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Aflatoxin Biosynthetic Pathway and Pathway Genes

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1. Introduction

Among the over 185 known species within the genus *Aspergillus, Aspergillus flavus* is the most economically important because it produces the toxic and carcinogenic aflatoxins. Its non-aflatoxigenic relative *A. oryzae* is used extensively for food fermentations (Jelinek et al., 1989). It is one of the most abundant soil-borne molds on earth. *A. flavus* fungus is a saprobe mold that is capable of surviving on many organic nutrient sources like plant debris, tree leaves, decaying wood, animal fodder, cotton, compost piles, dead insects and animal carcasses, stored grains, and even immunocompromised humans and animals (Klich, 1998). It has the ability to survive temperatures ranging from 12°C to 48°C, but the optimal growth temperature ranges from 28°C to 37°C. Its ability to grow at relatively high temperatures contributes to its pathogenicity toward humans and other warm blooded animals. For most of its lifecycle, the fungus exists in the form of mycelium or asexual spores known as conidia. Under adverse conditions such as lack of adequated nutrients or water, the fungal mycelium will transform to resistant structures called sclerotia which can survive extremely harsh environmental conditions. The fungus overwinters either as spores, sclerotia, or as mycelium in debris. When conditions become favorable the sclerotia germinate directly to produce new colonies or conidiophores with conidia (Bennett et al., 1986; Cotty, 1988; Chang et al., 2002).

Aflatoxins were first identified as the cause of a severe animal poisoning incident in England in 1960 called the Turkey X disease (Allcroft et al., 1961; Lancaster et al., 1961). *A. flavus* produces aflatoxin B_1 and B_2 whereas *A. parasiticus*, produces aflatoxins B_1, B_2, G_1, and G_2. These four major aflatoxins are named based on their blue (B) or green (G) fluorescence under ultraviolet light, and their relative mobility by thin-layer chromatography on silica gel. Aflatoxin M_1 is a hydroxylated derivative metabolized from aflatoxin B_1 by cows and secreted in milk (Van Egmond, 1989). In addition to aflatoxins B_1 and B_2, *A. flavus* also produces many other mycotoxins such as cyclopiazonic acid, kojic acid, beta-nitropropionic acid, aspertoxin, aflatrem and aspergillic acid (Goto et al., 1996).

The disease caused by ingestion of aflatoxins in contaminated food or feed is called aflatoxicosis. Acute aflatoxicosis occurs when aflatoxins are consumed at moderate to high levels. Depending on the level and duration of exposure, aflatoxins possess both hepatotoxic and carcinogenic properties. Symptoms in humans include vomiting, abdominal pain, alteration in digestion, limb and pulmonary edema, convulsions, rapid progressive jaundice,

swollen liver, high fever, coma, and death. The predominant damage is to the liver (Scholl & Groopman, 1995); (Fung & Clark, 2004; Lewis et al., 2005), but acute damage to the kidneys and heart have been found (Richard & Payne, 2003). In liver aflatoxins irreversibly bind to protein and DNA to form adducts such as aflatoxin B_1-lysine in albumin and a guanyl-N7 adduct in DNA (Skipper & Tannenbaum, 1990). Disruption of the proteins and DNA bases in hepatocytes causes the toxicity (Tandon et al., 1978; Azziz-Baumgartner et al., 2005). Major outbreaks of acute aflatoxicosis from contaminated food in humans were reported in developing countries (Centers for Disease Control and Prevention, 2004; Lewis et al., 2005). For example, in western India in 1974, 108 persons died among 397 people affected with aflatoxin poisoning in more than 150 villages (Krishnamachari et al., 1975). A more recent incident of aflatoxin poisoning occurred in Kenya in July 2004 leading to the death of 125 people among 317 reported with illness due to consumption of aflatoxin contaminated maize (corn) (Centers for Disease Control and Prevention, 2004; Lewis et al., 2005). Acute toxicosis is not the only concern. World health authorities warn that low doses and long term dietary exposure to aflatoxins is also a major risk as chronic exposure can lead to hepatocellular carcinoma (Bressac et al., 1991; Hsu et al., 1991; Wogan, 1992; Fung & Clark, 2004).

Among the four major types of aflatoxins, aflatoxin B_1 is the most toxic and the most potent carcinogen in humans and animals including nonhuman primates, birds, fish, and rodents. Chronic exposure can result in suppressed immune response, malnutrition, proliferation of the bile duct, centrilobular necrosis and fatty infiltration of the liver, hepatic lesions, and even hepatomas. In animal models, aflatoxin B_1 is modified into a more toxic and carcinogenic by-product during detoxification by a cytochrome P450 monooxygenase in liver (Ngindu et al., 1982; Hsieh, 1989; Eaton & Gallagher, 1994; Lewis et al., 2005). The epoxide form of aflatoxin binds to guanine residues in DNA, forms guanyl-N7 adducts, and induces mutations. One mutation, a G to T transversion (Baertschi et al., 1989; Bressac et al., 1991) at the third base of codon 249, a mutation hot spot of the p53 tumor suppressor gene, is generally believed to be the mechanism for initiating hepatocarcinoma formation (Busby & Wogan, 1981; Hsu et al., 1991; Ozturk, 1991; Coursaget et al., 1993). The p53 gene encodes a transcription factor involved in cell cycle regulation. It is commonly mutated in human liver cancers (Groopman et al., 1994). Aflatoxin B_1 is also a potential immunosuppressive agent (Raisuddin et al., 1993). Chronic low level exposure of growing vertebrates to aflatoxins may enhance their susceptibility to infection and tumorigenesis (Raisuddin et al., 1993). AFB_1 also affects other organs and tissues, such as the lungs and the entire respiratory system (Kelly et al., 1997). Human hepatocarcinomas are also associated with hepatitis B virus (HBV) and C virus (HCV) infections (Peers et al., 1987; Hsieh, 1989; Wild et al., 1992). Together with aflatoxins these viruses significantly increased the risk of hepatoma in hepatitis patients (Chen et al., 1996a; Chen et al., 1996b; McGlynn et al., 2003; Arsura & Cavin, 2005). In developing countries, many children are exposed to aflatoxin before birth (Turner et al., 2007), while nursing (Polychronaki et al., 2007) and after weaning (Gong et al., 2004). An association of hepatocellular carcinoma and dietary exposure with aflatoxins has been established from patients living in high-risk areas of China, Kenya, Mozambique, Phillippines, Swaziland, Thailand, Transkei of South Africa (Lancaster et al., 1961; Zuckerman et al., 1967; Wong et al., 1977; Hsieh et al., 1985; Zhu et al., 1987; Huang & Hsieh, 1988; Wilson, 1989; Wogan, 1992; Eaton & Gallagher, 1994; Lewis et al., 2005).

Aspergillus flavus can grow in immunocompromised warm blooded mammals and can cause invasive and non-invasive aspergillosis in humans and animals (Denning et al., 1991; Denning, 1998; Mori et al., 1998; Denning et al., 2003). *A. flavus* is the second leading cause of

aspergillosis slightly behind *A. fumigatus.* The incidence of aspergillosis caused by Aspergilli is rising due to the increase of immunocompromised patients in the population due to HIV infection (Denning, 1998; Nierman et al., 2005; Ronning et al., 2005).
A. flavus is a weak and opportunistic plant pathogen, affecting many agricultural crops such as maize (corn), cotton, groundnuts (peanuts), as well as tree nuts such as Brazil nuts, pecans, pistachio nuts, and walnuts. Preharvest contamination of these crops with aflatoxins is common. *A. flavus* also causes the spoilage of post harvest grains during storage. Because *A. flavus* lacks host specificity (St Leger et al., 2000) and can attack seeds of both monocots and dicots, and seeds produced both above ground (corn) as well as below the ground (peanuts). Under weather conditions favorable for its growth, *A. flavus* can cause ear rot on maize, resulting in significant economic losses to farmers (Robens, 2001; Richard & Payne, 2003; Robens & Cardwell, 2005).

2. Economic significance

Due to the toxic and carcinogenic properties of aflatoxins, only extremely low levels of aflatoxins in foods and feeds is allowed (Council for Agricultural Science and Technology, 2003; Fung & Clark, 2004). The International Agency for Research on Cancer (IARC) has designated aflatoxin as a human liver carcinogen (Van Egmond, 1989; van Egmond & Jonker, 2005; van Egmond et al., 2007). To minimize potential exposure to aflatoxins, maximum levels of aflatoxins in many commodities have been set at levels below 20 ppb by most countries (Van Egmond, 1989; van Egmond & Jonker, 2005; van Egmond et al., 2007). Regulatory guidelines of the U.S. Food and Drug Administration (FDA) specifically prevent the sale of commodities if contamination by aflatoxins exceeds 20 ppb total aflatoxins for interstate commerce of food and feedstuff and 0.5 ppb aflatoxin M_1 in milk. The European Commission has set the limits on groundnuts subject to further processing at 15 ppb for total aflatoxins and 8 ppb for aflatoxin B_1, and for nuts and dried fruits subject to further processing at 10 ppb for total aflatoxins and 5 ppb for aflatoxin B_1. The aflatoxin standards for cereals, dried fruits, and nuts intended for direct human consumption are even more stringent, and the limit for total aflatoxins is 4 ppb and 2 ppb for aflatoxin B_1 (van Egmond & Jonker, 2005).
Aflatoxin contamination of agricultural commodities poses a potential risk to livestock and human health (Lancaster et al., 1961; Bennett & Lee, 1979; Bennett, 1987; Jelinek et al., 1989; Cleveland & Bhatnagar, 1992; Eaton & Groopman, 1994; Hall & Wild, 1994; Bhatnagar et al., 2002; Bennett & Klich, 2003; Richard & Payne, 2003). It is not only a serious food safety concern, but it has significant economic implications for the agricultural industry worldwide because of restrictions limiting the trade of contaminated crops.
Since its discovery, extensive efforts have been made and expense incurred worldwide to monitor aflatoxin occurrence and to develop control strategies (Bennett, 1970; Bennett & Goldblatt, 1973; Bennett et al., 1976b; Papa, 1976; Papa, 1979; Papa, 1984). The hallmark discovery of a color mutant that accumulates the brick-red pigment, norsolorinic acid (NOR), in *A. parasiticus* marked a milestone in the understanding the chemistry of aflatoxin biosynthesis (Bennett et al., 1971; Bennett et al., 1976a; Bennett, 1979; Bennett et al., 1983). Since NOR is the earliest and the first stable aflatoxin precursor in the aflatoxin biosynthetic pathway (Hsieh et al., 1976; Dutton, 1988; Bennett et al., 1997), this discovery led to the identification of other key aflatoxin intermediates and established the primary metabolites in the aflatoxin pathway. It provided the opportunity to isolate the first aflatoxin pathway

gene that encodes a reductase for the conversion from NOR to eventually aflatoxins (Hsieh & Mateles, 1970; Hsieh et al., 1973; Hsieh et al., 1976; Chang et al., 1992)Dutton, 1982 #357;Dutton, 1985 #313}. After the cloning of several important aflatoxin pathway genes, a 75 kb aflatoxin pathway gene cluster was established in *A. parasiticus* and *A. flavus* (Yu et al., 1995a). Discovery of the cluster promoted renewed interest in understanding aflatoxin biosynthesis by scientists all over the world. Significant progress has been made in elucidating the biosynthetic pathway, the pathway intermediates, genes, corresponding enzymes, and regulatory mechanisms (Bennett & Lee, 1979; Bennett et al., 1981; Cleveland et al., 1987; Bennett & Papa, 1988; Bhatnagar et al., 1992; Chang et al., 1993; Keller et al., 1993; Chang et al., 1995a; Chang et al., 1999a; Ehrlich et al., 1999a; Bennett & Klich, 2003; Chang, 2004; Yu et al., 2004c; Crawford et al., 2008a; Ehrlich, 2009; Ehrlich & Yu, 2009). At least 27 enzymatic steps have been characterized or proposed to be involved in bioconversion of aflatoxin intermediates to aflatoxins (Ehrlich, 2009). In this chapter, we focus on the aflatoxin biosynthetic pathway and the function of aflatoxin cluster genes. For detailed historical information on the aflatoxin pathway genes and gene cluster discovery, please refer to previous reviews (Yabe & Nakajima, 2004; Yu et al., 2004a; Yu et al., 2004c; Yu et al., 2011).

3. Aflatoxin biosynthetic pathway and genes involved

Attempts to decipher the aflatoxin biosynthetic pathway began shortly after the determination of the structure of these toxins (Goldblatt, 1969; Singh & Hsieh, 1977; FAO, 1995). The discovery of a colored mutant in *A. parasiticus* that accumulates norsolorinic acid (NOR) (Bennett et al., 1971; Lee et al., 1971; Bennett et al., 1997) paved the road for the establishment of aflatoxin biosynthetic pathway. With the rapid gene cloning and enzyme characterization, the enzymatic steps for biosynthesis of the 15 structurally defined aflatoxin pathway intermediates have been identified (Cleveland & Bhatnagar, 1987; Bhatnagar et al., 1989; Cleveland & Bhatnagar, 1990; Bhatnagar & Cleveland, 1991; Cleveland & Bhatnagar, 1991; Bhatnagar et al., 1992; Trail et al., 1995a; Yu et al., 1995a; Minto & Townsend, 1997; Townsend, 1997; Yu et al., 1997; Yu et al., 1998; Keller et al., 2000; Yu et al., 2004b; Crawford et al., 2008b). There are estimated to be 27 enzymatic steps in the aflatoxin biosynthesis (Ehrlich, 2009). As many as 30 genes are potentially involved in aflatoxin biosynthesis (Figure 1). The genes and corresponding enzymes have been extensively studied (Bennett et al., 1971; Lee et al., 1971; Yabe & Nakajima, 2004; Yu et al., 2004a; Yu et al., 2004c). In *A. flavus* and *A. parasiticus* the aflatoxin pathway genes are clustered within a 75-kb region of the fungal genome on chromosome III roughly 80 kb away from telomere (Wilson, 1989; Trail et al., 1995a; Trail et al., 1995b; Yu et al., 1995a; Townsend, 1997; Yu et al., 2004a; Yu et al., 2004c; Chang et al., 2005).

3.1 Acetate to norsolorinic acid (NOR)

Norsolorinic acid (NOR) was confirmed to be the first stable aflatoxin precursor (Bennett et al., 1971; Bennett, 1981; Bennett et al., 1983). A hexanoyl starter unit is the initial substrate for aflatoxin formation (Hsieh & Mateles, 1970). Two fatty acid synthases (FAS) and a polyketide synthase (NR-PKS, PksA) are involved in the synthesis of the polyketide from a hexanoyl starter unit. Seven iterative, malonyl-derived ketide extensions are required to produce norsolorinic acid anthrone (noranthrone) (Wilson, 1989; Trail et al., 1995a; Trail et al., 1995b; Brown et al., 1996a; Brown et al., 1996b; Watanabe et al., 1996; Watanabe & Townsend, 2002; Yabe & Nakajima, 2004; Crawford et al., 2006; Crawford et al., 2008a;

Crawford et al., 2008b). Mahanti et al. (Mahanti et al., 1996) cloned, by genetic complementation, a 7.5-kb large transcript which is required for NOR formation in a blocked *A. parasiticus* mutant. Its protein has high degree of similarity (67%) and identity (48%) to the beta-subunit of FASs (FAS1) of *Saccharomyces cerevisiae* and *Yarrowia lipolytica*. Metabolite feeding and gene disruption experiments further confirmed that *uvm8* encodes a subunit of a novel fatty acid synthase (FAS) directly involved in the backbone formation of the polyketide precursor of NOR during aflatoxin biosynthesis, therefore, on the basis of its function, the *uvm8* gene was renamed *fas-1A*. In the revised naming scheme, the *fas-1A* gene was renamed as *fas-1*, it encodes fatty acid synthase-1 in the aflatoxin biosynthetic pathway gene cluster (Figure 1). Another large transcript (*fas-2A*) which encodes an alpha-subunit of fatty acid synthase in the aflatoxin gene cluster was reported (Mahanti et al., 1996). The gene *fas-1A* and *fas-2A* were renamed *fas-1* and *fas-2*. They encode two fatty acid synthases (FASα and FASβ) (Payne, 1998). In *A. nidulans* the involvement of FASs in sterigmatocystin (ST) biosynthesis was also confirmed and were named *stcJ* and *stcK* in the ST cluster (Brown et al., 1996a; Brown et al., 1996b). The biochemical evidence for the role of a fatty acid synthase and a polyketide synthase (PKS) in the biosynthesis of aflatoxin was demonstrated (Watanabe & Townsend, 1996). Further details on the early stage of aflatoxin biosynthesis involving fatty acid synthases and polyketide synthases were reported (Watanabe et al., 1996; Hitchman et al., 2001; Watanabe & Townsend, 2002; Crawford et al., 2006). The N-acetylcysteamine thioester of hexanoic acid was incorporated into NOR in a *fas-1* disrupted transformant. A polyketide synthase gene (*pksA*) in *A. parasiticus* was demonstrated by gene disruption to be required for aflatoxin biosynthesis (Chang et al., 1995a). The predicted amino acid sequences of these PKSs contain the typical four conserved domains commonly found in other known PKS proteins: β-ketoacyl synthase (KS), acyltransferase (AT), acyl carrier protein (ACP), and thioesterase (TE) (Chang et al., 1995a). Townsend's group has dissected the functional domains of the PKS for aflatoxin biosynthesis (Crawford et al., 2008a; Crawford et al., 2008b; Crawford et al., 2008c). These include domains for the starter unit acyl transferase (SAT) which recognizes hexanoyl CoA and the N-acetylcysteamine thioester of hexanoic acid, the acyl carrier protein (ACP) , ketosynthase (KS), malonyl-CoA:ACP transacylase (MAT), product template (PT) allowing the iterative steps in forming the polyketide, and a thioesterase/Claisen-like cyclase (TE/CLC) (Crawford et al., 2008a). The predicted product converted by PksA is noranthrone. The conversion of noranthrone to NOR, the first stable intermediate in the pathway (Bennett et al., 1971; Lee et al., 1971; Papa, 1979; Bennett et al., 1981; Papa, 1982; Bennett et al., 1994; Bennett et al., 1997), is poorly defined, but it has been proposed to be catalyzed by a noranthrone oxidase, a monooxygenase, or to occur spontaneously (Dutton, 1988). Sequence analysis and enzymatic studies supports the contention that the *hypC* (a gene in the intergenic region of *pksA* and *nor-1*) gene product is the required noranthrone oxidase involved in the catalysis of the orxidation of norsolorinic acid anthrone to NOR (Ehrlich, 2009) The *fas-1*, *fas-2*, and *pksA* genes were renamed as *aflA*, *aflB* and *aflC* respectively (Wilson, 1989; Yu et al., 2004a; Yu et al., 2004c) (Figure 1). The *aflA*, *aflB* and *aflC* gene homologues in *A. nidulans* are *stcJ*, *stcK*, and *stcA*, respectively (Brown et al., 1996b).

3.2 Norsolorinic acid (NOR) to averantin (AVN)

The first stable AF intermediate was identified as NOR produced in *A. parasiticus* uv-generated disruption mutants (Bennett et al., 1971; Lee et al., 1971; Detroy et al., 1973;

Bennett et al., 1981) and in *A. flavus* (Papa, 1979; Papa, 1982) The NOR-accumulating mutants are leaky mutants whose aflatoxin biosynthesis is not completely blocked. By genetic complementation, the gene, *aflD* (*nor-1*), encoding a reductase was cloned (Chang et al., 1992). A recombinant Nor-1 protein expressed in *E. coli* catalyzed the reduction of NOR. Therefore, *aflD* (*nor-1*) encodes the ketoreductase needed for the conversion of the 1'-keto group in NOR to the 1'-hydroxyl group of AVN (Zhou & Linz, 1999). Disruption of the *aflD* (*nor-1*) gene also confirmed its involvement in conversion of NOR to AVN in aflatoxin biosynthesis (Trail et al., 1994). The *aflD* (*nor-1*) homologous gene in *A. nidulans* is *stcE* (Brown et al., 1996b). Genes homologous to *aflD* (*nor-1*), in the AF cluster, such as *aflE* (*norA*) and *aflF* (*norB*) are predicted to encode short chain aryl alcohol dehydrogenases. These proteins may also be able to catalyze the reduction of NOR to AVN depending on the reductive environment of the cell and may explain the leakiness of the *nor-1* mutation if they are able to complement Nor-1's function (Cary et al., 1996),

3.3 Averantin (AVN) to 5'-hydroxyaverantin (HAVN)

Radioisotope incorporation experiments established the earliest evidence for the conversion of AVN to HAVN (Bennett et al., 1980; McCormick et al., 1987). In these studies, three enzymatic steps can account for the conversion of NOR to averufin (AVF) (Yabe et al., 1991a). They are (i) NOR to AVN catalyzed by a reductase, (ii) NOR to HAVN catalyzed by a monooxygenase, and (iii) HAVN to AVF catalyzed by a second dehydrogenase. It was also proposed that the oxidation reactions are reversible and that NADPH was the preferred cofactor (Yabe et al., 1991b). The gene previously named *ord-1* encoding a P-450 monooxygenase was cloned and disrupted (Yu et al., 1997). Substrate feeding studies of the *ord*-1 mutant confirmed that HAVN is the intermediate in the conversion of AVN to AVF. The *ord-1* gene, which has a high degree of sequence similarity to *A. nidulans stcF* (Brown et al., 1996b), was renamed *aflG* (*avnA*).

3.4 5'-Hydroxyaverantin (HAVN) to oxoaverantin (OAVN), and averufin (AVF)

Numerous studies have established averufin as one of the key intermediates in aflatoxin formation (Lee et al., 1971; Hsieh, 1973; Lin & Hsieh, 1973; Lin et al., 1973; Fitzell et al., 1975; Singh & Hsieh, 1977; Keller et al., 2000). Several intermediates were reported to be involved in the conversion from AVN to AVF (Lin & Hsieh, 1973; Bhatnagar et al., 1992). One of these averufanin (AVNN), based on later studies was considered a shunt metabolite and not a genuine aflatoxin intermediate (Sakuno et al., 2003; Yabe & Nakajima, 2004). Chang et al. (Lee et al., 1971; Lin & Hsieh, 1973; Chang et al., 2000) characterized the cluster gene *aflH* (*adhA*) in *A. parasiticus* which encodes an alcohol dehydrogenase. It was showed that *adhA* deletion mutants accumulated predominantly HAVN and after prolonged growth the mutants were able to produce small amounts of AVNN consistant with AVNN being a shunt metabolite. Thus, HAVN might be converted directly to AVF or indirectly to AVF by an additional cytosolic enzyme. Sakuno et al. (Sakuno et al., 2003) characterized two cytosolic enzymes and a new aflatoxin intermediate named 5'-oxoaverantin (OAVN) as an intermediate between HAVN and AVF. The enzyme for the conversion from HAVN to OAVN is encoded by the *aflH* (*adhA*) gene. The *adhA* gene deletion mutant is leaky indicating that additional enzyme(s) or gene(s) may be involved in the conversion from OAVN to AVF. The enzymatic steps for aflatoxin biosynthesis and the possible involvement of additional enzymes have also been described (Townsend, 1997; Ehrlich, 2009; Ehrlich et

al., 2010). Woloshuk and Payne (Woloshuk & Payne, 1994) identified an alcohol dehydrogenase gene, *adh1*, in *A. flavus*, expressed concurrently with aflatoxin pathway genes. No further report is made on the role of *A. flavus adh1* gene in aflatoxin synthesis. The *aflH* (*adhA*) gene in *A. flavus* and the *adhA* gene in *A. parasiticus* share no significant homology at either the DNA or the amino acid level.

3.5 Averufin (AVF) to versiconal hemiacetal acetate (VHA)

VHA was identified as an aflatoxin precursor formed by oxidation of AVF (Fitzell et al., 1977). The conversion of AVF to VHA involves the cytochrome P450 monooxidase, CypX, and another gene, *aflI* (*avfA*). Although *aflI* is required for the conversion, its oxidative role is unclear (Yu et al., 2000b). *A. nidulans* also has an *aflI* gene homolog (*stcO*) (Brown et al., 1996b; Yu et al., 2000b). Complementation of an averufin-accumulating mutant, *A. parasiticus* SRRC 165, with the *aflI* gene of *A. flavus* restored the strain's ability to convert AVF to VHA and to produce aflatoxins (Yu et al., 2000b). It is likely that the *aflI* (*avfA*) encoded protein along with CypX gene product is involved in the ring-closure step in the formation of hydroxyversicolorone. It is possible that the *avfA* gene product is assocated with the P450 monooxygenase to carry out the conversion as no additional intermediates other that AVF result from the disruption of either gene.

3.6 Versiconal hemiacetal acetate (VHA) to versiconal (VHOH, also abbreviated as VAL)

Several research groups have demonstrated that an esterase is involvement in the conversion of VHA to VHOH (VAL) (Schroeder et al., 1974; Yao & Hsieh, 1974; Bennett et al., 1976b; Fitzell et al., 1977; Hsieh et al., 1989; Yabe et al., 1991a; Yabe et al., 1991b; Kusumoto & Hsieh, 1996). The esterase was purified in *A. parasiticus* (Hsieh et al., 1989; Kusumoto & Hsieh, 1996). An esterase gene, *aflJ* (*estA*), in the aflatoxin gene cluster was identified (Yu et al., 2002). The homologous gene in the *A. nidulans* ST biosynthetic gene cluster is *stcI*. In the *A. parasiticus aflJ* (*estA*) deletion mutants, the accumulated metabolites were mainly VHA and versicolorin A (VERA) (Chang et al., 2004). A small amount of versiconol acetate (VOAc) and other downstream aflatoxin intermediates, including VHOH and versicolorin B also accumulated. A metabolic grid containing VHA, VOAc, VHOH, and versiconol (VOH) was previously described and it was suggested that the reactions from VHA to VHOH and from VOAc to VOH are catalyzed by the same esterase (Yabe et al., 1991a). Later, another metabolic grid containing versicolorone (VONE), VOAc, and VHA was identified (Yabe et al., 2003). Indeed, it has now been proven that the *estA*-encoded esterase catalyzes the conversion of both VHA to VHOH and VOAc to VOH during aflatoxin biosynthesis (Chang et al., 2004).

3.7 Versiconal (VHOH) to versicolorin B (VER B)

The enzymatic evidence that VHOH is converted toVERB by a cyclase was first provided by Lin and Anderson (Lin & Anderson, 1992). This enzyme was identified as versicolorin B synthase and was studied intensively by Townsend's laboratory (Zuckerman et al., 1967; Hsieh, 1973; McGuire et al., 1996; Silva et al., 1996; Silva & Townsend, 1997). The gene was cloned and named *vbs* (Zuckerman et al., 1967; Hsieh, 1973; Silva et al., 1996). The expected cyclase activity was demonstrated by the expressed recombinant protein of the *vbs* gene (Silva et al., 1996; Silva & Townsend, 1997). The VHOH cyclase (Lin & Anderson, 1992) and

VER B synthase (McGuire et al., 1996) were independently isolated from *A. parasiticus*. The enzyme catalyzes the side chain cyclodehydration of racemic VHA to VER B. This is another key step in aflatoxin formation since it closes the bisfuran ring of aflatoxin, the moiety ultimately responsible for aflatoxin's toxicity andcarcinogenicity. . The *vbs* gene was re-named *aflK* (*vbs*) a (Yu et al., 2004c). The homologous gene in the *A. nidulans* ST biosynthetic gene cluster is *stcN*.

3.8 Versicolorin B (VER B) to versicolorin A (VER A)

VER B is a critical branch point leading to the formation of either AFB_1/AFG_1 or AFB_2/AFG_2. Similar to AFB_2/AFG_2, VER B contains a tetrahydrobisfuran ring and, like AFB_1/AFG_1, VERA contains a dihydrobisfuran ring. The conversion of VER B to VER A requires desaturation of the bisfuran ring of VER B by an unstable microsomal enzyme that requires NADPH (Yabe et al., 1993). Disruption of *stcL* in *A. nidulans* (Kelkar et al., 1997) abolished ST synthesis and resulted in the accumulation of VER B. The *stcL* gene encodes a cytochrome P-450 monooxygenase. The homologue, *aflL* (*verB*), is present in the aflatoxin gene cluster of *A. parasiticus* and *A. flavus* strains. Cultural conditions appear to markedly affect the activity of VER B desaturase and thereby, the final ratio of AFB_1 to AFB_2 and AFG_1 to AFG_2 (Yabe & Nakajima, 2004).

3.9 Versicolorin A (VER A) to demethylsterigmatocystin (DMST) and versicolorin B (VER B) to demethyldihydrosterigmatocystin (DMDHST)

The formation of DMST and the biochemical conversion steps from VERA to DMST (and VerB to DHDMST) have been described in great detail (Henry & Townsend, 2005). The *aflM* (*ver-1*) gene (Skory et al., 1992), cloned by genetic complementation of VER A-accumulating *A. parasiticus* CS10, was shown to be responsible for the conversion of VER A to an intermediate that has not been isolated. The *aflM* (*ver-1*) gene was predicted to encode a ketoreductase, similar Nor-1. The *ver-1* homologue, *stcU*, (previously named *verA*) was identified in *A. nidulans* (Keller et al., 1994). Double mutation of *stcU* and *stcL* resulted in accumulation of only VER A (Keller et al., 1994). The *stcS* gene (previously named *verB*), another cytochrome P-450 monooxygenase gene, was also identified and studies showed that it is also involved in the conversion of VER A to an intermediate in the formation of DMST (possibly the first intermediate, which is then acted upon by Ver-1). Disruption of *stcS* resulted in the accumulation of VER A as did disruption of Ver-1 (Keller et al., 1995). Thus, both *stcU* and *stcS* are required for the conversion of VER A to DMST. The *stcS* homologue in *A. parasiticus*, named *aflN* (*verA*), has also been identified (Yu et al., 2004a; Yu et al., 2004c). A third enzyme is required for the conversion: hypA (aflY). This gene is predicted to encode a Baeyer-Villiger monooxygenase. Disruption of this gene also led to accumulation of VERA suggesting that, like VER-1, it acts as part of an enzyme complex without allowing the formation of an intermediate. A fourth enzyme, OrdB has also been implicated in the conversion, and like AvfA, its homolog, may be a helper protein for the monooxygenase, CypX.

3.10 Demethylsterigmatocystin (DMST) to sterigmatocystin (ST) and demethyldihydrosterigmatocystin (DMDHST) to dihydrosterigmatocystin (DHST)

Enzyme purification studies revealed that two *O*-methyltransferases, I and II, are involved in aflatoxin biosynthesis (Yabe et al., 1989). *O*-methyltransferase I catalyzes the transfer of

the methyl from S-adenosylmethionine (SAM) to the hydroxyls of DMST and DHDMST to produce ST and DHST, respectively. This 43-kDa enzyme was purified from *A. parasiticus* and characterized (Yabe et al., 1998; Yabe et al., 1999). The corresponding gene, *dmtA*, was isolated from *A. parasiticus* based on a partial amino acid sequence of the purified enzyme (Motomura et al., 1999). Yu et al. (Yu et al., 2000b) concurrently isolated the same gene but named it *aflO* (*omtB*) (for *O*-methyltransferase B) from *A. parasiticus*, *A. flavus* and *A. sojae*. The predicted *dmtA*-encoded protein contains a consensus SAM-binding motif (Motomura et al., 1999). The *aflO* (*omtB*) homolog in *A. nidulans* was identified as *stcP*. This gene is required for the conversion of DMST to ST in *A. nidulans* as shown by gene disruption (Kelkar et al., 1996).

3.11 Sterigmatocystin (ST) to *O*-methylsterigmatocystin (OMST) and demethylsterigmatocystin (DMST) to dihydro-*O*-methylsterigmatocystin (DHOMST)

The gene for *O*-methyltransferase required for the conversion of ST to OMST and DHST to DHOMST was first cloned (Yu et al., 1993) from *A. parasiticus* by reverse genetics using antibodies raised against the purified *A. parasiticus O*-methyltransferase A (Keller et al., 1993). This gene was initially named *omt-1*, then *omtA* and finally renamed *aflP* (*omtA*) (Yu et al., 1993). The recombinant enzyme was expressed in *E. coli* and its activity to convert ST to OMST was demonstrated by substrate feeding studies (Yu et al., 1993). *O*-methyltransferase A has strict substrate-specificity and cannot methylate DMST or DHDMST. Thus, the *O*-methyltransferases A encoded by *aflP* (*omtA*) is the enzyme responsible for the conversion of ST to OMST and DMST to DHOMST. The genomic DNA sequence of this gene (*omtA*) was cloned from *A. parasiticus* and *A. flavus* (Yu et al., 1995b). This *aflP* (*omtA*) gene homologue was also detected in other aflatoxigenic and non-aflatoxigenic *Aspergillus* species (Klich et al., 1995). The absence of the *aflP* orthologue in *A. nidulans* is the reason that *A. nidulans* produces ST as the end product instead of aflatoxins.

3.12 *O*-methylsterigmatocystin (OMST) to aflatoxin B_1 (AFB_1) and aflatoxin G_1 (AFG_1) and demethyldihydrosterigmatocystin (DMDHST) to aflatoxin B_2 (AFB_2) and aflatoxin G_2 (AFG_2)

The relationship between B-group and G-group aflatoxin formation was proposed based on feeding experiments (Yabe et al., 1988). A P-450 monooxygenase gene in *A. flavus* named *ord-1* was shown to be necessory for this reaction (Prieto et al., 1996; Prieto & Woloshuk, 1997). This P-450 monooxygenase gene, *aflQ* (*ordA*), was cloned in *A. parasiticus* and demonstrated in a yeast system that it is involved in the conversion of OMST to AFB_1/AFG_1, and DHOMST to AFB_2/AFG_2 (Yu et al., 1998). Whether *aflQ* (*ordA*) gene product, OrdA, catalyzes two successive monooxygenase reactions in the later steps of aflatoxin biosynthesis is not clear. Studies (Yu et al., 1998) suggested that additional enzyme(s) is required for the synthesis of G-group aflatoxins. After the cloning and characterization of the *cypA* gene, it is clear that *cypA* encoded a cytochrome P450 monooxygenase for the formation of G-group aflatoxins (Ehrlich et al., 2004). Most recently, the *nadA* gene, which was shown, by gene profiling studies using microarray, to be a member of the aflatoxin gene cluster (Price et al., 2006; Yu et al., 2011) rather than belonging to the adjoining sugar utilization cluster as originally proposed (Yu et al., 2000a), was found to play a role in AFG_1/AFG_2 formation. Yabe's group recently disrupted the *nadA* gene and reported that NadA is a cytosolic enzyme for the conversion from a new aflatoxin intermediate named NADA, which is between OMST and AFG_1, to

AFG_1 (Cai et al., 2008). The *aflE* (*norA*) gene was initially believed to be involved in the conversion of NOR due to certain degree of sequence similarity to the *aflD* (*nor-1*) gene (Cary et al., 1996). However, recent studies support the hypothesis that the *aflE* (*norA*) is involved in the final two steps in AFB_1 formation (Ehrlich, 2009). In the same report, the transcript, *hypB*, a homolog of *hypC*, may be involved in one of the oxidation steps in the conversion of OMST to aflatoxins. *A. flavus* produces only AFB_1 and AFB_2, whereas *A. parasiticus* produces all four major aflatoxins, AFB_1, AFB_2, AFG_1, and AFG_2. Coincidentally, only the G-group aflatoxin producer, *A. parasiticus*, has intact *nadA* and *norB* genes. Preliminary data suggests that *norB* encodes another enzyme predominantly involved in AFG_1/ AFG_2 formation (Ehrlich et al., 2008).

4. Regulation of aflatoxin biosynthesis

The aflatoxin pathway genes are found to be clustered in the genome of *A. flavus* and *A. parasiticus* (Yu et al., 1995a; Woloshuk & Prieto, 1998; Yu et al., 2004a; Yu et al., 2004c). These genes are expressed concurrently except for the regulatory gene *aflR*. In this gene cluster, a positive-acting regulatory gene, *aflR*, is located in the middle of the gene cluster. Adjacent to *aflR* a divergently transcribed gene, *aflS* (*aflJ*), was also found to be involved in the regulation of transcription (Meyers et al., 1998; Chang, 2004). Other physically unrelated genes, such as *laeA* and *veA*, also have been shown to exhibit a "global" regulatory role on aflatoxin biosynthesis (Kato et al., 2003; Bok & Keller, 2004; Calvo et al., 2004; Perrin et al., 2007).

4.1 Regulation by *aflR*

The *aflR* gene, encoding a 47 kDa sequence-specific zinc-finger DNA-binding protein is required for transcriptional activation of most, if not all, the structural genes of the aflatoxin gene cluster (Chang et al., 1993; Payne et al., 1993; Woloshuk et al., 1994; Chang et al., 1995b; Yu et al., 1996a; Yu et al., 1996b; Flaherty & Payne, 1997; Ehrlich et al., 1998; Chang et al., 1999a; Chang et al., 1999b). Like other Gal4-type regulatory proteins that bind to palindromic sequences, functional AflR probably binds as a dimer. It binds to the palindromic sequence 5'-TCGN5CGR-3' in the promoter regions of the structural genes (Ehrlich et al., 1999a; Ehrlich et al., 1999b). The AflR-binding motifs are found to be located from –80 to –600 bp, with the majority at the –100 to –200 bp, 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 binding motif, only one of them is the preferred binding site such as in the case of *aflC* (*pksA*) (Ehrlich et al., 1999a; Ehrlich et al., 1999b). The more upstream motif is found to belong to another gene for turning on the expression of *hypC* (Ehrlich, unpublished observation). Deletion of *aflR* in *A. parasiticus* abolishes the expression of other aflatoxin pathway genes (Cary et al., 2000). Overexpression of *aflR* in *A. flavus* up-regulates aflatoxin pathway gene transcription and aflatoxin accumulation (Flaherty & Payne, 1997) in a fashion similar to that reported for *A. parasiticus* (Chang et al., 1995b). These results demonstrate that AflR is specifically involved in the regulation of aflatoxin biosynthesis. Indeed, all 23 upregulated genes, identified by transcription profiling using DNA microarray assays comparing wild-type and *aflR*-deleted *A. parasiticus* strains, have the consensus AflR binding motif in their promoter regions (Meyers et al., 1998; Price et al., 2006; Wilkinson et al., 2007a; Wilkinson et al., 2007b).

4.2 Regulation by *aflS* (*aflJ*)

The *aflS* (*aflJ*) gene, although not demonstrating significant homology with any other encoded proteins found in databases, is necessary for aflatoxin formation. In the *A. parasiticus aflR* transformants, the production of aflatoxin pathway intermediates was significantly enhanced in transformants that contained an additional *aflR* plus *aflS* (Chang et al., 1995b). Quantitative PCR showed that in the *aflS* knockout mutants, the lack of *aflS* transcript is associated with 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*). The mutants lost the ability to synthesize aflatoxin intermediates and no aflatoxins were produced (Meyers et al., 1998). However, deletion of *aflS* (*aflJ*) did not have a discernible effect on *aflR* transcription, and vice versa. Du et al. (Du et al., 2007) showed that overexpression of *A. flavus aflS* (*aflJ*) did not result in elevated transcription of *aflM* (*ver-1*), *aflP* (*omtA*), or *aflR*, but it appears to have some effect on *aflC* (*pksA*), *aflD* (*nor-1*), *aflA* (*fas-1*), and *aflB* (*fas-2*) (Du et al., 2007), which are required for the biosynthesis of the early aflatoxin pathway intermediate, averantin. The mechanism(s) by which *aflS* modulates transcription of these pathway genes in concert with *aflR* is under investigation by gene profiling analysis using microarray technology.

4.3 Regulation by *laeA*

The novel global regulatory gene, *laeA* (for **l**ack of ***a****flR* **e**xpression), was first identified from *A. nidulans* (Bok & Keller, 2004). This gene is well conserved in fungi as shown by its presence in the genomes of all fungi so far sequenced. LaeA is a nuclear protein which contains an S-adenosylmethionine (SAM) binding motif and activates transcription of several other secondary metabolism gene clusters in addition to the AF cluster. Examples include the sterigmatocystin and penicillin clusters in *A. nidulans*, the gliotoxin cluster in *A. fumigatus*, and aflatoxin cluster in *A. flavus* (Bok & Keller, 2004; Bouhired et al., 2007). It also regulates genes required for virulence of *A. fumigatus* (Sugui et al., 2007). Perrin et al. (Perrin et al., 2007) carried out a whole-genome comparison of the transcriptional profiles of wild-type and *laeA*-deleted *A. fumigatus* strains and found that LaeA positively controls the expression of 20% to 40% of major classes of secondary metabolite biosynthesis genes. It also regulates some genes not associated with secondary metabolite clusters. Similar results were confirmed in gene expression profiling in *A. flavus* using microarrays to study the genetic mechanism of sclerotia formation (Yu, personal communication). The exact mechanism of how LaeA regulates secondary metabolism gene clusters is not yet known. Interestingly, when an unrelated gene such as *argB* was placed within the boundary of the ST gene cluster, it was co-regulated with other genes in the cluster. But, when a gene in the cluster, such as *aflR* was placed elsewhere in the genome, its regulation was not affected by LaeA (Bok et al., 2006). One proposed regulatory mechanism is that LaeA differentially methylates histone protein and it alters the chromatin structure for gene expression. Unlike the mentioned signaling factors, the primary role of LaeA is to regulate metabolic gene clusters, not sporulation, because *laeA*-deleted strains produced wild-type levels of conidia (Bok & Keller, 2004). Most recent analyses of nonaflatoxigenic *A. parasiticus sec-* (for secondary metabolism negative) variants generated through serial transfer of mycelia of the *sec+* parents show that *laeA* was expressed in both *sec+* and *sec-* strains (Kale et al., 2007). This result suggests that LaeA only exerts its effect on aflatoxin biosynthesis at a certain level and is independent of other regulatory pathways that are involved in fungal development.

4.4 Regulation by *veA*

The *veA* gene in *A. nidulans* (Mooney & Yager, 1990) is a gene initially found to be crucial for light-dependent conidiation. The light dependence is abolished by a mutation (*veA1*) which allows conidiation of *A. nidulans* to occur in the dark. A comparison of the light effect on sterigmatocystin production by *A. nidulans veA*+ and *veA1* strains showed that both strains produced sterigmatocystin but the highest amount was produced by the *veA*+ strain grown in darkness. However, *veA*-deleted *A. flavus* and *A. parasiticus* strains completely lost the ability to produce aflatoxin regardless of the illumination conditions (Duran et al., 2007; Stinnett et al., 2007). Under normal growth conditions, some *A. flavus* and all *A. parasiticus* strains produce conidia in both dark and light conditions. Stinnett et al. (Stinnett et al., 2007) showed that VeA contains a bipartite nuclear localization signal (NLS) motif and its migration to the nucleus is light-dependent and requires the importin α carrier protein. In the dark VeA is located mainly in the nucleus; under light it is located both in cytoplasm and nucleus. VeA has no recognizable DNA-binding seuqences and likely exerts its effect on sterigmatosyctin and aflatoxin production through protein-protein interactions with other regulatory factors. Post-translational modifications such as phosphylation and dephosphorylation may modulate its activity. Lack of VeA production in the *veA*-deleted *A. flavus* and *A. parasiticus* strains consequently abolishes aflatoxin production because a threshold concentration of nuclear VeA might be necessary to initiate aflatoxin biosynthesis.

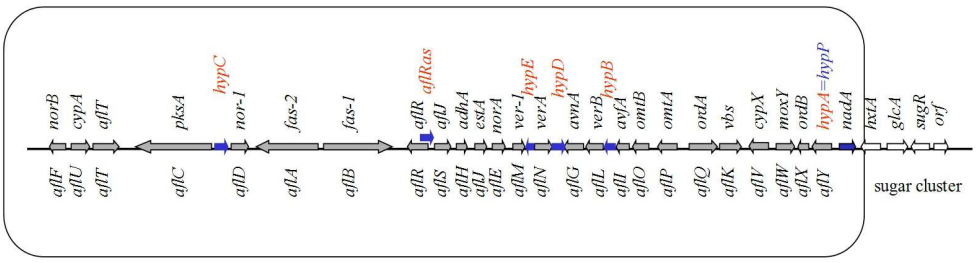


Fig. 1. Aflatoxin pathway gene cluster in *A. flavus.* This figure shows the order and location of the 30 aflatoxin pathway genes plus an *aflR* antisense gene clustered together in about 80 kb DNA region. The old gene names are labeled on top of the line and the new gene names sysmatically renamed according to gene convention are labeled below the line (Yu et al., 2004c). The transcripts of *hypA*, *hypB*, *hypC*, *hypD*, *hypE* and *aflRas* are identified through *Aspergillus flavus* EST. Arrows indicate the direction of gene transcription.

5. Conclusions

Aflatoxins are toxic and carcinogenic secondary metabolites produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus* that contaminate preharvest crops and post harvest grains. Scientists worldwide have extensively studied biosynthesis of aflatoxins for more than 50 years. Aflatoxin biosynthesis is a complex process involving many intermediates and enzymes. Regulation of aflatoxin gene expression occurs at multiple levels and by multiple regulatory components. There are genetic factors, biotic and abiotic elements that affect aflatoxin formation. Recent studies have shed more light on the functions of the enzymes involved in each of the steps of aflatoxin biosynthesis, the genes encoding those enzymes,

and the regulatory mechanisms of aflatoxin formation. Better understanding of the mechanisms of aflatoxin biosynthesis helps to identify natural inhibitors of fungal growth aflatoxin formation, and eventually will allow design of effective strategies to can reduce or eliminate aflatoxin contamination of food and feed commodities.

6. References

Allcroft, R., Carnaghan, R.B.A., Sargeant, K. & O'Kelly, J. (1961). A toxic factor in Brazilian groundnut meal, *Vet. Rec.* 73, 428-429.

Arsura, M. & Cavin, L.G. (2005). Nuclear factor-kappaB and liver carcinogenesis, *Cancer Lett.* 229, 157-169.

Azziz-Baumgartner, E., Lindblade, K., Gieseker, K., Rogers, H.S., Kieszak, S., Njapau, H., Schleicher, R., McCoy, L.F., Misore, A., DeCock, K., Rubin, C. & Slutsker, L. (2005). Case-control study of an acute aflatoxicosis outbreak, Kenya, 2004, *Aflatoxin Investigative Group. Environ. Health Perspect.* 113, 1779-1783.

Baertschi, S.W., Raney, K.D., Shimada, T., Harris, T.M. & Guengerich, F.P. (1989). Comparison rates of enzymatic oxidation of aflatoxin B1, aflatoxin G1, and sterigmatocystin and activities of the epoxides in forming guanyl-N adducts and inducing different genetic responses, *Chem. Res. Toxicol.* 2, 114-122.

Bennett, J.W. (1970). Microbiological aspects of the aflatoxin problem, *Amer Ass Feed Microscop Offic Proc* 118-131.

Bennett, J.W. (1979). Aflatoxins and anthraquinones from diploids of *Aspergillus parasiticus*, *J. Gen. Microbiol.* 113, 127-136.

Bennett, J.W. (1981). Loss of norsolorinic acid and aflatoxin production by a mutant of *Aspergillus parasiticus*, *J. Gen. Microbiol.* 124, 429- 432.

Bennett, J.W. (1987). Mycotoxins, mycotoxicoses, mycotoxicology and mycopathologia, *Mycopathologia* 100, 3-5.

Bennett, J.W., Bhatnagar, D. & Chang, P.K. (1994). The molecular genetics of aflatoxin biosynthesis, *FEMS Symp.* 51-58.

Bennett, J.W., Chang, P.K. & Bhatnagar, D. (1997). One gene to whole pathway: the role of norsolorinic acid in aflatoxin research, *Adv. Appl. Microbiol.* 45, 1-15.

Bennett, J.W. & Goldblatt, L.A. (1973). The isolation of mutants of *Aspergillus flavus* and *A. parasiticus* with altered aflatoxin producing ability, *Sabouraudia* 11, 235-241.

Bennett, J.W. & Klich, M. (2003). Mycotoxins, *Clin. Microbiol. Rev.* 16, 497-516.

Bennett, J.W., Kronberg, F. & Gougis, G. (1976a). Pigmented isolates from anthraquinone-producing mutants of *Aspergillus parasiticus*, *American Society for Microbiology* 76, 6.

Bennett, J.W., Kronberg, F.G., Goodman, L.A. & Seltman, M.A. (1983). Isolation of an anthraquinoe-accumulating mutant of *Aspergillus parasiticus* and partial characterization by dry column chromatography, *Mycologia* 75, 202-208.

Bennett, J.W. & Lee, L.S. (1979). Mycotoxins - their biosynthesis in fungi: aflatoxins and other bisfuranoids, *J Food Protection* 42, 805-809.

Bennett, J.W., Lee, L.S. & Cucullu, A.F. (1976b). Effect of dichlorvos on aflatoxin and versicolorin A production in *Aspergillus parasiticus*, *Bot. Gaz.* 137, 318-324.

Bennett, J.W., Lee, L.S., Shoss, S.M. & Boudreaux, G.H. (1980). Identification of averantin as an aflatoxin B1 precursor: placement in the biosynthetic pathway, *Appl. Environ. Microbiol.* 39, 835-839.

Bennett, J.W., Lee, L.S. & Vinnett, C.H. (1971). The correlation of aflatoxin and norsolorinic acid production, *J. American Oil Chemists Society* 48, 368-370.

Bennett, J.W., Leong, P.M., Kruger, S.J. & Keyes, D. (1986). Sclerotial and low aflatoxigenic morphological variants from haploid and diploid *Aspergillus parasiticus Experientia* 42, 848-851.

Bennett, J.W. & Papa, K.E. (1988). The aflatoxigenic *Aspergillus* spp., *Adv. Plant Pathol.* 6, 265-279.

Bennett, J.W., Silverstein, R.B. & Kruger, S.J. (1981). Isolation and characterization of two nonaflatoxigenic classes of morphological variants of *Aspergillus parasiticus*, *J. American Oil Chemists Society* 952.

Bhatnagar, D., Brown, R., Ehrlich, K. & Cleveland, T.E. (2002). Mycotoxins Contaminating Cereal Grain Crops: Their Occurrence and Toxicity. In: Khachatourians, G.G. & Arora, D.K. (Eds.), *Applied Mycology and Biotechnology*. Elsevier B.V., New York, pp. 171-196.

Bhatnagar, D. & Cleveland, T.E. (1991). Aflatoxin biosynthesis: developments in chemistry, biochemistry, and genetics, *Res. Bull. Iowa State Univ. Agric. Home Econ. Exp. Stn.* 391-405.

Bhatnagar, D., Cleveland, T.E. & Lillehoj, E.B. (1989). Enzymes in aflatoxin B1 biosynthesis: strategies for identifying pertinent genes, *Mycopathologia* 107, 75-83.

Bhatnagar, D., Ehrlich, K.C. & Cleveland, T.E. (1992). Oxidation- reduction reactions in biosynthesis of secondary metabolites. In: Bhatnagar, D., Lillehoj, E.B. & Arora, D.K. (Eds.), *Mycotoxins in Ecological Systems*. Marcel Dekker, New York, pp. 255-285.

Bok, J.-W. & Keller, N.P. (2004). LaeA, a regulator of secondary metabolism in *Aspergillus* spp., *Eukaryot. Cell* 3, 527-535.

Bok, J.W., Noordermeer, D., Kale, S.P. & Keller, N.P. (2006). Secondary metabolic gene cluster silencing in *Aspergillus nidulans*, *Mol. Microbiol.* 61, 1636-1645.

Bouhired, S., Weber, M., Kempf-Sontag, A., Keller, N.P. & Hoffmeister, D. (2007). Accurate prediction of the *Aspergillus nidulans* terrequinone gene cluster boundaries using the transcriptional regulator LaeA, *Fungal Genet. Biol.* 44, 1134-1145.

Bressac, B., Kew, M., Wands, J. & Ozturk, M. (1991). Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa, *Nature* 350, 429-431.

Brown, D.W., Adams, T.H. & Keller, N.P. (1996a). *Aspergillus* has distinct fatty acid synthases for primary and secondary metabolism, *Proc. Natl. Acad. Sci. U. S. A.* 93, 14873-14877.

Brown, D.W., Yu, J.H., Kelkar, H.S., Fernandes, M., Nesbitt, T.C., Keller, N.P., Adams, T.H. & Leonard, T.J. (1996b). Twenty-five coregulated transcripts define a sterigmatocystin gene cluster in *Aspergillus nidulans*, *Proc. Natl. Acad. Sci. U. S. A.* 93, 1418-1422.

Busby, W.F., Jr. & Wogan, G.N. (1981). Aflatoxins. In: Shank, R.C. (Ed.), *Mycotoxins and N-nitrosocompounds: Environmental Risks*. CRC Press, Boca Raton, pp. 3-45.

Cai, J., Zeng, H., Shima, Y., Hatabayashi, H., Nakagawa, H., Ito, Y., Adachi, Y., Nakajima, H. & Yabe, K. (2008). Involvement of the *nadA* gene in formation of G-group aflatoxins in *Aspergillus parasiticus*, *Fungal Genet Biol* 45, 1081-1093.

Calvo, A.M., Bok, J.-W., Brooks, W. & Keller, N.P. (2004). VeA is required for toxin and sclerotial production in *Aspergillus parasiticus*, *Appl. Environ. Microbiol.* 70, 4733-4739.

Cary, J.W., Ehrlich, K.C., Wright, M., Chang, P.K. & Bhatnagar, D. (2000). Generation of aflR disruption mutants of *Aspergillus parasiticus*, *Appl. Microbiol. Biotechnol.* 53, 680-684.

Cary, J.W., Wright, M., Bhatnagar, D., Lee, R. & Chu, F.S. (1996). Molecular characterization of an *Aspergillus parasiticus* dehydrogenase gene, *norA*, located on the aflatoxin biosynthesis gene cluster, *Appl. Environ. Microbiol.* 62, 360-366.

Centers for Disease Control and Prevention (2004). Outbreak of aflatoxin poisoning - eastern and central province, Kenya, *MMWR Morb Mortal Weekly Report* 53, 790-792.

Chang, P.K. (2004). Lack of interaction between AFLR and AFLJ contributes to nonaflatoxigenicity of *Aspergillus sojae*, *J. Biotechnol.* 107, 245-253.

Chang, P.K., Bennett, J.W. & Cotty, P.J. (2002). Association of aflatoxin biosynthesis and sclerotial development in *Aspergillus parasiticus*, *Mycopathologia* 153, 41-48.

Chang, P.K., Cary, J.W., Bhatnagar, D., Cleveland, T.E., Bennett, J.W., Linz, J.E., Woloshuk, C.P. & Payne, G.A. (1993). Cloning of the *Aspergillus parasiticus apa-2* gene associated with the regulation of aflatoxin biosynthesis, *Appl. Environ. Microbiol.* 59, 3273-3279.

Chang, P.K., Cary, J.W., Yu, J., Bhatnagar, D. & Cleveland, T.E. (1995a). The *Aspergillus parasiticus* polyketide synthase gene *pksA*, a homolog of *Aspergillus nidulans wA*, is required for aflatoxin B1 biosynthesis, *Mol. Gen. Genet.* 248, 270-277.

Chang, P.K., Ehrlich, K.C., Yu, J., Bhatnagar, D. & Cleveland, T.E. (1995b). 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.

Chang, P.K., Horn, B.W. & Dorner, J.W. (2005). Sequence breakpoints in the aflatoxin biosynthesis gene cluster and flanking regions in nonaflatoxigenic *Aspergillus flavus* isolates, *Fungal Genet. Biol.* 42, 914-923.

Chang, P.K., Skory, C.D. & Linz, J.E. (1992). Cloning of a gene associated with aflatoxin B1 biosynthesis in *Aspergillus parasiticus*, *Curr. Genet.* 21, 231-233.

Chang, P.K., Yabe, K. & Yu, J. (2004). The *Aspergillus parasiticus estA*- encoded esterase converts versiconal hemiacetal acetate to versiconal and versiconol acetate to versiconol in aflatoxin biosynthesis, *Appl. Environ. Microbiol.* 70, 3593-3599.

Chang, P.K., Yu, J., Bhatnagar, D. & Cleveland, T.E. (1999a). 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.

Chang, P.K., Yu, J., Bhatnagar, D. & Cleveland, T.E. (1999b). Repressor- AFLR interaction modulates aflatoxin biosynthesis in *Aspergillus parasiticus*, *Mycopathologia* 147, 105-112.

Chang, P.K., Yu, J., Ehrlich, K.C., Boue, S.M., Montalbano, B.G., Bhatnagar, D. & Cleveland, T.E. (2000). *adhA* in *Aspergillus parasiticus* is involved in conversion of 5'-hydroxyaverantin to averufin, *Appl. Environ. Microbiol.* 66, 4715-4719.

Chen, C.J., Wang, L.Y., Lu, S.N., Wu, M.H., You, S.L., Zhang, Y.J., Wang, L.W. & Santella, R.M. (1996a). Elevated aflatoxin exposure and increased risk of hepatocellular carcinoma, *Hepatology* 24, 38- 42.

Chen, C.J., Yu, M.W., Liaw, Y.F., Wang, L.W., Chiamprasert, S., Matin, F., Hirvonen, A., Bell, D.A. & Santella, R.M. (1996b). Chronic hepatitis B carriers with null genotypes of glutathione S transferase MI and TI polymorphisms who are exposed to aflatoxin are at increased risk of hepatocellular carcinoma, *Am. J. Human Genet.* 59, 128-134.

Cleveland, T.E. & Bhatnagar, D. (1987). Individual reaction requirements of two enzyme activities, isolated from Aspergillus parasiticus, which together catalyze conversion of sterigmatocystin to aflatoxin B1, *Can. J. Microbiol.* 33, 1108-1112.

Cleveland, T.E. & Bhatnagar, D. (1990). Evidence for de novo synthesis of an aflatoxin pathway methyltransferase near the cessation of active growth and the onset of aflatoxin biosynthesis in *Aspergillus parasiticus* mycelia, *Can. J. Microbiol.* 36, 1-5.

Cleveland, T.E. & Bhatnagar, D. (1991). Molecular regulation of aflatoxin biosynthesis. In: Bray, G.A. & Ryan, D.H. (Eds.), *Proc Symp Mycotoxins Cancer Health*, pp. 270-287.

Cleveland, T.E. & Bhatnagar, D. (1992). Molecular strategies for reducing aflatoxin levels in crops before harvest. In: Bhatnagar, D. & Cleveland, T.E. (Eds.), *Molecular Approaches to Improving Food Quality and Safety*. Van Nostrand Reinhold, New York, pp. 205- 228.

Cleveland, T.E., Lax, A.R., Lee, L.S. & Bhatnagar, D. (1987). Appearance of enzyme activities catalyzing conversion of sterigmatocystin to aflatoxin B1 in late-growth-phase Aspergillus parasiticus cultures, *Appl. Environ. Microbiol.* 53, 1711-1713.

Cotty, P. (1988). Aflatoxin and sclerotial production by *Aspergillus flavus*: influence of pH, *Phytopathol.* 78, 1250-1253.

Council for Agricultural Science and Technology (2003), Mycotoxins: Risk in plant, animal, and human systems, Ames, IA.

Coursaget, P., Depril, N., Chabaud, M., Nandi, R., Mayelo, V., LeCann, P. & Yvonnet, B. (1993). High prevalence of mutations at codon 249 of the p53 gene in hyptocellular carcinomas from Senegal, *Br. J. Cancer* 67, 1395-1397.

Crawford, J.M., Dancy, B.C., Hill, E.A., Udwary, D.W. & Townsend, C.A. (2006). Identification of a starter unit acyl-carrier protein transacylase domain in an iterative type I polyketide synthase, *Proc. Natl. Acad. Sci. U. S. A.* 103, 16728-16733.

Crawford, J.M., Thomas, P.M., Scheerer, J.R., Vagstad, A.L., Kelleher, N.L. & Townsend, C.A. (2008a). Deconstruction of iterative multidomain polyketide synthase function, *Science* 320, 243-246.

Crawford, J.M., Vagstad, A.L., Ehrlich, K.C. & Townsend, C.A. (2008b). Starter unit specificity directs genome mining of polyketide synthase pathways in fungi, *Bioorg. Chem.* 36, 16-22.

Crawford, J.M., Vagstad, A.L., Whitworth, K.P., Ehrlich, K.C. & Townsend, C.A. (2008c). Synthetic strategy of nonreducing Iterative polyketide synthases and the origin of the classical "Starter-Unit Effect", *Chembiochem.*

Denning, D.W. (1998). Invasive aspergillosis, *Clin. Infect. Dis.* 26, 781-805.

Denning, D.W., Follansbee, S.E., Scolaro, M., Norris, S., Edelstein, H. & Stevens, D.A. (1991). Pulmonary aspergillosis in the acquired immunodeficiency syndrome, *N. Engl. J. Med.* 324, 654-662.

Denning, D.W., Riniotis, K., Dobrashian, R. & Sambatakou, H. (2003). Chronic cavitary and fibrosing pulmonary and pleural aspergillosis: Case series, proposed nomenclature and review, *Clin. Infect. Dis.* 37, S265-280.

Detroy, R.W., Freer, S. & Ciegler, A. (1973). Aflatoxin and anthraquinone biosynthesis by nitrosoguanidine-derived mutants of *Aspergillus parasiticus*, *Can. J. Microbiol.* 19, 1373-1378.

Du, W., Obrian, G.R. & Payne, G.A. (2007). Function and regulation of *aflJ* in the accumulation of aflatoxin early pathway intermediate in *Aspergillus flavus*, *J. Food Addit. Contam.* 24, 1043-1050.

Duran, R.M., Cary, J.W. & Calvo, A.M. (2007). Production of cyclopiazonic acid, aflatrem, and aflatoxin by *Aspergillus flavus* is regulated by *veA*, a gene necessary for sclerotial formation, *Appl. Microbiol. Biotechnol.* 73, 1158-1168.

Dutton, M.F. (1988). Enzymes and aflatoxin biosynthesis, *Microbiol. Rev.* 52, 274-295. Eaton, D. & Gallagher, E. (1994). Mechanisms of aflatoxin carcinogenesis, *Annu. Rev. Pharmacol. Toxicol.* 34, 135-172.

Eaton, D.L. & Groopman, J.D. The toxicology of aflatoxins: human health, veterinary, and agricultural significance. Academic Press, San Diego, CA, 1994. Ehrlich, K.C. (2009). Predicted roles of uncharacterized clustered genes in aflatoxin biosynthesis, *Toxins* 1, 37-58.

Ehrlich, K.C., Cary, J.W. & Montalbano, B.G. (1999a). Characterization of the promoter for the gene encoding the aflatoxin biosynthetic pathway regulatory protein AFLR, *Biochim. Biophys. Acta.* 1444, 412-417.

Ehrlich, K.C., Chang, P.-K., Scharfenstein, J.S.L., Cary, J.W., Crawford, J.M. & Townsend, C.A. (2010). Absence of the aflatoxin biosynthesis gene, *norA*, allows accumulation of deoxyaflatoxin B1 in *Aspergillus flavus* cultures, *FEMS Microbiol. Lett.* 305, 65-70.

Ehrlich, K.C., Chang, P.K., Yu, J. & Cotty, P.J. (2004). Aflatoxin biosynthesis cluster gene *cypA* is required for G aflatoxin formation, *Appl. Environ. Microbiol.* 70, 6518-6524.

Ehrlich, K.C., Montalbano, B.G., Bhatnagar, D. & Cleveland, T.E. (1998). Alteration of different domains in AFLR affects aflatoxin pathway metabolism in *Aspergillus parasiticus* transformants, *Fungal Genet. Biol.* 23, 279-287.

Ehrlich, K.C., Montalbano, B.G. & Cary, J.W. (1999b). Binding of the C6- zinc cluster protein, AFLR, to the promoters of aflatoxin pathway biosynthesis genes in *Aspergillus parasiticus*, *Gene* 230, 249-257.

Ehrlich, K.C., Scharfenstein, J.S.L., Montalbano, B.G. & Chang, P.-K. (2008). Are the genes nadA and norB involved in formation of aflatoxin G1, *International Journal of Molecular Sciences* 9, 1717- 1729.

Ehrlich, K.C. & Yu, J. (2009). Aflatoxin-like gene clusters and how they evolved. In: Varma, A.K. & Rai, M.K. (Eds.), *Mycotoxins in Food, Feed, and Bioweapons*. Springer Verlag, Heidelberg, Dordrecht, London, New York, pp. 65-76.

FAO (1995), Worldwide regulations for mycotoxins 1995, A Compendium. Italy, Rome.

Fitzell, D.L., Hsieh, D.P., Yao, R.C. & La Mar, G.N. (1975). Biosynthesis of averufin from acetate by A*spergillus parasiticus*, *J. Agric. Food Chem.* 23, 442-444.

Fitzell, D.L., Singh, R., Hsieh, D.P. & Motell, E.L. (1977). Nuclear magnetic resonance identification of versiconal hemiacetal acetate as an intermediate in aflatoxin biosynthesis, *J. Agric. Food. Chem.* 25, 1193-1197.

Flaherty, J.E. & Payne, G.A. (1997). Overexpression of *aflR* leads to upregulation of pathway gene expression and increased aflatoxin production in *Aspergillus flavus*, *Appl. Environ. Microbiol.* 63, 3995-4000.

Fung, F. & Clark, R.F. (2004). Health effects of mycotoxins: a toxicological overview, *J. Toxicol. Clin. Toxicol.* 42, 217-234. Goldblatt, L.A. (1969). Aflatoxin-scientific background, control and implications. Acaderitic Press, New York.

Gong, Y., Hounsa, A., Egal, S., Turner, P.C., Sutcliffe, A.E., Hall, A.J., Cardwell, K. & Wild, C.P. (2004). Postweaning exposure to aflatoxin results in impaired child growth: a longitudinal study in Benin, West Africa, *Environ Health Perspect* 112, 1134-1138.

Goto, T., Wicklow, D.T. & Ito, Y. (1996). Aflatoxin and cyclopiazonic acid production by a sclerotium-producing *Aspergillus tamarii* strain, *Appl. Environ. Microbiol.* 62, 4036-4038.

Groopman, J.D., Wogan, G.N., Roebuck, B.D. & Kensler, T.W. (1994). Molecular biomarkers for aflatoxins and their application to human cancer prevention, *Cancer Res.* 54, 190-191.

Hall, A.J. & Wild, C.P. (1994). Epidemiology of aflatoxin-related disease. In: Eaton, D.L. & Groopman, J.D. (Eds.), *The Toxicology of Aflatoxins: Human Health, Veterinary, and Agricultural Significance*. Academic Press, San Diego, pp. 233-258.

Henry, K.M. & Townsend, C.A. (2005). Ordering the reductive and cytochrome P450 oxidative steps in demethylsterigmatocystin formation yields general insights into the biosynthesis of aflatoxin and related fungal metabolites, *J. Am. Chem. Soc.* 127, 3724-3733.

Hitchman, T.S., Schmidt, E.W., Trail, F., Rarick, M.D., Linz, J.E. & Townsend, C.A. (2001). Hexanoate synthase, a specialized type I fatty acid synthase in aflatoxin B1 biosynthesis, *Bioorg. Chem.* 29, 293-307.

Hsieh, D.P. (1973). Inhibition of aflatoxin biosynthesis of dichlorvos, *J. Agric. Food Chem.* 21, 468-470.

Hsieh, D.P., Cullen, J.M., Hsieh, L.S., Shao, Y. & Ruebner, B.H. (1985). Cancer risks posed by aflatoxin M1, *Princess Takamatsu Symp.* 16, 57-65.

Hsieh, D.P., Lin, M.T. & Yao, R.C. (1973). Conversion of sterigmatocystin to aflatoxin B1 by *Aspergillus parasiticus*, *Biochem. Biophys. Res. Commun.* 52, 992-997.

Hsieh, D.P., Lin, M.T., Yao, R.C. & Singh, R. (1976). Biosynthesis of aflatoxin. Conversion of norsolorinic acid and other hypothetical intermediates into aflatoxin B1, *J. Agric. Food Chem.* 24, 1170-1174.

Hsieh, D.P. & Mateles, R.I. (1970). The relative contribution of acetate and glucose to aflatoxin biosynthesis, *Biochem. Biophys. Acta.* 208, 482- 486.

Hsieh, D.P., Wan, C.C. & Billington, J.A. (1989). A versiconal hemiacetal acetate converting enzyme in aflatoxin biosynthesis, *Mycopathologia* 107, 121-126.

Hsieh, D.P.H. (1989). Potential human health hazards of mycotoxins. In: Natori, S., Hashimoto, H. & Ueno, Y. (Eds.), *Mycotoxins and Phycotoxins*. Elsevier, Amsterdam, pp. 69-80.

Hsu, I.C., Metcalf, R.A., Sun, T., Welsh, J.A., Wang, N.J. & Harris, C.C. (1991). Mutational hotspot in the p53 gene in human hepatocellular carcinomas, *Nature* 350, 427-428.

Huang, J.H. & Hsieh, D.P. (1988). Comparative study of aflatoxins M1 and B1 production in solid-state and shaking liquid cultures, *Proc. Natl. Sci. Counc. Repub. China* 12, 34-42.

Jelinek, C.F., Pohland, A.E. & Wood, G.E. (1989). Worldwide occurrence of mycotoxins in food and feeds--an update, *J. Assoc. Off. Anal. Chem.* 72, 223-230.

Kale, S.P., Cary, J.W., Hollis, N., Wilkinson, J.R., Bhatnagar, D., Yu, J., Cleveland, T.E. & Bennett, J.W. (2007). Analysis of aflatoxin regulatory factors in serial transfer-induced non-aflatoxigenic *Aspergillus parasiticus*, *J. Food Addit. Contam.* 24, 1061-1069.

Kato, N., Brooks, W. & Calvo, A.M. (2003). The expression of sterigmatocystin and penicillin genes in *Aspergillus nidulans* is controlled by *veA*, a gene required for sexual development, *Eukaryot. Cell* 2, 1178-1186.

Kelkar, H.S., Keller, N.P. & Adams, T.H. (1996). *Aspergillus nidulans stcP* encodes an *O*-methyltransferase that is required for sterigmatocystin biosynthesis, *Appl. Environ. Microbiol.* 62, 4296- 4298.

Kelkar, H.S., Skloss, T.W., Haw, J.F., Keller, N.P. & Adams, T.H. (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.

Keller, N.P., Dischinger, H.C., Jr., Bhatnagar, D., Cleveland, T.E. & Ullah, A.H. (1993). Purification of a 40-kilodalton methyltransferase active in the aflatoxin biosynthetic pathway, *Appl. Environ. Microbiol.* 59, 479-484.

Keller, N.P., Kantz, N.J. & Adams, T.H. (1994). *Aspergillus nidulans verA* is required for production of the mycotoxin sterigmatocystin, *Appl. Environ. Microbiol.* 60, 1444-1450.

Keller, N.P., Segnar, S., Bhatnagar, D. & Adams, T.H. (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.

Keller, N.P., Watanabe, C.M., Kelkar, H.S., Adams, T.H. & Townsend, C.A. (2000). Requirement of monooxygenase-mediated steps for sterigmatocystin biosynthesis by *Aspergillus nidulans*, *Appl. Environ. Microbiol.* 66, 359-362.

Kelly, J.D., Eaton, D.L., Guengerich, F.P. & Coulombe, R.A., Jr. (1997). Aflatoxin B1 activation in human lung, *Toxicol. Appl. Pharmacol.* 144, 88-95.

Klich, M.A. (1998). Soil fungi of some low-altitude desert cotton fields and ability of their extracts to inhibit *Aspergillus flavus*, *Mycopathologia* 142, 97-100.

Klich, M.A., Yu, J., Chang, P.K., Mullaney, E.J., Bhatnagar, D. & Cleveland, T.E. (1995). Hybridization of genes involved in aflatoxin biosynthesis to DNA of aflatoxigenic and non-aflatoxigenic aspergilli, *Appl. Microbiol. Biotechnol.* 44, 439-443.

Krishnamachari, K.A., Bhat, R.V., Nagarajan, V. & Tilak, T.B. (1975). Hepatitis due to aflatoxicosis: an outbreak of hepatitis in parts of western India, *Lancet.* 1, 1061-1063.

Kusumoto, K.I. & Hsieh, D.P.H. (1996). Purification and characterization of the esterases involved in aflatoxin biosynthesis in *Aspergillus parasiticus*, *Can. J. Microbiol.* 42, 804-810.

Lancaster, M.D., Jenkins, F.P. & Philip, J.M. (1961). Toxicity associated with certain samples of ground nuts, *Nature* 192, 1095-1096.

Lee, L.S., Bennett, J.W., Goldblatt, L.A. & Lundin, R.E. (1971). Norsolorinic acid from a mutant strain of *Aspergillus parasiticus*, *J. American Oil Chemists Society* 48, 93-94.

Lewis, L., Onsongo, M., Njapau, H., Schurz-Rogers, H., Luber, G., Kieszak, S., Nyamongo, J., Backer, L., Dahiye, A.M., Misore, A., DeCock, K. & Rubin, C. (2005). Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in eastern and central Kenya, *Environ Health Perspect* 113, 1763- 1767.

Lin, B.K. & Anderson, J.A. (1992). Purification and properties of versiconal cyclase from *Aspergillus parasiticus*, *Arch. Biochem. Biophys.* 293, 67-70.

Lin, M.T. & Hsieh, D.P. (1973). Averufin in the biosynthesis of aflatoxin B, *J. Am Chem. Soc.* 95, 1668-1669.

Lin, M.T., Hsieh, D.P., Yao, R.C. & Donkersloot, J.A. (1973). Conversion of averufin into aflatoxins by *Aspergillus parasiticus*, *Biochemistry* 12, 5167-5171.

Mahanti, N., Bhatnagar, D., Cary, J.W., Joubran, J. & Linz, J.E. (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.

McCormick, S.P., Bhatnagar, D. & Lee, L.S. (1987). Averufanin is an aflatoxin B1 precursor between averantin and averufin in the biosynthetic pathway, *Appl. Environ. Microbiol.* 53, 14-16.

McGlynn, K.A., Hunter, K., LeVoyer, T., Roush, J., Wise, P., Michielli, R.A., Shen, F.M., Evans, A.A., London, W.T. & Buetow, K.H. (2003). Susceptibility to aflatoxin B1-related primary hepatocellular carcinoma in mice and humans, *Cancer Res.* 63, 4594-4601.

McGuire, S.M., Silva, J.C., Casillas, E.G. & Townsend, C.A. (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.

Meyers, D.M., Obrian, G., Du, W.L., Bhatnagar, D. & Payne, G.A. (1998). Characterization of *aflJ*, a gene required for conversion of pathway intermediates to aflatoxin, *Appl. Environ. Microbiol.* 64, 3713-3717.

Minto, R.E. & Townsend, C.A. (1997). Enzymology and molecular biology of aflatoxin biosynthesis, *Chem. Rev.* 97, 2537-2556.

Mooney, J.L. & Yager, L.N. (1990). Light is required for conidiation in *Aspergillus nidulans*, *Genes Dev.* 4, 1473-1482.

Mori, T., Matsumura, M., Yamada, K., Irie, S., Oshimi, K., Suda, K., Oguri, T. & Ichinoe, M. (1998). Systemic aspergillosis caused by an aflatoxin-producing strain of *Aspergillus flavus*, *Med. Mycol.* 36, 107-112.

Motomura, M., Chihaya, N., Shinozawa, T., Hamasaki, T. & Yabe, K. (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.

Ngindu, A., Johnson, B.K., Kenya, P.R., Ngira, J.A., Ocheng, D.M., Nandwa, H., Omondi, T.N., Jansen, A.J., Ngare, W., Kaviti, J.N., Gatei, D. & Siongok, T.A. (1982). Outbreak of acute hepatitis caused by aflatoxin poisoning in Kenya., *Lancet.* 1, 1346-1348.

Nierman, W.C., Pain, A., Anderson, M.J., Wortman, J.R., Kim, H.S., Arroyo, J., Berriman, M., Abe, K., Archer, D.B., Bermejo, C., Bennett, J., Bowyer, P., Chen, D., Collins, M.,

Coulsen, R., Davies, R., Dyer, P.S., Farman, M., Fedorova, N., Fedorova, N., Feldblyum, T.V., Fischer, R., Fosker, N., Fraser, A., Garcia, J.L., Garcia, M.J., Goble, A., Goldman, G.H., Gomi, K., Griffith-Jones, S., Gwilliam, R., Haas, B., Haas, H., Harris, D., Horiuchi, H., Huang, J., Humphray, S., Jimenez, J., Keller, N., Khouri, H., Kitamoto, K., Kobayashi, T., Konzack, S., Kulkarni, R., Kumagai, T., Lafon, A., Latge, J.P., Li, W., Lord, A., Lu, C., Majoros, W.H., May, G.S., Miller, B.L., Mohamoud, Y., Molina, M., Monod, M., Mouyna, I., Mulligan, S., Murphy, L., O'Neil, S., Paulsen, I., Penalva, M.A., Pertea, M., Price, C., Pritchard, B.L., Quail, M.A., Rabbinowitsch, E., Rawlins, N., Rajandream, M.A., Reichard, U., Renauld, H., Robson, G.D., Rodriguez de Cordoba, S., Rodriguez-Pena, J.M., Ronning, C.M., Rutter, S., Salzberg, S.L., Sanchez, M., Sanchez-Ferrero, J.C., Saunders, D., Seeger, K., Squares, R., Squares, S., Takeuchi, M., Tekaia, F., Turner, G., Vazquez de Aldana, C.R., Weidman, J., White, O., Woodward, J., Yu, J.H., Fraser, C., Galagan, J.E., Asai, K., Machida, M., Hall, N., Barrell, B. & Denning, D.W. (2005). Genomic sequence of the pathogenic and allergenic filamentous fungus *Aspergillus fumigatus*, *Nature* 438, 1151-1156.

Ozturk, M. (1991). p53 mutation in hepatocellular carcinoma after aflatoxin exposure, *Lancet.* 338, 1356-1359.

Papa, G. (1982). Norsolorinic acid mutant of *Aspergillus flavus*, *J. Gen. Microbiol.* 128, 1345-1348.

Papa, K.E. (1976). Linkage groups in *Aspergillus flavus*, *Mycologia* 68, 159-165.

Papa, K.E. (1979). Genetics of *Aspergillus flavus*: complementation and mapping of aflatoxin mutants, *Genet. Res.* 34, 1-9.

Papa, K.E. (1984). Genetics of *Aspergillus flavus*: linkage of aflatoxin mutants, *Can. J. Microbiol.* 30, 68-73.

Payne, G.A. (1998). Process of contamination by aflatoxin-producing fungi and their impacts on crops. In: Sinha, K.K. & Bhatnagar, D. (Eds.), *Mycotoxins in Agriculture and Food Safety*. Marcel Dekker, New York, pp. 279-306.

Payne, G.A., Nystrom, G.J., Bhatnagar, D., Cleveland, T.E. & Woloshuk, C.P. (1993). Cloning of the *afl-2* gene involved in aflatoxin biosynthesis from *Aspergillus flavus*, *Appl. Environ. Microbiol.* 59, 156-162.

Peers, F., Bosch, X., Kaldor, J., Linsell, A. & Pluijmen, M. (1987). Aflatoxin exposure, hepatitis B virus infection and liver cancer in Swaziland, *Int. J .Cancer* 39, 545-553.

Perrin, R.M., Fedorova, N.D., Bok, J.W., Cramer, R.A., Wortman, J.R., Kim, H.S., Nierman, W.C. & Keller, N.P. (2007). Transcriptional regulation of chemical diversity in *Aspergillus fumigatus* by LaeA, *PLoS Pathog.* 3, e50.

Polychronaki, N., West, R.M., Turner, P.C., Amra, H., Abdel-Wahhab, M., Mykkänen, H. & El-Nezami, H. (2007). A longitudinal assessment of aflatoxin M1 excretion in breast milk of selected Egyptian mothers, *Food Chem Toxicol* 45, 1210-1215.

Price, M.S., Yu, J., Nierman, W.C., Kim, H.S., Pritchard, B., Jacobus, C.A., Bhatnagar, D., Cleveland, T.E. & Payne, G.A. (2006). The aflatoxin pathway regulator AflR induces gene transcription inside and outside of the aflatoxin biosynthetic cluster, *FEMS Microbiol. Lett.* 255, 275-279.

Prieto, R. & Woloshuk, C.P. (1997). *ord1*, an oxidoreductase gene responsible for conversion of *O*- methylsterigmatocystin to aflatoxin in *Aspergillus flavus*, *Appl. Environ. Microbiol.* 63, 1661-1666.

Prieto, R., Yousibova, G.L. & Woloshuk, C.P. (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.

Raisuddin, S., Singh, K.P., Zaidi, S.I., Paul, B.N. & Ray, P.K. (1993). Immunosuppressive effects of aflatoxin in growing rats, *Mycopathologia* 124, 189-194.

Richard, J.L. & Payne, G.A. (2003), Mycotoxins: Risks in Plant, Animal and Human Systems, Council for Agricultural Science and Technology (CAST), Ames, IA. Robens, J.F. (2001). The costs of mycotoxin management to the USA: Management of aflatoxins in the United States, *APSnet Feature* *http://www.apsnet.org/online/feature/mycotoxin/top.html* 2-8.

Robens, J.F. & Cardwell, K. (2005). The cost of mycotoxin management in the United States. In: Abbas, H.K. (Ed.), *Aflatoxin and Food Safety*. CRC Press, Boca Raton, FL, pp. 1-12.

Ronning, C.M., Fedorova, N.D., Bowyer, P., Coulson, R., Goldman, G., Kim, H.S., Turner, G., Wortman, J.R., Yu, J., Anderson, M.J., Denning, D.W. & Nierman, W.C. (2005). Genomics of *Aspergillus fumigatus*, *Rev. Iberoam Micol. (RIAM)* 22, 223-228.

Sakuno, E., Yabe, K. & Nakajima, H. (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.

Scholl, P. & Groopman, J.D. (1995). Epidermiology of human exposures and its relationship to liver cancer. In: Eklund, M., Richard, J.L. & Mise, K. (Eds.), *Molecular Approaches to Food Safety: Issues Involving Toxic Microorganisms*. Alaken, Inc., Fort Collins, Co., pp. 169-182.

Schroeder, H.W., Cole, R.J., Grigsby, R.D. & Hein, H., Jr. (1974). Inhibition of aflatoxin production and tentative identification of an aflatoxin intermediate "versiconal acetate" from treatment with dichlorvos, *Appl. Microbiol.* 27, 394-399.

Silva, J.C., Minto, R.E., Barry, C.E., 3rd, Holland, K.A. & Townsend, C.A. (1996). Isolation and characterization of the versicolorin B synthase gene from *Aspergillus parasiticus*. Expansion of the aflatoxin B1 biosynthetic gene cluster, *J. Biol. Chem.* 271, 13600-13608.

Silva, J.C. & Townsend, C.A. (1997). Heterologous expression, isolation, and characterization of versicolorin B synthase from *Aspergillus parasiticus*. A key enzyme in the aflatoxin B1 biosynthetic pathway, *J. Biol. Chem.* 272, 804-813.

Singh, R. & Hsieh, D.P. (1977). Aflatoxin biosynthetic pathway: elucidation by using blocked mutants of *Aspergillus parasiticus*, *Arch. Biochem. Biophys.* 178, 285-292. Skipper, P.L. & Tannenbaum, S.R. (1990). Protein adducts in the molecular dosimetry of chemical carcinogens, *Carcinogenesis* 11, 507-518.

Skory, C.D., Chang, P.K., Cary, J. & Linz, J.E. (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.

St Leger, R.J., Screen, S.E. & Shams-Pirzadeh, B. (2000). Lack of host specialization in *Aspergillus flavus*, *Appl. Environ. Microbiol.* 66, 320-324.

Stinnett, S.M., Espeso, E.A., Cobeno, L., Araujo-Bazan, L. & Calvo, A.M. (2007). *Aspergillus nidulans* VeA subcellular localization is dependent on the importin alpha carrier and on light, *Mol. Microbiol.* 63, 242-255.

Sugui, J.A., Pardo, J., Chang, Y.C., Zarember, K.A., Nardone, G., Galvez, E.M., Mullbacher, A., Gallin, J.I., Simon, M.M. & Kwon- Chung, K.J. (2007). Gliotoxin is a virulence factor of *Aspergillus fumigatus*: *gliP* deletion attenuates virulence in mice immunosuppressed with hydrocortisone, *Eukaryot. Cell* 6, 1562- 1569.

Tandon, H.D., Tandon, B.N. & Ramalingaswami, V. (1978). Epidemic of toxic hepatitis in India of possible mycotoxic origin, *Arch. Pathol. Lab. Med.* 102, 372-376.

Townsend, C.A. (1997). Progress towards a biosynthetic rationale of the aflatoxin pathway, *Pure Appl. Chem.* 58, 227-238.

Trail, F., Chang, P.-K., Cary, J. & Linz, J.E. (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.

Trail, F., Mahanti, N. & Linz, J. (1995a). Molecular biology of aflatoxin biosynthesis, *Microbiol.* 141, 755-765.

Trail, F., Mahanti, N., Rarick, M., Mehigh, R., Liang, S.H., Zhou, R. & Linz, J.E. (1995b). 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.

Turner, P.C., Collinson, A.C., Cheung, Y.B., Gong, Y., Hall, A.J., Prentice, A.M. & Wild, C.P. (2007). Aflatoxin exposure in utero causes growth faltering in Gambian infants, *Int J Epidemiol* 36, 1119-1125.

van Egmond, H.P. (1989). Current situation on regulations for mycotoxins. Overview of tolerances and status of standard methods of sampling and analysis, *J. Food Addit. Contam.* 6, 139-188.

van Egmond, H.P. & Jonker, M.A. (2005). Worldwide regulations on aflatoxins. In: Abbas, H.K. (Ed.). CRC Press, Taylor and Francis Group, Boca Raton, FL, pp. 77-93.

van Egmond, H.P., Schothorst, R.C. & Jonker, M.A. (2007). Regulations relating to mycotoxins in food: Perspectives in a global and European context, *Anal Bioanal Chem* 389, 147-157.

Watanabe, C.M. & Townsend, C.A. (1996). Incorporation of molecular oxygen in aflatoxin B1 biosynthesis, *J. Org. Chem.* 61, 1990-1993.

Watanabe, C.M. & Townsend, C.A. (2002). Initial characterization of a type I fatty acid synthase and polyketide synthase multienzyme complex NorS in the biosynthesis of aflatoxin B1, *Chem. Biol.* 9, 981-988.

Watanabe, C.M., Wilson, D., Linz, J.E. & Townsend, C.A. (1996). Demonstration of the catalytic roles and evidence for the physical association of type I fatty acid synthases and a polyketide synthase in the biosynthesis of aflatoxin B1, *Chem. Biol.* 3, 463-469.

Wild, C.P., Shrestha, S.M., Anwar, W.A. & Montesano, R. (1992). Field studies of aflatoxin exposure, metabolism and induction of genetic alterations in relation to HBV infection and hepatocellular carcinoma in The Gambia and Thailand, *Toxicol. Lett.* 64-65, 455-461.

Wilkinson, J.R., Yu, J., Abbas, H.K., Scheffler, B.E., Kim, H.S., Nierman, W.C., Bhatnagar, D. & Cleveland, T.E. (2007a). Aflatoxin formation and gene expression in response to carbon source media shift in *Aspergillus parasiticus*, *J. Food Addit. Contam.* 24, 1051-1060.

Wilkinson, J.R., Yu, J., Bland, J.M., Nierman, W.C., Bhatnagar, D. & Cleveland, T.E. (2007b). Amino acid supplementation reveals differential regulation of aflatoxin biosynthesis in *Aspergillus flavus* NRRL 3357 and *Aspergillus parasiticus* SRRC 143, *Appl. Microbiol. Biotechnol.* 74, 1308-1319.

Wilson, D.M. (1989). Analytical methods for aflatoxins in corn and peanuts, *Arch. Environ. Contam. Toxicol.* 18, 308-314. Wogan, G.N. (1992). Aflatoxins as risk factors for hepatocellular carcinoma in humans, *Cancer Res.* 52, 2114-2118.

Woloshuk, C.P., Foutz, K.R., Brewer, J.F., Bhatnagar, D., Cleveland, T.E. & Payne, G.A. (1994). Molecular characterization of *aflR*, a regulatory locus for aflatoxin biosynthesis, *Appl. Environ. Microbiol.* 60, 2408-2414.

Woloshuk, C.P. & Payne, G.A. (1994). The alcohol dehydrogenase gene *adh1* is induced in *Aspergillus flavus* grown on medium conducive to aflatoxin biosynthesis, *Appl. Environ. Microbiol.* 60, 670-676.

Woloshuk, C.P. & Prieto, R. (1998). Genetic organization and function of the aflatoxin B1 biosynthetic genes, *FEMS Microbiol. Lett.* 160, 169- 176.

Wong, J.J., Singh, R. & Hsieh, D.P. (1977). Mutagenicity of fungal metabolites related to aflatoxin biosynthesis, *Mutat. Res.* 44, 447- 450.

Yabe, K., Ando, Y. & Hamasaki, T. (1988). Biosynthetic relationship among aflatoxins B1, B2, G1, and G2, *Appl. Environ. Microbiol.* 54, 2101-2106.

Yabe, K., Ando, Y. & Hamasaki, T. (1991a). A metabolic grid among versiconal hemiacetal acetate, versiconol acetate, versiconol and versiconal during aflatoxin biosynthesis, *J. Gen. Microbiol.* 137, 2469-2475.

Yabe, K., Ando, Y., Hashimoto, J. & Hamasaki, T. (1989). Two distinct *O*-methyltransferases in aflatoxin biosynthesis, *Appl. Environ. Microbiol.* 55, 2172-2177.

Yabe, K., Chihaya, N., Hamamatsu, S., Sakuno, E., Hamasaki, T., Nakajima, H. & Bennett, J.W. (2003). Enzymatic conversion of averufin to hydroxyversicolorone and elucidation of a novel metabolic grid involved in aflatoxin biosynthesis, *Appl. Environ. Microbiol.* 69, 66-73.

Yabe, K., Matsushima, K., Koyama, T. & Hamasaki, T. (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.

Yabe, K., Matsuyama, Y., Ando, Y., Nakajima, H. & Hamasaki, T. (1993). Stereochemistry during aflatoxin biosynthesis: conversion of norsolorinic acid to averufin, *Appl. Environ. Microbiol.* 59, 2486- 2492.

Yabe, K. & Nakajima, H. (2004). Enzyme reactions and genes in aflatoxin biosynthesis, *Appl. Microbiol. Biotechnol.* 64, 745-755.

Yabe, K., Nakamura, M. & Hamasaki, T. (1999). Enzymatic formation of G-group aflatoxins and biosynthetic relationship between G- and B- group aflatoxins, *Appl. Environ. Microbiol.* 65, 3867-3872.

Yabe, K., Nakamura, Y., Nakajima, H., Ando, Y. & Hamasaki, T. (1991b). Enzymatic conversion of norsolorinic acid to averufin in aflatoxin biosynthesis, *Appl. Environ. Microbiol.* 57, 1340-1345.

Yao, R.C. & Hsieh, D.P. (1974). Step of dichlorvos inhibition in the pathway of aflatoxin biosynthesis, *Appl. Microbiol.* 28, 52-57.

Yu, J., Bhatnagar, D. & Cleveland, T.E. (2004a). Completed sequence of aflatoxin pathway gene cluster in *Aspergillus parasiticus*, *FEBS Lett.* 564, 126-130.

Yu, J., Bhatnagar, D. & Cleveland, T.E. (2004b). Genetics and biochemistry of aflatoxin formation and genomics approach for eliminating aflatoxin contamination. In: Romeo, J.T. (Ed.), *Recent Advances in Phytochemistry* Elsevier, New York, pp. 224-242.

Yu, J., Cary, J.W., Bhatnagar, D., Cleveland, T.E., Keller, N.P. & Chu, F.S. (1993). Cloning and characterization of a cDNA from *Aspergillus parasiticus* encoding an *O*-methyltransferase involved in aflatoxin biosynthesis, *Appl. Environ. Microbiol.* 59, 3564-3571.

Yu, J., Chang, P.K., Bhatnagar, D. & Cleveland, T.E. (2000a). Cloning of a sugar utilization gene cluster in *Aspergillus parasiticus*, *Biochim. Biophys. Acta.* 1493, 211-214.

Yu, J., Chang, P.K., Bhatnagar, D. & Cleveland, T.E. (2002). Cloning and functional expression of an esterase gene in *Aspergillus parasitcus*, *Mycopathologia* 156, 227-234.

Yu, J., Chang, P.K., Cary, J.W., Bhatnagar, D. & Cleveland, T.E. (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.

Yu, J., Chang, P.K., Cary, J.W., Wright, M., Bhatnagar, D., Cleveland, T.E., Payne, G.A. & Linz, J.E. (1995a). Comparative mapping of aflatoxin pathway gene clusters in *Aspergillus parasiticus* and *Aspergillus flavus*, *Appl. Environ. Microbiol.* 61, 2365-2371.

Yu, J., Chang, P.K., Ehrlich, K.C., Cary, J.W., Bhatnagar, D., Cleveland, T.E., Payne, G.A., Linz, J.E., Woloshuk, C.P. & Bennett, J.W. (2004c). Clustered pathway genes in aflatoxin biosynthesis, *Appl. Environ. Microbiol.* 70, 1253-1262.

Yu, J., Chang, P.K., Ehrlich, K.C., Cary, J.W., Montalbano, B., Dyer, J.M., Bhatnagar, D. & Cleveland, T.E. (1998). Characterization of the critical amino acids of an *Aspergillus parasiticus* cytochrome P- 450 monooxygenase encoded by *ordA* that is involved in the biosynthesis of aflatoxins B1, G1, B2, and G2, *Appl. Environ. Microbiol.* 64, 4834-4841.

Yu, J., Chang, P.K., Payne, G.A., Cary, J.W., Bhatnagar, D. & Cleveland, T.E. (1995b). Comparison of the *omtA* genes encoding *O*-methyltransferases involved in aflatoxin biosynthesis from *Aspergillus parasiticus* and *A. flavus*, *Gene* 163, 121-125.

Yu, J., Fedorova, F., Montalbano, B.G., Bhatnagar, D., Cleveland, T.E., Bennett, J.W. & Nierman, W.C. (2011). Mycotoxin gene expression in *Aspergillus flavus* in response to temperature as detected by RNA- Sequencing *FEMS Microbiol. Lett.* submitted.

Yu, J., Woloshuk, C.P., Bhatnagar, D. & Cleveland, T.E. (2000b). Cloning and characterization of *avfA* and *omtB* genes involved in aflatoxin biosynthesis in three *Aspergillus* species, *Gene* 248, 157- 167.

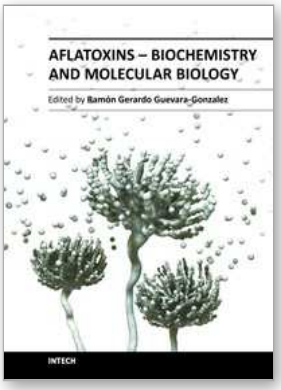
Yu, J.H., Butchko, R.A., Fernandes, M., Keller, N.P., Leonard, T.J. & Adams, T.H. (1996a). Conservation of structure and function of the aflatoxin regulatory gene *aflR* from *Aspergillus nidulans* and *A. flavus*, *Curr. Genet.* 29, 549-555.

Yu, J.H., Wieser, J. & Adams, T.H. (1996b). The *Aspergillus* FlbA RGS domain protein antagonizes G protein signaling to block proliferation and allow development, *EMBO J.* 15, 5184-5190.

Zhou, R. & Linz, J.E. (1999). Enzymatic function of the nor-1 protein in aflatoxin biosynthesis in Aspergillus parasiticus, *Appl. Environ. Microbiol.* 65, 5639-5641.

Zhu, J.Q., Zhang, L.S., Hu, X., Xiao, Y., Chen, J.S., Xu, Y.C., Fremy, J. & Chu, F.S. (1987). Correlation of dietary aflatoxin B1 levels with excretion of aflatoxin M1 in human urine, *Cancer Res.* 47, 1848-1852.

Zuckerman, A.J., Rees, K.R., Inman, D. & Petts, V. (1967). Site of action of aflatoxin on human liver cells in culture, *Nature* 214, 814-815.



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Aflatoxins â€“ Biochemistry and Molecular Biology is a book that has been thought to present the most significant advances in these disciplines focused on the knowledge of such toxins. All authors, who supported the excellent work showed in every chapter of this book, are placed at the frontier of knowledge on this subject, thus, this book will be obligated reference to issue upon its publication. Finally, this book has been published in an attempt to present a written forum for researchers and teachers interested in the subject, having a current picture in this field of research about these interesting and intriguing toxins.

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