JOURNAL OF BACTERIOLOGY, Feb. 2001, p. 1434–1440 Vol. 183, No. 4
0021-9193/01/$04.00+0 DOI: 10.1128/JB.183.4.1434–1440.2001
Copyright © 2001, American Society for Microbiology. All Rights Reserved.

Multiple Catalase Genes Are Differentially Regulated in *Aspergillus nidulans*

LAURA KAWASAKI AND JESÚS AGUIRRE*

Departamento de Genética Molecular, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, 04510 México, D. F., Mexico

Received 4 August 2000/Accepted 21 November 2000

Detoxification of hydrogen peroxide is a fundamental aspect of the cellular antioxidant responses in which catalases play a major role. Two differentially regulated catalase genes, *catA* and *catB*, have been studied in *Aspergillus nidulans*. Here we have characterized a third catalase gene, designated *catC*, which predicts a 475-amino-acid polypeptide containing a peroxisome-targeting signal. With a molecular mass of 54 kDa, CatC shows high similarity to other small-subunit monofunctional catalases and is most closely related to catalases from other fungi, *Archaea*, and animals. In contrast, the CatA (~84 kDa) and CatB (~79 kDa) enzymes belong to a family of large-subunit catalases, constituting a unique fungal and bacterial group. The *catC* gene displayed a relatively constant pattern of expression, not being induced by oxidative or other types of stress. Targeted disruption of *catC* eliminated a constitutive catalase activity not detected previously in zymogram gels. However, a catalase activity detected in *catA catB* mutant strains during late stationary phase was still present in *catC* and *catABC* null mutants, thus demonstrating the presence of a fourth catalase, here named catalase D (CatD). Neither *catC* nor *catABC* triple mutants showed any developmental defect, and both mutants grew as well as wild-type strains in H_2O_2-generating substrates, such as fatty acids, and/or purines as the sole carbon and nitrogen sources, respectively. CatD activity was induced during late stationary phase by glucose starvation, high temperature, and, to a lesser extent, H_2O_2 treatment. The existence of at least four differentially regulated catalases indicates a large and regulated capability for H_2O_2 detoxification in filamentous fungi.

Several studies indicate that reactive oxygen species play crucial roles in various aspects of cell physiology, such as cellular defense (45), life span (38), stress signaling (22), development (19), apoptosis (30), and pathology (33). The hydrogen peroxide formed during aerobic metabolism is capable of generating other reactive oxygen species, which can damage many cellular components (18). Catalases and peroxidases are the most important enzymatic systems used to degrade H_2O_2. There are three separate families of catalases: Mn-catalases, bifunctional catalase-peroxidases, and monofunctional, or "true," catalases. The last group is the one best characterized and corresponds to homotetrameric heme-containing enzymes present in eubacteria and eukaryotes and recently also found in the *Archaea* (34). Within this family of catalases, two clearly distinct classes can be recognized: the small-subunit (50- to 65-kDa) and the large-subunit (~80-kDa) enzymes. The first class includes a large number of catalases from bacteria, plants, fungi, and animals. An increasing number of catalases of the second class have been identified in bacteria and filamentous fungi (5, 8, 13, 15, 23–25, 27, 37) but not in higher eukaryotes.

The core sequence of the true catalases is composed of 360 to 390 amino acid residues (24, 48), while the large-subunit enzymes typically have ~70 and ~150 additional residues at the N and C termini, respectively. These terminal sequences seem to confer increased stability on the enzymes (6, 24).

Our studies focused on the antioxidant response in eukaryotes and its possible connections to cellular development (19), through the detailed analysis of catalase gene regulation in *Aspergillus nidulans*. Well-characterized sexual and asexual development processes in this filamentous fungus are amenable to genetic analysis (1, 40). In *A. nidulans* the catalase genes *catA* and *catB* have been characterized, both encoding large-subunit (~84- and ~79-kDa, respectively) true catalases (23, 27). The *catA* and *catB* genes are evolutionarily divergent, as judged from the relatively low similarity among the encoded polypeptides (40% identity) and the different exon structures (23). The *catA* mRNA accumulates during sporulation as well as in response to multiple types of stress, and its translation is connected to asexual and sexual spore formation, resulting in the high levels of catalase A activity in spores. This regulation is mediated by the *catA* 5′ untranslated mRNA region (26). In contrast, the *catB* gene is induced and translated in growing and developing hyphae and in response to oxidative and other types of stress. Both catalases provide protection against H_2O_2 at different stages of the *A. nidulans* life cycle, and CatA, and to a lesser extent CatB, protects germlings from heat shock (23, 27, 28). Here, we present the characterization of a third catalase gene, the *catC* gene, and present evidence for the existence of a fourth catalase (CatD) in *A. nidulans.* Unlike *catA* and *catB*, *catC* encodes a small-subunit catalase with a peroxisomal targeting sequence which is closely related to catalases from other fungi, animals, and *Archaea*. The *catC* gene is not essential for fatty acid and/or purine utilization, and its expression is constitutive, overlapping in time with the expression of the other catalase genes. On the other hand, the CatD activity was induced under a narrow set of conditions, such as the late stationary phase, glucose starvation, high temperature, and H_2O_2 treatment.

* Corresponding author. Mailing address: Departamento de Genética Molecular, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Apartado Postal 70-242, 04510 México, D. F., Mexico. Phone: (525) 622-5651. Fax: (525) 622-5630. E-mail: jaguirre@ifisiol.unam.mx.

TABLE 1. Strains used in this work[a]

Strain	Genotype	Reference or source[b]
FGSC26	*biA1 veA1*	FGSC
RMS011	*pabaA1 yA2 ΔargB::trpCΔB veA1 trpC801*	36
CLK20	*biA1 ΔcatA::argBΔA metG1 ΔcatB::argBΔB veA1*	Progeny from cross TRN1 × CLK15 (this work) (FGSC strain A1055 and ATCC MYA-116)
TLK61	*pabaA1 yA2 ΔcatC::argBΔC ΔargB::trpCΔB trpC801 veA1*	Obtained by transforming strain RMS011 with linear pLK20 (this work)
TLK12	*pabaA1 yA2 ΔargB::trpCΔB ΔcatB::argBΔB trpC801 veA1*	23 (FGSC strain A1054 and ATCC MYA-118)
CLK35	*pabaA1 yA2 biA1 ΔcatC::argBΔC ΔcatA::argBΔA ΔcatB::argBΔB veA1*	Progeny from cross CLK20 × TLK61 (this work)
CLK14	*biA1 ΔcatA::argBΔA metG1 ΔcatB::argBΔB veA1*	Progeny from cross CLK12 × TLK12 (this work)
CLK15	*biA1 metG1 ΔcatB::argBΔB veA1*	Progeny from cross CLK12 × TLK12 (this work)
RYC17	*ΔargB::trpCΔB ΔcatA::argB veA1*	Partial genotype 7
RYC16	*ΔargB::trpCΔB ΔcatA::argB ΔcatB::argB veA1*	Partial genotype 7
CLK36	*pabaA1 biA1 ΔcatC::argBΔC ΔcatA::argBΔA metG1 ΔcatB::argBΔB veA1*	Progeny from cross CLK20 × TLK61 (this work)

[a] To obtain triple *catA catB catC* mutants, strains CLK20 and TLK61 were crossed. Master plates containing progeny from this cross were screened for the lack of CatB, using 20 mM H_2O_2, as described previously (23). Of 94 strains, 37 lacked *catB* and used to extract genomic DNA. DNA samples were screened for *catC* disruption by PCR, using oligonucleotides catC10 (5′AAGATTGGGTCGAAGCGG3′) and argB1 (5′CATAAGTCCGCCAGCAGG3′). The lack of CatA and CatB activities was confirmed by Zymogram analysis.

[b] FGSC, Fungal Genetics Stock Center.

MATERIALS AND METHODS

Strains, media, transformation, and growth conditions. The *A. nidulans* strains used in this work are shown in Table 1. All strains were grown in supplemented minimal-nitrate or minimal-ammonium (20 mM ammonium tartrate) medium (21). When carbon sources other than glucose were used, the concentrations were 100 mM (50 mM in solid medium) sodium acetate, 0.5% Tween 80, 200 mM ethanol, 200 mM methanol, 1% glycerol, and 6 mM oleate in 1% Tergitol NP-10. Nitrogen sources other than nitrate or ammonium were 2.2 mM adenine or 0.8 mg of uric acid/ml. Developmental cultures were induced as previously described (2). To disrupt the *catC* gene, strain RMS011 was transformed with plasmid pLK20 by using standard techniques (46).

Catalase induction by different types of stress. Wild-type strain FGSC26 was used to study *catC* gene expression under different conditions. Liquid cultures were inoculated with 5×10^5 spores/ml and grown for 12 h (nitrate as the nitrogen source) or 14 h (ammonium as the nitrogen source) at 37°C and 300 rpm. Then mycelia were incubated under different conditions or filtered through Miracloth and transferred to different media. Stress conditions were heat shock (42°C), 5 mM paraquat, 0.5 mM H_2O_2 (added every 30 min), 1 M sorbitol, and 1 M NaCl. Cultures were incubated under these conditions for 2 to 6 h. Mycelia were harvested and frozen in liquid nitrogen. Total RNA was extracted using Trizol (Gibco-BRL) and Northern blotting analysis was performed using standard techniques using *catC* as a probe.

Cloning of *catC*, sequencing, and plasmid construction. Oligonucleotides catC1 (5′CTAGGTACCGAGCGAGTGGTCCATGCC3′) and catC2 (5′AGTAGATCTCGGGATTCTCGTCAAGG3′) were designed based upon a 1,085-bp *A. nidulans* genomic sequence (contig ANIC10430), predicting a catalase fragment different from CatA and CatB, provided by Cereon Genomics, LLC. These primers were used to amplify by PCR a 770-bp DNA fragment, using total *A. nidulans* DNA as the template. This PCR product was cloned into PCRII (pLK12) vector (Invitrogen) and subsequently used to probe an *A. nidulans* chromosome-specific cosmid library (4). Eight cosmids belonging to chromosome I were identified: L9E07, L28G03, W6C12, W9009, W10009, W11609, W17G01, and W28001. Restriction analysis of cosmids W6C12, W9009, W28001, and W17G01 indicated that they represent the same chromosomal region. Cosmid W17G01 was used as a template to fully sequence both DNA strands of the *catC* gene, by automatic fluorescent sequencing in an ABI PRISM 310 from Perkin-Elmer. After DNA sequencing was completed, primers catC8 (5′TTCCTCAATGCTTAGTGC3′) and catC9 (5′TCCCGGGAACTTTAAGGCATGTTAG3′) were used to amplify by PCR a 2,200-bp fragment containing the complete *catC* gene, using cosmid W17G01 as the template. This 2,200-bp fragment was cloned into PCRII to originate plasmid pLK17. The *catC KpnI-NotI* fragment from pLK17 was ligated into pBluescript II KS(+) (Stratagene) to generate plasmid pLK19. pLK19 was digested with *Xho*I and *Hin*cII and ligated to the *argB Xho*I-*Sma*I fragment from plasmid pDC1 to generate pLK20, which was used to transform strain RMS011.

Hybridization analyses and nucleic acid isolation. Genomic DNA was isolated as reported previously (39). Total RNA was isolated with the Trizol reagent (Gibco-BRL), fractionated in formaldehyde-agarose gels, transferred to Hybond-N nylon membranes (Amersham), and hybridized by using standard techniques. The *Eco*RI fragment from pLK17 was used as a *catC*-specific probe, and the *Bam*HI-*Nru*I fragment from pDC1 was used as an *argB*-specific probe. Both were labeled with ^{32}P using the BRL random priming labeling kit. Transformants containing the desired *catC* disruption were identified by Southern blotting, using first the *catC Xho*I-*Hin*cII internal fragment from pLK19 and then the entire *catC Eco*RI fragment from pLK17 as probes.

Catalase activity determination. Mycelial samples from 50-ml cultures were filtered through Whatman paper, dried by passing ~200 ml of cold acetone through the mycelia, and stored at −75°C until used. Acetone-dried mycelia were ground with mortar and pestle by using dry ice, until a fine powder was obtained. Ground mycelia were used to prepare protein extracts, which were used to determine catalase activity in zymograms (23) or by O_2 evolution, using an oxygen electrode (11).

Nucleotide sequence accession number. The sequence obtained for *catC* has been deposited in GenBank under accession number AF316033.

RESULTS

Cloning and characterization of the *catC* gene. Using catalase activity zymograms, we detected the presence of a third catalase activity in mycelial samples obtained from several *catA catB* null mutants grown for 48 h. This novel activity, which was later

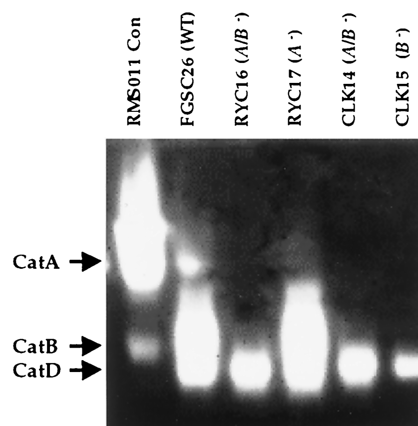

FIG. 1. A catalase activity is detected in zymogram analysis of mutants carrying different deletions in the *catA* and *catB* genes. Protein extracts (55 μg) obtained from mycelia grown for 48 h in minimal-nitrate liquid media were fractionated in a native polyacrylamide gel and stained for catalase activity. Catalase activity from isolated conidia (strain RMS011) is shown as a reference.

```
                 1                                                                                    |*                   110
M.barkeri        ~~~~mg.... .......... .......... ..eknsskvl ttgfgipvgd dq.nsltaG. nrGPvLmQDv hLlDklsHFd hErIPeRvvH AkGaGAgGyF Evt.aDvtky
D.discoideum     ~~~~m..... .......... .......... .....sapvl ttssgspi.d nnlnsmtaGv n.GPiLiQDf tLiDklaHFd rErIPeRvvH AkGaGAhGyF EvtssDvpkw
A.capsulatus     ~~~~mgaddt fnsyry.... .......... .kdt...pty tdsngcpvmd pes.sqrvGe n.GPlLlQDf hLiDllaHFd rErIPeRvvH AkGaGAyGeF Evld.Disdi
A.nidulans       ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~md pqa.sqrvGp n.GPlLlQDf nLiDllaHFd rErIPeRvvH AkGaGAyGeF Evtd.Disdi
S.pombe          ~nKll..... fn..rfassq skdsnisemn skdsntvpvy ttntgcpifn pma.aarvGk g.GPvLlQDs hLiDvfqHFd rErIPeRvvH AkGsGAfGeF Ectd.Ditky
S.cerev CTA1     msK.lgqek. .nevny.... ...sdvre.. ..dr....vv tnstgnpine pfv.tqriGe h.GPlLlQDy nLiDslaHFn rEnIPqRnpH AhGsGAfGyF Evtd.Ditdi
Consensus        ~~~~------ ---------- ---------- ---------- ---------- --------G- --GP-L-QD- -L-D---HF- -E-IP-R--H A-G-GA-G-F E----D----
                 111                        |       *                           *      |       |                           220
M.barkeri        tka..kflse .iGKrTev.. fvRFSTVGGe kGsaDsaRDP RGFavKFYTe dGnyDlVgNN TPVFFiRDPl KFPdFIHTQK RNPatnckDp dMFWDfLslt p...esiHQV
D.discoideum     cka..kflnk .vGKrTpi.. ftRFSTVGGe kGssDseRDP RGFavKFYTe eGnfDmVgNN TPVFFiRDPs KFPdFIHTQK RNPqtnckDp nMFWDfLgqt p...estHQV
A.capsulatus     t..tinml.k gvGKkTklvt ..RFSTVGGe kGsaDsaRDP RGFstKFYTe eGnwDwVfNN TPVFFlRDPs KFPlFIHTQK RNPqtnlkDa tMFWDyLs.t ..hqeaiHQV
A.nidulans       t..vidml.k gvGKkTk..t fvRFSTVGGe kGspDsaRDP RGFacKFYTe eGnwDwVfNN TPVFFlRDPs KFPmFIHTQK RNPqtnlkDa tMFWDyLs.t ..hqeavHQV
S.pombe          tkht..mfsk .vGKkTpmv. .aRFSTVGGe rGtpDtaRDP RGFalKFYTd eGifDmVgNN TPVFFlRDPa KFPlFIHTQK RNPqndmkDa tMFWDyLs.q ..naesiHQV
S.cerev CTA1     cgsa..mfsk .iGKrTkclt ..RFSTVGGd kGsaDtvRDP RGFatKFYTe eGnlDwVyNN TPVFFiRDPs KFPhFIHTQK RNPqtnlrDa dMFWDfLt.t penqvaiHQV
Consensus        ---------- --GK-T---- --RFSTVGG- -G--D--RDP RGF--KFYT- -G--D-V-NN TPVFF-RDP- KFP-FIHTQK RNP-----D- -MFWD-L--- -------HQV
                 221                                                                                                       330
M.barkeri        tiLFSD.rGT PatYRnMnGy SsHTyKwyn. ekGe.yfwvq yHfktdqGik nlTleEAeki g...gsdPD. hatrDLyeaI kkGdyPsWtl emQimtpeqa ed...yrfdi
D.discoideum     siLFSD.rGT PksYRhMhGf SsHTlKfvn. aqGkpy.wvk lHftsetGiq nyTaeEAakm s...mndPD. satrDLfetI akGgePaWkv siQlm...ef edalkyrfnp
A.capsulatus     mhLFSD.rGT PysYRhMnGy SgHTfKwltp d.Gg.fnyvq iHlktdqGsk tlTneEAtkl aa...enPDw ht.eDLfraI erGeyPsWtc yvQvlspqqa e...kfrwni
A.nidulans       mhLFSD.rGT PysYRhMnGy SgHTyKwikp d.Gt.fnyvq lHlktgqGnk tfTdaEAtrl aa...enPDw ht.qDLfnaI arGeyPsWtc yvQtlspeqa e...kfrwni
S.pombe          miLFSDlgGT PysYRfMdGf SsHTyKfvn. dkGe.fyyck wHfitnqGtk glTneEA... aaldgsnPD. harqDLfeaI erGdyPsWtl yvQvmtpqea e...kyryni
S.cerev CTA1     miLFSD.rGT PanYRsMhGy SgHTyKwsnk n.Gd.whyvq vHiktdqGik nlTieEAtki a...gsnPD. ycqqDLfeaI qnGnyPsWtv yiQtmterda k...klpfsv
Consensus        --LFSD--GT P--YR-M-G- S-HT-K---- --G------- -H-----G-- --T--EA--- -------PD- ----DL---I --G--P-W-- --Q------- ----------
                 331                                             |             |    |                                      440
M.barkeri        rDiTKvWphg dfPtmkiGkl vLNrNPtNyF AevEQAaFsP anlVPgigiS pDkmLQgRvF SYhDtHihRL G.pNynlIPV N...aP.... ........ky spensyqRDG
D.discoideum     fDvTKiWshk dyPliqiGrm vLNrNPeNyF AevEQAaFsP shmVPgiepS pDkmLQgRlF SYpDtHrhRL G.vNyqqIPV N...cP.... .favkggvk. ...n.yqRDG
A.capsulatus     fDlTKvWphs evPlrrfGrl vLNkNPqNyF AemEQAaFsP shlVPgvepS aDpvLQsRlF SYpDtHrhRL Gv.NyqqIPV N...cPl..r af........ ...npyqRDG
A.nidulans       fDlTKvWpqs evPlrrfGrf tLNkNPeNyF AevEQAaFsP shlVPgvepS aDpvLQaRlF SYpDtHrhRL GtsNyqsIPV N...cPl..r af........ ...tpfhRDG
S.pombe          fDlTKvWphk dvPmqrvGrf tLNqNPtNfF AdiEQAgFsP shmVPgievS aDpvLQvRtF SYpDtHrhRL G.aNfeqIPV NspkcPv... .f........ ...n.ysRDG
S.cerev CTA1     fDlTKvWpqg qfPlrrvGki vLNeNPlNfF AqvEQAaFaP sttVPyqeaS aDpvLQaRlF SYaDaHryRL G.pNfhqIPV N...cPyask ff........ ...npaiRDG
Consensus        -D-TK-W--- --P----G-- -LN-NP-N-F A--EQA-F-P ---VP----S -D--LQ-R-F SY-D-H--RL G--N---IPV N----P---- ---------- -------RDG
                 441                                                                                                       550
M.barkeri        fMrVdaN.Gg sgPnYwpnsf .......... ....ggPspd svylep.p.. ....fgvsG. .laartly.. .thpnD.... .DFvQagnly rdvmtdyd.. .r........
D.discoideum     fMaVngN.Gg kgPnYqpnsf .......... ....ggP... ....ephpef aqhkfdvsG. .faarqpy.. .nhpnD.... .DFvQpgdly r.lmse.dak sr.......f
A.capsulatus     aMaVngNyGa n.PnY.pstf rrm....ny. .....mPvka sq..e.h.e. ...kw..tGa vlakqlp... vt...De... .DFvQanglw q.vl...... grqpgqqanf
A.nidulans       aMsVngNhGa n.PnY.pstf rpl....qy. .....kPvka sq..e.h.e. ...kw..aGs vvteqlp... vt...De... .DFvQanglw k.vl...... grqpgqqenf
S.pombe          pMnVngNqG. nwPnY.pssi rplak.vqy. .....eP... .d..egh.e. ...kw..vG. ....q..... vtyhmDeitd vDFeQprafw qnvl...... gkkpgqqdnf
S.cerev CTA1     pMnVngNfGs e.PtY..... ..landksyt yiqqdrPi.. .q..q.hqe. ...vw..nGp ai....pyhw atspgD.... vDFvQarnly r.vl...... gkqpgqqknl
Consensus        -M-V--N-G- --P-Y----- ---------- ------P--- ---------- --------G- ---------- -----D---- -DF-Q----- ---------- ----------
                 551                                                                                        636
M.barkeri        .eNlvGnivs Hlsaaq.kri qlrqtal..F fkadrdygsr vakgleldik everla...n mtneerarat er~~~~~~~~ ~~~~~~
D.discoideum     vsNlvG.... Hmsgvtikei qvr..avsnF ykadkdlgar lckglgidvn dvikfaarsn l~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
A.capsulatus     vkNvaG.... Hlcna.eqkv ..rkaaygmF irvnkdlgss ies....... .....stea. lv.asqaqs. ......qPRL ~~~~~~
A.nidulans       vgNvaG.... Hlcna.hprv ..rqatygmF rrvnadlgkr iek....... .....atek. .k.ateara. ........RL ~~~~~~
S.pombe          vkNvaG.... Hlsgaisp.v ..rerqygvF trvdselgrr ire....... .....ateae vk.kmeekap kpinkgePhm fqgSs~
S.cerev CTA1     ayNi.G...i Hvegac.pqi ..qqrvydmF arvdkglsea ikk....... .....vaea. .khase.... .........L ssnSkf
Consensus        --N--G---- H--------- ---------F ---------- ---------- ---------- ---------- ---------- ------
```

FIG. 2. Comparison of *A. nidulans* CatC with the most similar catalases. CatC was aligned with catalases from *Ajellomyces capsulatus* (GenBank accession number AF189369), *Schizosaccharomyces pombe* (47), *Saccharomyces cerevisiae* (10), *D. discoideum* (14), and *M. barkeri* (34). Conserved amino acids that form part of the active (*) and heme coordination (|) sites are indicated. The alignment was performed using the programs PILEUP and PRETTY (12).

named CatD (see below), migrated slightly faster than CatB and was not present in asexual spores (Fig. 1). This result led us to contact Cereon Genomics, LLC (Cambridge, Mass.), for possible catalase gene sequences, different from *catA* and *catB*, present in their *A. nidulans* genomic sequence database. We used a 1,085-bp sequence identified in the database to design primers to amplify a 770-bp DNA fragment, using *A. nidulans* genomic DNA as the template. The cloned PCR product was confirmed by sequencing and used to probe a chromosome-specific library (4), which identified eight cosmid clones from chromosome I. Restriction analysis of four of these cosmids indicated that they represent the same chromosomal region. Using one of the cosmid clones, W17G01, the region of interest was sequenced in both strands. The resulting genomic sequence contains an uninterrupted open reading frame encoding a 475-amino-acid polypeptide. Because the predicted amino acid sequence showed high similarity to known catalases, this newly identified gene and its protein product were named *catC* and CatC, respectively.

***catC* encodes a small-subunit monofunctional catalase with a putative peroxisomal targeting signal.** The *catC* gene predicts a protein with a molecular mass of 54,128 Da. CatC is highly similar to catalase P from the fungal human pathogen *Ajellomyces* (*Histoplasma*) *capsulatus* (84% identity), Ctt1 from *Schizosaccharomyces pombe* (61% identity), CAT1 (43) from *Candida albicans* (61% identity), and the peroxisomal catalase Cta1p from *Saccharomyces cerevisiae* (58% identity). CatC also shows high similarity to CatA (14) from the slime mold *Dictyostelium discoideum* (52% identity) and catalases from the strict anaerobic methanogenic archaeon *Methanosarcina barkeri* (52% identity) and animals (data not shown). All of these enzymes belong to the family of small-subunit (50- to 65-kDa) monofunctional catalases. As shown in Fig. 2, amino acid sequences that form part of the active and heme coordination sites are conserved in CatC and these enzymes.

The CatC carboxy terminus contains the tripeptide ARL, which fits the consensus ([S/C/A][K/R/H]L) peroxisomal tar-

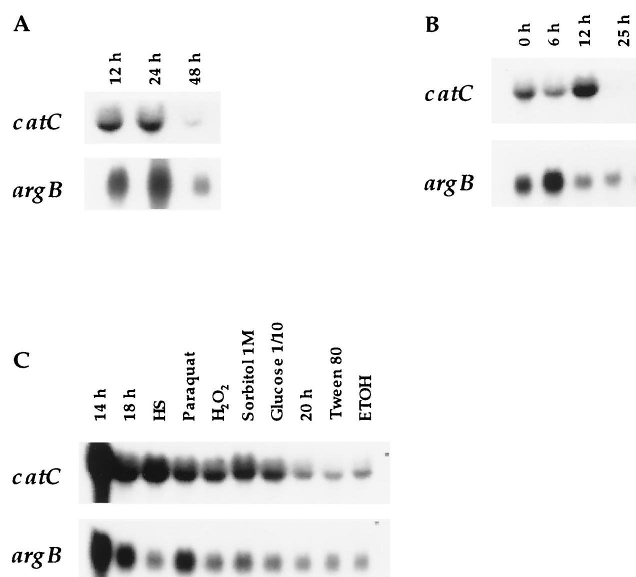

FIG. 3. *catC* expression during growth, asexual development, and stress. Total RNA extracted from wild-type strain FGSC26 mycelia, subject to the indicated conditions, was fractionated in a formaldehyde-agarose gel and used for Northern blot analyses. (A) Regulation during growth and stationary phase. RNA samples were from mycelia harvested at 12, 24, and 48 h of growth in liquid minimal-nitrate medium. (B) Expression during asexual development. RNA samples obtained from mycelia grown for 18 h in liquid minimal nitrate medium (0 h) and from mycelia induced to conidiate for the indicated time. (C) *catC* expression under different nutritional and stress conditions. Strain FGSC26 was grown in liquid minimal-ammonium medium for 14 h and then incubated at 42°C (HS) or in the presence of 5 mM paraquat or 0.5 mM H_2O_2 for 4 h. In the case of 1 M sorbitol, 0.1% glucose, 0.5% Tween 80, and 200 mM ethanol, the 14-h-grown mycelia were filtered, washed, and transferred to the indicated media for 4 h (sorbitol and glucose) or 6 h. Glucose (0.1%), Tween 80, and ethanol were used as the sole carbon sources. The *Eco*RI *catC* fragment from pLK12 was used as the probe. The same blots probed with *argB* are shown as RNA loading controls.

geting signal type I. This signal has been shown to be both necessary and sufficient to direct proteins to peroxisomes (17, 20). It is present in other enzymes from filamentous fungi that very likely are peroxisomal, such as monoamino oxidase (ARL) and urate oxidase (AKL) (29, 32).

***catC* expression is constant under different conditions.** We have reported that the *catA* and *catB* genes are differentially regulated during the *A. nidulans* life cycle as well as in response to different types of stress (23, 26, 27). With *argB* mRNA and rRNA staining (not shown) as loading controls, we examined the expression of *catC* by Northern blot analysis, using RNA samples from the wild-type strain FGSC26 grown under different conditions. As shown in Fig. 3A, the *catC* mRNA was detected in young hyphae as early as 12 h of growth, and its level was relatively constant up to 48 h of growth. This result suggested either that *catC* did not encode the catalase activity that appears after 48 h of growth (Fig. 1) or that it was subject to some type of posttranscriptional control. During asexual development, the *catC* message level showed little change up to 6 h, increased slightly by 12 h, and declined thereafter (Fig. 3B), to become barely detectable in isolated conidia (not shown).

The *catC* mRNA level was virtually unaffected by several stress and nutritional conditions, including oxidative stress, osmotic stress, and growth for 6 h in Tween 80 or ethanol as the sole carbon source (Fig. 3C). A slight induction was noticeable only during heat shock (Fig. 3C) and growth in uric acid as the sole nitrogen source (not shown).

Targeted disruption of *catC* revealed the existence of an unidentified catalase gene. To determine if *catC* encoded a catalase different from the one previously detected in *catA catB* double null mutants (Fig. 1), we designed plasmid pLK20 to perform a targeted disruption of *catC*. In pLK20 a central region of 740 bp from the *catC* gene was replaced by the *argB* gene as a selectable marker. This resulted in the deletion of amino acid residues 94 to 341 from CatC. Linear pLK20 was used to transform strain RMS011 to arginine independence. Forty-one Arg^+ transformants were analyzed by Southern blotting using the *catC* internal *Xho*I-*Hin*cII fragment deleted in pLK20. Among these, 12 transformants gave no hybridization signal, indicating deletion of the corresponding *catC* fragment. Genomic DNA from three of these *catC* mutants was digested with *Bam*HI, *Eco*RI, and *Sal*I and analyzed by Southern blotting using the entire *catC* gene as the probe. All three transformants gave hybridization patterns identical to the one shown in Fig. 4B for strain TLK61. This pattern is consistent with the double recombination event and consequent disruption of the *catC* gene, depicted in Fig. 4A.

To analyze zymogram catalase activity patterns in a more conclusive way, we created triple *catABC* mutant strains (Table 1). Both *catA catB* (strain CLK20) and *catA catB catC* (strain CLK36) mutants were grown for 12, 24, and 48 h, and corresponding protein extracts were used to detect catalase activity in zymograms. As shown in Fig. 5, the catalase activity detected previously in 48-h samples from the *catAB* double mutants (Fig. 1) was unaffected by the deletion of the *catC* gene, demonstrating that this catalase, which we have designated CatD, is encoded by an as-yet-unidentified gene.

Samples from the *catA catB* double mutant showed a catalase activity smear at the gel wells and a more defined band right below the concentrator gel. These activities were totally absent in samples from the *catABC* triple mutant (Fig. 5), indicating that the *catC*-encoded catalase was not detected in our previous zymogram gel system. This was supported when catalase-specific activity was assayed by O_2 evolution in the samples used for the zymogram. As shown at the bottom of Fig. 5, ~250 U of catalase activity was detected in samples from strain CLK20 grown for 12 h, which remained constant at 24 h of growth. In contrast, catalase activity was negligible in 12- and 24-h samples from strain CLK36. The 48-h sample of CLK36 contained 92 U of catalase activity, which would correspond to the catalase D activity detected in the zymogram. A slight decrease in CatC activity in the CLK20 48-h sample may explain why the total catalase-specific activity remained around 250 U, despite the contribution of CatD activity. These results confirm that *catC* encodes a novel catalase activity that remains relatively unchanged during 12 to 48 h of growth. This pattern of CatC activity is consistent with the *catC* mRNA levels detected during the same period of growth (Fig. 3A).

Catalase C activity is not required for asexual or sexual development or for fatty acid and/or purine utilization. We observed no obvious defect during asexual development of *A.*

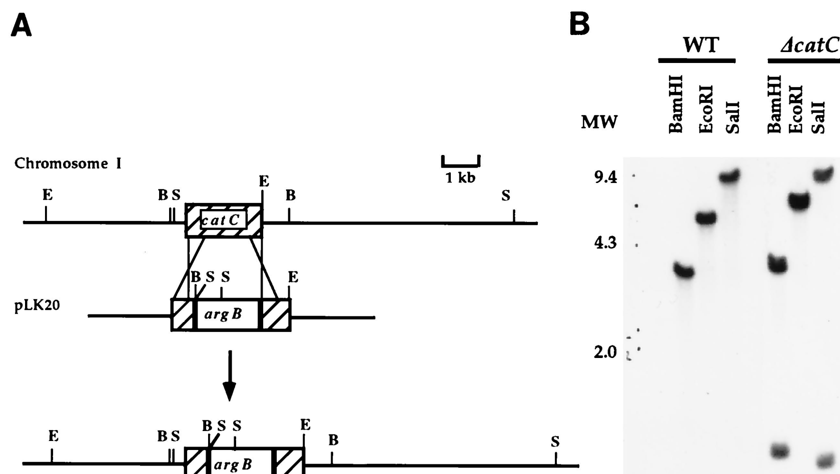

FIG. 4. *catC* gene targeted disruption. (A) Plasmid pLK20 was constructed by replacing a central region of 740 bp from the *catC* gene with the *argB* gene, used as a selectable marker. The CatC region removed corresponds to amino acids 94 to 341 (Fig. 2). Linear pLK20 was used to transform strain RMS011 to arginine independence. Restriction sites: B, *Bam*HI; E, *Eco*RI; S, *Sal*I. (B) Total DNA extracted from recipient strain RMS011 and the Δ*catC* strain TLK61 was digested with indicated restriction enzymes and used for Southern blot analysis. The probe used was the *Eco*RI *catC* fragment from pLK17. The same membrane probed with *argB* (not shown) gave a hybridization pattern fully consistent with the illustrated integration event. MW, molecular weight (weights are in thousands); WT, wild type.

nidulans catC mutants. However, Berteaux-Lecellier et al. (3) reported that peroxisomal function is necessary for caryogamy and sexual development in *Podospora anserina*. We found that wild-type strain FGSC26 and *catAB* (CLK20), *catB* (TLK12), *catC* (TLK61), and *catABC* (CLK35) null mutant strains were all able to differentiate sexual fruiting bodies (cleistothecia) in similar amounts and produced viable sexual spores.

A. nidulans can utilize oleate as the sole carbon source and purines as the sole nitrogen source. The degradation of these compounds appears to occur in peroxisomes and involve H_2O_2 generation (31, 42). We tested the growth response of CLK20, TLK12, TLK61, and CLK35 mutant strains in media containing different carbon and/or nitrogen sources (see Material and Methods). In particular, we tested oleate and Tween 80 as the sole carbon sources, adenine and uric acid as the sole nitrogen sources, and combinations of both carbon and nitrogen sources. All four catalase mutants grew as well as the wild-type strain in all tested media, indicating that CatC function is dispensable for growth in these substrates.

Catalase D is induced during late stationary phase, by glucose starvation, and heat shock. We used *catA catB* double mutants to examine CatD activity under different conditions. As shown in Fig. 5 and 6, CatD activity was not detectable before 48 h of growth. After 48 h, a slight increase was observed by 72 h (data not shown). Under our growing conditions, glucose in the medium becomes exhausted by 36 h (35). Therefore, 48 to 72 h of growth corresponds to a very late stationary phase under severe nutrient starvation. To analyze CatD activity under different growth and stress conditions, strain CLK20 was grown for 24 h, and then mycelia were shifted to different media for 10 to 12 h. Alternatively, 24-h mycelia were exposed for 10 h to osmotic stress, high temperature, or the oxidative stress caused by paraquat and H_2O_2 treatments. Figure 6 shows that glucose starvation and incubation at 42°C resulted in a clear induction of CatD activity, while H_2O_2 produced a modest induction. All other treatments, including nitrate starvation, failed to induce CatD.

DISCUSSION

The *catC* gene encodes a small-subunit monofunctional catalase, likely localized in peroxisomes. Here we have shown that *A. nidulans catC* encodes a catalase more related to small-subunit catalases from other fungi, a slime mold, *Archaea*, and

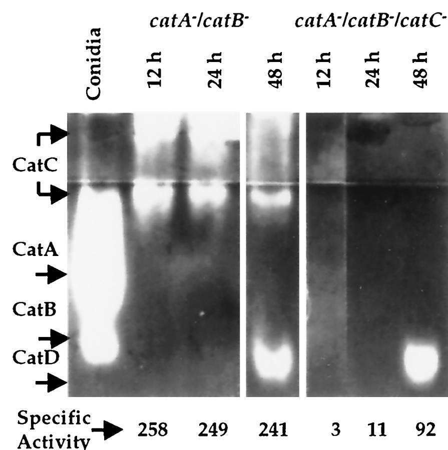

FIG. 5. *A. nidulans* contains at least four different catalases. Protein extracts (40 μg) prepared from strains CLK20 (*catA*$^-$ *catB*$^-$) and CLK36 (*catA*$^-$ *catB*$^-$ *catC*$^-$) grown for 12, 24 and 48 h were fractionated in a native polyacrylamide gel that was stained to detect catalase activity. A protein sample from isolated conidia (strain FGSC26) is shown as a catalase A and B reference. Numbers below the zymogram are catalase-specific activities (in units per milligram of protein per milliliter) in each sample, measured by O_2 evolution (11). Data are the averages of two determinations, with a maximum variation of 13% with respect to the average.

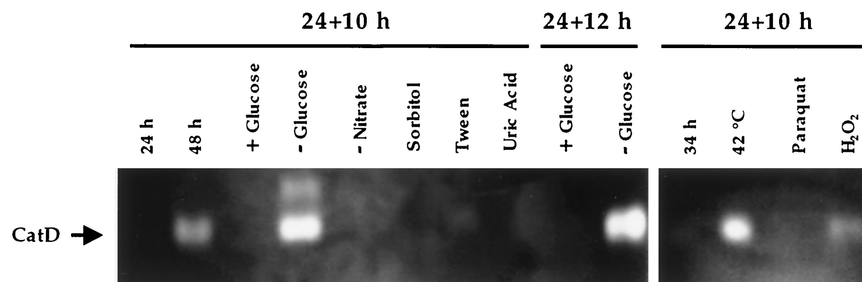

FIG. 6. Catalase D is induced during late stationary phase, by glucose starvation, heat shock, and H_2O_2. Strain CLK20 (*catA*$^-$ *catB*$^-$) was grown in minimal-nitrate medium for 24, 34, and 48 h. Mycelia grown for 24 h were incubated at 42°C, in the presence of 1 M sorbitol, 5 mM paraquat, or 0.5 mM H_2O_2 for 10 h. Where indicated, 24-h mycelia were shifted for 10 to 12 h to fresh media containing or lacking glucose, lacking nitrate, or containing 0.5% Tween 80 or 0.8 mg of uric acid/ml as the sole carbon and nitrogen sources, respectively. A total of 100 μg of protein was loaded in each lane.

animals than to catalases from eubacteria and plants. In contrast, CatA and CatB, along with other enzymes up to now found only in filamentous fungi and eubacteria, form the large-subunit catalase family (5, 8, 13, 15, 23, 24, 27, 37, 48). In fact, endosymbiosis and horizontal gene transfer mechanisms have been invoked to explain the grouping of these fungal and bacterial catalases (24). It seems clear that *catC* and *catAB* genes have different evolutionary origins, as judged from their sequence and size disparity and the *catC* lack of introns.

The *catC* gene was expressed at relatively constant levels under several growth, stress, and nutritional conditions, the most noticeable change being a gradual decrease during conidiation. This constitutive expression correlates well with the CatC activity detected during 12 to 48 h of growth (Fig. 5). CatC activity was not detected previously due to its extremely low migration rate in our zymogram gel system. This can be explained by the high isoelectric point (8.69) predicted for CatC, perhaps the most basic reported for a catalase, with our starting electrophoresis conditions at pH 8.5. A slight change in pH during electrophoresis may account for the CatC activity that enters the zymogram gel. Our attempt to resolve and/or detect CatC using electrofocusing gels was unsuccessful, while CatA and CatB were well separated and detected under the same conditions. This result could be explained by a higher stability of CatA and CatB than of the smaller CatC enzyme. In fact, CatB has been found to be resistant to 9 M urea, 2% sodium dodecyl sulfate, and ethanol-chloroform treatment (6).

Several lines of evidence suggest that CatC may be a peroxisomal enzyme. First, it contains the peroxisome-targeting signal ARL. Second, our preliminary cell fractionation experiments using cell extracts from *catA catB* double mutant grown for 18 h showed that at least 20% of the total CatC activity is contained within the subcellular particle pellet, along with high activity levels of the peroxisomal marker isocitrate lyase (41) and the mitochondrial marker fumarase. Third, a catalase activity has been cytochemically localized in microbodies from young growing hyphae, and cosedimentation of catalase activity and peroxisome marker enzymes has also been shown in *A. nidulans* (42). It is unlikely that the reported peroxisome-associated catalase (42) corresponds to CatA, CatB, or CatD. CatA and CatB do not contain peroxisome-targeting signals (23, 27). CatA activity is largely associated with spores (26) and has been immunolocalized in the asexual spore cell wall and cytosol (R. E. Navarro and J. Aguirre, unpublished data), whereas CatB has been immunolocalized in the cell wall and cytosol from hyphae (L. Kawasaki and J. Aguirre, unpublished data). CatD has been shown here to be present in old and high-temperature-grown hyphae.

Multiple catalases and other H_2O_2 detoxification enzymes in *A. nidulans*. Although there is some overlap, CatA and CatB are present at different stages of the *A. nidulans* life cycle and protect different cell types from H_2O_2 or other types of oxidative stress (23, 26, 27) and heat shock stress (28). The fact that the *catC* gene is expressed at relatively constant levels suggests that CatC activity overlaps CatA or CatB activity. However, confirmation of a peroxisomal location for CatC would argue against such functional overlap or redundancy. CatD seems repressed by glucose and is induced during late stationary phase, showing a partial overlap with CatB expression. No other catalase genes besides *catA*, *-B*, and *-C* were found in the *A. nidulans* genome database, suggesting that the database is not complete or that CatD does not belong to the monofunctional catalase family.

The fact that CatC is dispensable for growth in oleic acid as the sole carbon source and/or in purines as the sole nitrogen sources suggests the presence of alternative peroxisomal H_2O_2 detoxification systems. A search of an *A. nidulans* cDNA partial sequence database (http://www.genome.ou.edu/fungal.html) for genes encoding enzymes involved in H_2O_2 detoxification identified two genes in addition to *catA* and *catB*. Clone r2g02a1 predicts a protein with high similarity to fungal and mammalian PMP20 peroxisomal peroxidases (9, 16, 44). Clone c7g02a1 predicts a protein with high similarity to glutathione peroxidases. The existence of two putative thiol-dependent peroxidases and at least four catalases suggests a large and regulated capability for H_2O_2 degradation in filamentous fungi.

ACKNOWLEDGMENTS

This work was supported by grants IN206097 and IN214199, both from DGAPA-UNAM (PAPIIT).

We are grateful to Thomas Adams and Vicky Gavrias from Cereon Genomics, LLC, for the *catC* fragment DNA sequence. We thank Fabiola Méndez for experimental support and the IFICE-UNAM Molecular Biology Unit for DNA sequencing and oligonucleotide synthesis. We are indebted to Wilhelm Hansberg for helpful discussions and Kazuhiro Shiozaki for critical reading of the manuscript.

REFERENCES

1. **Adams, T. H., J. K. Wieser, and J. H. Yu.** 1998. Asexual sporulation in *Aspergillus nidulans*. Microbiol. Mol. Biol. Rev. **62:**35–54.
2. **Aguirre, J.** 1993. Spatial and temporal controls of the *Aspergillus brlA* developmental regulatory gene. Mol. Microbiol. **8:**211–218.
3. **Berteaux-Lecellier, V., M. Picard, C. Thompson-Coffe, D. Zickler, A. Panvier-Adoutte, and J. M. Simonet.** 1995. A nonmammalian homolog of the PAF1 gene (Zellweger syndrome) discovered as a gene involved in caryogamy in the fungus *Podospora anserina*. Cell **81:**1043–1051.
4. **Brody, H., J. Griffith, A. J. Cuticchia, J. Arnold, and W. E. Timberlake.** 1991. Chromosome-specific recombinant DNA libraries from the fungus *Aspergillus nidulans*. Nucleic Acids Res. **19:**3105–3109.
5. **Calera, J. A., S. Paris, M. Monod, A. J. Hamilton, J. P. Debeaupuis, M. Diaquin, R. Lopez-Medrano, F. Leal, and J. P. Latge.** 1997. Cloning and disruption of the antigenic catalase gene of *Aspergillus fumigatus*. Infect. Immun. **65:**4718–4724.
6. **Calera, J. A., J. Sanchez-Weatherby, R. Lopez-Medrano, and F. Leal.** 2000. Distinctive properties of the catalase B of *Aspergillus nidulans*. FEBS Lett. **475:**117–120.
7. **Chang, Y. C., B. H. Segal, S. M. Holland, G. F. Miller, and K. J. Kwon-Chung.** 1998. Virulence of catalase-deficient *Aspergillus nidulans* in p47(phox)−/− mice. Implications for fungal pathogenicity and host defense in chronic granulomatous disease. J. Clin. Investig. **101:**1843–1850.
8. **Cho, Y. H., E. J. Lee, and J. H. Roe.** 2000. A developmentally regulated catalase required for proper differentiation and osmoprotection of *Streptomyces coelicolor*. Mol. Microbiol. **35:**150–160.
9. **Choi, H. J., S. W. Kang, C. H. Yang, S. G. Rhee, and S. E. Ryu.** 1998. Crystal structure of a novel human peroxidase enzyme at 2.0 A resolution. Nat. Struct. Biol. **5:**400–406.
10. **Cohen, G., W. Rapatz, and H. Ruis.** 1988. Sequence of the *Saccharomyces cerevisiae* CTA1 gene and amino acid sequence of catalase A derived from it. Eur. J. Biochem. **176:**159–163.
11. **del Rio, L. A., M. Gomez Ortega, A. Leal Lopez, and J. Lopez Gorge.** 1977. A more sensitive modification of the catalase assay with the Clark oxygen electrode. Anal. Biochem. **80:**409–415.
12. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12:**387–395.
13. **Fowler, T., M. W. Rey, P. Vaha-Vahe, S. D. Power, and R. M. Berka.** 1993. The *catR* gene encoding a catalase from *Aspergillus niger*: primary structure and elevated expression through increased gene copy number and use of a strong promoter. Mol. Microbiol. **9:**989–998.
14. **Garcia, X. M., C. Foote, S. van Es, P. N. Devreotes, S. Alexander, and H. Alexander.** 2000. Differential developmental expression and cell type specificity of *Dictyostelium* catalases and their response to oxidative stress and UV-light. Biochim. Biophys. Acta **1492:**295–310.
15. **Garre, V., U. Muller, and P. Tudzynski.** 1998. Cloning, characterization, and targeted disruption of cpcat1, coding for an in planta secreted catalase of *Claviceps purpurea*. Mol. Plant-Microbe Interact. **11:**772–783.
16. **Godon, C., G. Lagniel, J. Lee, J. M. Buhler, S. Kieffer, M. Perrot, H. Boucherie, M. B. Toledano, and J. Labarre.** 1998. The H2O2 stimulon in *Saccharomyces cerevisiae* J. Biol. Chem. **273:**22480–22489.
17. **Gould, S. J., G. A. Keller, N. Hosken, J. Wilkinson, and S. Subramani.** 1989. A conserved tripeptide sorts proteins to peroxisomes. J. Cell Biol. **108:**1657–1664.
18. **Halliwell, B., and J. M. C. Gutteridge.** 1989. Free radicals in biology and medicine, 2nd ed. Clarendon Press and Oxford University Press, Oxford, United Kingdom.
19. **Hansberg, W., and J. Aguirre.** 1990. Hyperoxidant states cause microbial cell differentiation by cell isolation from dioxygen. J. Theor. Biol. **142:**201–221.
20. **Hettema, E. H., B. Distel, and H. F. Tabak.** 1999. Import of proteins into peroxisomes. Biochim. Biophys. Acta **1451:**17–34.
21. **Käfer, E.** 1977. Meiotic and mitotic recombination in *Aspergillus* and its chromosomal aberrations. Adv. Genet. **19:**33–131.
22. **Kamata, H., and H. Hirata.** 1999. Redox regulation of cellular signalling. Cell. Signal. **11:**1–14.
23. **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.
24. **Klotz, G. M., G. R. Klassen, and P. C. Loewen.** 1997. Phylogenetic relationships among prokaryotic and eukaryotic catalases. Mol. Biol. Evol. **14:**951–958.
25. **Lledias, F., P. Rangel, and W. Hansberg.** 1998. Oxidation of catalase by singlet oxygen. J. Biol. Chem. **273:**10630–10637.
26. **Navarro, R. E., and J. Aguirre.** 1998. Posttranscriptional control mediates cell type-specific localization of catalase A during *Aspergillus nidulans* development. J. Bacteriol. **180:**5733–5738.
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. **Noventa-Jordao, A. M., R. M. Couto, M. H. Goldman, J. Aguirre, S. Iyer, A. Caplan, H. F. Terenzi, and G. H. Goldman.** 1999. Catalase activity is necessary for heat-shock recovery in *Aspergillus nidulans* germlings. Microbiology **145:**3229–3234.
29. **Oestreicher, N., and C. Scazzocchio.** 1993. Sequence, regulation, and mutational analysis of the gene encoding urate oxidase in *Aspergillus nidulans*. J. Biol. Chem. **268:**23382–23389.
30. **Quillet-Mary, A., J. P. Jaffrezou, V. Mansat, C. Bordier, J. Naval, and G. Laurent.** 1997. Implication of mitochondrial hydrogen peroxide generation in ceramide-induced apoptosis. J. Biol. Chem. **272:**21388–21395.
31. **Scazzocchio, C.** 1994. The purine degradation pathway, genetics, biochemistry and regulation, p. 221–251. *In* S. D. Martinelli and J. R. Kinghorn (ed.), *Aspergillus*: 50 years on, vol. 29. Elsevier, Amsterdam, The Netherlands.
32. **Schilling, B., and K. Lerch.** 1995. Cloning, sequencing and heterologous expression of the monoamine oxidase gene from *Aspergillus niger*. Mol. Gen. Genet. **247:**430–438.
33. **Sheikh, F. G., K. Pahan, M. Khan, E. Barbosa, and I. Singh.** 1998. Abnormality in catalase import into peroxisomes leads to severe neurological disorder. Proc. Natl. Acad. Sci. USA **95:**2961–2966.
34. **Shima, S., A. Netrusov, M. Sordel, M. Wicke, G. C. Hartmann, and R. K. Thauer.** 1999. Purification, characterization, and primary structure of a monofunctional catalase from *Methanosarcina barkeri*. Arch. Microbiol. **171:** 317–323.
35. **Skromne, I., O. Sanchez, and J. Aguirre.** 1995. Starvation stress modulates the expression of the *Aspergillus nidulans brlA* regulatory gene. Microbiology **141:**21–28.
36. **Stringer, A. M., 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.
37. **Takasuka, T., N. M. Sayers, M. J. Anderson, E. W. Benbow, and D. W. Denning.** 1999. *Aspergillus fumigatus* catalases: cloning of an *Aspergillus nidulans* catalase B homologue and evidence for at least three catalases. FEMS Immunol. Med. Microbiol. **23:**125–133.
38. **Taub, J., J. F. Lau, C. Ma, J. H. Hahn, R. Hoque, J. Rothblatt, and M. Chalfie.** 1999. A cytosolic catalase is needed to extend adult lifespan in *C. elegans* daf-C and clk-1 mutants. Nature **399:**162–166.
39. **Timberlake, W. E.** 1980. Developmental gene regulation in *Aspergillus nidulans*. Dev. Biol. **78:**497–510.
40. **Timberlake, W. E., and A. J. Clutterbuck.** 1994. Genetic regulation of conidiation, p. 383–427. *In* S. D. Martinelli and J. R. Kinghorn (ed.), *Aspergillus*: 50 years on, vol. 29. Elsevier, Amsterdam, The Netherlands.
41. **Valenciano, S., J. R. De Lucas, I. Van der Klei, M. Veenhuis, and F. Laborda.** 1998. Characterization of *Aspergillus nidulans* peroxisomes by immunoelectron microscopy. Arch. Microbiol. **170:**370–376.
42. **Valenciano, S., J. R. D. Lucas, A. Pedregosa, I. F. Monistrol, and F. Laborda.** 1996. Induction of beta-oxidation enzymes and microbody proliferation in *Aspergillus nidulans*. Arch. Microbiol. **166:**336–341.
43. **Wysong, D. R., L. Christin, A. M. Sugar, P. W. Robbins, and R. D. Diamond.** 1998. Cloning and sequencing of a *Candida albicans* catalase gene and effects of disruption of this gene. Infect. Immun. **66:**1953–1961.
44. **Yamashita, H., S. Avraham, S. Jiang, R. London, P. P. Van Veldhoven, S. Subramani, R. A. Rogers, and H. Avraham.** 1999. Characterization of human and murine PMP20 peroxisomal proteins that exhibit antioxidant activity in vitro. J. Biol. Chem. **274:**29897–29904.
45. **Yang, Y., J. Shah, and D. F. Klessig.** 1997. Signal perception and transduction in plant defense responses. Genes Dev. **11:**1621–1639.
46. **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.
47. **Yoshioka, S., K. Kato, K. Nakai, H. Okayama, and H. Nojima.** 1997. Identification of open reading frames in *Schizosaccharomyces pombe* cDNAs. DNA Res. **4:**363–369.
48. **Zamocky, M., and F. Koller.** 1999. Understanding the structure and function of catalases: clues from molecular evolution and in vitro mutagenesis. Prog. Biophys. Mol. Biol. **72:**19–66.