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Cloning of the *Aspergillus parasiticus apa-2* Gene Associated with the Regulation of Aflatoxin Biosynthesis

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An *Aspergillus parasiticus* gene, designated *apa-2*, was identified as a regulatory gene associated with aflatoxin biosynthesis. The *apa-2* gene was cloned on the basis of overproduction of pathway intermediates following transformation of fungal strains with cosmid DNA containing the aflatoxin biosynthetic genes *nor-1* and *ver-1*. Transformation of an *O*-methylsterigmatocystin-accumulating strain, *A. parasiticus* SRRC 2043, with a 5.5-kb *Hin*dIII-*Xba*I DNA fragment containing *apa-2* resulted in overproduction of all aflatoxin pathway intermediates analyzed. Specific enzyme activities associated with the conversion of norsolorinic acid and sterigmatocystin were increased approximately twofold. The *apa-2* gene was found to complement an *A. flavus afl-2* mutant strain for aflatoxin production, suggesting that *apa-2* is functionally homologous to *afl-2*. Comparison of the *A. parasiticus apa-2* gene DNA sequence with that of the *A. flavus afl-2* gene (G. A. Payne, G. J. Nystorm, D. Bhatnagar, T. E. Cleveland, and C. P. Woloshuk, Appl. Environ. Microbiol. 59:156–162, 1993) showed that they shared >95% DNA homology. Physical mapping of cosmid subclones placed *apa-2* approximately 8 kb from *ver-1*.

Aflatoxins are a group of polyketide-derived, structurally related secondary metabolites that are produced by *Aspergillus flavus* and *Aspergillus parasiticus*. These compounds are potent toxins and carcinogens that contaminate food and feed worldwide (23). The implications of aflatoxins in hepatocellular carcinoma have been reported (10, 11). Aflatoxin contamination has an enormous impact on economic cost which arises from crop and livestock losses due to federal regulatory guidelines for sale of contaminated crops (23). Various efforts to eliminate aflatoxins from the human food supply are under way. Postharvest control relies on successful detection and removal of aflatoxins by physical, chemical, or biological means (32). Our laboratories have focused on preharvest elimination of aflatoxins through molecular approaches (for reviews, see references 14, 27, and 32). The aflatoxin B1 biosynthetic pathway is generally accepted to be as follows: acetate→polyketide→norsolorinic acid (NOR)→averantin (AVN)→averufanin→averufin (AVF)→hydroxyversicolorone→versiconal hemiacetal acetate→versicolorin B→versicolorin A (VA)→sterigmatocystin (ST)→*O*-methylsterigmatocystin (OMST)→aflatoxin B1 (AFB_1) (for reviews, see references 8 and 19). To date, only a few pathway enzymes have been purified (28, 30, 44). De novo synthesis of *O*-methyltransferase that catalyzes ST→OMST has been shown to be correlated with aflatoxin production (13). The establishment of fungal transformation systems in *A. flavus* and *A. parasiticus* has provided researchers with a useful approach for understanding molecular regulation of the complex aflatoxin pathway by cloning structural and regulatory genes (12, 36, 39). Two aflatoxin pathway structural genes of *A. parasiticus* that are associated with the conversion of NOR and VA, *nor-1* and *ver-1*, have been cloned (12, 39). The gene for a methyltransferase involved in the conversion ST→OMST has also been cloned (45). Recently, several cDNA clones believed to play a role in aflatoxin biosynthesis have been isolated (21), but their functions have yet to be determined. A correlation between fungal growth and time-course gene expression data suggests that aflatoxin biosynthesis is regulated at the transcriptional level (40). Despite this progress, the molecular and biochemical mechanisms that govern aflatoxin biosynthesis remain unclear. More recently, a putative regulatory gene of *A. flavus* (*afl-2*) was cloned (36). The *afl-2* gene was shown to restore several aflatoxigenic enzyme activities in a blocked, nonaflatoxigenic strain to levels comparable to those of wild-type, aflatoxigenic *A. flavus*. This finding suggested that the entire aflatoxin pathway may be under the control of a common regulator. In this study, we report the cloning of a regulatory gene, *apa-2*, in *A. parasiticus* which increases levels of aflatoxin precursors as well as aflatoxin production in the transformed strains. The *apa-2* gene showed a high degree of DNA homology to the *A. flavus afl-2* gene. We also investigated possible functional roles of this gene with respect to fungal growth.

MATERIALS AND METHODS

Fungal strains and media. *A. parasiticus* SRRC 2043 is a non-aflatoxin-producing strain that naturally accumulates OMST and lacks the enzyme (oxidoreductase) that converts OMST to AFB_1 (8, 9). *A. parasiticus* RHN1 is a nitrate reductase (*niaD*) mutant of this strain and was kindly provided by P. J. Cotty of the Southern Regional Research

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Center, Agricultural Research Service, U.S. Department of Agriculture. *A. parasiticus* SU1-N3 is a *niaD* mutant derived from wild-type aflatoxigenic *A. parasiticus* SU-1 (4). Fungal strains were maintained on potato dextrose agar or grown on 5/2 agar (5% V8 juice, 2% agar, pH 5.2) for the production of conidia. Czapek solution agar (Difco, Detroit, Mich.) supplemented with 0.6 M KCl was used as the protoplast regeneration medium (CZR). Coconut agar medium (18) was used for detection of aflatoxin production. Adye and Mateles (AM) growth medium (1) was used to grow submerged fungal cultures for pigment profile analysis. Cove's minimal salt medium (17) containing 10 mM nitrate as the sole nitrogen source was used for testing the repressive effect of nitrate on the production of precursors and aflatoxins.

Cosmids. A cosmid library containing wild-type *A. parasiticus* SU-1 genomic DNA fragments, with the *pyrG* (orotidine monophosphate decarboxylase) gene as the selectable marker (41), was screened with probes generated from the aflatoxin pathway genes *nor-1* and *ver-1*. Five *pyrG* cosmids (NA, NB, V2, V3, and V4) containing sequences flanking *nor-1* and/or *ver-1* were used in this study.

Subcloning. A *niaD* vector, pHD62, was used for most of the subcloning work. It was constructed as follows: a 6.2-kb *Hin*dIII DNA fragment from pSL82 (22) containing the *A. parasiticus niaD* gene was blunt ended and ligated to the *Sma*I site in the pUC18 multiple cloning region. This plasmid provided four unique restriction sites, *Hin*dIII, *Sph*I, *Sal*I, and *Xba*I, for inserting desired DNA fragments. DNA manipulation and bacterial transformation were performed by routine methods as described earlier (33).

Transformation. Fungal protoplasts were transformed in the presence of polyethylene glycol-$CaCl_2$ as previously described (12, 22). Cotransformation procedures were also used for screening aflatoxin-producing *A. parasiticus* RHN1 transformants. In cotransformation experiments, each *pyrG*-containing cosmid DNA was used in combination with the *A. parasiticus niaD* vector, pSL82, to transform protoplasts to nitrate utilization. The molar ratio of each cosmid to pSL82 was approximately 2:1 in the transformation mixtures.

Analysis of aflatoxin pathway intermediates. A total of 100 ml of liquid AM medium in 250-ml flasks was inoculated with fungal spore suspensions to a final concentration of approximately 10^5 spores per ml. Cultures were incubated without shaking for 7 days at 30°C. Mycelial mats and culture medium were extracted with acetone and chloroform as described earlier (15). Pigments were separated by thin-layer chromatography with an ether-methanol-water (96:3:1, vol/vol/vol) solvent system (for OMST and aflatoxins) or a toluene-ethyl acetate-acetic acid (50:30:4, vol/vol/vol) solvent system (for NOR, AVN, AVF, and VA). Thin-layer chromatography plates were analyzed by scanning densitometry (UV adsorption at 310 nm) to quantify the compounds as described earlier (36).

Enzyme assays. Four-day-old fungal cultures grown in AM medium were used for cell extract preparation. The mycelia were collected on Miracloth and washed extensively with distilled water. Fresh mycelia were ground to a fine powder under liquid nitrogen in a Waring blender. The powdered mycelia were suspended in buffer A (0.05 M potassium phosphate [pH 7.5], 10% [vol/vol] glycerol, and 2 mM β-mercaptoethanol) and stirred for 1 h. Enzyme assays were carried out at room temperature. The following substrates were added to each extract: (i) for the reductase assay, 27 μM NOR and 12 μM NADPH were used; (ii) for the methyltransferase assay, 23 μM *S*-adenosylmethionine and 31 μM ST were used. The reaction mixture had a final volume of 1 ml in a 10-ml unsealed glass vial. The reactions were carried out for 0.5 h with constant shaking and terminated by addition of chloroform, followed by vigorous shaking in capped assay vials. The resultant mixture was extracted, and the reaction products were identified and analyzed as described above.

Sequence analysis. Primer-walking, double-stranded sequencing of a 1.9-kb *Bam*HI-*Bam*HI genomic DNA fragment was performed. Sanger's dideoxy chain termination method (37) was performed with Sequenase version II (U.S. Biochemical Corp., Cleveland, Ohio) according to the manufacturer's instructions. Synthetic oligonucleotide primers were purchased from The Midland Certified Reagent Company (Midland, Tex.).

Nucleotide sequence accession number. The genomic DNA nucleotide sequence data of the *apa-2* gene reported in this paper have been submitted to GenBank and assigned the accession number L22177.

RESULTS

Analysis of *A. parasiticus* RHN1 transformants. Recent evidence has shown that the *A. parasiticus* aflatoxin biosynthetic genes, *nor-1* and *ver-1*, are clustered (39). In an attempt to clone the gene(s) responsible for the conversion of OMST to aflatoxin B1, five *pyrG* cosmids that contained regions of the *A. parasiticus* genome flanking the *nor-1* or *ver-1* gene were used in genetic complementation studies. Of the five cosmids, three (cosmids V2, V3, and V4) contained *ver-1*, one (cosmid NB) contained *nor-1*, and the other (cosmid NA) contained both *ver-1* and *nor-1*. These *pyrG*-containing cosmids were used separately along with a *niaD*-containing plasmid, pSL82, to cotransform a *niaD* mutant strain, *A. parasiticus* RHN1, to nitrate utilization. With each cosmid, approximately 90 to 120 transformants were obtained in total from two cotransformation experiments. These nitrate-utilizing transformants were transferred onto coconut agar plates to verify aflatoxin production. No aflatoxin-producing transformants were obtained. It is probable that a complete DNA fragment containing the gene(s) associated with the conversion of OMST to aflatoxins is not present on any one of the five cosmids. However, 5 of 90 transformant colonies generated from the cosmid NA had unique, somewhat pale orange mycelia on the Czapek Dox regeneration plates (CZR) unlike the white mycelia of the parent strain, SRRC 2043. When these transformants were subcultured onto coconut agar plates and examined closely under long-wave-length UV light, they appeared blue-yellow with decreased levels of fluorescent yellow pigments compared with the levels commonly produced by *A. parasiticus* SRRC 2043. This phenomenon was investigated further. Three transformants were grown in AM medium for pigment profile analyses. The results showed that they produced elevated quantities of known aflatoxin pathway intermediates, including NOR, AVN, AVF, VA, and OMST, in comparison with the control strain, SRRC 2043 (data not shown). The reasons for this overproduction phenomenon were examined.

Cloning of a DNA fragment responsible for pigment overproduction. Transformation of *A. parasiticus* RHN1 with a *niaD* vector, pHH, that contained a 12-kb *Hin*dIII DNA fragment isolated from the cosmid NA resulted in approximately 10% of the transformants having the pale orange colony morphology (Fig. 1). Further subcloning and transformation of *A. parasiticus* RHN1 with a 5.5-kb *Hin*dIII-*Xba*I DNA fragment (pHX1) that contained neither *nor-1* nor

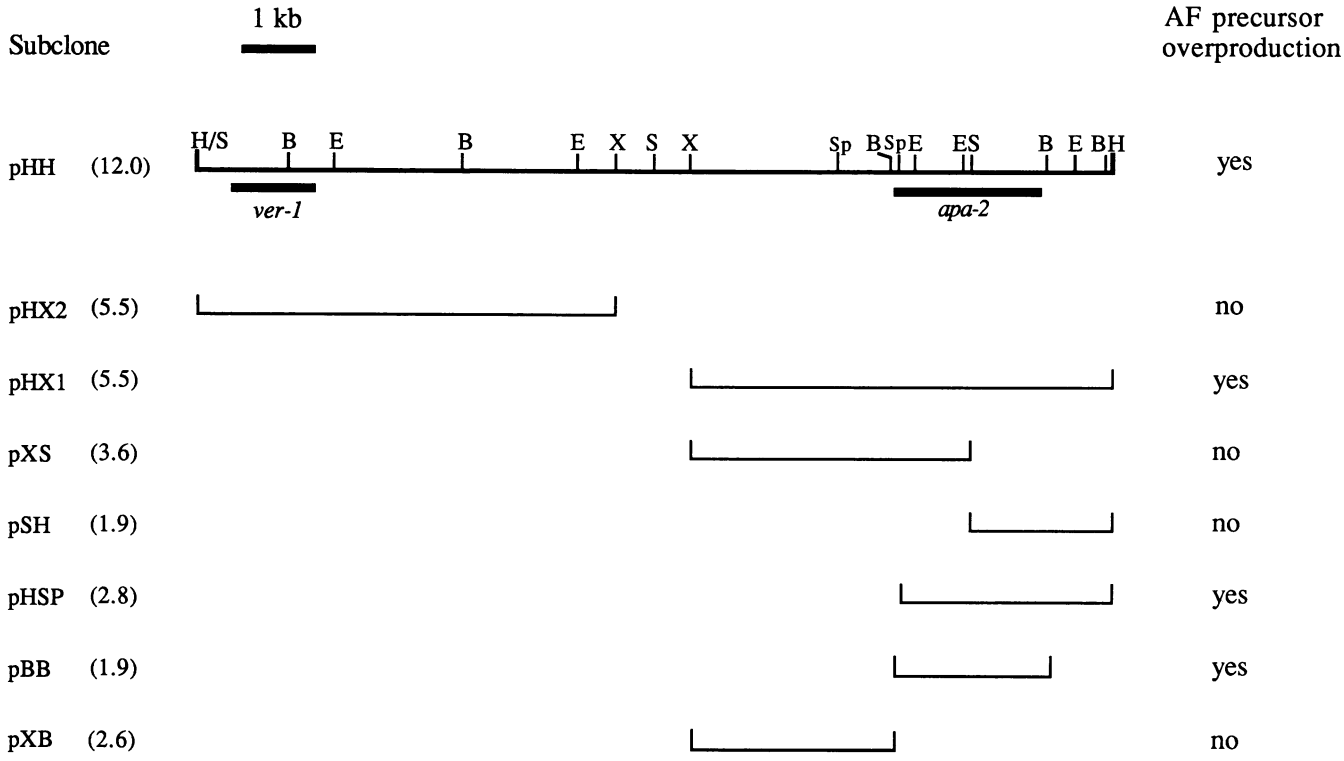

FIG. 1. Localization of the *A. parasiticus apa-2* gene based on overproduction of aflatoxin precursors through transformation of *A. parasiticus* RHN1 with various constructs. The 12-kb *Hin*dIII fragment was isolated from the cosmid NA. The position of *nor-1* is not shown here. Only the *Sph*I sites in the insert of pHX1 are shown in this map. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; S, *Sal*I; Sp, *Sph*I; X, *Xba*I.

ver-1 (Fig. 1) resulted in an elevated orange-red pigmentation in the mycelia of 50% of the nitrate-utilizing transformants on CZR. In contrast, transformation with pHX2, which contained *ver-1*, did not generate any pigmented transformants. Because the extent of mycelial pigmentation was similar among all pigmented RHN1(pHX1) transformants, two transformants were analyzed for the pathway intermediate profile. The result showed that levels of NOR, AVN, and VA were increased approximately four-, nine-, and threefold, respectively, in comparison with those of the wild-type, untransformed strain, SRRC 2043 (Table 1). Specific enzyme activities of the transformed strain associated with the conversion of NOR→AVN and ST→OMST were increased approximately twofold (Table 2). In light of this phenomenon, *A. parasiticus* RHN1 was used as the recipient strain, and pigment (intermediate) overproduction was used as an indicator for subsequent cloning work.

Localization of the putative gene responsible for intermediate overproduction. Subclones used in transformation of *A. parasiticus* RHN1 to localize the putative gene are shown in Fig. 1. It appeared that plasmid pHSP, a 2.8-kb *Hin*dIII-*Sph*I subclone of pHX1, increased pigmentation to a lesser extent in transformants grown on CZR than pHX1 did. However, thin-layer chromatography analyses indicated that the aflatoxin pathway intermediate profile of RHN1(pHSP) remained the same as that of RHN1(pHX1) transformants. Interestingly, transformation of RHN1 with constructs pXS and pSH (Fig. 1), which together encompass the 5.5-kb *Hin*dIII-*Xba*I region, did not generate any pigmented transformants on CZR. This result seems to exclude a possibility that intermediate overproduction was due to an unknown gene that resides very close to *apa-2*. Nevertheless, cotransformation of RHN1 with pXB and pHSP resulted in pig-

TABLE 1. Aflatoxin precursors produced by OMST-accumulating *A. parasiticus* strains

Metabolite	μg produced/g (dry wt) of mycelium in strain[a]:	
	SRRC 2043	RHN1(pHX1)[b]
NOR	0.03	0.14
AVN	0.09	0.83
AVF	Trace[c]	0.09
VA	0.02	0.07
Versicolorin B	Trace	0.01
ST	Trace	0.08
OMST	0.62	1.54

[a] The variation was never greater than 20%.
[b] Means of two transformants are shown.
[c] Trace, <0.01 μg.

TABLE 2. Enzyme activities in cell extracts of two strains of *A. parasiticus*

Reaction	Enzyme activity	Enzyme activity in extracts of strain:[a]	
		SRRC 2043	RHN1(pHX1)
NOR→AVN	Reductase	0.02 ± 0.005	0.06 ± 0.01
ST→OMST	Methyltransferase	0.08 ± 0.010	0.13 ± 0.03

[a] Expressed as micromoles of product per milligram of total protein per minute.

mented transformants with pigmentation levels similar to those with pHX1 (data not shown). Currently, we are examining the possibility that another putative gene may function additively or synergistically with *apa-2* and contribute to the observed elevated intermediate accumulation in *A. parasiticus* RHN1(pHX1). Transformation of *A. parasiticus* RHN1 with another construct, pBB, generated transformants with pigmentation levels similar to those with pHSP. These results indicate that the putative gene is located within the 1.8-kb *Bam*HI-*Sph*I DNA fragment. The functionality of this putative gene is very similar to that of *A. flavus afl-2* (36) as revealed by the increase in specific enzyme activities associated with the conversion of NOR→AVN and ST→OMST in *A. parasiticus* strains tested. Therefore, we speculated that it should share a certain degree of DNA homology with *afl-2*. This postulation was subsequently confirmed by Southern hybridization analysis of pHX1 with probes generated from *A. flavus afl-2*, which as expected hybridized strongly with 0.6- and 1.3-kb *Eco*RI-*Eco*RI DNA fragments (data not shown).

Sequence comparison of the *A. parasiticus apa-2* region with the *A. flavus* DNA containing *afl-2*. The region of DNA responsible for encoding the putative *A. flavus afl-2* gene product was localized to a 1.8-kb *Eco*RV-*Bam*HI DNA fragment (42). To determine the degree of DNA sequence homology between *apa-2* and *afl-2*, the majority of the 1.9-kb insert of pBB was sequenced. Comparison of both the nucleotide and amino acid levels showed that the region containing the putative *apa-2* gene had a striking similarity to the *A. flavus afl-2* gene (Fig. 2), with more than 95% DNA homology. Although a complete open reading frame for *afl-2* has not been identified yet, transformation of an *A. flavus afl-2* mutant strain, 650-33, with *apa-2* restored it to aflatoxin production, suggesting that the *A. parasiticus apa-2* gene is functionally homologous to the *A. flavus afl-2* gene. At this moment, attempts are being made to isolate full-length cDNA clones and to further characterize the *apa-2* gene.

Effects of elevated temperature and nitrate on aflatoxin pathway. Temperature is one of the most important environmental factors that influence aflatoxin production in toxigenic *A. flavus* and *A. parasiticus* strains (35, 38). A number of compounds and substances have also been shown to partially or effectively inhibit the production of aflatoxins (6, 25, 35, 46). One of these compounds, nitrate, has been demonstrated to completely repress the production of aflatoxin precursors, AVF, and versicolorins (6, 25). In this study, we investigated the effect of incubation at 37°C and nitrate on aflatoxin biosynthesis by analyzing the production of pathway intermediates and/or aflatoxins in *A. parasiticus* strains, RHN1 and SU1-N3, transformed with an *apa-2*-containing construct. Three strains, the OMST-accumulating SRRC 2043; RHN1(pSL82) (a *niaD* transformant), which was morphologically and nutritionally the same as SRRC 2043; and the wild-type, aflatoxigenic SU-1, were included as controls. For nitrate effect experiments, Cove's minimal salt medium containing 10 mM nitrate was used. For temperature effect experiments, aflatoxin-permissive AM medium was used. Stationary cultures were incubated at designated temperatures for up to 7 days. Pathway intermediates and aflatoxins were extracted and separated as described in the Materials and Methods section. For qualitative determination, appropriate aflatoxin or intermediate standards were included in each thin-layer chromatography analysis. The results (Table 3) indicate that the region of DNA containing the *apa-2* gene locus is responsible for the abolishment of the repressive effect of nitrate on aflatoxin production. However, no loss of repression by incubation at 37°C was seen in the transformants.

DISCUSSION

The development of transformation systems for *A. parasiticus* (22, 41) and *A. flavus* (43) has made genetic complementation a useful tool for cloning genes associated with aflatoxin biosynthesis. The established protocol allows the cloning of genes at specific blocked steps through functional complementation. However, for *A. parasiticus*, the number of blocked mutant strains available is limited compared with *A. flavus* (5). Although antibody screening of a cDNA library provides an alternative approach, pathway enzyme purification appears to be a great challenge to most researchers, because of the extremely short-lived nature of these enzymes (7, 19). *A. parasiticus* SRRC 2043 does not produce aflatoxins but accumulates the last known intermediate (OMST) in aflatoxin biosynthesis (9). This strain accumulates small amounts of pigmented aflatoxin pathway intermediates such as NOR, AVN, AVF, and VA in mycelia; it also shows higher enzyme activities in early steps compared with the wild type and other blocked strains (9). On the basis of the assumption of intermediate overproduction and hence change in mycelial color, it is possible to clone a gene encoding a putative regulatory factor that activates an entire pathway. The strategy of gene augmentation has been proven feasible, as is shown in the cloning of the *apa-2* gene through transformation of the OMST-accumulating strain, *A. parasiticus* SRRC 2043.

Clustering of aflatoxin biosynthetic genes has been reported (39). Complementation with sequences flanking known pathway genes such as *nor-1* and *ver-1* not only curtails a substantial amount of screening work, it facilitates the isolation of other clustered structural or regulatory genes. Conservation in *apa-2* and *afl-2* and their flanking regions suggests that organization of other pathway genes might be the same for *A. parasiticus* and *A. flavus*. Furthermore, the conservation may be extended to other aspergilli: for instance, the *ver-A* gene in *Aspergillus nidulans* has recently been identified through cross-hybridization with *A. parasiticus ver-1* (26). The relative positions of *nor-1*, *ver-1*, and *apa-2* have been mapped (this study and reference 31). This information should facilitate the cloning of the corresponding pathway genes in *A. flavus* and nonaflatoxigenic aspergilli such as *A. nidulans* and *Aspergillus versicolor*, which produce common aflatoxin pathway intermediates. Sequence comparison would also provide information concerning phylogenetic relationships among these aspergilli.

It is well established that elevated temperature (37°C) inhibits aflatoxin production in *A. flavus* and *A. parasiticus* (35, 38). Feng et al. (21) suggested that lack of aflatoxin production at 37°C was due to the inhibition of transcription of aflatoxin biosynthetic genes. In this study, the aflatoxin pathway was inhibited in untransformed and transformed strains at 37°C as evidenced by lack of production of pathway precursors and aflatoxins, suggesting a global effect of elevated temperature on the aflatoxin pathway (secondary metabolism). Similarly, nitrate has been shown to repress the aflatoxin pathway of *A. parasiticus* strains (6, 25). However, introduction of *apa-2* to either a blocked or a wild-type strain overcame the repressive effect of nitrate, suggesting an interrelatedness of aflatoxin biosynthesis and nitrate assimilation. It is probable that both processes are regulated in a concerted, antagonistic fashion through a common regulatory factor. The presence of an extra copy of

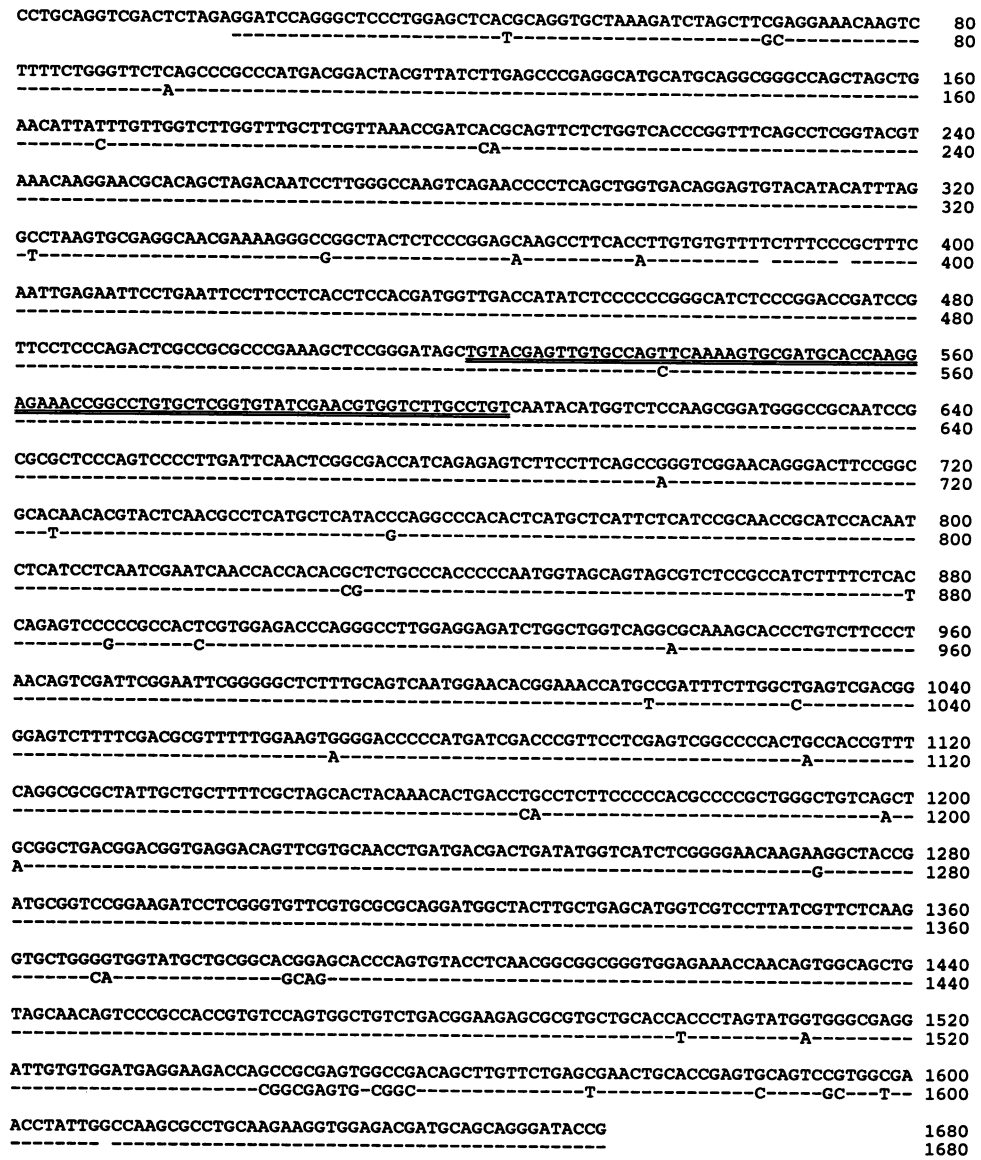

FIG. 2. Sequence comparison between the *A. parasiticus Bam*HI-*Bam*HI DNA fragment and the *A. flavus Eco*RV-*Bam*HI DNA fragment containing *afl-2*. The sequence of *A. parasiticus* is shown in the upper strand, and the sequence of *A. flavus* is shown in the lower strand. The region encoding a putative binuclear-type zinc finger motif (see Discussion) is double underlined. Nucleotides 1 to 20 belong to pUC18. Dashes indicate the same nucleotides in both fragments.

TABLE 3. Effects of nitrate and 37°C incubation on the aflatoxin biosynthetic pathway of *apa-2*-transformed *A. parasiticus* strains

Strain[a]	Nitrate[b]	37°C[c]
SRRC 2043	−	−
RHN1(pSL82)	−	−
RHN1(pHSP)	+	−
SU-1	−	−
SU1-N3(pHSP)	+	−

[a] See Results for description of strains.

[b] Cove's minimal salt medium containing 10 mM nitrate as the sole nitrogen source. −, pathway inactive (no precursors and/or aflatoxins detected); +, pathway active (precursors and/or aflatoxins detected).

[c] AM medium was prewarmed to 37°C prior to inoculation. −, no precursors and/or aflatoxins detected.

apa-2 (or an oversupply of *apa-2* gene product) may directly or indirectly deregulate the interrelated pathways; hence, it abolishes the repressive role of nitrate. Preliminary Northern (RNA) hybridization data showed that extremely low levels of transcripts of *apa-2*, *nor-1*, *ver-1*, and *omt-1* (encoding *O*-methyltransferase) in the wild-type SU-1 strain were detected only after 5 days' growth in Cove's nitrate medium (data not shown). This finding seems to be consistent with the notion that nitrate exerts its repressive effect on aflatoxin biosynthesis at the transcriptional level. However, we do not exclude the possibility that the nitrate effect may be posttranslational, as proposed by Niehaus and Jiang (35), such that increased transcription of the *apa-2* gene might overcome nitrate repression by a simple mass balance effect.

Comparison of the *apa-2* transcript (approximately 2.0 kb) and DNA sequence with an *A. nidulans* polyketide synthase

(PKS) and a putative *A. parasiticus* PKS excluded its identity as a PKS or part of a PKS (31, 34). It is highly probable that *apa-2* is a regulatory gene because the predicted amino acid sequence from one genomic DNA strand contains a cysteine-rich region (Cys-2-Cys-6-Cys-6-Cys-2-Cys-6-Cys, Fig. 2), homologous to the C_6 subgroup of binuclear-type zinc finger DNA binding motifs found in many fungal and *Saccharomyces cerevisiae* regulatory genes (2, 20, 24). Although no suitable *A. parasiticus* mutant strains defective in *apa-2* are available, introduction of *apa-2* into *A. parasiticus* SRRC 2043 resulted in the increase of specific enzyme activities associated with the conversion of NOR→AVN and ST→OMST, suggesting that *apa-2* enhances an existing gene function. Furthermore, complementation studies showed that *apa-2* and *afl-2* are functionally homologous. The *afl-2* gene has been shown to restore 80 to 95% of enzyme activities in an *afl-2* mutant of *A. flavus* (36). Taking into account the above observations, it is probable that aflatoxin pathway genes are regulated directly or indirectly through a common element, that is, *apa-2–afl-2*.

The appearance of sclerotia in aflatoxigenic *A. parasiticus* strains may enhance their survivability and competitiveness in harsh environments. Several studies have shown that aflatoxin production and sclerotial formation are interrelated (3, 16). In this laboratory, we are examining the effects of *apa-2* on the morphological and physiological aspects of *A. parasiticus*. Preliminary results indicate that in OMST-accumulating and wild-type *A. parasiticus*, sclerotial development was morphologically affected by the presence of an additional *apa-2* gene (data not shown). Currently, we are using gene disruption to elucidate the possible interrelatedness of aflatoxin biosynthesis, nitrate assimilation, and sclerotial morphogenesis.

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ADDENDUM IN PROOF

This study has demonstrated that the *apa-2* and *afl-2* genes from *A. parasiticus* and *A. flavus* have identical functions since *apa-2* can complement an *afl-2*-defective mutant of *A. flavus*. Therefore, in keeping with the genetic nomenclature routinely utilized by *Aspergillus* researchers (A. J. Clutterbuck, Genet. Res. (Cambridge), **21**:291–296, 1973), *apa-2* should be referred to as *aflR*, unless in subsequent studies differences are established between their specific roles in aflatoxin biosynthesis.

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