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The *Aspergillus nidulans snt* Genes Are Required for the Regulation of Septum Formation and Cell Cycle Checkpoints

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ABSTRACT

In *Aspergillus nidulans*, germinating conidia undergo multiple rounds of nuclear division before forming a septum. Previous genetic results suggest that the ability to separate nuclear division and septum formation depends upon a threshold level of activity of the cyclin-dependent kinase NIMX^{cdk1}. Mutations in *nimX* and *nimT*, the gene encoding the NIMX^{cdk1}-activating phosphatase, have revealed that Tyr-15 phosphorylation is important for determining the timing of the formation of the first septum. Here, we describe a screen for suppressors of *nimT23* (*snt*), designed to identify additional components of the pathway regulating septum formation. We show that a subset of the *snt* mutants are defective in the temporal regulation of septum formation and in cell cycle checkpoint responses. Molecular characterization of *sntA* shows that it is allelic to the previously described *ankA* gene, which encodes the NIMX^{cdk1} Tyr-15 kinase. Additional experiments described in this study show that nutritional conditions modulate the timing of septum formation and alter the phenotypes displayed by the *snt* mutants. A model that suggests that the timing of septum formation is influenced by DNA damage and glucose availability via the *sntA* and *sntB* gene products is proposed.

THE coordination of cytokinesis with growth and nuclear division is essential for the proper development of all eukaryotic organisms. Mechanisms for ensuring that cytokinesis is coordinated with growth and nuclear division have been described in complex eukaryotes such as Drosophila (reviewed in Neufeld and Edgar 1998) and also in the simple eukaryotes *Saccharomyces cerevisiae* (reviewed in Lew 2000) and *Schizosaccharomyces pombe* (reviewed in Cerutti and Simanis 2000). A common feature of these mechanisms is the regulation of the cell cycle machinery, including modulating the activity of cyclin-dependent kinases. The coordination of cytokinesis with nuclear division represents a unique problem in filamentous fungi such as *Aspergillus nidulans*. Following spore germination, hyphal cells will undergo several rounds of nuclear division prior to septum formation (Harris *et al.* 1994). Previous results have shown that septum formation is delayed in these cells until a size control is satisfied and a subsequent nuclear division occurs (Wolkow *et al.* 1996). The cyclin-dependent kinase (cdk) NIMX^{cdk1} is required for both mitosis and septum formation in *A. nidulans*, yet the ability of predivisional cells (germlings that have not yet formed septa; Harris 1997) to undergo mitosis in the absence of septum formation suggests that these effects are separable (Harris and Kraus 1998). Genetic evidence suggests that the ability to separate mitosis and septum formation depends upon a threshold level of NIMX^{cdk1} activity (Harris and Kraus 1998). Specifically, levels of NIMX^{cdk1} activity may be assessed at a defined point during the cell cycle of predivisional cells. Only if activity exceeds the threshold does septation occur following the subsequent round of mitosis. This model is supported by the observation that inhibition of cdk activity by activation of the DNA damage checkpoint results in a delay in septum formation (Harris and Kraus 1998).

Activation of cdks is controlled by the phosphorylation of specific amino acid residues. The most extensively characterized mechanism of mitotic regulation is the inhibitory phosphorylation of the fission yeast cdk Cdc2p on Tyr-15 (Russell and Nurse 1986, 1987; Gould and Nurse 1989). This phosphorylation is catalyzed by the Wee1p and Mik1p tyrosine kinases and is removed by the tyrosine phosphatase Cdc25p (Millar *et al.* 1991; McGowan and Russell 1993; Lee *et al.* 1994). The timing of activation of Cdc2p, a requirement for mitotic entry, is determined by the relative activities of the inhibitory Wee1p/Mik1p pathway and the activating Cdc25p pathway. The activity of the DNA damage and the replication checkpoint pathways of fission yeast are also regulated by inhibitory phosphorylation of Cdc2p (Rhind *et al.* 1997; Zeng *et al.* 1998). In the filamentous fungus *A. nidulans*, mitotic entry is regulated in a similar fashion. The activation of NIMX^{cdk1}, the *A. nidulans* ortholog of fission yeast Cdc2p, is required for mitotic entry (Osmani *et al.* 1991). Inhibitory phosphorylation on Tyr-15 of NIMX^{cdk1} is catalyzed by the tyrosine kinase ANKA and removed by the tyrosine phospha-

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tase NIMT, both of which are orthologs of fission yeast Wee1p and Cdc25p, respectively (O'Connell *et al.* 1992; Ye *et al.* 1997). As in fission yeast, the DNA damage checkpoint is regulated by Tyr-15 phosphorylation of NIMX^{cdk1} (Ye *et al.* 1997).

One prediction of the threshold model of regulating septum formation is that cdk activity would be inhibited at some point during the predivisional cell cycle. A possible mechanism for preventing NIMX^{cdk1} activity from exceeding the threshold level required to initiate septum formation is to regulate NIMT activation. This possibility was investigated by screening for suppressors of the heat-sensitive (Ts) *nimT23* mutant to identify potential negative regulators of NIMT. Here, we describe the isolation and characterization of mutants that suppress the *nimT23* growth defect. As expected, the subset of mutants we chose to analyze displayed defects in the regulation of septum formation. Molecular characterization of one of the genes identified in this screen revealed that it encodes the Wee1p tyrosine kinase ortholog ANKA. We also provide evidence that the timing of septum formation is modulated by nutrient availability and that growth conditions can also affect checkpoint responses. On the basis of our results, we propose a model for the regulation of septum formation in *A. nidulans*.

MATERIALS AND METHODS

Strains and growth conditions: All strains used in this study are listed in Table 1. Media used were YGV (2% glucose, 0.5% yeast extract, 0.01% vitamins), MNV (1% glucose, nitrate salts, trace elements, and 0.01% vitamins), MNV-EtOH (MNV with 20 ml/liter ethanol as the only carbon source), MNV-glycerol (MNV with 10 ml/liter glycerol as the only carbon source), MNV-OAc (MNV with 100 mm acetate as the only carbon source), and CM (1% glucose, 0.1% yeast extract, 0.2% peptone, 0.1% casamino acids, nitrate salts, trace elements, and 0.01% vitamins, pH 6.5). Nitrate salts, trace elements, and vitamins are described in the appendix to Kafer (1977). Strains carrying the *argB2* mutation were supplemented with 0.02% arginine. Strains carrying the *pyrG89* mutation were grown in MAG (2% malt extract, 2% glucose, 0.2% peptone, trace elements, and vitamins) supplemented with 5 mm uridine and 10 mm uracil. For solid media, 1.5% agar was added. Hydroxyurea (HU; Sigma Chemical, St. Louis) was prepared as a 2 m stock solution and added to media after autoclaving. Wild-type strains were incubated at 28° or 32°, and Ts strains were incubated at 42° or 43.5°. Diepoxyoctane (DEO; Aldrich Chemical, Milwaukee) was added to liquid media at the time of inoculation at a concentration of 0.025%. 5-Fluoroorotic acid (5-FOA; U.S. Biological) and 3-amino-1,2,4-triazole (3AT; Sigma Chemical) were added to solid media after autoclaving at concentrations of 1 mg/ml and 0.5 mm, respectively.

Mutagenesis and isolation of *snt* mutations: A suspension of 10^6 conidia from the strain MO73 was plated on CM plates and irradiated with UV, such that the survival rate was 10%. The plates were incubated for 3 days at the nonpermissive temperature of 43.5°. Colonies that formed were patched in grids on master CM plates and incubated at 28° for 3 days. Five hundred colonies were then retested for growth at 43.5° and for hypersensitivity to 5 mm HU. Candidates for further study were subjected to genetic crosses to determine if the suppression was due to a mutation in a single gene and if the HU hypersensitivity and the suppression cosegregated.

Staining, microscopy, and measurements: Growth of strains on coverslips, fixation of samples, staining with Calcofluor White and Hoechst 33258, and mounting of coverslips on glass slides were performed as previously described (Harris *et al.* 1994). Microscopy, photography, and measurements were performed as previously described (Harris and Kraus 1998). Cell size was determined by measuring hyphal length using a calibrated eyepiece micrometer. Since fungal hyphae grow solely at the tip, the length of a hypha generally indicates the extent to which it has grown. Under the conditions used in these experiments, the average hyphal diameters of all strains were similar although slight variations were observed. The nonparametric Mann-Whitney test was used to determine if two strains differed significantly in cell length and cell width (Zar 1984). To use this test, measurements were ranked and the U and U' values calculated. These values take the number of measurements and the sum of the ranks into consideration. The larger of the two values was compared to the respective critical value. If the U or U' value was greater than or equal to the respective critical value, the null hypothesis (that no significant difference in cell length or cell width exists) was rejected. The septation index (SI) represents the percentage of germlings that contain at least one septum ($n \geq 200$). The chromosome mitotic index (CMI) represents the percentage of cells that contain condensed mitotic chromatin ($n = 200$).

Protein extraction and Y15-phosphorylation assays: Strains were inoculated in YGV at 5×10^6–1×10^7 conidia/ml at 28°. Mycelia were harvested by filtration, rinsed in stop buffer (Moreno *et al.* 1989), pressed dry between paper towels, and frozen in liquid nitrogen. We extracted protein from frozen mycelia in HK buffer (Osmani *et al.* 1991) using a ground glass dounce homogenizer at 4°. Samples were centrifuged for 10 min at $14{,}000 \times$ g and the supernatant was removed. We used 2 mg of protein for affinity purification of NIMX^{cdk1} using p13^{suc1} agarose beads (Oncogene Research Products). For Western blots, proteins were separated by SDS-PAGE and transferred to Immobilon-P membranes (Millipore, Bedford, MA) by electroblotting with a semidry apparatus (Hoefer Scientific Instruments, San Francisco). Western analysis was performed by enhanced chemiluminescence (ECL; Amersham Life Sciences, Buckinghamshire, UK) according to the manufacturer's specifications. Membranes were probed with Y15 phospho-specific anti-Cdc2 antibodies (New England Biolabs, Beverly, MA) and anti-PSTAIRE antibodies (Santa Cruz Biotechnology) as a loading control and were used at dilutions of 1:1000 and 1:2000, respectively.

Cloning and identification of *sntA*: To clone the *sntA* gene, we used a genomic plasmid library that contains a sequence (AMA1) that allows autonomous replication in *A. nidulans* (Osherov and May 2000). Use of this autonomously replicating plasmid greatly increases transformation efficiency and facilitates recovery of the plasmid from transformants for subsequent cloning steps. Transformants were recovered on 5 mm HU and streak purified twice in the presence of HU. Genomic DNA was prepared from transformants and used to transform electrocompetent *Escherichia coli* cells, and 24 ampicillin-resistant colonies were analyzed for the recovery of the vector containing a genomic insert. Plasmids that contained a genomic insert were confirmed by the ability to retransform to HU resistance. Fragments of genomic inserts were subcloned into the plasmid pBluescript (Stratagene, La Jolla, CA) and sequenced at the Molecular Core Facility at the University of Connecticut Health Center. Partial sequence of a 3-kb *Xba*I genomic fragment was identical to the previously described *ankA* gene (accession no. U25693). In addition, a sequence was obtained that is likely to encode the small subunit of the *A. nidulans* ribonucleotide reductase and was designated *rnrA* (accession no. AF310625; see below).

TABLE 1

***Aspergillus nidulans* strains**

Strain	Genotype	Source[a]
ASH35	*sepA1; argB2; yA2*	Lab stock
ASH60	*sepB3; pabaA6; yA2*	Lab stock
ASH202	*sepB3; uvsB110; pabaA1; nicA2*	Lab stock
ASH244	*nimX3; argB2; wA3*	Lab stock
ASH278	*nimT23; argB2; wA3*	Lab stock
ASH288	*sepB3; nimX^{cdc2AF}; pabaA6*	Lab stock
AAH1	*nimA5; uvsB110; wA3*	Lab stock
A28	*pabaA6; biA1*	1
A781	*nimA5; wA3*	1
FRY20	*nimX^{cdc2AF}; wA3; pyrG89; pyroA4; pyr4^{+}*	2
GR5	*pyrG89; pyroA4; wA3*	1
MO73	*nimT23; pabaA6*	2
SO65	*nimX3; pyroA4; riboA1; wA3; yA2*	2
APK35	*sntA1; pabaA6*	This study
APK47	*sepB3; sntC1*	This study
APK53	*sepB3; sntB1; pabaA6; yA2*	This study
APK56	*sntB1; pabaA6*	This study
APK61	*sepB3; sntA1; pabaA6*	This study
APK64	*sntC1; pabaA6*	This study
APK66	*nimX3; sntB1; wA3*	This study
APK68	*nimX3; sntA1; wA3*	This study
APK69	*sepA3; sntA1; argB2; yA2*	This study
APK70	*sepA3; sntB1; argB2*	This study
APK72	*nimA5; sntA1; pabaA6; wA3*	This study
APK73	*nimA5; sntB1; pabaA6; wA3*	This study
APK107	*sntA1; pyrG89; chaA1*; [pAMA1-*ankA*]	This study
APK110	*sntA1/+*; *sntB1/+*	This study
APK111	*sntA1/+*; *sntB1/+*	This study

[a] Key to sources: 1, Fungal Genetics Stock Center, Department of Microbiology, University of Kansas Medical Center, Kansas City, KS 66160-7420; 2, Stephen Osmani, Weis Center for Research, Pennsylvania State University College of Medicine, Danville, PA 17822.

RESULTS

Isolation of Ts^{+} suppressors of the heat-sensitive *nimT23* mutation: Suppressors of the heat sensitivity caused by the *nimT23* mutation were isolated as described in MATERIALS AND METHODS. Conidia from the strain MO73 were plated on CM plates and irradiated with UV light. Five hundred revertants were then retested for growth at 43.5° and for hypersensitivity to 5 mM HU. The rationale was to facilitate future cloning of the suppressor genes by complementation of the HU hypersensitivity. We chose HU hypersensitivity as a selectable phenotype because mutants that are able to bypass the G_2 cell cycle arrest of the *nimT23* mutant might also be defective in cell cycle checkpoint function.

Of the 500 *snt* mutants (*s*uppressor of *nimT23*) isolated by the ability to grow at 43.5°, 90 did not form a colony on CM + 5 mM HU plates. These were then crossed with the *nimT23* strain ASH278 to determine if the HU hypersensitivity cosegregated with suppression of the Ts phenotype. For 20 of the 90 mutants, suppression was linked to the HU hypersensitivity because all Ts^{+} segregants were HU^{s}. Fifteen extragenic suppressors were then isolated by the appearance of Ts segregants after crossing each mutant with the wild-type strain GR5. One class of mutants we expected to isolate from this screen was loss-of-function alleles of *ankA*, the gene encoding the *A. nidulans* ortholog of Wee1p, which is responsible for the inhibitory Tyr-15 phosphorylation of NIMX^{cdk1}. The *ankA* gene is tightly linked to the *wA* locus (X. YE, personal communication); therefore, any suppressors that were linked to the *wA* locus after the cross with ASH278 were likely to contain a mutation in the *ankA* gene. Although one extragenic suppressor (A86, *sntA* linkage group) appeared to display loose linkage to *wA* (6/36 recombinants), we continued with its characterization. In addition, the suppressors were also crossed with the strain ASH244 to verify that they did not have a mutation in the *nimX* gene.

The 15 extragenic suppressors were examined microscopically to determine if septum formation was occurring prematurely. For these experiments, the SI was determined and compared to that of a wild-type strain. Since *A. nidulans* hyphae grow solely at the tip and the hyphal diameter of all strains was constant, cell size could be determined by measuring hyphal length. Seven *snt* mutants were chosen for subsequent linkage analysis on the basis that they showed a significant decrease in cell size at the time of septation compared to the wild-type control (see below), whereas the other eight mutants showed no such difference. These mutants sorted into three linkage groups, designated *sntA*, *sntB*, and *sntC*. These *snt* mutations are recessive because heterozygous diploids are able to form a colony on 5 mM HU (P. R. KRAUS and S. D. HARRIS, unpublished results). *sntA* and *sntB* define distinct complementation groups because diploids constructed from *sntA* and *sntB* parent strains were able to form a colony on plates containing 5 mM HU (Figure 1). No diploids were able to be constructed between *sntC* and *sntA* or *sntB* mutants. The *sntA* and *sntC* linkage groups contain only one isolate each, designated *sntA1* and *sntC1*, respectively. The other five suppressors comprise the *sntB* linkage group. The D80 isolate, designated *sntB1*, was chosen for further study because its HU^{s} phenotype was strongest. Due to the inability to assign *sntC* to a complementation group, only preliminary phenotypic characterization was performed. The viability curves for *sntA1*, *sntB1*, and *sntC1* mutants plated on media containing HU are shown in Figure 1.

***sntA1*, *sntB1*, and *sntC1* mutations cause deregulation of septum formation:** In wild-type *A. nidulans* germlings, septum formation does not occur until the hyphae have grown longer than 45–50 μm and have completed three or four rounds of nuclear division (HARRIS *et al.* 1994; WOLKOW *et al.* 1996). It has previously been shown that NIMX^{cdk1} activity is required for septum formation and that septum formation occurs inappropriately when NIMX^{cdk1} is deregulated (HARRIS and KRAUS 1998). Since

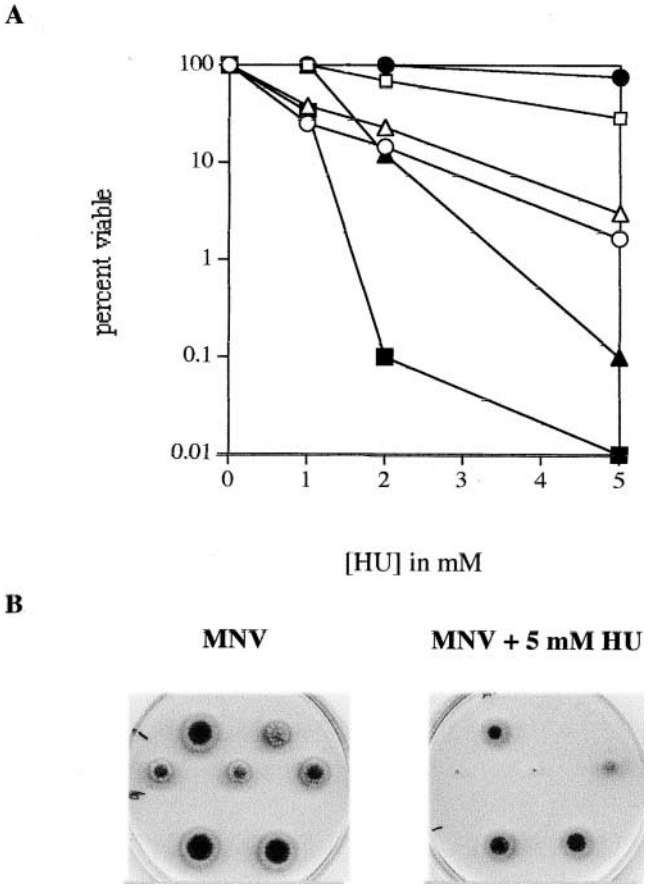

FIGURE 1.—*sntA1, sntB1*, and *sntC1* mutants are hypersensitive to HU. (A) Conidia from strains A28 (wild type, solid circles), APK35 (*sntA1*, open circles), APK56 (*sntB1*, open triangles), APK64 (*sntC1*, open squares), ASH201 (*uvsB110*, solid triangles), and FRY20 ($nimX^{cdc2AF}$, solid squares) were diluted and plated at 100 conidia per plate on CM media containing the indicated concentration of HU. The number of survivors on each plate was determined after 3 days incubation at 32°. Percent viability represents the percentage of colonies remaining on the treated plates as compared to the untreated control plates. Each data point represents the average of four plates. Experiments were repeated twice, and representative data are shown. (B) Conidia from the indicated strains were point inoculated on MNV and MNV + 5 mM HU and incubated for 3 days at 32°. (Top row) A28 (wild type) and FRY20 ($nimX^{cdc2AF}$); (middle row) APK35 (*sntA1*), APK56 (*sntB1*), and APK64 (*sntC1*); (bottom row) APK110 (*sntA1*/+; *sntB1*/+ diploid) and APK111 (*sntA1*/+; *sntB1*/+ diploid).

the NIMT tyrosine phosphatase is required for the activation of $NIMX^{cdk1}$, loss-of-function mutations in genes whose products negatively regulate NIMT might result in premature activation or elevated levels of $NIMX^{cdk1}$ activity. Accordingly, a subset of the *snt* mutants may display a premature septum formation phenotype. As predicted, cells harboring the *sntA1*, *sntB1*, and *sntC1* mutations underwent septum formation at a smaller size and with fewer nuclei than did wild-type cells (Figure 2). The mean length of wild-type cells possessing one septum (61.7 ± 15.2 μm) differs significantly from that of the *sntA1* mutant (24.5 ± 7.3 μm; $Z = 0.001$, $Z = 5.87$), the *sntB1* mutant (24.4 ± 6.7 μm; $\alpha = 0.001$, $Z = 5.93$), and the *sntC1* mutant (25.6 ± 7.8 μm; $\alpha = 0.001$, $Z = 5.89$) on the basis of the nonparametric Mann-Whitney test. Furthermore, 10–15% of *sntA1*, *sntB1*, and *sntC1* cells underwent septation at a size smaller than 25 μm, whereas no wild-type cells formed septa at this size. In addition, between 16 and 26% of *sntA1*, *sntB1*, and *sntC1* cells contained four or fewer nuclei when the first septum formed (Figure 3), a condition that is never observed in wild-type cells (HARRIS *et al.* 1994). A significant percentage of *sntA1*, *sntB1*, and *sntC1* cells also contained nuclei that were bisected by a septum (5–16%) or contained a double septum (2–5%; Figure 3), which is similar to the effect caused by the $nimX^{cdc2AF}$ mutant (HARRIS and KRAUS 1998).

Previous results have shown that general perturbations of DNA metabolism in *A. nidulans* germlings inhibit or delay the formation of the first septum (HARRIS and KRAUS 1998). The Ts *sepB3* mutation causes defects in normal chromosomal DNA metabolism at the nonpermissive temperature, and the formation of the first septum is blocked (HARRIS and HAMER 1995). In *sepB3* mutants that are defective in cell cycle checkpoint functions due to the presence of either the *uvsB110* or the $nimX^{cdc2AF}$ mutations, the inhibition of septum formation is relieved and the SI approaches wild-type levels (HARRIS and KRAUS 1998). To test whether the *sntA1*, *sntB1*, and *sntC1* mutations cause a similar effect, *sepB3 snt* double mutants were constructed and tested for the ability to undergo septum formation at the restrictive temperature. *sepB3 snt* double mutants were found to be Ts for growth and hypersensitive to 5 mM HU at the permissive temperature. Despite the presence of the *sepB3* mutation, the *sepB3 snt* double mutants were all able to form septa at the restrictive temperature of 42° (Table 2). The septation index in *sepB3 sntA1* mutants was 40.0 ± 7.5, while the septation indices in *sepB3 sntB1* and *sepB3 sntC1* mutants were ~70, which is similar to that of the *sepB3* $nimX^{cdc2AF}$ mutant (see DISCUSSION). In addition, cells possessing the *sntA1*, *sntB1*, and *sntC1* mutations were able to undergo septum formation when grown in the presence of 0.025% DEO, a bifunctional alkylating agent (P. R. KRAUS and S. D. HARRIS, unpublished results). The possibility that the *sntA1*, *sntB1*, and *sntC1* mutations can bypass the normal controls that regulate septum formation was eliminated by showing that *sepA3 snt* double mutants do not undergo septum formation at restrictive temperature. The product of the *sepA* gene is thought to be required for organization of the actin ring at the division site (HARRIS *et al.* 1997). These results show that the regulatory mechanisms that restrain septum formation when hyphal cells are either too small or have sustained DNA damage are abrogated by the *sntA1*, *sntB1*, and *sntC1* mutations.

The DNA damage checkpoint pathway is compromised in *sntA1* and *sntB1* mutants: The results reported above show that *sntA1* and *sntB1* mutants do not delay septum

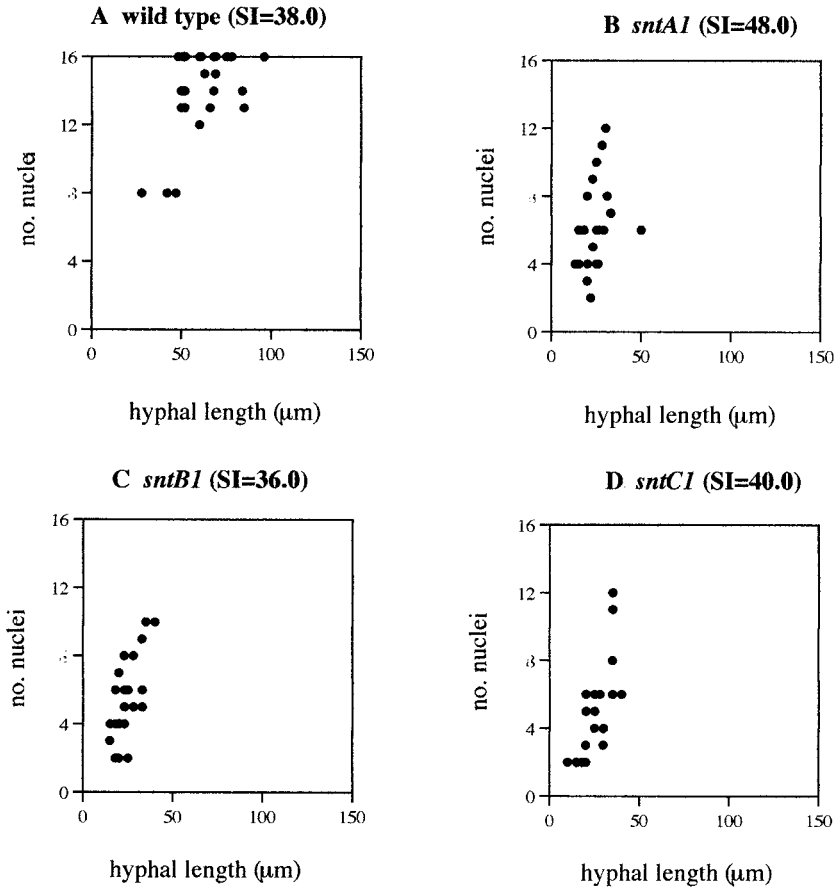

FIGURE 2.—*sntA1*, *sntB1*, and *sntC1* mutants undergo septum formation at a smaller size and with fewer nuclei than do wild-type cells. Conidia of the indicated genotypes were incubated on coverslips for 11 hr (wild type) or 12 hr (*snt* mutants) at 28°, such that the septation indices of the populations were similar. Coverslips were stained with Calcofluor White and Hoechst 33258 to visualize septa and nuclei, respectively. For each strain, the length and nuclear number of 25 cells possessing one septum were measured and plotted as a scatter graph. In addition, the SI was determined for a randomly selected population of 200 cells. The strains analyzed were the following: (A) A28, (B) APK35, (C) APK56, and (D) APK64.

formation in response to DNA damage as do wild-type cells. To test whether the DNA damage checkpoint pathway is nonfunctional in *sntA1* and *sntB1* mutants, we tested their ability to inhibit nuclear division in the presence of DNA damage. The *sntA1* and *sntB1* mutations were crossed into a background containing the Ts *nimA5* mutation, which causes a G_2 arrest at restrictive temperature (OSMANI *et al.* 1987). The *nimA5 snt* double

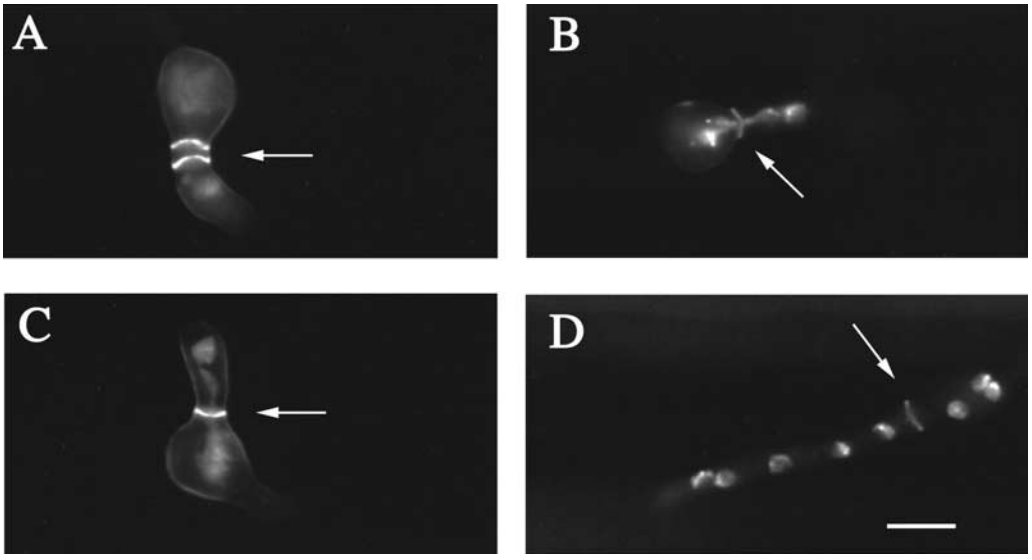

FIGURE 3.—Septum formation is deregulated in *sntA1*, *sntB1*, and *sntC1* mutants. Conidia were germinated on coverslips for 11–12 hr at 28°. Coverslips were stained with Calcofluor White and Hoechst 33258 to visualize septa and nuclei, respectively. (A) APK35 (*sntA1*) shows a cell with a double septum. (B) APK56 (*sntB1*) and (C) APK64 (*sntC1*) show cells in which the nuclear material is bisected by a septum ("cut" phenotype). (D) A28 (wild type). Septa are denoted by arrows. Hyphal widening occurred in *snt* mutant strains at a low frequency; however, the average hyphal diameters of *snt* strains were not significantly different from the diameter of wild-type strains as determined by the Mann-Whitney test ($\alpha = 0.001$; see MATERIALS AND METHODS). Bar, 10 μm.

TABLE 2

Septation indices of *sepB3 snt* double mutants

Strain	Genotype	Septation index
A28	Wild type	84.2 ± 2.2
ASH60	*sepB3*	1.7 ± 1.2
ASH288	*sepB3 nimX*^{cdc2AF}	69.8 ± 2.8
APK61	*sepB3 sntA1*	40.0 ± 4.4
APK53	*sepB3 sntB1*	69.5 ± 1.0
APK47	*sepB3 sntC1*	68.2 ± 1.9
APK66	*nimX3 sntA1*	0
APK67	*nimX3 sntB1*	0
APK69	*sepA1 sntA1*	0
APK70	*sepA1 sntB1*	0

Conidia from the indicated strains were germinated on coverslips for 12–16 hr at 42°. Coverslips were stained with Calcofluor White and Hoechst 33258 to visualize septa and nuclei, respectively. For each strain, 200 germlings were scored for the presence of septa. The SI represents the percentage of cells that contain one or more septa. Experiments were repeated three times; data shown are the mean SI and the standard error of the mean.

mutants were incubated at the restrictive temperature of 43.5° for 8 hr and then shifted to permissive temperature in either YGV or YGV containing 0.025% DEO. Cells harboring the *nimA5* mutation displayed a significant increase in the CMI after release into the control media, whereas the CMI remained low when they were released into media containing DEO (Figure 4A). In contrast, *nimA5 sntA1* and *nimA5 sntB1* cells showed a significant increase in the CMI when released into media containing DEO (Figure 4, B and C). The failure to delay nuclear division in the presence of DNA damage in the *nimA5 snt* double mutants is very similar to the behavior of the *nimA5 uvsB110* mutant (Figure 4D), which no longer has a functional DNA damage checkpoint (Hofmann and Harris 2000). These results show that the checkpoint that prevents mitotic entry due to the presence of DNA damage is compromised in *sntA1* and *sntB1* mutants.

The *sntA1* and *sntB1* mutations have different effects on cell cycle progression in the presence of the *sepB3* mutation: Conidia that harbor the *sepB3* mutation display a progressive cell cycle delay when incubated at restrictive temperature (Harris and Hamer 1995). This delay may allow repair of DNA damage that is caused by the lack of a functional *sepB* gene. The delay is presumably caused by the DNA damage or replication checkpoint, since it is abolished by the *nimX*^{cdc2AF} and *uvsB110* mutations (Hofmann and Harris 2000). To ask if the *sntA1* and *sntB1* mutations similarly abolished the cell cycle delay imposed by the loss of *sepB* function, we examined the nuclear division kinetics of *sepB3 snt* double mutants. We found that the *snt* mutations had different effects on the cell cycle delay caused by the presence of the *sepB3* mutation (Figure 5). The *sepB3 sntA1* double mutant resembled *sepB3 nimX*^{cdc2AF} and *sepB3 uvsB110* cells in that the cell cycle delay was abrogated (*i.e.*, hyphal cells accumulated four nuclei with kinetics similar to wild type). In contrast, the *sepB3 sntB1* double mutant showed a cell cycle delay similar to that of *sepB3* cells. These results show that the *sntB1* mutant is distinct from the *sntA1* mutant in that it is not dysfunctional in all checkpoints involving *uvsB* or Tyr-15 phosphorylation of NIMX^{cdk1}.

Reduced Tyr-15 phosphorylation in *sntA1* and *sntB1* mutants: Activation of the G_2/M DNA damage checkpoint in *A. nidulans* requires phosphorylation of Tyr-15 of NIMX^{cdk1} (Ye *et al.* 1997). One reason that the *sntA1* and *sntB1* mutants might be defective in the DNA damage checkpoint is that the removal of the phosphate group at position 15 by the NIMT phosphatase may not be prevented when the checkpoint is activated. Alternatively, the *sntA1* and *sntB1* mutants may not be able to undergo the inhibitory phosphorylation event even under normal conditions. To test this, we assessed the phosphorylation state of NIMX^{cdk1} in wild-type and *sntA1* and *sntB1* mutant cells by affinity purifying NIMX^{cdk1} from exponentially growing cells incubated in YGV or in YGV + 0.025% DEO. The phosphorylation state of NIMX^{cdk1} was assayed by Western blot using phosphospecific anti-Cdc2 antibodies. Exponentially growing *sntA1* and *sntB1* cells that have not been treated with DEO show greatly reduced phosphorylation when compared with wild-type cells (Figure 6). In addition, no induction of Tyr-15-phosphorylated NIMX^{cdk1} occurs after growth in the presence of DEO for 1 hr. Although the effects of adding DEO to wild-type cultures are presumably obscured by the asynchrony of the culture, these results are consistent with the notion that the *sntA1* and *sntB1* mutants either fail to undergo Tyr-15 phosphorylation or fail to prevent Tyr-15 dephosphorylation.

***sntA* encodes the cell cycle regulator ANKA:** *sntA* was cloned by complementation of the HU hypersensitivity using an autonomously replicating AMA1-based genomic library (Osherov and May 2000). Transformation of the *snt* mutants using this library yielded two types of plasmids. One type of plasmid contained the *A. nidulans* gene encoding the small subunit of ribonucleotide reductase, which we named *rnrA* (accession no. AF310625). The small subunit of ribonucleotide reductase is the target of HU (Desany *et al.* 1998), which suggests that multiple copies of the *rnrA* gene complement only the HU sensitivity of the *sntA1* and *sntB1* mutants. Consistent with this notion, *rnrA* introduced in single copy did not complement the HU hypersensitivity of either *sntA1* or *sntB1*, and *sntA1* and *sntB1* mutants containing the multicopy *rnrA* plasmid still formed septa inappropriately (P. R. Kraus and S. D. Harris, unpublished results). The other type of plasmid contained sequences identical to the previously identified *ankA* gene (Ye *et al.* 1996). *ankA* encodes a tyrosine kinase that is responsible for catalyzing the inhibitory phosphorylation of NIMX^{cdk1} on tyrosine 15. In *A. nidu-*

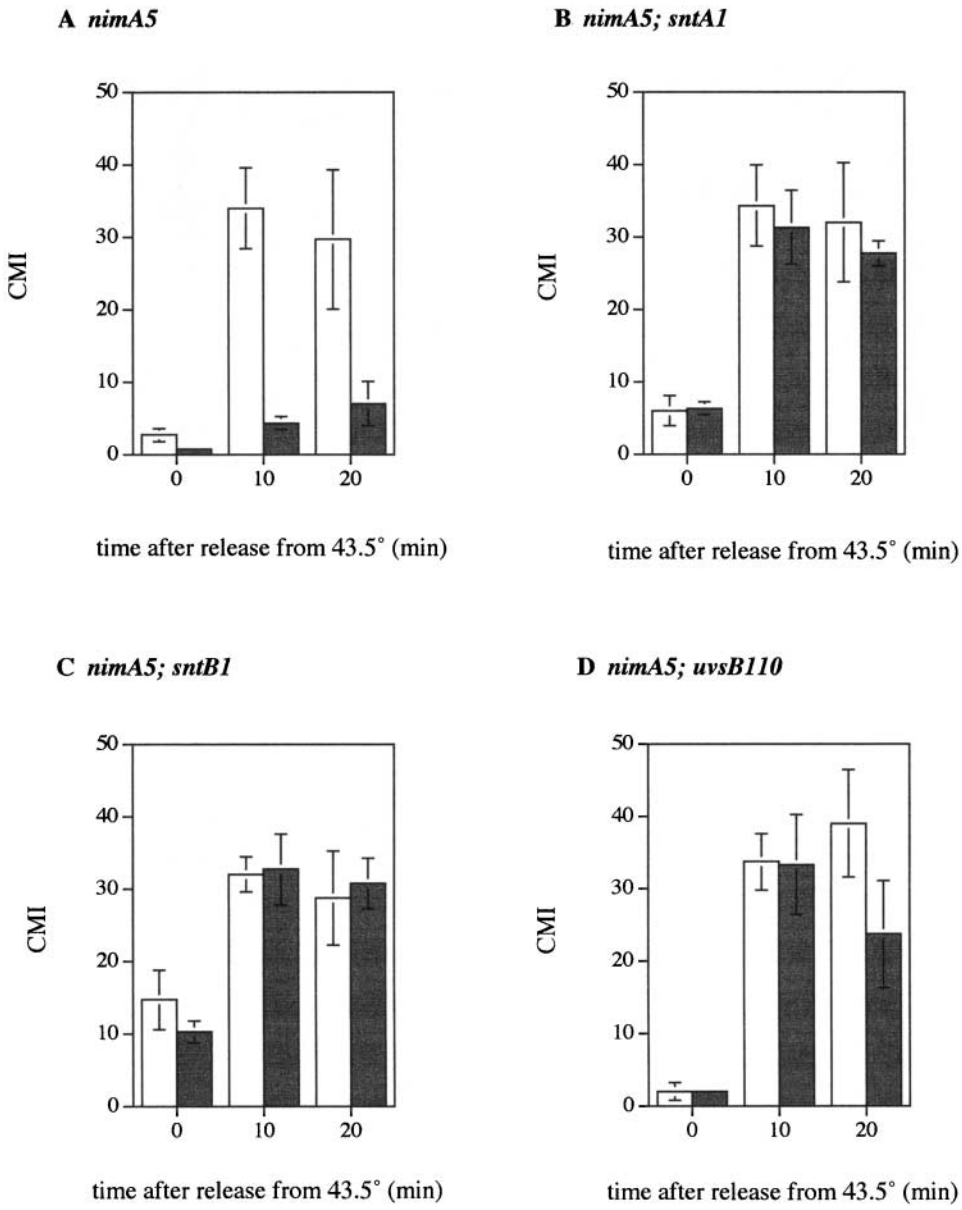

FIGURE 4.—The DNA damage checkpoint is abrogated in *sntA1* and *sntB1* mutants. Conidia from strains with the indicated genotype were germinated in YGV at 43.5° for 8 hr and released at 28° into YGV (open bars) or YGV + 0.025% DEO (solid bars). Samples were taken at the indicated time points and the CMI was determined for each sample by counting 200 cells and determining the percentage that contain condensed mitotic chromatin ($n = 3$). Data shown are the mean CMI and the standard error of the mean. Strains used were (A) A781, (B) APK72, (C) APK73, and (D) AAH1.

lans, the Δ*ankA* mutant has been shown to be defective in the slowing of S-phase checkpoint (YE *et al.* 1996), the DNA damage checkpoint (YE *et al.* 1997), and the regulation of septum formation (DE SOUZA *et al.* 1999). The *sntB1* mutant is also complemented, but to a slightly lesser extent, by the *ankA* gene when it is present on the AMA1-based plasmid. The loose linkage of *sntA* to *wA* suggests that *sntA* is an allele of *ankA*, whereas *sntB* is likely to be rescued by the introduction of multiple copies of *ankA*. To test this notion, *sntA1* and *sntB1* strains were transformed with an integrating plasmid containing *ankA*. A 3.8-kb *Eco*RI fragment containing *ankA* was subcloned into pRG3, which contains the *Neurospora crassa pyr-4* gene (WARING *et al.* 1989). Pyr^+ transformants were tested for the ability to grow in the presence of 5 mM HU. To screen for gene replacement events in these transformants, these strains were cured of the plasmid by growth on nonselective media containing 5-FOA. The resulting 5-FOA-resistant strains were tested for the ability to grow in the presence of 5 mM HU. A total of 59% ($n = 124$) of the 5-FOA-resistant strains derived from the *sntA1* strain were able to grow in the presence of 5 mM HU, indicating that the *sntA1* lesion is repaired by loss of the *ankA*-containing plasmid. No such HU-resistant colonies were found with 5-FOA-resistant strains derived from *sntB1* transformants ($n = 100$). We conclude that *sntA1* is an allele of *ankA*, while the *sntB1* mutation is in a different gene and can be complemented by *ankA* in multiple copies. Repeated attempts to clone *sntB* failed to identify a plasmid specific to *sntB1* complementation.

Growth conditions affect the regulation of septum formation and the *sntA1* and *sntB1* phenotypes: Previous experiments that characterized the regulation of sep-

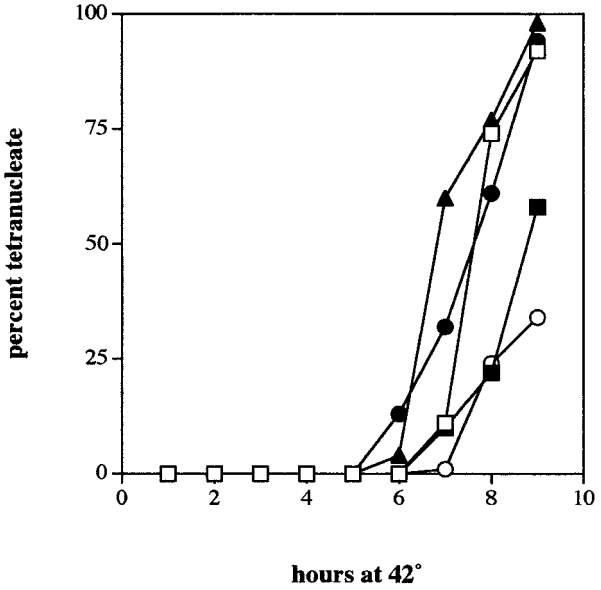

FIGURE 5.—*sntA1* and *sntB1* mutants have different effects on cell cycle progression in the *sepB3* background. Conidia from strains A28 (wild type, open squares), ASH60 (*sepB3*, solid squares), ASH202 (*sepB3 uvsB110*, solid triangles), APK53 (*sepB3 sntB1*, open circles), and APK61 (*sepB3 sntA1*, solid circles) were germinated on coverslips for 4 hr at 42°. Thereafter, one coverslip was removed every hour over a 5-hr period and processed for microscopy. Coverslips were stained with Calcofluor White and Hoechst 33258 to visualize septa and nuclei, respectively. For each sample, 200 germlings were scored for the number of nuclei present. Data points represent the percentage of germlings possessing four or more nuclei. The experiment was repeated three times, and representative data are shown.

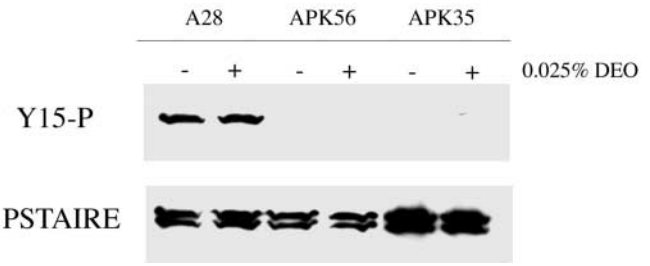

FIGURE 6.—Reduced Tyr-15 phosphorylation in *sntA1* and *sntB1* mutants. Conidia from strains A28 (wild type), APK 56 (*sntB1*), and APK35 (*sntA1*) were inoculated in YGV and grown for 12 hr at 28°. One-half of the cultures were harvested for protein extraction and the remaining one-half was treated with 0.025% DEO for 1 hr before harvesting. Protein was extracted and $NIMX^{cdk1}$ was affinity purified using $p13^{suc1}$ agarose beads. Proteins were separated by SDS-PAGE and transferred to Immobilon-P by electroblotting with a semidry apparatus. Western analysis was performed by ECL according to the manufacturer's specifications. Membranes were probed with Y15 phospho-specific anti-Cdc2 antibodies at 1:1000, stripped, and reprobed with anti-PSTAIRE antibodies at 1:2000.

tum formation in *A. nidulans* were performed in rich media (HARRIS *et al.* 1994; WOLKOW *et al.* 1996). To assess the role of growth conditions and/or nutrient availability in the regulation of septum formation, we performed a series of experiments using minimal media containing different carbon sources. Conidia from wild-type and *snt* mutants were incubated on coverslips in YGV for 8 hr and shifted to MNV-glycerol, and the timing of septum formation was determined relative to nuclear division and cell length. Wild-type cells that have been shifted to MNV-glycerol underwent septum formation at a significantly smaller size and with fewer nuclei than did the cells that remained in YGV for the duration of the experiment (Table 3). However, *sntA1* and *sntB1* mutants underwent septation at a small size irrespective of whether they were shifted to MNV-glycerol or remained in YGV. These data suggest that nutrient availability influences the timing of septum formation and that the *sntA* and *sntB* genes are required to delay septum formation under rich media conditions.

To further assess the effect of nutritional conditions on the *sntA1* and *sntB1* phenotype, the effects of altering the carbon source on the HU hypersensitivity of the *sntA1* and *sntB1* mutants were investigated. The viability of the *sntA1* and *sntB1* mutants following incubation on MNV, MNV-OAc, and MNV-EtOH media in the presence of varying concentrations of HU was determined. When incubated on MNV, *sntA1* and *sntB1* mutants displayed a significant loss of viability in the presence of HU compared to the wild type (Figure 7). However, the viability of the *sntA1* and *sntB1* mutants was substantially improved at even the highest concentrations of HU when incubated on MNV-OAc or MNV-EtOH (Figure 7). In particular, *sntA1* mutants were almost fully viable in the presence of HU when incubated on MNV-OAc. These observations suggest that the *sntA* and *sntB* gene products are partially dispensable for the checkpoint that responds to low concentrations of HU when hyphal cells are incubated on media containing acetate or ethanol as the sole carbon source. This effect appears to be specific to the carbon source, since amino acid starvation induced by 3AT (0.5 mM) did not increase the viability of the *sntA1* and *sntB1* mutants in the presence of HU (P. R. KRAUS and S. D. HARRIS, unpublished results).

While demonstrating that the *ankA* gene could complement all phenotypes caused by the *sntA1* mutation, we were surprised to observe that *sntA1* mutants containing the AMA1-*ankA* plasmid were extremely hypersensitive to HU when incubated on MNV-EtOH (compare solid squares in Figure 7, B and D). In contrast, these transformants displayed similar viability to wild-type cells when incubated on MNV (Figure 7D). These results are consistent with a model that suggests that ANKA levels are reduced under conditions where nutrients are scarce (see below).

DISCUSSION

Previous studies have shown that germinating *A. nidulans* conidiospores are not competent to undergo septa-

TABLE 3

Growth conditions affect the timing of septum formation

Strain		MNV-glycerol	YGV
A28	Length (μm)	29.5 ± 8.5	60.5 ± 16.9
	No. nuclei (mode)	8	16
APK35	Length (μm)	24.8 ± 10.3	25.0 ± 9.8
	No. nuclei (mode)	8	4
APK56	Length (μm)	23.5 ± 8.3	22.4 ± 7.3
	No. nuclei (mode)	8	8
FRY20	Length (μm)	26.5 ± 10.3	24.1 ± 8.4
	No. nuclei (mode)	8	8

Conidia from the indicated strains were inoculated on coverslips and incubated at 28° for 8 hr in YGV. The modal average of the nuclear number for all strains after the initial incubation was 4 (≥90%). After 8 hr, the coverslips were washed and shifted to either YGV or MNV-glycerol. After the shift, coverslips were processed every hour for 6 hr. Coverslips were stained with Calcofluor White and Hoechst 33258 to visualize septa and nuclei, respectively. Samples for further analysis were chosen based on the SI, which was 35–40 for all strains tested. For each strain, the length of 25 cells possessing one septum was measured and the nuclei were counted. The modal average of the nuclear number was determined for all strains, and the values represent 55–95% of the sample size. Data were subjected to the Mann-Whitney test and only the populations of A28 cells shifted to YGV and MNV-glycerol were significantly different ($\alpha = 0.001$). Lengths given represent the average and the standard error of the mean.

tion until they attain a specific cell size and complete at least one round of mitosis (Harris *et al.* 1994; Wolkow *et al.* 1996). Further study suggested that the timing of septum formation is controlled by the activity of the cyclin-dependent kinase NIMX^{cdk1} (Harris and Kraus 1998). To gain further insight into the molecular mechanisms underlying the regulation of septum formation, we performed a genetic screen for mutants in which septation was deregulated. Here, we report that (i) the *sntA1*, *sntB1*, and *sntC1* mutants display defects in the regulation of septum formation and in cell cycle checkpoint responses; (ii) the *sntA1* and *sntB1* mutants affect the Tyr-15 phosphorylation state of NIMX^{cdk1}; and (iii) the timing of septum formation and the defects caused by the *sntA1* and *sntB1* mutations can be modulated by nutrient availability.

The *sntA* and *sntB* genes are required for the regulation of septum formation and for cell cycle checkpoint responses: Previous genetic observations suggested that, in *A. nidulans*, predivisional hyphae are not able to form septa until they accumulate a threshold level of interphase NIMX^{cdk1} activity (Harris and Kraus 1998). These observations were consistent with a model in which the Tyr-15 phosphorylation state of NIMX^{cdk1} was maintained to prevent kinase activation until the appropriate cell size had been attained. Growth signals could conceivably cause this effect by downregulation of NIMT (the Tyr-15 phosphatase) function, upregulation of ANKA (the Tyr-15 kinase) function, or both. To investigate these possibilities and identify additional components of the regulatory pathway that controls the timing of septum formation, we screened for suppressors of the Ts *nimT23* mutation. Since the same pathway may also modulate cell cycle checkpoint responses (Ye *et al.* 1996, 1997; Harris and Kraus 1998; De Souza *et al.* 1999; Hofmann and Harris 2000), we characterized the subset of suppressors that were HU hypersensitive. The suppressor mutations satisfying our criteria identified three genes: *sntA*, *sntB*, and *sntC*. It is likely that additional gene products that function in this pathway, particularly those that specifically regulate septation, could be identified by analyzing the suppressors that retain wild-type HU resistance (*i.e.*, do not display checkpoint defects).

As predicted, the *sntA1*, *sntB1*, and *sntC1* mutants display defects in the regulation of septum formation. Similar to the *nimX*^{cdc2AF} mutant (Harris and Kraus 1998), predivisional hyphae possessing the *sntA1*, *sntB1*, and *sntC1* mutations undergo septation at a smaller size and with fewer nuclei than wild type. Furthermore, they retain the ability to form septa despite the presence of DNA damage. An additional feature shared by the *nimX*^{cdc2AF} mutant and the *sntA1* and *sntB1* mutants is abrogation of the G_2/M DNA damage checkpoint (Ye *et al.* 1997). Although other checkpoint-defective mutants can form septa in the presence of DNA damage (Harris and Kraus 1998; De Souza *et al.* 1999), they must still satisfy the cell size requirement. These observations suggest that the *sntA* and *sntB* gene products control the timing of septum formation by integrating multiple sensory inputs to regulate NIMX^{cdk1} activity (Figure 8).

It should be noted that the checkpoint defects displayed by the *sntA1* and *sntB1* mutants are not identical. Whereas the G_2/M DNA damage checkpoint and the slowing of the S-phase checkpoint are dysfunctional in *sntA1* and *sntB1* mutants (*i.e.*, the replication checkpoint defect is presumably the cause of the HU hypersensitivity), only *sntA1* mutants are defective in the checkpoint that responds to loss of SEPB function. Although the basis of the latter checkpoint is not clear, these results suggest that the *sntA* gene product performs a global function in modulating checkpoint responses. In contrast, the *sntB* gene product may be required only for checkpoints that regulate mitotic entry. Alternatively, the *sntB1* mutation may indirectly affect these checkpoint responses by perturbing a growth signaling pathway, as has been observed in *S. pombe* (Humphrey and Enoch 1998).

The *sntA* and *sntB* gene products control the Tyr-15 phosphorylation state of NIMX^{cdk1}: Two observations reported here suggest that the *sntA* and *sntB* gene products regulate septum formation and cell cycle checkpoint responses by influencing the Tyr-15 phosphorylation state of NIMX^{cdk1}. First, tyrosine phosphorylation of NIMX^{cdk1} could not be detected in the *sntA1* and *sntB1* mutants grown under normal conditions or after exposure to a DNA-damaging agent. Second, molecular characterization of *sntA* demonstrates that it encodes

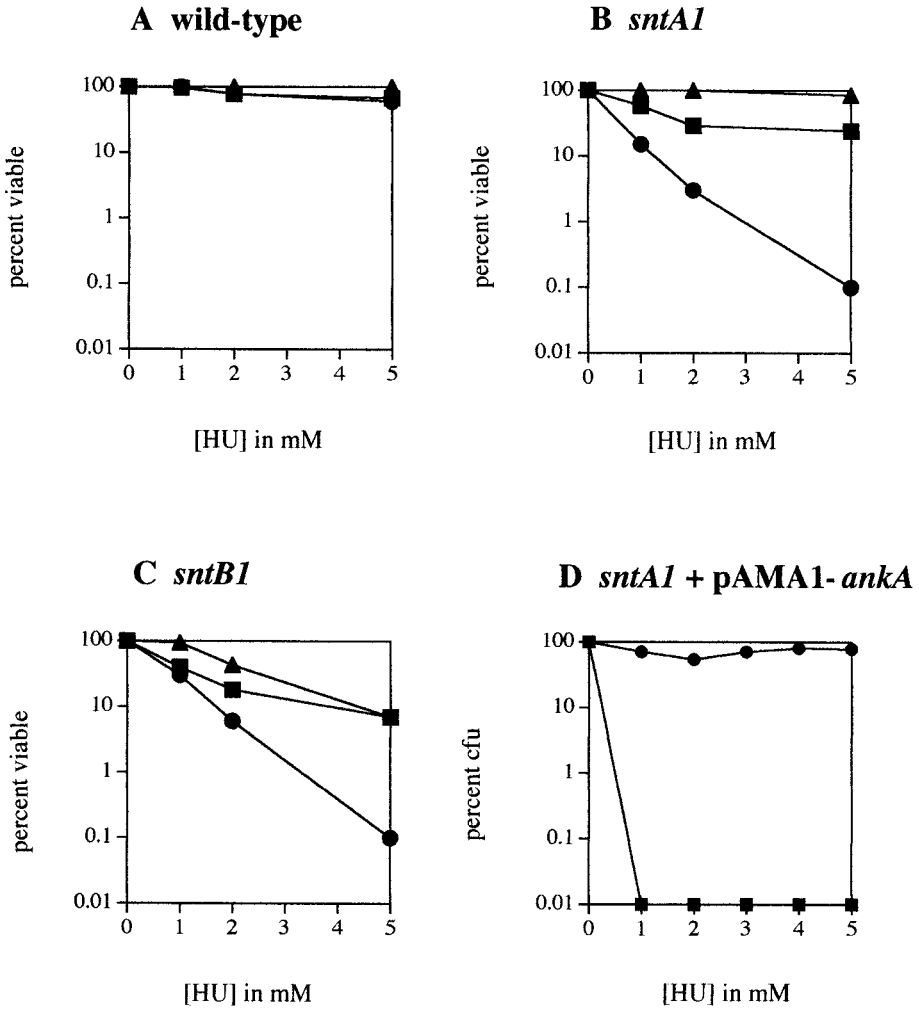

FIGURE 7.—Growth conditions affect the HU hypersensitivity of *sntA1* and *sntB1* mutants. Conidia from strains with the indicated genotype were diluted and plated at 100 conidia per plate on MNV (circles), MNV-OAc (triangles), and MNV-EtOH (squares) containing the indicated concentration of HU. The number of survivors on each plate was determined after 3 days incubation at 32°. "Percent viability" represents the percentage of colonies remaining on the treated plates as compared to the untreated control plates. Each data point represents the average of four plates. Experiments were repeated twice, and representative data are shown. Note that the *y*-axis for D is labeled "percent cfu," where cfu represents colony forming units. Viable colonies were observed (D) after an overlay of MNV agar lacking HU was applied and incubation continued for an additional 3 days. Strains used were the following: (A) A28, (B) APK35, (C) APK56, and (D) APK107.

ANKA, the *A. nidulans* ortholog of the *S. pombe* Tyr-15 kinase Wee1p (YE *et al.* 1996). Consistent with the phenotypes caused by the *sntA1* mutation, ANKA has been previously shown to be required for the slowing of S phase and DNA damage checkpoints in *A. nidulans* (YE *et al.* 1996, 1997) and has also been implicated in the regulation of septation (DE SOUZA *et al.* 1999). The recovery of a mutation in ANKA as a suppressor of *nimT23* was not surprising, since *wee1* mutations have been shown to suppress the growth defects of Tyr-15 phosphatase mutants in the yeasts *S. pombe* and *S. cerevisiae* (FANTES 1981; BOOHER *et al.* 1993). Presumably, the loss of Wee1p kinase activity restores cdk function in *nimT23* mutants by lowering the overall level of Tyr-15 phosphorylation.

The ability of multiple copies of ANKA to suppress the *sntB1* mutation implies that the *sntB* gene product also affects Tyr-15 phosphorylation of $NIMX^{cdk1}$. One possibility is that *sntB* encodes an upstream activator of ANKA. For example, *sntB* could encode a component of a signal transduction pathway that integrates growth signals with ANKA activity. Several protein kinases that negatively regulate Wee1p activity have been characterized in both *S. pombe* and *S. cerevisiae* (WU and RUSSELL 1993; MA *et al.* 1996), and the *sntB* gene product could conceivably antagonize the function of these kinases. Alternatively, *sntB* could encode a protein kinase that functions in parallel with ANKA. For example, in *S. pombe*, Mik1p is a Tyr-15 kinase that appears to function redundantly with Wee1p (LEE *et al.* 1994). If *sntB* does encode a Mik1p ortholog, our observations suggest that its function must have diverged to some extent from that of ANKA. In particular, the *sntB1* mutation has a much greater effect on septation in a *sepB3* background than does the *sntA1* mutation. In contrast, *sntA1* mutants display a broader spectrum of cell cycle checkpoint defects than *sntB1* mutants. These observations could be explained by proposing that ANKA has a more significant role in inhibiting mitotic cdk complexes, whereas the primary function of *A. nidulans* Mik1p is to inhibit the cdk complex that controls septum formation.

The nature of the $NIMX^{cdk1}$ complex that regulates septum formation in *A. nidulans* remains unknown. NIME is a B-type cyclin that associates with $NIMX^{cdk1}$ to control

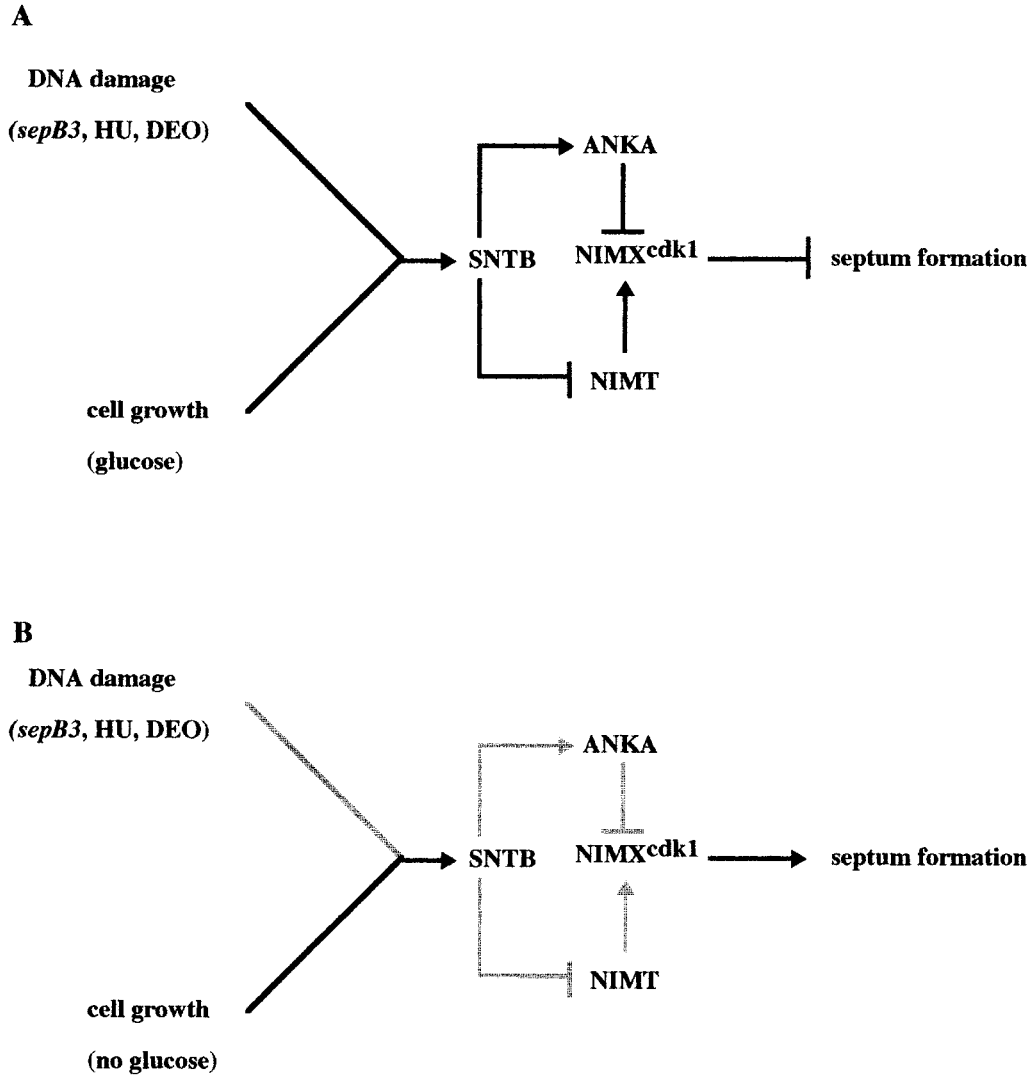

FIGURE 8.—A branched model for the regulation of septum formation. (A) Both DNA damage and glucose influence the Tyr-15 phosphorylation state of NIMX^{cdk1} through the action of SNTB, which we propose modulates ANKA and NIMT (solid lines). As a result, NIMX^{cdk1} activity is reduced and septum formation is delayed. (B) In the absence of glucose, the function of SNTB may be reduced (shaded lines). As a result, NIMX^{cdk1} activity increases and septum formation occurs earlier with respect to cell size. Under these conditions, the ability of DNA damage to affect NIMX^{cdk1} activity is curtailed.

mitotic entry in *A. nidulans* (O'CONNELL *et al.* 1992). Although the timing of septum formation is affected by the *nimE6* mutation (HARRIS and KRAUS 1998), we cannot rule out the existence of an additional septation-specific cyclin. If such a cyclin does exist in *A. nidulans*, it must be present at sufficient levels to promote septation well before predivisional hyphal cells have attained the cell size threshold. Otherwise, the *sntA1*, *sntB1*, and *nimX*^{cdc2AF} mutants would not be able to septate prematurely with respect to wild-type cells. We propose that complexes between the septation cyclin and NIMX^{cdk1} may be maintained in an inactive state by Tyr-15 phosphorylation. Alternatively, if there is no septation-specific cyclin, the timing of septum formation may be influenced by the localization of NIMX^{cdk1} (*i.e.*, cytoplasmic *vs.* nuclear). In this case, the localization of the *snt* gene products may permit the mitotic cdk complex to be active while the septation complex is inhibited.

The regulation of septum formation is modulated by growth conditions: The observation that wild-type predivisional hyphae cannot septate until they attain a specific cell size suggested that the timing of septum formation is coordinated with cellular growth (WOLKOW *et al.* 1996). We confirmed this notion by showing that predivisional hyphae shifted from rich glucose media to a poorer carbon source undergo septation at a significantly smaller size compared to hyphae that remain in glucose. We propose that septation is delayed in hyphae growing on rich glucose media so that individual hyphal cells can establish the appropriate volume of cytoplasm per nucleus. Furthermore, since the *sntA1* and *sntB1* mutants fail to delay septation on rich glucose media, we suggest that the *sntA* and *sntB* gene products play an active role in restraining septum formation (Figure 8). For example, glucose may activate a signal transduction pathway that inhibits activation of the relevant cdk complex by triggering the accumulation of ANKA (and/or a decrease in NIMT). In contrast, on poorer carbon sources, the relevant cdk complex may be activated by reducing the levels of ANKA (and/or the *sntB*

gene product), perhaps by proteolysis (Sia *et al.* 1998). This would allow septation to occur at a smaller cell size compared to cells grown on glucose (Figure 8).

The relationship between cell cycle progression and cellular growth: The ability of poorer carbon sources (*i.e.*, ethanol or acetate) to rescue the HU hypersensitivity of *sntA1* and *sntB1* mutants was an unexpected observation. Moreover, suppression of *S. pombe wee1* mutant defects by poor growth media has not been previously reported. Although minimal glucose medium has been shown to extend the G_1 phase of the *A. nidulans* cell cycle (Bergen and Morris 1983), we propose that poorer carbon sources may also delay S-phase or G_2 progression. Furthermore, we suggest that this delay is enacted by a mechanism that is independent of Tyr-15 phosphorylation of $NIMX^{cdk1}$ (*i.e.*, accumulation of a cdk inhibitor). Accordingly, when exposed to HU in the presence of a poorer carbon source, activation of this mechanism may delay S-phase or G_2 progression to a sufficient extent to allow the *sntA1* and *sntB1* mutants to respond to the lethal effects of HU. In contrast, when *sntA1* mutants containing a multicopy *ankA* plasmid are incubated under these conditions, activation of this mechanism combined with excess ANKA may prevent colony formation by blocking mitotic entry. This idea is supported by the observation that 50% of *sntA1* cells containing the multicopy *ankA* plasmid fail to enter mitosis in the presence of 5 mm HU, compared to <10% of wild-type cells (P. R. Kraus and S. D. Harris, unpublished observations).

The modulation of HU-hypersensitive phenotypes by growth conditions has been previously observed in *S. pombe* (Humphrey and Enoch 1998). In this case, the normal response to HU was restored to a checkpoint-defective mutant by inactivation of a stress-activated mitogen-activated protein kinase pathway. Our observations lend further support to the notion that regulatory mechanisms that normally act to maintain cell cycle fidelity, such as checkpoint responses, may become dispensable under adverse environmental conditions. This may have the beneficial effect of allowing the accumulation of potentially advantageous mutations.

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