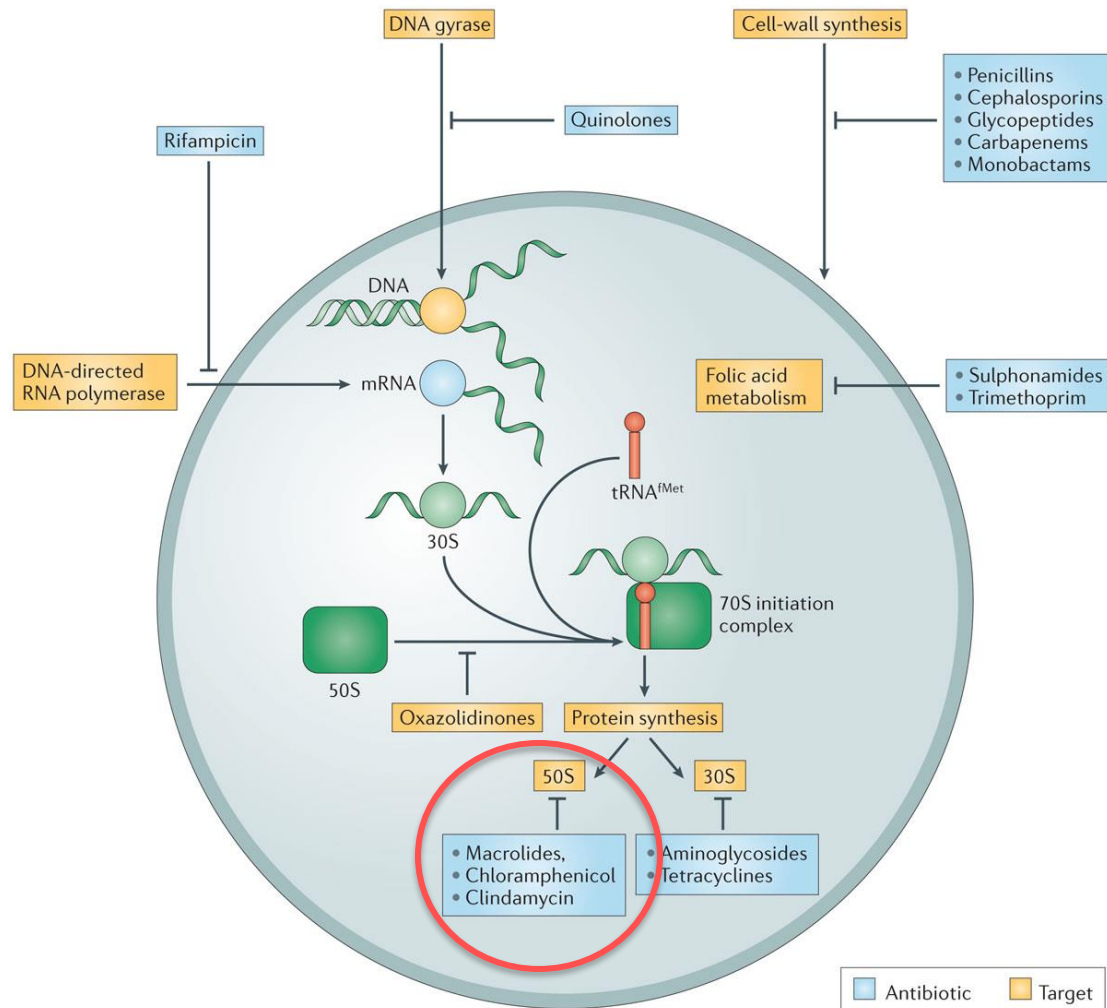
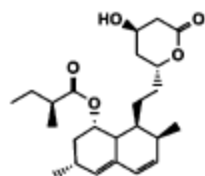


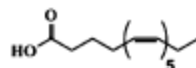
Complessi multienzimatici nella
biosintesi dei polichetidi:
le polichetide sintasi (PKS)

Bersagli degli antibiotici

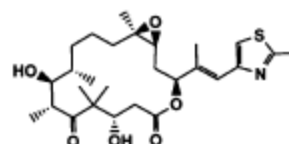




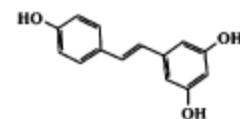
lovastatin
Aspergillus terreus
anticholesterol polyketide



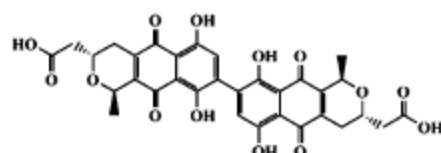
eicosapentanoic acid
Shewanella onedensis
fatty acid supplement



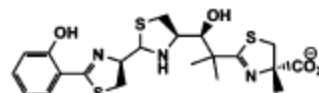
epothilone B
Sorangium cellulosum
anticancer polyketide-peptide



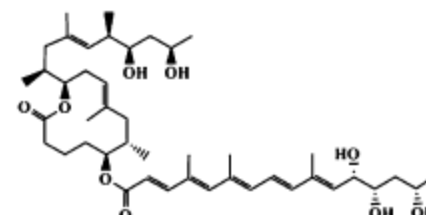
resveratrol
several plants including *Vitis*
polyketide antioxidant



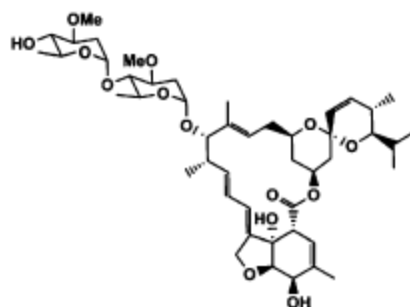
actinorhodin
Streptomyces coelicolor
polyketide pigment



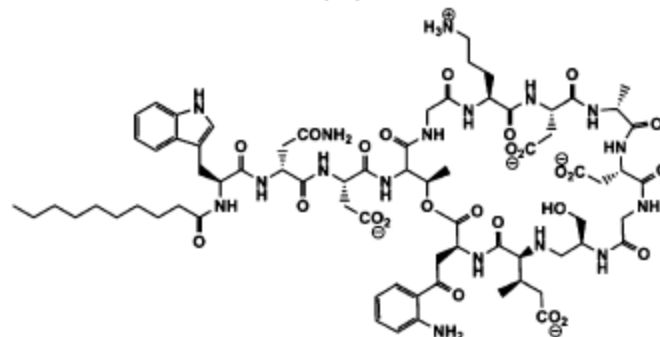
yersiniabactin
Yersinia pestis
nonribosomal peptide virulence factor



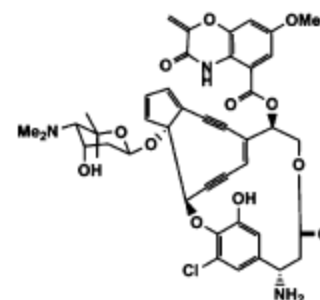
mycolactone B
Mycobacterium ulcerans
polyketide virulence factor



avermectin B1b
Streptomyces avermitilis
polyketide antiparasitic

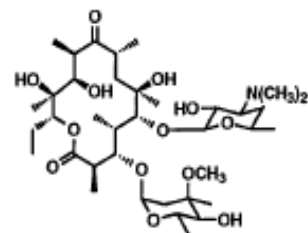


daptomycin
Streptomyces roseosporus
lipopeptide antibiotic

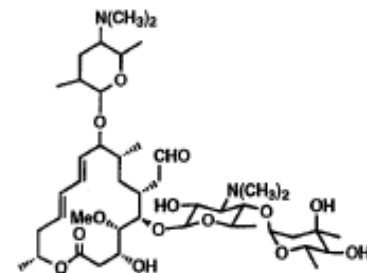


C-1027
Streptomyces globisporus
anticancer polyketide

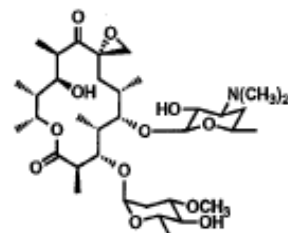
Fig. 1 Structures, producers, and biological activities of some notable fatty acid, polyketide, and nonribosomal peptide natural products.



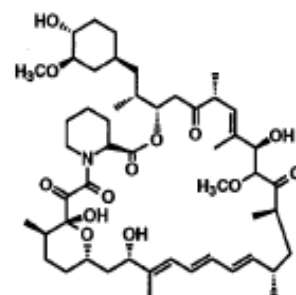
Erythromycin A (1)



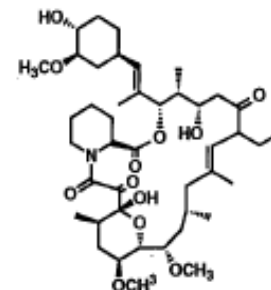
Spiramycin I (3)



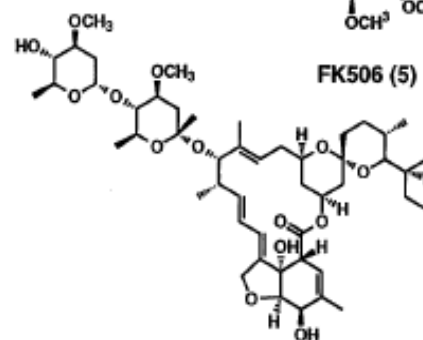
Oleandomycin (2)



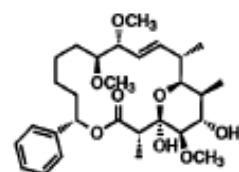
Rapamycin (4)



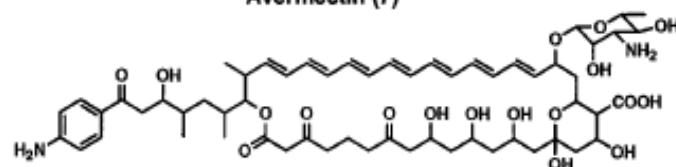
FK506 (5)



Avermectin (7)



Soraphen A (6)



Candicidin D (8)

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

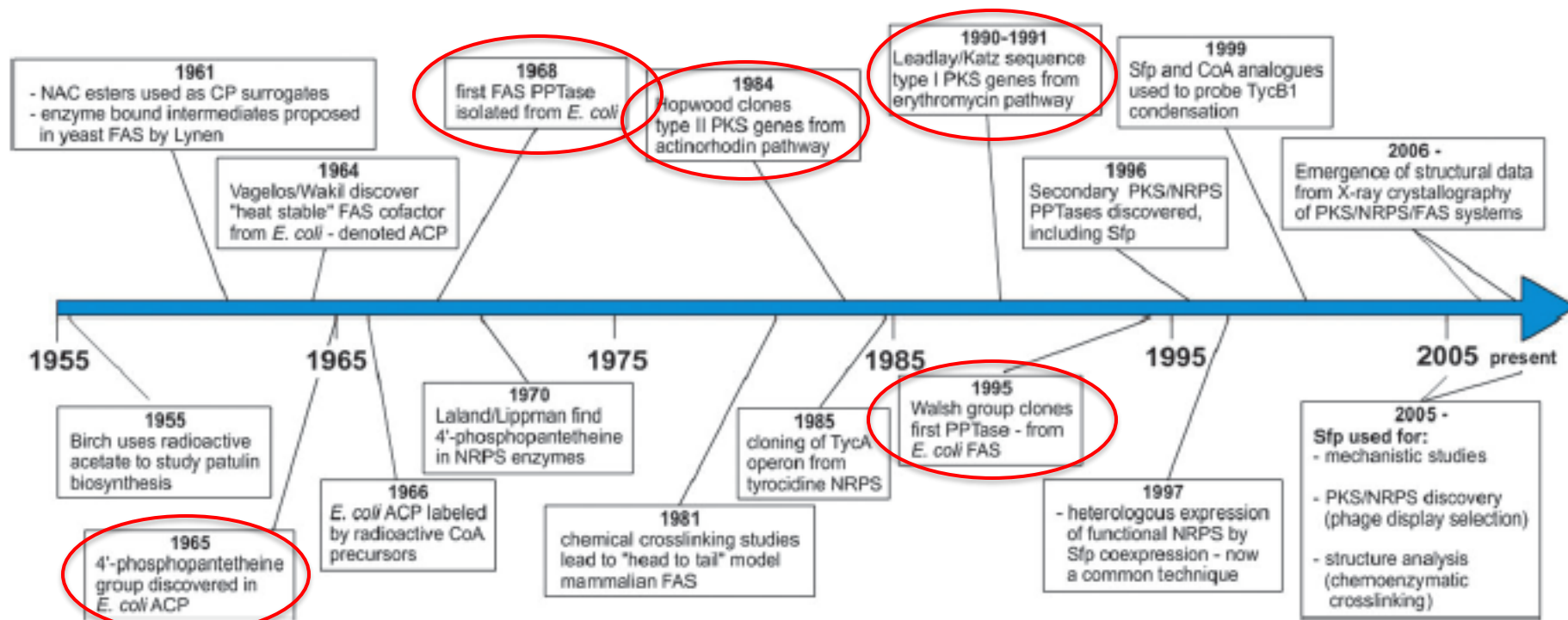


Fig. 2 Timeline of some notable events in the study of carrier-protein-mediated FAS, PKS, and NRPS biosynthetic enzymes.

Organizzazione strutturale dei sistemi multienzimatici FAS (fatty acid synthase), PKS e NRPS

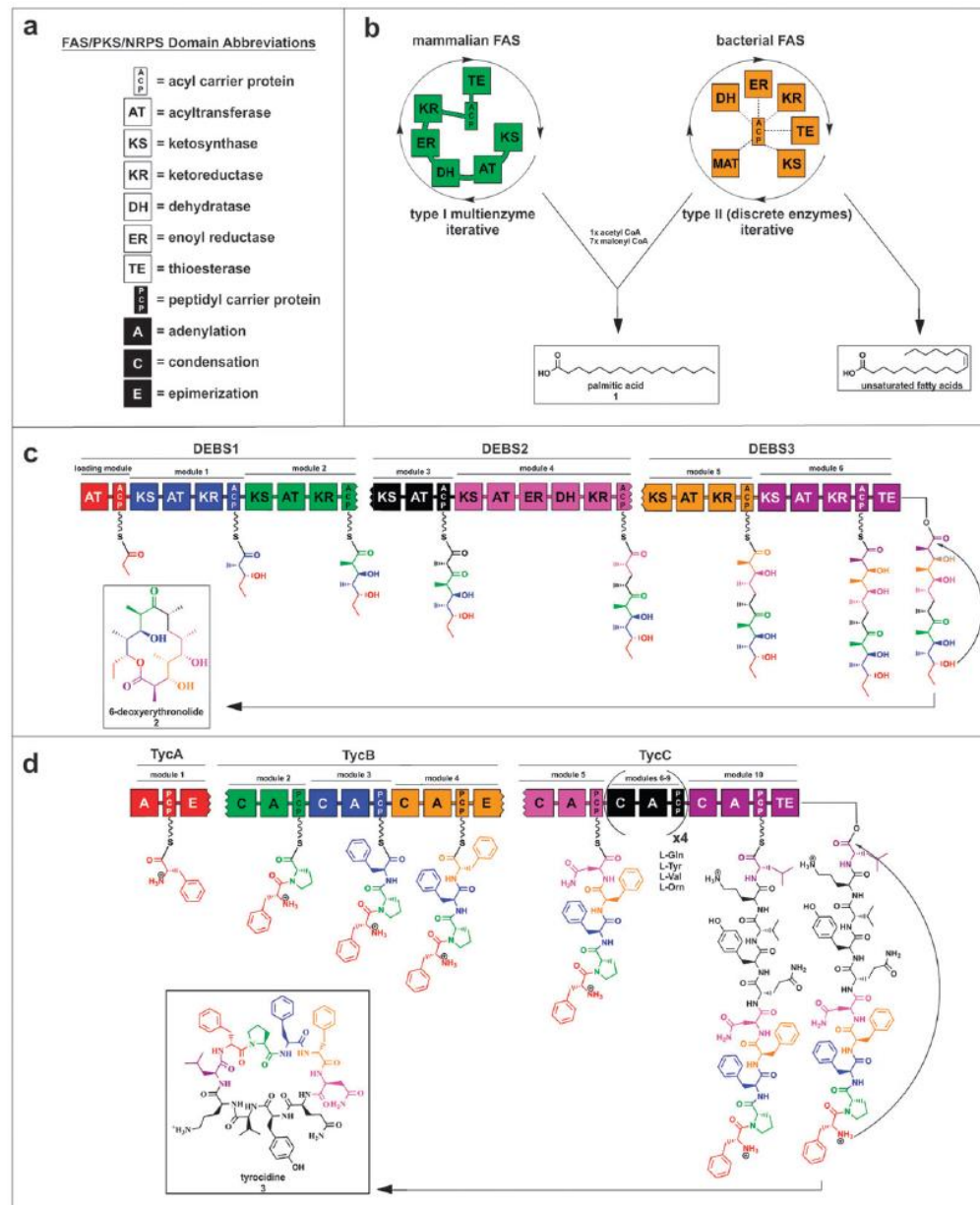
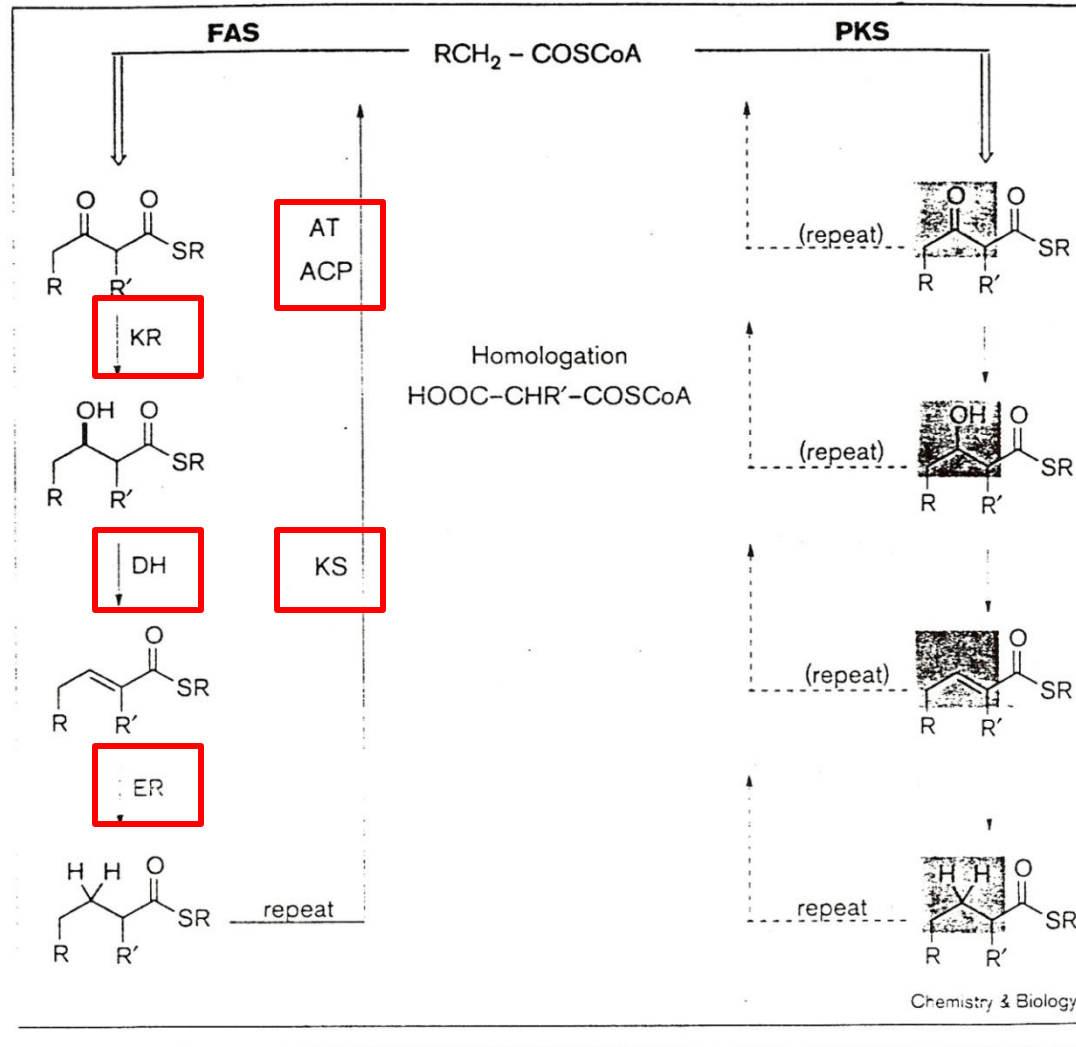


Fig. 3 Domain organization for FAS, PKS, and NRPS enzymes. (a) Domain abbreviations used in this manuscript. (b) Mammalian and bacterial FAS. Both produce the primary metabolite palmitic acid (1), but while mammalian FAS houses each necessary enzymatic activity on a single multienzyme, in bacteria they 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.

Analogie tra biosintesi dei polichetidi (PKS) e biosintesi degli acidi grassi (FAS)

FAS
Unità d'inizio:
Acetil-CoA
Unità di
allungamento:
Malonil-CoA



PKS
Unità d'inizio:
Acetil-CoA
Propionil-CoA,
etc
Unità di
allungamento:
Malonil-CoA
Metil-malonil-
CoA, etc

Biosintesi dei polichetidi

Analogie tra acido grasso sintasi (FAS) e polichetide sintasi (PKS)

- FAS tipo I funghi e vertebrati
iterative 'modulari': le attività catalitiche **AT-KS-ACP-KR-DH-ER** sono domini di una singola catena polipeptidica.
- FAS tipo II batteri e piante
iterative non modulari: ogni attività catalitica si trova su subunità separate
- PKS tipo I 'modulari': il numero di moduli è uguale al numero di cicli di condensazione
- PKS tipo II 'iterative': gli stessi domini catalizzano i successivi cicli di condensazione (spesso sintetizzano polichetidi aromatici)

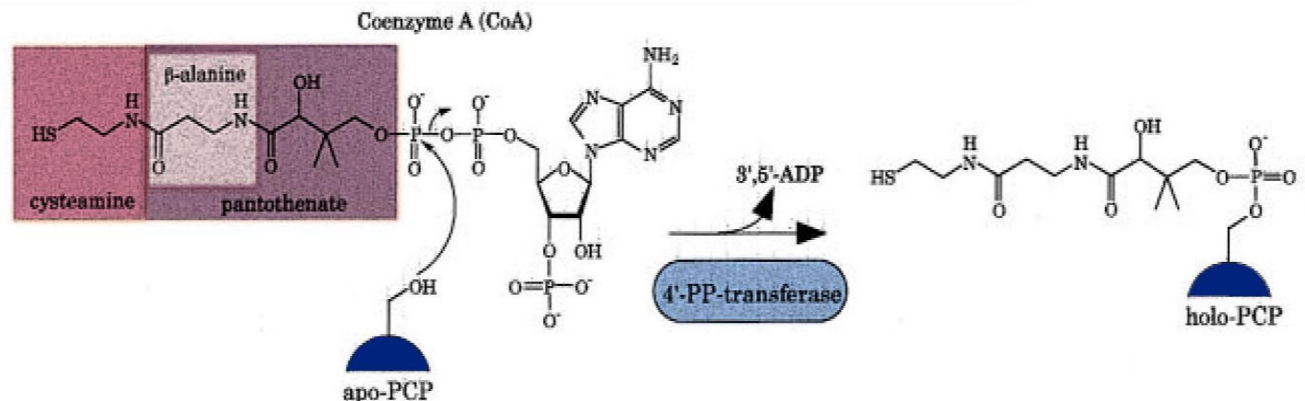
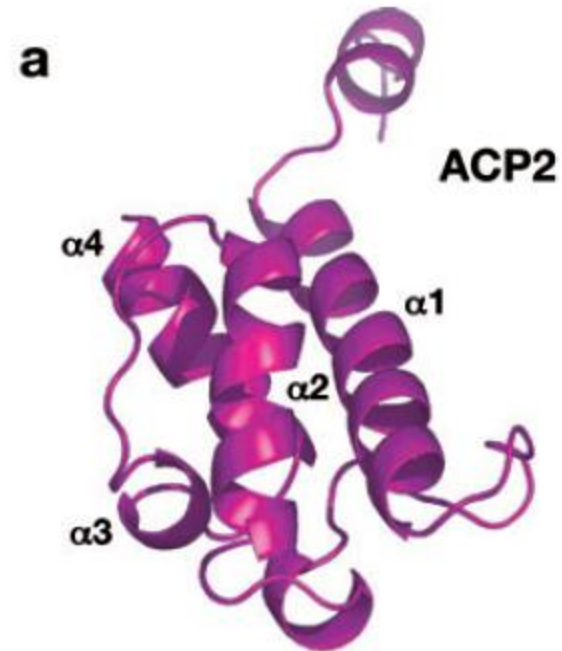
Biosintesi dei polichetidi

Versatilità delle PKS nella scelta di:

- Unità di inizio
- Natura e numero delle unità di allungamento
- Controllo dello stato di riduzione del β -chetogruppo
- Stereochimica delle catene laterali
- Ciclizzazione

Il dominio ACP

- Il dominio ACP contiene 4-fosfopanteteina che deriva dal coenzima A ed è legata covalentemente ad un residuo di serina.
- La 4-fosfopanteteina lega covalentemente (legame tioestere) il substrato e fornisce flessibilità e lunghezza (circa 2 nm) facilitando la comunicazione tra i siti attivi.



Interazioni del dominio ACP con i domini KS e AT

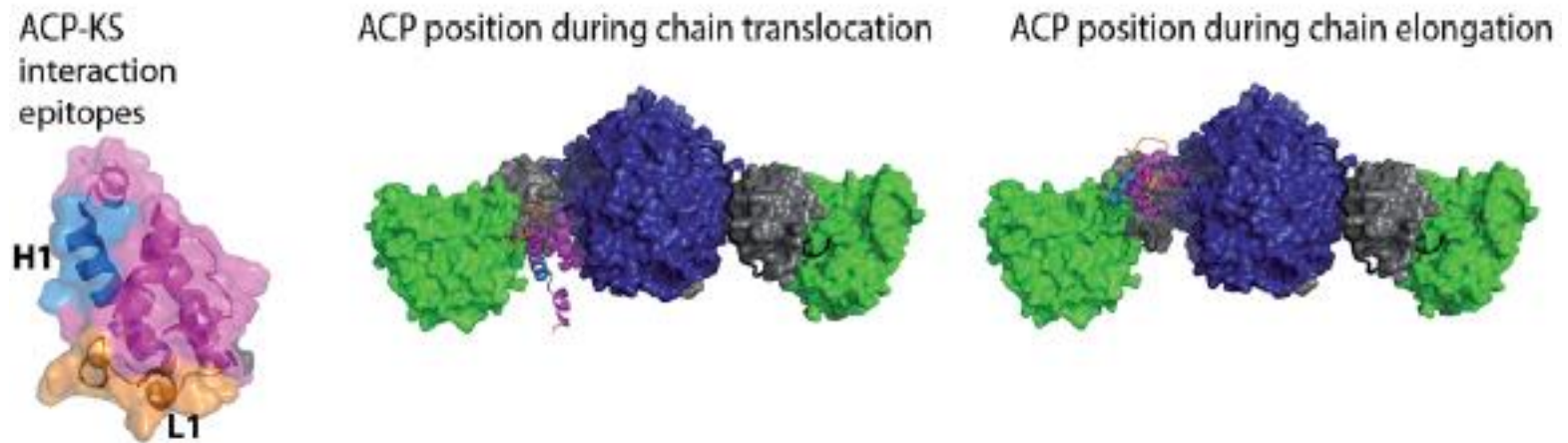


Fig. 3 Important interfaces for engineering ACP-KS and AT domain exchanges. (A) Interface of ACP-[KS-AT] according to ref. 40 and 41. Residues of ACP that are responsible for chain translocation in helix 1 are highlighted in blue. Loop 1 (orange) was found as the main determinant in chain elongation.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

KS: ketosintasi

condensazione

AT: acil-trasferasi

caricamento

Biosintesi dell'eritromicina

L'eritromicina A è stata isolata nel 1957 dal batterio Gram-positivo *Saccharopolyspora erythraea*, ha attività antibiotica verso i batteri Gram-positivi.

Inibisce la sintesi proteica legandosi all'RNA ribosomiale 23S nella cavità del sito peptidil-trasferasico.

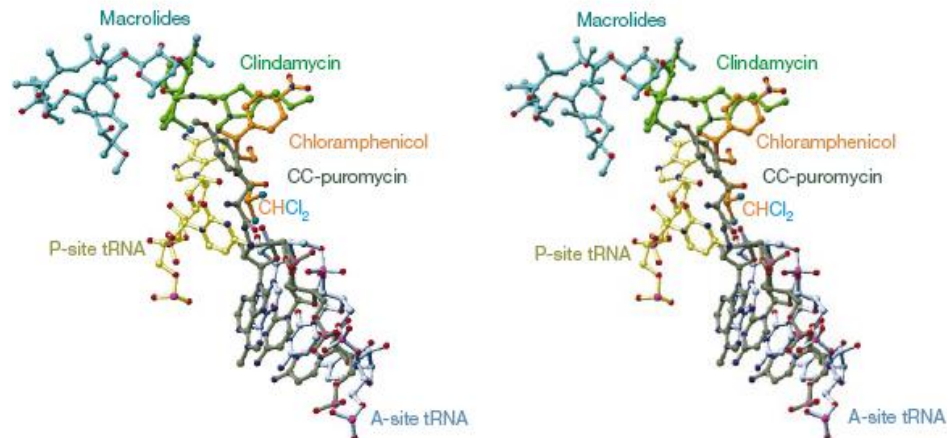
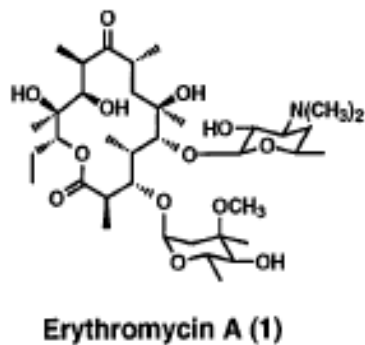


Figure 4 Relative position of chloramphenicol, clindamycin and macrolides with respect to CC-puromycin and the 3'-cytosine-adenine (CA) end of P-site and A-site tRNAs. The location of CC-puromycin was obtained by docking the position reported by ref. 2 into the peptidyl transferase centre of *D. radiodurans*. 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. CHCl₂ indicates the location of the dichloromethyl moiety of chloramphenicol.

Geni coinvolti nella biosintesi dell'eritromicina

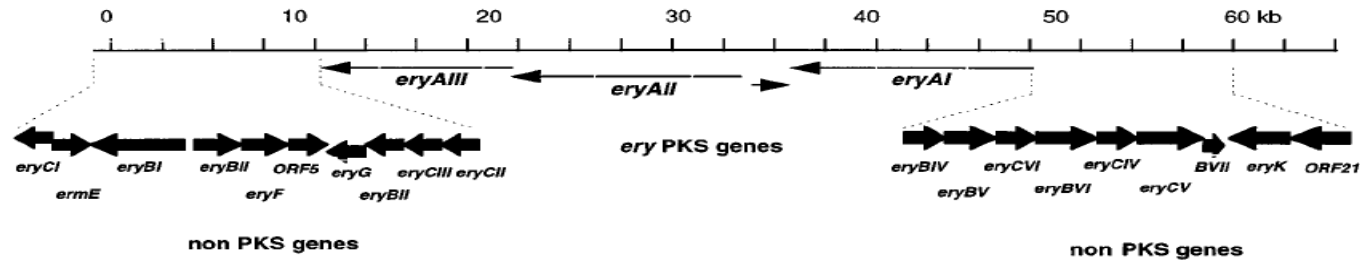
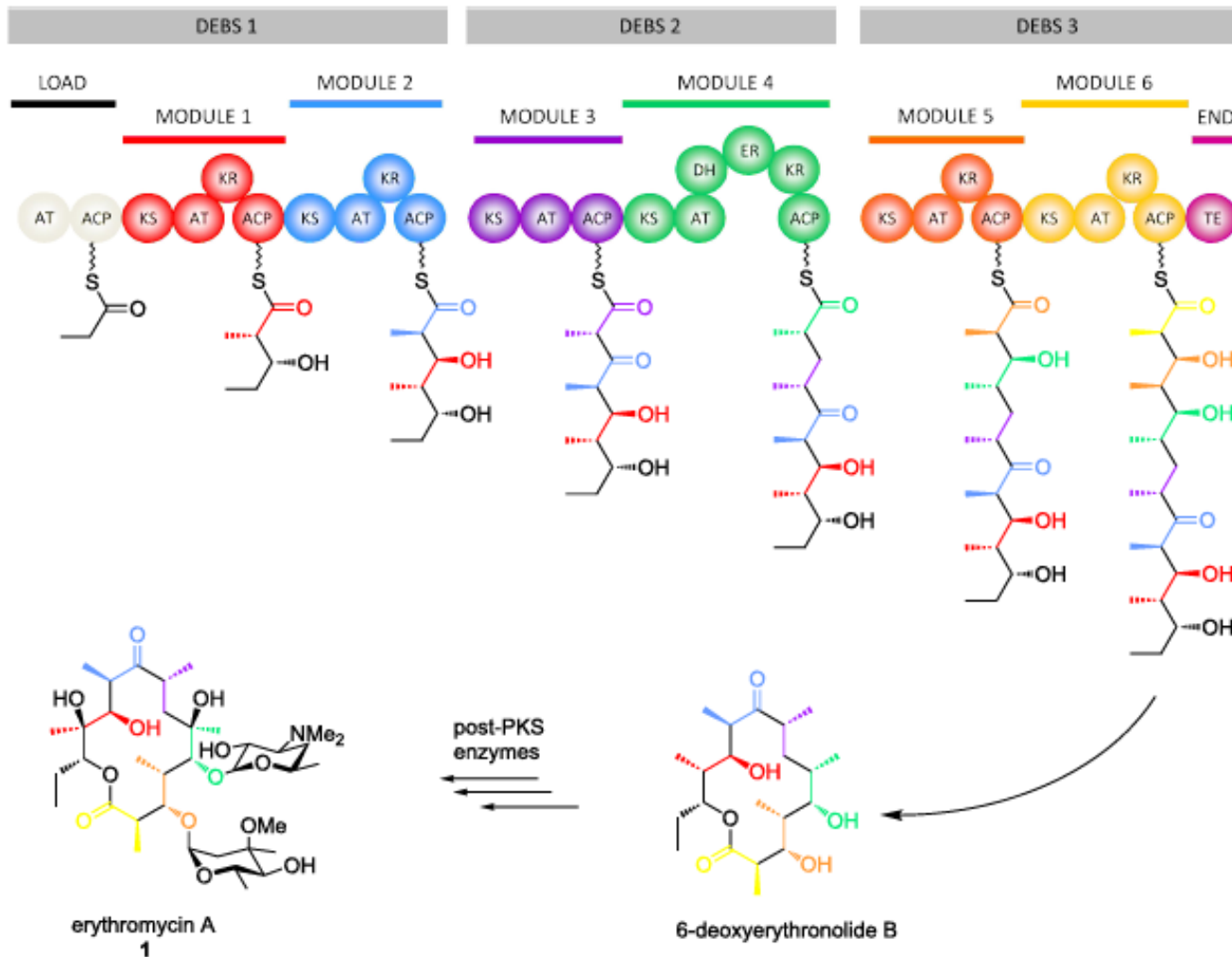


Figure 2. Map of regions of the *S. erythraea* genome containing genes associated with the late stages of the erythromycin biosynthetic pathway.

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

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

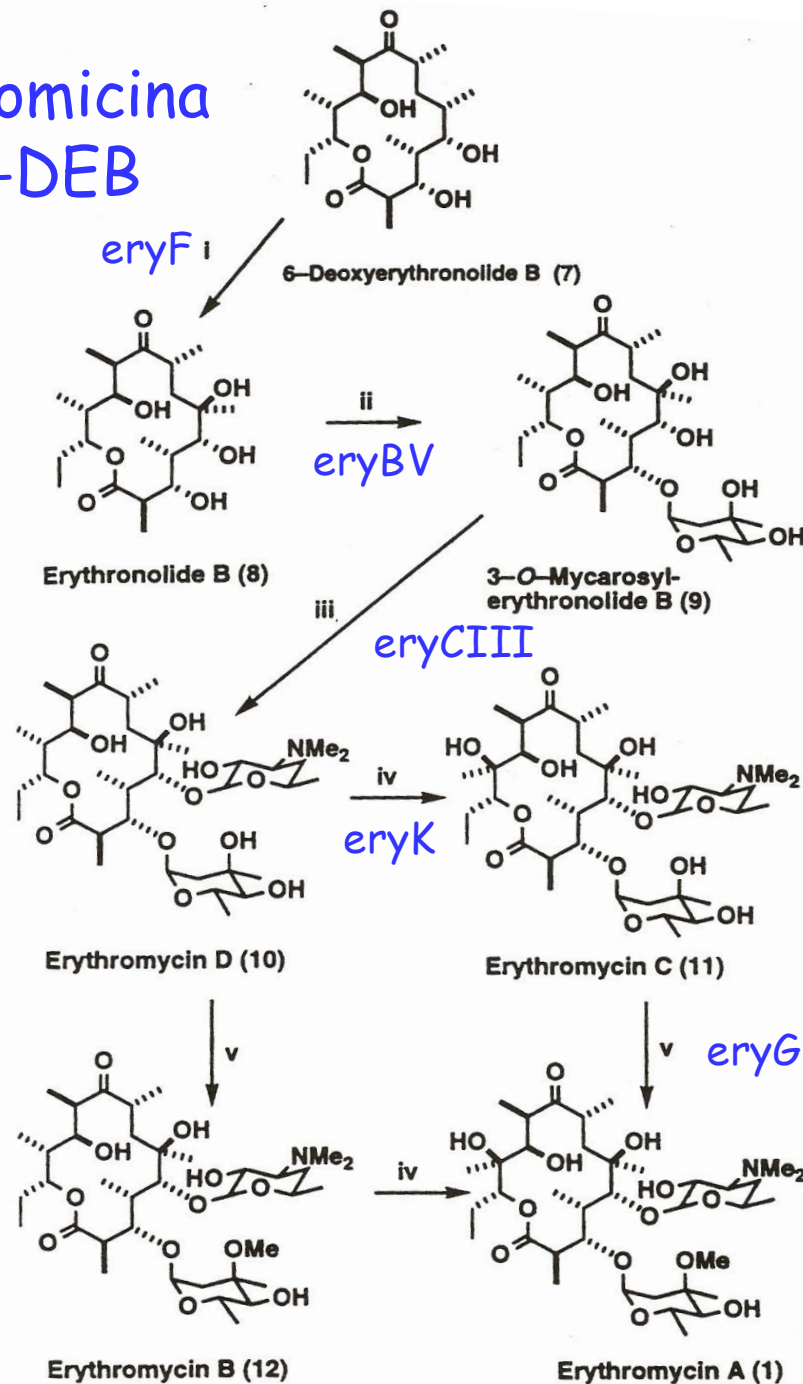
Organizzazione modulare della PKS che sintetizza lo scheletro polichetidico dell'eritromicina, il 6-deossieritronolide B (6-DEB)



Unità d'inizio:
Propionil-CoA
Unità di
allungamento:
2-S-Metil-
malonil-CoA

Biosintesi dell'eritromicina

Modificazioni del 6-DEB



L'eritromicina D
è il primo
intermedio a
mostrare attività
antibiotica

Interazioni tra eritromicina e rRNA 23S

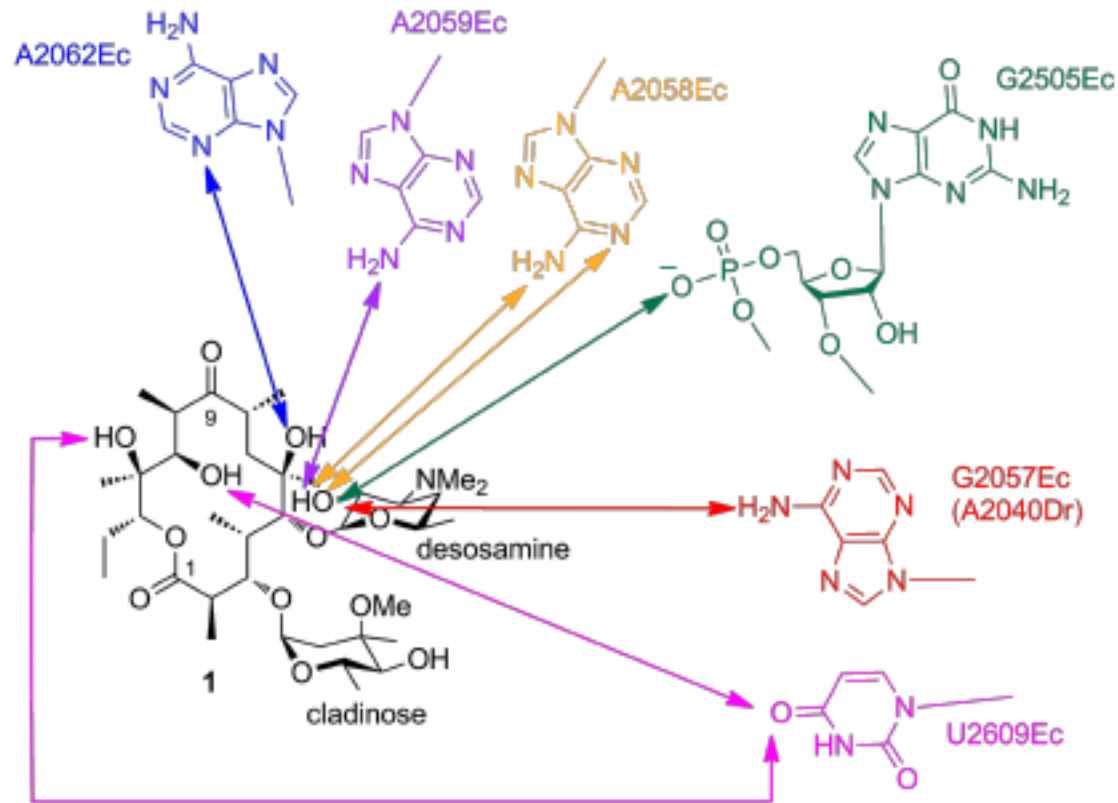


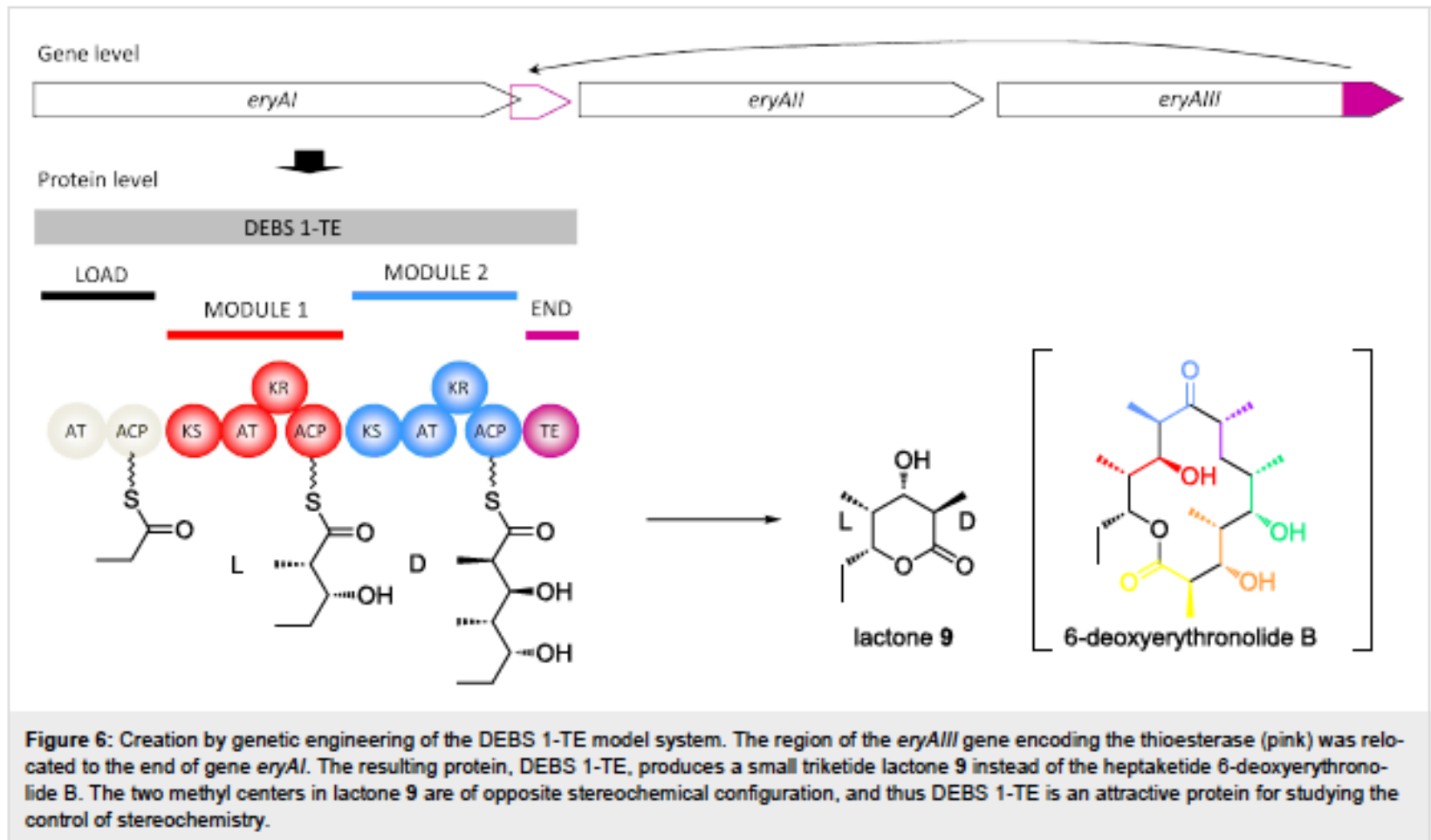
Figure 2: Schematic of erythromycin A (1) bound to 23S ribosomal RNA of the 50S subunit of the *Deinococcus radiodurans* (Dr) ribosome. The interactions between the polyketide and the nucleotides (*Escherichia coli* (Ec) numbering) are indicated with colored arrows (reactive groups are less than 4.4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

Espressione DEBS ricombinanti per studi strutturali e funzionali

- Problemi espressione PKS ricombinanti:
 - Grandi dimensioni delle proteine
 - Folding e modifiche post-traduzionali (fosfopanteteinilazione)
 - Presenza di precursori appropriati

L'ospite più utilizzato per l'espressione è il ceppo di *Streptomyces coelicolor* CH999 al quale manca (per delezione) il cluster genico che produce il polichetide aromatico actinorodina

Per facilitare studi funzionali delle PKS è stata creata una variante semplificata costituita da DEBS1 a cui è stato aggiunto il dominio TE, questa proteina è stata espressa in *S. coelicolor*.



Controllo della stereochimica dei gruppi metilici laterali

Mini-PKS costituita dal dominio di caricamento e due moduli (DEBS1-TE).

Due cicli di condensazione che danno origine a catene laterali con stereochimica diversa a partire da 2S-metil-malonil-CoA.

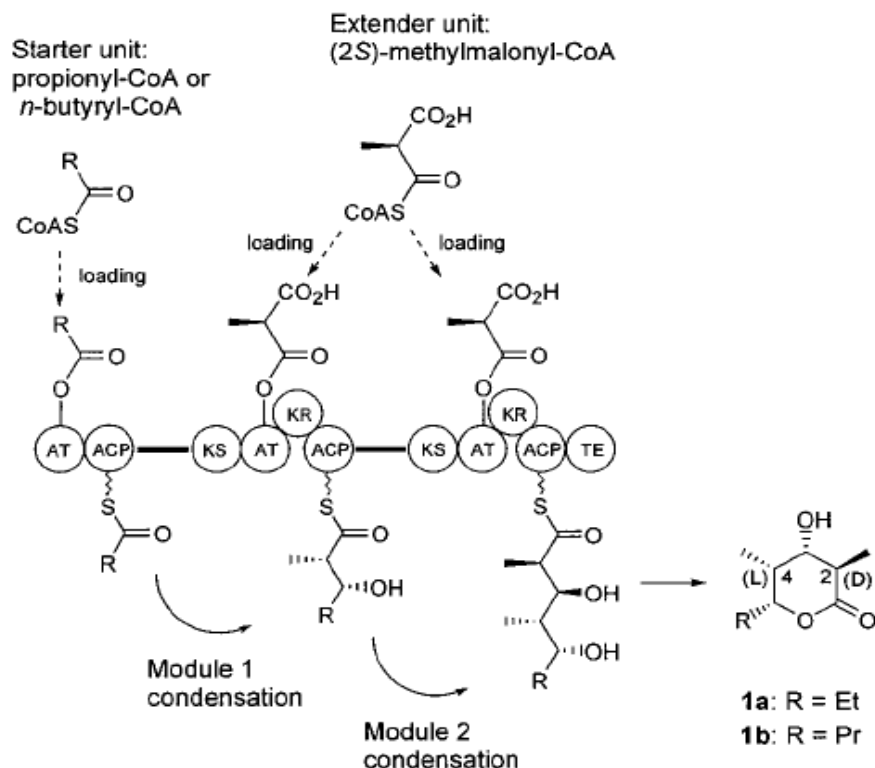


FIGURE 1: Operations to carry out two chain extension cycles by the “triketide lactone synthase”, 6-deoxyerythronolide B synthase 1-thioesterase (DEBS 1-TE). DEBS 1-TE is organized into two modules which catalyze the stereospecific condensation of an extender unit onto the growing chain and set the reduction 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 (2S)-stereoisomer of the chain-extending methylmalonyl-CoA, DEBS 1-TE produces the δ -lactones **1a** and **1b**, respectively.

Controllo della stereochimica dei gruppi metilici laterali

Il meccanismo corretto è stato identificato analizzando i prodotti con spettrometria di massa ed NMR

L'epimerizzazione è mediata dal dominio KR

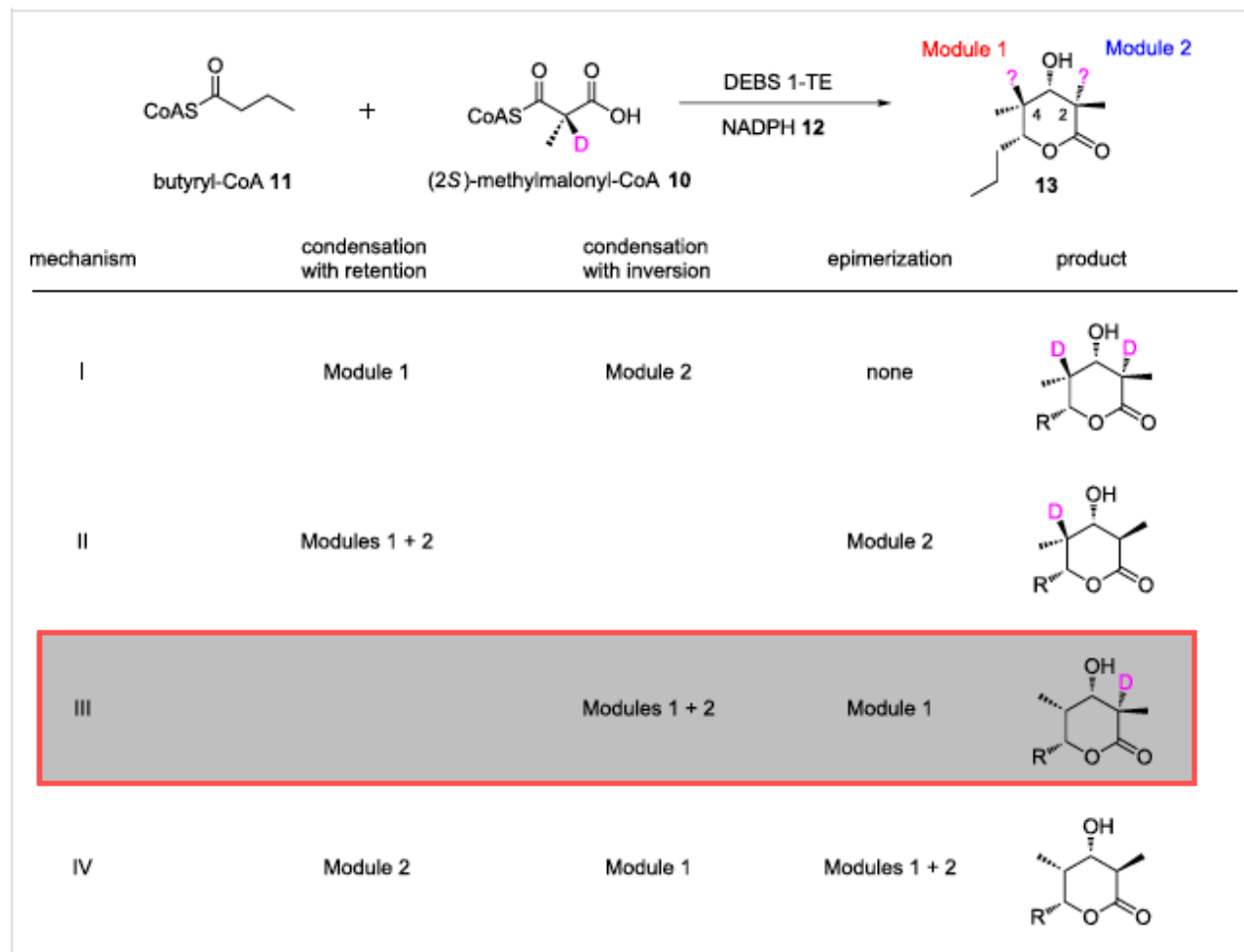


Figure 9: Experiment in vitro to determine the stereochemistry of condensation in modular PKS [46]. Use of specifically C-2-deuterium labeled extender unit **10** during biosynthesis with DEBS 1-TE (alongside starter unit butyryl-CoA **11** and NADPH **12**), resulted in a labeling pattern in the triketide 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.

Produzione di analoghi strutturali di polichetidi

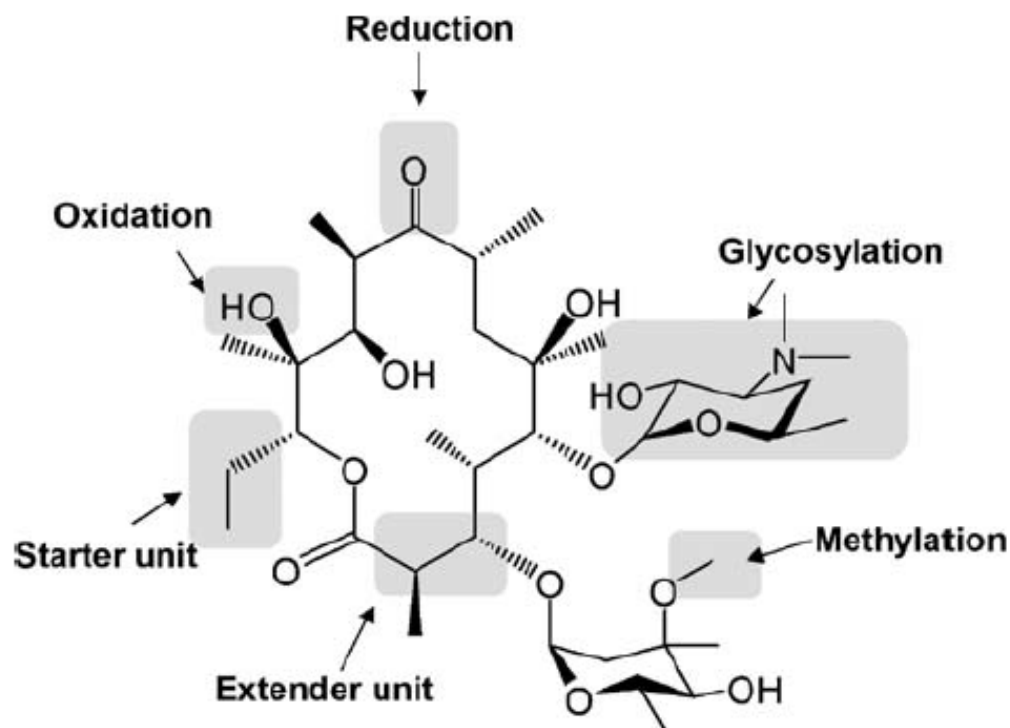


Fig. 3 Several biosynthetic targets for genetic engineering to generate novel macrolides. A wide variety of structural changes can be introduced into macrolactone ring biosynthesis by genetic engineering as follows: alteration of starter and extender units required for PKS and alteration of the extent 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

Inattivazione del gene *eryF* per rendere l'eritromicina stabile a basso pH

Il gruppo OH sul C-6 è coinvolto nella formazione dell'anidro-eritromicina (biologicamente inattiva) a pH acido.

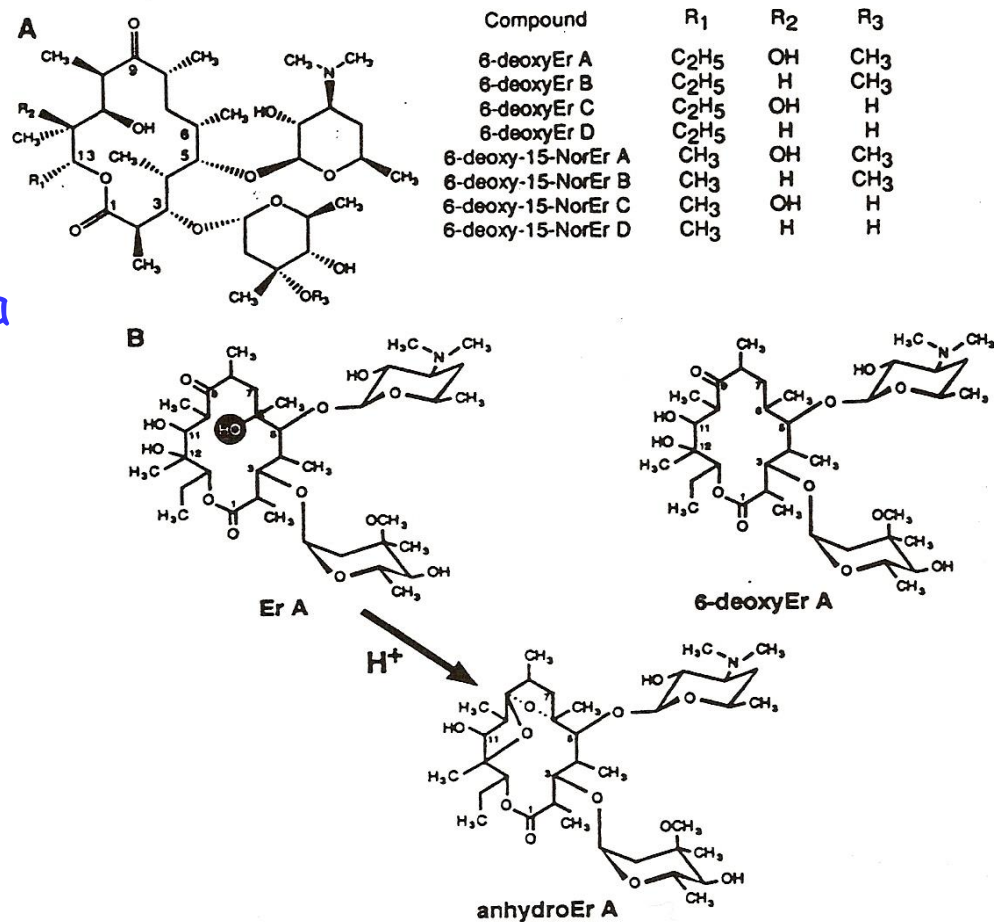


Fig. 2. (A) Structure (indicating stereochemistry) of 6-deoxyEr A and its biosynthetic precursors, 6-deoxyEr B, C, and D. Also shown are 6-deoxy-15-norEry A and its biosynthetic precursors. (B) Acid decomposition pathway of Er A to form anhydroEr A (17, 18), which involves the C-6 hydroxyl group (highlighted).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

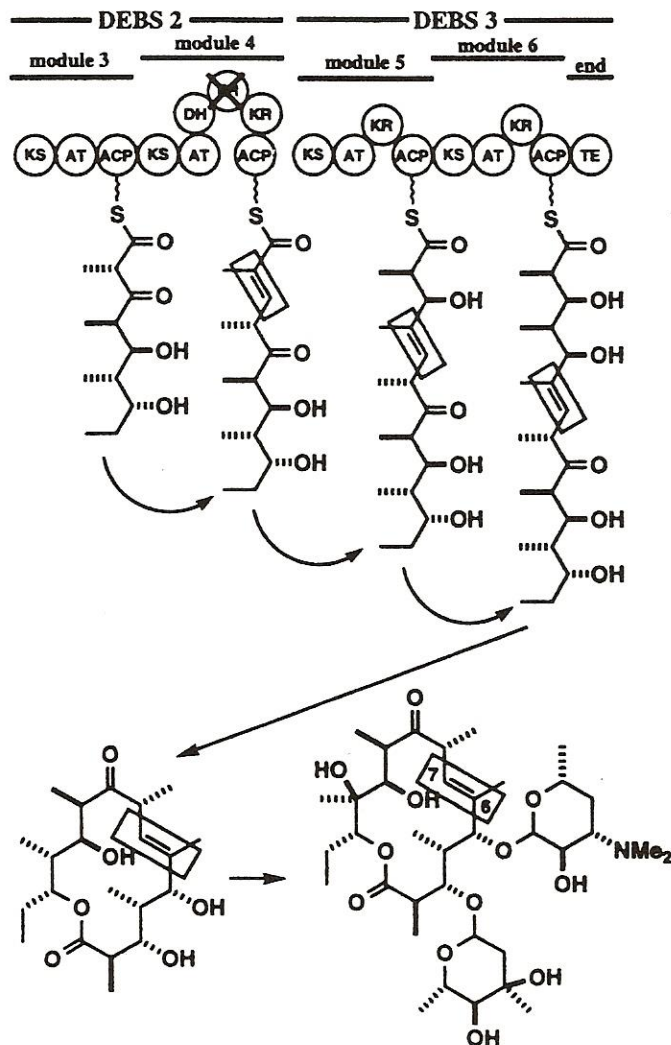
Inattivazione del dominio ER4

Il dominio ER4 è stato inattivato modificando il sito di legame per il cofattore NADPH

WT HAAAGGVGMA
Mut HAAASPVGMA

L'inattivazione di ER4 causa il mantenimento del doppio legame tra C-6 e C-7

Scheme 8



Biosintesi diretta da precursore per la produzione di analoghi strutturali dell'eritromicina

KS1 inattivato per eliminare la competizione con l'unità starter naturale propionil-CoA: mutazione della cisteina catalitica Cys→Ala

Le nuove unità starter vengono fornite come tioesteri dell'*N*-acetilcisteammina (SNAC): analoghi del substrato (acil-CoA)

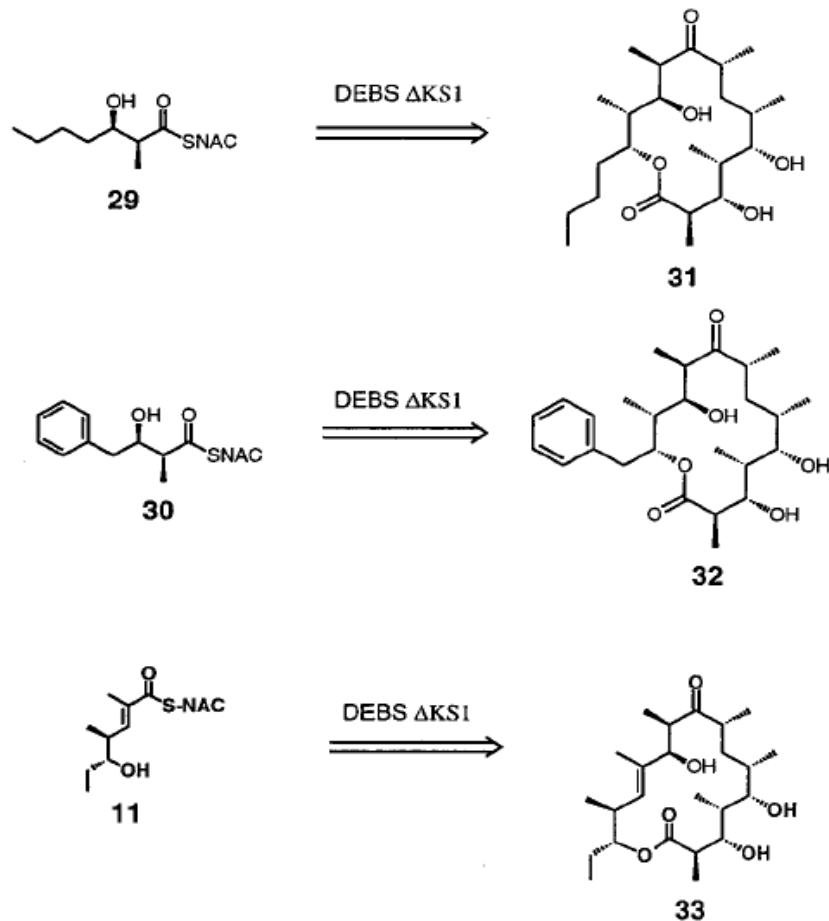


Figure 14. Precursor-directed biosynthesis of novel polyketides using genetically blocked modular polyketide synthases.

Biosintesi diretta da precursore per la produzione di analoghi strutturali dell'eritromicina

Gli analoghi strutturali del 6-DEB vengono aggiunti al terreno di un ceppo di *S. erythraea* non produttore di eritromicina.

Vengono processati e se ne valuta l'attività antibiotica.

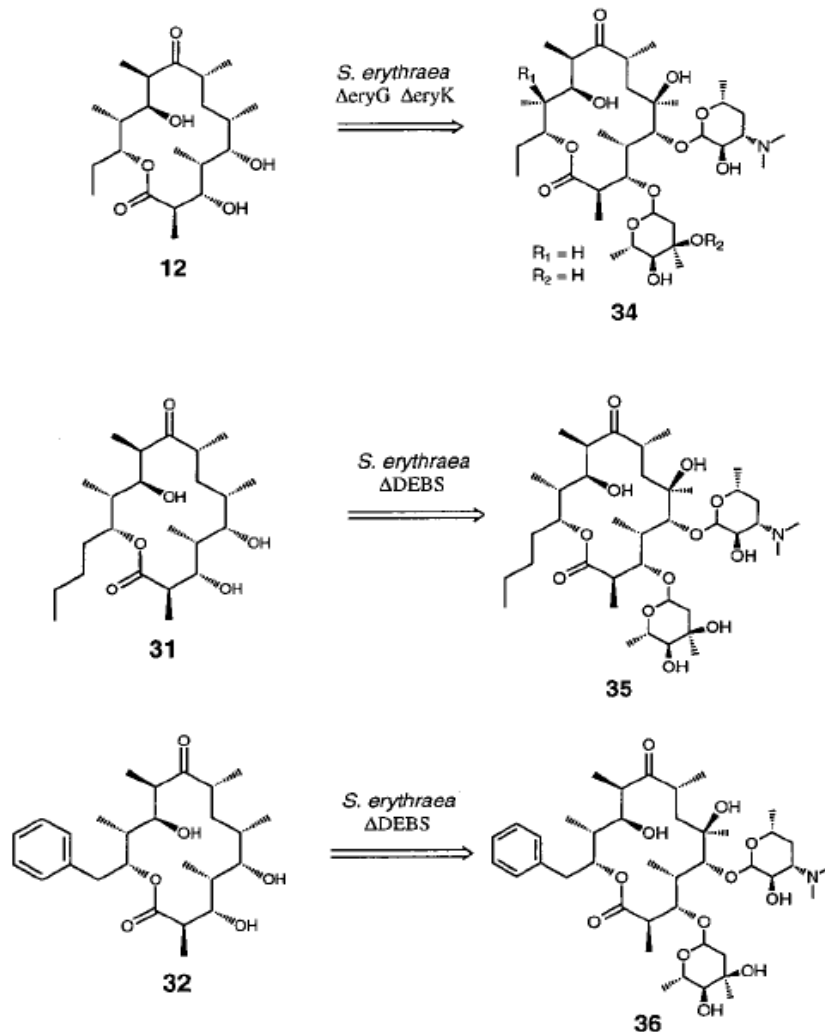
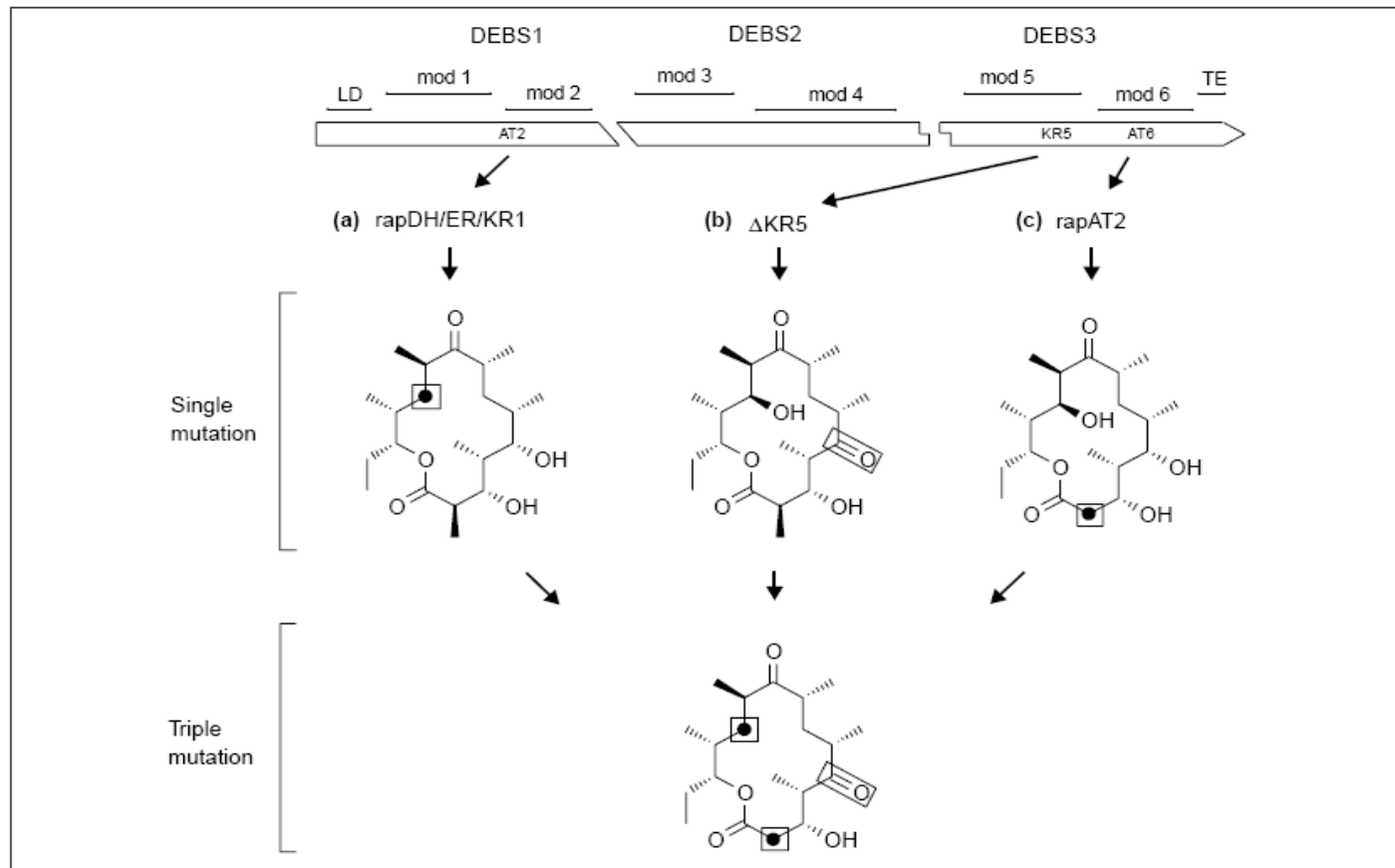


Figure 15. Bioconversion of structurally altered polyketide aglycons into novel erythromycin analogs.

Approccio combinatoriale per la produzione di analoghi strutturali dell'eritromicina: inattivazione di domini e scambio di domini



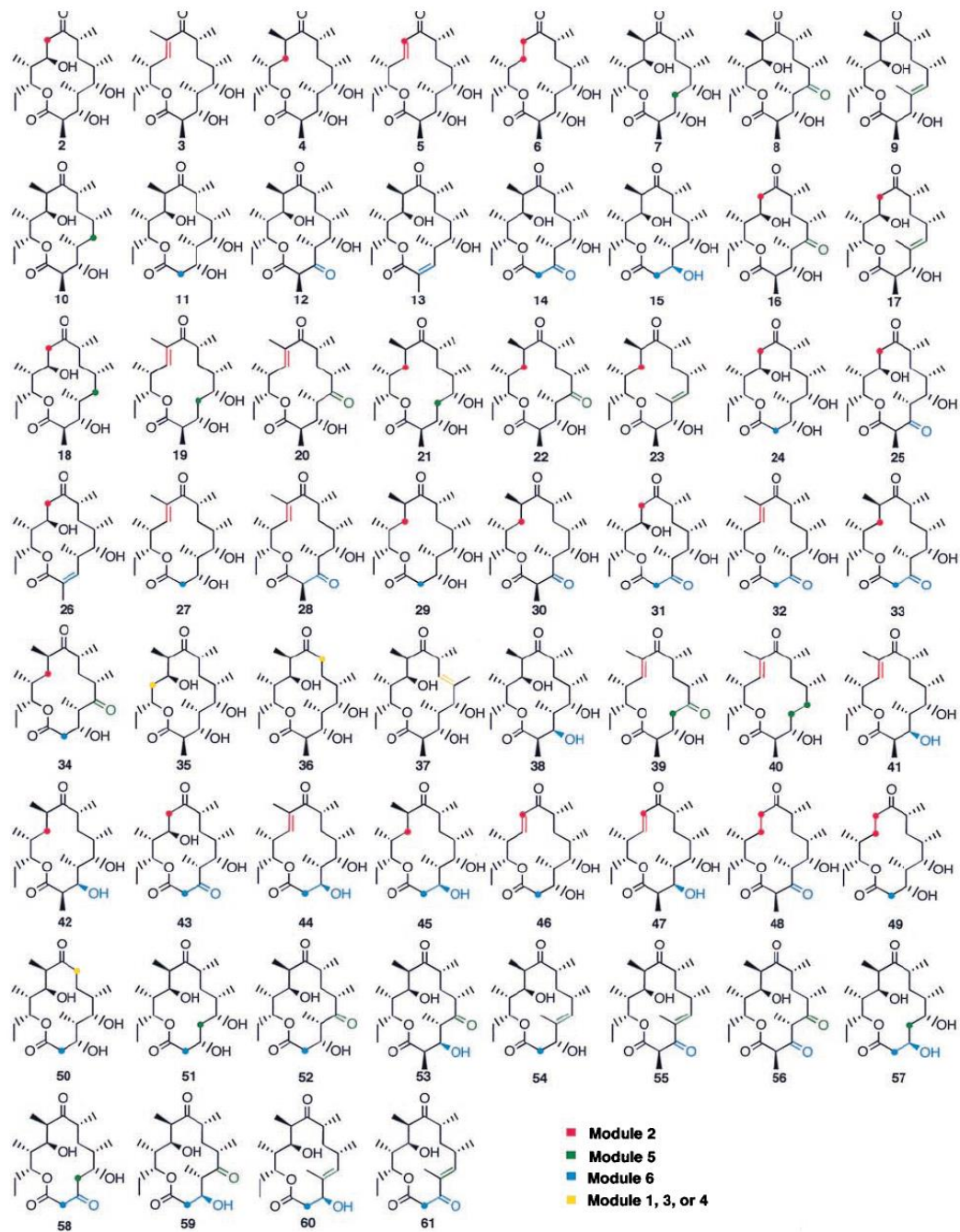
Combinatorial manipulation of DEBS. Single domain alterations that result in a functional PKS can be combined to generate multiple mutant PKSs. In this example, taken from [10**], three different single mutants could be combined to yield a functional triple mutant. The three mutations are:

(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.

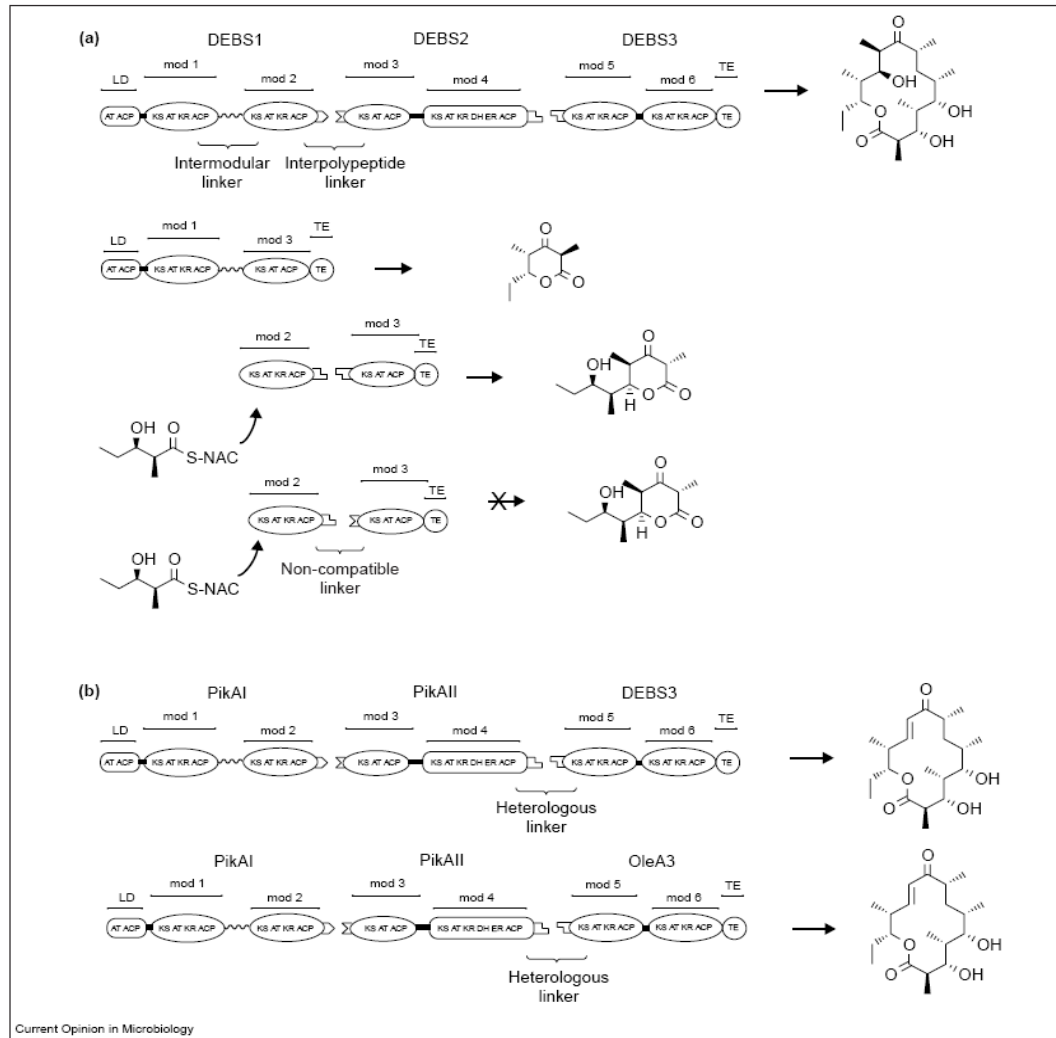
Table 2. Combinatorial double and triple substitutions and polyketide products

Mutation		6dEB analog product	Compound no.
Module 2	Module 5 or 6		
Module 2 × module 5 double mutants			
<i>rapAT2</i>	AT/ACP linker	5-deoxy-5-oxo-10-desmethyl	16
<i>rapAT2</i>	<i>rapDH/KR4</i>	4,5-anhydro-10-desmethyl	17
<i>rapAT2</i>	<i>rapDH/ER/KR1</i>	5-deoxy-5-oxo-10-desmethyl; 5-deoxy-10-desmethyl	16, 18
<i>rapDH/KR4</i>	<i>rapAT2</i>	4-desmethyl-10,11-anhydro	19
<i>rapDH/KR4</i>	AT/ACP linker	5-deoxy-5-oxo-10,11-anhydro	20
<i>rapDH/KR4</i>	<i>rapDH/ER/KR1</i>	NP	
<i>rapDH/ER/KR1</i>	<i>rapAT2</i>	4-desmethyl-11-deoxy	21
<i>rapDH/ER/KR1</i>	AT/ACP linker	5,11-dideoxy-5-oxo	22
<i>rapDH/ER/KR1</i>	<i>rapDH/KR4</i>	4,5-anhydro-11-deoxy	23
Module 2 × module 6 double mutants			
<i>raoAT2</i>	<i>rapAT2</i>	2,10-didesmethyl	24
<i>rapAT2</i>	AT/ACP linker	3-deoxy-3-oxo-10-desmethyl	25
<i>rapAT2</i>	<i>rapDH/KR4</i>	2,3-anhydro-10-desmethyl	26
<i>rapDH/KR4</i>	<i>rapAT2</i>	2-desmethyl-10,11-anhydro	27
<i>rapDH/KR4</i>	AT/ACP linker	3-deoxy-3-oxo-10,11-anhydro	28
<i>rapDH/ER/KR1</i>	<i>rapAT2</i>	2-desmethyl-11-deoxy	29
<i>rapDH/ER/KR1</i>	AT/ACP linker	3-deoxy-3-oxo-11-deoxy	30
Module 2 × module 6 triple mutants			
<i>rapAT14</i>	<i>rapAT2</i> +AT/ACP linker	2,10-didesmethyl-3-deoxy-3-oxo	31
<i>rapDH/KR4</i>	<i>rapAT2</i> +AT/ACP linker	2-desmethyl-3-deoxy-3-oxo-10,11-anhydro	32
<i>rapDH/ER/KR1</i>	<i>rapAT2</i> +AT/ACP linker	2-desmethyl-3,11-dideoxy-3-oxo	33
Module 2 × module 5 × module 6 triple mutant			
KR2→ <i>rapDH/ER/KR1</i>	KR5→AT/ACP linker, AT6→ <i>rapAT2</i>	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 produced at ≈ 0.2 mg/liter. NP, no product.



Riconoscimento molecolare tra moduli



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.

Riconoscimento molecolare tra moduli

Ruolo del dominio ACP
nel 'channeling' del
substrato al dominio KS

Il rilascio del substrato
è veloce quando ACP-TE
in *cis*

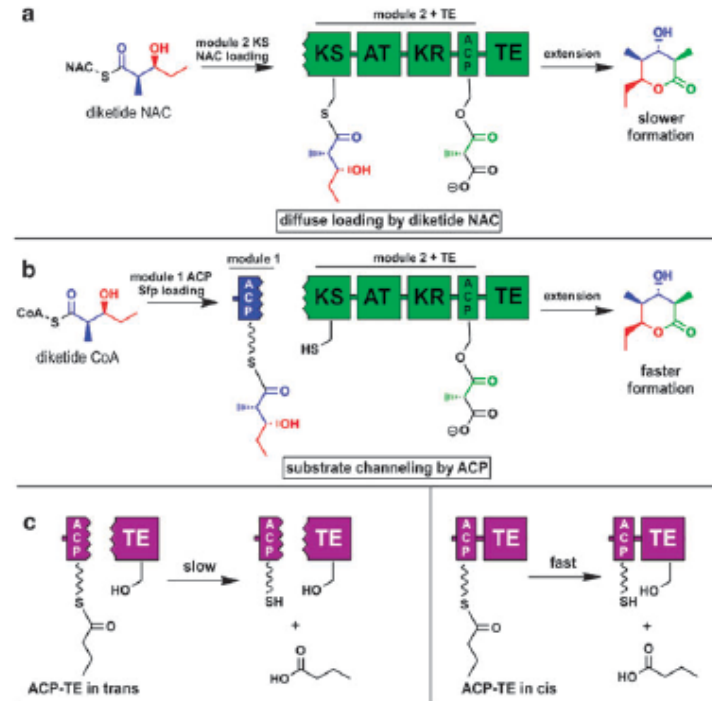
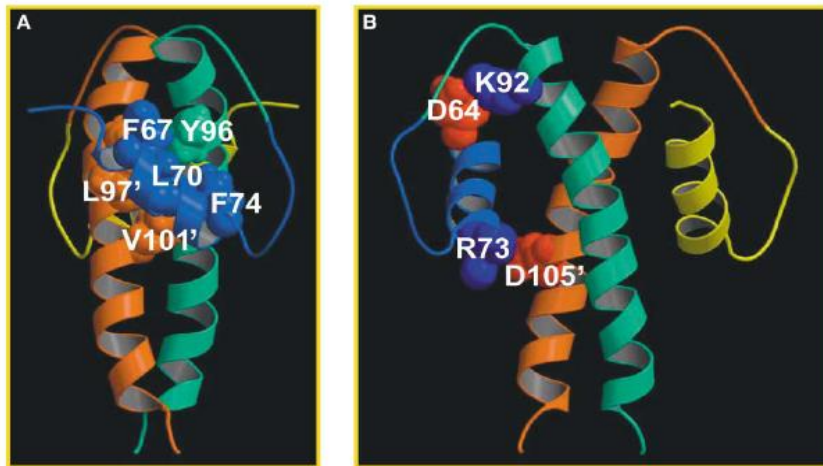


Fig. 10 Acyl CoA probes of PKS condensation and chain termination. (a) Incubation of DEBS module 2 + TE with a diketide-NAC results in diffuse loading of the KS cysteine, followed by condensation and chain cleavage to yield a triketide lactone. (b) Incubation of DEBS module 2 + TE with an Sfp loaded 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).

Struttura delle regioni linker tra moduli delle PKS DEBS2 e DEBS3



La complementarietà di carica è probabilmente alla base del riconoscimento tra moduli

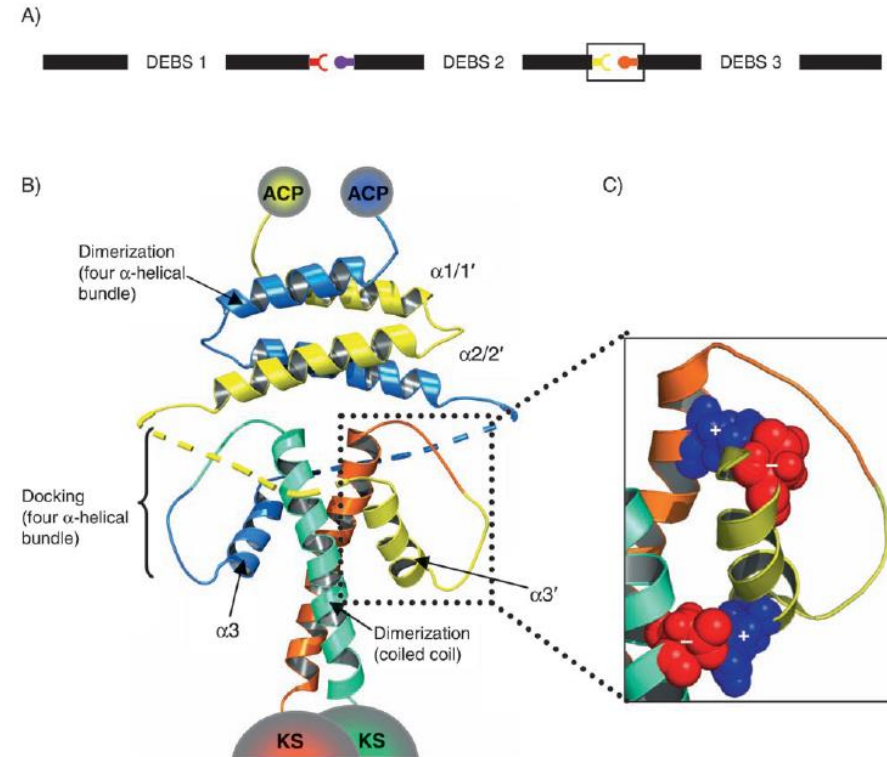


Figure 13. The structural basis for docking in modular PKS. A) PKS docking domains are located at the extreme C and N termini of the subunits. The complex of docking domains solved by NMR spectroscopy models the junction between polypeptides DEBS 2 and DEBS 3 in the erythromycin PKS. B) NMR solution 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.

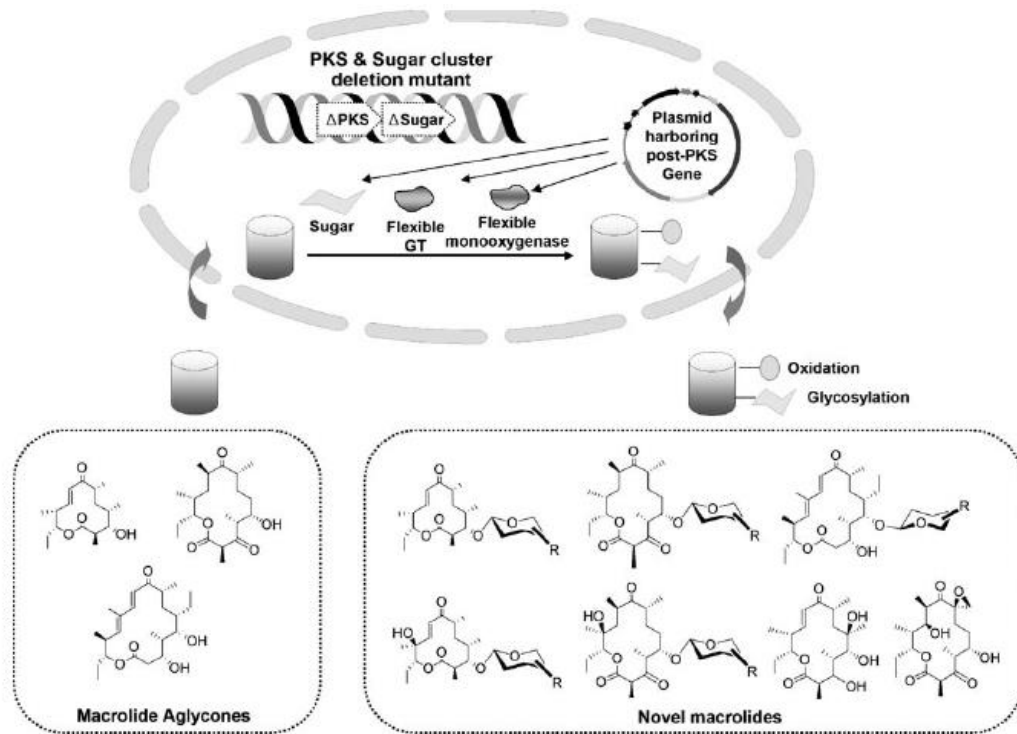


Fig. 5 Concept of combinatorial biosynthesis of post-PKS modification steps. Combinatorial biosynthesis is the application of genetic engineering to the modification of natural product biosynthetic pathways in order to produce unnatural or hybrid natural products.

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

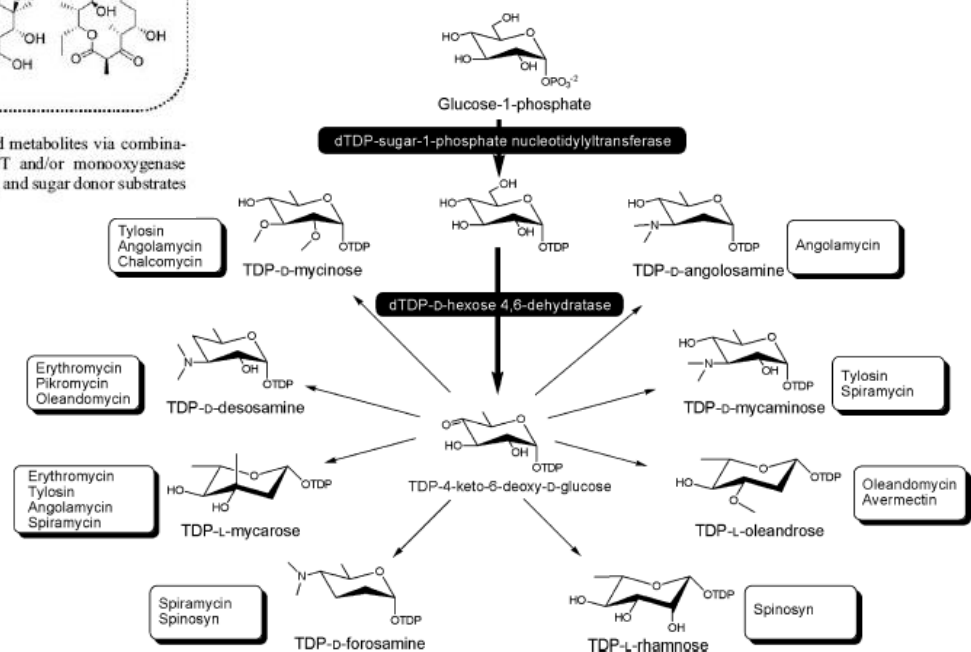
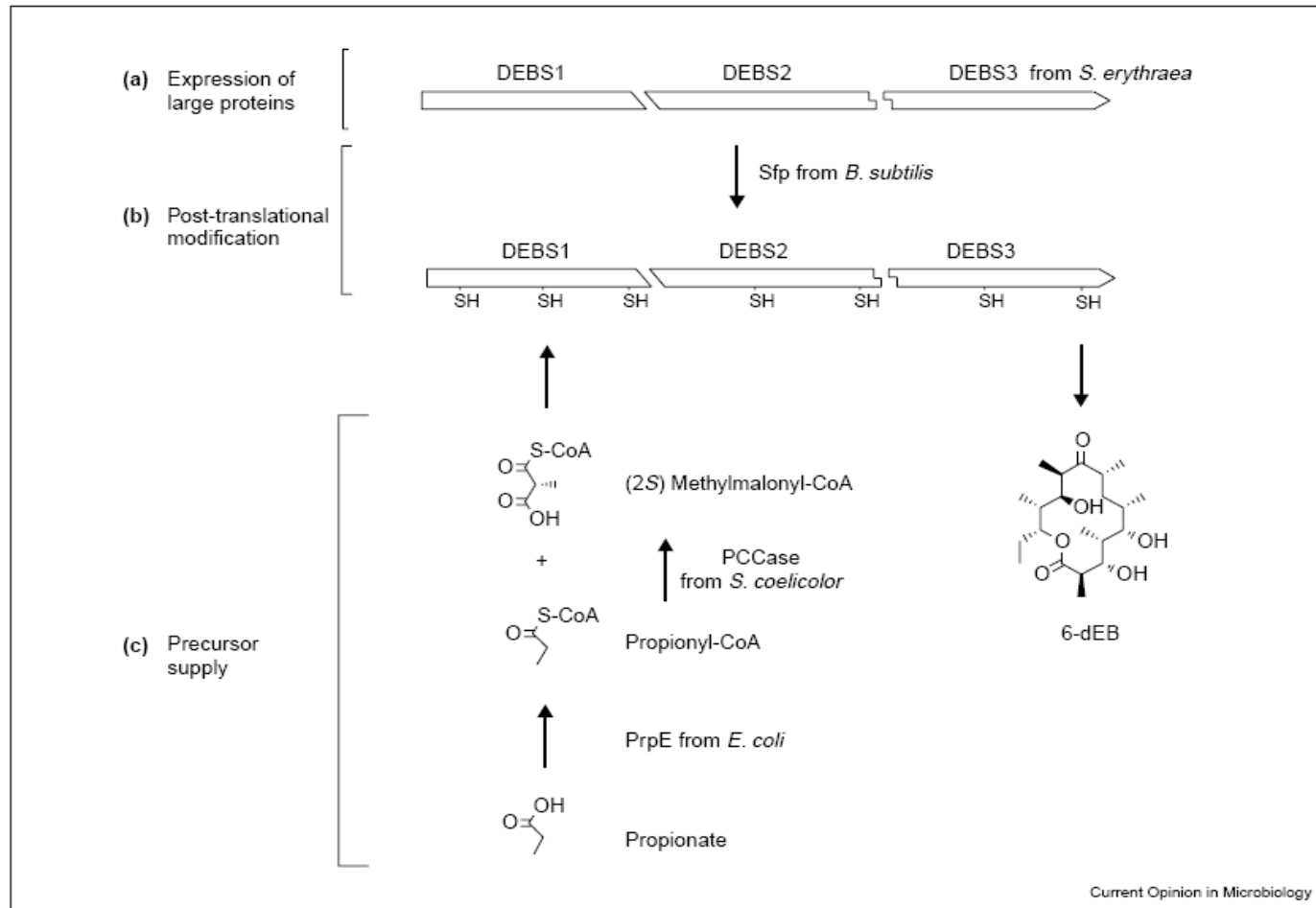


Fig. 4 Schematic representation of the pathways for 6-deoxysugar nucleotide biosynthesis. Two early enzymatic steps in the biosynthesis of the common intermediate dTDP-4-keto-6-deoxy-D-glucose are

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

Approccio combinatoriale per la produzione di analoghi strutturali dei polichetidi: modificazione degli zuccheri. Glicosiltrasferasi e monossigenasi con ampia specificità di substrato

Ingegneria metabolica in *E. coli* per la produzione di polichetidi

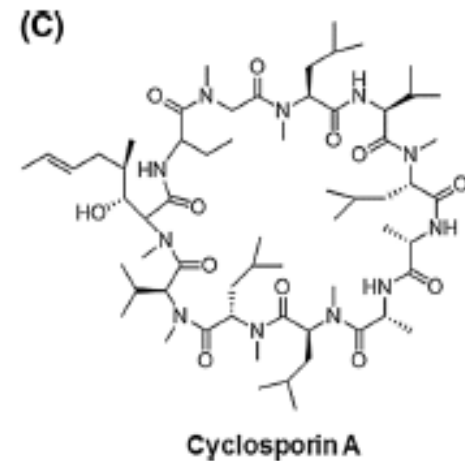
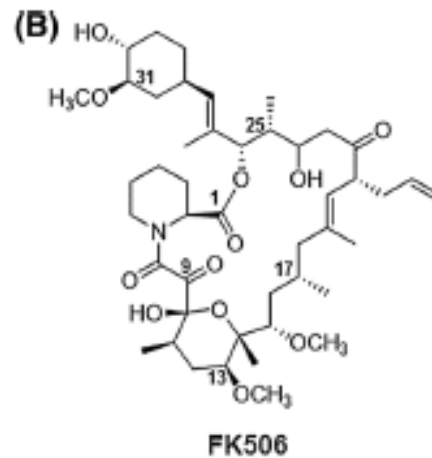
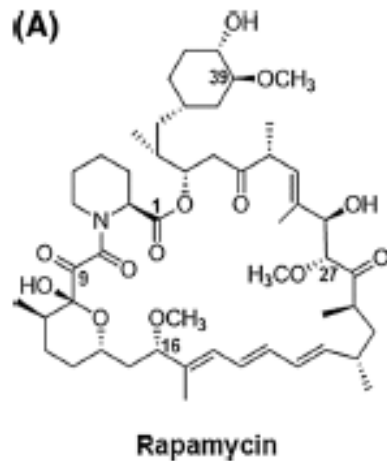


Engineering *E. coli* to produce polyketides. (a) Conditions for expression of large protein complexes, (b) a suitable phosphopantetheinyl transferase (Sfp), and (c) native PrpE

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*.

Immunosoppressori

Biosintesi della Rapamicina



La rapamicina è stata isolata nel 1975 da *Streptomyces hygroscopicus*, ha attività antifungina, antitumorale e immunosoppressoria.

Rappresenta un esempio di metabolita in cui lo scheletro polichetidico è legato ad un amminoacido

Meccanismo di azione della Rapamicina

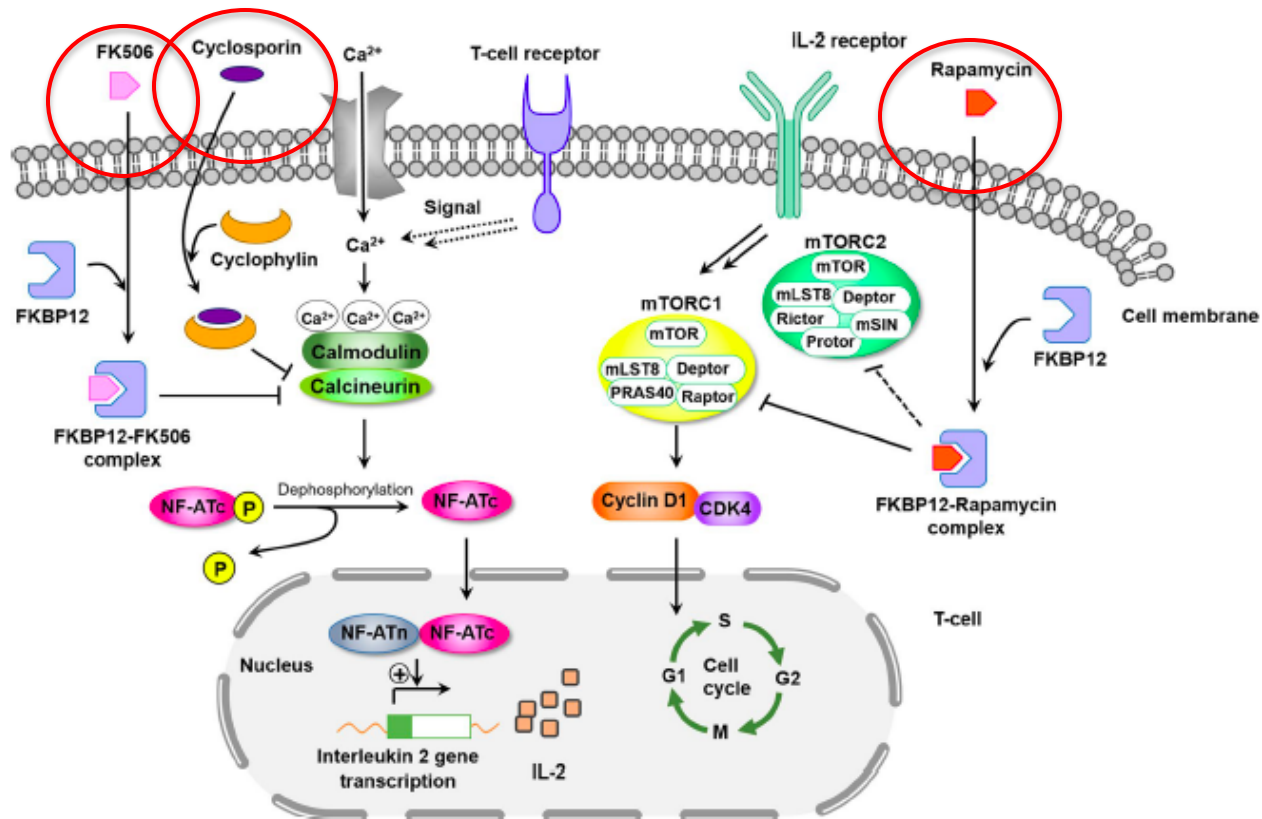
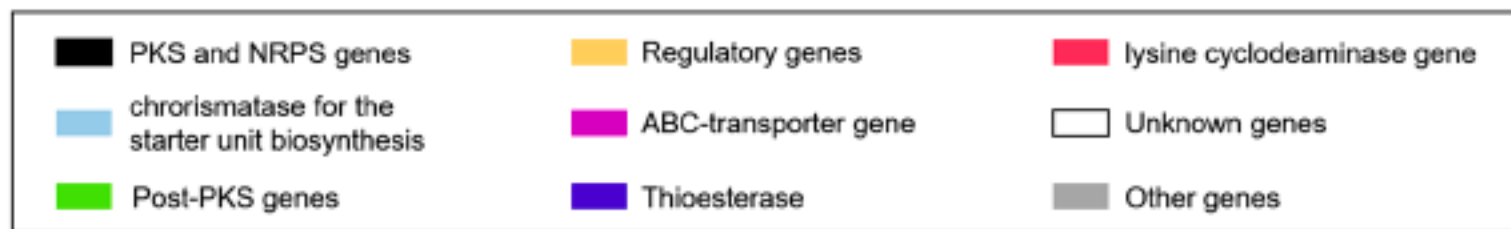
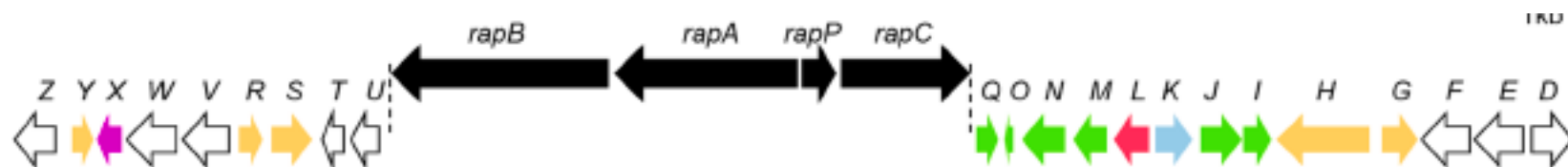
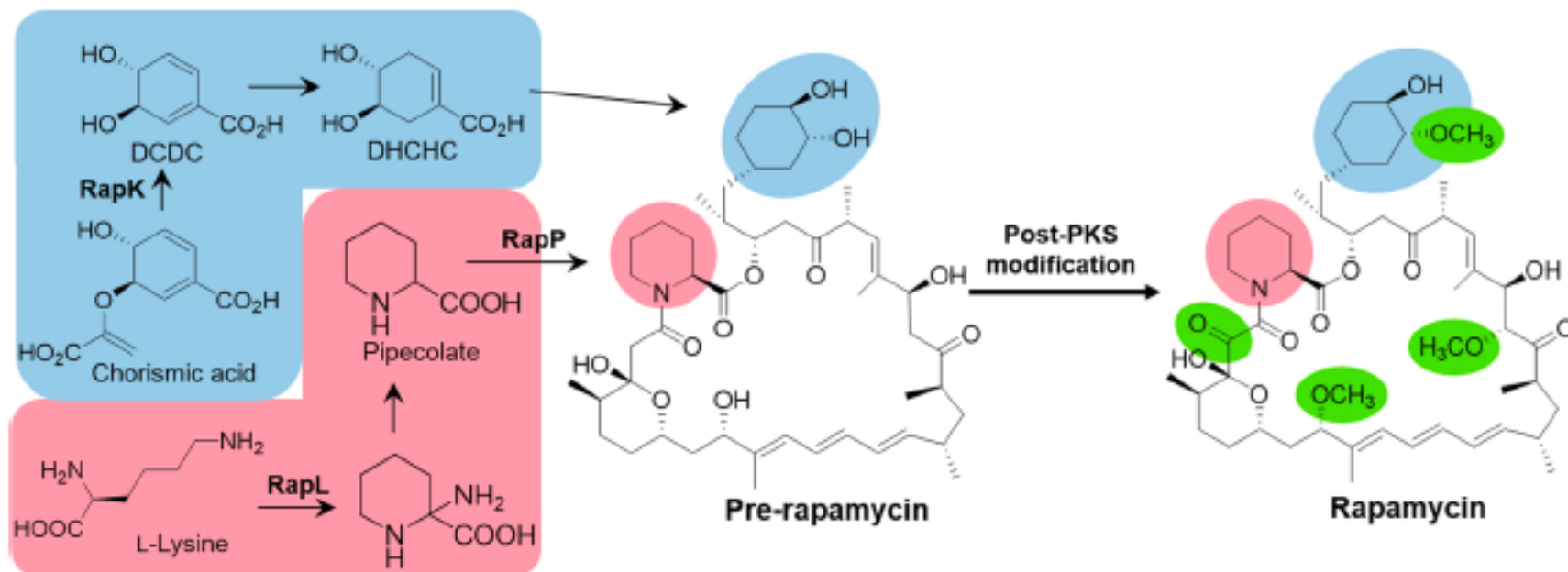


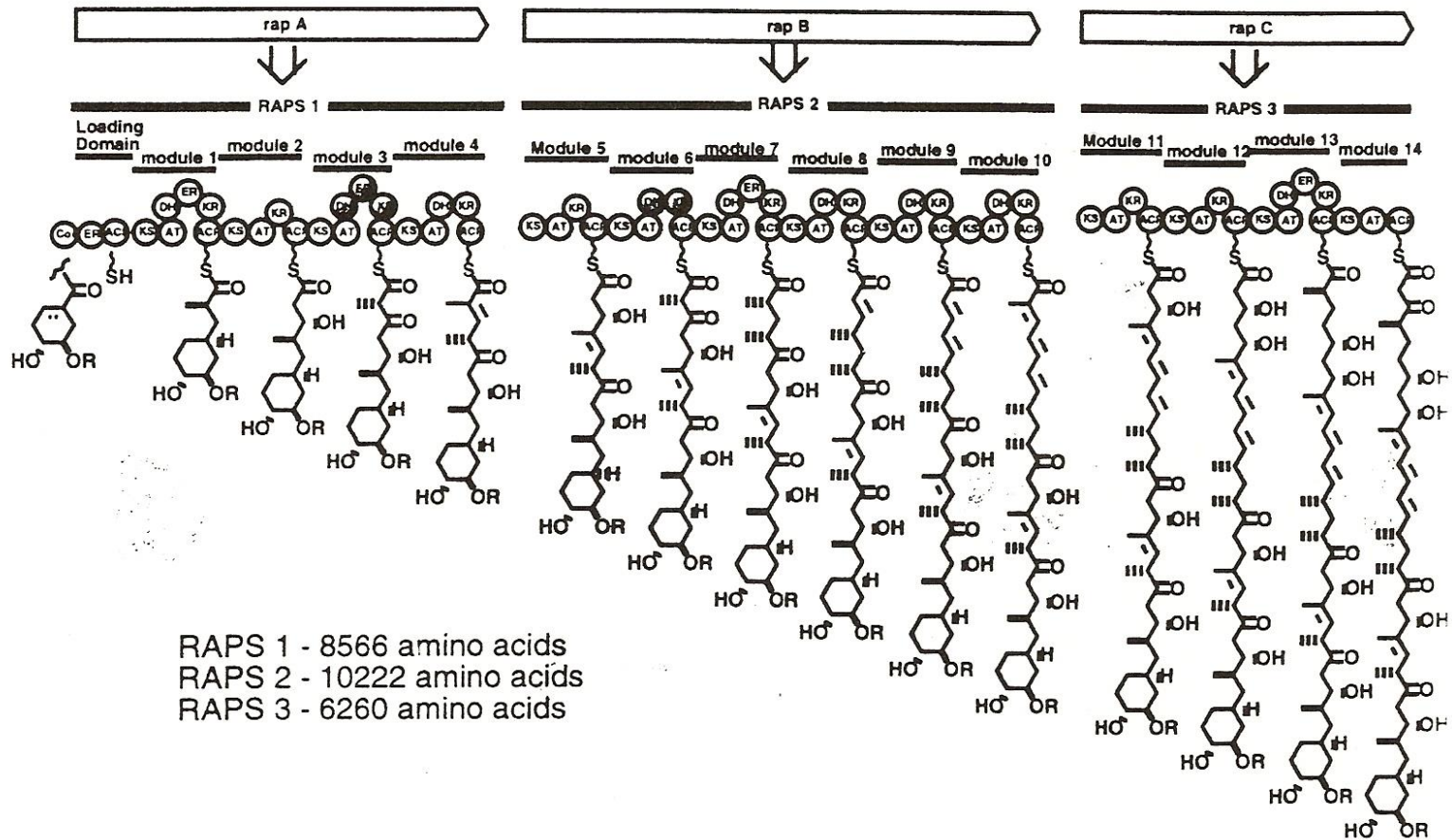
Fig. 2 Mechanism of action of immunosuppressive agents, such as rapamycin, FK506, and cyclosporin. Rapamycin inhibits mammalian target of rapamycin (mTOR) by binding to its intracellular receptor FK506-binding protein 12 (FKBP12). mTOR is the catalytic subunit of two structurally distinct complexes, 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 cyclophilin, respectively, and the resulting complexes block the calcineurin that is required for

dephosphorylation 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

Biosintesi della Rapamicina



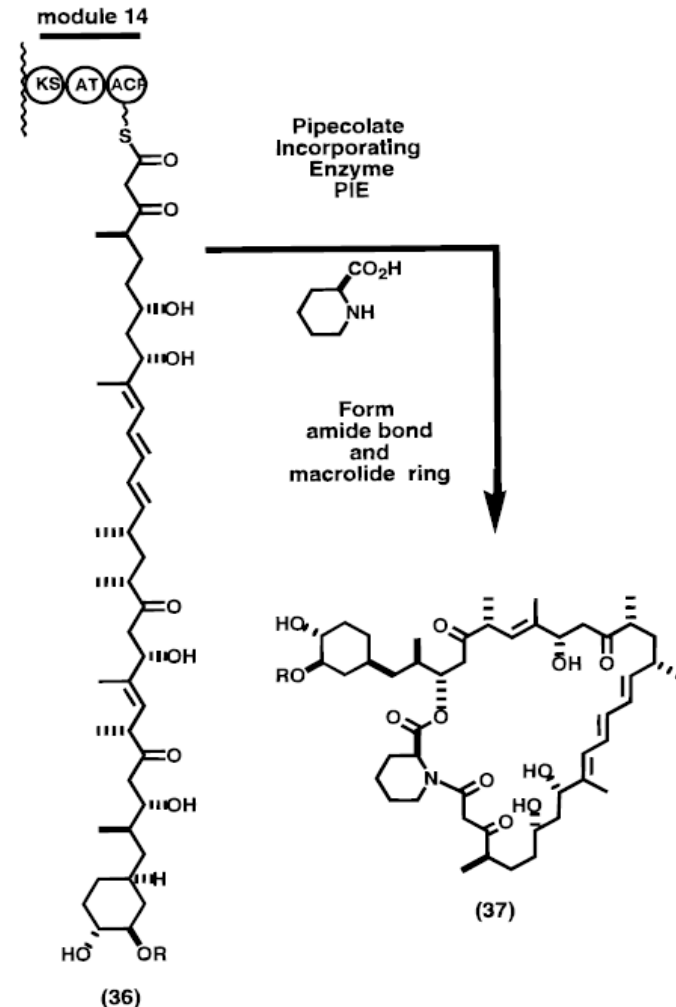
Organizzazione della PKS che sintetizza la Rapamicina



Biosintesi della Rapamicina

Il rilascio del polichetide dall'ultimo modulo di RAPS3 è catalizzato da rapP, che incorpora il pipecolato. Questo enzima ha elevata omologia con le NRPS

Esistono sistemi multienzimatici ibridi PKS-NRPS



Organizzazione strutturale di sistemi multienzimatici: PKS, NRPS e ibridi NRPS-PKS

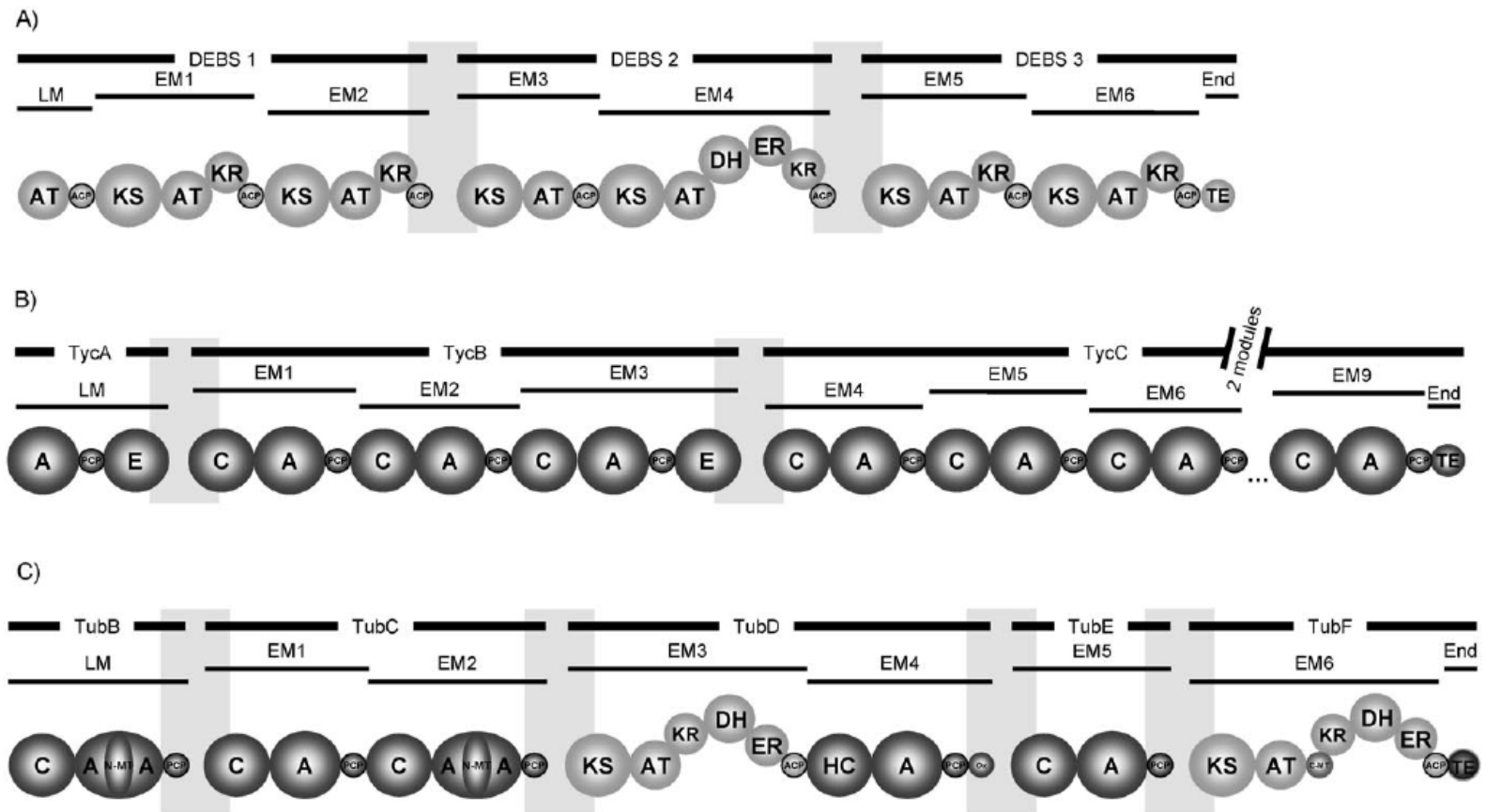
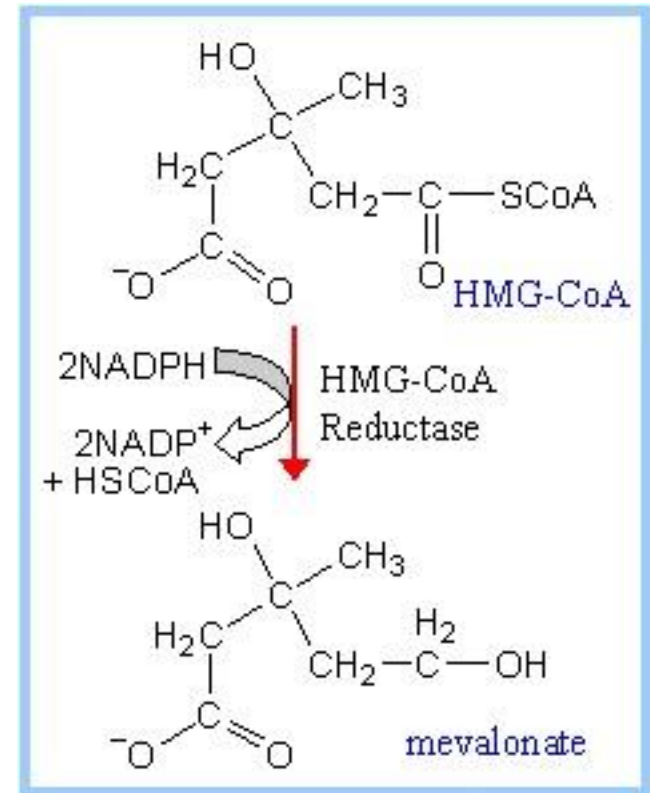
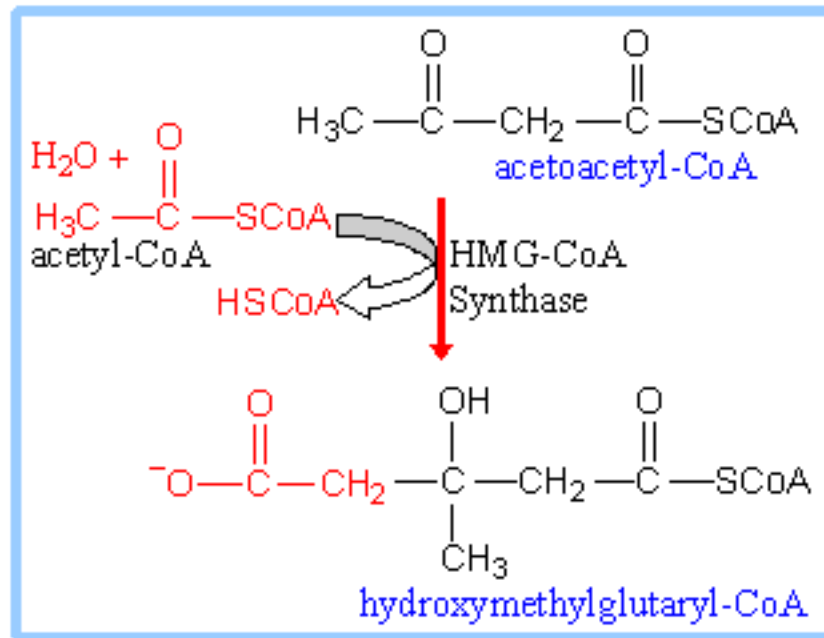


Figure 12. Multi-multienzyme organization of PKS, NRPS, and mixed PKS–NRPS systems. A) The 6-deoxyerythronolide B synthase (DEBS) responsible for erythromycin biosynthesis incorporates three subunits, DEBS 1, 2, and 3. Two intermodular junctions are formed across intersubunit interfaces (boxed gray regions). 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.

Sintesi del mevalonato, il precursore del colesterolo



Ruolo delle statine: inibitori della HMG-CoA reduttasi

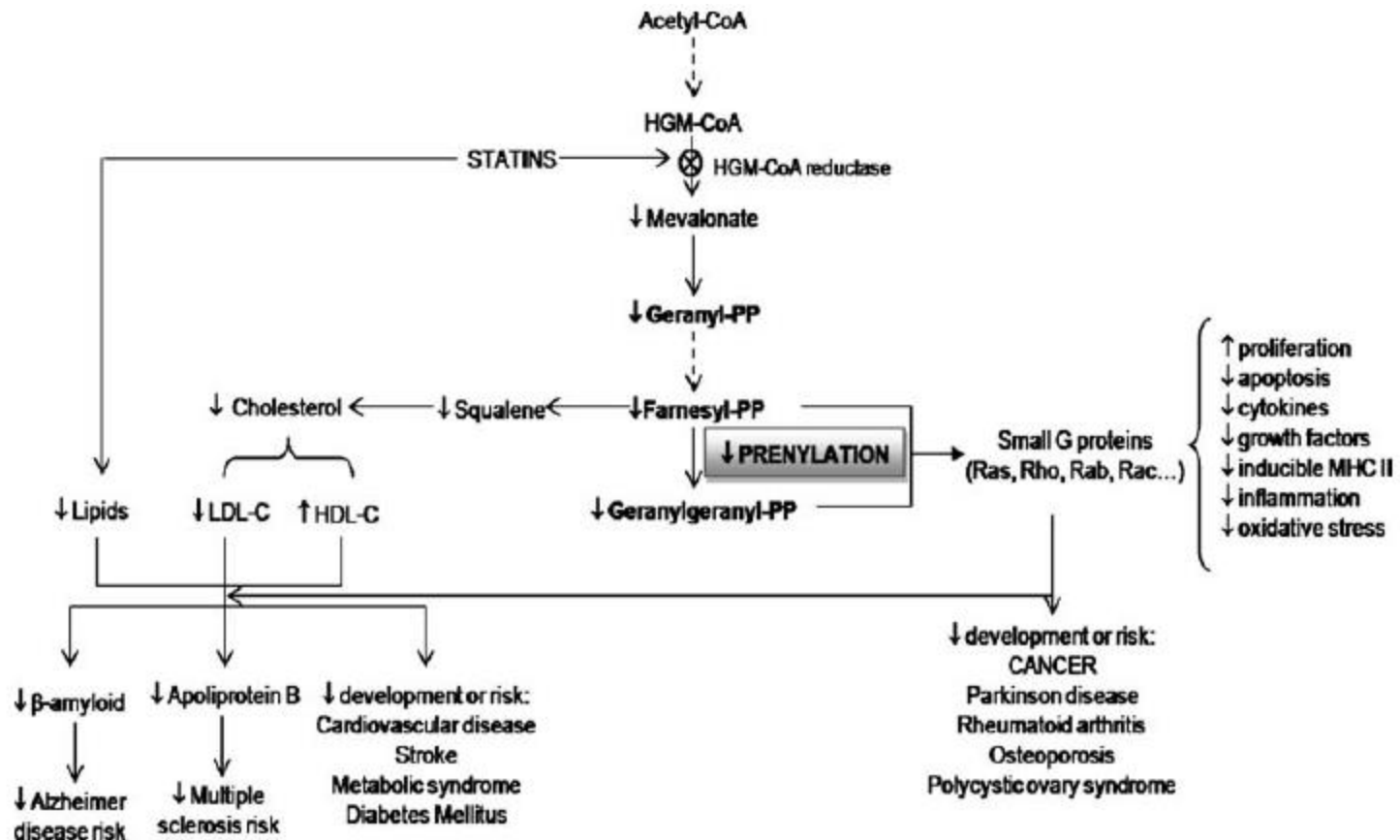


Fig. 5 Model explaining the great variety of biological effects of statins; and hence, current and potential uses. Inhibition of HMG-CoA reductase not only reduces cholesterol levels, but also of isoprenoid intermediates, affecting G proteins (i.e., Ras) prenylation. This can result in the modulation of signal transduction from receptors to gene

expression, directly affecting proliferation/apoptosis balance, inflammatory chemokines, and the cytogenic messages mediated by G proteins. Modified from: Cummings and Bauer 2000; Davignon and Leiter 2005; Massy and Gujjarro 2001

Struttura di HMG-CoA reduttasi con il substrato o una statina

Le statine sono inibitori
competitivi di HMG-CoA,
non di NADPH

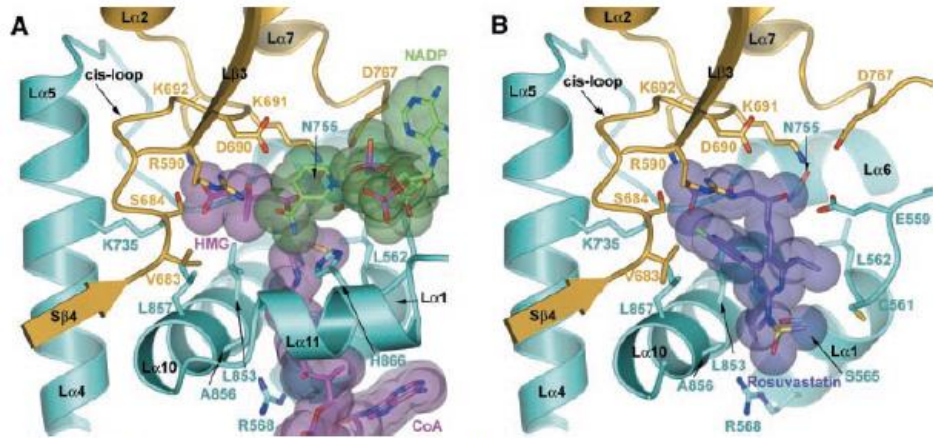
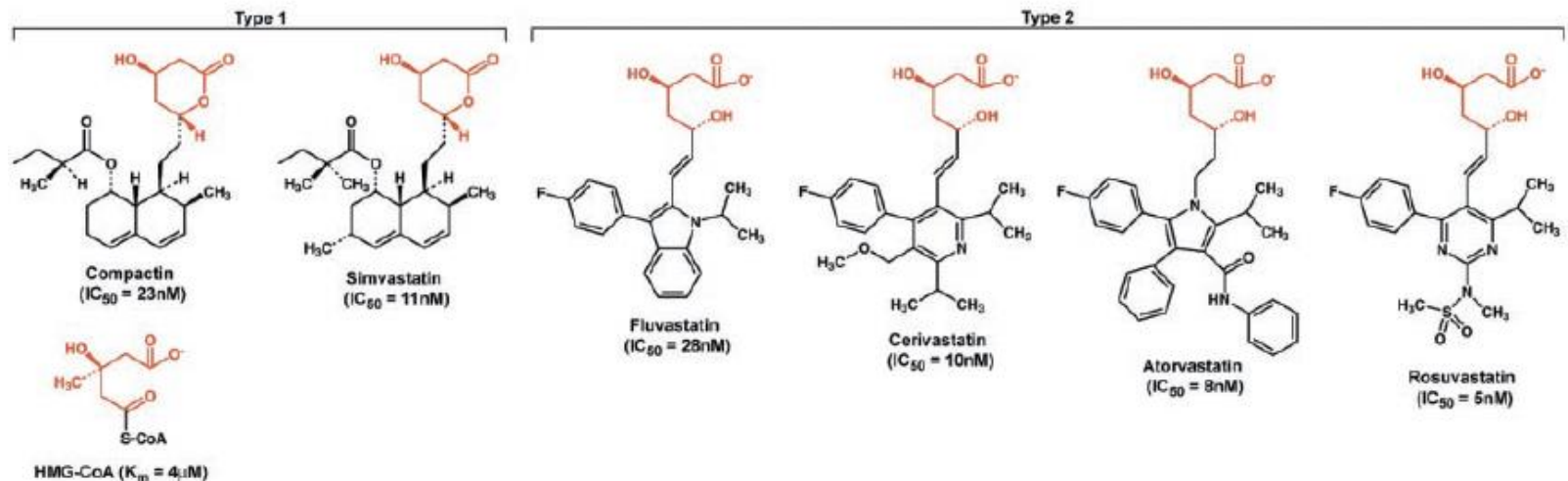
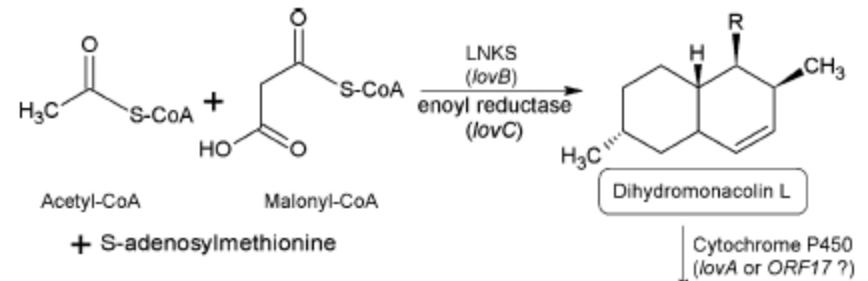
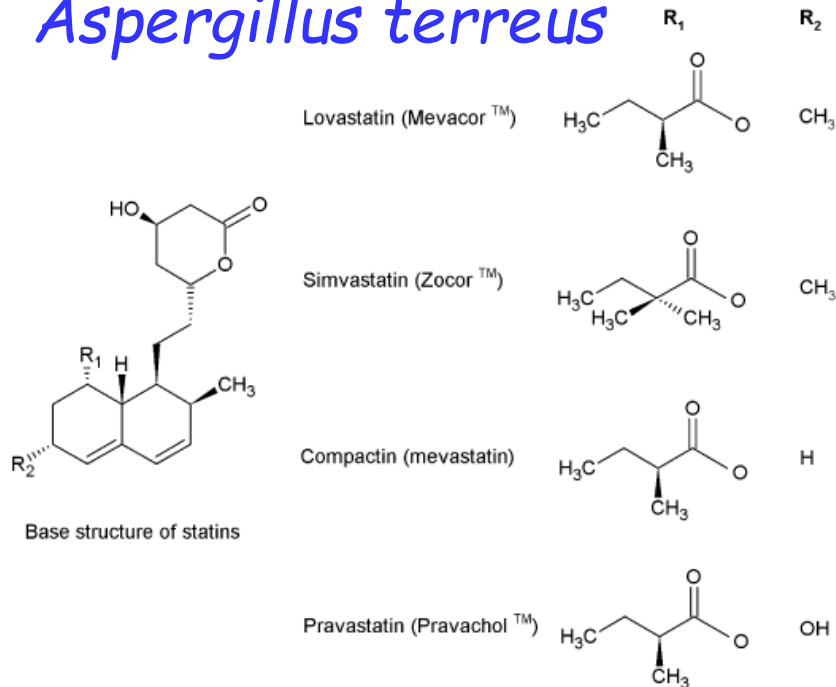


Fig. 2. Statins exploit the conformational flexibility of HMGR to create a hydrophobic binding pocket near the active site. (A) Active site of human HMGR in complex with HMG, CoA, and NADP. The active site is located at a monomer-monomer interface. One monomer is colored yellow, the other monomer is in blue. Selected side chains of residues that contact the substrates or the statin are shown in a ball-and-stick representation (20). Secondary structure elements are marked by black labels. HMG and CoA are colored in magenta; NADP is colored in green. To illustrate the molecular volume occupied by the substrates, transparent spheres with a radius of 1.6 Å are laid over the ball-and-stick representation of the substrates or the statin. (B) Binding of rosuvastatin to HMGR. Rosuvastatin is colored in purple; other colors and labels are as in (A). This figure and Figs. 3 and 4 were prepared with Bobscript (22), GLR (23), and POV-Ray (24).



Biosintesi delle statine: lovastatina prodotta da *Aspergillus terreus*



lovB è una PKS iterativa
con domini KS-MAT-DH-
MT-KR-ACP-CON

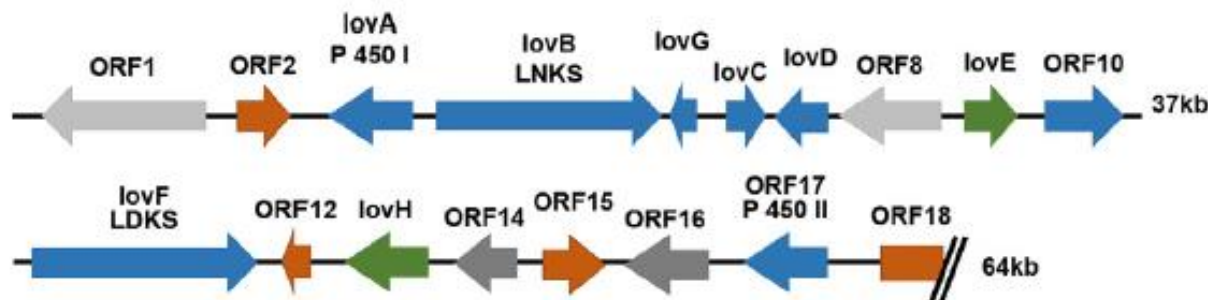
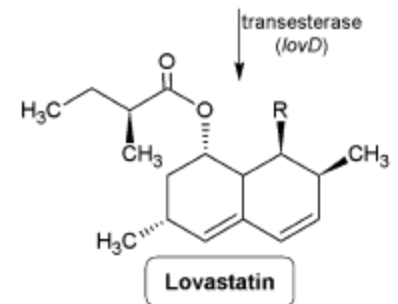
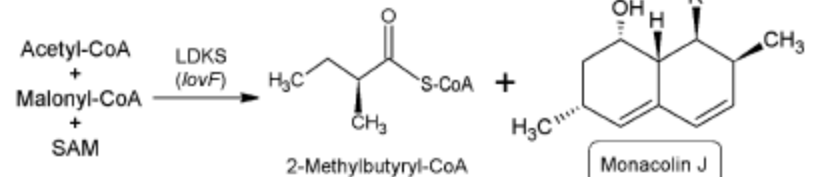


Fig. 4. Lovastatin biosynthetic gene cluster. The blue genes are encoding enzymes that operate in the metabolic pathway of lovastatin; green genes are encoding carriers; orange genes encode regulatory molecules; gray genes are encoding proteins involved in drug resistance and red genes have unknown function.

Biosintesi della lovastatina

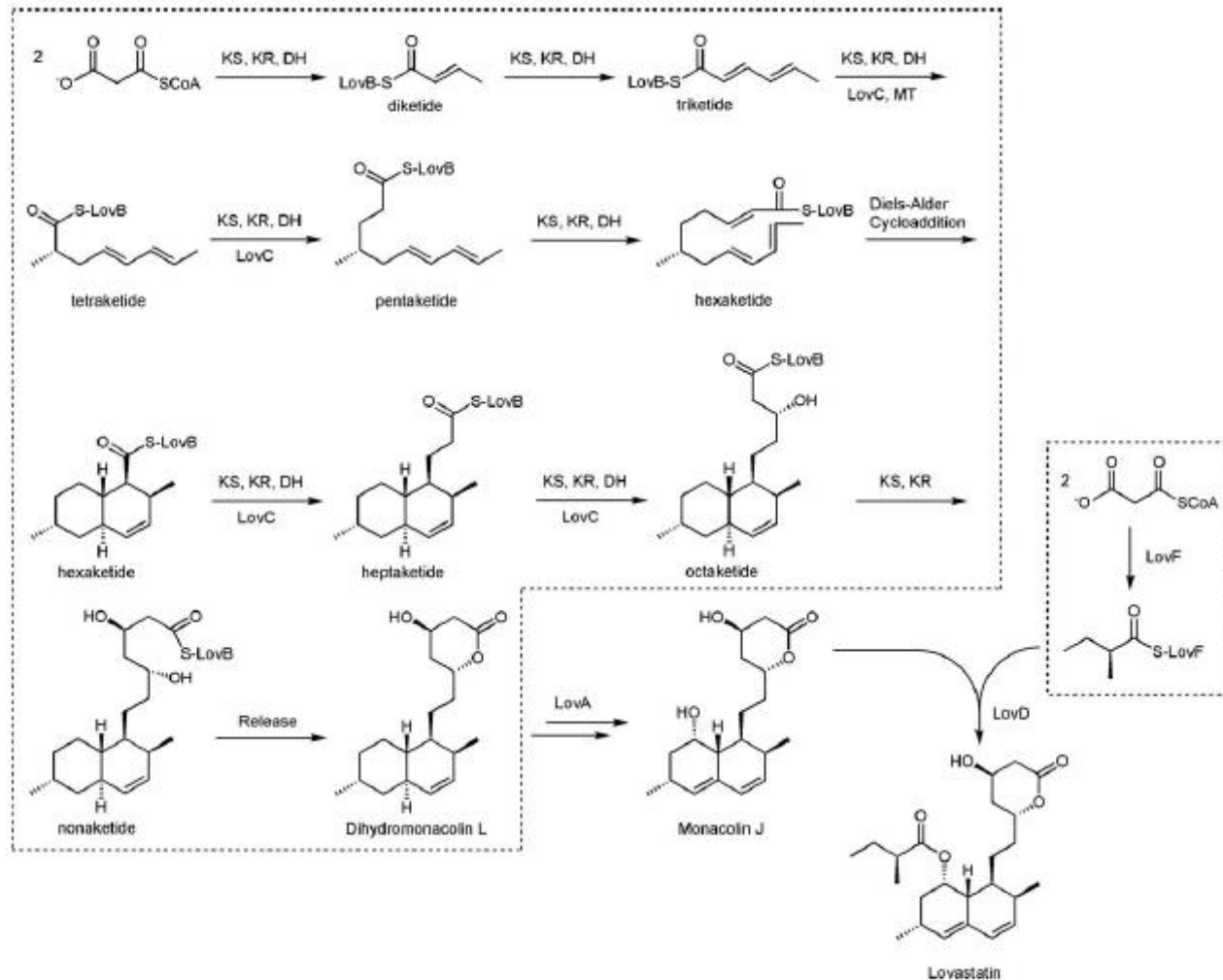


FIGURE 4 The proposed biosynthesis of lovastatin. The PKS steps are outlined by the dotted areas.