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Posttranscriptional Control Mediates Cell Type-Specific Localization of Catalase A during *Aspergillus nidulans* Development

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Two differentially regulated catalase genes have been identified in the fungus *Aspergillus nidulans*. The *catA* gene belongs to a class whose transcripts are specifically induced during asexual sporulation (conidiation) and encodes a catalase accumulated in conidia. Using a developmental mutant affected in the *brlA* gene, which is unable to form conidia but capable of producing sexual spores (ascospores), we demonstrated that the *catA* mRNA accumulated during induction of conidiation but did not produce CatA protein. In contrast, high levels of catalase A activity were detected in the ascospores produced by this mutant, indicating that the *catA* gene is posttranscriptionally regulated. The same type of regulation was observed for a *catA*::*lacZ* translational gene fusion, suggesting that the *catA* message 5′ untranslated region could be involved in translational control during development. In a wild-type strain, β-galactosidase activity driven from the *catA*::*lacZ* gene fusion was low in hyphae and increased 50-fold during conidiation and 620-fold in isolated conidia. Consistent with this finding spatial expression of the reporter gene was restricted to metulae, phialides, and conidia. Conidium-associated expression was maintained in a *stuA* mutant, in which the conidiophore cell pattern is severely deranged. *catA* mRNA accumulation was also observed when vegetative mycelia was subject to oxidative, osmotic, and nitrogen or carbon starvation stress. Nevertheless, catalase A activity was restricted to the conidia produced under nutrient starvation. Our results provide support for a model in which translation of the *catA* message, accumulated during conidiation or in response to different types of stress, is linked to the morphogenetic processes involved in asexual and sexual spore formation. Our findings also indicate that *brlA*-independent mechanisms regulate the expression of genes encoding spore-specific products.

The asexual sporulation (conidiation) pathway of the fungus *Aspergillus nidulans* represents an excellent model system for studying the mechanisms controlling development and pattern formation in multicellular eukaryotes. The formation of the asexual reproductive apparatus is initiated when nondifferentiated hyphae are exposed to air or starved for nutrients in liquid culture (11, 30, 34). The asexual spores (conidia) are produced by the conidiophore, a multicellular structure composed of a basal foot cell, an aerial stalk terminating in a multinucleate vesicle, a layer of uninucleate cells called metulae, and a layer of uninucleate, sporogenous cells or phialides (28). This developmental pathway is dependent on the *brlA* regulatory gene (1, 11), which is necessary for expression of most of the conidiation-specific genes that have been identified (35).

Although conidiophore differentiation involves the activation of several hundred genes (21, 34, 35), the functions of only a few have been elucidated. The *yA* gene, encoding a conidial laccase (5, 12), the *wA* gene, encoding a polyketide synthase necessary for conidium pigmentation (22), the *rodA* and *dewA* genes, which encode conidial cell wall-associated hydrophobic proteins (32, 33), and the *catA* gene, encoding the conidium-associated catalase A (27), are examples of known functions related to conidial attributes. In contrast to the *yA*, *wA*, *rodA*, and *dewA* genes, *catA* mRNA accumulation is not dependent on the *brlA* gene (27).

Two divergent and differentially regulated catalase genes have been found in *A. nidulans* (19, 27). Conidia from *catA* null mutants are H_2O_2 sensitive (27), whereas *catB* null mutants, unable to produce the vegetative catalase B, are H_2O_2 sensitive at the hyphal stage (18). More recently, a catalase C has been detected in *catA catB* double mutants (18a). The mechanisms that mediate the differential regulation of these catalases during development and oxidative stress are not known.

In this work, we studied the mechanisms responsible for the cell-type-specific localization of catalase A. We present evidence indicating that the *catA* message accumulates in a translationally inactive form under a variety of stress conditions and that *catA* translation is linked to morphogenetic processes involved in formation of metulae, phialides, and asexual or sexual spores. We found that regulatory sequences present in the *catA* message 5′ untranslated region (5′UTR) and first four codons are sufficient to confer *catA*-like regulation to the reporter gene *lacZ* under different conditions.

The operation of *brlA*-independent mechanisms regulating the expression of a gene encoding a spore-specific product such as catalase A suggests that this could represent a general mechanism during development in *A. nidulans* and other fungi.

MATERIALS AND METHODS

Strains and growth manipulations. The genotypes of *A. nidulans* strains used in this study are listed in Table 1. Strains CRN1 and CRN8 are sexual progeny from TRN3 × CRN10, CRN10 is from cross AJC9.47 (A. J. Clutterbuck), CRN2 is from TRN3 × UI-7, and CRN6 is from TRN1 × CRN10. All strains were grown in supplemented minimal-nitrate or minimal-ammonium (20 mM ammonium tartrate) medium (18). Developmental cultures were conducted as described previously (27). For *brlA* mutants, mycelia were scraped from 5-day-old colonies in petri dishes, fragmented, and used to inoculate liquid cultures (3). Standard genetic (29) and transformation (36) techniques were used.

Plasmids. A ~6-kb *Eco*RI *catA*-containing fragment from cosmid SW22C01 (27) was cloned into Bluescript KS− (Stratagene, La Jolla, Calif.) to generate pREN3. pREN3 was digested with *Spe*I and religated to remove most of the *catA*

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TABLE 1. *A. nidulans* strains used in this work

Strain	Genotype	Reference or source
AJC7.1	*biA1; brlA1 veA1*	8
CRN1	*catA (~1400 p/l)::lacZ (argB$^+$/argB::CAT); metG1; niiA4 brlA17 veA1*	This work
CRN10	*biA1; argB2; pyroA4; niiA4 brlA17 veA1*	A. J. Clutterbuck
CRN2	*stuA1 pabaA1 catA (~1400 p/l)::lacZ (argB$^+$/argB::CAT); trpC801 veA1*	This work
CRN6	*pabaA1 ΔargB::trpCΔB catA::argB trpC801 niiA4 brlA17 veA1*	This work
CRN8	*pabaA1; catA (~1400 p/l)::lacZ (argB$^+$/argB::CAT); niiA4 brlA17 veA1*	This work
FGSC-26	*biA1; veA1*	Fungal Genetic Stock Center
PW1	*biA1; argB2; metG1; veA1*	P. Weglenski
RMS011	*pabaA1 yA2; ΔargB::trpCΔB; veA1 trpC801*	32
TLK12	*pabaA1 yA2; ΔargB::trpCΔB; ΔcatB trpC801 veA1*	18
TRN1	*pabaA1 yA2; ΔargB::trpCΔB catA::argB; trpC801 veA1*	This work
TRN3	*biA1; catA (~1400 p/l)::lacZ (argB$^+$/argB::CAT); metG1; veA1*	This work
UI-7	*stuA1 yA2 pabaA1; trpC801 veA1*	B. Miller

coding region and generate plasmid pREN7. A ~1.4-kb *Kpn*I-*Not*I fragment containing the putative *catA* upstream regulatory sequences was obtained from pREN7 and used to replace the *yA* promoter in plasmid pRA42 (6), to generate pREN8. This results in a gene fusion consisting of *catA* upstream regulatory sequences, the first four codons of *catA*, and two extra codons (Ser and Arg) derived from Bluescript sequences, fused to the *lacZ* region contained in pRA42 (see Fig. 2). Plasmid pREN5, used to transform strain RMS011 (32) to generate the *catA*-disrupted strain TRN1, was made by cloning the ~600-bp *Bam*HI *catA* fragment from pOS1A (27) into plasmid pDC1 (4).

Nucleic acid isolation, manipulation, and hybridization analysis. Total RNA was isolated by using TRIZOL (GIBCO BRL), fractionated in formaldehyde-agarose gels, transferred to Hybond-N nylon membranes (Amersham), and hybridized as suggested by the manufacturer. Radioactive probes were ^{32}P labeled by using random primers (GIBCO BRL). Probes were the 1.5-kb *Pst*I fragment from pCAN5 (27) for *catA*, the 2-kb *Kpn*I-*Bam*HI fragment from pSF5 (14) for actin, the 3-kb *Pst*I-*Eco*RI fragment from pREN8 for *lacZ*, and the 1.7-kb *Eco*RI fragment from pDC1 for *argB*. Southern blot analysis was used to select transformants carrying a single copy of pREN8 integrated at the *argB* locus. Total DNA was isolated as described by Timberlake (34). Transcription initiation sites for *catA* and *catA*::*lacZ* genes were determined by primer extension reactions using oligonucleotide prenu4707 (5′ tgcggccgccatgaggatcgatcaga 3′).

Enzyme activity determination. Catalase activity was determined in native polyacrylamide gels (20 to 40 μg of protein) as described previously (27). β-Galactosidase activity in protein extracts and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) staining were determined as reported elsewhere (3). Total protein was determined by the method of Bradford (9) or Smith et al. (31) for diluted samples.

Submerged sporulation. For starvation experiments, 50-ml cultures in 250-ml flasks were grown for 18 h, filtered through Miracloth, washed once with minimal-glucose nitrate-free medium, resuspended in 50 ml of either glucose-free or nitrogen-free medium (250 ml flask), and incubated further as reported elsewhere (30).

RESULTS

***catA* mRNA translation is linked to spore formation.** We have reported that the *catA* mRNA accumulates during conidiation in *A. nidulans* developmental mutants affected in the *brlA* gene (27). Except for the stalk, *brlA* null mutants fail to produce all conidiophore cell types. However, they are able to undergo sexual development and produce meiotic spores called ascospores (11). Results in Fig. 1A show that the *catA* message was virtually undetectable during growth (0 h of conidiation) in either wild-type or *brlA1* null mutant strains, whereas high levels of *catA* mRNA were apparent in both strains after 25 h of conidiation. When protein extracts from these samples were used to determine catalase activity in native gels, CatA activity was detected in 25-h samples from the wild-type strain but not from the *brlA1* mutant (Fig. 1B). In contrast, ascospores formed by a *brlA* null mutant, which requires several days of incubation, contained high levels of a catalase activity that comigrated with CatA. This activity was absent in ascospores from a *brlA17 catA* double mutant, thus confirming that CatA accumulates to high levels in sexual spores (Fig. 1B) just as it does in asexual spores (27). An antibody that recognizes CatA failed to detect the CatA antigen in a Western blot analysis using 25-h protein samples from the *brlA1* mutant (not shown), arguing against the presence of an inactive form of CatA in those samples.

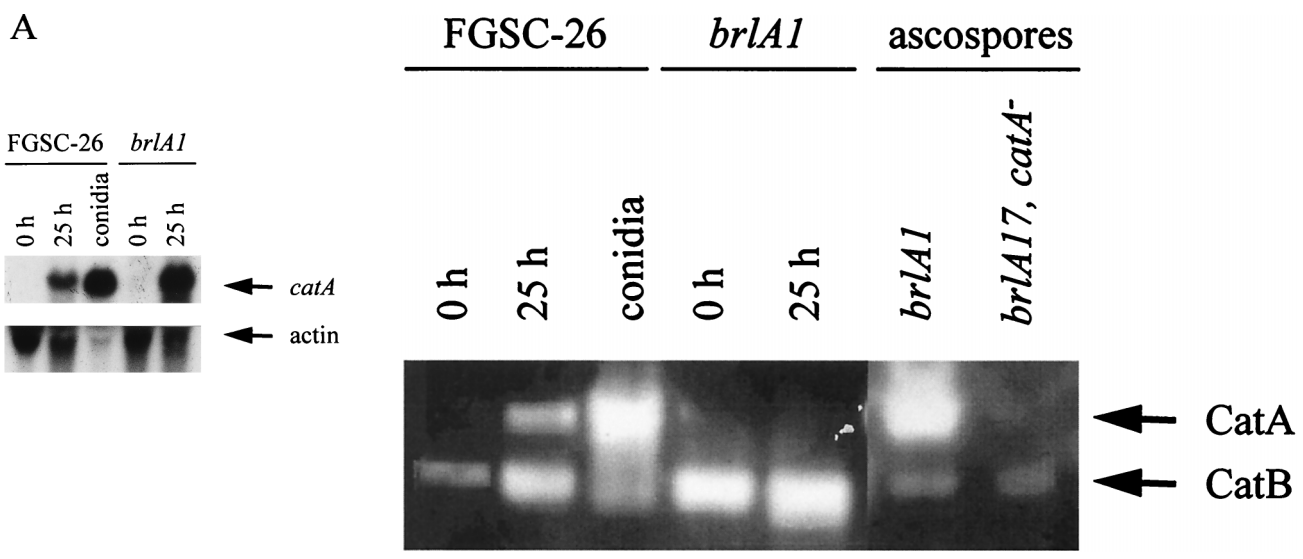

FIG. 1. *catA* mRNA accumulation and catalase activity during growth and conidiation in wild-type and *brlA1* mutant strains. (A) Total RNA extracted from growing mycelia (0 h of conidiation), mycelia induced to conidiate for 25 h, or isolated conidia were fractionated in formaldehyde-agarose gels, transferred to a nylon membrane, and hybridized to the *catA Pst*I fragment from pCAN5 and an actin-specific probe from pSF5. (B) Cell-free soluble protein extracts, prepared from samples at 0 and 25 h of conidiation, isolated conidia (20 μg), or ascospores (40 μg), were separated in a native polyacrylamide gel and stained for catalase activity (27). Samples corresponding to strains FGSC-26 (wild type), AJC7.1 (*brlA1*), and CRN6 (*brlA17 catA*) are indicated. Catalase A and B positions are shown by arrows.

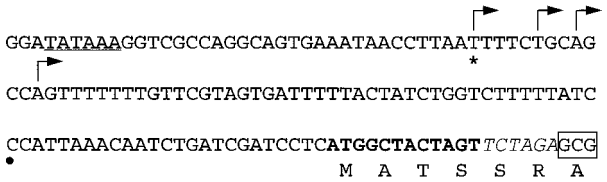

FIG. 2. *catA* regulatory and coding sequences present in the *catA*::*lacZ* gene fusion included in pREN8. Arrows indicate transcription initiation sites determined by primer extension previously (27) or in this work (*). A black dot indicates the 5′ end of *catA* cDNA clone C1g07a1.r2 reported in the *A. nidulans* expressed sequence tag database (29a). *catA* putative coding sequences are shown in boldface; a putative TATA box is underlined (27). The first codon from *E. coli lacZ* containing plasmid pRA42 (6) is boxed.

These results suggested that the *catA* mRNA detected in a *brlA* mutant induced to conidiate is not translated unless spores are formed, in this case by the alternative sexual developmental pathway.

A *catA*::*lacZ* fusion containing *catA* message 5′ UTR is regulated during sporulation and highly expressed in spores. To further understand the regulation of the *catA* gene, we constructed plasmid pREN8, containing *catA* upstream sequences extending from the proposed fourth codon to ca. bp 1400 fused to the *Escherichia coli lacZ* gene (Fig. 2). pREN8 also contained the *argB*::*CAT* fusion to direct integration of this construct to the *argB* locus (17). Eighteen Arg^+ transformants obtained after transforming strain PW1 with plasmid pREN8 were subjected to Southern blot analysis. Strain TRN3, which contains a single copy of pREN8 integrated at *argB*, was chosen for further analysis. Results in Fig. 3A show low levels of β-galactosidase specific activity (7 to 14 U) in samples from 0 to 12 h of development. In contrast, high levels of activity were detected by 25 (733 U) and 49 (670 U) h of conidiation, and much higher levels were found in isolated conidia (9,430 U). This pattern of β-galactosidase activity matched that reported for catalase A activity during conidiation (27). Control strain PW1 contained virtually undetectable levels of β-galactosidase activity during growth and conidiation.

To evaluate if the *catA*::*lacZ* fusion was regulated as the bona fide *catA* gene in a *brlA* null mutant background, the reporter gene was introduced by genetic crosses into a *brlA17* background, to generate strain CRN1. As shown in Fig. 3A, 34 U of β-galactosidase activity was detected before induction of conidiation. Virtually no increase in enzyme activity was observed after 25 h of conidiation, and only a minor increase was detected after 49 h (Fig. 3A), whereas the *catA*::*lacZ* message was accumulated in samples from both CRN1 and the wild-type strain TRN3 after 25 (not shown) and 49 (Fig. 3B) h. Ascospores formed by the *brlA17* null mutant strain CRN8 contained high levels of β-galactosidase activity (884 U), as opposed to ascospores formed by a *brlA17* mutant lacking the *catA*::*lacZ* fusion, which showed undetectable levels of enzyme activity.

The transcription initiation sites of the *catA*::*lacZ* reporter gene were determined by primer extension using RNA from wild-type and *brlA17* mutant strains, and no differences were detected between them. An initiation site in addition to those previously reported (27) was detected in both *catA* and *catA*::*lacZ* (Fig. 2). Therefore, the *catA*::*lacZ* fusion used here contains the sequences necessary for proper regulation during development. The *catA* message 5′ UTR is likely responsible for coupling translation to either asexual or sexual spore formation.

Spatial expression of the *catA*::*lacZ* fusion in wild-type and *stuA* mutant strains. The spatial expression of the *catA*::*lacZ* reporter was determined by in situ β-galactosidase activity detection using the chromogenic substrate X-Gal. Results in Fig. 4 show that conidiophore cell types corresponding to metulae, phialides, and spores were all stained in strain TRN3 (Fig. 4A), whereas no staining was detected in control strain PW1 (Fig. 4B). This staining pattern is consistent with the time course results shown in Fig. 3, since the increase in β-galactosidase activity by 25 h corresponded with the presence of fully developed conidiophores, containing metulae, phialides, and conidiospores.

The *stuA* gene encodes a transcriptional repressor (13) necessary for the proper spatial expression of the *brlA* regulatory gene (3) and for normal conidiophore pattern formation and ascosporogenesis (11, 25). We examined the spatial expression of the *catA*::*lacZ* reporter in a *stuA* mutant background. *stuA* null mutants produce short conidiophores often lacking metulae and phialides but are able to produce conidia (3, 11). Results in Fig. 4C show that β-galactosidase activity was mainly detected in conidia formed directly from vesicles or in some cases from abnormal phialides. The β-galactosidase specific activity in isolated conidia was 393 U, which corresponded to an 8.3-fold increase over the level in samples from 0 h of conidiation. These results indicate that at least part of the conidium-specific expression of the *catA*::*lacZ* gene fusion can occur in the absence of a functional *stuA* gene.

Different types of stress induce *catA* mRNA accumulation but not catalase A activity. Two catalase genes have been identified in *A. nidulans* (19, 27). The activity of the enzyme encoded by the *catB* gene is induced by oxidative and other types of stress (19). When the same kinds of stresses (Fig. 5) were applied for 3 h to the *catB*-deleted strain TLK12 grown for 12 h, no catalase A activity was detected (not shown), despite the fact that osmotic stress caused by NaCl or sorbitol, starva-

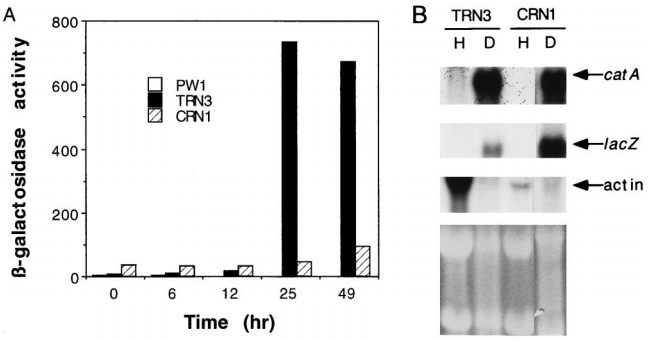

FIG. 3. Expression of *catA* and *catA*::*lacZ* reporter fusion in wild-type and *brlA* mutant strains during conidiation. (A) Plasmid pREN8, containing *catA* gene upstream sequences extending from the fourth proposed codon fused to the *E. coli lacZ* gene, was integrated at *argB* by transformation of the developmentally wild-type strain PW1. Transformant TRN3, containing a single copy of pREN8 integrated at *argB*, was crossed to *brlA17* mutant strain CRN10 to produce the *brlA17* mutant strain CRN1, containing the *catA*::*lacZ* fusion (Table 1). Both strains were grown in liquid medium for 18 h and induced to conidiate. Water-soluble protein extracts were prepared from samples harvested at the indicated times and assayed for β-galactosidase specific activity (24). The different times of development correspond to the following morphologies: 0 h of development (18 h of growth), undifferentiated hyphae; 6 h, conidiophore stalks; 12 h, conidiophores and first immature conidia; 25 h, mature conidiophores and conidia. β-Galactosidase activity in isolated conidia corresponded to 9,430 U. β-Galactosidase activities corresponding to strains TRN3 and CRN1 shown here and those indicated in the text are mean values from two independent experiments, with a maximum variation of 21% with respect to the mean. (B) Total RNA extracted from growing hyphae (H; 18 h of growth) or developmental cultures (D; 49 h of conidiation) was fractionated in formaldehyde-agarose gels, transferred to a nylon membrane, and hybridized to *catA*-, *lacZ*-, and actin-specific probes. The bottom part shows rRNA bands in the ethidium bromide-stained gel used to prepare the blot.

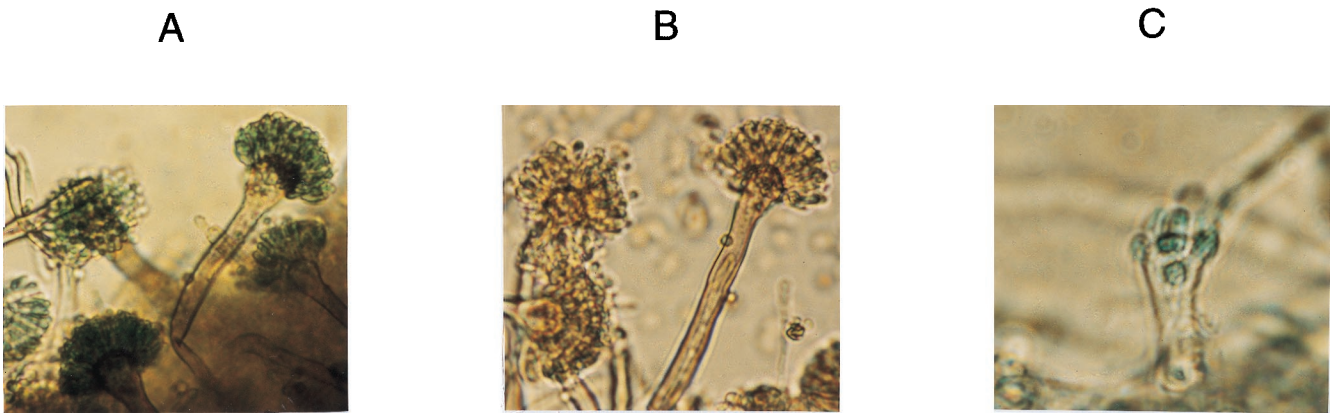

FIG. 4. Spatial expression of the *catA*::*lacZ* reporter gene in wild-type strains and a *stuA* developmental mutant during conidiation. Developmentally wild-type strains TRN3 (A) and PW1 (B) and *stuA* mutant strain CRN2 (C) were grown in petri dishes until colonies conidiated, stained with X-Gal, and examined microscopically as reported previously (2, 3). Blue-stained cell types in conidiophores shown in panel A correspond to metulae, phialides, and conidia. Blue-stained cell types in panel C correspond to conidia formed on top of the vesicle of a stunted conidiophore. Magnifications: ×240 (A and B) and ×480 (C).

tion for carbon or nitrogen, and to lesser extent H_2O_2 or paraquat treatment all induced *catA* mRNA accumulation (Fig. 5). These results show that in addition to air exposure (Fig. 1), other types of stress that do not result in spore production can lead to *catA* mRNA accumulation without catalase A activity production.

A. nidulans conidiates in liquid culture after 24 h of carbon and/or nitrogen starvation (30). Since a 3-h starvation for these nutrients resulted in *catA* message accumulation (Fig. 5, lanes 10 and 11), we used strain TRN3 to investigate if catalase A activity was detectable in mycelia starved for glucose during longer times or, if in this case, catalase A activity would also be restricted to conidia. Although the *catA* message was detected in mycelia starved for carbon during 24 h (Fig. 6A, lane 2), catalase A activity was confined to the conidia produced under those conditions (Fig. 6B, lane 3). In agreement with these results, we detected 45 U of β-galactosidase activity in mycelia starved for glucose during 24 h, compared with 468 U in isolated spores.

Taken together, our results provide support for a model in which translation of the *catA* message, which accumulates in response to induction of conidiation or during exposure to different types of stress, is linked to the morphogenetic processes involved in the formation of metulae, phialides, and asexual or sexual spores. In this model, translational regulation would be mediated by the 5′ UTR sequences present at the *catA* message.

DISCUSSION

The *A. nidulans* catalase A activity and corresponding mRNA are highly accumulated in conidiospores, where the enzyme provides protection against exogenous H_2O_2 (27). The results presented in this report show that the *catA* gene is subject to posttranscriptional controls and that translational regulation seems to play a major role in the cell-type-specific localization of catalase A.

brlA mutants blocked in asexual but not in sexual sporulation accumulated *catA* mRNA after exposure to air but failed to produce a CatA polypeptide. Nevertheless, ascospores formed by a *brlA* mutant contained high levels of catalase A activity, suggesting that translation of the *catA* message did not occur until either asexual or sexual spores started to be formed. This interpretation was further supported by the fact that temporal and spatial expression of a *catA*::*lacZ* translational fusion during development in both wild-type and *brlA* mutants paralleled catalase A activity. Because the transcription initiation sites of the *catA*::*lacZ* reporter corresponded to those of *catA*, elements present in the *catA* message 5′ UTR would be responsible for translational regulation during development. The *catA*::*lacZ* fusion used here contained the first four predicted CatA codons (Fig. 2). It remains to be determined if they play any specific role in translational control.

Conidial localization of β-galactosidase derived from *catA*::*lacZ* was maintained in a mutant affected in the *stuA* gene (Fig. 4C). However, enzyme activity in samples from 0 h of conidiation was higher in the *stuA* mutant than in a wild-type strain (47 and 9 U, respectively).

Exposure of nondifferentiated mycelia to different stress conditions, particularly to carbon or nitrogen starvation, also led to *catA* mRNA accumulation (Fig. 5). However, translational repression still restricted catalase A to the conidia produced under carbon starvation (Fig. 6). In these conditions, β-galactosidase activities derived from the *catA*::*lacZ* gene were ~10- and 20-fold higher in isolated conidia than in starved or nonstarved mycelia, respectively. Differences in β-galactosidase activity levels were observed in comparisons of conidia and ascospores or of conidia produced in air and those formed in liquid, which might result from differences in the sporulation

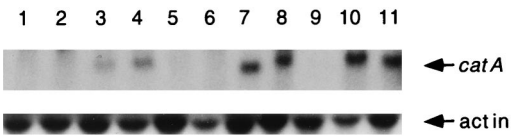

FIG. 5. *catA* mRNA accumulation during different stress conditions. Mycelia from strain TLK12, grown for 12 h in minimal medium at 37°C, were transferred to minimal medium and subjected to the following treatments: lanes 1 and 2, 2 and 3 h in minimal medium, respectively (controls); lane 3, 5 mM paraquat for 2 h; lane 4, 0.5 mM hydrogen peroxide for 2 h; lane 5, 0.8 mg of uric acid per ml as the sole nitrogen source for 2 h; lane 6, 42°C for 3 h; lane 7, 1 M sorbitol for 3 h; lane 8, 1 M sodium chloride for 3 h; lane 9, 4% ethanol as the sole carbon source for 3 h; lane 10, minimal medium lacking glucose for 3 h; lane 11, minimal medium lacking nitrate for 3 h. Total RNA from the indicated conditions was fractionated in formaldehyde-agarose gels, transferred to a nylon membrane, and hybridized to a *catA*-specific probe. The same membrane was hybridized to an actin probe as a loading control.

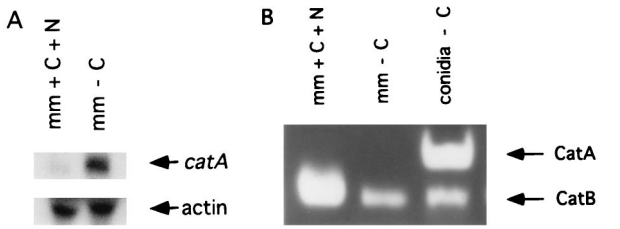

FIG. 6. *catA* mRNA and catalase activity accumulation during starvation-induced submerged sporulation. Strain TRN3 was grown for 18 h in glucose medium and shifted to standard medium (mm + C + N) or medium lacking glucose (mm − C). Samples taken at 24 h were filtered through Miracloth to retain mycelia. The resulting filtrate was passed and rinsed through 0.22-μm-pore-size Millipore membranes to collect the conidia produced during starvation. (A) Total RNA obtained from starved mycelia was subjected to Northern blot analysis using a *catA*- or actin-specific probe. (B) Corresponding protein extracts were fractionated in a native polyacrylamide gel and used to determine catalase activity (27).

process per se. Despite these differences, β-galactosidase activity was always severalfold higher in spores than in mycelia.

We propose that the cell-type-specific localization of catalase A is mediated by the 5′ UTR of the *catA* mRNA and occurs in a two-step process. First, the *catA* message would accumulate in a translationally inactive form, in response to different stressful conditions, including exposure to air. This can result from increased *catA* transcription and/or message stabilization. Preliminary results indicate that the stability of the *catA* message changes under different physiological conditions (26a). In a second step, accumulated *catA* mRNA would be targeted to the proper location (metulae, phialides, conidiospores, and ascospores), where it would be translated. Such a process could be related to cytoskeleton remodeling during the shift from polar to budding growth associated with conidiation. The moderate increase in β-galactosidase activity observed in a *brlA* null mutant (Fig. 3A) indicates that translational repression of the *catA*::*lacZ* mRNA was not complete. It remains to be resolved if the *catA* mRNA 3′ UTR sequences play a role in translational repression or other aspects of *catA* posttranscriptional control.

Notable examples of mRNA localization and translational regulation during development are represented by the *bicoid* and *nanos* mRNAs. The translation of both messages at their respective locations is crucial to embryonic polarity in *Drosophila*. For both mRNAs, *cis*-acting determinants have been confined to the 3′ UTR and microtubules have been implicated in their polarized distribution (reviewed in references 15 and 23). Asymmetrical distribution of mRNA and protein occurs during *Saccharomyces cerevisiae* mating-type switching, which requires the *HO* gene. *HO* transcription is prevented in daughter cells by the preferential accumulation of the unstable transcriptional repressor Ash1p and the *ASH1* mRNA. This process is dependent on actin, myosin, and a *cis*-acting element present at the 3′ UTR of the *ASH1* mRNA (20, 26).

Translational regulation of the ferritin mRNA is mediated by *cis*-acting sequences included at the 5′ UTR, which form a stable hairpin structure termed the iron-responsive element, where a protein binds to inhibit translation (7). Recently, Gu and Hecht (16) reported that a 65-kDa protein binds to the 5′ UTR of a testis-specific Cu/Zn superoxide dismutase mRNA and specifically inhibits its in vitro translation. Also, the redox-sensitive binding of a protein to the 3′ UTR of mouse and human catalase mRNAs has been reported (10). BLAST searches using the primary sequence of the 81- to 69-nucleotide pyrimidine-rich *catA* mRNA 5′ UTR (27) (Fig. 2) found neither clear similarities to known sequences nor short upstream open reading frames that could mediate translational regulation. On the other hand, secondary structure analysis using the computer programs FOLD and SQUIGGLES showed only a low stability stem-loop structure (minimum free energy of −6.4), whose significance remains to be studied.

Further research is required to understand the specific mechanisms by which the *catA* gene is posttranscriptionally regulated and to what extent it is regulated at the transcription level. *catA* provides the first example of a gene encoding a conidium-specific product whose mRNA accumulates independently from the *brlA* regulatory gene, but it could represent a general mechanism for other genes such as those corresponding to cDNA clones CAN65, CAN11, CAN77, and CAN32 (8).

A. nidulans also contains the *catB*-encoded catalase B. It is interesting that different types of stress result in the accumulation of both *catA* and *catB* messages but that only the *catB* mRNA is readily translated (17a, 18). It is not clear why catalase A is so tightly regulated, but its targeting to spores produced by two very different developmental pathways suggests a fundamental role in spore protection.

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REFERENCES

1. **Adams, T. H., M. T. Boylan, and W. E. Timberlake.** 1988. *brlA* is necessary and sufficient to direct conidiophore development in *Aspergillus nidulans*. Cell **54:**353–362.
2. **Aguirre, J., T. H. Adams, and W. E. Timberlake.** 1990. Spatial control of developmental regulatory genes in *Aspergillus nidulans*. Exp. Mycol. **14:**290–293.
3. **Aguirre, J.** 1993. Spatial and temporal controls of the *Aspergillus brlA* developmental regulatory gene. Mol. Microbiol. **8:**211–218.
4. **Aramayo, R., T. H. Adams, and W. E. Timberlake.** 1989. A large cluster of highly expressed genes is dispensable for growth and development in *Aspergillus nidulans*. Genetics **122:**65–71.
5. **Aramayo, R., and W. E. Timberlake.** 1990. Sequence and molecular structure of the *Aspergillus nidulans yA* (laccase I) gene. Nucleic Acids Res. **18:**3415.
6. **Aramayo, R., and W. E. Timberlake.** 1993. The *Aspergillus nidulans yA* gene is regulated by abaA. EMBO J. **12:**2039–2048.
7. **Basilion, J. P., T. A. Rouault, C. M. Massinople, R. D. Klausner, and W. H. Burgess.** 1994. The iron-responsive element-binding protein: localization of the RNA-binding site to the aconitase active-site cleft. Proc. Natl. Acad. Sci. USA **91:**574–578.
8. **Boylan, M. T., P. M. Mirabito, C. E. Willett, C. R. Zimmerman, and W. E. Timberlake.** 1987. Isolation and physical characterization of three essential conidiation genes from *Aspergillus nidulans*. Mol. Cell. Biol. **7:**3113–3118.
9. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
10. **Clerch, L. B.** 1995. A 3′ untranslated region of catalase mRNA composed of a stem-loop and dinucleotide repeat elements binds a 69-kDa redox-sensitive protein. Arch. Biochem. Biophys. **317:**267–274.
11. **Clutterbuck, A. J.** 1969. A mutational analysis of conidial development in *Aspergillus nidulans*. Genetics **63:**317–327.
12. **Clutterbuck, A. J.** 1972. Absence of laccase from yellow-spored mutants of *Aspergillus nidulans*. J. Gen. Microbiol. **70:**423–435.
13. **Dutton, J. R., S. Johns, and B. L. Miller.** 1997. StuAp is a sequence-specific transcription factor that regulates developmental complexity in *Aspergillus nidulans*. EMBO J. **16:**5710–5721.
14. **Fidel, S., J. H. Doonan, and N. R. Morris.** 1988. *Aspergillus nidulans* contains a single actin gene which has unique intron locations and encodes a γ-actin. Gene **70:**283–293.
15. **Gavis, E. R.** 1997. Expeditions to the pole: RNA localization in *Xenopus* and *Drosophila*. Trends Cell Biol. **7:**485–492.
16. **Gu, W., and N. R. Hecht.** 1996. Translation of a testis-specific Cu/Zn superoxide dismutase (SOD-1) mRNA is regulated by a 65-kilodalton protein which binds to its 5′ untranslated region. Mol. Cell. Biol. **16:**4535–4543.
17. **Hamer, J. E., and W. E. Timberlake.** 1987. Functional organization of the

Aspergillus nidulans trpC promoter. Mol. Cell. Biol. **7:**2352–2359.
18. **Käfer, E.** 1977. Meiotic and mitotic recombination in *Aspergillus* and its chromosomal aberrations. Adv. Genet. **19:**33–131.
18a. **Kawasaki, L., and J. Aguirre.** Unpublished data.
19. **Kawasaki, L., D. Wysong, R. Diamond, and J. Aguirre.** 1997. Two divergent catalase genes are differentially regulated during *Aspergillus nidulans* development and oxidative stress. J. Bacteriol. **179:**3284–3292.
20. **Long, R. M., R. H. Singer, X. Meng, I. Gonzalez, K. Nasmyth, and R. P. Jansen.** 1997. Mating type switching in yeast controlled by asymmetric localization of ASH1 mRNA. Science **277:**383–387.
21. **Martinelli, S. D., and A. J. Clutterbuck.** 1971. A quantitative survey of conidiation mutants in *Aspergillus nidulans*. J. Gen. Microbiol. **69:**261–268.
22. **Mayorga, M. E., and W. E. Timberlake.** 1992. The developmentally regulated *Aspergillus nidulans wA* gene encodes a polypeptide homologous to polyketide and fatty acid synthases. Mol. Gen. Genet. **235:**205–212.
23. **Micklem, D. R.** 1995. mRNA localisation during development. Dev. Biol. **172:**377–395.
24. **Miller, K. Y.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
25. **Miller, K. Y., J. Wu, and B. L. Miller.** 1992. StuA is required for cell pattern formation in Aspergillus. Genes Dev. **6:**1770–1782.
26. **Nasmyth, K. and R. Jansen.** 1997. The cytoskeleton in mRNA localization and cell differentiation. Curr. Opin. Cell Biol. **9:**396–400.
26a. **Navarro, R. E., and J. Aguirre.** Unpublished data.
27. **Navarro, R. E., M. A. Stringer, W. Hansberg, W. E. Timberlake, and J. Aguirre.** 1996. *catA*, a new *Aspergillus nidulans* gene encoding a developmentally regulated catalase. Curr. Genet. **29:**352–359.
28. **Oliver, P. T. P.** 1972. Conidiophore and spore development in *Aspergillus nidulans*. J. Gen. Microbiol. **73:**45–54.
29. **Pontecorvo, G., J. A. Roper, L. M. Hemmons, K. D. MacDonald, and A. W. J. Bufton.** 1953. The genetics of *Aspergillus nidulans*. Adv. Genet. **5:**141–283.
29a. **Roe, B. A., D. Kupfer, S. Clifton, and R. Prade.** *Aspergillus nidulans* cDNA sequencing project. http://www.genome.ou.edu/asper.html.
30. **Skromne, I., O. Sanchez, and J. Aguirre.** 1995. Starvation stress modulates the expression of the *Aspergillus nidulans brlA* regulatory gene. Microbiology **141:**21–28.
31. **Smith, P. K., R. I. Krohn, G. T. Hermanson, A. K. Mallia, F. H. Gartner, M. D. Provenzano, E. K. Fujimoto, N. M. Goeke, B. J. Olson, and D. C. Klenk.** 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. **150:**76–85.
32. **Stringer, M. A., R. A. Dean, T. C. Sewall, and W. E. Timberlake.** 1991. Rodletless, a new *Aspergillus* developmental mutant induced by directed gene inactivation. Genes Dev. **5:**1161–1171.
33. **Stringer, M. A., and W. E. Timberlake.** 1995. *dewA* encodes a fungal hydrophobin component of the Aspergillus spore wall. Mol. Microbiol. **16:**33–44.
34. **Timberlake, W. E.** 1980. Developmental gene regulation in *Aspergillus nidulans*. Dev. Biol. **78:**497–510.
35. **Timberlake, W. E., and A. J. Clutterbuck.** 1994. Genetic regulation of conidiation. Prog. Ind. Microbiol. **29:**383–427.
36. **Yelton, M. M., J. E. Hamer, and W. E. Timberlake.** 1984. Transformation of *Aspergillus nidulans* by using a *trpC* plasmid. Proc. Natl. Acad. Sci. USA **81:** 1470–1474.