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ENGINEERING OF POLYKETIDE SYNTHASES: HOW CLOSE ARE WE TO THE REALITY?*

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Introduction

Microbial metabolites, especially the antibiotics, have contributed enormously to the improvement in quality of healthcare over the last fifty years. However, the efficacy of such medicines has been eroded continually by the speed with which clinical infections acquire resistance to antibiotics. It is therefore necessary to continue to discover and develop new drugs to combat the 'antibiotic resistance problem'. Recent discoveries reinforce the view that some microbes make metabolites that have other useful therapeutic properties, e.g. immune-suppressants, cholesterol-lowering drugs and anti-parasitics. This adds to the momentum to drive the discovery of new microbial metabolites. The filamentous soil bacteria, *Streptomyces*, are the source of over half of all clinically useful microbial metabolites. The molecular genetics of this important genus has developed rapidly over the last 20 years – to the point where molecular techniques can now be used to subvert strains into making novel metabolites, not seen previously in nature.

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Using molecular biology to derive novel secondary metabolites

The *Streptomyces* are a prolific sourced different polyketide metabolites – from antibiotics such as tetracycline and erythromycin to therapeutic agents such as the immunosuppressant, FK506 and rapamycin, and the anti-parasitics, monensin and avermectin (Fig. 1).

The biosynthesis of all polyketides follows a unified chemistry: activated carboxylic acids (such as malonyl-CoA and methylmalonyl-CoA) are condensed end-to-end, with each condensation accompanied by a decarboxylation event (see Figs. 2 and 3 [1]).

Thus malonyl-CoA (three carbons) contributes a two carbon unit to the final structure, whereas branched methylmalonyl-CoA (four carbons) contributes a branched three carbon unit. After each condensation, a keto-group is present – hence the term 'polyketide'. That keto-group may be modified subsequently to form hydroxy, unsaturated or fully-reduced versions. The vast diversity of polyketide structures is derived in three ways: (1) from the nature and number of extender groups that are condensed together during any biosynthesis, (2) the extent to which each is reduced and (3) the stereo-chemistry of the reactions at each step.

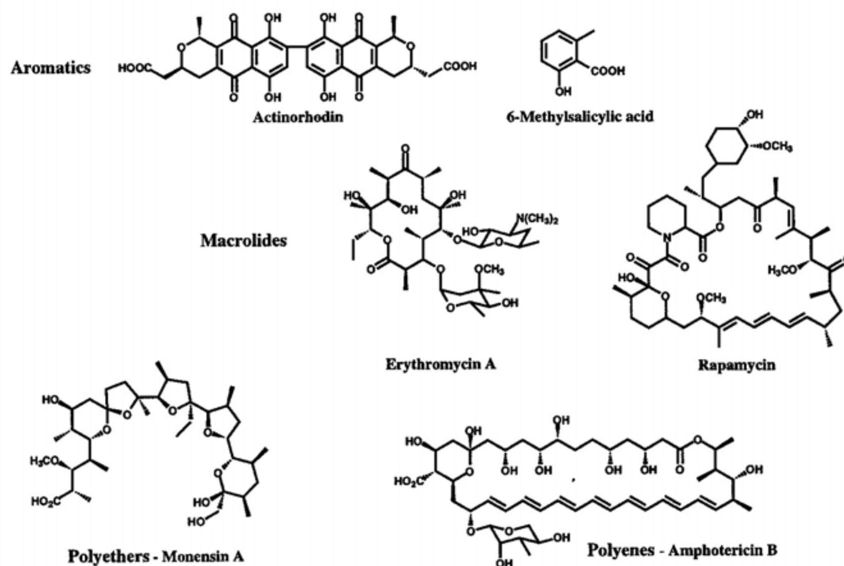

Fig. 1. Structures of some aromatic and complex polyketides

Although they have a unified chemistry, there is a dichotomy in the genetic programming of biosynthesis of polyketides. The polyketide derived compounds produced by *Streptomyces* and related filamentous soil *Actinomyces* could be classified in two main groups – simple aromatic polyketides (Type II) and complex polyketides, also called Type I polyketides.

Simple polyketides

Simple polyketides usually have an acetate starter unit and are composed entirely of two-carbon extender units (derived from malonyl-CoA). The keto-groups generated at each round of condensation remain unmodified while the nascent polyketide chain is being formed (Fig. 2). Simple polyketides are made by an array of different monofunctional polypeptides. The same catalytic centers are believed to be used again and again to extend the polyketide chain by iterative cycles. Each enzyme activity is encoded by a separate gene, leading to the classification of these enzymes as 'Type II polyketide synthases', through the parallel with fatty acid biosynthesis in *E. coli* which is encoded by a similar set of genes.

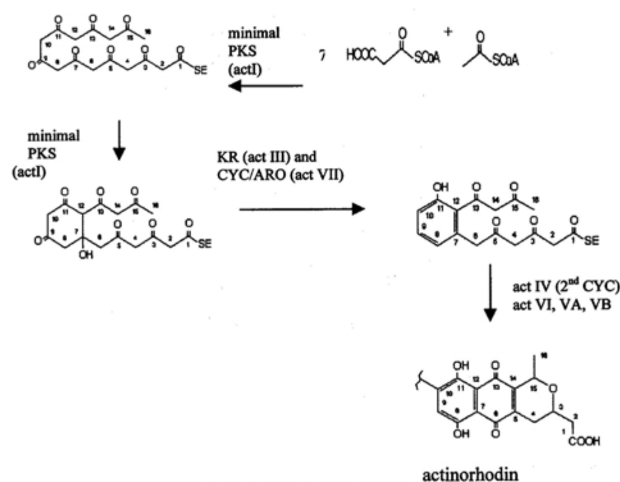

Fig. 2. Proposed pathway for biosynthesis of the polyketide intermediates of actinorhodin

For Type II synthases, the paradigm is the biosynthesis of actinorhodin (Fig. 2 [2]). Matrix experiments, in which combinations of genes for biosynthesis of related polyketides have been recombined in a single host and the structures of the resulting polyketides elucidated [3], have gone some way to assign some of the key enzyme

activities that determine which backbone structure is made. According to the "minimal PKS" hypothesis [3], the catalytic centre for condensation is located on a monofunctional 'ketosynthase-α' subunit, while its catalytically-incompetent β-homologue determines the number of cycles and hence the length of chain that is made. Lately, it has been shown that the ketosynthase-β subunit possesses decarboxylase activity [4] and also that other components of the multienzyme PKS complex (cyclase, hydroxylase) may well have a profound influence on the polyketide chain length [5, 6].

After the nascent polyketide chain is made, a concerted series of carbon–carbon bond formations is undertaken by 'cyclases', which substantially determine the ring structures. If a cyclase also contains an aromatase activity, then that ring gains aromatic functionality. Functional groups (e.g. methyl, hydroxyl) are introduced after the skeleton has been formed.

Despite this information, the design rules to invent new structures are still somewhat undefined. Nevertheless, manipulation of Type II polyketide synthases has provided a rich, if somewhat empirical source, of novel pro-drug structures.

Complex polyketides

Complex polyketides (by contrast) may contain different starter units. They may incorporate different extender units at each step of condensation. Each new keto-group may be modified chemically in up to four steps towards full reduction before the next extender group is added [1]. The genetic programming of such complicated structures is encoded by huge genes, which encode multi-domain polypeptides, often over 700 kDa in size. The domains occur in the same linear order as the enzymatic reactions take place and each domain is involved only once as a catalytic centre in the biosynthesis of each polyketide molecule (Fig. 3 [7]). Thus, the identities and linear order of these domains dictate, which structure is made. These systems are designated 'Type I polyketide synthases', by analogy with the mammalian fatty acid synthases which have a similar multi-domain architecture.

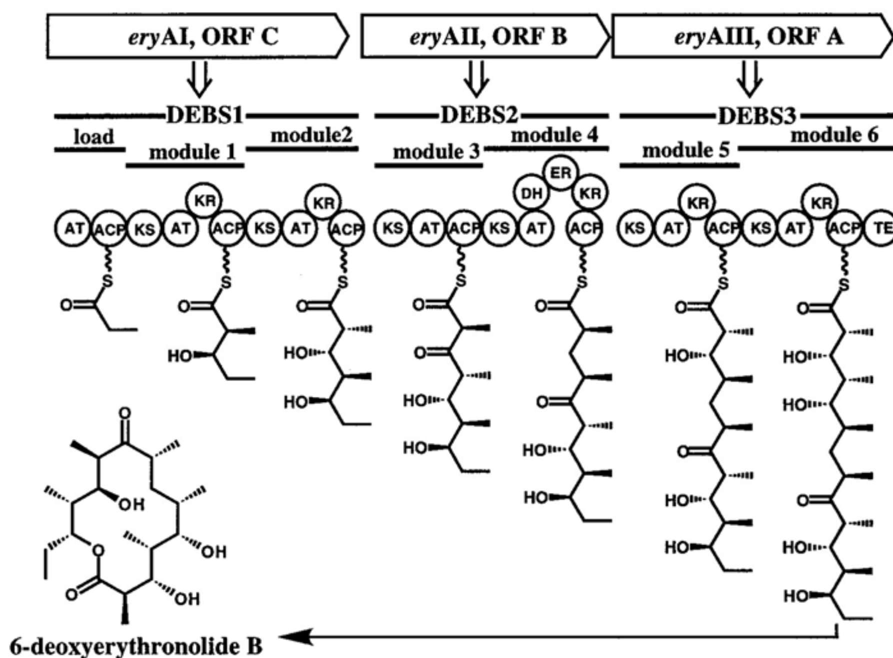

Fig. 3. Model hypothesis of the "programmed" assembly of 6-deoxyerythronolide B (6DEB) on the three multifunctional proteins, encoded by ORFs 1, 2 and 3 of the *eryA* locus of *Sac. erythraea*

"Designing" new structures

Knowledge of the genetic basis of programming of polyketide biosynthesis affords the opportunity to reprogram microbes to make novel structures. Paradoxically, more information is available on the programming of the complex Type I synthases, than their simple Type II counterparts (and more success has been achieved on reprogramming of Type I systems). Using the paradigm of biosynthesis of the 14-membered polyketide macrolide backbone of erythromycin by a Type I synthase, several approaches have resulted in novel structures [8]. It has been possible to change the identity of the extender group added at a particular step (from methylmalonyl-CoA to malonyl-CoA) resulting in that particular carbon in the finished structure no longer having a branched structure [9, 10]. It has been possible to reprogram the nature of reduction of the keto-group at specific points in biosynthesis, so that macrolides with different functional groups result [8]. The discovery that the linear series of reactions is terminated by a thioesterase domain (which specifies the nature of the macrolide ring that is formed) has made it possible, by repositioning the thioesterase domain earlier in

the linear sequence, to make novel polyketides with shorter chain length and smaller ring structures (Fig. 4 [11, 12]).

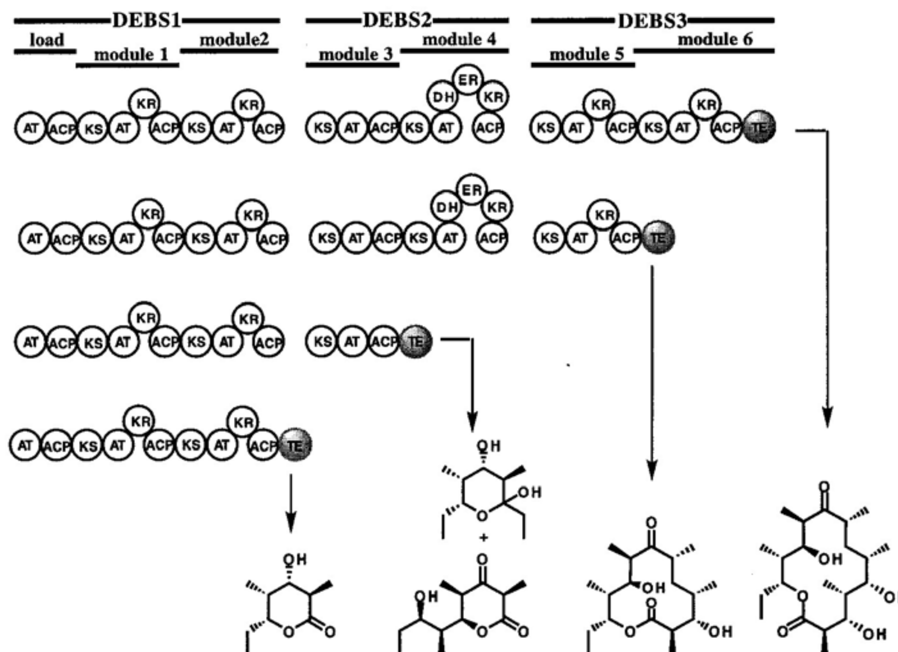

Fig. 4. Summary of genetic manipulations of erythromycin PKS genes and corresponding products generated by thioesterase/cyclase repositioning

Lastly, the nature of the starter unit chosen to prime biosynthesis of the chain has been shown to be dictated largely by a ‘loading domain’, which is located early in the linear sequence. This domain for erythromycin biosynthesis has been substituted by the analogous domain for biosynthesis of avermectin (another macrolide made by a Type I synthase [13, 14]). The avermectin domain has a much-relaxed specificity for the nature of starter unit that it will incorporate. Thus, it has been possible to create novel erythromycins with “non-natural” starter units.

As mentioned earlier, different type I PKS gene clusters consist of a number of “modules”. Each module catalyses the extension of one C2 unit. It also determines the degree of keto-group reduction. The exchange, contraction or module(s) expansion seems to be the obvious way for polyketide backbone modifications and generation of novel compounds [15].

In addition to the PKS genes, the complex polyketide gene clusters also contain genes involved in post PKS modifications like methylases, hydroxylases, and the genes

involved in glycoside formation and its attachment to the basic backbone of the polyketide. These groups usually posses a very high influence on the activity of the polyketide compounds. A number of modifications to the basic backbone of various polyketides have been introduced [8, 16].

Finally, a "combinatorial" approach which would include a large number of modifications in the single experiment, generating a large number of novel polyketides is one of the final goals "in the field" which until now has only been partially fulfilled [17, 18].

Conclusion

The molecular biology of *Streptomyces* is now well developed. Gene technology has been used to derive novel secondary metabolite structures, which may have utility as new anti-infectives or therapeutic agents. Our level of knowledge of these systems means that, in principle, agenetic blueprint can be drawn up to program the biosynthesis of the backbone of any macrolide structure. The same techniques may be used to boost the productivity of fermentation processes by up-regulating the entire antibiotic pathway, specifically enhancing a rate-limiting step, or altering the balance of central metabolism so that more precursors flow into the biochemical pathway for antibiotic biosynthesis (Baltz [19]). Molecular biology has unambiguously become an important tool in the drug discovery process.

References

1. Katz,L., Donadio,S.: Polyketide synthesis: prospects for hybrid antibiotics. Ann Rev Microbiol **47**: 875–912 (1993).
2. Hopwood,D.A.: Genetic contributions to understanding polyketide synthases. Chem Rev **97**: 2465–2497 (1997).
3. McDaniel,R., Ebert-Khosla,S., Hopwood,D.A., Khosla,C.: Rational design of aromatic polyketide natural products by recombinant assembly of enzyme subunits. Nature **375**: 549–554 (1995).
4. Bisang,C., Long,P.F., Cortes,J., Westcott,J., Crosby,J., Matharu,A.L., Cox,R.J., Simpson,T.J., Staunton,J., Leadlay,P.F.: A chain initiation factor common to both modular and aromatic polyketide synthases. Nature, **401**: 502-505 (1999).
5. Shen,Y.M., Yoon,P., Yu,T.W., Floss,H.G., Hopwood,D.A., Moore,B.S.: Ectopic expression of the minimal *WhiE* polyketide synthase generates a library of aromatic polyketides of different sizes and shapes. Proc Natl Acad Sci USA **96**: 3622–3627 (1999).

6. Petkovic,H., Thamchaipenet,A., Zhou,L.H., Hranueli,D., Raspor,P., Waterman,P.G., Hunter,I.S.: Disruption of an aromatase/cyclase from the oxytetracycline gene cluster of *Streptomyces rimosus* results in production of novel polyketides with shorter chain lengths. J Biol Chem **274**: 32829–32834 (1999).
7. Leadlay,P.F., Staunton,J., Aparacio,J.F., Bevitt,D.J., Caffrey,P., Cortes,J., Marsden,A., Roberts,G.A.: Erythromycin-producing polyketide synthase. Biochem Soc Trans **21**: 218–222 (1993).
8. Katz,L.: Modulation of modular polyketide synthases. Chem Rev **97**: 2557–2575 (1997).
9. Oliynyk,M., Grown,M.J.B., Leadlay,P.F.: Engineering of polyketide biosynthesis by domain replacements in a modular polyketide synthase. FASEB Journal **11**: 2644 (1997).
10. Ruan,X., Pereda,A., Stassi,D., Zeidner,D., Summers,R.G., Jackson,M., Shivakumar,A., Kakavas,S., Staver,M.J., Donadio,S., Katz,L.: Acyltransferase domain substitutions in erythromycin polyketide synthase yield novel erythromycin derivatives. J Bac **179**: 6416–6425 (1997).
11. Cortes,J., Weismann,K.E.H., Roberts,G.A., Brown,M.J.B., Staunton,J., Leadlay,P.F.: Repositioning of a modular polyketide synthase to promote specific chain cleavage. Science **268**: 1487–1489 (1995).
12. Pieper,R., Gokhale,R.S., Luo,G., Cane,D.E., Khosla,C.: Purification and characterization of bimodular and trimodular derivatives of the erythromycin polyketide synthase. Biochemistry **36**: 1846–1851 (1997).
13. Staunton,J., Wilkinson,B.: Biosynthesis of erythromycin and rapamycin. Chem Rev **97**: 2611–2629 (1997).
14. Marsden,A.F.A., Wilkinson,B., Cortes,J., Dunster,N.J., Staunton,J., Leadlay,P.F.: Engineering broader specificity into an antibiotic-producing polyketide synthase. Science **179**: 199–202 (1989).
15. Tang,L., Fu,H., McDaniel,R.: Formation of functional heterologous complex using subunits from the picromycin, erythromycin and oleandromycin polyketide synthases. Chem Biol **7**: 77–84 (2000).
16. Gassier,S., Rather,J., Wirtz,G., Kellenberger,L., Staunton,J., Leadlay,P.F.: A defined system for hybrid macrolide biosynthesis in *Saccharopolyspora erythraea*. Mol Microbiol **36**: 391–401 (2000).
17. McDaniel,R., Thamchaipenet,A., Gustafsson,C., Fu,H., Betlach,M., Betlach,M., Ashley,G.: Multiple gentic modifications of the erythromycin polyketide synthase to produce a library of novel “unnatural” natural products. Proc Natl Acad Sci USA **96**: 1846–1851 (1999).
18. Leadlay,P.F.: Combinatorial approaches to polyketide biosynthesis. Curr Op Chem Biol **1**: 162–168 (1997).
19. Baltz,R.H.: Genetic manipulation of antibiotic-producing *Streptomyces*. Trends Microbiol **6**: 76–83 (1998).