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Type I Polyketide Synthases That Require Discrete Acyltransferases

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Abstract

The diverse structures of polyketide natural products are reflected by the equally diverse polyketide biosynthetic enzymes, namely polyketide synthases (PKSs). Three major classes of PKSs are known—noniterative type I PKSs, iterative type II PKSs and acyl carrier protein-independent type III PKSs, each of which consists of additional variants. One such variant is the noniterative type I PKS in which each PKS module lacks the cognate acyltransferase (AT) domain. The essential AT activity is instead provided by a discrete AT in *trans*. Termed “AT-less” type I PKSs, the loading of the malonate extender units by the discrete AT enzyme LnmG to each of the AT-less PKS modules of LnmI and LnmJ was confirmed experimentally for biosynthesis of the anticancer antibiotic leinamycin (LNM). The LNM PKS has since served as a model for the continuous discovery of numerous additional AT-less type I PKSs incorporating variable extender units. However, biochemical characterization of AT-less type I PKSs remains very limited, and the mechanism by which AT-less type I PKSs accommodate multiple extender units is unknown. This chapter provides the protocols used to establish and characterize the LNM PKS. Application of these methods to other AT-less type I PKSs should aid the biochemical characterization and hence possible exploitation of these unique PKSs for polyketide natural product structural diversity by combinatorial biosynthetic methods.

1. Introduction

Polyketides constitute one of the largest families of natural products and many are biosynthesized by noniterative type I polyketide synthases (PKSs). Type I PKSs are multifunctional enzymes organized into modules, each of which harbors a set of distinct domains responsible for the catalysis of one cycle of polyketide chain elongation. Prototypically, a type I PKS elongation module contains minimally three domains—an acyltransferase (AT), an acyl carrier protein (ACP) and a β -ketoacyl synthase (KS)—that select, activate, and catalyze a decarboxylative Claisen condensation between the extender unit and the growing polyketide chain, generating a β -ketoacyl-S-ACP intermediate.

Optional domains are found between the AT and ACP domains, which carry out the variable set of reductive modifications of the β -keto group before the ensuing cycle of chain extension (Shen, 2003; Staunton and Weissman, 2001). For a prototypic type I PKS, as exemplified by the 6-deoxyerythromycin B synthase (DEBS) for biosynthesis of the aglycone of the polyketide antibacterial antibiotic erythromycin A (Donadio and Katz, 1992), an AT domain is an integral part of every PKS module, and each AT domain functions only once, for the module in which it resides. Integral AT domains in such prototypic type I PKSs were thus termed cognate ATs (Cheng *et al.*, 2003) (Fig. 8.1A).

A distinct variant of the noniterative type I PKSs contains no cognate ATs but has a short segment of remnant AT sequence in some or all modules, depending on the pathway. This subclass of type I PKSs was named the AT-less type I PKSs and the remnant AT segment the AT docking domain (Fig. 8.1B and C). The essential AT activities are provided in *trans* by discrete AT enzymes encoded by genes that are physically separated from the PKS genes. Therefore AT-less type I PKSs require discrete ATs acting in *trans* to select the extender unit and load it onto the ACP domain (Cheng *et al.*, 2003) (Figs. 8.1C and 8.2).

The AT-less class of type I PKSs was first discovered during genetic and biochemical studies of leinamycin (LNM) biosynthesis in *Streptomyces atroolivaceus* S-140 (Cheng *et al.*, 2002; Cheng *et al.*, 2003; Tang *et al.*, 2004). LNM is a hybrid peptide-polyketide natural product that shows potent antitumor activity, most significantly against tumor cell lines that are resistant to clinically important anticancer drugs (Hara *et al.*, 1989a,b). The *lnm* biosynthetic gene cluster consists of multiple genes encoding modular nonribosomal peptide synthetases (NRPSs), type I PKSs, and other components. The most striking feature of LNM PKSs is the lack of cognate AT domains in all six modules of the LnmI and LnmJ PKS proteins; instead, only a segment of remnant AT sequence was found in five of the six PKS modules after each KS domain (Figs. 8.1C and 8.2). Bioinformatic and genetic analyses identified *lnmG* as an essential AT-encoding gene, and biochemical studies subsequently confirmed LnmG as a discrete AT enzyme capable of loading the malonyl group from the extender unit substrate malonyl CoA onto the ACP domains in all six modules of the LnmI and LnmJ PKSs. On the basis of these findings, LnmI and LnmJ were hence proposed as the first AT-less type I PKSs, LnmG as the required *trans*-acting, discrete AT enzyme, and the remnant AT segments as AT docking domains (Figs. 8.1 and 8.2). Numerous additional AT-less type I PKSs have since been discovered (Fig. 8.3 and Table 8.1). However, unequivocal biochemical evidence validating this emerging subclass of AT-less type I PKSs remains scarce.

Noniterative type I PKSs use malonyl CoA, methylmalonyl CoA, ethylmalonyl CoA, methoxymalonyl ACP, and in rare cases, hydroxymalonyl ACP or aminomalonyl ACP, as extender units in the biosynthesis of diverse polyketide natural products (Chan *et al.*, 2006; Khosla *et al.*, 1999). (See also Chapter 7 of this volume.) Biochemical studies (for LnmG) and substrate specificity predictions (for all others) suggest that most, if not all, discrete ATs known for AT-less type I PKSs use malonyl CoA as a substrate (Table 8.1 and Fig. 8.4). Possible exceptions to this finding include EtnB from the etnangien biosynthetic pathway (Menche *et al.*, 2008) and KirCII from the kirromycin biosynthetic pathway (Weber *et al.*, 2008), which appear to use succinyl CoA and ethylmalonyl CoA, respectively. An AT-less

type I PKS that incorporates methoxymalonyl ACP as an extender unit has also been proposed, for the biosynthesis of oxazolomycin, the predicted discrete AT (OzmC) for which, however, shows little sequence homology to typical ATs known for PKSs. With the exception of LnmG, the actual substrate specificities for all discrete ATs identified for AT-less type I PKSs to date remain to be experimentally proven.

This chapter describes protocols for biochemical characterization of AT-less type I PKSs, as previously applied to establish and characterize the LNM PKS. They include (1) heterologous expression and overproduction of ACPs from AT-less type I PKS modules; (2) *in vitro* preparation of holo-ACPs; (3) heterologous expression and overproduction of discrete ATs; (4) *in vitro* assay of AT substrate specificity; and (5) *in vitro* assay of AT-catalyzed loading of extender unit substrate onto holo-ACPs. The biochemical reactions examined are depicted in Fig. 8.5.

2. Methods

2.1. Heterologous expression and overproduction of apo-ACPs from AT-less PKS modules

1. Conduct sequence analysis (e.g., by ClustalW program [Thompson *et al.*, 2002]) to determine the boundaries of individual ACP domains from AT-less type I PKS modules (Donadio and Katz, 1992).
2. Choose a host/vector system for heterologous gene expression [e.g., *Escherichia coli* BL21(DE3)/pET28a or pET37b from Novagen for overproduction of the gene product as His₆-tagged fusion protein] and a subsequent protein purification system (e.g., by affinity chromatography on Ni-NTA resin from Qiagen).
3. Design and synthesize PCR primers for the amplification of DNA encoding the target ACP domains. Restriction sites (e.g., CATATG for *Nde*I and AAGCTT for *Hind*III) are routinely added to the primers to facilitate directional cloning.
4. Amplify the DNA by PCR with a high-fidelity DNA polymerase (e.g., Pfu Turbo DNA polymerase from Stratagene), clone the amplicon into pET28a or pET37b, and sequence the construct to verify DNA amplification fidelity.
5. Introduce individual expression constructs into host strain *E. coli* BL21 (DE3) cells by transformation or electroporation. Select for transformants on LB agar supplemented with 25 mg/ml of kanamycin.
6. Optimize culture conditions on a small scale for overproduction of soluble ACPs by following the manufacturer's recommendations (e.g., the QIAexpressionist from Qiagen).
7. Scale up the culture volume for protein overproduction and proceed to purify sufficient amounts of individual ACPs for assays. We routinely grow 1 to 4 l of culture at 18 to 25° for 0.5 to 2 days and add 1 to 100 μ M (final concentration) isopropyl β -D-1-thiogalactopyranoside (IPTG) to induce gene expression. We also routinely employ lysozyme and sonication to lyse cells, and use gravity Ni-NTA affinity chromatography and subsequently anion exchange FPLC (e.g., the ÄKTA system from Amersham Pharmacia) to obtain pure ACPs.

8. Examine protein fractions by SDS-PAGE and pool together the fractions with at least 95% purity.
9. Dialyze pooled protein samples against 25 mM Tris-HCl, pH 8.0, 25 mM NaCl, 2 mM DTT and 10% glycerol.
10. Re-examine the quality of the purified ACPs by SDS-PAGE (Fig. 8.6A), and determine their concentrations by Bradford assay (Bradford, 1976).
11. Aliquot (e.g., 500 μ l per 1.5-ml tube) and store the ACP samples at -80° until use.

2.2. *In vitro* preparation of holo-ACPs

PKS ACPs overproduced in *E. coli* under the conditions described above are generally in their nonphosphopantetheinylated apo-form. Apo-ACPs need to be converted into their functional holo-forms by attachment of the 4'-phosphopantetheine group from CoA to the active-site Ser residue. This post-translational modification of apo-ACPs to holo-ACPs can be carried out *in vitro* with promiscuous 4-phosphopantetheinyl transferases (PPTases) such as Svp (Fig. 8.5) and followed by HPLC, MS, or autoradiography analysis (Lambalot *et al.*, 1996; Sanchez *et al.*, 2001).

2.2.1. *In vitro* preparation of holo-ACPs from apo-ACPs

1. Set up an analytical *in vitro* phosphopantetheinylation reaction in 100- μ l volume as follows: (1) 25 μ l of 4 X reaction buffer (400 mM Tris · HCl, pH 7.5, 50 mM $MgCl_2$, 10 mM DTT); (2) 10 μ l of 2.5 mM CoA stock (final concentration 250 μ M) (use 3H -labeled CoA for autoradiography analysis); (3) 10 μ l of apo-ACP (final concentration 10 μ M); (4) 2 μ l of Svp (final concentration 2 μ M); and (5) add H_2O to a total reaction volume of 100 μ l.
2. Incubate the reaction at 25° for 30 to 60 min.
3. Add 900 μ l of acetone to quench the reaction, and mix briefly by vortexing.
4. Freeze sample tube at -80° for at least 1 h to precipitate proteins, and pellet the proteins by centrifugation at 14,000 RPM for 20 min at 4° .
5. Decant supernatant, and dry pellet briefly in the air with the cap open.
6. Redissolve pellet in 30 μ l of 25 μ M Tris-HCl, pH 7.5, 25 μ M NaCl, 2 mM DTT and 10% glycerol.
7. Separate apo- and holo-ACPs from the reactions by HPLC with a Jupiter C-18 column (5 μ M, 300Å, 250 $\times$ 4.6 mm, Phenomenex), and a gradient elution from 85% buffer A (H_2O + 0.1% formic acid) to 90% buffer B (acetonitrile + 0.1% formic acid) in 25 min at a flow rate of 1 ml/min, with UV detection at 220 nm (Fig. 8.6B). Individual protein peaks are collected, lyophilized, and redissolved in 50 μ l of H_2O .
8. Determine the apo- and holo-ACPs by ESI-MS analysis in positive ionization mode on an Agilent 1000 HPLC-MSD SL instrument under standard operational conditions (Table 8.2).

9. Scale up the reaction volume three- to five-fold for preparative reactions, and aliquot and store the holo-ACP samples from Step 6 at -80° until use.

2.3. Heterologous expression and overproduction of discrete ATs

1. The majority of genes encoding discrete ATs for AT-less type I PKSs encode a single AT domain, but a few are predicted to encode tandem AT domains (see Table 8.1). For those that encode a single AT domain, use the entire open reading frame for heterologous expression. For those that encode tandem AT domains, conduct sequence analysis [e.g., by ClustalW program (Thompson *et al.*, 2002)] to determine the boundaries of individual AT domains for heterologous expression, taking as much advantage as possible of their natural N- or C-terminus (Donadio and Katz, 1992).
2. Follow Steps 2 through 8 as described in Section 2.1, but replace the target genes with those encoding discrete ATs.
3. Dialyze pooled protein samples against 25 μ M Tris-HCl, pH 7.0, 25 μ M NaCl, 2 μ M DTT and 10% glycerol.
4. Re-examine the quality of the purified ATs by SDS-PAGE (Fig. 8.6C), and determine their concentrations by Bradford assay (Bradford, 1976).
5. Aliquot (e.g., 500 μ l per 1.5-ml tube) and store the AT samples at -80° until use.

2.4. *In vitro* assay for AT substrate specificity

The protocol for a quasi-kinetic assay of AT substrate specificity was modified from previously published procedures (Liou *et al.*, 2003; Szafranska *et al.*, 2002). Acyl CoAs (use 14 C-labeled acyl CoAs only autoradiography analysis) used include malonyl CoA, methylmalonyl CoA, butyryl CoA, propionyl CoA, and acetyl CoA.

1. Set up a typical *in vitro* AT self-acylation reaction in a 200- μ l volume for each acyl CoA as follows: (1) 100 μ l of 2 X reaction buffer (100 μ M phosphate buffer, pH 7.0, 0.4 μ M DTT, 0.2 μ M EDTA); (2) 10 μ l of acyl CoA and (final acyl CoA concentration 20 μ M and $\sim 8 \times 10^8$ DPM if 14 C-labeled acyl CoA is included); (3) 5 μ l of LnmG (final concentration 2 μ M and add in Step 3 to initiate reaction); and (4) add H₂O to a total reaction volume of 200 μ l
2. Equilibrate all reagents on ice (0°) for 15 min or longer, and perform assay on ice to slow down the reaction.
3. Initiate reaction by adding LnmG and quickly mix by pipetting up and down several times.
4. Transfer 25 μ l of reaction mixture to a tube containing 50 μ l of ice-cold 10% TCA at each of the seven time points: 0.5, 1, 2, 4, 8, 16 and 32 min.
5. Add 25 μ l of 10-mg/ml BSA to each tube as a carrier protein and allow proteins to precipitate on ice for 15 min.
6. Pellet proteins by centrifugation at 14,000 RPM for 20 min at 4° .

7. Wash protein pellet twice with 100 μ l of 10% ice-cold TCA.
8. Solubilize the pellet in 100 μ l of 10 μ M NaCl, 2% SDS solution at ambient temperature.
9. Mix with 5 μ l of scintillation cocktail and count radioactivity in a scintillation counter (Beckman Coulter).
10. Plot the radioactivity counts as a function of time for each acyl CoA. Use the count of a 25- μ l fraction directly from the original reaction (Step 4) as 100% DPM value. Normalize the 100% DPM values of individual acyl CoAs to that of malonyl CoA (Fig. 8.7A).
11. Similarly, assays can be performed with a fixed amount of AT enzyme (2 μ M of LnmG) and variable concentrations of acyl CoA in 25- μ l reaction for 2 min on ice (Fig. 8.7B).

2.5. *In vitro* assay of AT-catalyzed loading of acyl CoA extender substrate to holo-ACPs

This assay can be carried out in two completely separate steps (continuing from the holo-ACPs prepared from Step 9, Section 2.2) or in two consecutive steps in a single tube (continuing from the holo-ACPs prepared from Step 2, Section 2.2). The protocol described below was modified from Lambalot *et al.* (1996) and Sanchez *et al.* (2001) and was developed for a typical analytical reaction. The loading of the acyl group from the acyl CoA substrate (malonyl CoA in the current example) onto holo-ACP can be followed by HPLC, MS or autoradiography analysis. Only the protocol for assay in a single tube is provided, which could be readily adapted for assay in two separate steps. For preparative reactions, scale up reaction volumes three- to five-fold.

- 1 Add to the reaction tube from the Step 2 of Section 2.2 a 25- μ l solution containing 2 μ M LnmG and 200 μ M [2- 14 C] malonyl CoA ($\sim 5 \times 10^8$ DPM).
- 2a For assaying one ACP in a time-course (Fig. 8.8A), incubate the reactions at 25° and quench the reactions by adding 900 μ l of acetone at various time points (1, 2, 4, 8, 16, 32, 64, and 128 min). Mix briefly by vortexing.
- 2b For assaying multiple ACPs at a fixed time point (Fig. 8.8B), incubate the reactions at 25° and quench by adding 900 μ l of acetone at a fixed time point (2 min). Mix briefly by vortexing.
- 3 Freeze samples at -80° for at least 1 h to precipitate proteins and pellet the proteins by centrifugation at 14,000 RPM for 30 min at 4°.
- 4 Decant supernatant, and dry pellet briefly in the air with the cap open.
- 5 Follow Steps 6 to 8 of Section 2.2 to analyze the resultant acyl-S-ACPs by HPLC (Fig. 8.6B) and ESI-MS (Table 8.2).
- 6 Alternatively, redissolve protein pellets in 25 μ l of 1 $\times$ sampler buffer.
- 7 Analyze protein samples on a 4 to 15% SDS-PAGE gel (Bio-Rad), visualize proteins by Coomassie blue staining and record the gel image (Fig. 8.8).

- 8 Air dry the gel and expose it to a low-energy (LE) phosphor screen (Amersham Pharmacia) at ambient temperature for 3 to 7 days to acquire autoradiography with a phosphorimager (Molecular Dynamics) (Fig. 8.8).

3. Conclusion

Bioinformatics analysis of gene clusters continues to unveil candidates encoding AT-less type I PKSs for natural product biosynthesis (Fig. 8.3 and Table 8.1). The LNM PKS, however, remains the only AT-less type I PKS that has been confirmed experimentally, with LnmG acting in *trans* as a discrete AT responsible for loading the extender substrate malonyl CoA to all six modules of the LnmI and LnmJ PKS proteins (Cheng *et al.*, 2003). A significant knowledge gap exists between the predicted and experimentally validated involvement of AT-less type I PKSs in natural product biosynthesis, yet such information is absolutely required to realize the full potential of this subclass of novel PKSs in combinatorial biosynthesis for natural product structural diversity. It is hoped that methods devised for studies of the LNM PKS will be applicable to other AT-less type I PKSs, thereby aiding their biochemical and mechanistic characterization. Although gene expression in heterologous hosts such as *E. coli* is intrinsically unpredictable, overproduction of functional ACPs and discrete ATs generally does not constitute an insurmountable problem. ACPs overproduced in *E. coli* are often in the apo-form, but conversion to the functional holo-form is readily achievable thanks to the availability of promiscuous PPTases such as Svp (Sanchez *et al.*, 2001) and Sfp (Lambalot *et al.*, 1996). While conceptually straightforward, care must be taken in executing assays for the substrate specificity of discrete ATs, since AT self-acylation reaction is often rapid and followed by a gradual spontaneous hydrolysis, and assays for discrete AT-catalyzed loading of extender substrates to holo-ACPs, because the general protocol often requires specific optimization, and acylated ACPs also undergo slow hydrolysis under the assay conditions (Fig. 8.5).

AT-less type I PKSs provide tremendous opportunities for PKS engineering. For instance, given that LnmG is responsible for the loading of the malonyl CoA extender unit to all six modules of the LnmI and LnmJ PKS, it was reasoned that LnmG could be a rate-limiting factor for LNM biosynthesis. Subsequent overexpression of *lnmG* under a strong promoter in the LNM-producing-strain *S. atroolivaceus* S-140 indeed resulted in improved LNM titers by three- to five-fold (Cheng *et al.*, 2003; Tang *et al.*, 2004). It is also very tempting to envision the application of *lnmG* and other discrete AT-encoding genes to either prototypical type I PKSs or AT-less type I PKSs to alter the extender-unit specificity by combinatorial methods, thereby further expanding the size and diversity of polyketide natural products.

Finally, it is exciting to consider how these established methods designed with LNM in mind will also be applicable to decipher the mechanism by which AT-less type I PKSs accommodate multiple extender units. Take for instance the recently discovered discrete ATs from the etnangien biosynthetic pathway (EtnB) (Menche *et al.*, 2008), the kirromycin biosynthetic pathway (KirCII) (Weber *et al.*, 2008), and the oxazolomycin biosynthetic pathway (OzmC) (Zhao *et al.*, 2009), whose predicted substrate specificities are for succinyl CoA, ethylmalonyl CoA, and methoxymalonyl ACP, respectively, in addition to other

dedicated discrete ATs for malonyl CoA in the same pathway. If experimentally proven, EtnB, KirCII, and OzmC, together with their corresponding AT-less PKS machinery, would provide additional opportunities to introduce uncommon extender units for engineering novel natural products by combinatorial biosynthesis methods.

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Figure 8.1.

Modular organization of type I polyketide synthases (PKSs). (A) A prototypical type I PKS module contains the cognate AT domain and other domains. (B) An AT-less type I PKS module lacks the cognate AT domain but often contains a short segment of remnant AT residues. (C) The remnant AT segment lacks critical catalytic motifs (GHSxG and AFHS) and is therefore catalytically inactive, but may serve as a docking domain mediating the interactions between the discrete AT (LnmG) and AT-less PKS modules in LnmI and LnmJ (Tang *et al.*, 2004).

Figure 8.2.

A model for LNM biosynthesis featuring the LNM hybrid NRPS-PKS megasynthetase with the discrete ATenzyme LnmG that loads the malonyl CoA extender units onto all six modules on the LnmI and LnmJ PKSs (Cheng *et al.*, 2003; Tang *et al.*, 2004).

Figure 8.3.

Natural products whose biosynthetic pathways are predicted to feature AT-less type I PKSs.

Figure 8.4.

Phylogenetic analysis of cognate and discrete acyltransferases (ATs), highlighting substrate specificities. Phylogenetic analysis was performed by the neighbor-joining method (Bruno *et al.*, 2000). DiscreteATs form a loose but distinctive clade distant from the cognate ATs of prototypical PKSs. For discrete ATs, see Table 8.1 for references. For cognate ATs, NCBI accession numbers are given in parentheses.

Figure 8.5.

Scheme depicting the reactions assayed by the protocols provided. (A) Self-acylation of a discrete AT at the active site Ser residue with the extender substrate malonyl CoA. (B) Conversion of apo-ACP to holo-ACP by attaching the 4'-phosphopantetheine group from CoA to the active site Ser residue by PPTases such as Svp. (C) Transfer of activated substrate from acylated AT to the SH-group of holo-ACP. (D) Spontaneous hydrolysis of acylated AT or acylated ACP.

Figure 8.6.

(A) Purified LnmI and LnmJACPs on a 4 to 15% SDS-PAGE: lane 1, molecular weight standards; lane 2, ACP3; lane 3, ACP4; lane 4, ACP5; lane 5, ACP6-1; lane 6, ACP6-2; lane 7, ACP7; lane 8, ACP8. The numbers after the ACPs refer to the PKS modules from which they are derived, with 6-1 and 6-2 to indicate the first and second ACPs, respectively, for PKS module 6. (B) HPLC analysis of carrier protein modification and LnmG-catalyzed loading of the extender substrate onto holo-ACP, as exemplified by ACP3: (I) apo-ACP3 (●); (II) Svp-catalyzed conversion of apo-ACP to holo-ACPs (◆); and (III) LnmG-catalyzed loading of malonyl CoA onto holo-ACP, showing holo-ACP3 (◆) converted to malonyl-S-ACP3 (▼). (C) Purified discrete AT (LnmG) on a 4 to 15% SDS-PAGE: lane 1, molecular weight standards; lane 2, LnmG.

Figure 8.7.

Determination of malonyl CoA as the preferred substrate of the discrete AT (LnmG): (A) time-course assays of AT self-acylation with malonyl CoA (•), methylmalonyl CoA (◆), butyryl CoA (▲), propionyl CoA (★), or acetyl CoA (○); and (B) assays with a fixed amount of AT enzyme (2 μM of LnmG) and variable concentrations of malonyl CoA (•) or methylmalonyl CoA (◆).

Figure 8.8.

In vitro assays of LnmG-catalyzed loading of malonyl CoA onto holo-ACPs of the LnmI and LnmJ PKSs: (A) Complete assay of individual ACPs in reactions with Svp, CoA, LnmG and [2-¹⁴C]malonyl CoA, as visualized on a 4 to 15% SDS-PAGE (I) and by phosphorimaging (II) (lane 1, molecular mass standards and lanes 2 to 8, ACP3 to ACP8); and (B) time course of LnmG-catalyzed loading of [2-¹⁴C]malonyl CoA onto holo-ACP3, as visualized on a 4 to 15% SDS-PAGE (I) and by phosphorimaging (II).

Table 8.1

A compilation of natural products biosynthesized by AT-less type I PKSs, predicted enzyme properties, and respective producing organisms

Compound (Reference)	PKS module	Discrete AT protein	Substrate specificity ^a	Gene cluster status	Organism
Albicidin (Huang <i>et al.</i> , 2001; Royer <i>et al.</i> , 2004)	2 PKS modules on AlbI (Xabb)	1 AT on AlbXIII	Malonyl CoA	Cluster completed and confirmed	<i>Xanthomonas albilineans</i>
Bacillaene (Butcher <i>et al.</i> , 2007; Chen <i>et al.</i> , 2006; Kunst <i>et al.</i> , 1997; Straight <i>et al.</i> , 2007)	13 PKS modules on PksJLMNR (also known as BaeJLMNR)	1 AT on PksC, 1 AT on PksD, and 1 AT on PksE (also known as BaeCDE)	Malonyl CoA	Cluster completed and confirmed	<i>Bacillus subtilis</i> (B. <i>amyloliquefaciens</i> FZB 42)
Bryostatins (Hildebrand <i>et al.</i> , 2004; Sudek <i>et al.</i> , 2007)	13 PKS modules (1 nonfunctional) on BryABCD	2 ATs on BryP	Malonyl CoA	Putative cluster complete	" <i>Candidatus</i> Endobugula sertula" (bacterial symbiont from bryozoan <i>Bugula neritina</i>)
Chivosazol (Perlova <i>et al.</i> , 2006)	15 PKS modules on ChiBCDEF	1 AT on ChiA	Malonyl CoA	Cluster completed and confirmed	<i>Sorangium cellulosum</i> So ce56
Difficidin (Chen <i>et al.</i> , 2006)	13 PKS modules on DifFGHIJKL	1 AT on DifA	Malonyl CoA	Cluster completed and confirmed	<i>Bacillus amyloliquefaciens</i> FZB 42
Disorazol (Carvalho <i>et al.</i> , 2005; Kopp <i>et al.</i> , 2005)	10 PKS modules (3 nonfunctional) on DisABC (also known as DszABC)	1 AT on DisD (also known as DszD)	Malonyl CoA	Cluster completed and confirmed	<i>Sorangium cellulosum</i> So ce12
Emangien (Menche <i>et al.</i> , 2008)	20 PKS modules on EmDEFGHI	1 AT on EtmB and 2 ATs on EtmK	Malonyl CoA Succinyl CoA	Cluster completed and confirmed	<i>Sorangium cellulosum</i> So ce1045
FK228 (depsipeptide) (Cheng <i>et al.</i> , 2007)	2 PKS modules on DepBC	No discrete AT identified	Malonyl CoA	Cluster confirmed but possibly incomplete	<i>Chromobacterium violaceum</i> No. 968
Iso-Migrastatin (Farnet <i>et al.</i> , 2002)	10 PKS modules on MgsEFG	2 ATs on MgsB and 1 AT on MgsH	Malonyl CoA	Cluster completed and confirmed	<i>Streptomyces plantensis</i>
Kirromycin (Weber <i>et al.</i> , 2008)	14 PKS modules on KirAI-AVI with 2 AT domains on KirAVI	2 ATs on KirCI and 1 AT on KirCII	Malonyl CoA Ethylmalonyl CoA	Cluster completed and confirmed	<i>Streptomyces collinus</i> Tü 365
Lactimidomycin (Farnet <i>et al.</i> , 2002)	10 PKS modules on LtmEFG	2 ATs on LtmB and 1 AT on LtmH	Malonyl CoA	Cluster completed and confirmed	<i>Streptomyces amphibiosporus</i>
Lankacidin (Arakawa <i>et al.</i> , 2005; Mochizuki <i>et al.</i> , 2003)	5 PKS modules; on LkcACFG	1 AT on LkcD	Malonyl CoA	Cluster completed and confirmed	<i>Streptomyces rochei</i> 7434AN4
Leinamycin (Cheng <i>et al.</i> , 2002, 2003; Tang <i>et al.</i> , 2004, 2006)	6 PKS modules on LnmIJ	1 AT on LnmG	Malonyl CoA	Cluster completed and confirmed	<i>Streptomyces atroolivaceus</i> S-140
Macrolactin (polyketide 2) (Chen <i>et al.</i> , 2006; Schneider <i>et al.</i> , 2007)	11 PKS modules on MinBCDEFGH	1 AT on MinA	Malonyl CoA	Cluster completed and confirmed	<i>Bacillus amyloliquefaciens</i> FZB 42
Mupirocin (pseudomonic acidA) (El-Sayed <i>et al.</i> , 2003)	8 PKS modules on MmpABD	2 ATs on MmpC (deposited as MmpIII)	Malonyl CoA	Cluster completed and confirmed	<i>Pseudomonas fluorescens</i> NCIMB 10586
Myxovirescin (antibiotic TA) (Paitan <i>et al.</i> , 1999, 2001; Simunovic <i>et al.</i> , 2006)	15 PKS modules (2 inactive) on TaiL, Tai-1 and TaiOP	2 ATs on TaiV	Malonyl CoA	Cluster completed and confirmed	<i>Myxococcus xanthus</i> DK1622

Compound (Reference)	PKS module	Discrete AT protein	Substrate specificity ^a	Gene cluster status	Organism
Neocarzinil (Otsuka <i>et al.</i> , 2004)	4 PKS modules on ORF4/5/6 with 2 AT domains	No discrete AT identified	Malonyl CoA	Cluster completed and confirmed	<i>Streptomyces carzinostaticus</i> var. F-41
Onnamide (Piel <i>et al.</i> , 2004c, 2005)	7 PKS modules on OmnB and OmnI. Several PKS modules still missing	No discrete AT identified	Malonyl CoA	Putative cluster incomplete	Bacterial symbiont from sponge <i>Theonella swinhoei</i>
Oxazolomycin (Song <i>et al.</i> , 2008; Zhao <i>et al.</i> , 2006, 2009)	10 PKS modules on OzmHIJKNQ	2 ATs on OzmM and 1 AT on OzmC	Malonyl CoA Methoxymalonyl ACP	Cluster completed and confirmed	<i>Streptomyces albus</i> JA3453
Pederin (Piel, 2002; Piel, <i>et al.</i> , 2004a, 2004b, 2004c, 2004d, 2005)	10 PKS modules on PedI and PedF; 4 optional PKS modules on PedH	2 ATs on PedC and 1 AT on PedD	Malonyl CoA	Putative cluster complete	Bacterial symbiont of <i>Paederus</i> ssp. beetles
Rhizoxin (Partida-Martinez and Hertweck, 2007)	12 PKS modules on RhiABCDEF (also known as RzxABCDEF)	2 ATs on RhiG (also known as RzxG)	Malonyl CoA	Cluster completed and confirmed	<i>Burkholderia rhizoxina</i>
Rhizoxin S2 and analogues (Brendel <i>et al.</i> , 2007)	12 PKS modules on RzxABCDEF	2 ATs on RzxG	Malonyl CoA	Cluster completed and confirmed	<i>Pseudomonas fluorescens</i> Pf-5
Unknown (Fritzler and Zhu, 2007; Zhu <i>et al.</i> , 2002)	7 PKS modules with 2 AT domains on CpPKS1	No discrete AT identified	Malonyl CoA	Putative cluster incomplete	<i>Cryptosporidium parvum</i>
Virginiamycin M (Pulsawat <i>et al.</i> , 2007)	7 PKS modules on VirAFGH	1 AT on VirI	Malonyl CoA	Cluster completed and confirmed	<i>Streptomyces virginiae</i>

^a Only the substrate specificity of LnmG has been experimentally confirmed.

Table 8.2

ESI-MS analysis of apo-, holo-, and malonyl-S-ACPs of the LnmI and LnmJ PKSs

ACPs ^a	apo-ACP [M + H] ⁺		holo-ACP [M + H] ⁺		Malonyl-S-ACP [M + H] ⁺	
	Calculated	Found	Calculated	Found	Calculated	Found
LnmI-ACP3	11,702	11,700	12,042	12,040	12,128	12,126
LnmJ-ACP4	12,245	12,241	12,585	12,582	12,671	12,669
LnmJ-ACP5	12,520	12,517	12,860	12,857	12,946	12,943
LnmJ-ACP6-1	12,209	12,206	12,549	12,546	12,635	12,632
LnmJ-ACP6-2	12,151	12,147	12,491	12,486	12,577	12,572
LnmJ-ACP7	12,322	12,318	12,662	12,665	12,748	12,751
LnmJ-ACP8	12,090	12,087	12,430	12,427	12,516	12,512

^aThe numbers after ACPs refer to the LNM PKS modules from which they are derived, with 6-1 and 6-2 to indicate the first and second ACPs, respectively, for LNM PKS module 6.