engagement of the CSN complex in *A. nidulans* S-phase progression and/or recovery.

As an initial step toward understanding the observed S-phase checkpoint deficiencies and *npkA* interaction with the *csnD/csnE* inactivation mutants, we identified the *A. nidulans* RNR genes. As in most eukaryotes (Jordan and Reichard 1998), *A. nidulans* also has two RNR-encoding genes, *rnrA* and *rnsA*, which encode the small and large subunits, respectively. These two genes have increased mRNA accumulation when *A. nidulans* is grown in the presence of DNA-damaging agents. Interestingly, the mRNA expression of these genes is reduced in both Δ*csnD*/Δ*csnE* and the double mutants Δ*csnD* Δ*npka* and Δ*csnE* Δ*npkA* upon DNA damage caused by CPT. These results suggest that the RNR genes could also play a role in the CSN-mediated DNA damage response in *A. nidulans*. Additionally, the defect in the ribonucleotide reductase expression in the double mutants Δ*csnD* Δ*npka* and Δ*csnE* Δ*npkA* reinforces the hypothesis that it may act in a common checkpoint pathway that also regulates the ribonucleotide reductase gene expression.

In conclusion, our data are consistent with the possibility that the CSN complex is involved in cell cycle and checkpoint response upon DNA damage in *A. nidulans*. NpkA and UvsB^{ATR} appear to play an important role in signalosome biological interactions because the corresponding double mutants with Δ*csnD* and Δ*csnE* have deficiencies in several aspects of DNA damage response.

This research was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo and the Conselho Nacional de Desenvolvimento Científico e Tecnológico, São Paulo, Brazil (to G.H.G.) and the Deutsche Forschungsgemeinschaft (German Research Council) to G.H.B.

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Communicating editor: J. J. Loros