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REVIEW

Biosynthesis of sphinganine-analog mycotoxins

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Abstract Sphinganine-analog mycotoxins (SAMT) are polyketide-derived natural products produced by a number of plant pathogenic fungi and are among the most economically important mycotoxins. The toxins are structurally similar to sphinganine, a key intermediate in the biosynthesis of ceramides and sphingolipids, and competitive inhibitors for ceramide synthase. The inhibition of ceramide and sphingolipid biosynthesis is associated with several fatal diseases in domestic animals and esophageal cancer and neural tube defects in humans. SAMT contains a highly reduced, acyclic polyketide carbon backbone, which is assembled by a single module polyketide synthase. The biosynthesis of SAMT involves a unique polyketide chain-releasing mechanism, in which a pyridoxal 5′-phosphate-dependent enzyme catalyzes the termination, offloading and elongation of the polyketide chain. This leads to the introduction of a new carbon–carbon bond and an amino group to the polyketide chain. The mechanism is fundamentally different from the thioesterase/cyclase-catalyzed polyketide chain releasing found in bacterial and other fungal polyketide biosynthesis. Genetic data suggest that the ketosynthase domain of the polyketide synthase and the chain-releasing enzyme are important for controlling the final product structure. In addition, several post-polyketide modifications have to take place before SAMT become mature toxins.

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Introduction

Mycotoxins are fungal secondary metabolites produced by a large number of phytopathogenic and food spoilage fungi, for example, *Aspergillus*, *Penicillium, Fusarium*, and *Alternaria* species. These species produce some of the most important mycotoxins, including trichothecenes, aflatoxins, sterigmatocystin, fumonisins, and AAL-toxins [1, 23, 73]. Extensive research has been devoted to aflatoxins, sterigmatocystin, and trichothecenes, and their biosynthesis is relatively well understood [11, 23, 39, 53]. In recent years, significant research efforts have also been given to studying the biosynthetic mechanism for fumonisins and AAL-toxins (Fig. 1) [28, 77]. Fumonisins and AAL-toxins together are called sphinganine-analog mycotoxins (SAMT) due to their structural similarity to sphinganine (dihydrosphingosine, DHS), which is the backbone precursor of sphingolipids (Fig. 1) [9]. Fumonisins are produced by the fungus *F. verticillioides* (synonym *F. moniliforme*, teleomorph *Gibberella moniliformis*, synonym *G. fujikuroi* Mating Population A), which is a widespread pathogen of corn and contaminates maize-based food and feed worldwide [50, 56]. AAL-toxins are produced by tomato pathogen *Alternaria alternata* f. sp. *Lycopersici* [7, 8, 37]. The mechanism for SAMT to execute their toxicity is through the competitive inhibition of sphinganine *N*-acetyltransferase (ceramide synthase) [50, 52]. This leads to the obstruction of complex sphingolipid biosynthesis, such as the important second messenger ceramide in animal systems, and the accumulation of sphinganine. The inhibition of this enzyme leads to various diseases in animals and humans as ceramides and

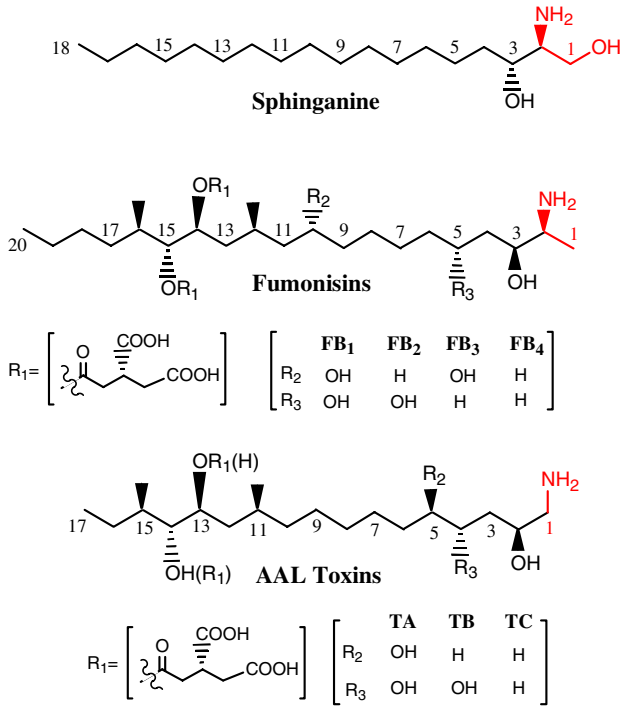

Fig. 1 Chemical structure of sphinganine and sphinganine-analog mycotoxins (SAMT), fumonisins and AAL toxins. Only the most abundant B-series of fumonisins and three most common AAL-toxins are shown

sphingolipids are ubiquitous constituents of eukaryotic cells and involved in crucial signal transduction of numerous cellular processes [50, 65]. It is known that fumonisins can cause leukoencephalomalacia and pulmonary edema syndrome in animals and are associated to human esophageal cancer and neural tube defects [50]. SAMT are also found to induce apoptosis [78]. In addition to their animal toxicity, AAL-toxins are known as the primary determinants of phytopathogenicity as in the case of stem-canker disease in tomatos [82]. In this review, we examine our current understanding on the biosynthesis of SAMT, with a focus on the biosynthetic mechanism for the polyketide chain.

The biosynthetic origins of sphinganine-analog mycotoxins

The biosynthetic origins of SAMT have been studied by several groups using isotope-labeled precursors (Fig. 2). For fumonisins, the 18-carbon backbone from C-3 to C-20 is derived from acetate, and C-1 and C-2, as well as the C-2 amino, are derived from alanine [5, 10]. The two methyl groups at C-12 and C-16 are derived from methionine [59]. Although multiple hydroxyls are present on the polyketide chain, only the hydroxyl at C-3 is derived from acetate; the

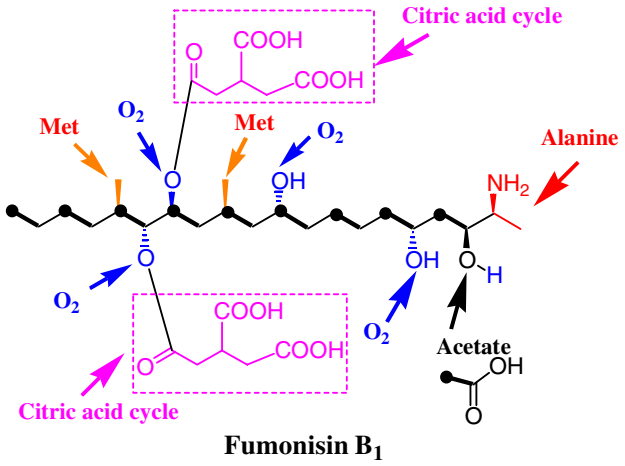

Fig. 2 The biosynthetic origins of fumonisin B1. Fumonisins C3–20 carbon backbone is originated from a highly reduced polyketide, whereas C1–2 and the amino group are from decarboxylated L-alanine. AAL-toxins have similar origins, except that their polyketide backbone is 2-carbon shorter, C-1 and the amino are derived from glycine, and only one tricarballylic ester is present

hydroxyls at C-5, C-10, C-14, and C-15 of FB_1 are derived from molecular oxygen [16]. Therefore, the initially synthesized carbon chain is a highly reduced polyketide. The origin of the two tricarballylic esters is not certain, but most likely from the citric acid cycle [5, 87]. For AAL-toxins, the biosynthetic origin is similar to that of fumonisins, except that the acetate-originated carbon chain (from C-2 to C-17) is two carbons shorter, C-1 and the amino group are derived from glycine, and only one tricarballylic ester is present (either at C-13 or C-14 hydroxyl) (Fig. 1). Biosynthetic origins, as well as the presence of methyl groups on the carbon backbone, suggest that the carbon backbone of SAMT is assembled via a polyketide biosynthetic mechanism.

Polyketides and polyketide synthases (PKS)

Polyketides are a large, structurally diverse family of metabolites [72]. They are biosynthesized through sequential decarboxylative condensations of short carboxylic acids, which are catalyzed by polyketide synthases (PKS) [19, 27, 32, 40, 67]. Throughout the biosynthesis, the growing polyketide intermediates remain covalently attached to the ACP (acyl carrier protein) domain via the 4′-phosphopantetheine cofactor [49, 76]. In bacterial modular PKSs (Type I), the mechanism by which PKS control structural variations in the products is relatively well understood. The order, number, and domain-composition of the modules dictate the length and reduction level of the polyketide carbon backbone [32, 40, 72]. Mediated by the hydrolytic function of the thioesterase (TE) domain located at the end

of the modules, the product is released with a length depending on the number of elongation cycles. For example, the three bi-modular PKS, 6-deoxyerythronolide B synthases (DEBS-1, DEBS-2, and DEBS-3) that make the precursor for erythromycins in *Saccharopolyspora erythraea*, catalyze six cycles of decarboxylative condensation from one molecule of propionyl-CoA and six molecules of 2-methylmalonyl-CoA to synthesize the 15-carbon backbone of erythromycins [19, 26, 27, 44, 74]. Because the position of the modules dictates the course of catalytic events in the bacterial modular PKS, the pathways can be modified in semi-predictable fashion by moving, deleting, or adding modules [17, 18, 43, 45, 51, 54]. Novel polyketides with complex structures have been produced by manipulating the biosynthetic machinery.

Fungal polyketides and fungal PKS

So far, most of the biosynthetic investigations have been carried out on polyketides that are isolated from soil bacteria, especially in the order of Actinomycetes. Although filamentous fungi are known to produce numerous polyketide natural products, our understanding of the biosynthetic mechanism for these products is still at the infant stage. Many fungal polyketides are biologically active, economically important natural products [20]. These polyketides have significant impacts on human health because many of the fungal producers are unavoidable species in our daily lives. For example, aflatoxins produced by the peanut pathogens *Aspergillus flavus* and *A. parasiticus* are very potent carcinogens [53, 57], fumonisins produced by the corn pathogen *F. verticillioides* cause several fatal diseases in livestock and are associated with human esophageal cancer and neural tube defects [50, 65], fusarin C produced by *F. verticillioides* is a potent mycotoxin [70], zearalenones produced by the wheat pathogens *F. graminearum* and *F. roseum* are potent estrogens [60], melanins produced by the human pathogen *A. fumigatus* are virulence factors for this fungus to cause pneumonia and invasive disseminated diseases [75, 80], T-toxins produced by the corn pathogen *Cochliobolus heterostrophus* [84] and AAL-toxins by the tomato pathogen *Alternaria alternata* f. sp. *Lycopersici* [37] are the pathogenicity factors essential for the fungi to infect plants, equisetin produced by the corn/wheat pathogen *F. heterosporum* is an HIV-1 integrase inhibitor [69], squalestatin produced by *Phoma sp*. is a potent inhibitor for mammalian squalene synthase [21], tenellin produced by the insect pathogen *Beauveria bassiana* is toxic to mammalian erythrocytes and probably important for this fungus to cause white muscardine disease in the domestic silk worm [29, 83], 6-methylsalicylic acid produced by several pathogenic *Penicillium* sp. is a precursor of the mycotoxin patulin [24, 71], and lovastatin/compactin produced by *A. terreus* is an important cholesterol-lowering drug [42, 68].

Like bacterial type I PKS, fungal PKS are modular enzymes, but typically contain only a single set of domains [72] (Fig. 3). Therefore, the single module must be iteratively used during the polyketide chain elongation. Fungal PKS are divided into three groups based on the reduction level of their products, non-reducing PKS (NR-PKS), partially reducing PKS (PR-PKS), and highly reducing PKS (HR-PKS) [4, 48, 58]. NR-PKSs synthesize aromatic polycyclic compounds, such as tetrahydroxy naphthalene synthase (THNS) in *Colletotrichum lagenarium* [33] and *Wangiella dermatitidis* [30]. Their domain architecture has recently been redefined, which includes several unique domains [22] (Fig. 3a). The domains consist of SAT (starter unit ACP transacylase), KS (β-ketoacylsynthase), AT (acyl transferase), PT (product template), ACP (acyl carrier protein), and TE/CLC (thiolesterase/Claisen-like cyclase). KS-AT-ACP are the "minimal PKS" found in all PKS systems and required for chain extension. The studies of aflatoxin PKS (norsolorinic acid synthase) showed that SAT controls chain initiation and PT involves chain length control [20] (Townsend, personal communication). TE/CLC controls cyclization and chain releasing and probably also chain-length determination [35, 79].

Fungal partially reducing PKS synthesize cyclic compounds like 6-methylsalicylic acid (6-MSA), which has an aromatic ring but results from a partially reduced intermediate (Fig. 3b). The domain architecture of PR-PKS differs considerably from NR-PKS [2, 34]. 6-MSAS consists of KS-AT-DH-Core-KR-ACP. It does not have SAT, PT, or TE/CLC, but has β-keto processing domains, KR (β-ketoacyl reductase), DH (dehydratase), which are selectively used during the biosynthesis to produce "partially" reduced intermediates. The so-called Core domain is probably involved in the subunit–subunit interaction, as 6-MSAS is a homotetramer [55].

Fungal highly reducing PKS synthesize non-aromatic compounds like fumonisins, AAL-toxins, and lovastatin (Fig. 3C). The domain architecture resembles a module of bacterial Type-I PKSs, typically consisting of KS-AT-DH-MT-ER-KR-ACP. Again, it does not have SAT, PT, or TE/CLC, but contains a complete set of β-keto processing domains, KR, DH, ER (enoylreductase), as well as a methyltransferase (MT) that is found only in HR-PKS among the three groups [20]. The mechanism for HR-PKS programming has been a mystery. It is not clear what controls the number of chain elongation and the reduction level of the carbon chain, as HR-PKS with identical domain architecture can synthesize products with huge variations in chain-lengths and reduction level [20]. In addition, the PKS does not have an obvious way of terminating and offloading the linear polyketide chain.

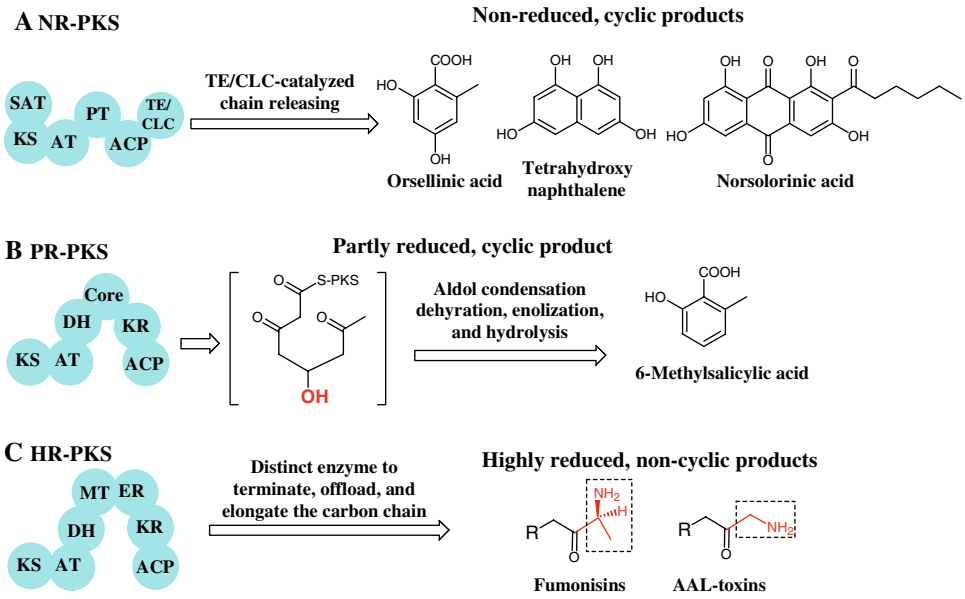

Fig. 3 The domain organization of the three types of fungal polyketide synthases and the structure of representative polyketides synthesized by the synthases. *NR-PKS* nonreducing polyketide synthase, *PR-PKS* partially-reducing polyketide synthase, HR-PKS highly-reducing polyketide synthase

Sphinganine-analog mycotoxins as a model system to study the biosynthesis of fungal highly reduced polyketides

The study of the biosynthetic mechanism for fungal highly reduced polyketides has been challenging due to the difficulties in detecting the intermediates with a linear, non-aromatic structure and in manipulating the multi-domain PKS genes in the filamentous fungi. Our laboratory has been studying the biosynthesis of SAMT and using it as a model system for the biosynthesis of fungal highly reduced polyketides [28, 88, 89]. This system has several advantages. Fumonisins and AAL-toxins are structurally similar, and the main difference is the length of polyketide carbon chain. Fumonisins have a dimethylated 18-carbon chain, while AAL-toxins have a dimethylated 16-carbon chain. The PKS for fumosinins (Fum1p, 2507 residues, encoded by *FUM1*) [62] and AAL-toxins (Alt1p, 2521 residues, encoded by *ALT1*) (personal communication with Dr. Motoichiro Kodama at Tottori University, Japan) have an identical domain architecture, KS-AT-DH-MT-ER-KR-ACP, and are highly homologous to each other (72%/60% similarity/identity for the entire amino acid sequence). However, Fum1p synthesizes an 18-carbon chain, while Alt1p makes a 16-carbon chain. Thus, a simple domain swapping experiment could reveal interesting insights into the mechanism for the chain-length control. Most intriguingly, Fum1p and Alt1p do not contain a thioesterase (TE) domain for releasing bacterial reduced polyketides [40] or a Claisen-like cyclase (CLC) domain for releasing fungal non-reduced, aromatic polyketides [35]. Furthermore, since the products are acyclic compounds, the polyketide-releasing through simultaneous cyclization, as seen in partially reducing PKS (Fig. 3b), cannot be involved. In addition, in fumonisin biosynthesis the linear 18-carbon chain is offloaded by condensing with the α-carbon of L-alanine, whereas in AAL-toxins, the linear 16-carbon is offloaded by condensing with the α-carbon of glycine (Fig. 3c). Therefore, the study of SAMT biosynthesis could also potentially reveal novel mechanisms for polyketide structure determination.

In order to study the biosynthetic mechanism, we first developed a genetic system that enables specific and functional manipulations of the PKS domains in *F. verticillioides* [86]. In bacteria, the genetic manipulation of PKS domains has proven a powerful way to elucidate the domain structure and function of PKS [40, 72]. In our system, we used a two-stage screening strategy, including both positive and negative screenings, to manipulate the domains of SAMT PKS. Since our goal is not only to specifically change the domains, but also to maintain the activity of the chimeric PKS, the one-stage protocol developed previously [62] to screen resistant colonies will not be appropriate for our purpose. In the first stage screening, hygromycin-resistant colonies were obtained by transforming fungal protoplasts with a properly constructed vector. Any of the single crossovers would result in a disrupted PKS gene, which should be nonfunctional. PCR was used to quickly distinguish putative homologous recombinants from those mutants resulted from random insertions. The putative homologous recombinants were further confirmed by Southern hybridization. In the second stage, the mutants were grown on a liquid medium without hygromycin for 4–5 generations to encourage the second crossover. The individual single colonies were then replica plated on two plates, one with hygromycin and one without. Colonies that did not grow on the plate with hygromycin but grew on the

plate without hygromycin were selected as putative domain replacement mutants, which were confirmed by PCR and Southern analysis.

Genetic manipulations of the PKS for sphinganine-analog mycotoxin biosynthesis

Using this genetic system, we replaced the KS domain of fumonisin *FUM1* in *F. verticillioides* with the KS domain of T-toxin *PKS1* from *C. heterostrophus*. KS is the catalytic domain responsible for the carbon–carbon formation during polyketide chain assembly. A replacement of this domain will most likely lead to structural variations in products. T-toxins are a family of mycotoxins with carbon chain lengths varying from 35 to 45, and the C41 analog is most abundant (50%) [47]. Fum1p and PKS1 share a sequence similarity of 44.1% and identity of 34.2% for the entire amino acid sequence [84]. When the KS domain of *FUM1* was replaced with the KS domain of *PKS1*, the *F. verticillioides* strain produced fumonisins [88]. The result shows that the heterologous KS is able to functionally interact with the six other domains of Fum1p. It also suggests that KS domain alone may not be sufficient to control the product's structure. This represents the first successful domain swapping in fungal HR-PKS. To further test if the whole fumonisin PKS could be functionally replaced by a PKS that has a similar domain architecture, we replaced entire *FUM1* with *PKS1* [88]. This *F. verticillioides* strain did not produce any fumonisin or new metabolites. Previous heterologous expression and in vitro assays of 6-methylsalicylic acid synthase [3, 41, 64] and squalestatin tetraketide synthase [21] have shown that each of the single module fungal PKS contains the necessary information for synthesizing a distinct polyketide product. The results obtained from fumonisin biosynthesis suggest that the intrinsic interactions between the intact PKS and downstream enzymes in the biosynthetic pathway may play a key role in the control of the biosynthesis of fungal reduced polyketides.

To obtain more insights, we replaced the KS domain of *FUM1* with the KS domain of *LovF*, which encodes lovastatin diketide synthase (LDKS) responsible for the 2-methylbutyryl side chain of lovastatin in *Aspergillus terreus* [42]. Since Fum1p, T-toxin PKS1 and LDKS synthesize very different polyketide chains, the outcome of this experiment is expected to reveal interesting new information on KS domain's role in product structure determination. The KS-replaced *F. verticillioides* strains produced four new compounds with a yield comparable to fumonisins in the wild type. HRMS and NMR data showed that these compounds are not linear, acyclic polyketides found in SAMT, T-toxins, or lovastatin, but are rather aromatic metabolites, dihydroisocoumarins [89]. The result is surprising because, as discussed earlier, the enzyme architecture for fungal aromatic (non-reduced) polyketides is very different from that for fungal highly reduced (non-aromatic) polyketides. A possible way for the dihydroisocoumarins to arise is through inter-molecular ring formation between two short (C8 and C10), linear polyketide intermediates [89]. A series of possibly spontaneous reactions including aldol condensation, Claisen condensation, aromatization, and lactonization between the intermediates would account for the formation of the aromatic products. These results show that fungal chimeric PKS with different KS domain makes different products, suggesting that the KS domain does have an effect on the product structure. It is possible that the substrate-binding pocket of the KS domain of T-toxin PKS1 is large enough to allow the chimeric PKS to synthesize a C18 chain required for fumonisin production. This is reasonable considering that PKS1 is involved in the synthesis of polyketides (T-toxins) larger than fumonisins. Thus, T-toxin KS within the context of Fum1p is able to produce a proper polyketide chain that can be processed by the downstream enzymes in the pathway, which eventually leads to fumonisin production in the fungus. On the other hand, the substrate-binding pocket of the KS domain of LDKS could be smaller than that of Fum1p or T-toxin PKS1, since LDKS synthesizes a short carbon chain (C4). Thus, the chimeric Fum1p with the KS from LDKS could only make "short" chains that cannot be processed by the downstream enzymes and thus "stuck" on the PKS. One possible way for the release of these stuck short chains is through inter-molecular ring formation, leading to the aromatic compounds. Therefore, both the KS domain and downstream processing enzymes are important for the production of a distinct group of products during fungal highly reduced polyketide biosynthesis [89].

The production of dihydroisocoumarins in *F. verticillioides* represents the first successful genetic manipulation of an iterative polyketide synthase gene to produce new, biologically active metabolites in a filamentous fungus. The dihydroisocoumarins are known to have anti-malarial, antifungal, and anti-tuberculosis activities [46] and had never been isolated from a *Fusarium* species. The new *F. verticillioides* strains not only stop producing mycotoxins but also produce a group of active metabolites. The fungal strains could have potential applications in agricultural biotechnology.

Polyketide chain-releasing in sphinganine-analog mycotoxin biosynthesis

The genetic manipulation of the KS domain of *FUM1* has revealed interesting results for SAMT biosynthesis. Both the genetic data and biosynthetic origins of SAMT suggest

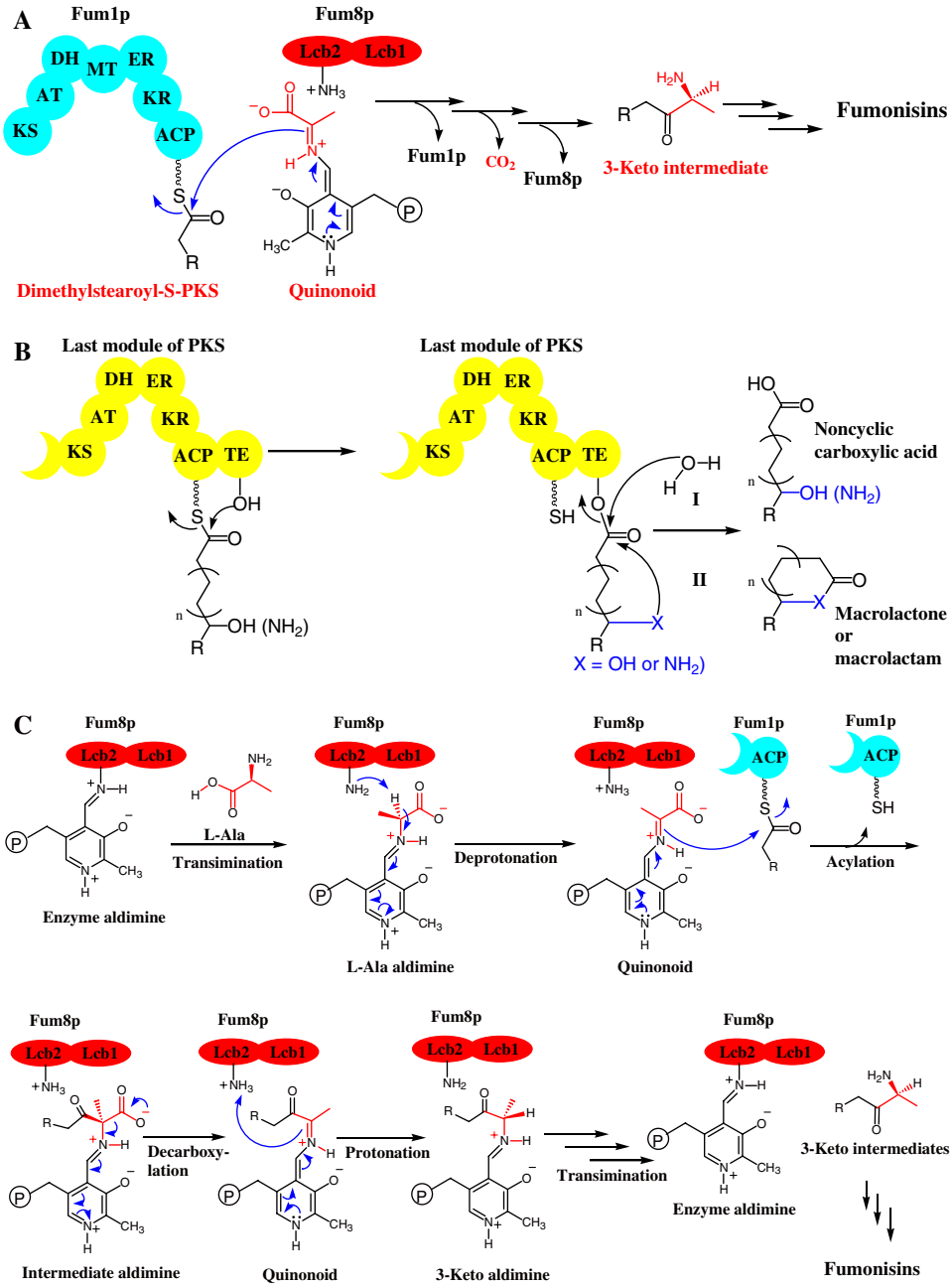

Fig. 4 A comparison of the Fum8p PLP-dependent polyketide chain-releasing mechanism proposed for SAMT biosynthesis (**a**) with the common TE (and CLC)-dependent chain-release mechanism in the biosynthesis of bacterial reduced polyketides and fungal aromatic polyketides (**b**). Fum8p contains the catalytic domain (Lcb2) and regulatory domain (Lcb1). (**c**) A more detailed description for the Fum8p PLP-dependent polyketide chain termination and offloading from PKS (Fum1p) during the biosynthesis of fumonisins. Note that only the acyl carrier protein (ACP) domain of Fum1p is shown

that the enzymes for terminating-offloading the SAMT polyketide chains are important in determining the structure of the final products. As shown in Fig. 3, the terminating–offloading of SAMT polyketide is accomplished by a new carbon–carbon bond formation, between C-3 and C-2 in fumonisins and C-2 and C-1 in AAL toxins. Thus, a distinct chain-releasing mechanism is expected for SAMT biosynthesis. Within the *FUM* gene cluster, *FUM8* is predicted to encode a homolog (Fum8p, 839 residues) of L-serine palmitoyltransferase, which is a member of the 2-oxoamino synthase family [66]. Similarly, in the *ALT* gene cluster, *ALT4* is predicted to encode a homolog (Alt4p, 871 residues) of the same family of enzymes (personal communication with Dr. Motoichiro Kodama at Tottori University, Japan). The amino acid sequence of Fum8p and Alt4p shares a similarity/identify of 50%/38% for the entire sequence.

2-Oxoamino synthases are a small family of enzymes involved in several very important primary biosynthetic pathways, including L-serine palmitoyltransferase (SPT) in ceramide-sphingolipid biosynthesis [38], 5-aminolevulinate synthase (ALAS) in heme biosynthesis [31], and 8-amino-7-oxononanoate synthase (AONS) in biotin biosynthesis [81]. The enzymes are known to use pyridoxal 5′-phosphate as cofactor to catalyze the acyltransfer reactions [38]. The new carbon–carbon bond formation during the terminating–offloading of SAMT polyketide is catalytically

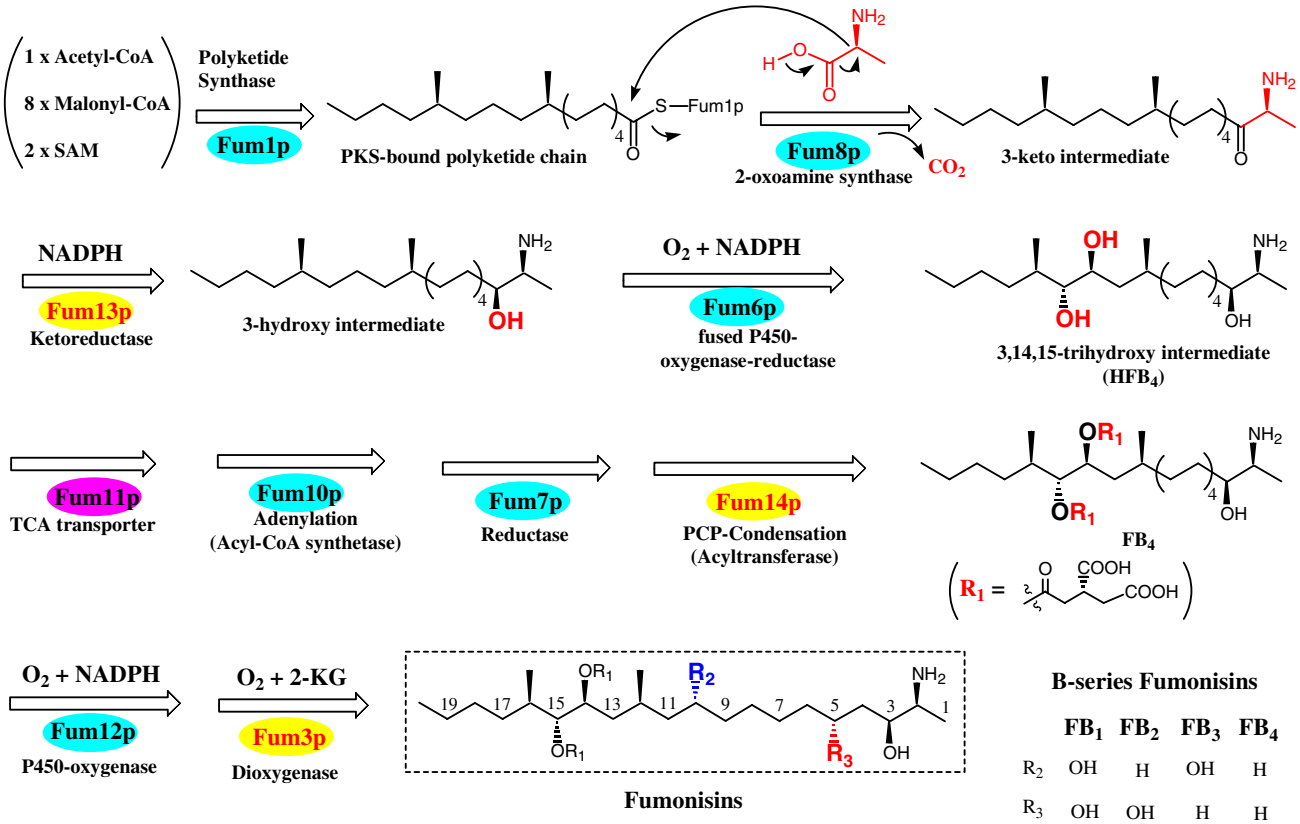

Fig. 5 A proposed biosynthetic pathway for fumonisins. *SAM* *S*-adenosyl methionine, *TCA* tricarballylic acid, *2-KG* 2-ketoglutarate

analogous to the reactions catalyzed by the 2-oxoamino synthases. Thus, Fum8p and Alt4p are most likely the enzymes responsible for the polyketide chain releasing in the biosynthesis of fumonisins and AAL-toxins, respectively. While ALAS and AONS are homodimers, SPT is a heterodimer of two separate subunits, Lcb1p and Lcb2p. Lcb2p contains the active site lysine residue to form a Schiff's base with pyridoxal 5′-phosphate [36]. Fum8p and Alt4p are natural fusions of Lcb2p and Lcb1p, with the *N*-terminal half being homologous to Lcb2p and the C-terminal half being homologous to Lcb1p.

Based on the general mechanism known for the 2-oxoamine synthases, we propose a pyridoxal 5′-phosphate-dependent releasing of polyketide acyl chain that is covalently attached to the 4′-phosphopantetheine cofactor of the ACP domain of PKS (Fum1p) (Fig. 4a). The reaction mechanism follows the sequence of deprotonation–condensation–decarboxylation–reprotonation. The PLP-enzyme uses a carbon nucleophile (carbanion derived from the α-carbon of L-alanine by deprotonation) to attack the carbonyl of polyketide acyl-S-ACP, which results in the release of the PKS (ACP-SH) and the formation of a new carbon–carbon bond in the PLP-bound aldimine intermediate (Fig. 4a). It is likely that the substrate specificity of the chain-releasing enzymes is important for controlling the size of the polyketide chain in the final products. This chain-releasing mechanism is fundamentally distinct from the TE/CLC-mediated polyketide chain releasing (Fig. 4b), which typically uses a nitrogen or oxygen nucleophile to attack the carbonyl and involves a catalytic triad (e.g. Ser-Asp-His) at the active site of TE/CLC [12, 35]. The subsequent decarboxylation, reprotonation, and transimination of this aldimine intermediate result in the regeneration of the PLP-enzyme and free 3-keto intermediate for fumonisins (Fig. 4c). The net results are (1) the termination and release of the polyketide chain from PKS, (2) the elongation of the polyketide acyl chain, and (3) the incorporation of the amino group.

Polyketide chain modifications in sphinganine-analog mycotoxin biosynthesis

On the PLP-dependent polyketide chain releasing, a 3-keto compound is produced as an intermediate. This 3-keto intermediate is stereospecifically reduced by the product of *FUM13* [14, 85]. We have expressed *FUM13* in *E. coli* and purified the 46-kDa Fum13p. When 3-keto FB_3 was incubated with Fum13p in the presence of NADPH, it was converted to FB_3. This demonstrates that Fum13p is an NADPH-dependent ketoreductase required for the reduction

of 3-keto to 3-hydroxy in fumonisins (Fig. 5). The further modifications of the SAMT polyketide chain include several hydroxylations and esterifications [6, 13–15, 25, 28, 61, 63, 77, 85, 87]. The hydroxylation at carbon C-14, C-15, and C-10 are probably catalyzed by the P450 monooxygenases (Fum6p and Fum12p) encoded by *FUM6* and *FUM12* [6, 63]. However, the nature and specificity of the enzymes have not been demonstrated in vitro. The esterifications of the hydroxylated intermediate are catalyzed by a nonribosomal peptide synthetase complex (Fum10p-Fum14p-Fum7p, note that the putative tricarboxylate transporter Fum11p is likely to involve in vivo) [15, 87]. We have expressed *FUM14* in *E. coli*. This gene was predicted to encode the peptidyl carrier protein (PCP) and condensation (C) domain of a nonribosomal peptide synthetase [87]. Fum14p was shown to convert HFB_3 and HFB_4, which are biosynthetic precursors of fumonisins lacking the tricarballylic esters, to the tricarballylic esters-containing fumonisins, FB_3 and FB_4, respectively, when incubated with tricarballylic thioester of *N*-acetylcysteamine. Interestingly, the nonribosomal peptide synthetase catalyzes a C–O bond (ester) formation, instead of the typical C–N bond (amide) formation. The final step of biosynthesis is the hydroxylation at C-5, which is catalyzed by the product of *FUM3* gene [13, 25]. We have expressed *FUM3* in *Saccharomyces cerevisiae* and purified the 33-kDa Fum3p. The enzyme was able to convert FB_3 to FB_1 in the presence of α-ketoglutarate, Fe^{2+}, ascorbic acid, and catalase. This demonstrates that Fum3p is a α-ketoglutarate-dependent dioxygenases required for C-5 hydroxylation of fumonisins (Fig. 5).

Conclusion

As an important group of mycotoxins, SAMT represent an unusual type of fungal highly reduced polyketides. Like other fungal polyketides, the biosynthesis of SAMT involves a single module PKS. The architecture of fungal HR-PKS is distinct from that of NR-PKS and PR-PKS. The study of SAMT biosynthesis is starting to reveal a unique chain-releasing mechanism that is not found in any other polyketides. Most notably, this mechanism allows the formation of a new carbon–carbon bond and introduction of a new functional group during the polyketide chain-releasing step, which is an unprecedented way of creating structural diversity of polyketides. Filamentous fungi produce numerous polyketide metabolites. Many of the metabolites have important biological activities (such as virulence factors, carcinogens, and therapeutics), fascinating molecular architectures (such as aflatoxins and T-toxins), and enormous commercial value (such as lovastatin). These polyketides play a significant role in human and animal health because of their presence in many indispensable fungal species that affect our everyday lives. The understanding of the molecular mechanism for SAMT biosynthesis will contribute to the development of new strategies toward mycotoxin reduction and elimination in food industry and agriculture.

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