APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 2004, p. 1253–1262 Vol. 70, No. 3
0099-2240/04/$08.00+0 DOI: 10.1128/AEM.70.3.1253–1262.2004

MINIREVIEW

Clustered Pathway Genes in Aflatoxin Biosynthesis

Jiujiang Yu,[1]* Perng-Kuang Chang,[1] Kenneth C. Ehrlich,[1] Jeffrey W. Cary,[1] Deepak Bhatnagar,[1] Thomas E. Cleveland,[1] Gary A. Payne,[2] John E. Linz,[3] Charles P. Woloshuk,[4] and Joan W. Bennett[5]

Southern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, New Orleans, Louisiana 70124[1]; Department of Plant Pathology, North Carolina State University, Raleigh, North Carolina 27695[2]; Department of Food Science and Human Nutrition, Michigan State University, East Lansing, Michigan 48824[3]; Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907[4]; and Department of Cell and Molecular Biology, Tulane University, New Orleans, Louisiana 70118[5]

Aflatoxins, a group of polyketide-derived furanocoumarins (Fig. 1), are the most toxic and carcinogenic compounds among the known mycotoxins. Among the at least 16 structurally related aflatoxins characterized, however, there are only four major aflatoxins, B_1, B_2, G_1, and G_2 (AFB_1, AFG_1, AFB_2, and AFG_2), that contaminate agricultural commodities and pose a potential risk to livestock and human health (2, 8, 13, 33, 34, 52, 62, 81). *Aspergillus flavus* produces AFB_1 and AFB_2. *Aspergillus parasiticus* produces AFB_1, AFG_1, AFB_2, and AFG_2. Some other species that produce aflatoxins are *Aspergillus nomius*, *Aspergillus pseudotamarii* (51), *Aspergillus bombycis* (82), *Aspergillus ochraceoroseus* (60; J. C. Frisvad and R. A. Samson, Abstr. 10th Int. Congr. Mycol., p. 24, 2002), and *Emericella venezuelensis* (M. Klich, personal communication). Aflatoxins were discovered in *A. flavus* (hence the name "a-fla-toxin") about 40 years ago after an outbreak of Turkey X disease in England (60). Other significant members of the aflatoxin family, M_1 and M_2, are oxidative forms of aflatoxin B_1 modified in the digestive tract of some animals and isolated from milk, urine, and feces (14). Of the four aflatoxins, aflatoxin B_1 is the most potent hepatocarcinogenic compound. There has been very detailed research on the natural occurrence, identification, characterization, biosynthesis, and genetic regulation of aflatoxins, as well as on the prevention and control of aflatoxin contamination of food and feed. Aflatoxin biosynthesis has been proposed to involve at least 23 enzymatic reactions. Thus far, at least 15 structurally well-defined aflatoxin intermediates have been identified in the aflatoxin biosynthetic pathway (reviewed in references 8, 14, 15, 35, 73, 80, 94, 120, and 123). It has been demonstrated that 25 identified genes clustered within a 70-kb DNA region in the chromosome are involved in aflatoxin biosynthesis (94, 114). Here, we propose a new naming scheme that follows the naming convention in *Aspergillus*. These genes and their enzymes involved in the aflatoxin biosynthetic pathway are reviewed. Sterigmatocystin (ST) and dihydrosterigmatocystin (DHST), produced by certain strains of *Aspergillus nidulans* (37), are the penultimate precursors of aflatoxins. The homologous genes of ST synthesis in *A. nidulans* and their involvement in the biochemical pathway common to aflatoxins and ST are discussed.

* Corresponding author. Mailing address: U.S. Department of Agriculture, Agricultural Research Service, Southern Regional Research Center, 1100 Robert E. Lee Blvd., New Orleans, LA 70124. Phone: (504) 286-4405. Fax: (504) 286-4419. E-mail: jiuyu@srrc.ars.usda.gov.

AFLATOXIN PATHWAY GENE CLUSTER

The completed 70-kb DNA sequence containing the 25 genes or open reading frames (ORFs) represents a well-defined aflatoxin pathway gene cluster (Fig. 1). On average, about 2.8 kb of chromosomal DNA contains one gene. Among these genes are large ones of about 5 to 7 kb each, encoding the fatty acid synthase (FAS) alpha (5.8 kb) and beta (5.1 kb) subunits (FASα and FASβ) and polyketide synthase (PKS; 6.6 kb). Excluding these three large genes, the average size of the other 22 genes is about 2 kb. In the 5′ end of the cluster sequence, an approximately 2-kb DNA region with no identifiable ORF was located. This sequence presumably marks the end of this cluster in this orientation. The 3′ end of this gene cluster is delineated by a well-defined sugar utilization gene cluster consisting of four genes (33). The 82,081-bp fully annotated DNA sequence in *A. parasiticus* containing the aflatoxin pathway gene cluster and the sugar utilization gene cluster has been submitted to the GenBank database (nucleotide sequence accession number AY371490).

NEW NAMING SCHEME FOR THE AFLATOXIN PATHWAY GENES

The first aflatoxin biosynthesis gene cloned was *nor-1* in *A. parasiticus* (23). The name of this gene, like those of many other genes in the pathway, is based on the substrate converted by the gene product. The genes named according to substrates include *nor-1* (norsolorinic acid [NOR]), *norA* (NOR), *norB* (NOR), *avnA* (averantin [AVN]), *avfA* (averufin [AVF]), *ver-1* (versicolorin A [VERA]), *verA* (VERA), and *verB* (versicolorin B [VERB]). Other genes were named according to their enzymatic functions. These include *fas-2* (FAS alpha subunit), *fas-1* (FAS beta subunit), *pksA* or *pksL1* (PKS), *adhA* (alcohol dehydrogenase), *estA* (esterase), *vbs* (VERB synthase), *dmtA* (*mt-I*) (*O*-methyltransferase I) or *omtB* (*O*-methyltransferase B), *omtA* (*O*-methyltransferase A), *ordA* (oxidoreductase A), *cypA* (cytochrome P450 monooxygenase), *cypX* (cytochrome P450 monooxygenase), and *moxY* (monooxygenase). *fas-1* was initially named *uvm8* since it was identified through UV mutation. The *fas-2* and *fas-1* genes were also named *hexA* and

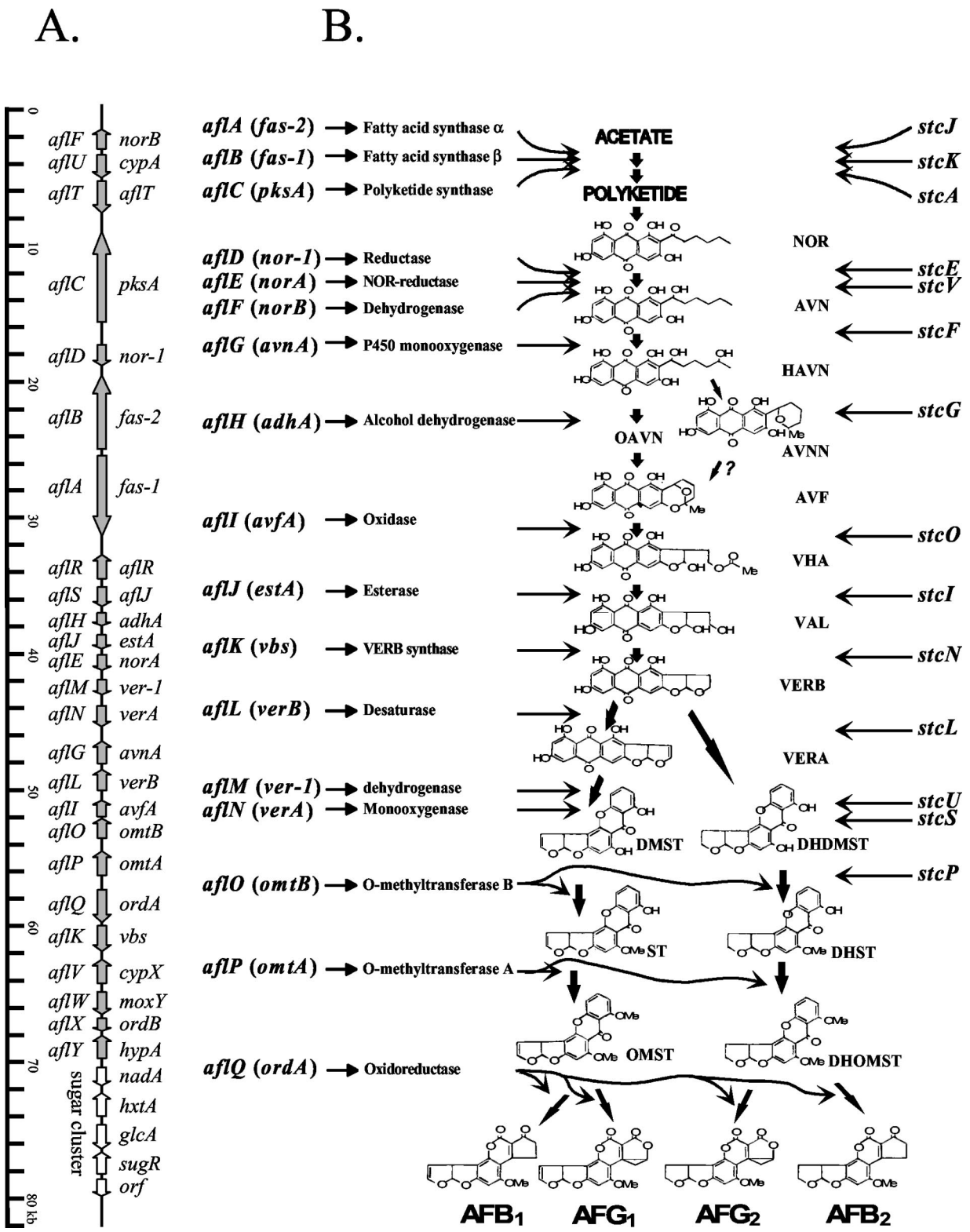

FIG. 1. Clustered genes (A) and the aflatoxin biosynthetic pathway (B). The generally accepted pathway for aflatoxin and ST biosynthesis is presented in panel B. The corresponding genes and their enzymes involved in each bioconversion step are shown in panel A. The vertical line represents the 82-kb aflatoxin biosynthetic pathway gene cluster and sugar utilization gene cluster in *A. parasiticus* and *A. flavus*. The new gene names are given on the left of the vertical line and the old gene names are given on the right. Arrows along the vertical line indicate the direction of gene transcription. The ruler at far left indicates the relative sizes of these genes in kilobases. The ST biosynthetic pathway genes in *A. nidulans* are indicated at the right of panel B. Arrows in panel B indicate the connections from the genes to the enzymes they encode, from the enzymes to the bioconversion steps they are involved in, and from the intermediates to the products in the aflatoxin bioconversion steps. Abbreviations: NOR, norsolorinic acid; AVN, averantin; HAVN, 5′-hydroxyaverantin; OAVN, oxoaverantin; AVNN, averufanin; AVF, averufin; VHA, versiconal hemiacetal acetate; VAL, versiconal; VERB, versicolorin B; VERA, versicolorin A; DMST, demethylsterigmatocystin; DHDMST, dihydrodemethylsterigmatocystin; ST, sterigmatocystin; DHST, dihydrosterigmatocystin; OMST, *O*-methylsterigmatocystin; DHOMST, dihydro-*O*-methylsterigmatocystin; AFB_1, aflatoxin B_1; AFB_2, aflatoxin B_2; AFG_1, aflatoxin G_1; AFG_2, aflatoxin G_2.

TABLE 1. Aflatoxin pathway cluster genes

Gene	Original name and other names used (accession no.)[a]	ST gene homolog[b]	Enzyme or product	Function in the pathway[e]
aflA	*fas-2* (*hexA*) (AF391094)	*stcJ*	FAS alpha subunit	Acetate → polyketide
aflB	*fas-1* (*hexB*) (AF391094), *uvm8, fas1, fas-1A* (L48183)	*stcK*	FAS beta subunit	Acetate → polyketide
aflC	*pksA* (Z47198), *pksL1* (L42765, L42766)	*stcA*	PKS	Acetate → polyketide
aflD	*nor-1* (L27801)	*stcE*	Reductase	NOR → AVN
aflE[c]	*norA, aad* (U24698), *adh-2* in *A. flavus* (U32377)	*stcV*	NOR reductase/dehydrogenase	NOR → AVN
aflF[c]	*norB*		Dehydrogenase	NOR → AVN
aflG	*avnA* (U62774), *ord-1* (L40839)	*stcF*	P450 monooxygenase	AVN → HAVN
aflH	*adhA* (U76621)	*stcG*	Alcohol dehydrogenase	HAVN → AVF or AVNN
aflI	*avfA* (AF154050), *ord-2* (L40840) (AF159789 in *A. flavus*)	*stcO*	Oxidase	AVF → VHA
aflJ	*estA* (AF417002)	*stcI*	Esterase	VHA → VAL
aflK	*vbs* (AF169016, U51327)	*stcN*	VERB synthase	VAL → VERB
aflL	*verB* (AF106958) (AF106959 and AF106960 in *A. flavus*)	*stcL*	Desaturase	VERB → VERA
aflM	*ver-1* (M91369)	*stcU*	Dehydrogenase/ketoreductase	VERA → DMST
aflN[c]	*verA*	*stcS* (*verA*)	Monooxygenase	VERA → DMST
aflO	*dmtA* (*mt-I*) (AB022905, AB022906), *omtB* (AF154050) (AF159789 in *A. flavus*)	*stcP*	*O*-Methyltransferase I or *O*-methyltransferase B	DMST → ST, DHDMST → DHST
aflP	*omtA* (L25834), *omt-1* cDNA (L22091), (L25836 in *A. flavus*)		*O*-Methyltransferase A or *O*-methyltransferase II	ST → OMST, DHST → DHOMST
aflQ	*ordA* (AF017151, AF169016), *A. flavus ord-1* (U81806, U81807)		Oxidoreductase/P450 monooxygenase	OMST → AFB_1 and AFG_1, DHOMST → AFB_2 and AFG_2
aflR	*aflR* (L26222), *apa-2* (L22177), *afl-2* (AF427616, AF441429)	*aflR*	Transcription activator	Pathway regulator
aflS	*aflJ* (AF002660) (AF077975 in *A. flavus*)	Unnamed	Transcription enhancer	Pathway regulator
aflT	*aflT* (AF268071)		Transmembrane protein	Unassigned
aflU	*cypA*		P450 monooxygenase	Unassigned
aflV	*cypX* (AF169016)	*stcB*	P450 monooxygenase	Unassigned
aflW	*moxY* (AF169016)	*stcW*	Monooxygenase	Unassigned
aflX	*ordB*	*stcQ*	Monooxygenase/oxidase	Unassigned
aflY	*hypA*		Hypothetical protein	Unassigned
aflR2[d]	*aflR2* (AF452809)	Second copy	Transcription activator	
aflS2	*aflJ2* (AF452809, AF295204)	Second copy	Transcription enhancer	
aflH2	*adhA2* (AF452809)	Second copy	Alcohol dehydrogenase	
aflJ2	*estA2* (AF452809)	Second copy	Esterase	
aflE2	*norA2* (AF452809)	Second copy	Dehydrogenase (early terminated)	
aflM2	*ver-1B* (AF452809)	Second copy	Dehydrogenase (missing N terminal)	
aflO2	*omtB2* (AF452809)	Second copy	Methyltransferase B (missing N terminal)	

[a] The accession number of the complete 82,081-bp aflatoxin gene cluster, including a sugar utilization gene cluster, in *A. parasiticus* is AY391490 and updates the sequences of the underlined accession numbers. The genes and their accession numbers are from *A. parasiticus* unless otherwise noted.

[b] The accession number of the ST gene cluster in *A. nidulans* is U34740, and the corresponding contig number is 1.132 (from 183018 to 242843) in the Whitehead database.

[c] The placements of *aflE* (*norA*), *aflF* (*norB*), and *aflN* (*verA*) in the pathway were based on their homologies to aflatoxin or ST genes and their functions have not been experimentally confirmed.

[d] The *aflR2, aflS2, aflH2, aflJ2, aflE2, aflM2*, and *aflO2* genes are partially duplicated cluster genes (second copy) in *A. parasiticus*, and their functions and chromosomal locations in the genome have not yet been clarified.

[e] Arrows signify conversion.

hexB for the hexanoate synthase alpha and beta subunits, respectively (GenBank accession no. AF391094). The *aflR* regulatory gene was initially named *afl-2* in *A. flavus* (79) and *apa-2* in *A. parasiticus* (24). This regulatory gene was later named *aflR* in both *A. flavus* and *A. parasiticus* as well as in *A. nidulans* for its function as a transcription activator. Another gene was demonstrated to be somehow involved in regulation and was named *aflJ* (72). For consistency and uniformity with the functions of the genes in the aflatoxin biosynthetic pathway, we institute here a consensus for gene naming in *Aspergillus* (4, 36). The three-letter code "*afl*" is used to represent aflatoxin pathway genes. A capital letter in alphabetical order from "A" to "Y" represents each individual gene confirmed to be or potentially involved in aflatoxin biosynthesis, e.g., *aflA* to *aflY* for all of the 25 genes and ORFs (Fig. 1) (Table 1). Those genes whose pathway involvement has already been characterized and confirmed or proposed on the basis of homologies to known genes in aflatoxin or ST synthesis are designated *aflA* to *aflQ* from the initial conversion of fatty acids to the final products, aflatoxins. *aflR* (retains the same name) and *aflS* (*aflJ*) are named for transcription regulators. Those genes whose pathway involvements are ambiguous or remain unclear at this point are designated *aflT* (retains the same name), *aflU* (*cypA*), *aflV* (*cypX*), *aflW* (*moxY*), *aflX* (*ordB*), and *aflY* (*hypA*)

TABLE 2. Aflatoxin cluster genes and their ST gene homologs

Gene (old name)	No. of aa[d]	Introns	$TCGN_5CGA$ position(s) (deviation)[a]	ST gene homolog	No. of aa for homolog	Introns in homolog	% aa identity (% of homologous aa)
aflA (*fas-2*)	1,671	2	−503 ($TCGN_5CGG$)	*stcJ*	1,559	1	48 (62)
aflB (*fas-1*)	1,888	3	−209 ($CCGN_5CGA$)	*stcK*	1,914	3	45 (61)
aflC (*pksA*)	2,109	5	−469, −118	*stcA* (*wA*)	2,181	2	60 (72)
aflD (*nor-1*)	271	3	−98	*stcE*	260	3	56 (73)
aflE (*norA*)	388	1	−150	*stcV*	387	2	66 (82)
aflF (*norB*)	382	0	−113				
aflG (*avnA*)	495	2	−171, −110 ($TCGN_5CGG$)	*stcF*	506	1	71 (84)
aflH (*adhA*)	278	0	−149	*stcG*	?	?	?
aflI (*avfA*)	285	0	Follow *omtB*?[b]	*stcO*	290 (297)[c]	0	55 (66)
aflJ (*estA*)	314	1	−144, −99	*stcI*	286	0	50 (64)
aflK (*vbs*)	643	1	−147	*stcN*	?	?	?
aflL (*verB*)	500	1	−86	*stcL*	500	1	82 (89)
aflM (*ver-1*)	262	2	−182, −149	*stcU* (*verA*)	264	2	90 (96)
aflN (*verA*)	492	1	−238	*stcS*	505	0	64 (75)
aflO (*omtB*)	386	3	−216	*stcP*	208 (379)[c]	3	75 (85)
aflP (*omtA*)	418	4	−174				
aflQ (*ordA*)	528	6	−114				
aflR (*aflR*)	444	0	−120, −249 (TTAGGCCTAA)	*aflR*	433	0	33 (45)
aflS (*aflJ*)	438	2	−74 ($CCGN_5CGA$)	Unnamed			
aflT (*aflT*)	514	5	−320 ($TCGN_5CGC$)				
aflU (*cypA*)	498	4	−176				
aflV (*cypX*)	508	2	−137	*stcB*	435	3	61 (73)
aflW (*moxY*)	481	0	−188, −170	*stcW*	488	3	69 (80)
aflX (*ordB*)	266	0	−145	*stcQ*	274	0	54 (68)
aflY (*hypA*)	495	2	−124				

[a] $TCGN_5CGA$ is the AflR-binding motif, and deviations from this typical motif are given. Confirmed AflR-binding sites (44) are underlined.
[b] No $TCGN_5CGA$ motif has been identified in the *aflI* (*avfA*) promoter region. It is likely cotranscribed with the *alfO* (*omtB*) gene.
[c] Based on a recent study of *A. parasiticus* (117), the correct number of amino acids in ST genes is suggested in parentheses.
[d] aa, amino acids.

(Table 1). We promote the use of this conventional naming system in the future. We also encourage the use of both the new name and the old name in parentheses, such as *aflA* (*fas-2*), the first time a gene designation appears in manuscripts to assist in understanding gene function.

The names of some of the duplicated aflatoxin genes (21, 30, 64) in *A. parasiticus* include the numeral "2," indicating second copy, such as *aflR2*, *aflS2* (*aflJ2*), *aflH2* (*adhA2*), *aflJ2* (*estA2*), *aflE2* (*norA2*), *aflM2* (*ver1B*), and *aflO2* (*omtB2*). The genes in this partial duplicated cluster are likely to be nonfunctional, possibly because of chromosomal location (31; J. Yu, unpublished observation). Recent evidence (J. E. Linz, unpublished observation) shows that the *aflM2* (*ver1B*) gene is expressed, but its translation remains to be investigated.

GENES IN AFLATOXIN BIOSYNTHESIS

Aflatoxins are polyketide-derived secondary metabolites produced via the following conversion path: acetate → polyketide → anthraquinones → xanthones → aflatoxins (3, 6, 11, 15, 102, 120). The genes involved in the major conversion steps from early precursors to aflatoxins and their functions are discussed below. The gene homologs in *A. nidulans* involved in the biosynthesis of ST are compared and discussed in Table 2.

***aflA* (*fas-2*), *aflB* (*fas-1*), and *aflC* (*pksA*) are involved in the conversion of acetate to NOR.** Molecular evidence has demonstrated that two FASs and a PKS are involved in the synthesis of a polyketide from the primary metabolite, acetate (16, 92). By complementation of an aflatoxin-blocked UV mutant, UVM8, Mahanti et al. (67) identified a gene initially named *uvm8* which is required for NOR biosynthesis and aflatoxin production in *A. parasiticus*. The predicted amino acid sequence of *uvm8* shares high degrees of similarity (67%) and identity (48%) to the beta subunit of FASs (FAS1) from *Saccharomyces cerevisiae* (96, 97). Complementation, metabolite feeding, and gene disruption experiments performed by Mahanti et al. (67) showed that the 7.5-kb transcript of the *uvm8* gene encodes one subunit of a novel FAS directly involved in the formation of the polyketide backbone prior to the conversion to the next stable metabolite, NOR, in aflatoxin synthesis. Because of its presumed function, the *uvm8* gene was renamed *fas-1A* or *fas-1* for the FAS beta subunit in aflatoxin biosynthesis. Additional sequence analyses of a cosmid clone found another FAS gene, *fas-2A*, encoding the alpha subunit of FAS (67). These names, *fas-1A* and *fas-2A*, were then simplified to *fas-1* and *fas-2* for the aflatoxin pathway gene cluster encoding FAS-1 (FASβ) and FAS-2 (FASα), respectively (80). The *fas-2* and *fas-1* genes were also named *hexA* and *hexB* for the hexanoate synthase alpha and beta subunits, respectively (GenBank accession no. AF391094). Brown et al. (16) proposed the involvement of FAS in ST biosynthesis in *A. nidulans*. They identified two genes, *stcJ* and *stcK*, encoding FASα and FASβ subunits (FAS-2 and FAS-1) in the ST cluster required for ST synthesis that are homologous to *fas-2* and *fas-1*, respectively. For consistency in nomenclature, these two genes, *fas-2* and *fas-1*, encoding the FAS alpha and beta subunits, have been designated *aflA* and *aflB*, respectively.

Watanabe et al. (99) reported the role of the FASs as well as the presence of a PKS in aflatoxin biosynthesis. Chang et al. (25) cloned the *pksA* gene encoding the PKS from *A. parasiticus*. Trail et al. (97) demonstrated, by using knockout experiments, that *pksA* is important for aflatoxin biosynthesis. The

pksA gene is weakly homologous to a PKS-encoding gene (*wA*) that was identified earlier in *A. nidulans* and involved in spore pigmentation (70). Feng and Leonard (45) also isolated a PKS gene, which they named *pksL1*, from *A. parasiticus*. Disruption of the *pksL1* gene produced neither aflatoxins nor any aflatoxin intermediates. *pksL1* was found to be identical to *pksA*, and they are most likely the same gene. Yu and Leonard (125) isolated a PKS gene, *pksST*, from *A. nidulans*. Its nucleotide sequence is identical to that of *stcA* from *A. nidulans* (17). However, no significant nucleotide sequence homology was found between *wA* (70) and *stcA* (*pksST*). The predicted amino acid sequences of these PKSs contain four conserved domains typical of other known PKS proteins: β-ketoacyl synthase, acyltransferase, acyl carrier protein, and thioesterase. The PKS gene from *A. parasiticus* (*pksA* or *pksL1*) was designated *pksA* (114) in the aflatoxin pathway gene cluster and its homolog in *A. nidulans* was designated *stcA* (17). Watanabe and Townsend (100) partially purified the roughly 1,400-kDa PKS NorS from *A. parasiticus*. NOR is the first stable intermediate in the pathway (1, 7, 76, 77). The conversion of noranthrone to NOR is poorly defined, but it has been proposed to occur via a noranthrone oxidase (98), a monooxygenase (12), or spontaneously (41). The *pksA* gene for this PKS is here renamed *aflC*.

***aflD* (*nor-1*), *aflE* (*norA*), and *aflF* (*norB*) are involved in the conversion of NOR to AVN.** By use of NOR-accumulating mutants, it was demonstrated by Papa (76, 77) in *A. flavus* and by Bennett (1) and Detroy et al. (40) in *A. parasiticus* that NOR is an intermediate in the aflatoxin biosynthetic pathway (7). It was found that the NOR-accumulating mutants are always leaky and that aflatoxin biosynthesis is not completely blocked (40). NOR is converted to AVN by a reductase/dehydrogenase enzyme, and this reaction is reversible depending on NADP(H) or NAD(H) (3, 12, 41, 106). Chang et al. (23) cloned the *nor-1* gene that complemented a NOR-accumulating mutant of *A. parasiticus*. It was demonstrated that this gene encoded a ketoreductase that was capable of converting NOR to AVN (90, 95). Cary et al. (19) cloned another possible allele of the NOR reductase gene, *norA*, which had about 70% homology to aryl-alcohol dehydrogenases. The *norA* gene may be involved in the conversion of NOR to AVN (19). However, deletion of *norA* did not impair the ability to convert NOR to AVN (20). This might be due to the presence of *aflD* (*nor-1*), *aflE2* (*norA2*), and other NOR reductase genes in the genome. The *norA* gene had no significant homology to the *nor-1* gene at either the DNA or amino acid level. An additional gene, *norB*, was identified in the aflatoxin gene cluster and was found to have no significant homology at the DNA level to either *nor-1* or *norA*. However, the homology to the *norA* protein at the amino acid level was as high as 68%. Attempts to delete the *norB* gene failed to generate mutants lacking aflatoxin production (Yu, unpublished). This might be due to the presence of the other two NOR reductase genes, *nor-1* and *norA*. The *nor-1* and *norA* gene homologs in *A. nidulans* are *stcE* and *stcV*, respectively. However, no *norB* gene homolog was identified in the ST gene cluster (17). The *norA* and *norB* genes were found in the *Aspergillus flavus* EST database to be expressed under aflatoxin-supportive medium conditions, indicating possible functional involvement in aflatoxin synthesis (Yu, unpublished). The enzymatic function and coordinated genetic regulation of the three genes are to be studied further. The *nor-1*, *norA*, and *norB* genes are renamed *aflD*, *aflE*, and *aflF*, respectively.

***aflG* (*avnA*) gene encodes a cytochrome P450 monooxygenase that converts AVN to HAVN.** Evidence that 5′-hydroxyaverantin (HAVN) is an intermediate in aflatoxin biosynthesis has been reported (5, 6). Yabe et al. (106) demonstrated for the first time that HAVN is a precursor of aflatoxins and that AVN is successively converted to HAVN and finally AVF by a microsome and cytosol enzyme, respectively. Yu et al. (116) cloned and characterized a gene that encoded a cytochrome P450 monooxygenase (originally reported as *ord-1* [114]). Gene disruption and substrate feeding studies (116) have demonstrated that HAVN and possibly an additional compound are the intermediate products in the conversion of AVN to AVF. This *avnA* gene is here renamed *aflG*.

***aflH (adhA)* is involved in conversion of HAVN to AVF.** In the scheme proposed by Yabe et al. (106, 108), averufanin (AVNN) was considered to be a shunt metabolite and not an aflatoxin intermediate. Bhatnagar et al. (12) proposed that both AVN and AVNN were intermediates in the pathway from NOR to AVF on the basis of radiolabeling experiments. However, Chang et al. (29) cloned a gene, *adhA*, which encodes an alcohol dehydrogenase. Disruption of *adhA* resulted in accumulation of HAVN in the fungal mycelia. These results suggested that HAVN is converted to AVF by the enzyme encoded by *adhA* (29). Sakuno et al. (85) recently succeeded in characterizing two cytosolic enzymes and an intermediate, 5′-oxoaverantin (OAVN), involved in this pathway from HAVN to AVF. The enzyme that converts HAVN to OAVN is consistent with the protein encoded by *adhA* (85). The gene for the second enzyme has yet to be identified. The *adhA* gene is here renamed *aflH*.

***aflI* (*avfA*) encodes an oxidase for conversion of AVF to VHA.** The conversion from AVF to versiconal hemiacetal acetate (VHA) is thought to involve an oxidase (12). Yu et al. (119) cloned a gene, *avfA*, from *A. parasiticus*, an *A. flavus* AVF-accumulating strain, and an *Aspergillus sojae* strain. Gene complementation experiments using the AVF-accumulating mutant strain demonstrated that the *avfA* gene encodes an enzyme (oxidase) that is necessary for the conversion of AVF to VHA. The *avfA* gene is here renamed *aflI*. A more recent study (111) identified an additional stable intermediate, hydroxyversicolorone (93), between AVF and VHA. In addition, several metabolites, versicolorone (9), versicolorol, versiconol acetate (VOAc), and versiconol (VOH), were found to be transiently accumulating as well (111). These metabolites might be involved in the shunt steps from hydroxyversicolorone to VHA and to versiconal (VAL). No genes responsible for these biological steps have thus far been identified in the aflatoxin cluster.

***aflJ* (*estA*) is involved in the conversion of VHA to VAL.** Evidence for the involvement of an esterase in the conversion of VHA to VAL in aflatoxin biosynthesis was found when *A. parasiticus* was treated with the organophosphorus pesticide dichlorvos (5, 48, 86, 105, 106, 112). The esterase was purified from *A. parasiticus* (50, 61), and the gene for an esterase, *estA*, was cloned (124). On the basis of its homology to *stcI* in the ST gene cluster in *A. nidulans* (17), this enzyme was proposed to be involved in the conversion of VHA to VAL in aflatoxin synthesis. Gene disruption demonstrated that *aflI* (*estA*) is

directly involved in the conversion of VHA to VAL and of VOAc to VOH in a separate conversion scheme of VHA to VOAc to VOH to VAL (P.-K. Chang et al., submitted for publication).

***aflK (vbs)* is involved in the conversion of VAL to VERB.** Conversion of VAL to VERB or versicolorin C in *A. parasiticus* has been shown to involve a versiconal cyclase (66). Yabe and Hamasaki (107) provided enzymatic evidence for the conversion. Silva et al. (88), Silva and Townsend (87), and McGuire et al. (71) cloned and demonstrated the function of the VERB synthase gene, *vbs*, for the conversion of VHA to VERB in *A. parasiticus*. This is a key step in aflatoxin formation since it closes the bisfuran ring of aflatoxin; this ring is required for binding to DNA and gives aflatoxin its mode of action as a mutagen. The *vbs* gene is here renamed *aflK*.

***aflL* (*verB*) is involved in the conversion of VERB to VERA.** Yabe and Hamasaki (107) have demonstrated that, in the aflatoxin biosynthetic pathway, the formation of VERA from VERB is a branch point separating biosynthesis of AFB_1 and AFG_1 from that of AFB_2 and AFG_2 (11, 13). The conversion of VERB to VERA has also been proposed and confirmed to require a desaturation of the bisfuran ring of VERB (107). Disruption of *stcL* in *A. nidulans* by Kelkar et al. (54) prevented ST synthesis and resulted in the accumulation of VERB, thereby showing that *stcL* encoding a P450 monooxygenase was required for the conversion. The *stcL* homolog, *aflL* (*verB*), from *A. parasiticus* and *A. flavus* was cloned in the aflatoxin pathway gene cluster (GenBank accession no. AF106958). The *aflL* gene encoding a cytochrome P450 monooxygenase/desaturase is presumed to be involved in the conversion of VERB to VERA in aflatoxin biosynthesis. The gene responsible for the conversion directly from VERB to demethyldihydrosterigmatocystin (DMDHST) and then to AFB_2 and AFG_2 has not been defined. It is possible that *aflL* is involved in conversion of both VERB to VERA and VERB to DMDHST.

***aflM* (*ver-1*) and *aflN* (*verA*) are involved in the conversion of VERA to DMST.** The *ver-1* gene involved in aflatoxin synthesis was first cloned in *A. parasiticus* (89). This gene was shown, by complementation of a *ver-1* mutant, to be required for the conversion of VERA to demethylsterigmatocystin (DMST) (65, 89). Keller et al. (56) identified a gene, *stcU* (formerly named *verA*), a homolog of *ver-1*, in *A. nidulans* that encodes a ketoreductase required for the conversion of VERA to DMST. Strains with mutations in both *stcU* and *stcL* showed accumulation of VERB only (56). Keller et al. (57) also identified *stcS* (formerly named *verB* [58]), encoding a cytochrome P450-type monooxygenase, which is involved in the conversion of VERA to DMST. Disruption of this gene resulted in the accumulation of VERA. Thus, both *stcU* and *stcS* are required for the conversion of VERA to DMST. The *verA* gene was recently identified in *A. parasiticus* SRRC 143. The *ver-1* gene is here renamed *aflM*, and *verA* is renamed *aflN*. The *aflM* homolog in the ST gene cluster is *stcU*, and *aflN* is now *stcS*. Sequence analysis indicated that the *aflN* gene encodes a cytochrome P450 monooxygenase and has high homology to *stcS*. It is presumed that both *aflM* and *aflN* are involved in the conversion of VERA to DMST in aflatoxin biosynthesis even though no significant sequence homology between *aflM* and *aflN* at either the DNA or amino acid level has been identified. It is interesting that some degree of amino acid sequence homology (45%) has been identified between *aflL* and *aflN*, but no sequence homology has been found between *aflL* and *aflM*. The exact function of *aflN* is yet to be determined.

***aflO* (*omtB*, *dmtA*) is involved in the conversion of DMST to ST and of DMDHST to DHST.** Yabe et al. (104) demonstrated two distinct *O*-methyltransferase activities in *A. parasiticus*. The enzyme for one of the two activities is named *O*-methyltransferase I for the conversion of DMST to ST (104); the same enzyme is also responsible for the conversion of DMDHST to DHST (104). This *O*-methyltransferase has been purified and characterized (109). The gene for this *O*-methyltransferase in *A. parasiticus* was cloned by Motomura et al. (74) and was named *dmtA* or *mt-I* for *O*-methyltransferase I. The same gene was concurrently cloned by Yu et al. (119) in *A. parasiticus*, *A. flavus*, and *A. sojae*. This gene was named *omtB* after the cloning of the *omtA* gene (113, 115; see below), so the enzyme encoded by this gene was named *O*-methyltransferase B. The gene homolog in *A. nidulans* is *stcP* (53). Disruption of *stcP* (53) demonstrated the requirement of this gene for the conversion from DMST to ST. *dmtA* or *omtB* is here renamed *aflO*.

***aflP (omtA)* is involved in the conversion of ST to OMST and DMST to DHOMST.** The involvement of an *O*-methyltransferase in the later step of aflatoxin formation has been studied extensively (10, 11, 55, 104). Yabe et al. (104) reported two methyltransferase activities involved in aflatoxin formation in *A. parasiticus*, *O*-methyltransferase I (mentioned above) and *O*-methyltransferase II for the conversion of ST to *O*-methylsterigmatocystin (OMST) and DHST to dihydro-*O*-methylsterigmatocystin (DHOMST) (104). The cDNA for the gene corresponding to this activity was cloned (named *omt-1*) from *A. parasiticus* by antibody screening of a cDNA expression library (113). The enzyme was expressed in *Escherichia coli*, and its activity for converting ST to OMST was demonstrated by substrate feeding studies (113). The genomic DNA sequence for this gene was also cloned (named *omtA*) from *A. parasiticus* and *A. flavus* (115). The *omt-1* or *omtA* gene is here renamed *aflP*. A gene disruption experiment by Lee et al. (63) unambiguously demonstrated the function of *omtA* in vivo.

***aflQ* (*ordA*) is involved in the conversion of OMST to AFB_1 and AFG_1 and of DMDHST to AFB_2 and AFG_2.** The biosynthetic relationship between B-group (AFB_1 and AFB_2) and G-group (AFG_1 and AFG_2) aflatoxins has been proposed (11, 32, 103). Enzymatic studies have demonstrated the involvement of an NADPH-dependent monooxygenase (12, 103, 110) in the conversion of OMST to AFB_1 in the late stages of aflatoxin biosynthesis. Cleveland (32) demonstrated in a substrate feeding study with two *A. parasiticus* mutant strains that DHOMST was converted to AFB_2. A cytochrome P450 monooxygenase gene, *ord-1*, was reported to be required for this reaction in *A. flavus* (83, 84). Yu et al. (117) cloned the *ord-1* gene (then named *ordA*, now renamed *aflQ*), encoding a cytochrome P450 monooxygenase, from *A. parasiticus* and *A. flavus*. It has been demonstrated by expression and substrate feeding in a yeast system that this gene is responsible for the conversion of OMST to AFB_1 and AFG_1 and of DHOMST to AFB_2 and AFG_2 (117); the critical amino acids for the enzymatic activity and heme-binding motif were identified by site-directed mutagenesis. It has also been demonstrated that the synthesis of G-group toxins (AFG_1 and AFG_2) requires en-

zymes in addition to those necessary for B-group aflatoxin synthesis (110, 117).

GENES INVOLVED IN PATHWAY REGULATION

***aflR* is involved in transcription activation.** In both the aflatoxin and ST gene clusters, there is a positive regulatory gene, *aflR* (originally named *afl-2* [79] and *apa-2* [24]), for activating pathway gene transcription. The *aflR* gene encodes a sequence-specific zinc binuclear DNA-binding protein, a Gal 4-type 47-kDa polypeptide, and has been shown to be required for transcriptional activation of most, if not all, of the structural genes (24, 26, 27, 28, 42, 49, 79, 101, 126). The transcription of aflatoxin pathway genes can be activated when the AflR protein binds to the palindromic sequence 5′-TCGN$_5$CGA-3′ (also called AflR-binding motif) in the promoter region of the structural genes (43, 44, 47) in *A. parasiticus*, *A. flavus*, and *A. nidulans*. The AflR-binding motifs are located from position −80 to position −600, with the majority at the −100 to −200 positions relative to the translation start site. AflR binds, in some cases, to a deviated sequence rather than the typical motif, such as in the case of *aflG* (*avnA*). When there is more than one such motif in the promoter region of a gene, only one is a preferred binding site, such as in the case of *aflC* (*pksA* [43, 44]). *A. sojae*, a nontoxigenic strain used in industrial fermentations, was found to contain a defective *aflR* gene in addition to other defects in the aflatoxin pathway structural genes (68, 69, 91). Thus, in the absence of the functional regulatory protein, no induction of aflatoxin can occur in this food grade *Aspergillus*.

***aflS (aflJ)* gene is involved in regulation of aflatoxin biosynthesis.** Adjacent to the *aflR* gene in the aflatoxin gene cluster, a divergently transcribed gene, *aflS* (originally named *aflJ*), was also found to be involved in the regulation of transcription (72). A recent study (22) has shown that *aflS* interacts with *aflR* but not the structural genes. In the *aflS* knockout mutants, the lack of *aflS* transcript is associated with a 5- to 20-fold reduction of expression of some aflatoxin pathway genes such as *aflC* (*pksA*), *aflD* (*nor-1*), *aflM* (*ver-1*), and *aflP* (*omtA*) and a loss of the ability to synthesize aflatoxin intermediates (72). The *aflS* homolog was located adjacent to the *aflR* gene in the ST gene cluster (U34740), but no name has yet been given to it (Daren Brown, personal communication). The exact mechanism by which *aflS* modulates transcription of these pathway genes in concert with *aflR* is presently being investigated in a USDA laboratory (Southern Regional Research Center, New Orleans, La.) by gene expression analysis using microarray technology.

CLUSTER GENES UNASSIGNED TO THE PATHWAY

Recently, additional genes have been identified in the gene cluster which are putatively involved in aflatoxin biosynthesis (Table 1). A typical AflR-binding motif was identified in the untranslated region (UTR) of all of these genes in the gene cluster, indicating that they are potential targets for AflR. In contrast, no AflR-binding motif was identified in the UTR of the four sugar utilization genes (*nadA*, *hxtA*, *glcA*, and *sugR*) that are found adjacent to the aflatoxin gene cluster (Table 2). More importantly, these new genes, like other characterized aflatoxin pathway genes, were found to be expressed under aflatoxigenic growth conditions in the *Aspergillus flavus* EST database, indicating possible functional involvement in aflatoxin synthesis (Yu, unpublished).

***aflT*.** In the aflatoxin pathway gene cluster, a gene named *aflT*, encoding a membrane-bound protein with homology to antibiotic efflux genes presumed to be involved in aflatoxin secretion, was discovered in *A. parasiticus* (P.-K. Chang et al., unpublished data). However, disruption of this gene does not affect aflatoxin formation (Chang et al., unpublished).

***aflU (cypA)*.** *aflU* encodes a polypeptide of 498 amino acids. A Blast search identified significant homologies to cytochrome P450-type monooxygenase enzymes in the GenBank database. A typical heme-binding motif of cytochrome P450 monooxygenase has been identified near the C terminus. Expression studies using reverse transcriptase PCR showed that the transcript was detected only under aflatoxin-conducive conditions (81, 113) and not on nonconducive medium (peptone medium) (Yu, unpublished). These observations support the possible involvement of this gene in aflatoxin biosynthesis.

***aflV* (*cypX*).** The *aflV* gene encodes another cytochrome P450 monooxygenase (118) and is homologous to *stcB* in *A. nidulans*. Gene knockout experiments have been performed extensively on the *aflV* gene. Unfortunately, no conclusive results have been obtained. Keller et al. (59) also disrupted *stcB*, the *aflV* homolog in *A. nidulans*, but were unable to demonstrate a clear role in biosynthesis.

***aflW* (*moxY*).** *aflW* encodes a monooxygenase (118) which is homologous to *stcW* in ST synthesis in *A. nidulans* (54). As with *aflV*, no conclusive results regarding aflatoxin synthesis could be asserted despite studies using disruption experiments in *A. parasiticus* and its homolog *stcW* in *A. nidulans*.

***aflX* (*ordB*).** Adjacent to *aflW*, an additional gene, *aflX* (*ordB*), was found. The *aflX* gene encodes a polypeptide of 266 amino acids with significant homology to an oxidase in the GenBank database. At the amino acid level, the *aflX* gene shows 54% identity and 68% similarity to *stcQ* in the ST gene cluster in *A. nidulans* (17). No intron has been identified in the coding region. We tentatively named it *ordB* due to its possible function as an oxidoreductase in aflatoxin synthesis. However, no pathway-specific involvement of this gene has yet been defined.

***aflY* (*hypA*).** Adjacent to the *aflX* gene, another new gene, *aflY* (*hypA*), which encodes a polypeptide with homology to a hypothetical protein, was also identified. The *aflY* gene encodes a polypeptide of 495 amino acids with unknown function. No *aflY* gene homolog was identified in the *A. nidulans* gene cluster.

CONCLUDING REMARKS

Genes involved in most of the bioconversion steps in the aflatoxin/ST biosynthetic pathway have been confirmed through either gene disruption or enzymatic studies. However, details of several biological conversion steps and of genes responsible for the reactions have not yet been deciphered. Among the 25 genes identified in the aflatoxin biosynthetic pathway gene cluster, the functions of 19 in aflatoxin biosynthesis have been assigned and the functions of 6 are unassigned. Among the genes assigned to the pathway steps, the

placements of *aflE* (*norA*), *aflF* (*norB*), and *aflN* (*verA*) were based on their homologies to aflatoxin or ST genes and their functions have not been experimentally confirmed (Table 1). It has been demonstrated (109, 110, 117) that additional enzymes are required for G-group toxin formation in *A. parasiticus*. However, an enzyme(s) or corresponding gene(s) for such reactions has yet to be identified. The possibility remains that one or more of these unassigned genes, such as *aflU*, *aflX*, and/or *aflY*, on the basis of their predicted enzymatic functions, might be involved in G-group toxin formation.

Aflatoxins and ST share almost identical biochemical pathways. The majority of the homologous genes and their enzymes involved in the two pathways, except for the last two steps, are identified (Table 1). However, no gene homologs are identified for aflatoxin cluster genes (*aflD*, *aflF*, *aflT*, *aflU*, and *aflY*) in the ST gene cluster and for ST cluster genes (*stcC*, *stcD*, *stcH*, *stcM*, *stcR*, *stcT*, and *stcX*) in the aflatoxin gene cluster. The *aflS* gene homolog was located in the ST gene cluster. However, its function is unclear and no name has yet been given (Daren Brown, personal communication). Note that *aflP* and *aflQ* are not essential to ST biosynthesis since ST is the final product in *A. nidulans*. These two genes are either nonfunctional or have been lost in the evolutionary process since no homologous genes have been identified either within or outside the ST gene cluster (Table 1). The possibility exists that some of the genes involved in aflatoxin and ST biosynthesis are located somewhere outside the gene clusters. The genetic control of aflatoxin biosynthesis in relation to primary metabolism and environmental stimuli is apparently beyond this defined gene cluster (18, 38, 39, 46, 49, 78, 80, 122). Identification of all of the genes and global regulators involved in and related to aflatoxin biosynthesis in the fungal system is a daunting challenge. *A. flavus* genomics and microarray technologies (75, 121, 122) will provide a new avenue for deciphering such mechanisms and unraveling these regulatory elements governing aflatoxin biosynthesis.

REFERENCES

1. **Bennett, J. W.** 1981. Loss of norsolorinic acid and aflatoxin production by a mutant of *Aspergillus parasiticus*. J. Gen. Microbiol. **124:**429–432.
2. **Bennett, J. W.** 1987. Mycotoxins, mycotoxicoses, mycotoxicology and *Mycopathologia*. Mycopathologia **100:**3–5.
3. **Bennett, J. W., and S. B. Christensen.** 1983. New perspectives on aflatoxin biosynthesis. Adv. Appl. Microbiol. **29:**53–92.
4. **Bennett, J. W., and L. L. Lasure.** 1985. Conventions for gene symbols, p. 538–544. *In* J. W. Bennett and L. L. Lasure (ed.), Gene manipulations in fungi. Academic Press, New York, N.Y.
5. **Bennett, J. W., L. S. Lee, and A. F. Cucullu.** 1976. Effect of dichlorvos on aflatoxin and versicolorin A production in *Aspergillus parasiticus*. Bot. Gaz. **137:**318–324.
6. **Bennett, J. W., L. S. Lee, S. M. Shoss, and G. H. Boudreaux.** 1980. Identification of averantin as an aflatoxin B_1 precursor: placement in the biosynthetic pathway. Appl. Environ. Microbiol. **39:**835–839.
7. **Bennett, J. W., P.-K. Chang, and D. Bhatnagar.** 1997. One gene to whole pathway: the role of norsolorinic acid in aflatoxin research. Adv. Appl. Microbiol. **45:**1–15.
8. **Bennett, J. W., and M. Klich.** 2003. Mycotoxins. Clin. Microbiol. Rev. **16:**497–516.
9. **Berger-Deguee, M., and Y. Bergar.** 1982. Structure of versicolorone isolated from *Aspergillus versicolor*. Phytochemistry **21:**1449–1451.
10. **Bhatnagar, D., A. H. J. Ullah, and T. E. Cleveland.** 1988. Purification and characterization of a methyltransferase from *Aspergillus parasiticus* SRRC 163 involved in aflatoxin biosynthetic pathway. Prep. Biochem. **18:**321–349.
11. **Bhatnagar, D., T. E. Cleveland, and D. G. I. Kingston.** 1991. Enzymological evidence for separate pathways for aflatoxin B1 and B2 biosynthesis. Biochemistry **30:**4343–4350.
12. **Bhatnagar, D., K. C. Ehrlich, and T. E. Cleveland.** 1992. Oxidation-reduction reactions in biosynthesis of secondary metabolites, p. 255–286. *In* D. Bhatnagar, E. B. Lillehoj, and D. K. Arora (ed.), Handbook of applied mycology: mycotoxins in ecological systems. Marcel Dekker, New York, N.Y.
13. **Bhatnagar, D., K. C. Ehrlich, and T. E. Cleveland.** 1993. Biochemical characterization of an aflatoxin B_2 producing mutant of *Aspergillus flavus*. FASEB J. **7:**A1234.
14. **Bhatnagar, D., J. Yu, and K. C. Ehrlich.** 2002. Toxins of filamentous fungi. Chem. Immunol. **81:**167–206.
15. **Bhatnagar, D., K. C. Ehrlich, and T. E. Cleveland.** 2003. Molecular genetic analysis and regulation of aflatoxin biosynthesis. Appl. Microbiol. Biotechnol. **61:**83–93.
16. **Brown, D. W., T. H. Adams, and N. P. Keller.** 1996. *Aspergillus* has distinct fatty acid synthases for primary and secondary metabolism. Proc. Natl. Acad. Sci. USA **19:**14873–14877.
17. **Brown, D. W., J.-H. Yu, H. S. Kelkar, M. Fernandes, T. C. Nesbitt, N. P. Keller, T. H. Adams, and T. J. Leonard.** 1996. Twenty-five coregulated transcripts define a sterigmatocystin gene cluster in *Aspergillus nidulans*. Proc. Natl. Acad. Sci. USA **93:**1418–1422.
18. **Calvo, A. M., R. A. Wilson, J. W. Bok, and N. P. Keller.** 2002. Relationship between secondary metabolism and fungal development. Microbiol. Mol. Biol. Rev. **66:**447–459.
19. **Cary, J. W., M. Wright, D. Bhatnagar, R. Lee, and F. S. Chu.** 1996. Molecular characterization of an *Aspergillus parasiticus* dehydrogenase gene, *norA*, located on the aflatoxin biosynthesis gene cluster. Appl. Environ. Microbiol. **62:**360–366.
20. **Cary, J. W., N. Barnaby, K. C. Ehrlich, and D. Bhatnagar.** 1999. Isolation and characterization of experimentally induced, aflatoxin biosynthetic pathway deletion mutants of *Aspergillus parasiticus*. Appl. Microbiol. Biotechnol. **51:**808–812.
21. **Cary, J. W., J. M. Dyer, K. C. Ehrlich, M. S. Wright, S. H. Liang, and J. E. Linz.** 2002. Molecular and functional characterization of a second copy of the aflatoxin regulatory gene, aflR-2, from *Aspergillus parasiticus*. Biochim. Biophys. Acta **1576:**316–323.
22. **Chang, P.-K.** 2003. The *Aspergillus parasiticus* protein *AFLJ* interacts with the aflatoxin pathway-specific regulator AFLR. Mol. Genet. Genomics **268:**711–719.
23. **Chang, P.-K., C. D. Skory, and J. E. Linz.** 1992. Cloning of a gene associated with aflatoxin B1 biosynthesis in *Aspergillus parasiticus*. Curr. Genet. **21:**231–233.
24. **Chang, P.-K., J. W. Cary, D. Bhatnagar, T. E. Cleveland, J. W. Bennett, J. E. Linz, C. P. Woloshuk, and G. A. Payne.** 1993. Cloning of the *Aspergillus parasiticus apa-2* gene associated with the regulation of aflatoxin biosynthesis. Appl. Environ. Microbiol. **59:**3273–3279.
25. **Chang, P.-K., J. W. Cary, J. Yu, D. Bhatnagar, and T. E. Cleveland.** 1995. *Aspergillus parasiticus* polyketide synthase gene, *pksA*, a homolog of *Aspergillus nidulans wA*, is required for aflatoxin B_1. Mol. Gen. Genet. **248:**270–277.
26. **Chang, P.-K., K. C. Ehrlich, J. Yu, D. Bhatnagar, and T. E. Cleveland.** 1995. Increased expression of *Aspergillus parasiticus aflR*, encoding a sequence-specific DNA binding protein, relieves nitrate inhibition of aflatoxin biosynthesis. Appl. Environ. Microbiol. **61:**2372–2377.
27. **Chang, P.-K., J. Yu, D. Bhatnagar, and T. E. Cleveland.** 1999. Repressor-AFLR interaction modulates aflatoxin biosynthesis in *Aspergillus parasiticus*. Mycopathologia **147:**105–112.
28. **Chang, P.-K., J. Yu, D. Bhatnagar, and T. E. Cleveland.** 1999. The carboxy-terminal portion of the aflatoxin pathway regulatory protein *AFLR* of *Aspergillus parasiticus* activates *GAL1*::*lacZ* gene expression in *Saccharomyces cerevisiae*. Appl. Environ. Microbiol. **65:**2508–2512.
29. **Chang, P.-K., J. Yu, K. C. Ehrlich, S. M. Boue, B. G. Montalbano, D. Bhatnagar, and T. E. Cleveland.** 2000. The aflatoxin biosynthesis gene *adhA* in *Aspergillus parasiticus* is involved in conversion of 5′-hydroxyaverantin to averufin. Appl. Environ. Microbiol. **66:**4715–4719.
30. **Chang, P.-K., and J. Yu.** 2002. Characterization of a partial duplication of the aflatoxin gene cluster in *Aspergillus parasiticus* ATCC 56775. Appl. Microbiol. Biotechnol. **58:**632–636.
31. **Chiou, C. H., M. Miller, D. L. Wilson, F. Trail, and J. E. Linz.** 2002. Chromosomal location plays a role in regulation of aflatoxin gene expression in *Aspergillus parasiticus*. Appl. Environ. Microbiol. **68:**306–315.
32. **Cleveland, T. E.** 1989. Conversion of dihydro-*O*-methylsterigmatocystin to aflatoxin B_2 by *Aspergillus parasiticus*. Arch. Environ. Contam. Toxicol. **18:**429–433.
33. **Cleveland, T. E., and D. Bhatnagar.** 1991. Molecular regulation of aflatoxin biosynthesis, p. 270–287. *In* G. A. Bray and D. H. Ryan (ed.), Mycotoxins, cancer and health. Pennington Center Nutrition Series, vol. 1. LSU Press, Baton Rouge, La.
34. **Cleveland, T. E., and D. Bhatnagar.** 1992. Molecular strategies for reducing aflatoxin levels in crops before harvest, p. 205–228. *In* D. Bhatnagar and T. E. Cleveland (ed.), Molecular approaches to improving food quality and safety. Van Nostrand Reinhold, New York, N.Y.
35. **Cleveland, T. E., J. W. Cary, R. L. Brown, D. Bhatnagar, J. Yu, P.-K. Chang, C. A. Chlan, and K. Rajasekaran.** 1997. Use of biotechnology to

eliminate aflatoxin in preharvest crops. Bull. Inst. Compr. Agric. Sci. Kinki Univ. **5:**75–90.
36. **Clutterbuck, A. J.** 1973. Gene symbols in *Aspergillus nidulans*. Genet. Res. **21:**201–296.
37. **Cole, R. J., and E. H. Cox.** 1987. Handbook of toxic fungal metabolites. Academic Press, New York, N.Y.
38. **Cotty, P. J.** 1988. Aflatoxin and sclerotial production by *Aspergillus flavus*: influence of pH. Phytopathology **78:**1250–1253.
39. **Demain, A. L.** 1972. Cellular and environmental factors affecting the synthesis and excretion of metabolites. J. Appl. Chem. Biotechnol. **22:**345–372.
40. **Detroy, R. W., S. Freer, and A. Ciegler.** 1973. Aflatoxin and anthraquinone biosynthesis by nitrosoquanidine-derived mutants of *Aspergillus parasiticus*. Can. J. Microbiol. **19:**1373–1378.
41. **Dutton, M. F.** 1988. Enzymes and aflatoxin biosynthesis. Microbiol. Rev. **52:**274–295.
42. **Ehrlich, K. C., B. G. Montalbano, D. Bhatnagar, and T. E. Cleveland.** 1998. Alteration of different domains in AFLR affects aflatoxin pathway metabolism in *Aspergillus parasiticus* transformants. Fungal Genet. Biol. **23:**279–287.
43. **Ehrlich, K. C., J. W. Cary, and B. G. Montalbano.** 1999. Characterization of the promoter for the gene encoding the aflatoxin biosynthetic pathway regulatory protein AFLR. Biochim. Biophys. Acta **1444:**412–417.
44. **Ehrlich, K. C., B. G. Montalbano, and J. W. Cary.** 1999. Binding of the C6-zinc cluster protein, AFLR, to the promoters of aflatoxin pathway biosynthesis genes in *Aspergillus parasiticus*. Gene **230:**249–257.
45. **Feng, G. H., and T. J. Leonard.** 1995. Characterization of the polyketide synthase gene (*pksL1*) required for aflatoxin biosynthesis in *Aspergillus parasiticus*. J. Bacteriol. **177:**6246–6254.
46. **Feng, G. H., and T. J. Leonard.** 1998. Culture conditions control expression of the genes for aflatoxin and sterigmatocystin biosynthesis in *Aspergillus parasiticus* and *A. nidulans*. Appl. Environ. Microbiol. **64:**2275–2277.
47. **Fernandes, M., N. P. Keller, and T. H. Adams.** 1998. Sequence-specific binding by *Aspergillus nidulans* AflR, a C6 zinc cluster protein regulating mycotoxin biosynthesis. Mol. Microbiol. **28:**1355–1365.
48. **Fitzell, D. L., R. Singh, D. P. H. Hsieh, and E. L. Motell.** 1977. Nuclear magnetic resonance identification of versicolor hemiacetal acetate as an intermediate in aflatoxin biosynthesis. Agric. Food Chem. **25:**1193–1197.
49. **Flaherty, J. E., and G. A. Payne.** 1997. Overexpression of *aflR* leads to upregulation of pathway gene expression and increased aflatoxin production in *Aspergillus flavus*. Appl. Environ. Microbiol. **63:**3995–4000.
50. **Hsieh, D. P., C. C. Wan, and J. A. Billington.** 1989. A versiconal hemiacetal acetate converting enzyme in aflatoxin biosynthesis. Mycopathologia **107:** 121–126.
51. **Ito, Y., S. W. Peterson, D. T. Wicklow, and T. Goto.** 2001. *Aspergillus pseudotamarii*, a new aflatoxin producing species in *Aspergillus* section *Flavi*. Mycol. Res. **105:**233–239.
52. **Jelinek, C. F., A. E. Pohland, and G. E. Wood.** 1989. Worldwide occurrence of mycotoxins in foods and feeds—an update. J. Assoc. Off. Anal. Chem. **72:**223–230.
53. **Kelkar, H. S., N. P. Keller, and T. H. Adams.** 1996. *Aspergillus nidulans stcP* encodes an *O*-methyltransferase that is required for sterigmatocystin biosynthesis. Appl. Environ. Microbiol. **62:**4296–4298.
54. **Kelkar, H. S., S. Hernant, T. W. Skloss, J. F. Haw, N. P. Keller, and T. H. Adams.** 1997. *Aspergillus nidulans stcL* encodes a putative cytochrome P-450 monooxygenase required for bisfuran desaturation during aflatoxin/sterigmatocystin biosynthesis. J. Biol. Chem. **272:**1589–1594.
55. **Keller, N. P., J. H. C. Dischinger, D. Bhatnagar, T. E. Cleveland, and A. H. J. Ullah.** 1993. Purification of a 40-kilodalton methyltransferase active in the aflatoxin biosynthetic pathway. Appl. Environ. Microbiol. **59:**479–484.
56. **Keller, N. P., N. J. Kantz, and T. H. Adams.** 1994. *Aspergillus nidulans verA* is required for production of the mycotoxin sterigmatocystin. Appl. Environ. Microbiol. **60:**1444–1450.
57. **Keller, N. P., S. Segner, D. Bhatnagar, and T. H. Adams.** 1995. *stcS*, a putative P-450 monooxygenase, is required for the conversion of versicolorin A to sterigmatocystin in *Aspergillus nidulans*. Appl. Environ. Microbiol. **61:**3628–3632.
58. **Keller, N. P., D. Brown, R. A. E. Butchko, M. Fernandes, H. Kelkar, C. Nesbitt, S. Segner, D. Bhatnagar, T. E. Cleveland, and T. H. Adams.** 1995. A conserved polyketide mycotoxin gene cluster in *Aspergillus nidulans*, p. 263–277. *In* J. L. Richard (ed.), Molecular approaches to food safety issues involving toxic microorganisms. Alaken, Fort Collins, Colo.
59. **Keller, N. P., C. M. H. Watanabe, H. S. Kelkar, T. H. Adams, and C. A. Townsend.** 2000. Requirement of monooxygenase-mediated steps for sterigmatocystin biosynthesis by *Aspergillus nidulans*. Appl. Environ. Microbiol. **66:**359–362.
60. **Klich, M. A., E. J. Mullaney, C. B. Daly, and J. W. Cary.** 2000. Molecular and physiological aspects of aflatoxin and sterigmatocystin biosynthesis by *Aspergillus tamarii* and *A. ochraceoroseus*. Appl. Microbiol. Biotechnol. **53:** 605–609.
61. **Kusumoto, K., and D. P. Hsieh.** 1996. Purification and characterization of the esterases involved in aflatoxin biosynthesis in *Aspergillus parasiticus*. Can. J. Microbiol. **42:**804–810.
62. **Lancaster, M. D., F. P. Jenkins, and J. M. Phillip.** 1961. Toxicity associated with certain samples of groundnuts. Nature **192:**1095–1096.
63. **Lee, L. W., C. H. Chiou, and J. E. Linz.** 2002. Function of native OmtA in vivo and expression and distribution of this protein in colonies of *Aspergillus parasiticus*. Appl. Environ. Microbiol. **68:**5718–5727.
64. **Liang, S.-H., C. D. Skory, and J. E. Linz.** 1996. Characterization of the function of the *ver-1A and ver-1B* genes, involved in aflatoxin biosynthesis in *Aspergillus parasiticus*. Appl. Environ. Microbiol. **62:**4568–4575.
65. **Liang, S.-H., T. S. Wu, R. Lee, F. S. Chu, and J. E. Linz.** 1997. Analysis of mechanisms regulating expression of the *ver-1* gene, involved in aflatoxin biosynthesis. Appl. Environ. Microbiol. **63:**1058–1065.
66. **Lin, B. K., and J. A. Anderson.** 1992. Purification and properties of versiconal cyclase from *Aspergillus parasiticus*. Arch. Biochem. Biophys. **293:**67–70.
67. **Mahanti, N., D. Bhatnagar, J. W. Cary, J. Joubran, and J. E. Linz.** 1996. Structure and function of *fas-1A*, a gene encoding a putative fatty acid synthetase directly involved in aflatoxin biosynthesis in *Aspergillus parasiticus*. Appl. Environ. Microbiol. **62:**191–195.
68. **Matsushima, K., P.-K. Chang, J. Yu, K. Abe, D. Bhatnagar, and T. E. Cleveland.** 2001. Pre-termination in *aflR* of *Aspergillus sojae* inhibits aflatoxin biosynthesis. Appl. Microbiol. Biotechnol. **55:**585–589.
69. **Matsushima, K., K. Yashiro, Y. Hanya, K. Abe, K. Yabe, and T. Hamasaki.** 2001. Absence of aflatoxin biosynthesis in koji mold (*Aspergillus sojae*). Appl. Microbiol. Biotechnol. **55:**771–776.
70. **Mayorga, M. E., and W. E. Timberlake.** 1992. The developmentally regulated *Aspergillus nidulans wA* gene encodes a polypeptide homologous to polyketide and fatty acid syntheses. Mol. Gen. Genet. **235:**205–212.
71. **McGuire, S. M., J. C. Silva, E. G. Casillas, and C. A. Townsend.** 1996. Purification and characterization of versicolorin B synthase from *Aspergillus parasiticus*. Catalysis of the stereodifferentiating cyclization in aflatoxin biosynthesis essential to DNA interaction. Biochemistry **35:**11470–11486.
72. **Meyers, D. M., G. O'Brian, W. L. Du, D. Bhatnagar, and G. A. Payne.** 1998. Characterization of *aflJ*, a gene required for conversion of pathway intermediates to aflatoxin. Appl. Environ. Microbiol. **64:**3713–3717.
73. **Minto, R. E., and C. A. Townsend.** 1997. Enzymology and molecular biology of aflatoxin biosynthesis. Chem. Rev. **97:**2537–2556.
74. **Motomura, M., N. Chihaya, T. Shinozawa, T. Hamasaki, and K. Yabe.** 1999. Cloning and characterization of the *O*-methyltransferase I gene (*dmtA*) from *Aspergillus parasiticus* associated with the conversions of demethylsterigmatocystin to sterigmatocystin and dihydrodemethylsterigmatocystin to dihydrosterigmatocystin in aflatoxin biosynthesis. Appl. Environ. Microbiol. **65:**4987–4994.
75. **O'Brian, G. R., A. M. Fakhoury, and G. A. Payne.** 2003. Identification of genes differentially expressed during aflatoxin biosynthesis in *Aspergillus flavus* and *Aspergillus parasiticus*. Fungal Genet. Biol. **39:**118–127.
76. **Papa, K. E.** 1979. Genetics of *Aspergillus flavus*: complementation and mapping of aflatoxin mutants. Genet. Res. **34:**1–9.
77. **Papa, K. E.** 1982. Norsolorinic acid mutant of *Aspergillus flavus*. J. Gen. Microbiol. **128:**1345–1348.
78. **Payne, G. A., and W. M. Hagler, Jr.** 1983. Effect of specific amino acids on growth and aflatoxin production by *Aspergillus parasiticus and Aspergillus flavus* in defined media. Appl. Environ. Microbiol. **46:**805–812.
79. **Payne, G. A., G. J. Nystrom, D. Bhatnagar, T. E. Cleveland, and C. P. Woloshuk.** 1993. Cloning of the *afl-2* gene involved in aflatoxin biosynthesis from *Aspergillus flavus*. Appl. Environ. Microbiol. **59:**156–162.
80. **Payne, G. A., and M. P. Brown.** 1998. Genetics and physiology of aflatoxin biosynthesis. Annu. Rev. Phytopathol. **36:**329–362.
81. **Payne, G. A.** 1998. Process of contamination by aflatoxin-producing fungi and their impacts on crops, p. 279–306. *In* K. K. Sinha and D. Bhatnagar (ed.), Mycotoxins in agriculture and food safety, vol. 9. Marcel Dekker, New York, N.Y.
82. **Peterson, S. W., Y. Ito, B. W. Horn, and T. Goto.** 2001. *Aspergillus bombycis*, a new aflatoxigenic species and genetic variation in its sibling species, *A. nomius*. Mycologia **93:**689–703.
83. **Prieto, R., and C. P. Woloshuk.** 1997. *ord1*, an oxidoreductase gene responsible for conversion of *O*-methylsterigmatocystin to aflatoxin in *Aspergillus flavus*. Appl. Environ. Microbiol. **63:**1661–1666.
84. **Prieto, R., G. L. Yousibova, and C. P. Woloshuk.** 1996. Identification of aflatoxin biosynthesis genes by genetic complementation in an *Aspergillus flavus* mutant lacking the aflatoxin gene cluster. Appl. Environ. Microbiol. **62:**3567–3571.
85. **Sakuno, E., K. Yabe, and H. Nakajima.** 2003. Involvement of two cytosolic enzymes and a novel intermediate, 5′-oxoaverantin, in the pathway from 5′-hydroxyaverantin to averufin in aflatoxin biosynthesis. Appl. Environ. Microbiol. **69:**6418–6426.
86. **Schroeder, H. W., R. J. Cole, R. D. Grigsby, and H. Hein, Jr.** 1974. Inhibition of aflatoxin production and tentative identification of an aflatoxin intermediate "versiconal acetate" from treatment with dichlorvos. Appl. Microbiol. **27:**394–399.
87. **Silva, J. C., and C. A. Townsend.** 1996. Heterologous expression, isolation,

and characterization of versicolorin B synthase from *Aspergillus parasiticus*. J. Biol. Chem. **272:**804–813.

88. **Silva, J. C., R. E. Minto, C. E. Barry, K. A. Holland, and C. A. Townsend.** 1996. Isolation and characterization of the versicolorin B synthase gene from *Aspergillus parasiticus*: expansion of the aflatoxin B_1 biosynthetic cluster. J. Biol. Chem. **271:**13600–13608.
89. **Skory, C. D., P.-K. Chang, J. Cary, and J. E. Linz.** 1992. Isolation and characterization of a gene from *Aspergillus parasiticus* associated with the conversion of versicolorin A to sterigmatocystin in aflatoxin biosynthesis. Appl. Environ. Microbiol. **58:**3527–3537.
90. **Skory, C. D., P.-K. Chang, and J. E. Linz.** 1993. Regulated expression of the *nor-1* and *ver-1* genes associated with aflatoxin biosynthesis. Appl. Environ. Microbiol. **59:**1642–1646.
91. **Takahashi, T., P.-K. Chang, K. Matsushima, J. Yu, Y. Koyama, K. Abe, D. Bhatnagar, and T. E. Cleveland.** 2002. Nonfunctionality of *Aspergillus sojae aflR* in a strain of *Aspergillus parasiticus* with a disrupted *aflR* gene. Appl. Environ. Microbiol. **68:**3737–3743.
92. **Townsend, C. A., S. B. Christensen, and K. Trautwein.** 1984. Hexanoate as a starter unit in polyketide synthesis. J. Am. Chem. Soc. **106:**3868–3869.
93. **Townsend, C. A., K. A. Plavcan, K. Pal, S. W. Brobst, M. S. Irish, E. W. Ely, and J. W. Bennett.** 1988. Hydroxyversicolorone: isolation and characterization of a potential intermediate in aflatoxin biosynthesis. J. Org. Chem. **53:**2472–2477.
94. **Townsend, C. A.** 1997. Progress towards a biosynthetic rationale of the aflatoxin pathway. Pure Appl. Chem. **58:**227–238.
95. **Trail, F., P.-K. Chang, J. Cary, and J. E. Linz.** 1994. Structural and functional analysis of the *nor-1* gene involved in the biosynthesis of aflatoxins by *Aspergillus parasiticus*. Appl. Environ. Microbiol. **60:**4078–4085.
96. **Trail, F., N. Manhanti, and J. Linz.** 1995. Molecular biology of aflatoxin biosynthesis. Microbiology **141:**755–765.
97. **Trail, F., N. Mahanti, M. Rarick, R. Mehigh, S. H. Liang, R. Zhou, and J. E. Linz.** 1995. Physical and transcriptional map of an aflatoxin gene cluster in *Aspergillus parasiticus* and functional disruption of a gene involved early in the aflatoxin pathway. Appl. Environ. Microbiol. **61:**2665–2673.
98. **Vederas, J. C., and T. T. Nakashima.** 1980. Biosynthesis of averufin by *Aspergillus parasiticus*: detection of 180-label by 13C NMR isotope shifts. J. Chem. Soc. Chem. Commun. **4:**183–185.
99. **Watanabe, C. M. H., D. Wilson, J. E. Linz, and C. A. Townsend.** 1996. Demonstration of the catalytic roles and evidence for the physical association of type I fatty acid syntheses and a polyketide synthase in the biosynthesis of aflatoxin B_1. Chem. Biol. **3:**463–469.
100. **Watanabe, C. M., and C. A. Townsend.** 2002. Initial characterization of a type I fatty acid synthase and polyketide synthase multienzyme complex NorS in the biosynthesis of aflatoxin B_1. Chem. Biol. **9:**981–988.
101. **Woloshuk, C. P., K. R. Foutz, J. F. Brewer, D. Bhatnagar, T. E. Cleveland, and G. A. Payne.** 1994. Molecular characterization of *aflR*, a regulatory locus for aflatoxin biosynthesis. Appl. Environ. Microbiol. **60:**2408–2414.
102. **Yabe, K.** 2003. Pathway and genes of aflatoxin biosynthesis, p. 227–251. *In* F. Fierro and J. Francisco (ed.), Microbial secondary metabolites: biosynthesis, genetics and regulation. Research Signpost, Trivandrum, India.
103. **Yabe, K., Y. Ando, and T. Hamasaki.** 1988. Biosynthetic relationship among aflatoxins B_1, B_2, G_1, and G_2. Appl. Environ. Microbiol. **54:**2101–2106.
104. **Yabe, K., Y. Ando, J. Hashimoto, and T. Hamasaki.** 1989. Two distinct *O*-methyltransferases in aflatoxin biosynthesis. Appl. Environ. Microbiol. **55:**2172–2177.
105. **Yabe, K., Y. Ando, and T. Hamasaki.** 1991. A metabolic grid among versiconal hemiacetal acetate, versiconol acetate, versiconol and versiconal during aflatoxin biosynthesis. J. Gen. Microbiol. **137:**2469–2475.
106. **Yabe, K., Y. Nakamura, H. Nakajima, Y. Ando, and T. Hamasaki.** 1991. Enzymatic conversion of norsolorinic acid to averufin in aflatoxin biosynthesis. Appl. Environ. Microbiol. **57:**1340–1345.
107. **Yabe, K., and T. Hamasaki.** 1993. Stereochemistry during aflatoxin biosynthesis: cyclase reaction in the conversion of versiconal to versicolorin B and racemization of versiconal hemiacetal acetate. Appl. Environ. Microbiol. **59:**2493–2500.
108. **Yabe, K., Y. Matsuyama, Y. Ando, H. Nakajima, and T. Hamasaki.** 1993. Stereochemistry during aflatoxin biosynthesis: conversion of norsolorinic acid to averufin. Appl. Environ. Microbiol. **59:**2486–2492.
109. **Yabe, K., K. Matsushima, T. Koyama, and T. Hamasaki.** 1998. Purification and characterization of *O*-methyltransferase I involved in conversion of demethylsterigmatocystin to sterigmatocystin and of dihydrodemethylsterigmatocystin to dihydrosterigmatocystin during aflatoxin biosynthesis. Appl. Environ. Microbiol. **64:**166–171.
110. **Yabe, K., M. Nakamura, and T. Hamasaki.** 1999. Enzymatic formation of G-group aflatoxins and biosynthetic relationship between G- and B-group aflatoxins. Appl. Environ. Microbiol. **65:**3867–3872.
111. **Yabe, K., N. Chihaya, S. Hamamatsu, E. Sakuno, T. Hamasaki, H. Nakajima, and J. W. Bennett.** 2003. Enzymatic conversion of averufin to hydroxyversicolorone and elucidation of a novel metabolic grid involved in aflatoxin biosynthesis. Appl. Environ. Microbiol. **69:**66–73.
112. **Yao, R. C., and D. P. H. Hsieh.** 1974. Step of dichlorvos inhibition in the pathway of aflatoxin biosynthesis. Appl. Microbiol. **28:**52–57.
113. **Yu, J., J. W. Cary, D. Bhatnagar, T. E. Cleveland, N. P. Keller, and F. S. Chu.** 1993. Cloning and characterization of a cDNA from *Aspergillus parasiticus* encoding an *O*-methyltransferase involved in aflatoxin biosynthesis. Appl. Environ. Microbiol. **59:**3564–3571.
114. **Yu, J., P.-K. Chang, J. W. Cary, M. Wright, D. Bhatnagar, T. E. Cleveland, G. A. Payne, and J. E. Linz.** 1995. Comparative mapping of aflatoxin pathway gene clusters in *Aspergillus parasiticus* and *Aspergillus flavus*. Appl. Environ. Microbiol. **61:**2365–2371.
115. **Yu, J., P.-K. Chang, G. A. Payne, J. W. Cary, D. Bhatnagar, and T. E. Cleveland.** 1995. Comparison of the *omtA* genes encoding *O*-methyltransferases involved in aflatoxin biosynthesis from *Aspergillus parasiticus* and *A. flavus*. Gene **163:**121–125.
116. **Yu, J., P.-K. Chang, J. W. Cary, D. Bhatnagar, and T. E. Cleveland.** 1997. *avnA*, a gene encoding a cytochrome P-450 monooxygenase is involved in the conversion of averantin to averufin in aflatoxin biosynthesis in *Aspergillus parasiticus*. Appl. Environ. Microbiol. **63:**1349–1356.
117. **Yu, J., P.-K. Chang, J. W. Cary, K. C. Ehrlich, B. Montalbano, J. M. Dyer, D. Bhatnagar, and T. E. Cleveland.** 1998. Characterization of the critical amino acids of an *Aspergillus parasiticus* cytochrome P450 monooxygenase encoded by *ordA* involved in aflatoxin B_1, G_1, B_2, and G_2 biosynthesis. Appl. Environ. Microbiol. **64:**4834–4841.
118. **Yu, J., P.-K. Chang, D. Bhatnagar, and T. E. Cleveland.** 2000. Genes encoding cytochrome P450 and monooxygenase enzymes define one end of the aflatoxin pathway gene cluster in *Aspergillus parasiticus*. Appl. Microbiol. Biotechnol. **53:**583–590.
119. **Yu, J., C. P. Woloshuk, D. Bhatnagar, and T. E. Cleveland.** 2000. Cloning and characterization of *avfA* and *omtB* genes involved in aflatoxin biosynthesis in three *Aspergillus* species. Gene **248:**157–167.
120. **Yu, J., D. Bhatnagar, and K. C. Ehrlich.** 2002. Aflatoxin biosynthesis. Rev. Iberoam. Micol. **19:**191–200.
121. **Yu, J., D. Bhatnagar, and T. E. Cleveland.** 2002. *Aspergillus flavus* genomics for elimination of aflatoxin contamination. Mycopathologia **155:**10.
122. **Yu, J., D. Bhatnagar, T. E. Cleveland, and W. C. Nierman.** 2002. *Aspergillus flavus* EST technology and its applications for eliminating aflatoxin contamination. Mycopathologia **155:**6.
123. **Yu, J.** Genetics and biochemistry of mycotoxin synthesis. *In* D. K. Arora (ed.), Handbook of fungal biotechnology, 2nd ed., in press. Marcel Dekker, New York, N.Y.
124. **Yu, J., P.-K. Chang, D. Bhatnagar, and T. E. Cleveland.** 2003. Cloning and functional expression of an esterase gene in *Aspergillus parasiticus*. Mycopathologia **156:**227–234.
125. **Yu, J.-H., and T. J. Leonard.** 1995. Sterigmatocystin biosynthesis in *Aspergillus nidulans* requires a novel type I polyketide synthase. J. Bacteriol. **177:**4792–4800.
126. **Yu, J.-H., R. A. Butchko, M. Fernandes, N. P. Keller, T. J. Leonard, and T. H. Adams.** 1996. Conservation of structure and function of the aflatoxin regulatory gene *aflR* from *Aspergillus nidulans* and *A. flavus*. Curr. Genet. **29:**549–555.