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Unraveling polyketide synthesis in members of the genus *Aspergillus*

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Abstract

Aspergillus species have the ability to produce a wide range of secondary metabolites including polyketides that are generated by multi-domain polyketide synthases (PKSs). Recent biochemical studies using dissected single or multiple domains from PKSs have provided deep insight into how these PKSs control the structural outcome. Moreover, the recent genome sequencing of several species has greatly facilitated the understanding of the biosynthetic pathways for these secondary metabolites. In this review, we will highlight the current knowledge regarding polyketide biosynthesis in *Aspergillus* based on the domain architecture of non-reducing, highly reducing, and partially reducing PKSs, and PKS-non-ribosomal peptide synthetases.

Keywords

Secondary metabolites; Fungi; Polyketide; Nonribosomal peptides

Introduction

Polyketides are structurally diverse secondary metabolites including polyphenols, polyenes, and macrolides. These natural products are produced by polyketide synthases (PKSs) from a

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variety of organisms including bacteria, fungi, and plants (Fischbach and Walsh 2006; Hertweck 2009). PKSs are categorized into three subtypes: types I, II, and III PKSs. As in the nomenclature of fatty acid synthases (FASs), type I PKSs refers to covalently linked multifunctional enzymes, whereas the catalytic components in type II PKS are free-standing. Furthermore, type III PKSs are homodimeric multi-functional enzymes distinguished from types I and II by the use of malonyl-CoA rather than malonyl-*S*-pantetheinyl-thiolation domain species as substrates (Austin and Noel 2003). Apart from these three subtypes, PKSs are also categorized as iterative or noniterative, distinguished by whether the substrate is used for one or more than one round of elongation.

Polyketides produced from fungal species have drawn much attention due to their important role in human health. These compounds include toxins such as sterigmatocystin (**1**) and aflatoxin B_1 (**2**); the virulence factor melanin; and the medicinally important anticholesterol drug lovastatin (**3**) (Fig. 1). Assembly of these fungal polyketide carbon skeletons are catalyzed by multi-domain type I polyketide synthases (PKSs) and all known fungal PKSs so far are iterative in nature except lovastatin diketide synthase (LovF) and its homolog compactin diketide synthase (MlcB) which are noniterative (*vide infra* and Abe et al. 2002). Recent fungal genome projects of *Aspergillus* species has revealed that these microorganisms have a surprisingly large number of secondary metabolite genes, the products of which are mostly currently unknown (Galagan et al. 2005). The sequencing of fungal genomes also revealed that biosynthesis genes of secondary metabolites in fungi are generally clustered (Galagan et al. 2005; Keller et al. 2005). For reasons still not well understood, it seems that most biosynthetic gene clusters are either silent or expressed at very low levels, and that their products are, thus, difficult to detect or identify. It appears that some chemical or environmental signals for triggering these “cryptic” pathways are missing in standard laboratory culture conditions (Bergmann et al. 2007; Chiang et al. 2008; Schroeckh et al. 2009). Finding the correct conditions to unlock cryptic natural product clusters will be important to decoding the complexity of the fungal secondary metabolome. Since reviews describing how to activate cryptic biosynthetic pathways have been published elsewhere (Chiang et al. 2009a; Scherlach and Hertweck 2009), this review will focus on the relationship of PKS domain architecture and the structure of the product of the PKS. Three excellent reviews concerning fungal PKSs have been published by Schumann and Hertweck; Cox; and Hoffmeister and Keller (Cox 2007; Hoffmeister and Keller 2007; Schumann and Hertweck 2006). We will try to extend previous understanding and highlight the most current knowledge of type I PKSs in *Aspergillus* species.

General domain architecture and reaction mechanisms of fungal PKSs

Type I multi-domain PKSs use substrates such as acetyl-CoA and malonyl-CoA (mal-CoA) in a similar fashion to multi-domain FASs but differ in the existence of incomplete reduction and/or dehydration steps. Table 1 lists the characterized polyketide genes and their cognate proteins, as well as domain organizations from *Aspergillus* species. These PKSs minimally contain ketosynthase (KS), acyl transferase (AT) and acyl carrier protein (ACP) domains (Fig. 2a). After translation, the ACP domain of PKSs requires post-translational modification through the addition of a phosphopantetheinyl group to the conserved Ser by phosphopantetheinyl transferase (PPTase). This generates a *holo*-ACP with an approximate 18 Å phosphopantetheine (PPT) arm from an *apo*-ACP. A single PPTase (CfwA/NpgA) has been identified and likely to be responsible for the activation of all PKSs in *A. nidulans* (Marquez-Fernandez et al. 2007). A malonyl extender unit is then transferred onto the terminal thiol of the phosphopantetheine prosthetic group of ACP, a reaction that is mostly catalyzed by the AT domain. In the presence of an acyl starter unit on a conserved cysteine residue of the KS domain, the KS catalyzes Claisen condensation of malonyl extender to generate a diketide. There are two major subclasses of fungal type I PKSs. They are non-

reducing (NR) and highly reducing (HR) PKSs distinguished basically by the existence of domains that can catalyze the reduction and/or dehydration steps (Table 1). In HR-PKSs, the α-carbon of the diketide may be methylated to generate α-methyl-β-ketothioester by the C-methyltrasferase (CMeT) domain which uses *S*-adenosylmethionine (SAM) as a substrate. In addition, the β-keto group may further be reduced to β-hydroxyl, α,β-unsaturated carbonyl, or α,β-saturated carbonyl functionalities by the catalytic activities of keto reductase (KR), dehydratase (DH), and enoyl reductase (ER) domains (Fig. 2b). The processed diketide can then transfer back to the KS and be extended further. After several rounds of elongation, the polyketide chain is released from the PKS. Polyketide structural diversity is determined by the optionally "programmed" activity of CMeT, KR, DH, and ER domains during each round of extension. However, how those multi-functional enzymes control the product chain length and the site of "programming" is largely unknown.

Two major subclasses of type I PKSs were also observed by Kroken et al. who performed phylogenetic analyses on amino acid sequences of KS domains of characterized fungal *PKS* genes and putative *PKS* genes from genomic sequences (Kroken et al. 2003). They showed that fungal NR-and HR-PKSs are the two main clades of microbial type I PKSs. They also proposed that *NR-PKSs* evolved by losing DH-ER-KR domains while gaining the TE/CLC (thioesterase/Claisen cyclase) domain from their "reducing" *PKSs* ancestors. Moreover, the Townsend group has recently identified SAT (starter unit-ACP transacylase) and PT (product template) domains that control the structural outcome of NR-PKSs. We will describe their elegant work before going on to the next section.

Discovery of SAT and PT domains

Fungal PKSs are large multi-domain proteins which make overexpression, functional purification and accurate examination of individual reactions difficult. In order to dissect the function of individual domains, Udwary, Merski, and Townsend developed an algorithm, UMA (Udwary-Merski algorithm) that combines primary sequence similarity, predicted secondary structure, and local hydrophobicity to predict interdomainal linker regions (Udwary et al. 2002). By using UMA, they identified two previously unidentified domains, SAT and PT domains, from the norsolorinic acid synthase (NSAS) family of NR-PKSs (Table 1). NSAS is a family of octaketide synthases that catalyze the assembling of a hexanoyl starter unit with seven mal-CoA followed by dehydration/aromatization and Claisen cyclization to generate norsolorinic acid anthrone (**4**) (Fig. 3a). It was found that **4** oxidized to norsolorinic acid (**5**) spontaneously during workup, although this oxidation might be catalyzed enzymatically in vivo, as is known to occur in emodin biosynthesis (Chen et al. 1995). After purifying different domains as recombinant proteins from *E. coli*, the Townsend group showed that hexanoyl-CoA could be transferred to the SAT domain directly but could only be transferred to the ACP domain in the presence of the SAT domain (Fig. 3b) (Crawford et al. 2008a). Ma et al. also noticed that the AT domain of NSAS could only transfer malonate from mal-CoA to ACP but not hexanoate or acetate from the cognate CoA (Ma et al. 2006). Thus, the SAT domain located in the N terminus of NSAS with high similarity to the mal-CoA/ACP transferase domain is responsible for selecting the hexanoate starter unit. The same group further aligned SAT domains of 76 NSAS family PKSs identified from Blastp searches of the NCBI non-redundant protein sequence database and realized that SAT domains are widespread among fungal NR-PKSs (Crawford et al. 2008b). Forty-nine protein sequences harbored the expected GXCXG active-site motif in the SAT domain, where the active site Cys tethers to the starter unit. Twelve sequences contained a GXSXG motif, which presumably use oxyester rather than thioester chemistry for transfering the starter unit. Fourteen sequences had a GXGXG motif, which might be inactive or have an unknown function. Interestingly, the residue directly upstream of the active site His in the catalytic dyad of SAT domain contains a conserved Ala for the known

hexanoyl acceptors and has either a bulky Phe or Tyr for the known acetyl acceptors. Thus, the SAT domains might use a steric approach to "selecting" the acyl starter unit.

The PT domain which is located between the AT and ACP domains has a critical role in specific aldol cyclization and aromatization. Without the PT domain, the SAT-KS-AT tridomain and the *holo*-ACP monodomain in NSAS can still produce polyketides of the correct chain length (**5** and **6**) but in very small amounts (Fig. 3c). However, the products markedly increase in the presence of the PT domain that drives the irreversible dehydration/aromatization reaction (Crawford et al. 2008a). Without the TE/CLC monodomain, **5** is the minor product and naphthopyrone **6** is the major product (Fig. 3a, pathway B). In the presence of all domains including the TE/CLC monodomain, **5** becomes the major product, supporting the idea that TE/CLC functions as a Claisen cyclase (Fig. 3a, pathway A). The crystal structure of the NSAS PT monodomain has recently been solved and displays a distinct "double hot dog" (DHD) fold (Crawford et al. 2009). The rigid octaketide binding pocket in the PT domain extends 30 Å from the surface to the bottom and can be divided into three regions; the PPT-binding region, cyclization chamber, and hexyl-binding region (Fig. 3d). The PPT-binding region extends 14 Å from the protein surface into the pocket and is proposed to bind the ~ 18 Å PPT arm. The cyclization chamber (8×13.5 Å) can accommodate two aromatic rings and contains the proposed catalytic dyad His^{1345} and Asp^{1543}. The hydrophobic hexyl-binding region (6×6 Å) lies at the bottom and is perfectly adapted to accept the substrate hexanoyl starter unit.

NR-PKSs containing the TE/CLC releasing domain

Aflatoxins are a group of potent environmental toxins and carcinogens produced by *Aspergillus* species and their biosynthesis has drawn much attention. Chang et al. and Feng et al. identified the *PKS* gene from *A. parasiticus* responsible for aflatoxin biosynthesis (the same gene was given different designations, *pksA* and *pksL1*, by the two groups) (Chang et al. 1995; Feng and Leonard 1995). Yu et al. identified *pksST* from *A. nidulans* required for sterigmatocystin (**1**) biosynthesis (Yu and Leonard 1995), originally designated *pksST* but renamed *stcA* by Brown et al. in order to simplify the nomenclature and reflect the fact that genes required for a specific secondary metabolite are usually clustered (Brown et al. 1996). The PKS responsible for sterigmatocystin (**1**) and aflatoxin biosynthesis, NSAS as discussed above, contains SAT, KS, AT, PT, ACP, and TE/CLC domains (Fig. 3). It is interesting to note that StcA contains two ACP domains (Table 1). NSAS forms a complex with HexA/HexB (α and β components of fungal FAS) and produces norsolorinic acid (**5**) in the presence of acetyl-CoA, mal-CoA, and NADPH. Watanabe et al. showed that hexanoyl-CoA is not a free intermediate produced by the NSAS/HexA/HexB complex suggesting that hexanoate must be passed directly to the SAT domain of NSAS after being synthesized by HexA/HexB (Watanabe and Townsend 2002).

Many fungi produce melanins, which are dark brown or black pigments formed by oxidative polymerization of phenolic compounds. Melanin is known to contribute to survival, cell wall rigidity and impermeability, and most importantly, is a virulence factor for pathogenic fungi (Gomez and Nosanchuk 2003). In contrast to melanin biosynthesis in brown and black fungi, little is known about pigment biosynthesis in green and bluish-green fungi. In *A. nidulans*, *wA* gene was identified to encode a PKS required of green pigment biosynthesis (Mayorga and Timberlake 1992). Watanabe et al. expressed the *wA* gene in *A. oryzae* strain M-2-3 and this led to the isolation of citreoisocoumarin (**7**), and its derivatives dehydrocitreoisocoumarin (**8**) and compound **9**, thus establishing that WA is a heptaketide synthase (Fig. 4a, pathway B) (Watanabe et al. 1998). Since there is no KR domain in WA, the secondary alcohol in the side chain of **7** and **9** is presumably reduced by a host reductase. However, it was later realized that the *wA* gene originally identified was missing one base

pair thus caused a frame shift and produced a 67 aa C-terminal truncated protein in their heterologous expression experiment (Fig. 4a, pathway B). The full length WA was later heterologously expressed and naphthopyrone YWA1 (**10**) was re-identified to be the real metabolite (Fig. 4a, pathway A) (Watanabe et al. 1999). This suggested that SAT, KS, AT, PT, and two ACP domains of WA produce a heptaketide and catalyze the first aromatic ring formation, and the C-terminal region of WA is involved in the cyclization of the second aromatic ring of YWA1 (**10**). Thus, the Ebizuka group first demonstrated that the C terminus of WA turns out to function as a Claisen cyclase (CLC) by using a heterologous expression system. A detailed functional analysis by a series of C-terminal deletions and site-directed mutagenesis showed that Ser^{1967} and His^{2129} residues in CLC domain of WA are responsible for naphthopyrone (**10**) formation and this leads to the proposed mechanism shown in Fig. 4b. Another feature of WA is that it contains two ACP domains. Site-directed mutagenesis of the Ser residue, a known phosphopantetheine anchor site, to Cys in either ACP domain produced YWA1 (**10**). This suggests that only a single ACP is necessary to catalyze the heptaketide formation (Fujii et al. 2001).

A pentaketide, 1,3,6,8-tetrahydroxynaphthalene (T4HN, **11**), is an initial precursor of melanin. In 1998, Tsai et al. and Langfelder et al. identified the *PKS* gene from *A. fumigatus* necessary for melanin biosynthesis (the same gene was designated *alb1* and *pksP* by the two groups) (Langfelder et al. 1998; Tsai et al. 1998). Heterologous expression of the *alb1* gene in *A. oryzae* M-2-3 resulted in the isolation of a heptaketide YWA1 (**10**) but not the pentaketide T4HN (**11**) (Watanabe et al. 2000). It was later realized that an enzyme, Ayg1p, catalyzed chain-length shortening to produce T4HN (**11**) (Fig. 4a, pathway A) (Fujii et al. 2004; Tsai et al. 2001). Notably, another *PKS* gene, *PKS1*, responsible for melanin biosynthesis was identified from *Colletotrichum lagenarium* in 1995 (Takano et al. 1995). When *PKS1* was heterologously expressed in *A. oryzae* M-2-3, a major pentaketide T4HN (**11**) together with other minor pentaketides including α-acetylorsellinic acid (**12**) were isolated as their acetylated derivatives, **13** and **14**, after acetylation in order to avoid oxidative polymerization (Fig. 5a) (Fujii et al. 1999). When isotope-labeled acetyl-CoA or mal-CoA was fed into a cell-free extract containing PKS1, only mal-CoA was able to incorporate into T4HN (**11**) and this led to the hypothesis that mal-CoA but not acetyl-CoA serves as the starter unit (Fig. 5a) (Fujii et al. 2000). The detailed product distribution of this heterologous expression system was later revisited by using LC-ESI-MS (liquid chromatography-electrospray ionization-mass spectrometry) (Fig. 5b) (Watanabe and Ebizuka 2004). Interestingly, when the TE/CLC domain was inactivated, PKS1 produced mainly 6,8-dihydroxy-3-(2-oxopropyl)-isocumarin (**18**), a hexaketide. The authors thus concluded that the TE/CLC domain has a critical role in chain-length determination. However, Crawford et al. demonstrated that when recombinant SAT domain of PKS1 was purified from *E. coli*, it was able to accept acetyl-CoA as a starter unit (Crawford et al. 2008c). Crawford et al. thus proposed an alternative mechanism, that PKS1 incorporates one acetyl-CoA and five mal-CoA to generate 2-acetyl-T4HN (**15**) which is later shortened by an ‘Ayg1p’ like enzyme in the heterologous host (Fig. 5c). This alternative mechanism also fulfills the observed phenomenon that isotope labeled acetyl-CoA is not incorporated into T4HN since it has been cleaved by the ‘Ayg1p’ like enzyme to become acetic acid. As a consequence, both chain-length control and dehydration/aromatization control would be carried out prior to the TE/CLC catalyzed Claisen cyclization. This provides a general pattern for iterative systems that contain SAT domains and a biochemical rationale for the classical acetyl “starter unit effect”. The function of each domain will promise to become clearer as data are accumulated in future experiments.

NR-PKSs with no releasing domain

Since most biosynthetic gene clusters are cryptic, we were curious whether SUMOylation might play a part in secondary metabolite regulation. Deletion of the *sumo* gene in *A. nidulans* dramatically increases asperthecin (**21**) production and this led us to identify the *apt* gene cluster through a series of targeted deletions (Fig. 6, Apt pathway) (Szewczyk et al. 2008). AptA is a TE/CLC less NR-PKS that catalyzes octaketide formation from one acetyl-CoA and seven mal-CoA. The lack of a TE/CLC domain in AptA and the presence, near *aptA* in the genome, of a β-lactamase, *aptB*, which is also essential for asperthecin biosynthesis led us to propose that AptB is responsible for releasing the octaketide from the NR-PKS, AptA. This hypothesis has recently been validated biochemically by Awakawa et al. who showed that atrochrysone carboxylic acid (**22**) is an unstable metabolite released from ACAS (atrochrysone carboxylic acid synthase) in the presence of ACTE (atrochrysone carboxyl ACP thioesterase, a β-lactamase-like protein) (Awakawa et al. 2009). Subsequent decarboxylation, dehydration, and oxidation at various positions resulted in the formation of **21**.

In response to the accruing evidence that a significant portion of the regulation of the expression of secondary metabolite gene clusters occurs at the chromatin level (Cichewicz 2010), we deleted the *A. nidulans cclA* gene, an ortholog of the *bre2* gene of *Saccharomyces cerevisiae* (Bok et al. 2009). *Bre2* encodes a critical member of the COMPASS (complex associated with Set1), a conserved eukaryotic transcriptional effector both facilitating and repressing chromatin-mediated processes through methylation of lysine 4 of histone H3 (Mueller et al. 2006; Sims and Reinberg 2006). Chemical profiling of a *cclA* deletant followed by genetic analysis led us to identify two silent gene clusters, one involved in monodictyphenone biosynthesis (Fig. 6, Mdp pathway) and the other required for F-9775 biosynthesis (*vide infra*). MdpG which has high similarity with AptA is also a TE/CLC less NR-PKS. MdpF, a β-lactamase-type TE nearby, thus presumably catalyzes product release. Subsequent decarboxylation, dehydration, and oxidation generate emodin (**23**) which is further processed by downstream enzymes to produce monodictyphenone (**24**).

NR-PKS containing the TE releasing domain

In addition to MdpG, another PKS, AN7909, responsible for F-9775 biosynthesis has also been identified from a *cclA* deletion strain (Bok et al. 2009). Notably, F-9775A and B (**25** and **26**) together with orsellinic acid (**27**) can be induced by growth in Czapek's media even in a wild-type *cclA* background and deletion of AN7909 not only eliminates **25** and **26** but also **27** production (Fig. 7) (Sanchez et al.). These results together with the structures of F-9775A and B, suggested that these compounds are derived from orsellinic acid (**27**). Interestingly, a theacitrin derivative (**28**) has been proposed to be generated from two molecules of a pyrogallol derivative (**29**) after oxidative dimerization, intramolecular cyclization and rearrangement (Davis et al. 1997). F-9775A and B, thus, could be biosynthesized from three molecules of **27**. Remarkably, co-cultivated *A. nidulans* with the bacterium *Streptomyces hygroscopicus* also resulted in the activation of AN7909 and the production of **25–27** (Schroeckh et al. 2009). Both approaches demonstrated that AN7909 encodes a long-searched for orsellinic acid synthase, OrsA.

NR-PKSs containing the reductase releasing domain

The presence of cluster-specific regulatory activators is a common feature of fungal secondary metabolite biosynthetic gene clusters (Chiang et al. 2009a). Bergmann et al. first demonstrated that ectopic overexpression of *apdR*, a regulatory gene within a cryptic hybrid PKS-non-ribosomal peptide synthetase (NRPS) gene cluster, resulted in the concerted activation of the gene cluster and production of two new cytotoxic metabolites (Fig. 13c,

vide infra). Using a similar approach, the endogenous promoter of a regulatory gene within the *afo* gene cluster was replaced with an inducible promoter (Chiang et al. 2009b). This led us to discover a new compound asperfuranone (**30**) and two PKSs, one HR-PKS (AfoG) and one NR-PKS (AfoE), necessary for asperfuranone biosynthesis (Fig. 8). Deletion of a key hydroxylase (*afoD*) in the asperfuranone biosynthetic pathway resulted in the accumulation of benzaldehyde derivative **31** suggesting that AfoG is responsible for catalyzing the biosynthesis of a tetraketide and then transfering the reduced tetraketide to the SAT domain of AfoE to generate the reduced and non-reduced hybrid octaketide. Interestingly, the C-terminal region of AfoE contains a reductase (R) domain and this matches with the reductive release mechanism to generate aldehyde **31**. The reductive release mechanism was first described by the Cox group who isolated 3-methylorcinaldehyde (**32**) by heterologous expression of 3-methylorcinaldehyde synthase (MOS) in *A. oryzae* M-2-3 (Bailey et al. 2007).

HR-PKSs

HR-PKSs found in filamentous fungi are the most interesting but the least understood of the PKS family members. Lovastatin (**3**, Mevacor, Merck) is a cholesterol lowering drug produced by *A. terreus*. It is also a precursor of a more potent anticholesterol drug simvastatin (Zocor, Merck) which was the second largest selling cholesterol lowering drug in the world before losing US patent protection (Maggon 2005). The biosynthesis of lovastatin has been extensively reviewed by Hill and Cox (Cox 2007; Hill 2006). Two HR-PKSs, LovB (lovastatin nonaketide sythases) and LovF, are involved in the formation of the lovastatin core structure. LovB and LovF both contain KS, AT, DH, CMeT, ER, KR, and ACP domains (Fig. 9). Unlike NR-PKSs, HR-PKSs analyzed to date do not contain SAT domains. It is likely that fungal HR-PKSs initiate polyketide synthesis through decarboxylation of malonyl-ACP or direct transfer of an acetyl unit, but with less efficiency, by the AT domain (Ma and Tang 2007). Interestingly, the ER domain of LovB is nonfunctional and needs to be compensated by the free-standing ER protein, LovC, to produce dihydromonacolin L (**33**). There is a condensation (CON) domain in the C terminus of LovB and this domain is hypothesized to be involved in product release. In vitro studies of LovB have been hampered by difficulties in purifying sufficient amounts of functional LovB from either *A. terreus* or a heterologous *Aspergillus* host. Recently, the Vederas and Tang groups were able to purify the 335-kD LovB by using an efficient expression system from an engineered *Saccharomyces cerevisiae* strain, BJ5464-NpgA, which contains a chromosomal copy of the *A. nidulans* PPTase gene (*npgA*) (Ma et al. 2009). Reconstitution experiments indicated that the CON domain is not involved in product release but does play a crucial role in the formation of **33**. This also suggested that other endogenous TEs might be involved in product release (Fig. 9). LovF is the only known noniterative fungal PKS identified from *Aspergillus* so far. It has similar domain architecture to LovB but there is no CON domain. The Tang group investigated the mechanism of product release from LovF and found that LovD, an acyl transferase, interacts with LovF and transfers (*S*)-2-methylbutyrate to monacolin J (**34**) to produce **3** (Xie et al. 2009).

In the course of screening for NRPS gene deletion mutants, EasB was identified to be involved in the biosynthesis of the side chains of emericellamides (**35–39**) (Fig. 10) (Chiang et al. 2008). Interestingly, the lack of a product release domain in EasB as well as the necessity of a free-standing acyl transferase (EasC) for emericellamide biosynthesis nearby EasB in the genome might indicate that EasC is involved in the product release. The long chain fatty acids released from EasB then incorporate into EasA, an NRPS, to generate **35–39**. Interestingly, when comparing EasA with AfoG, the HR-PKS responsible for asperfuranone biosynthesis (Fig. 8), AfoG does not have a product release domain. Notably,

the absence of a free-standing acyl transferase in the *afo* gene cluster suggests that the tetraketide synthesized by AfoG might transfer directly to the SAT domain of AfoE.

Critical insights by the Cox and Fujii groups have been obtained from heterologous expression of HR-PKSs using *A. oryzae* as a host. Examples include the tetraketide side chain of squalestatin S1 (**40**) biosynthesized by SQTKS (squalestatin tetraketide synthase) from Phoma sp. C2932; alternapyrone (**41**) catalyzed by PKSN; and aslanipyrone (**42**) and aslaniol (**43**) biosynthesized by PKSF from *Alternaria solani* (Table 1 and Fig. 11) (Cox et al. 2004; Fujii et al. 2005; Kasahara et al. 2006). Partial sequences of two different *PKS* genes, *pks* and *aoks1* involved in ochratosin A (**44**) biosynthesis, have been obtained by O'Callaghan et al. and Bacha et al.; and their roles in ochratosin A biosynthesis have been demonstrated through disruption of these genes (Bacha et al. 2009a; O'Callaghan et al. 2003). In combination, these studies indicate that two different PKSs are involved in the biosynthesis of **44**. Bacha et al. also showed that disruption of *apks1* did not abolish mullein (**45**) production arguing against the previous hypothesis that mullein (**45**) is a precursor of **44**.

Partially reducing PKSs

The 6-methylsalicylic acid synthase (MSAS) was the first PKS to be purified from *Penicillium patulum* (Dimroth et al. 1970) and also the first fungal *PKS* gene to be sequenced (Beck et al. 1990). By using the KS region of *MSAS* as a probe, Fujii et al. cloned a *PKS* gene, *atX*, from *A. terreus* (Fujii et al. 1996). Heterologous expression of *atX* in *A. nidulans* led to the isolation of 6-methylsalicylic acid (6-MSA, **46**) confirming that *atX* encodes MSAS (Fig. 12). MSAS has been comprehensively reviewed by Cox and it should be note that MSAS does not contain SAT, PT, and TE/CLC domains when compared with NR-PKSs (Cox 2007). It is not clear how the acetyl starter unit loads to the KS domain and how the 6-MSA off-loads from the ACP domain. Recently, the biosynthesis of two potent anti-bacterial and anti-fungal compounds, asperlactone (**47**) and isoasperlactone (**48**), identified from *A. westerdikiae* has been linked to the 6-MSA biosynthesis pathway (Fig. 12) (Bacha et al. 2009b).

PKS-NRPS hybrids containing the Dieckmann cyclase domain

As discussed above, structural diversity in *Aspergillus* secondary metabolites is often created by mixing PKSs with FASs, other PKSs, and NRPSs in a single biosynthesis pathway. Many filamentous fungi sequenced to date also contain at least one hybrid PKS-NRPS where a single enzyme contains both PKS and NRPS domains. α-Cyclopiazonic acid (CPA, **49**) is a pentacyclic indole tetramic acid mycotoxin produced by several *Aspergillus* and *Penicillium* strains. Because the dimethylallyltransferase involved in CPA biosynthesis is located between the aflatoxin biosynthesis gene cluster and the adjacent telomere of *A. oryzae*, Tokuoka et al. were able to identify *cpaA*, the PKS-NRPS gene responsible for CPA biosynthesis, by comparing the subtelomeric region of the CPA nonproducing strain *A. oryzae* RIB40 with that of the CPA-producing strains *A. oryzae* NBRC4177 and *A. flavus* NRRL3357 (Tokuoka et al. 2008). They found that NBRC 4177 has an additional 17–18 kb beyond the region corresponding to the telomere repeat in RIB40. The subtelomeric region of RIB40 near the aflatoxin biosynthesis gene cluster encodes only a portion of the 5′ region of the *cpaA* gene, but not the entire gene, and it could consequently be deduced that *cpaA* is necessary for CPA biosynthesis. The remaining genes involved in CPA biosynthesis are next to the aflatoxin biosynthesis gene cluster. (Chang et al. 2009; Tokuoka et al. 2008). Heterologous expression of CpaA in *A. oryzae* M-2-3 led to the isolation of *cyclo*-acetoacetyl-L-tryptophan (cAATrp, **50**) (Fig. 13a) (Seshime et al. 2009). This suggests that the PKS portion of CpaA catalyzes diketide formation and DH, CMeT, ER, and KR domains

are nonfunctional. The NRPS portion of CpaA containing condensation (C), adenylation (A), and peptidyl carrier protein (PCP) domains that catalyze the condensation of diketide and Trp. Two possible mechanisms of **50** release by the R domain located on the C terminus of CpaA have been proposed, including Dieckman cyclization (Fig. 13a, pathway A) and reductive releasing/cyclization/reoxidation (Fig. 13a, pathway B). By using recombinant PTP-R didomain, PTP, and R monodomain proteins, the Walsh group demonstrated that the R domain of CpaA does not function as a reductase because the Tyr in Ser-Tyr-Lys catalytic triad for the reduction is mutated to Leu (Liu and Walsh 2009). This provides direct evidence that the C-terminal R domain actually functions as a Dieckmann cyclase (DKC) but not a reductase. They also showed that the Tyr in Ser-Tyr-Lys catalytic triad in the R domain of TENS and ApdA is mutated to Phe. Therefore, Dieckman cyclization is the mechanism of releasing products from TENS and ApdA (*vide infra*).

TenS (tenellin synthetase) and the adjunct genes involved in tenellin (**51**) biosynthesis have been identified from the insect pathogenic fungus *Beauveria bassiana* by using degenerate primers for the CMeT domain (Fig. 13b) (Eley et al. 2007). Heterologous expression of *tenS* without *orf3*, encoding a *trans* ER, in *A. oryzae* M-2-3 led to the isolation of prototenellins (**52–54**) (Halo et al. 2008). This validated that the C-terminal domain of TENS does not function as a reductase but, rather, as a DKC. Coexpression of *tenS* with *orf3* led to the production of pretenellin A (**55**) suggesting that the ER activity is required at the first round of polyketide synthesis to generate a correctly elaborated polyketide.

By using a genomic-driven approach, Bergmann et al. induced aspyridone A (**56**) and B (**57**) production *via* the activation of the *apd* gene cluster regulator, ApdR, in *A. nidulans* (Fig. 13c) (Bergmann et al. 2007). Because ApdA lacks a functional ER domain, the free-standing ER, ApdC, is likely involved in the formation of the tetraketide that is then linked to Tyr by the NRPS portion of ApdA. Similar to TENS, a Dieckmann cyclization is likely to occur to generate intermediate **58**, which then converts to **56** and **57** after oxidative ring expansion.

PKS-NRPS hybrid containing the reductase domain

Pseurotin A (**59**) is a competitive inhibitor of chitin synthase and an inducer of nerve-cell proliferation. Maiya et al. identified a PKS-NRPS, PsoA which is involved in **59** biosynthesis, from *A. fumigatus* (Fig. 14) (Maiya et al. 2007). The PKS portion of PsoA contains KS, AT, DH, CMeT, KR, and ACP domains that catalyze pentaketide formation, presumably using a propionate starter. The NRPS portion then condenses L-Phe with the pentaketide. The R domain which has correct catalytic triad in the C terminus of PsoA might catalyze a reductive cyclization (intramolecular Knoevenagel condensation) to generate the first putative intermediate, **60**, that then serves as a precursor of **59**.

Perspective

Continued advances in genome sequencing and molecular genetic technology will ensure that reverse genetic approaches, i.e., analyzing the chemotype of a specific *PKS* null mutant, will continue to play a crucial role in elucidating secondary metabolite biosynthesis. Since the complexity in chemical structures seen in secondary metabolites is generated by enzymes and ultimately genes, scientists worldwide are now trying to predict the structure of the secondary metabolites from their cognate genes. A central question is how the “programming”, i.e., chain length determination, sites of reduction and cyclization, are achieved in an “iterative” system. By using heterologous expression systems and/or reconstitution of the catalytic domains, deduced with the UMA algorithm, as individual enzymes, it is now possible to dissect these megasynthases and understand the function of each domain(s). Taking advantage of fungal genome projects and modern molecular genetic techniques, fungal natural product researchers are now entering an exciting new era in

understanding the logic of polyketide biosynthesis and in engineering the biosynthetic pathways to produce novel chemical entities. It should be noted that there are no SAT and PT domains in PR- and HR-PKSs, and thus, the basis for their complex programming are unclear. Since fungal genome projects have made the sequences of hundreds of *PKS* genes available, analyzing PKS architecture or each PKS domain provides an avenue to discover new biosynthetic pathways.

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Reference

Abe Y, Suzuki T, Ono C, Iwamoto K, Hosobuchi M, Yoshikawa H. Molecular cloning and characterization of an ML-236B (compactin) biosynthetic gene cluster in *Penicillium citrinum*. Mol Genet Genomics. 2002; 267:636–646. [PubMed: 12172803]

Austin MB, Noel JP. The chalcone synthase superfamily of type III polyketide synthases. Nat Prod Rep. 2003; 20:79–110. [PubMed: 12636085]

Awakawa T, Yokota K, Funa N, Doi F, Mori N, Watanabe H, Horinouchi S. Physically discrete beta-lactamase-type thioesterase catalyzes product release in atrochrysone synthesis by iterative type I polyketide synthase. Chem Biol. 2009; 16:613–623. [PubMed: 19549600]

Bacha N, Atoui A, Mathieu F, Liboz T, Lebrihi A. *Aspergillus westerdijkiae* polyketide synthase gene “aoks1” is involved in the biosynthesis of ochratoxin A. Fungal Genet Biol. 2009a; 46:77–84. [PubMed: 18955151]

Bacha N, Dao HP, Atoui A, Mathieu F, O'Callaghan J, Puel O, Liboz T, Dobson AD, Lebrihi A. Cloning and characterization of novel methylsalicylic acid synthase gene involved in the biosynthesis of isoasperlactone and asperlactone in *Aspergillus westerdijkiae*. Fungal Genet Biol. 2009b; 46:742–749. [PubMed: 19589392]

Bailey AM, Cox RJ, Harley K, Lazarus CM, Simpson TJ, Skellam E. Characterisation of 3-methylorcinaldehyde synthase (MOS) in *Acremonium strictum*: first observation of a reductive release mechanism during polyketide biosynthesis. Chem Commun (Camb). 2007:4053–4055. [PubMed: 17912413]

Beck J, Ripka S, Siegner A, Schiltz E, Schweizer E. The multifunctional 6-methylsalicylic acid synthase gene of *Penicillium patulum*. Its gene structure relative to that of other polyketide synthases. Eur J Biochem. 1990; 192:487–498. [PubMed: 2209605]

Bergmann S, Schumann J, Scherlach K, Lange C, Brakhage AA, Hertweck C. Genomics-driven discovery of PKS-NRPS hybrid metabolites from *Aspergillus nidulans*. Nat Chem Biol. 2007; 3:213–217. [PubMed: 17369821]

Bok JW, Chiang YM, Szewczyk E, Reyes-Domingez Y, Davidson AD, Sanchez JF, Lo HC, Watanabe K, Strauss J, Oakley BR, Wang CC, Keller NP. Chromatin-level regulation of biosynthetic gene clusters. Nat Chem Biol. 2009; 5:462–464. [PubMed: 19448638]

Brown DW, Yu JH, Kelkar HS, Fernandes M, Nesbitt TC, Keller NP, Adams TH, Leonard TJ. Twenty-five coregulated transcripts define a sterigmatocystin gene cluster in *Aspergillus nidulans*. Proc Natl Acad Sci USA. 1996; 93:1418–1422. [PubMed: 8643646]

Chang PK, Cary JW, Yu J, Bhatnagar D, Cleveland TE. The *Aspergillus parasiticus* polyketide synthase gene *pksA*, a homolog of *Aspergillus nidulans wA*, is required for aflatoxin B1 biosynthesis. Mol Gen Genet. 1995; 248:270–277. [PubMed: 7565588]

Chang PK, Horn BW, Dorner JW. Clustered genes involved in cyclopiazonic acid production are next to the aflatoxin biosynthesis gene cluster in *Aspergillus flavus*. Fungal Genet Biol. 2009; 46:176–182. [PubMed: 19038354]

Chen Z-G, Fujii I, Ebizuka Y, Sankawa U. Purification and characterization of emodinanthrone oxygenase from *Aspergillus terreus*. Phytochemistry. 1995; 38:299–305.

Chiang YM, Szewczyk E, Nayak T, Davidson AD, Sanchez JF, Lo HC, Ho WY, Simityan H, Kuo E, Praseuth A, Watanabe K, Oakley BR, Wang CC. Molecular genetic mining of the *Aspergillus* secondary metabolome: discovery of the emericellamide biosynthetic pathway. Chem Biol. 2008; 15:527–532. [PubMed: 18559263]

Chiang YM, Lee KH, Sanchez JF, Keller NP, Wang CC. Unlocking fungal cryptic natural products. Nat Prod Commun. 2009a; 4:1505–1510. [PubMed: 19967983]

Chiang YM, Szewczyk E, Davidson AD, Keller N, Oakley BR, Wang CC. A gene cluster containing two fungal polyketide synthases encodes the biosynthetic pathway for a polyketide, asperfuranone, in *Aspergillus nidulans*. J Am Chem Soc. 2009b; 131:2965–2970. [PubMed: 19199437]

Cichewicz RH. Epigenome manipulation as a pathway to new natural product scaffolds and their congeners. Nat Prod Rep. 2010; 27:11–22. [PubMed: 20024091]

Cox RJ. Polyketides, proteins and genes in fungi: programmed nano-machines begin to reveal their secrets. Org Biomol Chem. 2007; 5:2010–2026. [PubMed: 17581644]

Cox RJ, Glod F, Hurley D, Lazarus CM, Nicholson TP, Rudd BA, Simpson TJ, Wilkinson B, Zhang Y. Rapid cloning and expression of a fungal polyketide synthase gene involved in squalestatin biosynthesis. Chem Commun (Camb). 2004:2260–2261. [PubMed: 15489970]

Crawford JM, Thomas PM, Scheerer JR, Vagstad AL, Kelleher NL, Townsend CA. Deconstruction of iterative multidomain polyketide synthase function. Science. 2008a; 320:243–246. [PubMed: 18403714]

Crawford JM, Vagstad AL, Ehrlich KC, Townsend CA. Starter unit specificity directs genome mining of polyketide synthase pathways in fungi. Bioorg Chem. 2008b; 36:16–22. [PubMed: 18215412]

Crawford JM, Vagstad AL, Whitworth KP, Ehrlich KC, Townsend CA. Synthetic strategy of nonreducing iterative polyketide synthases and the origin of the classical “starter-unit effect”. Chembiochem. 2008c; 9:1019–1023. [PubMed: 18338425]

Crawford JM, Korman TP, Labonte JW, Vagstad AL, Hill EA, Kamari-Bidkorpeh O, Tsai SC, Townsend CA. Structural basis for biosynthetic programming of fungal aromatic polyketide cyclization. Nature. 2009; 461:1139–1143. [PubMed: 19847268]

Davis AL, Lewis JR, Cai Y, Powell C, Davis AP, Wilkins JPG, Pudney P, Clifford MN. A polyphenolic pigment from black tea. Phytochemistry. 1997; 46:1397–1402.

Dimroth P, Walter H, Lynen F. Biosynthesis of 6-methylsalicylic acid. Eur J Biochem. 1970; 13:98–110. [PubMed: 4392442]

Eley KL, Halo LM, Song Z, Powles H, Cox RJ, Bailey AM, Lazarus CM, Simpson TJ. Biosynthesis of the 2-pyridone tenellin in the insect pathogenic fungus *Beauveria bassiana*. Chembiochem. 2007; 8:289–297. [PubMed: 17216664]

Feng GH, Leonard TJ. Characterization of the polyketide synthase gene (*pksL1*) required for aflatoxin biosynthesis in *Aspergillus parasiticus*. J Bacteriol. 1995; 177:6246–6254. [PubMed: 7592391]

Fischbach MA, Walsh CT. Assembly-line enzymology for polyketide and nonribosomal peptide antibiotics: logic, machinery, and mechanisms. Chem Rev. 2006; 106:3468–3496. [PubMed: 16895337]

Fujii I, Ono Y, Tada H, Gomi K, Ebizuka Y, Sankawa U. Cloning of the polyketide synthase gene *atX* from *Aspergillus terreus* and its identification as the 6-methylsalicylic acid synthase gene by heterologous expression. Mol Gen Genet. 1996; 253:1–10. [PubMed: 9003280]

Fujii I, Mori Y, Watanabe A, Kubo Y, Tsuji G, Ebizuka Y. Heterologous expression and product identification of *Colletotrichum lagenarium* polyketide synthase encoded by the PKS1 gene involved in melanin biosynthesis. Biosci Biotechnol Biochem. 1999; 63:1445–1452. [PubMed: 10501004]

Fujii I, Mori Y, Watanabe A, Kubo Y, Tsuji G, Ebizuka Y. Enzymatic synthesis of 1, 3, 6, 8-tetrahydroxynaphthalene solely from malonyl coenzyme A by a fungal iterative type I polyketide synthase PKS1. Biochemistry. 2000; 39:8853–8858. [PubMed: 10913297]

Fujii I, Watanabe A, Sankawa U, Ebizuka Y. Identification of Claisen cyclase domain in fungal polyketide synthase WA, a naphthopyrone synthase of *Aspergillus nidulans*. Chem Biol. 2001; 8:189–197. [PubMed: 11251292]

Fujii I, Yasuoka Y, Tsai HF, Chang YC, Kwon-Chung KJ, Ebizuka Y. Hydrolytic polyketide shortening by ayg1p, a novel enzyme involved in fungal melanin biosynthesis. J Biol Chem. 2004; 279:44613–44620. [PubMed: 15310761]

Fujii I, Yoshida N, Shimomaki S, Oikawa H, Ebizuka Y. An iterative type I polyketide synthase PKSN catalyzes synthesis of the decaketide alternapyrone with regio-specific octa-methylation. Chem Biol. 2005; 12:1301–1309. [PubMed: 16356847]

Galagan JE, Calvo SE, Cuomo C, Ma LJ, Wortman JR, Batzoglou S, Lee SI, Basturkmen M, Spevak CC, Clutterbuck J, Kapitonov V, Jurka J, Scazzocchio C, Farman M, Butler J, Purcell S, Harris S, Braus GH, Draht O, Busch S, D'Enfert C, Bouchier C, Goldman GH, Bell-Pedersen D, Griffiths-Jones S, Doonan JH, Yu J, Vienken K, Pain A, Freitag M, Selker EU, Archer DB, Peñalva MA, Oakley BR, Momany M, Tanaka T, Kumagai T, Asai K, Machida M, Nierman WC, Denning DW, Caddick M, Hynes M, Paoletti M, Fischer R, Miller B, Dyer P, Sachs MS, Osmani SA, Birren BW. Sequencing of *Aspergillus nidulans* and comparative analysis with *A. fumigatus* and *A. oryzae*. Nature. 2005; 438:1105–1115. [PubMed: 16372000]

Gomez BL, Nosanchuk JD. Melanin and fungi. Curr Opin Infect Dis. 2003; 16:91–96. [PubMed: 12734441]

Halo LM, Marshall JW, Yakasai AA, Song Z, Butts CP, Crump MP, Heneghan M, Bailey AM, Simpson TJ, Lazarus CM, Cox RJ. Authentic heterologous expression of the tenellin iterative polyketide synthase nonribosomal peptide synthetase requires coexpression with an enoyl reductase. Chembiochem. 2008; 9:585–594. [PubMed: 18266306]

Hendrickson L, Davis CR, Roach C, Nguyen DK, Aldrich T, McAda PC, Reeves CD. Lovastatin biosynthesis in *Aspergillus terreus*: characterization of blocked mutants, enzyme activities and a multifunctional polyketide synthase gene. Chem Biol. 1999; 6:429–439. [PubMed: 10381407]

Hertweck C. The biosynthetic logic of polyketide diversity. Angew Chem Int Ed Engl. 2009; 48:4688–4716. [PubMed: 19514004]

Hill AM. The biosynthesis, molecular genetics and enzymology of the polyketide-derived metabolites. Nat Prod Rep. 2006; 23:256–320. [PubMed: 16572230]

Hoffmeister D, Keller NP. Natural products of filamentous fungi: enzymes, genes, and their regulation. Nat Prod Rep. 2007; 24:393–416. [PubMed: 17390002]

Kasahara K, Fujii I, Oikawa H, Ebizuka Y. Expression of *Alternaria solani* PKSF generates a set of complex reduced-type polyketides with different carbon-lengths and cyclization. Chembiochem. 2006; 7:920–924. [PubMed: 16642517]

Keller NP, Turner G, Bennett JW. Fungal secondary metabolism—from biochemistry to genomics. Nat Rev Microbiol. 2005; 3:937–947. [PubMed: 16322742]

Kennedy J, Auclair K, Kendrew SG, Park C, Vederas JC, Hutchinson CR. Modulation of polyketide synthase activity by accessory proteins during lovastatin biosynthesis. Science. 1999; 284:1368–1372. [PubMed: 10334994]

Kroken S, Glass NL, Taylor JW, Yoder OC, Turgeon BG. Phylogenomic analysis of type I polyketide synthase genes in pathogenic and saprobic ascomycetes. Proc Natl Acad Sci USA. 2003; 100:15670–15675. [PubMed: 14676319]

Langfelder K, Jahn B, Gehringer H, Schmidt A, Wanner G, Brakhage AA. Identification of a polyketide synthase gene (*pksP*) of *Aspergillus fumigatus* involved in conidial pigment biosynthesis and virulence. Med Microbiol Immunol. 1998; 187:79–89. [PubMed: 9832321]

Liu X, Walsh CT. Cyclopiazonic acid biosynthesis in *Aspergillus* sp.: characterization of a reductase-like R* domain in cyclopiazonate synthetase that forms and releases cyclo-acetoacetyl-l-tryptophan. Biochemistry. 2009; 48:11032–11044. [PubMed: 19877600]

Ma SM, Li JW, Choi JW, Zhou H, Lee KK, Moorthie VA, Xie X, Kealey JT, Da Silva NA, Vederas JC, Tang Y. Complete reconstitution of a highly reducing iterative polyketide synthase. Science. 2009; 326:589–592. [PubMed: 19900898]

Ma SM, Tang Y. Biochemical characterization of the minimal polyketide synthase domains in the lovastatin nonaketide synthase LovB. FEBS J. 2007; 274:2854–2864. [PubMed: 17466016]

Ma Y, Smith LH, Cox RJ, Beltran-Alvarez P, Arthur CJ, Simpson FRST. Catalytic relationships between type I and type II iterative polyketide synthases: the *Aspergillus parasiticus* norsolorinic acid synthase. Chembiochem. 2006; 7:1951–1958. [PubMed: 17086560]

Maggon K. Best-selling human medicines 2002–2004. Drug Discov Today. 2005; 10:739–742. [PubMed: 15922927]

Maiya S, Grundmann A, Li X, Li SM, Turner G. Identification of a hybrid PKS/NRPS required for pseurotin A biosynthesis in the human pathogen *Aspergillus fumigatus*. Chembiochem. 2007; 8:1736–1743. [PubMed: 17722120]

Marquez-Fernandez O, Trigos A, Ramos-Balderas JL, Viniegra-Gonzalez G, Deising HB, Aguirre J. Phosphopantetheinyl transferase CfwA/NpgA is required for *Aspergillus nidulans* secondary metabolism and asexual development. Eukaryot Cell. 2007; 6:710–720. [PubMed: 17277172]

Mayorga ME, Timberlake WE. The developmentally regulated *Aspergillus nidulans wA* gene encodes a polypeptide homologous to polyketide and fatty acid synthases. Mol Gen Genet. 1992; 235:205–212. [PubMed: 1465094]

Mueller JE, Canze M, Bryk M. The requirements for COMPASS and Paf1 in transcriptional silencing and methylation of histone H3 in *Saccharomyces cerevisiae*. Genetics. 2006; 173:557–567. [PubMed: 16582434]

O'Callaghan J, Caddick MX, Dobson AD. A polyketide synthase gene required for ochratoxin A biosynthesis in *Aspergillus ochraceus*. Microbiology. 2003; 149:3485–3491. [PubMed: 14663081]

Sanchez JF, Chiang YM, Szewczyk E, Davidson AD, Ahuja M, Oakley CE, Bok JW, Keller NP, Oakley BR, Wang CC. Molecular genetic analysis of the orsellinic acid/F9775 gene cluster of *Aspergillus nidulans*. Mol Biosyst. 2010; 6:587–593. [PubMed: 20174687]

Scherlach K, Hertweck C. Triggering cryptic natural product biosynthesis in microorganisms. Org Biomol Chem. 2009; 7:1753–1760. [PubMed: 19590766]

Schroeckh V, Scherlach K, Nutzmann HW, Shelest E, Schmidt-Heck W, Schuemann J, Martin K, Hertweck C, Brakhage AA. Intimate bacterial-fungal interaction triggers biosynthesis of archetypal polyketides in *Aspergillus nidulans*. Proc Natl Acad Sci USA. 2009; 106:14558–14563. [PubMed: 19666480]

Schumann J, Hertweck C. Advances in cloning, functional analysis and heterologous expression of fungal polyketide synthase genes. J Biotechnol. 2006; 124:690–703. [PubMed: 16716432]

Seshime Y, Juvvadi PR, Tokuoka M, Koyama Y, Kitamoto K, Ebizuka Y, Fujii I. Functional expression of the *Aspergillus flavus* PKS-NRPS hybrid CpaA involved in the biosynthesis of cyclopiazonic acid. Bioorg Med Chem Lett. 2009; 19:3288–3292. [PubMed: 19410456]

Sims RJ 3rd, Reinberg D. Histone H3 Lys 4 methylation: caught in a bind? Genes Dev. 2006; 20:2779–2786. [PubMed: 17043307]

Szewczyk E, Chiang YM, Oakley CE, Davidson AD, Wang CC, Oakley BR. Identification and characterization of the asperthecin gene cluster of *Aspergillus nidulans*. Appl Environ Microbiol. 2008; 74:7607–7612. [PubMed: 18978088]

Takano Y, Kubo Y, Shimizu K, Mise K, Okuno T, Furusawa I. Structural analysis of PKS1, a polyketide synthase gene involved in melanin biosynthesis in *Colletotrichum lagenarium*. Mol Gen Genet. 1995; 249:162–167. [PubMed: 7500937]

Tokuoka M, Seshime Y, Fujii I, Kitamoto K, Takahashi T, Koyama Y. Identification of a novel polyketide synthase-nonribosomal peptide synthetase (PKS-NRPS) gene required for the biosynthesis of cyclopiazonic acid in *Aspergillus oryzae*. Fungal Genet Biol. 2008; 45:1608–1615. [PubMed: 18854220]

Trail F, Mahanti N, Rarick M, Mehigh R, Liang SH, Zhou R, Linz JE. 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. 1995; 61:2665–2673. [PubMed: 7618880]

Tsai HF, Chang YC, Washburn RG, Wheeler MH, Kwon-Chung KJ. The developmentally regulated alb1 gene of *Aspergillus fumigatus*: its role in modulation of conidial morphology and virulence. J Bacteriol. 1998; 180:3031–3038. [PubMed: 9620950]

Tsai HF, Wheeler MH, Chang YC, Kwon-Chung KJ. A developmentally regulated gene cluster involved in conidial pigment biosynthesis in *Aspergillus fumigatus*. J Bacteriol. 1999; 181:6469–6477. [PubMed: 10515939]

Tsai HF, Fujii I, Watanabe A, Wheeler MH, Chang YC, Yasuoka Y, Ebizuka Y, Kwon-Chung KJ. Pentaketide melanin biosynthesis in *Aspergillus fumigatus* requires chain-length shortening of a heptaketide precursor. J Biol Chem. 2001; 276:29292–29298. [PubMed: 11350964]

Udwary DW, Merski M, Townsend CA. A method for prediction of the locations of linker regions within large multifunctional proteins, and application to a type I polyketide synthase. J Mol Biol. 2002; 323:585–598. [PubMed: 12381311]

Watanabe A, Ebizuka Y. Unprecedented mechanism of chain length determination in fungal aromatic polyketide synthases. Chem Biol. 2004; 11:1101–1106. [PubMed: 15324811]

Watanabe A, Ono Y, Fujii I, Sankawa U, Mayorga ME, Timberlake WE, Ebizuka Y. Product identification of polyketide synthase coded by *Aspergillus nidulans wA* gene. Tetrahedron Lett. 1998; 39:7733–7736.

Watanabe A, Fujii I, Sankawa U, Mayorga ME, Timberlake WE, Ebizuka Y. Re-identification of *Aspergillus nidulans wA* gene to code for a polyketide synthase of naphthopyrone. Tetrahedron Lett. 1999; 40:91–94.

Watanabe A, Fujii I, Tsai H, Chang YC, Kwon-Chung KJ, Ebizuka Y. *Aspergillus fumigatus alb1* encodes naphthopyrone synthase when expressed in *Aspergillus oryzae*. FEMS Microbiol Lett. 2000; 192:39–44. [PubMed: 11040426]

Watanabe CM, Townsend CA. Initial characterization of a type I fatty acid synthase and polyketide synthase multienzyme complex NorS in the biosynthesis of aflatoxin B_1. Chem Biol. 2002; 9:981–988. [PubMed: 12323372]

Xie X, Meehan MJ, Xu W, Dorrestein PC, Tang Y. Acyltransferase mediated polyketide release from a fungal megasynthase. J Am Chem Soc. 2009; 131:8388–8389. [PubMed: 19530726]

Yu JH, Leonard TJ. Sterigmatocystin biosynthesis in *Aspergillus nidulans* requires a novel type I polyketide synthase. J Bacteriol. 1995; 177:4792–4800. [PubMed: 7642507]

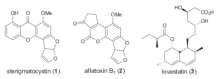

Fig. 1.
Three important secondary metabolites from *Aspergillus*

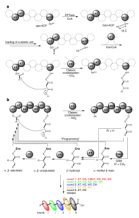

Fig. 2.
General reaction mechanisms catalyzed by iterative fungal PKSs. **a** Minimal fungal PKS containing KS, AT, and ACP domains. **b** Programming of CMeT, KR, DH, and ER domains in HR-PKS

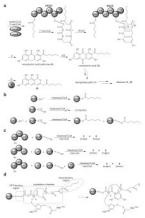

Fig. 3.

SAT and PT domains of NSAS in aflatoxin B_1 biosynthetic pathway. **a** Biosynthetic pathway of norsolorinic acid (**5**), sterigmatosystin (**1**), and aflatoxin B_1 (**2**) (pathway A); and naphthopyrone (**6**) formation in the absence of TE/CLC domain (pathway B). **b** In vitro hexanoyl transfer activity in the presence of SAT, ACP, or SAT and ACP monodomains. **c** Products detected after reconstituting different domain (s) of NSAS in vitro. **d** Proposed cyclization mechanism catalyzed by the NSAS PT domain

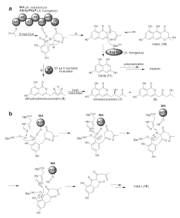

Fig. 4.

TE/CLC containing NR-PKSs involved in melanin biosynthesis. **a** Biosynthetic pathway of YWA1 (**10**), T4HN (**11**), and melanin (pathway A); and isocoumarin formation in the absence of TE/CLC domain (pathway B). **b** Proposed mechanism of Claisen cyclization by TE/CLC domain of WA

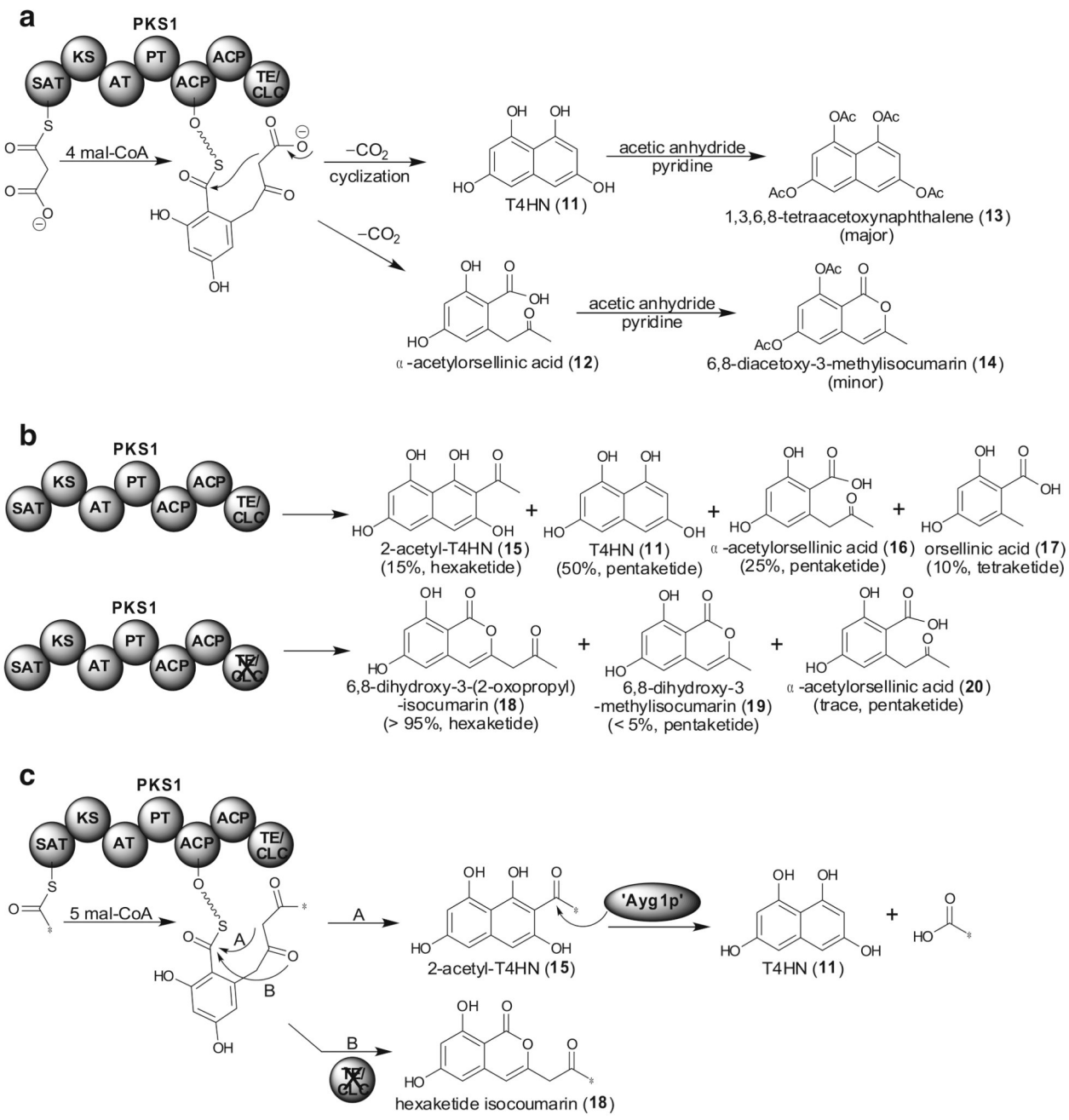

Fig. 5.

Two different proposed T4HN biosynthetic pathways catalyzed by PKS1. **a** Proposed biosynthetic pathway using mal-CoA as a starter unit and metabolites isolated from hetrologous expression of PKS1 in *A. oryzae* M-2-3. **b** Revisit the product distribution produced by wild type and TE/CLC less PKS1 by LC-ESI-MS. **c** Alternative proposed mechanism of T4HN (**11**) formation using acetyl-CoA as a starter unit. *: ^{14}C-labeled carbon form acetyl-CoA

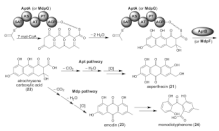

Fig. 6.
TE less NR-PKSs involved in asperthecin (**21**, Apt pathway) and monodictyphenone (**24**, Mdp pathway) biosynthesis

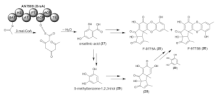

Fig. 7.
Proposed F9775 (**25** and **26**) and orsellinic acid (**27**) biosynthetic pathway catalyzed by TE containing NR-PKS, OrsA

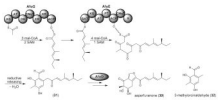

Fig. 8.
Biosynthetic pathway of asperfuraone (**30**) mediated by one HR-PKS (AfoG) and one NR-PKS (AfoE) that containing the reductase (R) releasing domain

Fig. 9.
Lovastatin (**3**) biosynthesis mediated by one iterative HR-PKS (LovB) and one noniterative HR-PKS (LovF)

Fig. 10.
Proposed functions of EasB (HR-PKS), EasC (AT), and EasA (NRPS) involved in the biosynthesis of emericellamides (**35–39**)

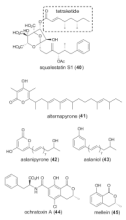

Fig. 11.
Chemical structures of compounds **40–45** synthesized by fungal HR-PKSs

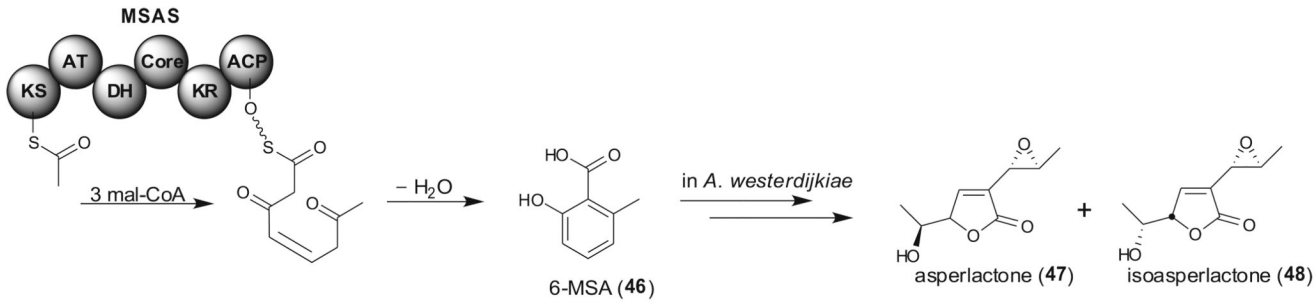

Fig. 12.
RP-PKS domain architecture of 6-MSA synthase (MSAS) and the connection of asperlactone (**47**) and isoasperlactone (**48**) biosynthesis in *A. westerdijkiae*

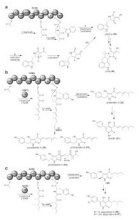

Fig. 13.

Fungal PKS-NRPSs containing the Dieckman cyclase (DKC) releasing domain. **a** Two possible product release mechanisms in cAATrp (**50**) formation in the CPA (**49**) biosynthetic pathway. Dieckman cyclization/releasing (pathway A) and reductive releasing/cyclization/reoxidation (pathway B). **b** Major compounds isolated from *A. oryzae* M-2-3 transformed to express *tenS* (**52–54**) or to co-express *tenS* and *orf3* (**55**). **c** Proposed biosynthesis and production of aspyridones (**56** and **57**) after activation of the regulator in *apd* gene cluster

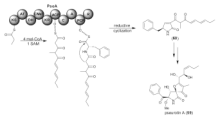

Fig. 14.
Proposed function of PsoA, a PKS-NRPS containing the reductase (R) releasing domain, involved in pseurotin A (**49**) biosynthesis Appl Microbiol Biotechnol

Table 1

Characterized multi-domain type I PKSs from *Aspergillus*

Origin	Gene	Protein	No. of aa[d]	Secondary metabolite	Domain architecture[e]	Ref.
NR-PKS						
A. parasiticus	*pksA*	PksA (NSAS)	2109	Aflatoxin B_1 (**2**)	SAT-KS-AT-PT-ACP-TE/CLC	(Chang et al. 1995; Trail et al. 1995)
A. parasiticus	*pksL1*	PKSL1 (NSAS)	2109	Aflatoxin B_1 (**2**)	SAT-KS-AT-PT-ACP-TE/CLC	(Feng and Leonard 1995)
A. nidulans	*pksST*, *stcA*	StcA (NSAS)	2181	Sterigmatocystin (**1**)	SAT-KS-AT-PT-ACP-ACP-TE/CLC	(Brown et al. 1996; Yu and Leonard 1995)
A. nidulans	*wA*[b]	WA[b]	1986	Isocoumarins (**7–9**)	SAT-KS-AT-PT-ACP-ACP	(Mayorga and Timberlake 1992; Watanabe et al. 1998)
A. nidulans	*wA*	WA	2157	YWA1 (**10**)	SAT-KS-AT-PT-ACP-ACP-TE/CLC	(Watanabe et al. 1999)
A. fumigatus	*alb1*	Alb1p	2146	YWA1 (**10**)	SAT-KS-AT-PT-ACP-ACP-TE/CLC	(Tsai et al. 1998, 1999; Watanabe et al. 2000)
A. fumigatus	*pksP*	PksP	2146	YWA1 (**10**)	SAT-KS-AT-PT-ACP-ACP-TE/CLC	(Langfelder et al. 1998)
C. lagenarium[a]	*PKS1*	PKS1	2187	T4HN (**11**)	SAT-KS-AT-PT-ACP-ACP-TE/CLC	(Fujii et al. 1999, 2000; Takano et al. 1995)
A. nidulans	*aptA*	AptA	1792	Asperthecin (**21**)	SAT-KS-AT-PT-ACP	(Szewczyk et al. 2008)
A. nidulans	*mdpG*	MdpG	1806	Monodictyphenone (**24**)	SAT-KS-AT-PT-ACP	(Bok et al. 2009)
A. nidulans	*orsA*	OrsA	2103	Orsellinic acid (**27**) and F9775 (**25** and **26**)	SAT-KS-AT-PT-ACP-ACP-TE	(Bok et al. 2009; Sanchez et al. 2010; Schroeckh et al. 2009)
A. nidulans	*afoE*	AfoE	2793	Asperfuranone (**30**)	SAT-KS-AT-PT-ACP-CMeT-R	(Chiang et al. 2009b)
Acremonium strictum[a]	*ASpks1*	MOS	2729	3-Methylorcinaldehyde (**32**)	SAT-KS-AT-PT-ACP-CMeT-R	(Bailey et al. 2007)
HR-PKS						
A. terreus	*lovB*	LovB	3038	Lovastatin (**3**)	KS-AT-DH-CMeT-(ER)-KR-ACP-C	(Hendrickson et al. 1999; Kennedy et al. 1999)
A. terreus	*lovF*	LovF	2532	Lovastatin (**3**)	KS-AT-DH-CMeT-ER-KR-ACP	(Kennedy et al. 1999)
A. nidulans	*easB*	EasB	2534	Emericellamides (**35–39**)	KS-AT-DH-CMeT-ER-KR-ACP	(Chiang et al. 2008)
A. nidulans	*afoG*	AfoG	2527	Asperfuranone (**30**)	KS-AT-DH-CMeT-ER-KR-ACP	(Chiang et al. 2009b)
Phoma sp. C2932[a]	*PhPKS1*	SQTKS	2714	Squalestatin tetraketide (**40**)	KS-AT-DH-CMeT-ER-KR-ACP	(Cox et al. 2004)
Alternaria solani[a]	*alt5*	PKSN	2551	Alternapyrone (**41**)	KS-AT-DH-CMeT-ER-KR-ACP	(Fujii et al. 2005)
Alternaria solani[a]	*pksF*	PKSF	2260	Aslanipyrone (**42**) and aslaniol (**43**)	KS-AT-DH-ER-KR-ACP	(Kasahara et al. 2006)

Origin	Gene	Protein	No. of aa[d]	Secondary metabolite	Domain architecture[e]	Ref.
A. ochraceus	*pks*[c]	PKS[c]	502	Ochratoxin A (**44**)		(O'Callaghan et al. 2003)
A. westerdijkiae	*aoks1*[c]	AoKS1[c]	654	Ochratoxin A (**44**)		(Bacha et al. 2009a)
PR-PKS						
A. terreus	*atX*	MSAS	1803	6-MSA (**46**)	KS-AT-DH-Core-KR-ACP	(Fujii et al. 1996)
A. westerdijkiae	*aomsas*	MSAS	1766	6-MSA (**46**), asperlactone (**47**) and isoasperlactone (**48**)	KS-AT-DH-Core-KR-ACP	(Bacha et al. 2009b)
PKS-NRPS						
A. oryzae	*cpaA*	CpaA	3907	CPA (**49**)	KS-AT-(DH)-(CMeT)-(ER)-(KR)-ACP-C-A-PCP-DKC	(Tokuoka et al. 2008)
A. flavus	*cpaA*	CpaA	3906	CPA (**49**)	KS-AT-(DH)-(CMeT)-(ER)-(KR)-ACP-C-A-PCP-DKC	(Chang et al. 2009; Seshime et al. 2009)
Beauveria bassiana[a]	*tenS*	TENS	4239	Tenellin (**51**)	KS-AT-DH-CMeT-(ER)-KR-ACP-C-A-PCP-DKC	(Eley et al. 2007; Halo et al. 2008)
A. nidulans	*apdA*	ApdA	3930	Aspyridones (**56** and **57**)	KS-AT-DH-CMeT-(ER)-KR-ACP-C-A-PCP-DKC	(Bergmann et al. 2007)
A. fumigatus	*psoA*	PsoA	3991	Pseurotin A (**59**)	KS-AT-DH-CMeT-KR-ACP-C-A-PCP-R	(Maiya et al. 2007)

[a] Identified by heterologous expression in *A. oryzae* M-2-3

[b] 67 aa C-terminal truncated WA

[c] Partial DNA sequences

[d] Predicted no. of aa if studied from genome mining

[e] Domains in parenthesis are non-functional