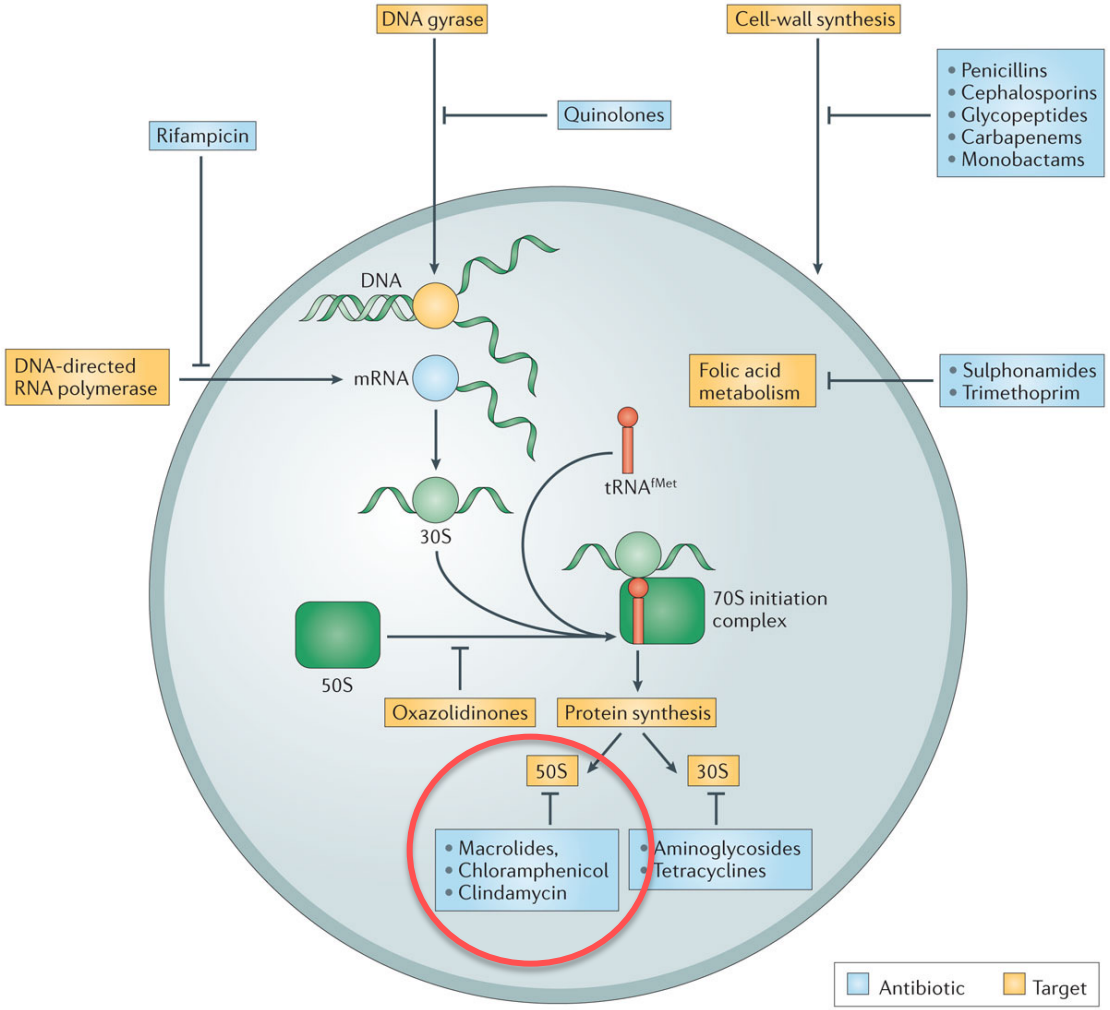
Multienzymatic complexes for the biosynthesis of polyketides: polyketide synthases (PKS)

Antibiotic targets



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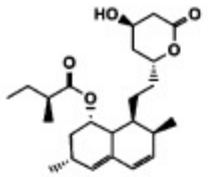
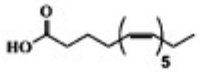
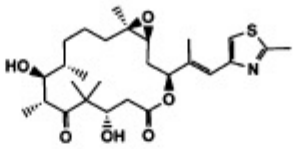


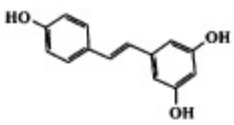
lovastatin
Aspergillus terreus
anticholesterol polyketide



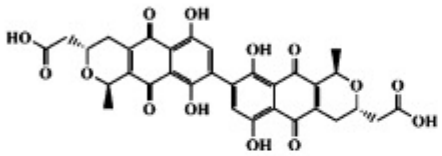
eicosapentanoic acid
Shewanella onedensis
fatty acid supplement



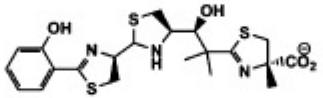
epothilone B
Sorangium cellulosum
anticancer polyketide-peptide



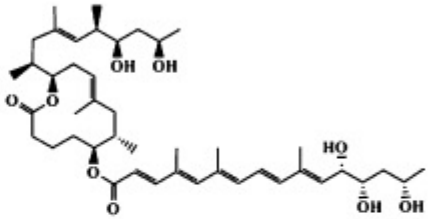
resveratrol
several plants including *Vitrus*
polyketide antioxidant



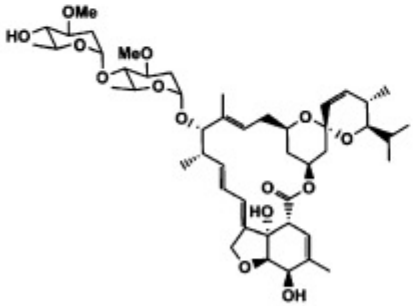
actinorhodin
Streptomyces coelicolor
polyketide pigment



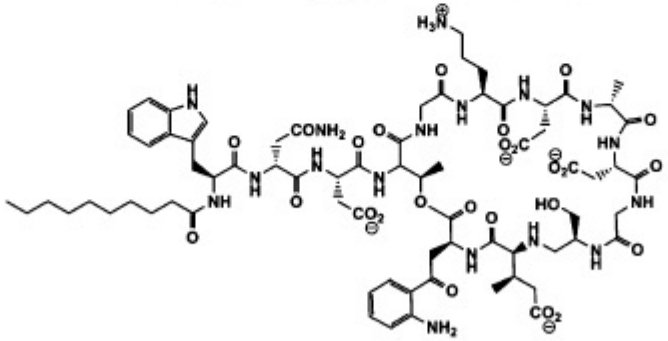
yersiniabactin
Yersinia pestis
nonribosomal peptide virulence factor



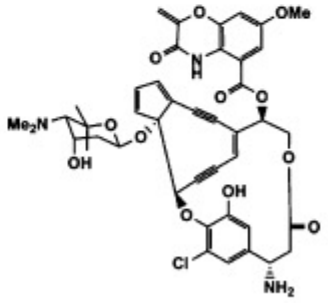
mycolactone B
Mycobacterium ulcerans
polyketide virulence factor



avermectin B1b
Streptomyces avermitilis
polyketide antiparasitic



daptomycin
Streptomyces roseosporus
lipopeptide antibiotic



C-1027
Streptomyces globisporus
anticancer polyketide

Fig. 1 Structures, producers, and biological activities of some notable fatty acid, polyketide, and nonribosomal peptide natural products.

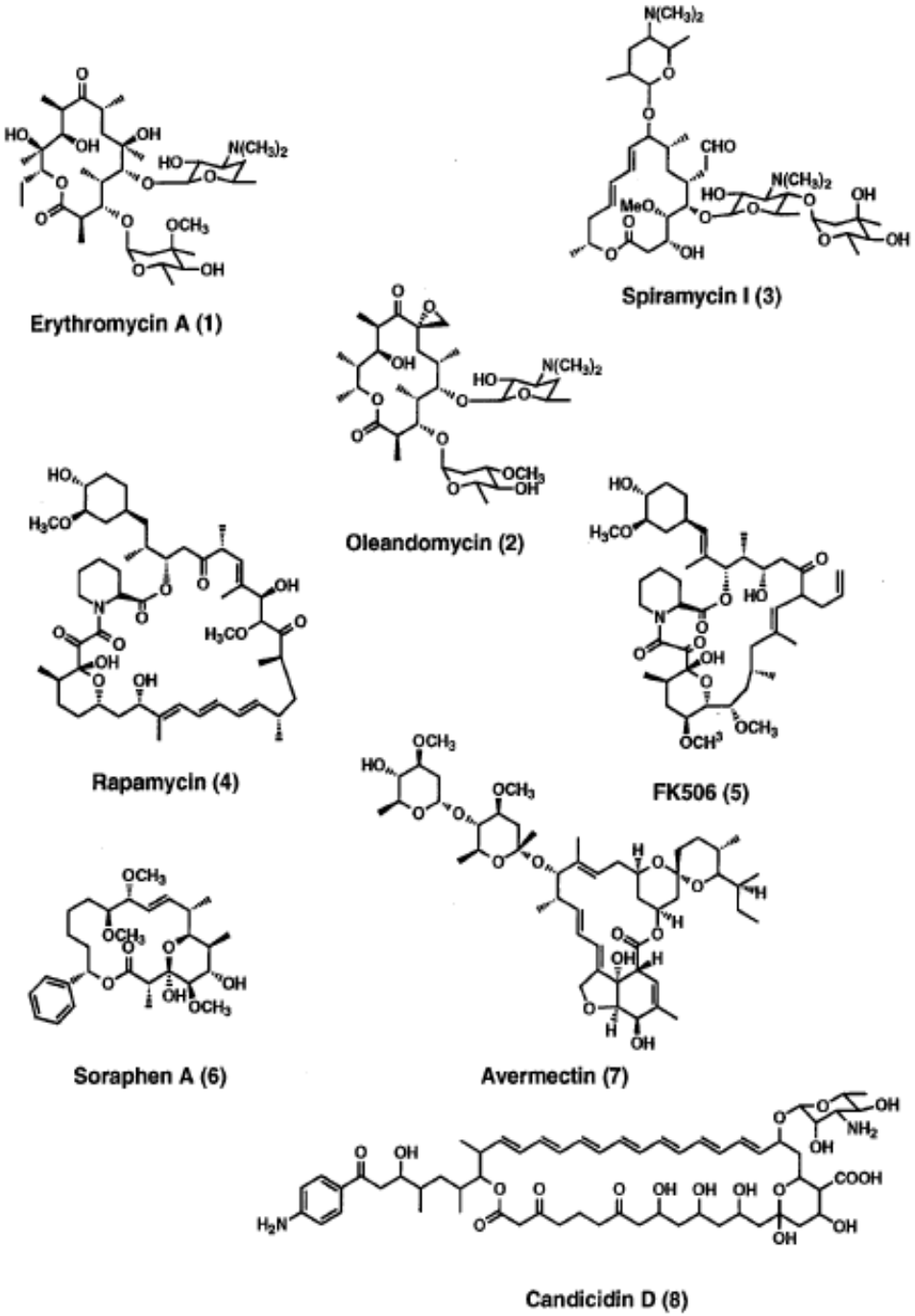


Figure 1. Structures of selected natural products derived from modular polyketide synthases.

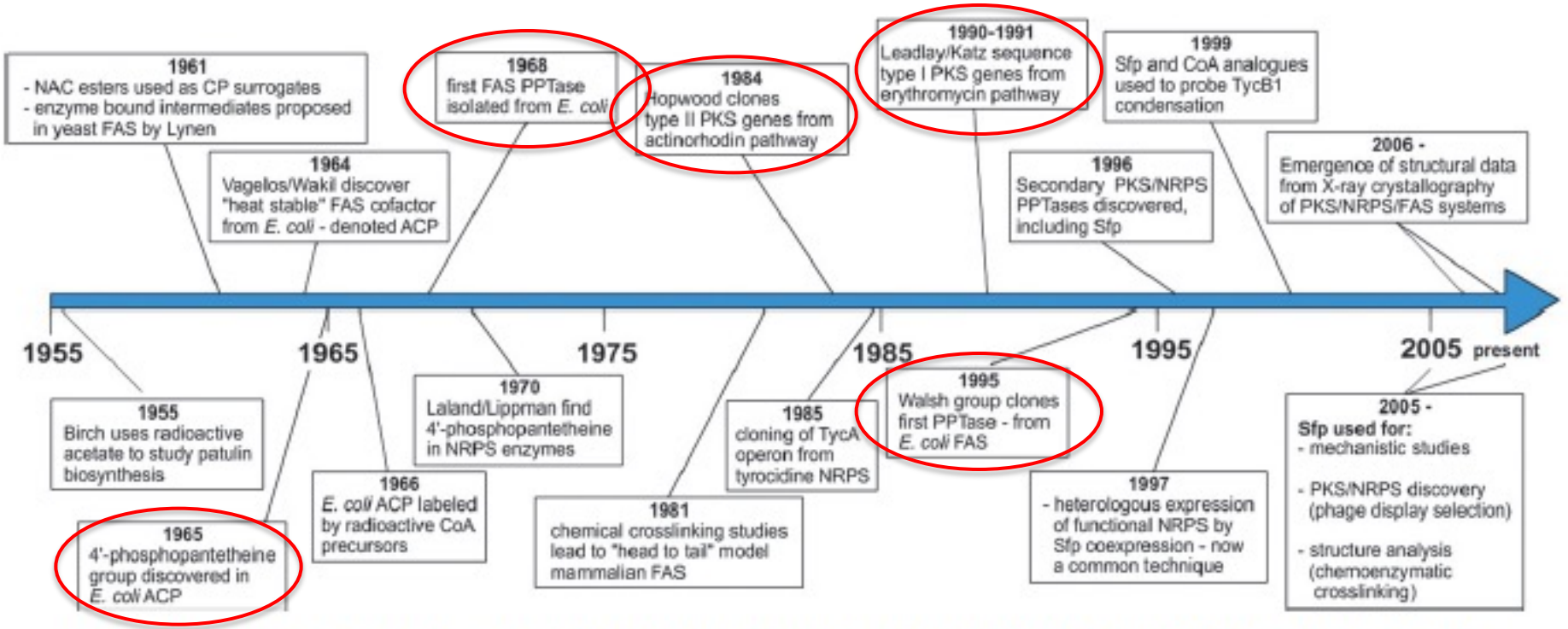


Fig. 2 Timeline of some notable events in the study of carrier-protein-mediated FAS, PKS, and NRPS biosynthetic enzymes.

Structural organization of multienzymatic complexes FAS (fatty acid synthase), PKS and NRPS

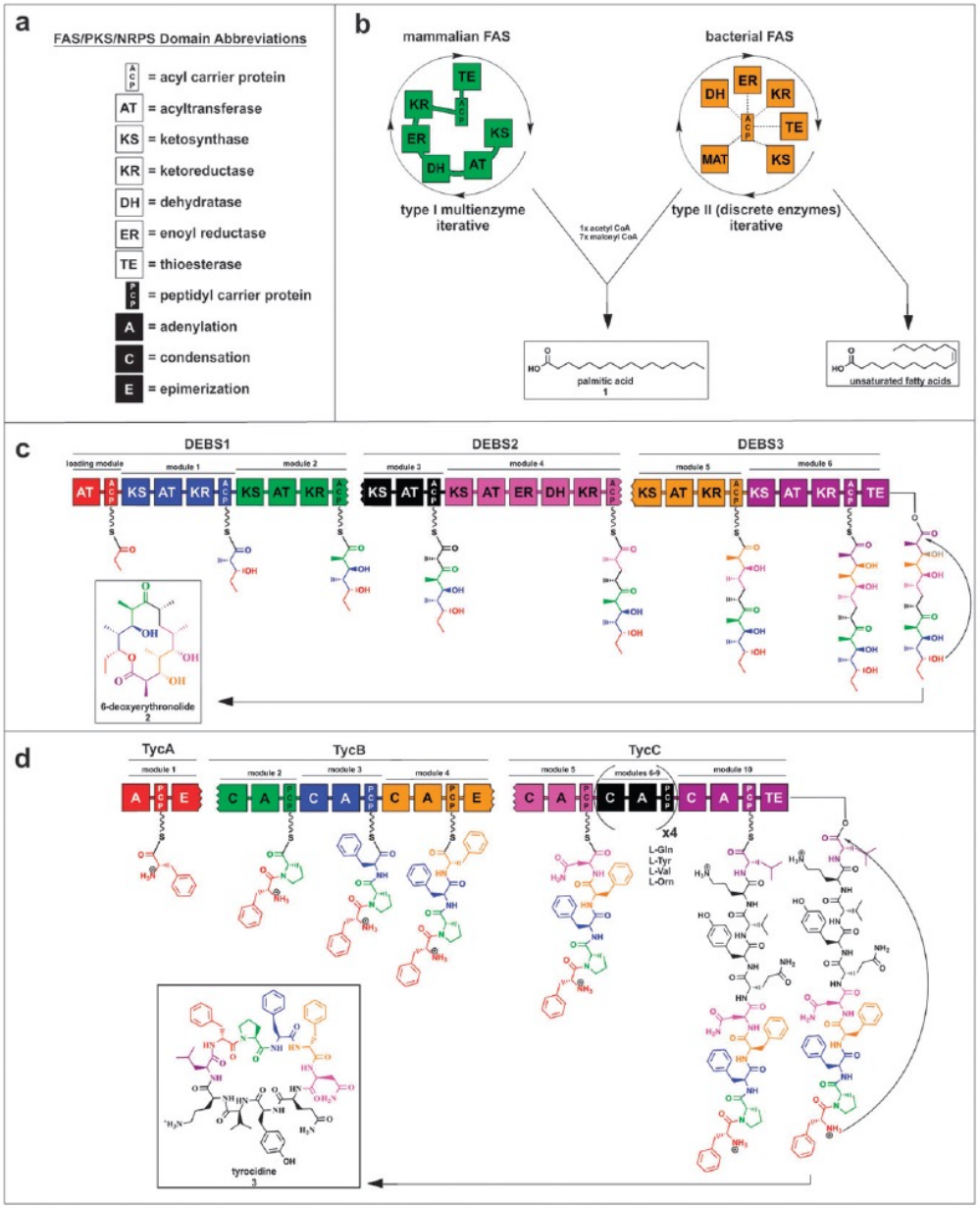
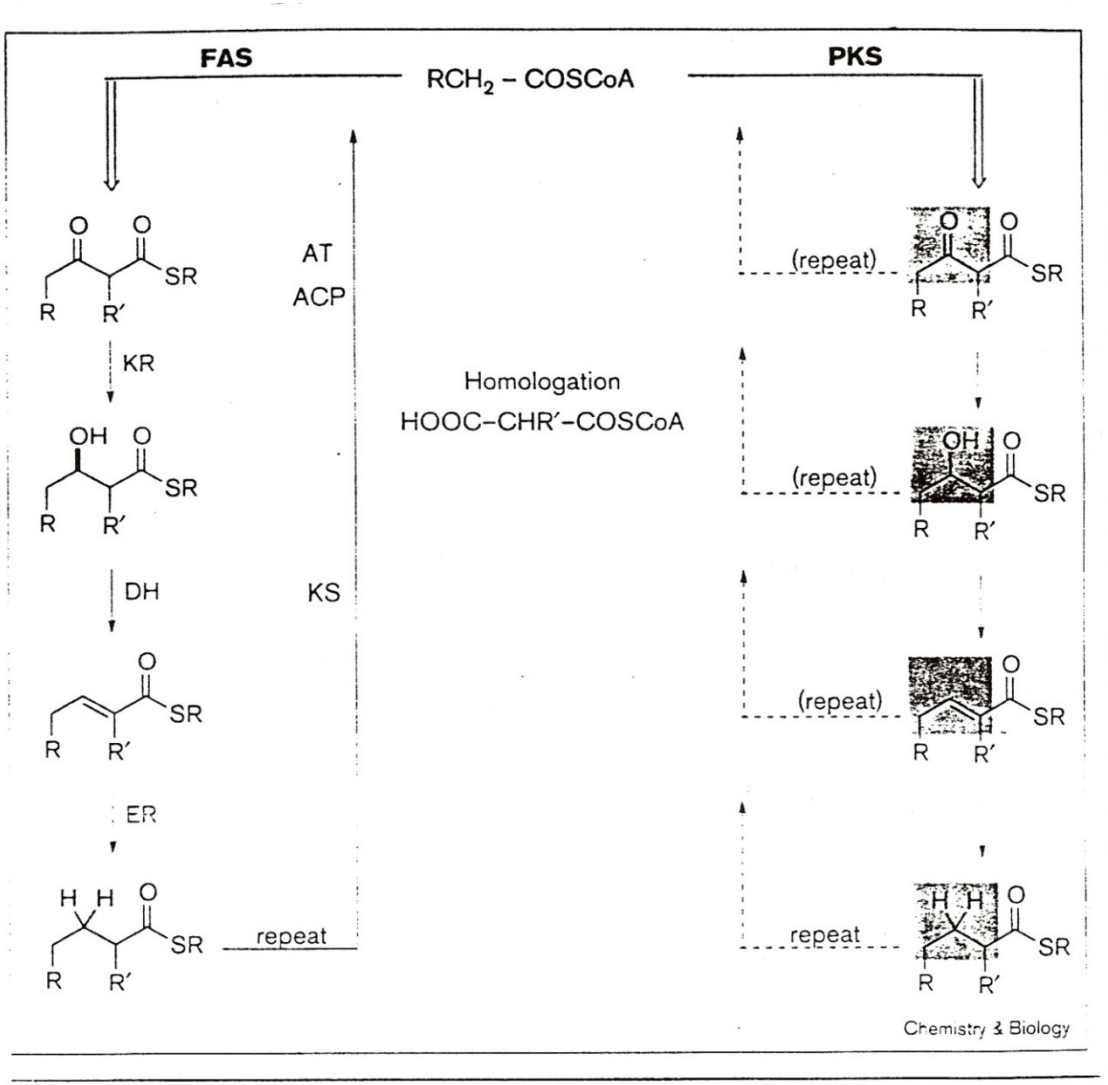


Fig. 3 Domain organization for FAS, PKS, and NRPS enzymes. (a) Domain abbreviations used in this manuscript. (b) Mammalian and bacterial FAS. Both produce the primary metabolite palmitic acid (1), but while mammalian FAS houses each necessary enzymatic activity on a single multienzyme, in bacteria they function in *trans*. (c) Type I PKS responsible for 6-deoxyerythronolide (2) production. Monomer units are color coded to indicate module of origin. Jagged ACP–KS junctions indicate intermodular communication. (d) Type I NRPS responsible for tyrocidine (3) production.

Analogies between polyketide (PKS) and fatty acid (FAS) biosynthesis

FAS
Starter unit:
Acetyl-CoA
Extender unit:
Malonyl-CoA



PKS
Starter unit:
Acetyl-CoA
Propionyl-CoA, etc
Extender unit:
Malonyl-CoA
Methyl-malonyl-CoA, etc

Biosynthesis of polyketides

Analogies between fatty acid synthase (FAS) and polyketide synthase (PKS)

- FAS type I fungi and vertebrates
  iterative 'modular': catalytic activities AT-KS-ACP-KR-DH-ER are domains of a single polypeptide chain.
- FAS type II bacteria and plants
  iterative non modular: each catalytic activity is on separate subunits

- PKS type I 'modular': the number of modules equals the number of elongation cycles, the type of domains determine the reduction state of the β-ketogroup
- PKS type II 'iterative': the same domains catalyze all condensation cycles (mostly synthesize aromatic polyketide products)

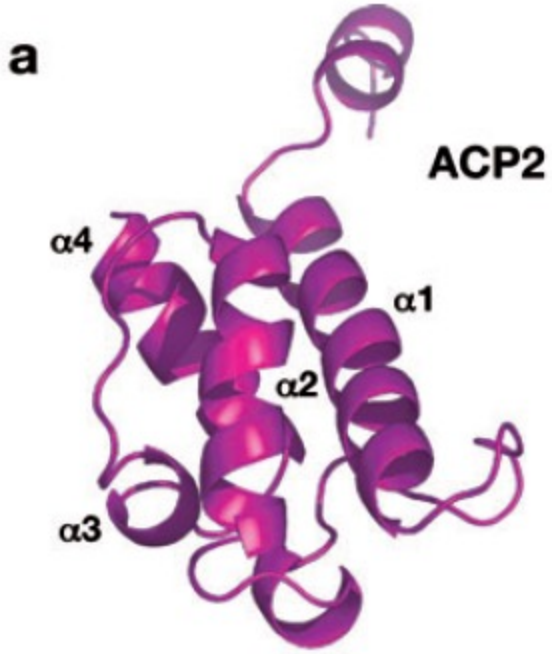
Biosynthesis of polyketides

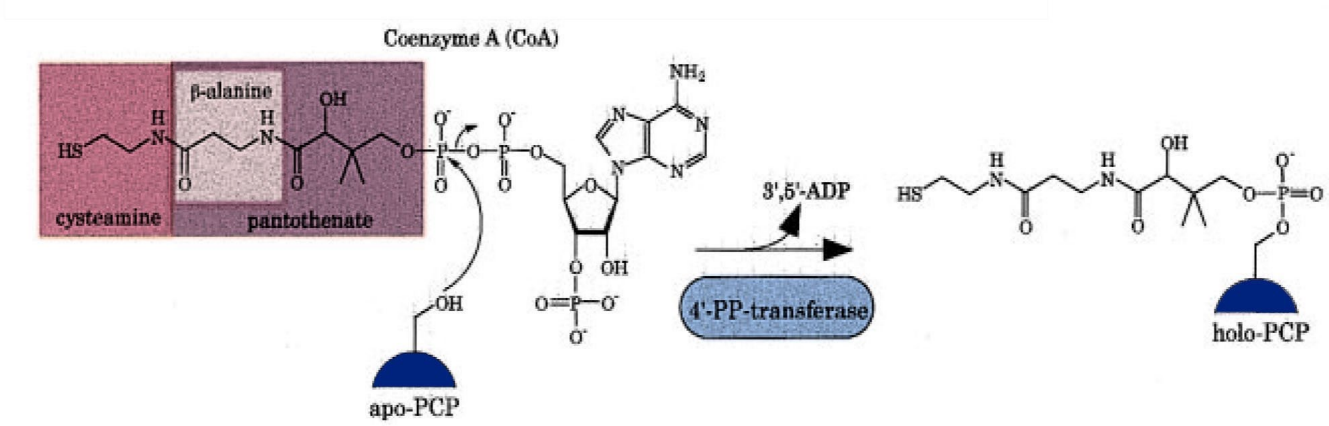
Versatility of PKS in the choice of:

- Starter unit
- Nature and number of extender units
- Control of the reduction state of the β-ketogroup
- Stereochemistry of the side-chains
- Cyclization

The ACP domain

- The ACP domain contains 4-phosphopantetheine derived from coenzyme A and covalently bound to a serine residue.
- 4-phosphopantetheine covalently binds (thioester bond) the substrate and provides flexibility and length (about 2 nm) facilitating communication among active sites.





Structural models of PKS domains

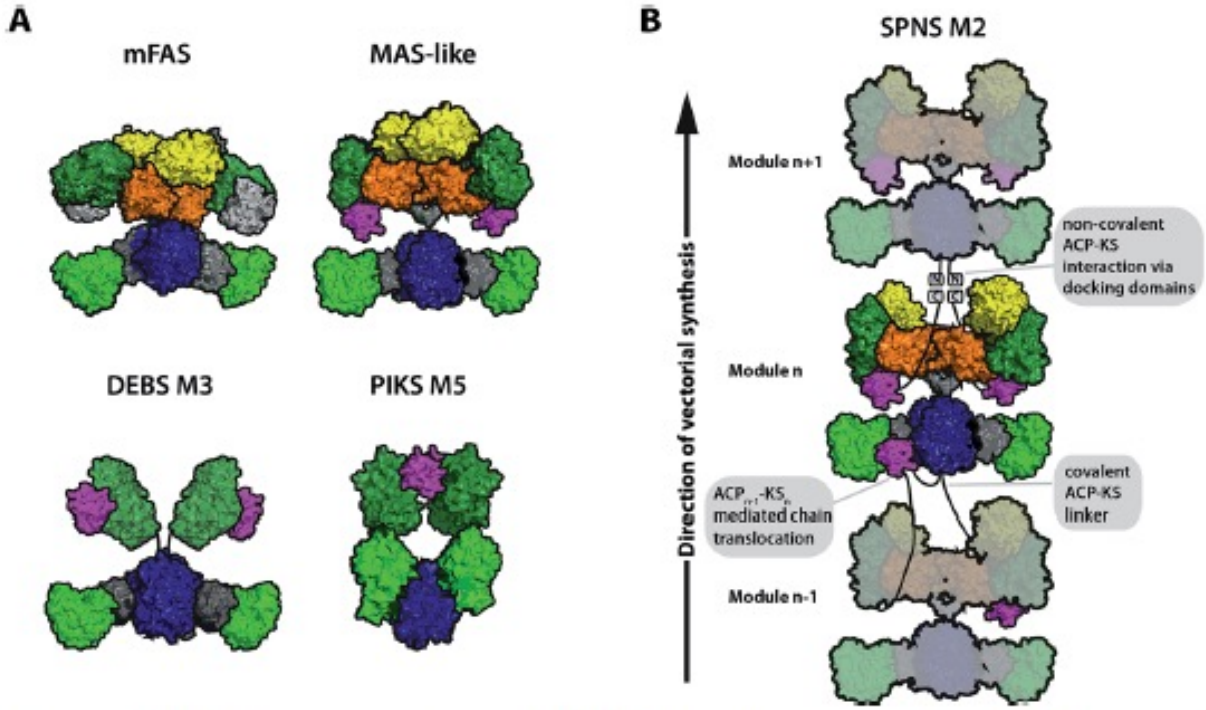


Fig. 2 Different models for assembly line PKSs in comparison to mFAS. (A) Schematic representation of mFAS (based on PDB 2VZ8),[28] a MAS-like PKS (based on PDB 5BP1 and 5BP4),[21] a model for the partially reducing DEBS M3 based on SAXS analysis,[33] and a model of the partially reducing pikromycin synthase module 5 (PIKS M5) based on cryo-EM analysis.[27] (B) Graphical representation of a PKS module assembly using the example of the proposed model for the spinomycin synthase module 2 (SPNS M2).[23] The SPNS M2 model was assembled from PDBs 5BP1 (KS–AT), 3SLK (ER–KR), and 5BP4 (DH) with KR of 3SLK superimposed on KR of 5BP4. PKS modules can either be connected through covalent ACP–KS linkers or through non-covalently interacting docking domains at the C- and N-termini. Additionally, chain translocation is mediated through specific interactions between the upstream ACP (ACP_{n-1}) and the downstream KS (KS_n), shown for one of the two ACPs in $module_{n-1}$. Domain coloring: KS (blue), AT (light green), KR (dark green), DH (orange), ER (yellow), ACP (magenta), linker regions such as the KS-AT linker (dark gray), and only for mFAS non-catalytic pseudo-methyltransferase domain (light gray).

Interactions of the ACP domain with KS and AT domains

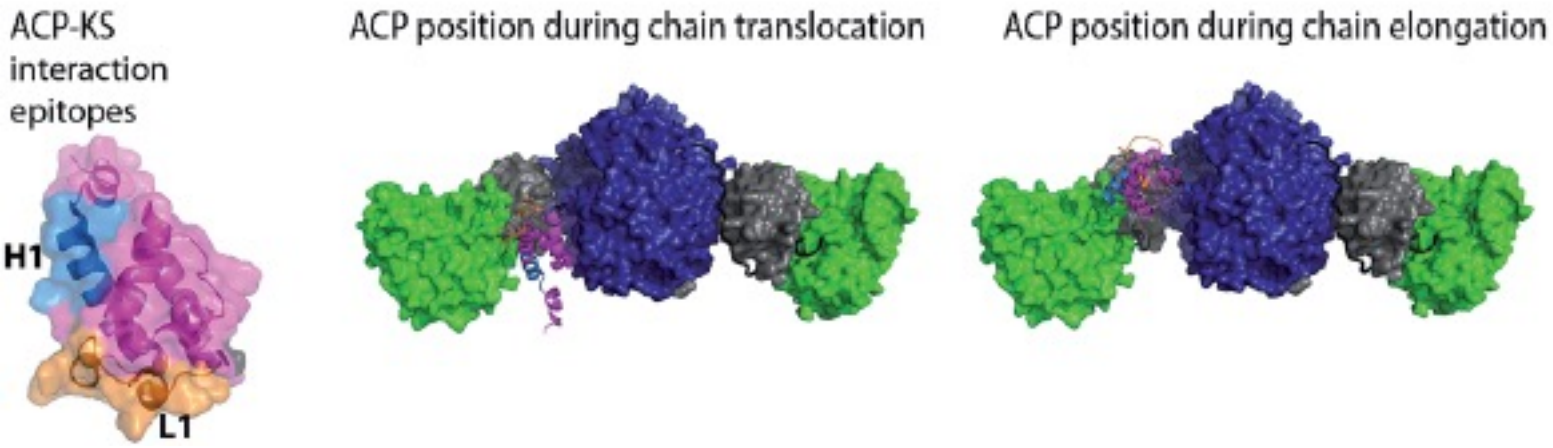


Fig. 3 Important interfaces for engineering ACP–KS and AT domain exchanges. (A) Interface of ACP–[KS–AT] according to ref. 40 and 41. Residues of ACP that are responsible for chain translocation in helix 1 are highlighted in blue. Loop 1 (orange) was found as the main determinant in chain elongation. The ACP docks to different positions into the small cleft of the KS–AT fold during chain translocation *vs.* chain elongation. (B)

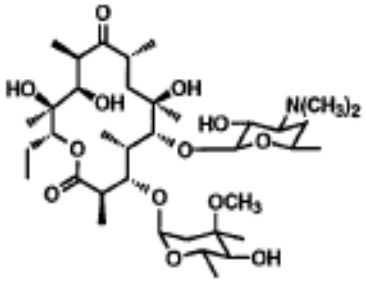
KS: ketosynthase
AT: acyl-transferase

condensation
loading

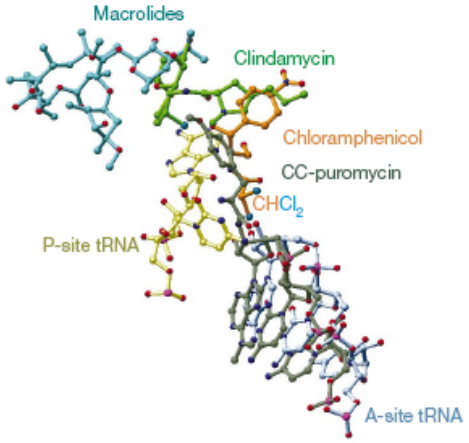
Erythromycin biosynthesis

Erythromycin A was isolated in 1957 from the Gram-positive bacterium *Saccharopolyspora erythraea*, it has antibiotic activity against Gram-positive bacteria.

It inhibits protein synthesis by binding ribosomal RNA 23S in the peptidyl-transferase site.



Erythromycin A (1)



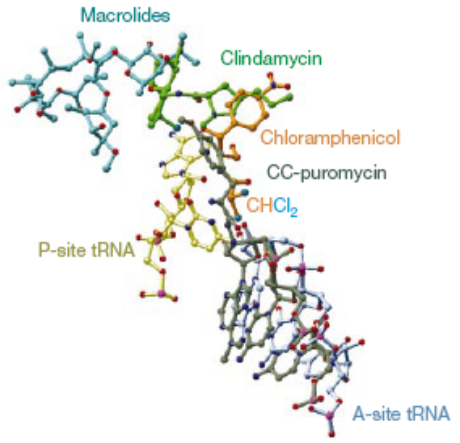


Figure 4 Relative position of chloramphenicol, clindamycin and macrolides with respect to CC-puromycin and the 3′-cytosine-adenine (CA) end of P-site and A-site tRNAs. The location of CC-puromycin was obtained by docking the position reported by ref. 2 into the peptidyl transferase centre of *D. radiodurans*. The location of the 3′-CA end of P- and A-site tRNAs was obtained by docking the position reported by ref. 42 into the peptidyl transferase centre of *D. radiodurans*. Light blue, 3′-CA end of A-site tRNA; light yellow, 3′-CA end of P-site tRNA; grey, puromycin; gold, chloramphenicol; green, clindamycin; cyan, macrolides (erythromycin). Oxygen atoms are shown in red and nitrogen atoms in dark blue. $CHCl_2$ indicates the location of the dichloromethyl moiety of chloramphenicol.

Genes involved in the biosynthesis of erythromycin

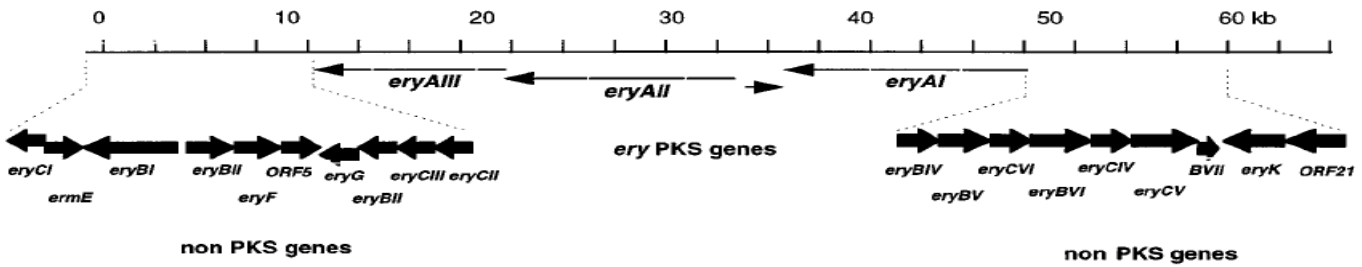
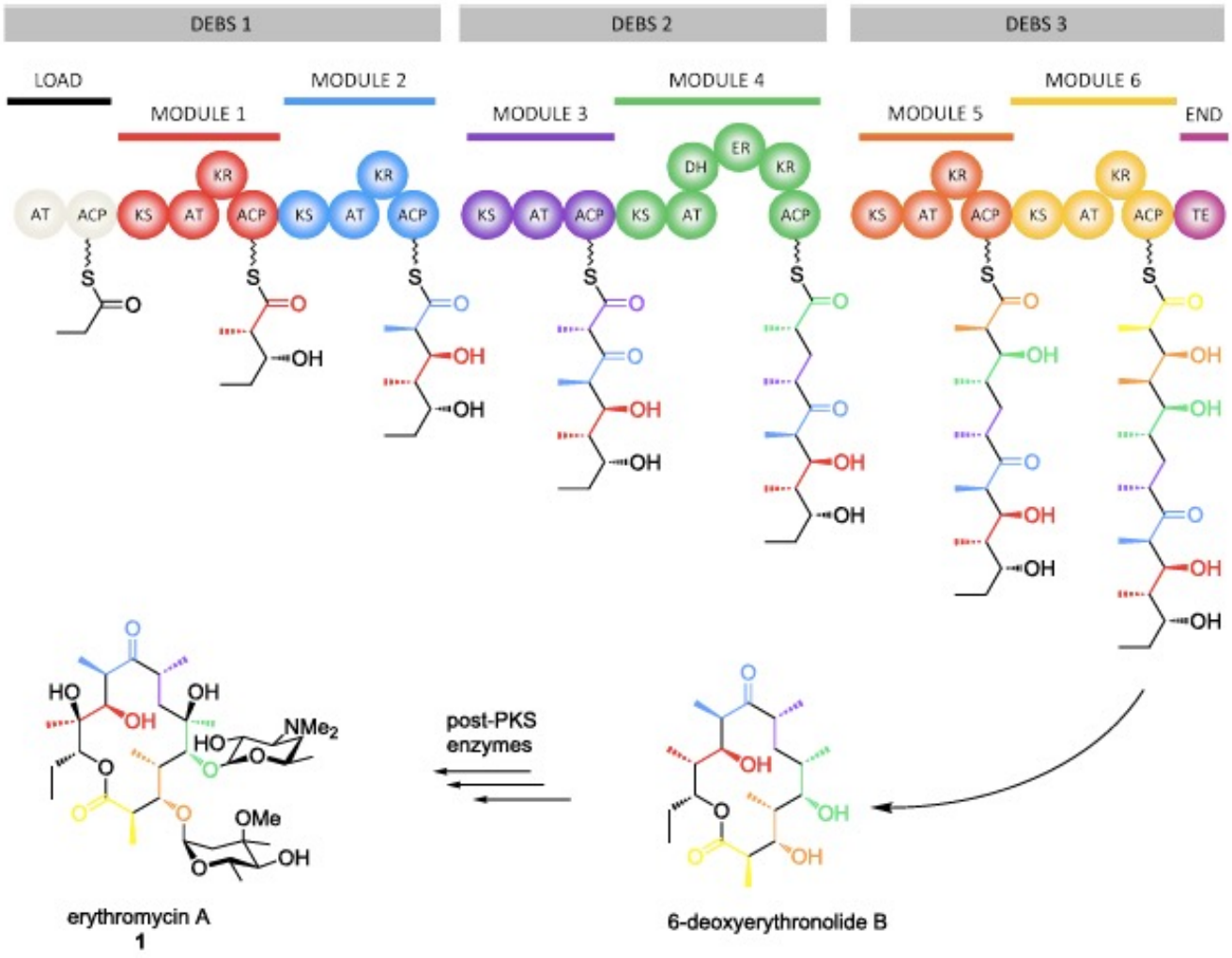


Figure 2. Map of regions of the *S. erythraea* genome containing genes associated with the late stages of the erythromycin biosynthetic pathway.

Table 1. Summary of Known or Proposed Functions of Erythromycin Biosynthesis Genes

locus	role in Er biosynthesis	function
eryAI	macrolactone synthesis	polyketide synthase
eryAII	macrolactone synthesis	polyketide synthase
eryAIII	macrolactone synthesis	polyketide synthase
eryBI	L-mycarose synthesis	not known
eryBII	L-mycarose synthesis	3-ketoreductase
eryBIII	L-mycarose synthesis	not known
eryBIV	L-mycarose synthesis	4-ketoreductase
eryBV	L-mycarose attachment	mycarosyltransferase
eryBVI	L-mycarose synthesis	not known
eryBVII	L-mycarose synthesis	5-epimerase
eryCI	D-desosamine synthesis	3-aminotransferase
eryCII	D-desosamine synthesis	not known
eryCIII	D-desosamine attachment	desosaminyltransferase
eryCIV	D-desosamine synthesis	3,4-dehydratase
eryCV	D-desosamine synthesis	not known
eryCVI	D-desosamine synthesis	3-aminodimethyltransferase
eryF	C-6 hydroxylation	P450 monooxygenase
eryG	C″-3 *O*-methylation	*O*-methyltransferase
eryI	not known	thioesterase
eryK	C-12 hydroxylation	P450 monooxygenase
ermE	resistance	*N*-methyltransferase

Modular organization of PKS that produces the polyketide scaffold of erythromycin, 6-deoxyerythronolide B (6-DEB)

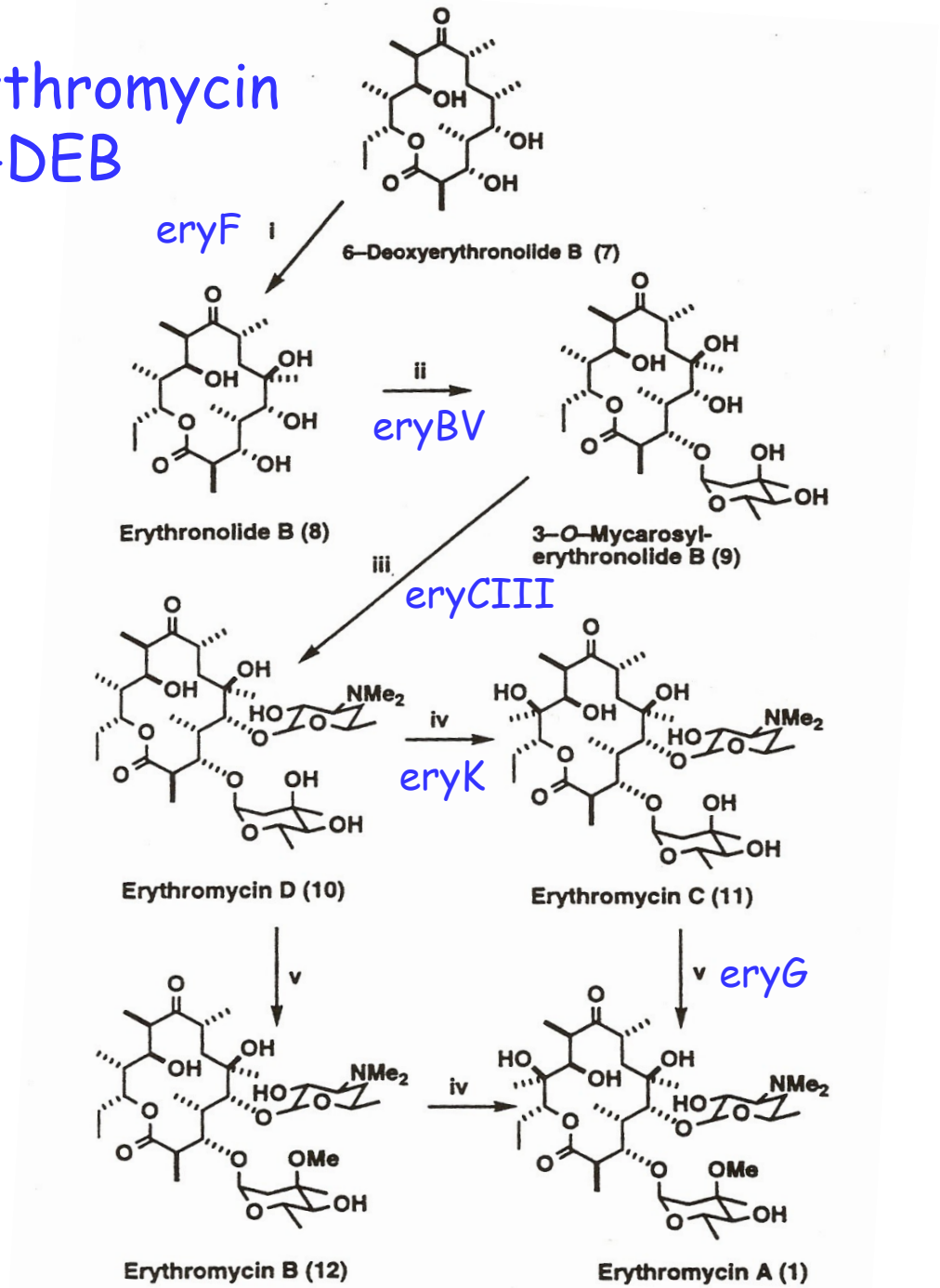


Starter unit:
Propionyl-CoA

Extender unit:
2-S-Methyl-malonyl-CoA

Biosynthesis of erythromycin
Modifications of 6-DEB

Erythromycin D is the first intermediate with antibiotic activity



Interactions between erythromycin and 23S rRNA

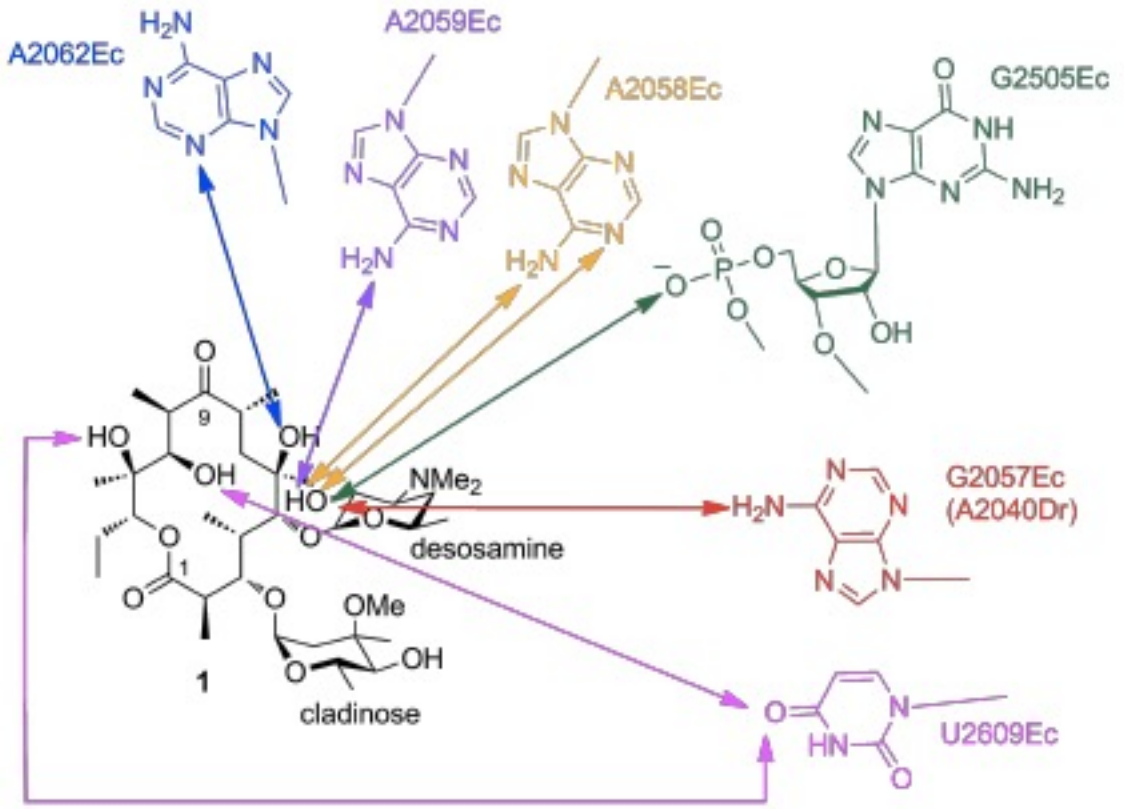


Figure 2: Schematic of erythromycin A (**1**) bound to 23S ribosomal RNA of the 50S subunit of the *Deinococcus radiodurans* (Dr) ribosome. The interactions between the polyketide and the nucleotides (*Escherichia coli* (Ec) numbering) are indicated with colored arrows (reactive groups are less than 4.4 Å apart). Adapted from [5].

Expression of recombinant DEBS for structural and functional studies

- Problems in the expression of recombinant PKS:
  - large proteins
  - folding and post-translational modifications (phosphopantetheinylation)
  - presence of appropriate precursors

The host that was used for expression is the *Streptomyces coelicolor* CH999 strain, which lacks the aromatic polyketide actinorhodin gene cluster

A simplified PKS formed by DEBS1 with the TE domain, has been created for structural and functional studies and it has been expressed in *S. coelicolor*.

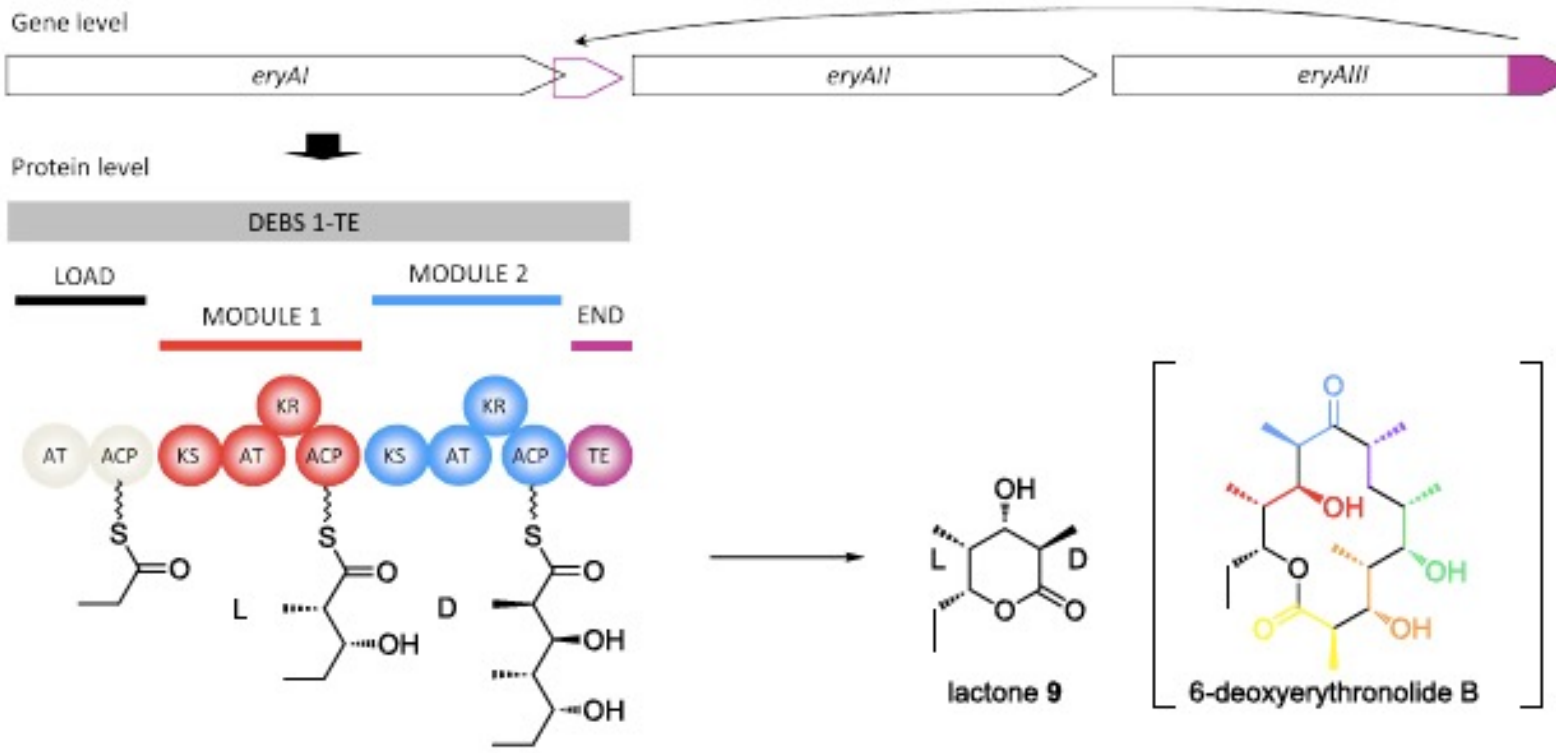


Figure 6: Creation by genetic engineering of the DEBS 1-TE model system. The region of the *eryAIII* gene encoding the thioesterase (pink) was relocated to the end of gene *eryAI*. The resulting protein, DEBS 1-TE, produces a small triketide lactone **9** instead of the heptaketide 6-deoxyerythronolide B. The two methyl centers in lactone **9** are of opposite stereochemical configuration, and thus DEBS 1-TE is an attractive protein for studying the control of stereochemistry.

Control of the methyl group side-chain stereochemistry

Mini-PKS formed by the loading domain and two modules (DEBS1-TE).

Two condensation cycles that produce side-chains with different stereochemistry starting from 2S-methyl-malonyl-CoA.

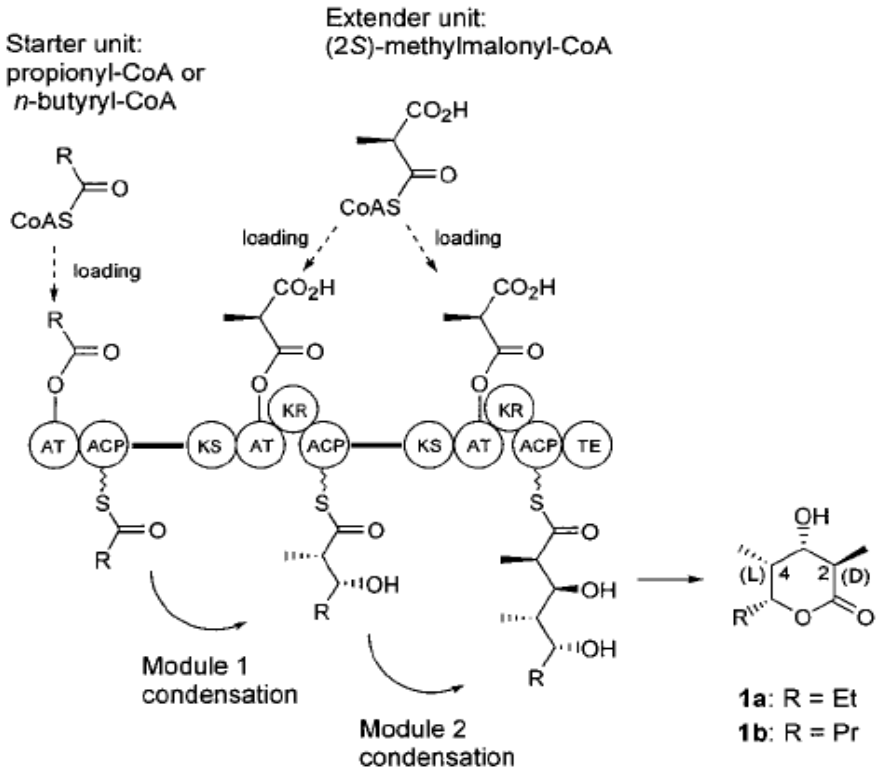
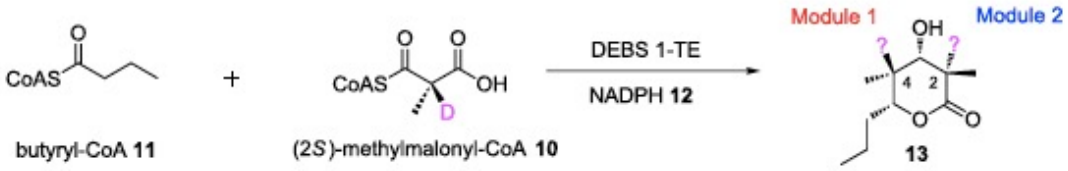


FIGURE 1: Operations to carry out two chain extension cycles by the "triketide lactone synthase", 6-deoxyerythronolide B synthase 1-thioesterase (DEBS 1-TE). DEBS 1-TE is organized into two modules which catalyze the stereospecific condensation of an extender unit onto the growing chain and set the reduction level of the β-keto group of the resulting intermediate. When supplied with NADPH and a suitable starter (*e.g.*, propionyl-CoA or *n*-butyryl-CoA) and the correct (2*S*)-stereoisomer of the chain-extending methylmalonyl-CoA, DEBS 1-TE produces the δ-lactones **1a** and **1b**, respectively.

Control of the methyl group side-chain stereochemistry

The correct mechanism was identified by analysis of the products with mass spectrometry and NMR

Epimerization is mediated by the KR domain



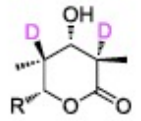
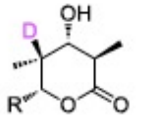
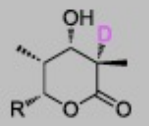
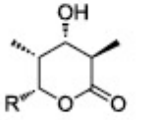
mechanism	condensation with retention	condensation with inversion	epimerization	product
I	Module 1	Module 2	none	
II	Modules 1 + 2		Module 2	
III		Modules 1 + 2	Module 1	
IV	Module 2	Module 1	Modules 1 + 2	

Figure 9: Experiment in vitro to determine the stereochemistry of condensation in modular PKS [46]. Use of specifically C-2-deuterium labeled extender unit **10** during biosynthesis with DEBS 1-TE (alongside starter unit butyryl-CoA **11** and NADPH **12**), resulted in a labeling pattern in the triketide lactone product **13**, which allowed discrimination between the four possible mechanisms for condensation in modules 1 and 2 of the PKS (the C-2 methyl center of the product is established by module 2 and the C-4 center by module 1). The obtained pattern (exclusive deuterium labeling at the C-2 position) was consistent with mechanism III (boxed) – inversion of stereochemistry in both modules as found for fatty acid synthase, with an additional epimerization occurring in module 1 to give the observed final configuration.

Production of polyketide structural analogues

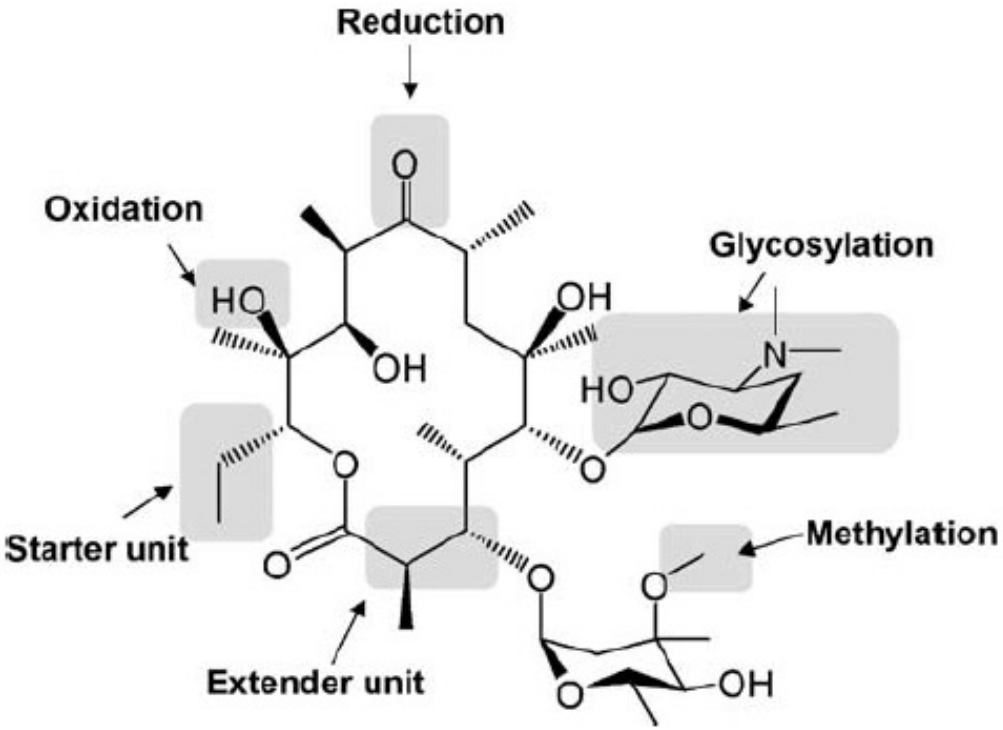
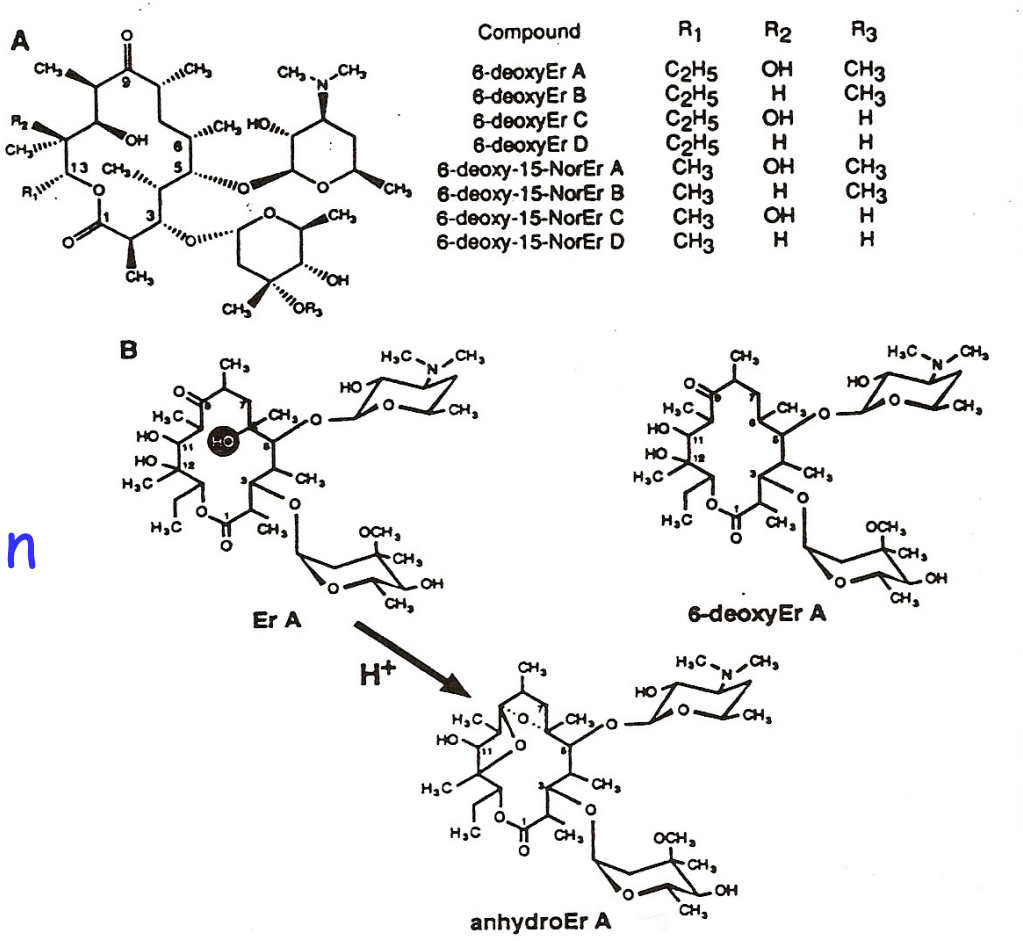


Fig. 3 Several biosynthetic targets for genetic engineering to generate novel macrolides. A wide variety of structural changes can be introduced into macrolactone ring biosynthesis by genetic engineering as follows: alteration of starter and extender units required for PKS and alteration of the extent of β-carbon processing and chain length. Modification of glycosylation and oxidation patterns by flexible GTs and monooxygenases, respectively, can lead to the generation of structurally-altered non-natural macrolides

Inactivation of the eryF gene to make erythromycin resistant to low pH

The C-6 OH group is involved in the formation of biologically inactive anhydro-erythromycin at acidic pH.



Compound	R_1	R_2	R_3
6-deoxyEr A	C_2H_5	OH	CH_3
6-deoxyEr B	C_2H_5	H	CH_3
6-deoxyEr C	C_2H_5	OH	H
6-deoxyEr D	C_2H_5	H	H
6-deoxy-15-NorEr A	CH_3	OH	CH_3
6-deoxy-15-NorEr B	CH_3	H	CH_3
6-deoxy-15-NorEr C	CH_3	OH	H
6-deoxy-15-NorEr D	CH_3	H	H

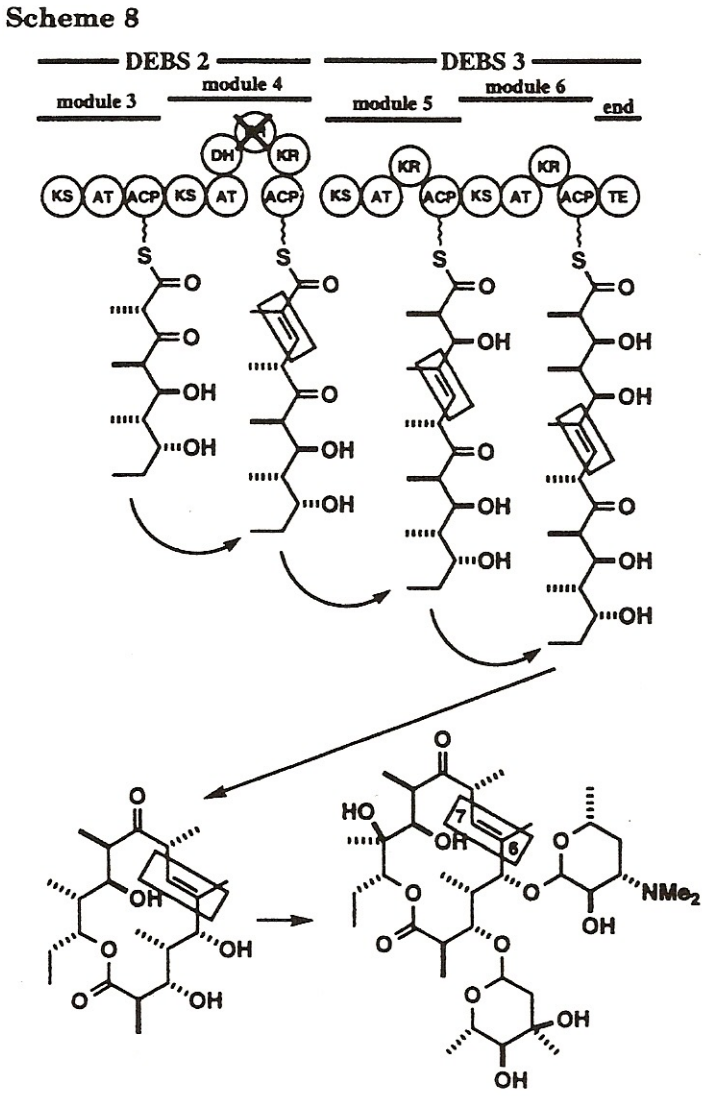
Fig. 2. (**A**) Structure (indicating stereochemistry) of 6-deoxyEr A and its biosynthetic precursors, 6-deoxyEr B, C, and D. Also shown are 6-deoxy-15-norEry A and its biosynthetic precursors. (**B**) Acid decomposition pathway of Er A to form anhydroEr A (*17, 18*), which involves the C-6 hydroxyl group (highlighted). 6-deoxy Er A is more resistant to acid inactivation because the C-6 hydroxyl group is absent (*21*).

Inactivation of the ER4 domain

The ER4 domain was inactivated by modifying the binding site for the NADPH cofactor

WT HAAAGGVGMA
Mut HAAASPVGMA

Inactivation of ER4 causes retention of the double bond between C-6 and C-7



Precursor-directed biosynthesis for production of structural analogues of erythromycin

KS1 is inactivated to avoid competition with the natural starter unit propionyl-CoA: mutation of the catalytic cysteine Cys→Ala

New starter units are supplied as N-acetylcisteamine thioesters (SNAC): substrate analogues (acyl-CoA)

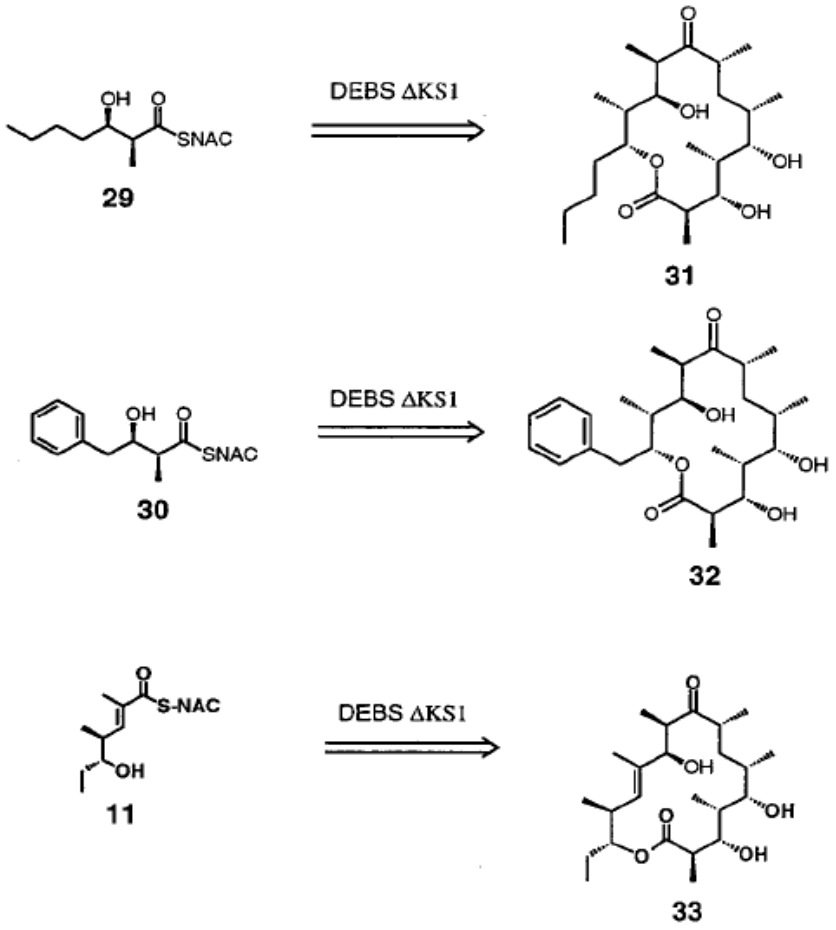


Figure 14. Precursor-directed biosynthesis of novel polyketides using genetically blocked modular polyketide synthases.

Precursor-directed biosynthesis for production of structural analogues of erythromycin

Structural analogues of 6-DEB are added to the growth medium of a *S. erythraea* strain that does not produce erythromycin.
They are processed and antibiotic activity is evaluated.

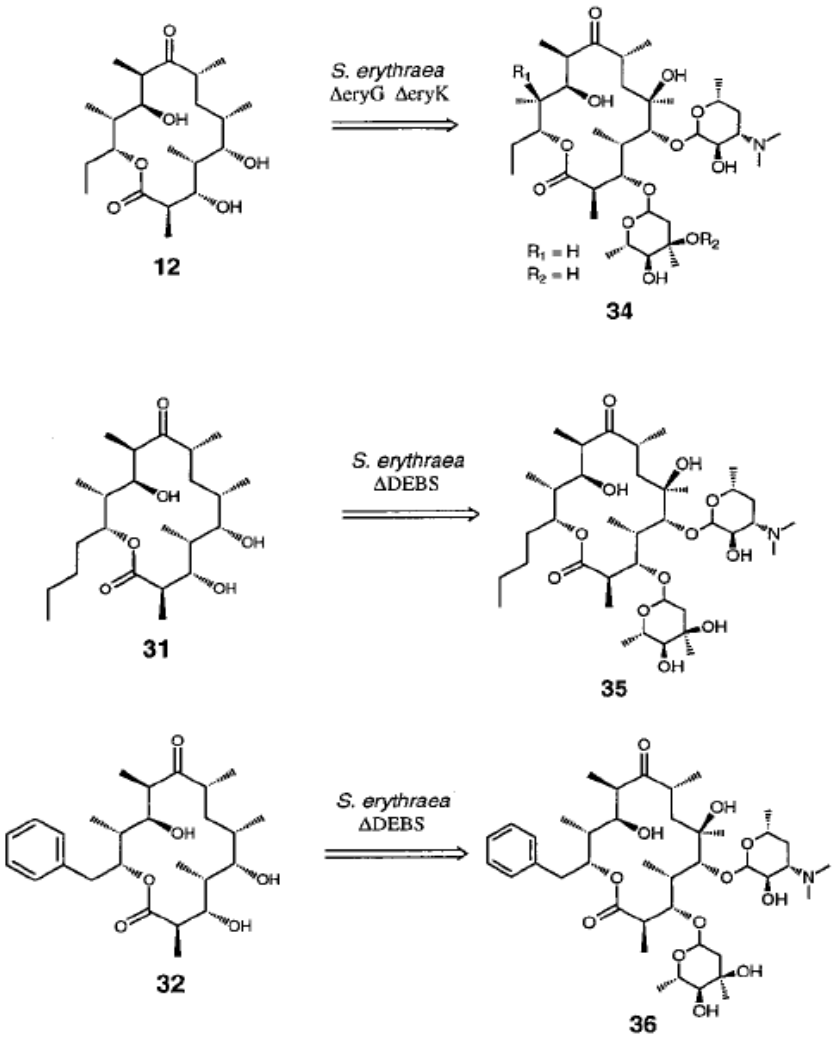
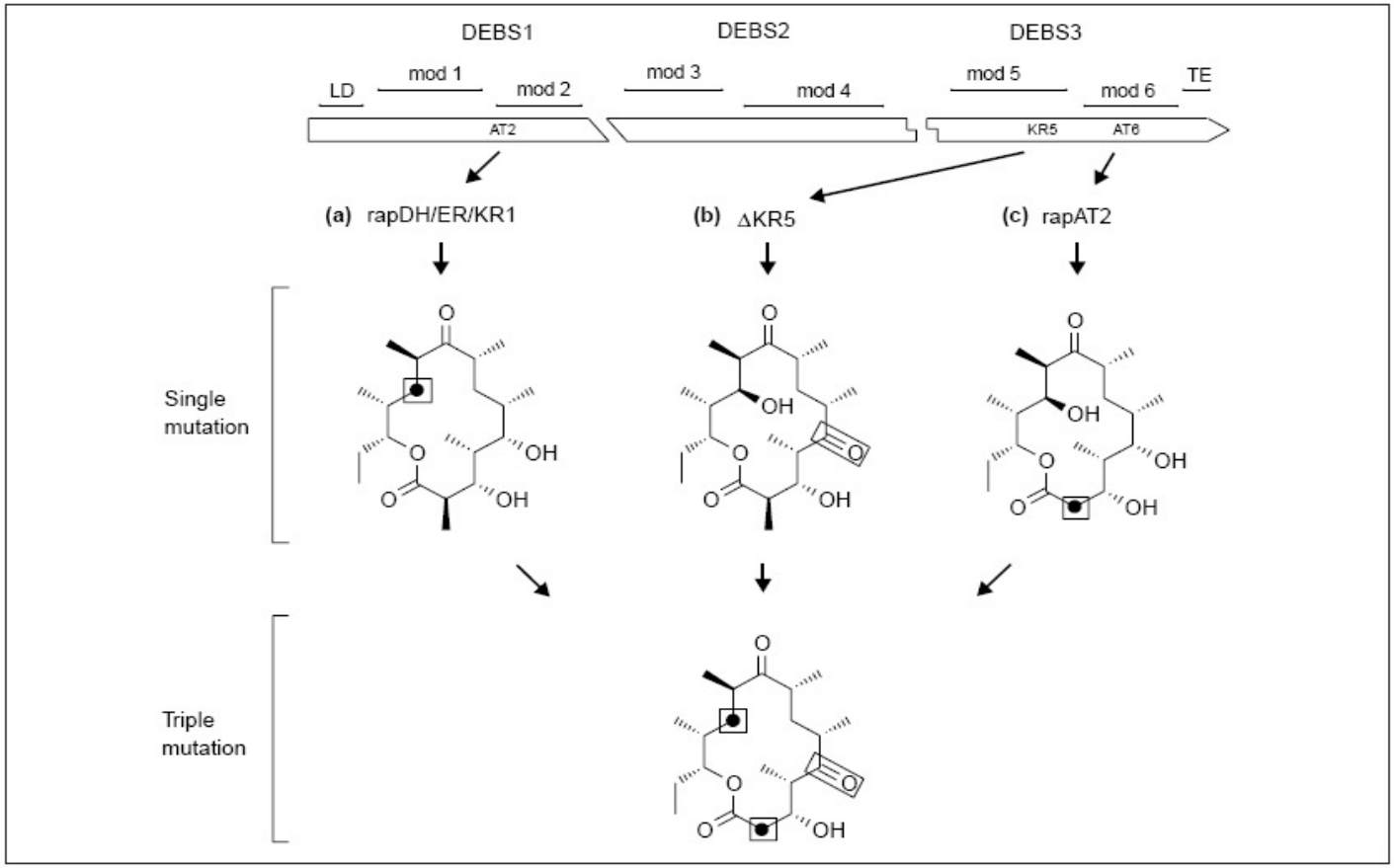


Figure 15. Bioconversion of structurally altered polyketide aglycons into novel erythromycin analogs.

Combinatorial approach for production of erythromycin structural analogues: domain inactivation and exchange

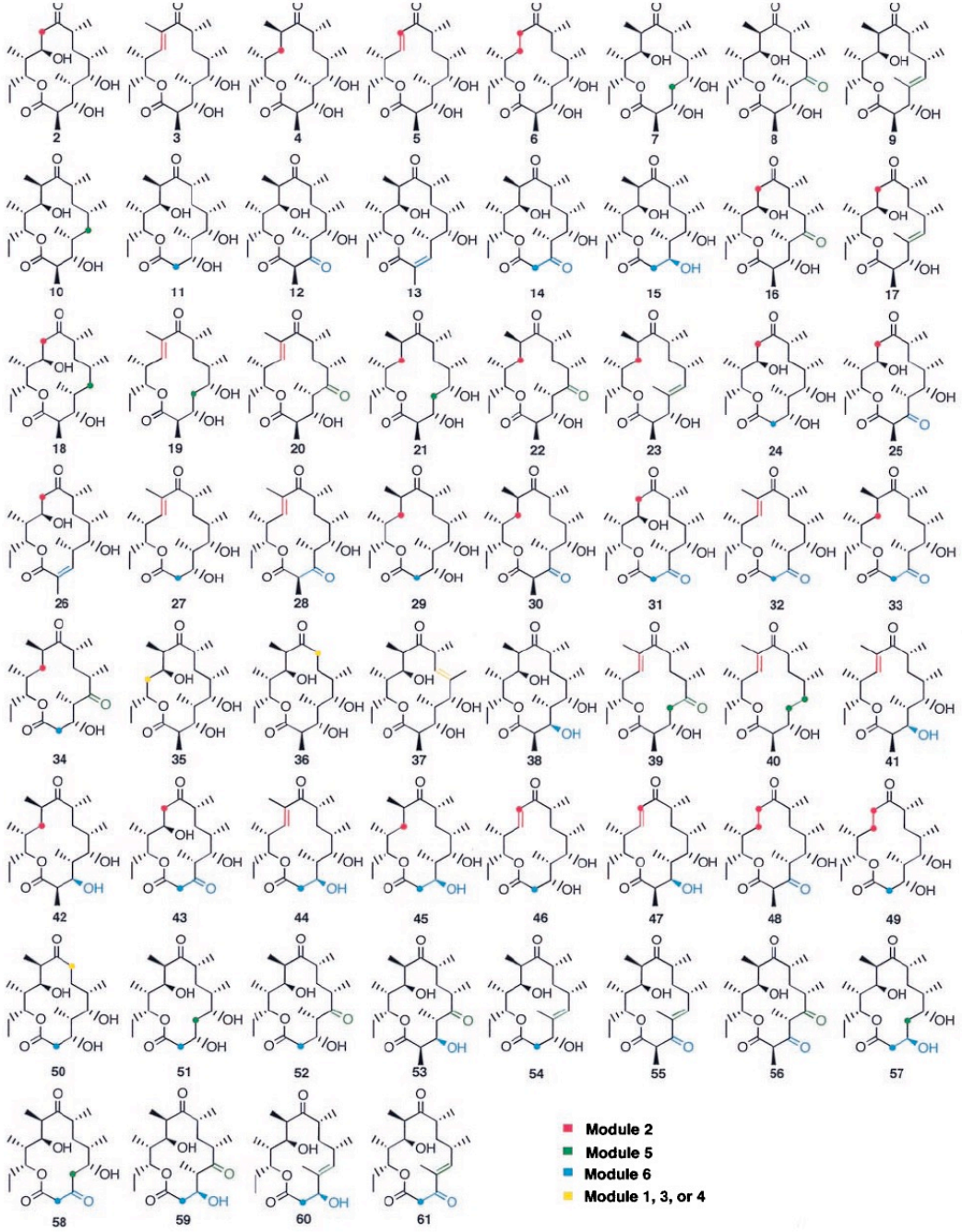


Combinatorial manipulation of DEBS. Single domain alterations that result in a functional PKS can be combined to generate multiple mutant PKSs. In this example, taken from [10••], three different single mutants could be combined to yield a functional triple mutant. The three mutations are: **(a)** substitution of the KR in module 2 with the DH/ER/KR domain from module 2 of the rapamycin (rap) PKS; **(b)** a KR deletion in module 5; and **(c)** substitution of the methylmalonyl-specific AT in module 6 with the malonyl-specific AT from module 2 of the rapamycin PKS.

Table 2. Combinatorial double and triple substitutions and polyketide products

Mutation			
Module 2	Module 5 or 6	6dEB analog product	Compound no.
Module 2 × module 5 double mutants			
*rap*AT2	AT/ACP linker	5-deoxy-5-oxo-10-desmethyl	**16**
*rap*AT2	*rap*DH/KR4	4,5-anhydro-10-desmethyl	**17**
*rap*AT2	*rap*DH/ER/KR1	5-deoxy-5-oxo-10-desmethyl; 5-deoxy-10-desmethyl	**16, 18**
*rap*DH/KR4	*rap*AT2	4-desmethyl-10,11-anhydro	**19**
*rap*DH/KR4	AT/ACP linker	5-deoxy-5-oxo-10,11-anhydro	**20**
*rap*DH/KR4	*rap*DH/ER/KR1	NP	
*rap*DH/ER/KR1	*rap*AT2	4-desmethyl-11-deoxy	**21**
*rap*DH/ER/KR1	AT/ACP linker	5,11-dideoxy-5-oxo	**22**
*rap*DH/ER/KR1	*rap*DH/KR4	4,5-anhydro-11-deoxy	**23**
Module 2 × module 6 double mutants			
*rao*AT2	*rap*AT2	2,10-didesmethyl	**24**
*rap*AT2	AT/ACP linker	3-deoxy-3-oxo-10-desmethyl	**25**
*rap*AT2	*rap*DH/KR4	2,3-anhydro-10-desmethyl	**26**
*rap*DH/KR4	*rap*AT2	2-desmethyl-10,11-anhydro	**27**
*rap*DH/KR4	AT/ACP linker	3-deoxy-3-oxo-10,11-anhydro	**28**
*rap*DH/ER/KR1	*rap*AT2	2-desmethyl-11-deoxy	**29**
*rap*DH/ER/KR1	AT/ACP linker	3-deoxy-3-oxo-11-deoxy	**30**
Module 2 × module 6 triple mutants			
*rap*AT14	*rap*AT2+AT/ACP linker	2,10-didesmethyl-3-deoxy-3-oxo	**31**
*rap*DH/KR4	*rap*AT2+AT/ACP linker	2-desmethyl-3-deoxy-3-oxo-10,11-anhydro	**32**
*rap*DH/ER/KR1	*rap*AT2+AT/ACP linker	2-desmethyl-3,11-dideoxy-3-oxo	**33**
Module 2 × module 5 × module 6 triple mutant			
KR2→ *rap*DH/ER/KR1	KR5→AT/ACP linker, AT6→*rap*AT2	2-desmethyl-5,11-dideoxy-5-oxo	**34**

Compound yields from all the multiple mutants fell to >0.1 mg/liter and could not be determined accurately by evaporative light scattering detection. The primary exception was compound **29**, which was producd at ≈0.2 mg/liter. NP, no product.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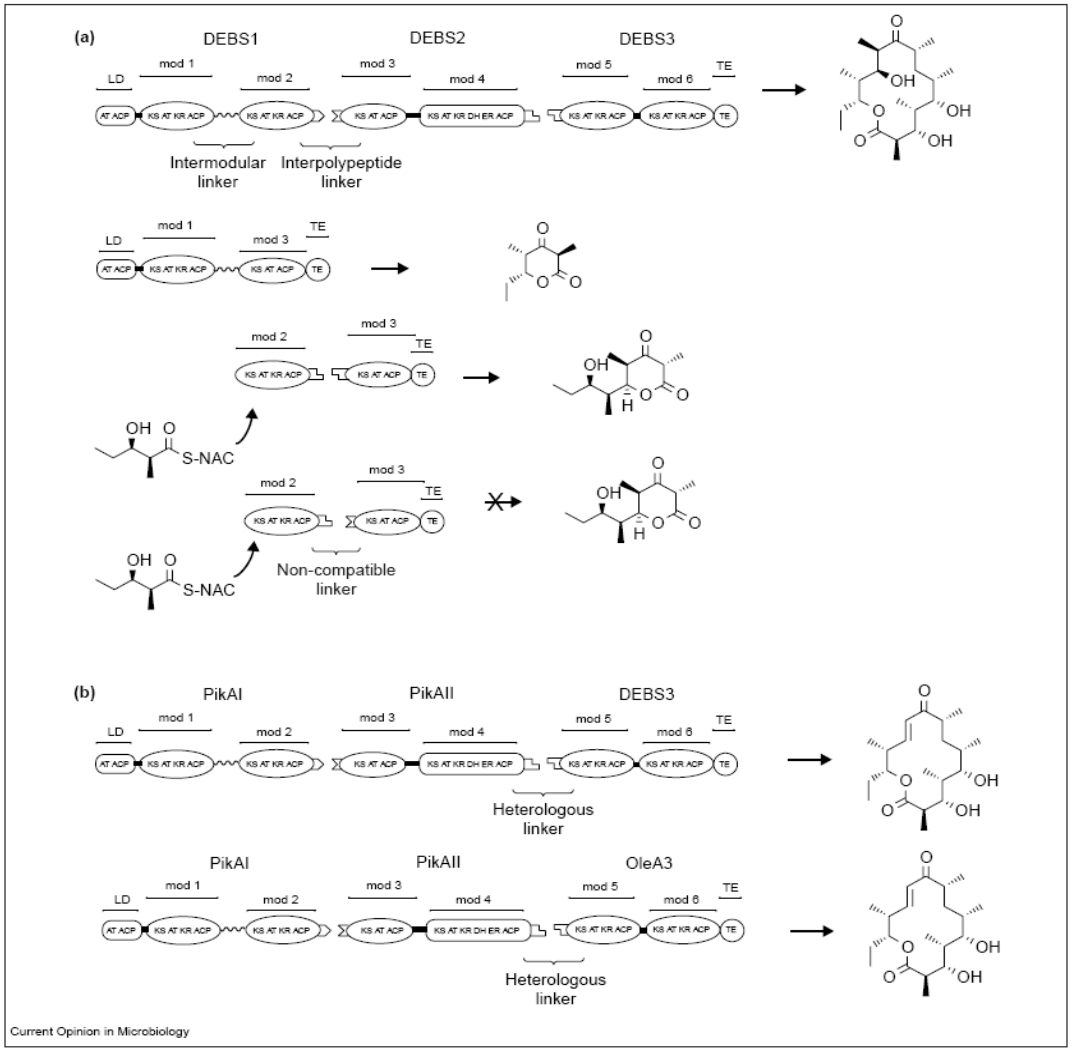
Module 2
Module 5
Module 6
Module 1, 3, or 4

Molecular recognition between modules



Directed chain transfer between modules and subunits in modular PKSs. **(a)** Distinct intermodular and interpolypeptide linkers have been identified and used to direct polyketide intermediates between modules. Compatible amino- and carboxy-terminal linker pairs must be used for productive association of modules. **(b)** Complete subunits encoding multiple modules from heterologous sources can be used in functional complementation. PKS subunits from the picromycin PKS (PikAI and PikAII) were combined with DEBS and oleandomycin PKS (OleA3) subunits. It is interesting that, in these cases, non-cognate interpolypeptide linkers remained compatible.

Molecular recognition between modules

Role of the ACP domain in substrate 'channeling' to the KS domain

Substrate release is fast when ACP-TE is in *cis*

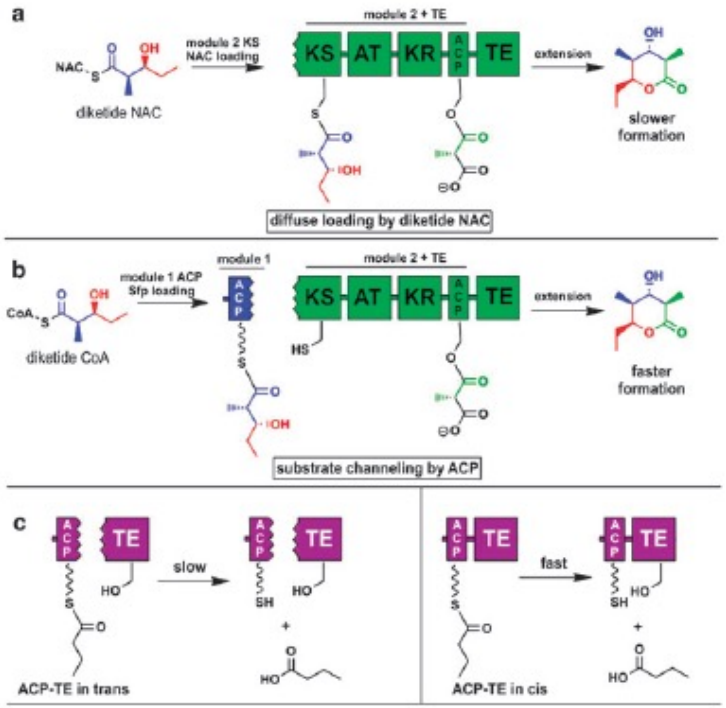


Fig. 10 Acyl CoA probes of PKS condensation and chain termination. (a) Incubation of DEBS module 2 + TE with a diketide-NAC results in diffuse loading of the KS cysteine, followed by condensation and chain cleavage to yield a triketide lactone. (b) Incubation of DEBS module 2 + TE with an Sfp loaded diketide-S-ACP substrate allows ACP-mediated substrate channeling of the diketide intermediate, resulting in faster formation of the triketide lactone and the ability to process noncognate substrates. (c) Kinetic studies of TE-mediated cleavage of an Sfp loaded butyryl-S-ACP in the DEBS system show significantly slower cleavage when the substrate is presented in *trans* (left) *versus* in *cis* on the same polypeptide (right).

Structure of linker regions between PKS DEBS2 and DEBS3 modules

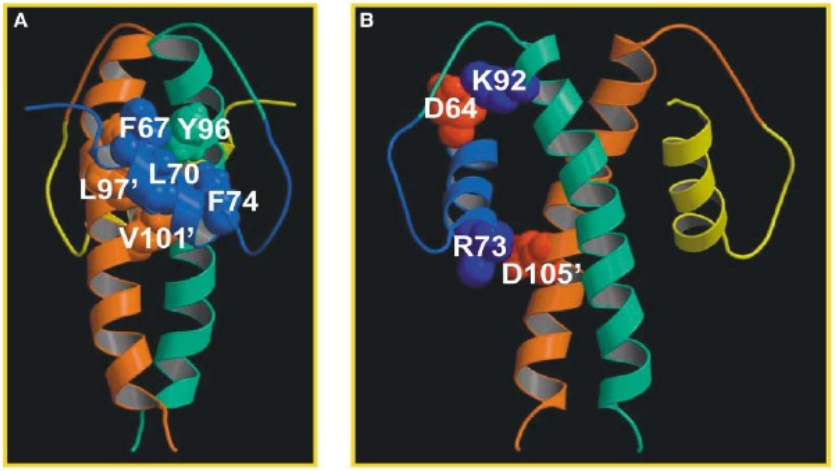


Figure 6. Residues Involved in Docking between DEBS 2 and DEBS 3

(A) The parallel four-helix bundle is held together by a series of hydrophobic interactions between helix 3 and 3′ and the coil by helices 4 and 4′.

(B) Partially buried salt bridges at the ends of helices 3 and 3′ may play a role in determining the specificity of docking in the

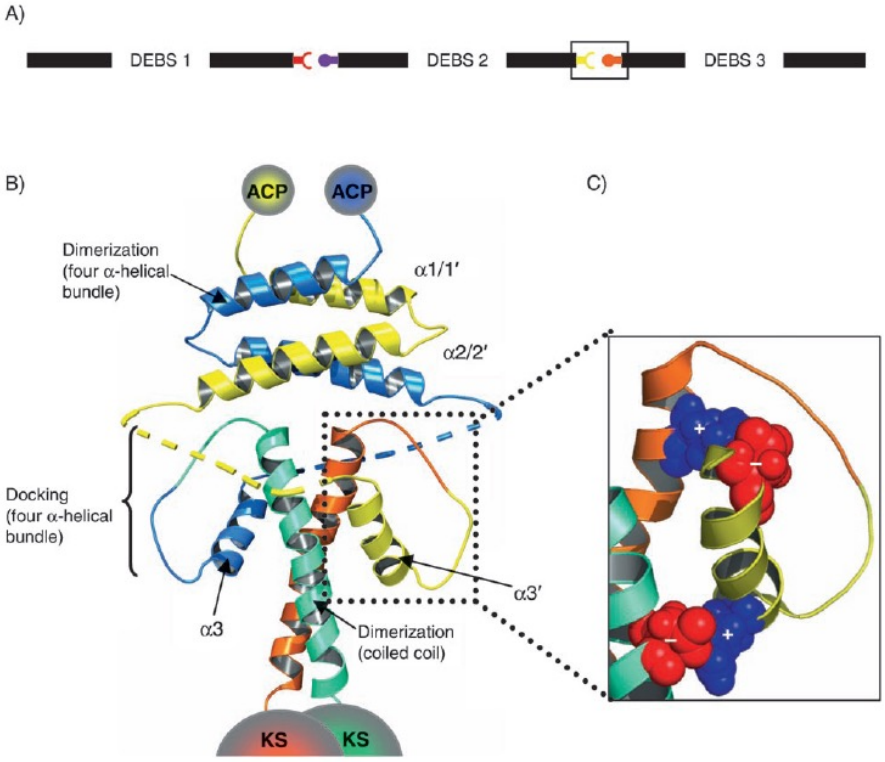


Figure 13. The structural basis for docking in modular PKS. A) PKS docking domains are located at the extreme C and N termini of the subunits. The complex of docking domains solved by NMR spectroscopy models the junction between polypeptides DEBS 2 and DEBS 3 in the erythromycin PKS. B) NMR solution structure of the DEBS docking complex. The dimeric C-terminal docking domain is shown in blue and yellow (three helices), while the dimeric N-terminal docking domain is shown in green and orange. Two dimerization elements are present, an intertwined four α-helical bundle formed by helices 1, 1′, 2, and 2′, and a coiled-coil motif formed by the N-terminal docking domain. Docking between the two domains, results in formation of a second four α-helical bundle, as indicated. The linker region between helices 2 and 3 is highly mobile, and therefore is represented as a dashed line in the structure. C) Charged residues located at critical positions in the interface (see box in B) are likely to contribute to the specificity of docking.

Charge complementarity is probably at the basis of module recognition

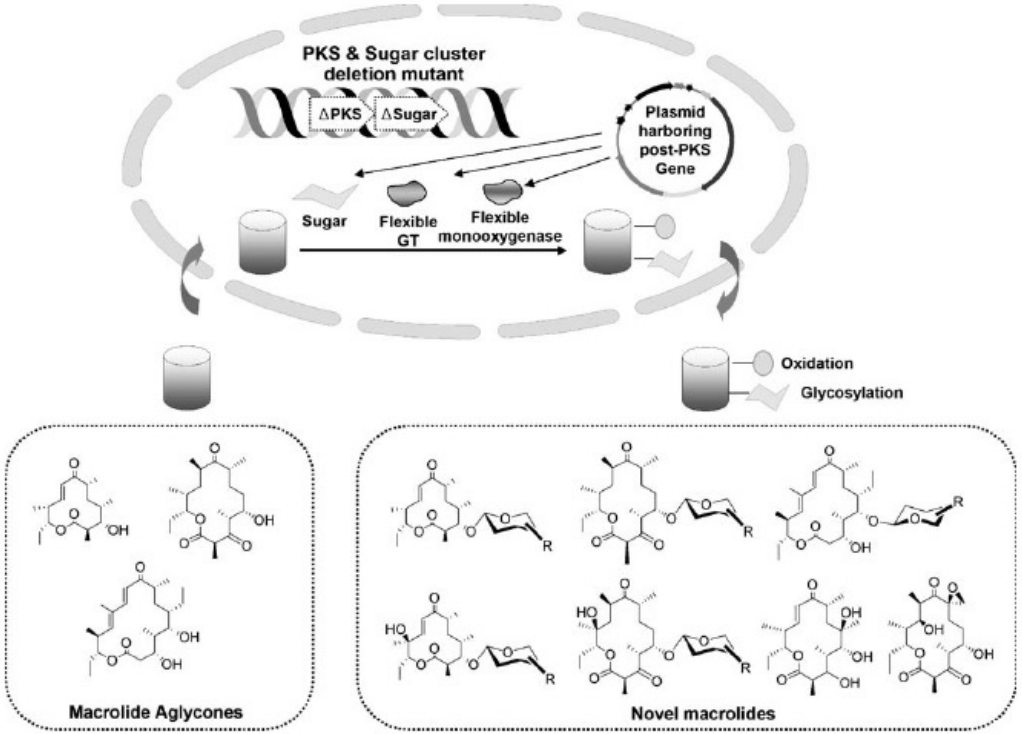


Fig. 5 Concept of combinatorial biosynthesis of post-PKS modification steps. Combinatorial biosynthesis is the application of genetic engineering to the modification of natural product biosynthetic pathways in order to produce unnatural or hybrid natural products. Generating glycosylated and/or oxygenated metabolites via combinatorial biosynthesis requires a flexible GT and/or monooxygenase active toward the sugar acceptor (aglycone) and sugar donor substrates

Combinatorial approach for production of polyketide structural analogues: modification of sugars. Glycosyltransferases and monoxygenases with broad substrate specificity

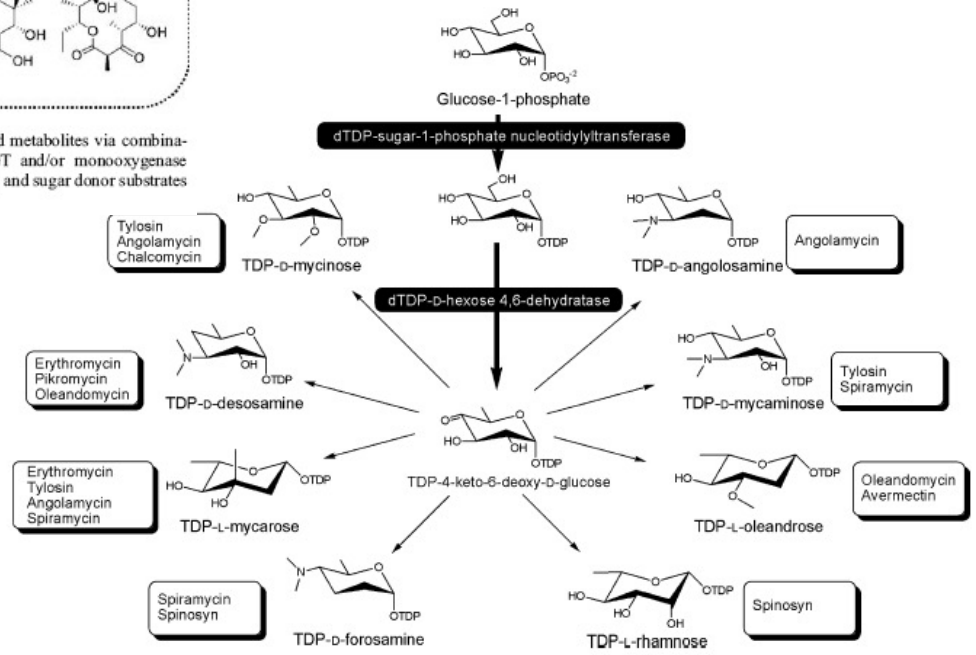
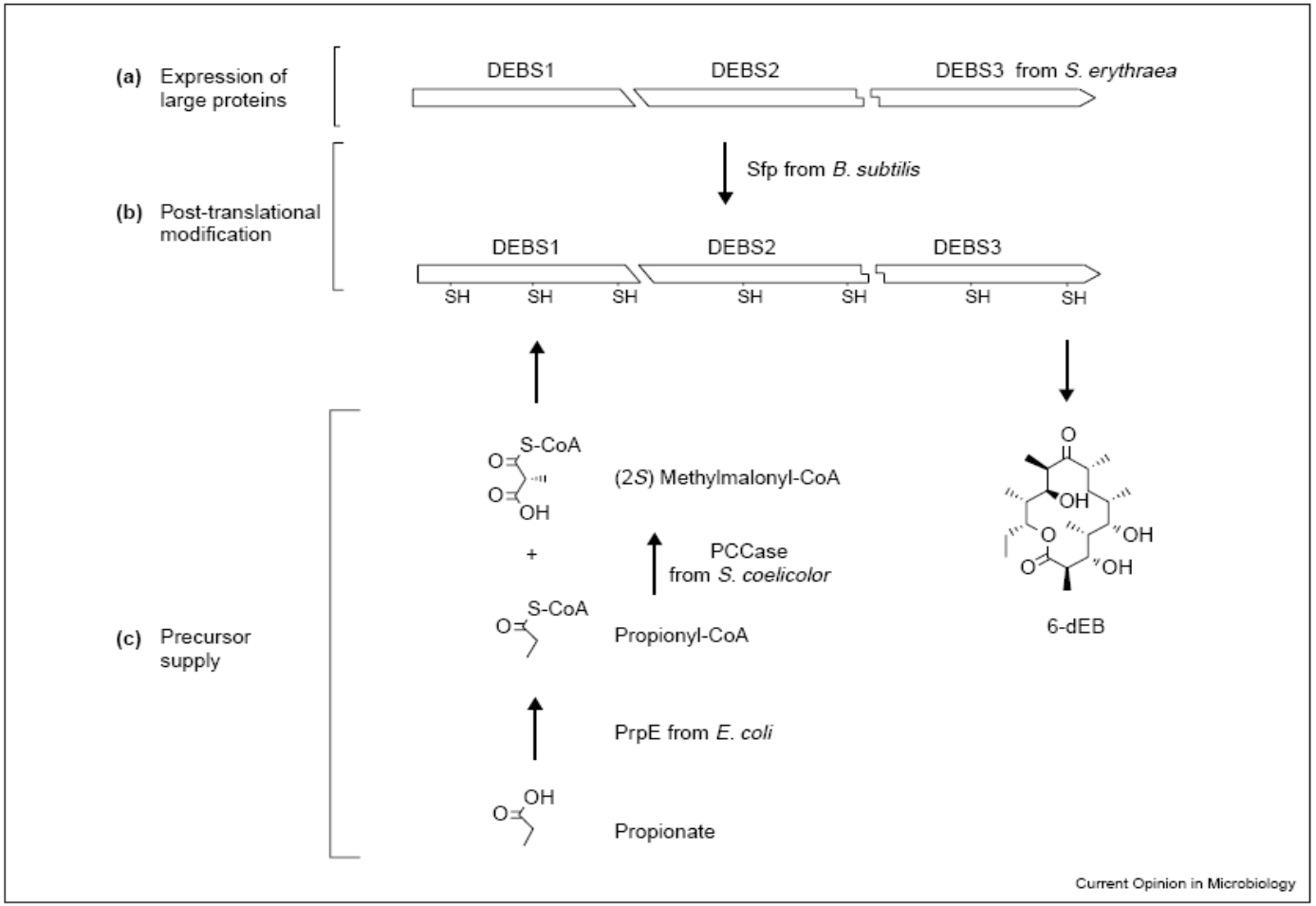


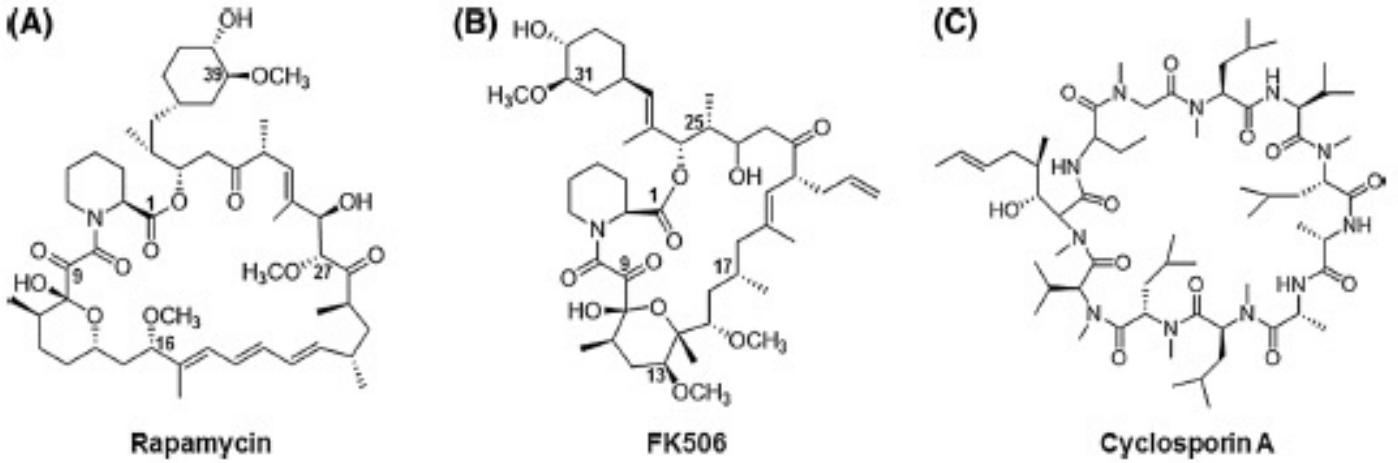
Fig. 4 Schematic representation of the pathways for 6-deoxysugar nucleotide biosynthesis. Two early enzymatic steps in the biosynthesis of the common intermediate dTDP-4-keto-6-deoxy-D-glucose are indicated. Structural diversity in the sugar family arises from further modifications of the 4-keto-6-deoxy intermediate through specific enzymatic reactions that affect different carbons of the hexose chain

Metabolic engineering in *E. coli* for production of polyketides



Engineering *E. coli* to produce polyketides. **(a)** Conditions for expression of large protein complexes, **(b)** a suitable phosphopantetheinyl transferase (Sfp), and **(c)** native PrpE propionyl-CoA synthase and PCC propionyl-CoA carboxylase from *S. coelicolor* required for appropriate precursor metabolism were all necessary to achieve biosynthesis of 6-dEB in *E. coli*.

Immunosuppressors: Rapamycin



Rapamycin was isolated in 1975 from *Streptomyces hygroscopicus*, it has antifungal, antitumour and immunosuppressive activity.
It is an example of a metabolite where the polyketide backbone is bound to an amino acid.

Mechanism of action of Rapamycin

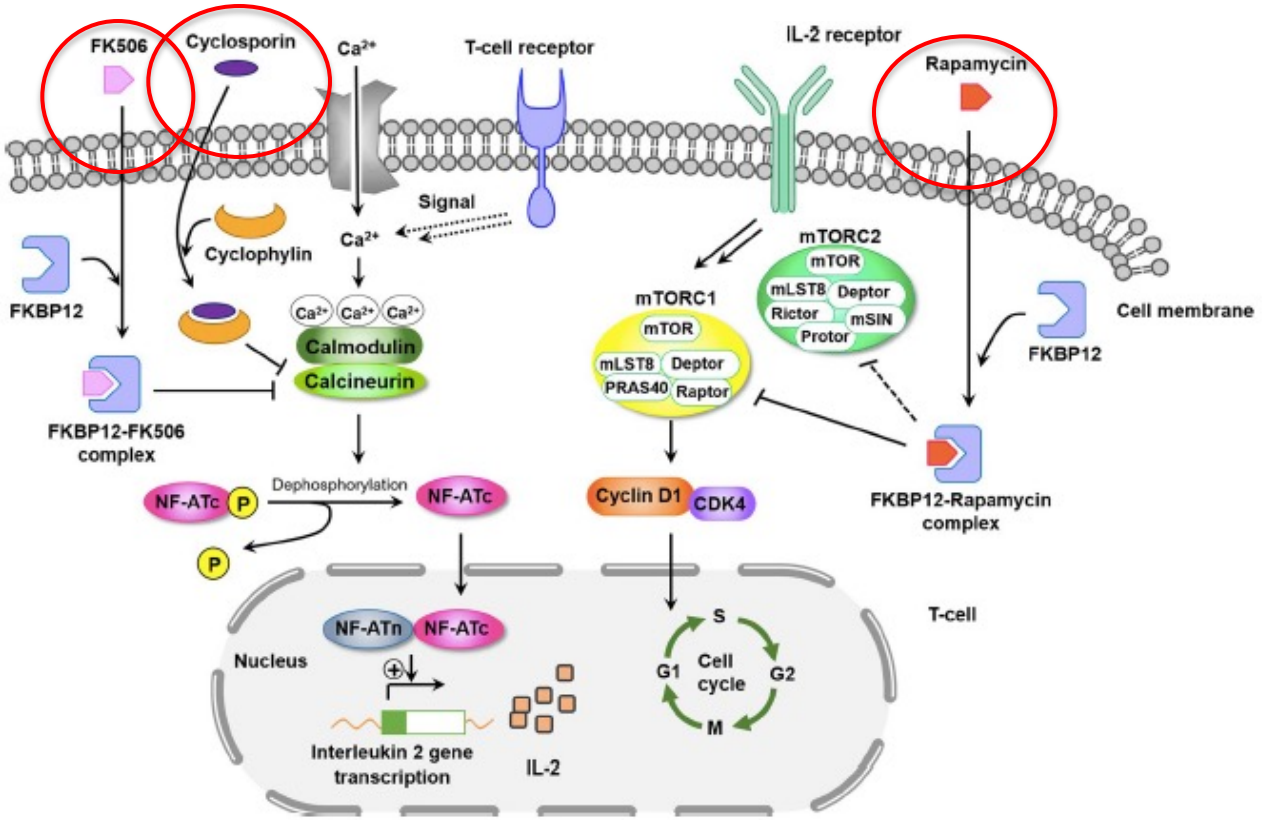
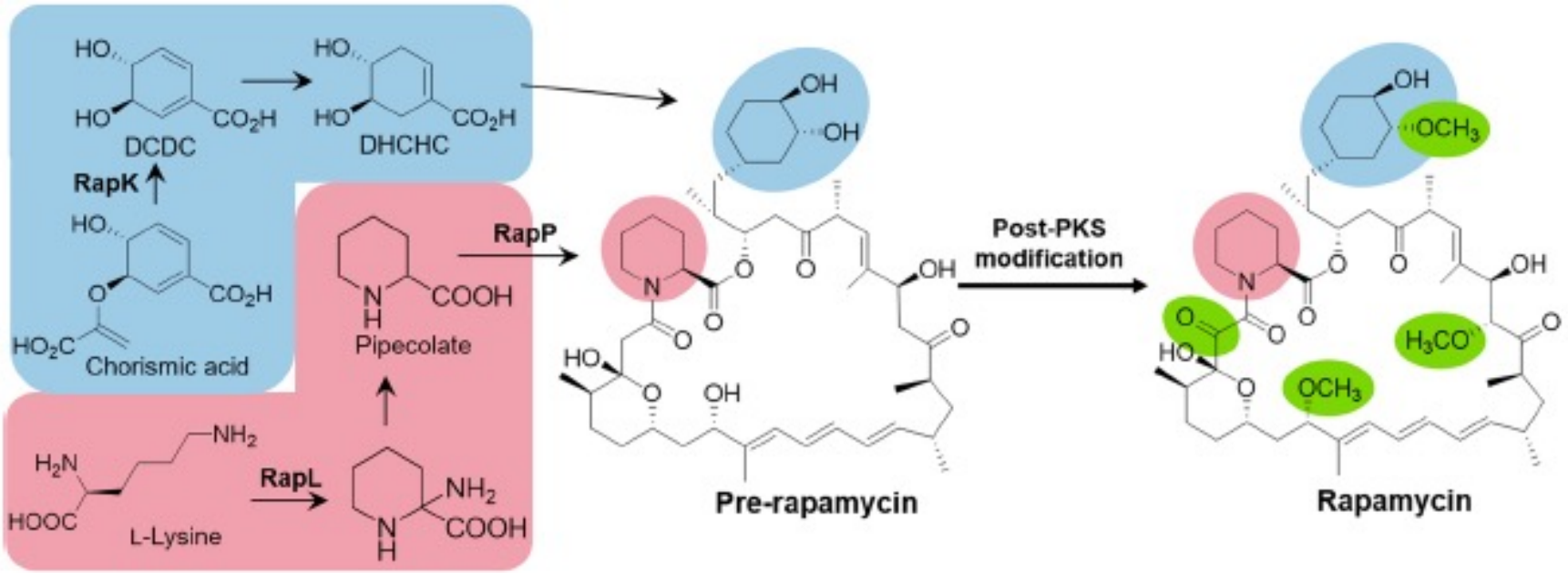
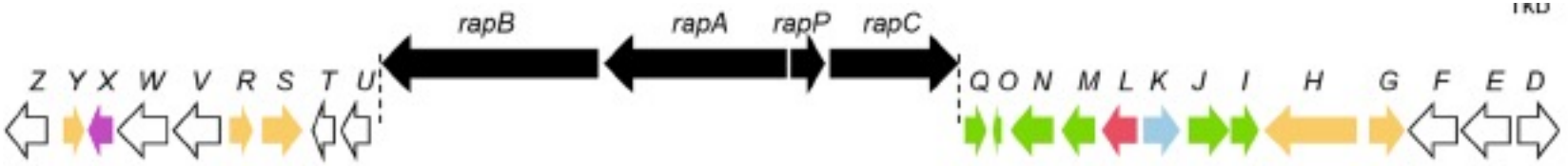


Fig. 2 Mechanism of action of immunosuppressive agents, such as rapamycin, FK506, and cyclosporin. Rapamycin inhibits mammalian target of rapamycin (mTOR) by binding to its intracellular receptor FK506-binding protein 12 (FKBP12). mTOR is the catalytic subunit of two structurally distinct complexes, such as mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). While the mTORC1 is rapamycin sensitive (*solid line*), mTORC2 is affected by chronic exposure (*dotted line*) to rapamycin. Rapamycin bound mTORC1 led to the inhibition of the cell cycle progression of T cells from G1 to S phases, thereby suppressing T-cell proliferation. In contrast, FK506 and cyclosporin bind to FKBP and cyclophylin, respectively, and the resulting complexes block the calcineurin that is required for dephosphrylation of the cytoplasmic component of the nuclear factor of activated T cells (NF-ATc). This results in the inhibition of the translocation of NF-ATc to the nucleus and the binding of NF-ATc to the nuclear component of the nuclear factor of activated T cells (NF-ATn) preventing interleukin 2 (IL-2) production and subsequent T-cell proliferation. Protein abbreviations: *mLST8* mammalian homolog of protein Lethal with SEC12, *Deptor* DEP-domain containing mTOR-interacting protein, *PRAS40* proline-rich AKT substrate of 40 kDa, *Raptor* regulatory-associated protein of mTOR, *mSIN* mitogen-activated protein kinase-associated protein, *Rictor* rapamycin-insensitive companion of TOR, *Protor* proline-rich protein, *CDK* cyclin dependent kinases

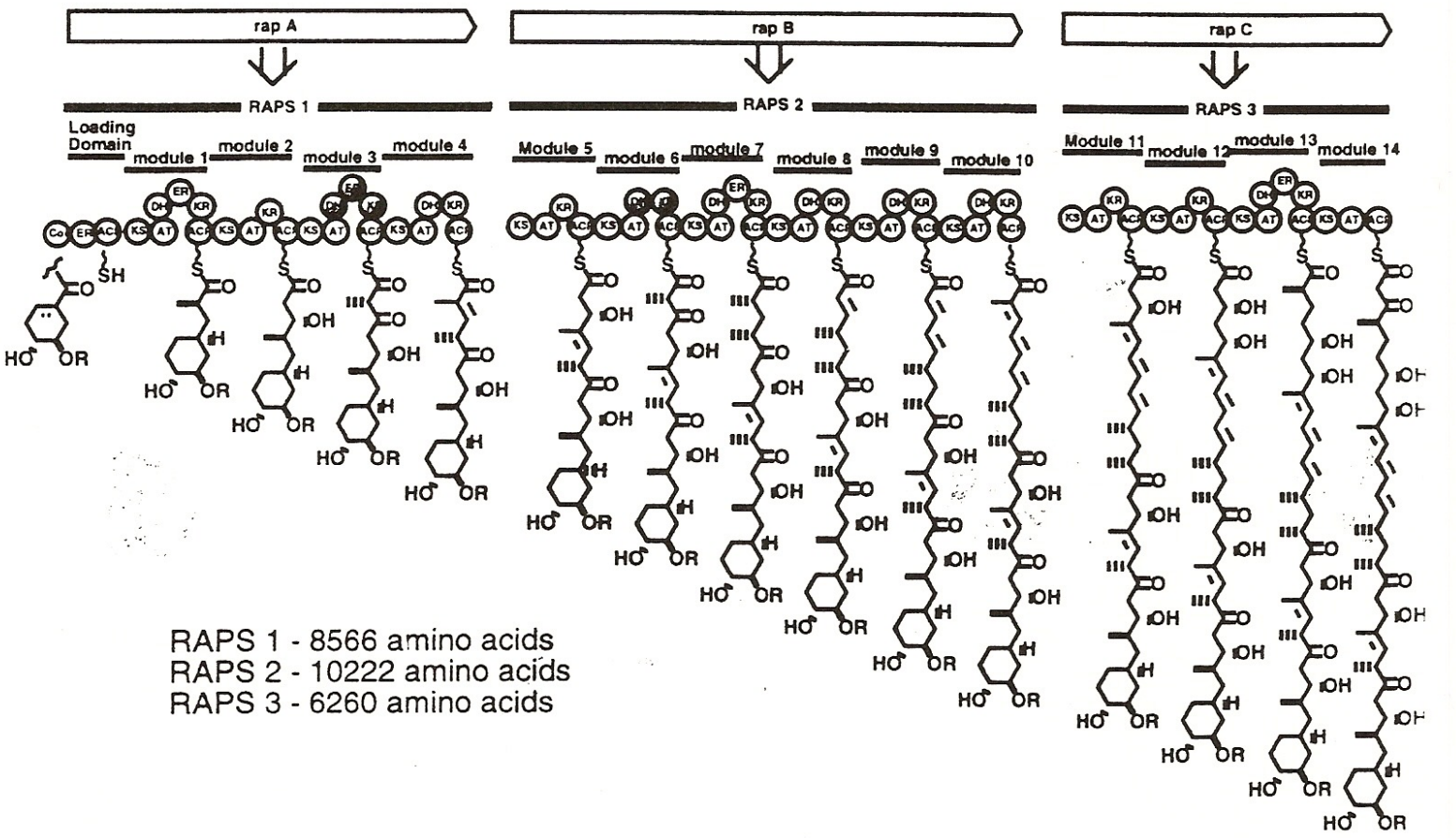
Biosynthesis of Rapamycin





PKS and NRPS genes
Regulatory genes
lysine cyclodeaminase gene
chrorismatase for the starter unit biosynthesis
ABC-transporter gene
Unknown genes
Post-PKS genes
Thioesterase
Other genes

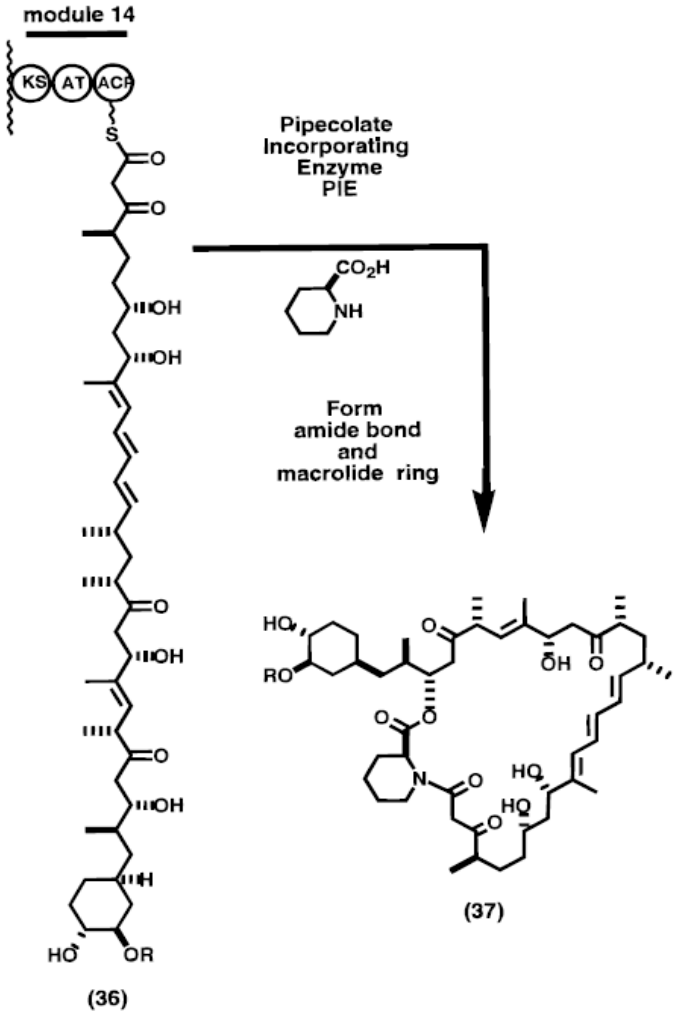
Organization of rapamycin PKS



Biosynthesis of Rapamycin

Polyketide release from the last module of RAPS3 is catalyzed by rapP, that incorporates pipecolate. This enzyme is highly homologous to NRPS

Hybrid multienzymatic systems PKS-NRPS



Structural organization of multienzymatic systems: PKS, NRPS and mixed NRPS-PKS

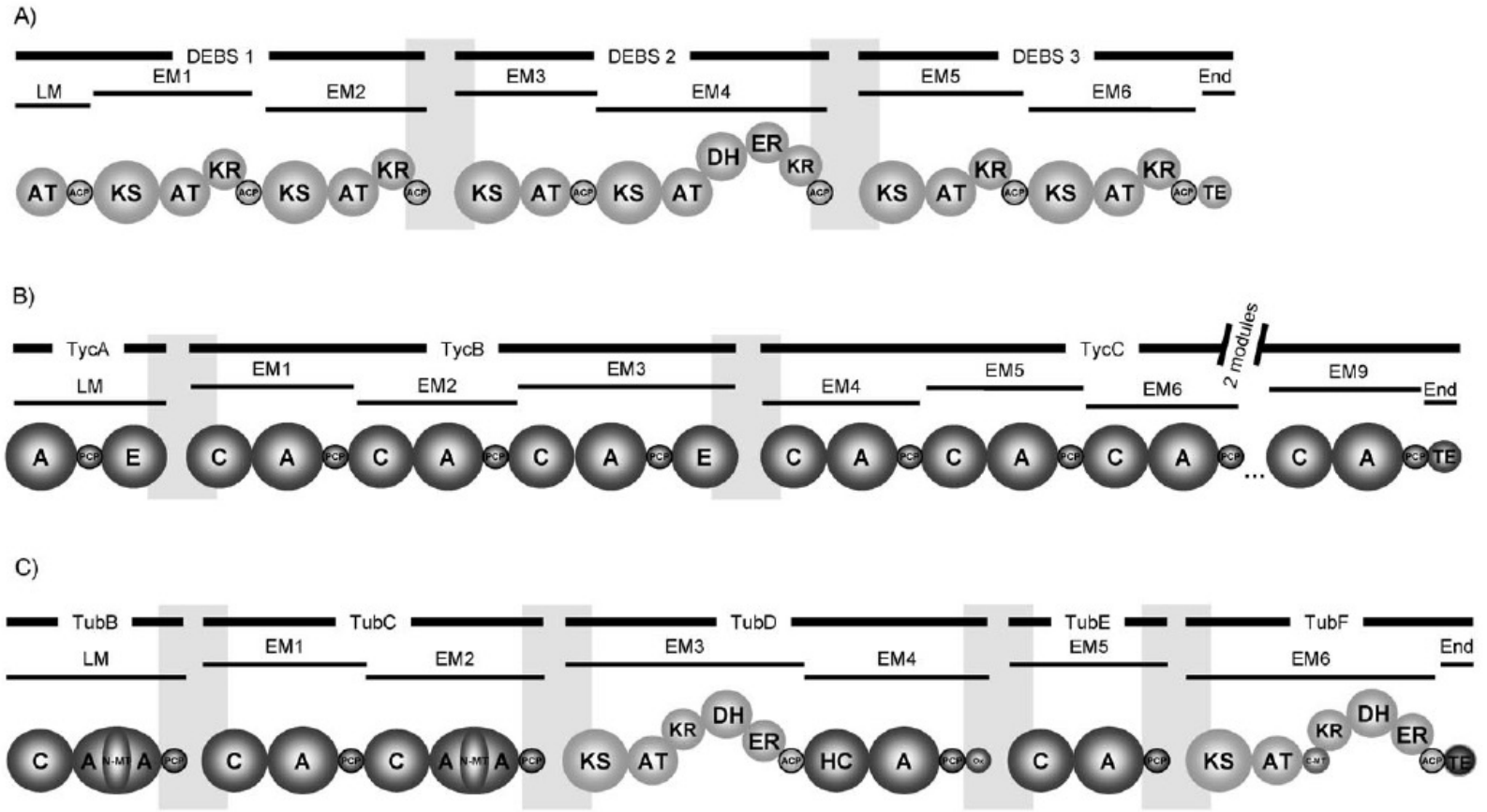


Figure 12. Multi-multienzyme organization of PKS, NRPS, and mixed PKS–NRPS systems. A) The 6-deoxyerythronolide B synthase (DEBS) responsible for erythromycin biosynthesis incorporates three subunits, DEBS 1, 2, and 3. Two intermodular junctions are formed across intersubunit interfaces (boxed gray regions). B) NRPS subunits TycA, TycB, and TycC cooperate to assemble the polypeptide tyrocidine. Chain extension occurs across two intersubunit interfaces (boxed gray regions). C) Tubulysin is assembled by a hybrid PKS–NRPS that incorporates three NRPS subunits (TubB, TubC, and TubE), a PKS subunit (TubF), and a mixed PKS–NRPS subunit (TubD). Both chain extension (NRPS–NRPS) and chain transfer (NRPS–PKS) are accomplished by domains located at intersubunit junctions (boxed gray regions). Abbreviations are: Ox, oxidase; C-MT, *C*-methyltransferase.