Biosynthesis – Inspiration for Drug Discovery

Biosynthesis of Fatty Acids & Polyketides

Alan C. Spivey
a.c.spivey@imperial.ac.uk

Imperial College
London

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Format & Scope of Lectures

- **What are fatty acids?**
  - 1° metabolites: fatty acids; 2° metabolites: their derivatives
  - biosynthesis of the building blocks: acetyl CoA & malonyl CoA

- **Fatty acid synthesis by Fatty Acid Synthases (FASs)**
  - the chemistry involved
  - the FAS protein complex & the dynamics of the iterative synthesis process

- **Fatty acid secondary metabolites**
  - eiconasiods: prostaglandins, thromboxanes & leukotrienes

- **What are polyketides?**
  - definitions & variety

- **Polyketide synthesis by PolyKetide Synthases (PKSs)**
  - the chemistry involved
  - the PKS protein complexes & the dynamics of the iterative synthesis process

- **Polyketide secondary metabolites**
  - Type I modular metabolites: macrolides – e.g. erythromycin
  - Type I iterative metabolites: e.g. mevinolin (=lovastatin®)
  - Type II iterative metabolites: aromatic compounds and polyphenols: e.g. actinorhodin
Fatty Acid Primary Metabolites

- **Primary metabolites:**
  - **fully saturated, linear carboxylic acids & derived (poly)unsaturated derivatives:**
    - constituents of essential natural waxes, seed oils, *glycerides* (fats) & phospholipids
    - **structural role** – *glycerides* & phospholipids are essential constituents of cell membranes
    - **energy storage** – *glycerides* (fats) can also be catabolised into acetate → citric acid cycle
    - **biosynthetic precursors** – for elaboration to secondary metabolites

**Saturated Acids** \[\text{MeCH}_2(\text{CH}_2)_n\text{CH}_2\text{CO}_2\text{H} \ (n = 2-8)\] e.g.

- caprylic acid \( (C8, n = 2) \)
- capric acid \( (C8, n = 3) \)
- lauric acid \( (C12, n = 4) \)

**Mono-unsaturated Acid Derivatives (MUFAs)** e.g.

- palmitoleic acid \( (C16, \Delta^9) \)
- oleic acid \( (C18, \Delta^9) \) \( (>80\% \text{ of fat in olive oil}) \)

**Poly-unsaturated Acid Derivatives (PUFAs)** e.g.

- arachidonic acid \( (AA) \) \( (C20, \Delta^5, \Delta^8, \Delta^{11}, \Delta^{14}) \)
- eicosapentaenoic acid (EPA) \( (C20, \Delta^5, \Delta^8, \Delta^{11}, \Delta^{14}, \Delta^{17}) \) \( \text{in cod liver oil} \)
Fatty Acids Derivatives – Secondary Metabolites

- **Secondary metabolites**
  - further *elaborated* derivatives of **polyunsaturated fatty acids (PUFAs)**
    - *e.g.* polyacetylenes & 'eicosanoids' (prostaglandins, thromboxanes & leukotrienes)

![Chemical Structures]

- **Polyacetylenes**
  - *e.g.* wyerone (anti-fungal)

- **Prostaglandins**
  - *e.g.* prostaglandin F\(_{2\alpha}\) (PGF\(_{2\alpha}\))

- **Thromboxanes**
  - *e.g.* thromboxane A\(_2\) (TXA\(_2\))

- **Leukotrienes**
  - *e.g.* leukotriene A\(_4\) (LTA\(_4\))

- **Obtucalene II**
  - Marine natural product in chamomile tea
Primary Metabolism - Overview

**Primary metabolism**

\[ \text{CO}_2 + \text{H}_2\text{O} \]

**PHOTOSYNTHESIS**
1) 'light reactions': \( \text{hv} \rightarrow \text{ATP} \) and \( \text{NADPH} \)
2) 'dark reactions': \( \text{CO}_2 \rightarrow \text{sugars} \) (Calvin cycle)

- **Glycolysis**
- **Glucose** & other 4,5,6 & 7 carbon sugars

**Primary metabolites**

- phosphoenol pyruvate
- erythrose-4-phosphate

**Secondary metabolites**

- oligosaccharides
- polysaccharides
- nucleic acids (RNA, DNA)

- SHIKIMATE METABOLITES
  - cinnamic acid derivatives
  - aromatic compounds
  - lignans

- ALKALOIDS
  - penicillins
  - cephalosporins
  - cyclic peptides

- FATTY ACIDS & POLYKETIDES
  - polyacetylenes
  - prostaglandins
  - aromatic compounds, polyphenols
  - macrolides

- ISOPRENOIDS
  - terpenoids
  - steroids
  - carotenoids

**Citric acid cycle (Krebs cycle)**

- Pyruvate
- Acetyl coenzyme A
- Malonyl coenzyme A
- Mevalonate
**Biosynthesis of Malonyl Coenzyme A**

- **Malonyl coenzyme A** is the key ‘**extender unit**’ for the biosynthesis of **fatty acids (& polyketides)**:
  - is formed by the **carboxylation** of **acetyl coenzyme A** mediated by a **biotin-dependent enzyme**
  - this is the **first committed step of fatty acid/polyketide biosynthesis** (& is a rate controlling step)

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**Primary metabolism**

- **pyruvate** → **GLYCOLYSIS**
  - oxidative decarboxylation
- **acetyl CoA** → **CITRIC ACID CYCLE**
  - acetyl CoA carboxylase (biotin-dependent)
- **mevalonate (MVA)** → **saturated fatty acids**
- **malonyl CoA** → **unsaturated fatty acids**
- **lipids**

**Secondary metabolism**

- **FATTY ACIDS & POLYKETIDES**
  - prostaglandins
  - polyacetylenes
  - aromatic compounds, polyphenols
  - macrolides
- **ISOPRENOIDS**
  - terpenoids
  - steroids
  - carotenoids
Biosynthesis of Malonyl Coenzyme A

- **Bicarbonate** is the source of the $CO_2$:
  - the bicarbonate is first *activated* via *phosphorylation* by ATP
  - then the *phosphorylated bicarbonate* carboxylates *biotin* to give *carboxybiotin*
  - then the *carboxybiotin* carboxylates the enolate of *acetyl CoA* to give *malonyl CoA*:

  - the carboxylation of biotin & acetyl CoA are mediated by a *single biotin-dependent enzyme (complex)* having both *biotin carboxylase* and *transcarboxylase active sites*
  - *NB.* coupling to ATP ‘hydrolysis’ provides *energy* to drive carboxylation processes
Biosynthesis of Fatty Acids – *Iterative Oligomerisation*

- **fatty acids** are biosynthesised from *acetyl CoA* as a *starter unit* by *iterative* ‘head-to-tail’ *oligomerisation* involving:
  - condensation with *malonyl CoA* as an *extender unit* (with loss of *CO₂*) – a *decarboxylative Claisen condensation*
  - 3-step *reduction* of the resulting *ketone* → *methylene*
- after *n = 2-8 iterations* the *C8-20 saturated fatty acid* is released from the enzyme(s):
The Decarboxylative Claisen Condensation (dCc)

- *in vitro* – the classical *Claisen condensation*:

![Chemical reaction diagram for in vitro Claisen condensation]

- *in vivo* - the *decarboxylative Claisen condensation* catalysed by a *ketosynthase (KS)*

![Chemical reaction diagram for in vivo decarboxylative Claisen condensation]

- the energy released upon loss of CO₂ provides a driving force for the condensation
- thioesters are also particularly reactive partners in this type of condensation...
The Claisen Condensation - Why Thioesters?

- recall the chemistry of coenzyme A (1st lecture) – properties of alkyl thioesters (cf. alkyl esters)
  - highly electrophilic carbonyl (~ ketone)
  - high acidity of protons $\alpha$ to the carbonyl of thioesters (cf. ester)
  - weak C-S bond (cf. C-O bond):
    - due to poor orbital overlap between the p-orbital lone pair on sulfur ($n_S$) [cf. $n_O$] and the carbonyl anti bonding orbital $\pi^*_\text{C=O}$; (i.e. minimal 'resonance' $n_S \rightarrow \pi^*_\text{C=O}$)

- good leaving group ability of $RS^-$ (cf. $RO^-$)
  - due to $pK_a$ (RSH) ~10 cf. $pK_a$ (ROH) ~16

\[
\begin{align*}
\text{polarised reactive carbonyl} & \quad \text{weak C-S bond} \\
\text{pKa = 20} & \quad \text{less effective} \\
\end{align*}
\]

\[
\begin{align*}
\text{pKa = 25} & \\
\end{align*}
\]
Ketone → Methylene - Reduction

- ketone → methylene reduction is achieved via a 3-step process:
  1. \( \text{NADPH}-\text{mediated ketone} \rightarrow \text{alcohol reduction} \) catalysed by a keto reductase (KR)
  2. syn-elimination of water catalysed by a dehydratase (DH)
  3. \( \text{NADPH}-\text{mediated hydrogenation} \) of the double bond catalysed by an enoyl reductase (ER)

- all steps are generally stereospecific but stereospecificity varies from organism to organism
  - indicated specificities are for human FAS
Biosynthesis of Fatty Acids – Overview of FAS

- The in vivo process by which all this takes place involves a ‘molecular machine’ - Fatty Acid Synthase (FAS)
  - **Type I FAS:** single multifunctional protein complex (e.g. in mammals incl. humans)
  - **Type II FAS:** set of discrete, dissociable single-function proteins (e.g. in bacteria)
  - **All FASs** comprise 8 components (ACP & 7× catalytic activities): ACP, KS, AT, MT, KR, DH, ER & [TE]:

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KS = keto synthase (also known as CE = condensing enzyme); AT = acetyl transferase; MT = malonyl transferase;
KR = keto reductase; DH = dehydratase; ER = enoyl reductase; TE = thioesterase; ACP = acyl carrier protein
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The Acyl Carrier Protein (ACP)

- The **Acyl Carrier Protein (ACP)** is the key protein that allows the growing oligomer to access the appropriate active sites.
- The ACP is first **primed** by the post-translational modification of one of its serine hydroxyl groups:
  - the introduction of a **phosphopantetheine ‘swinging-arm’** by reaction with **acetyl coenzyme A**:
Human Fatty Acid Synthase (FAS)

- The first three-dimensional structure of human fatty acid synthase (272 kDa) at 4.5 Å resolution by X-ray crystallography:

Structural overview. (A) Front view: FAS consists of a lower part comprising the KS (lower body) and MAT domains (legs) connected at the waist with an upper part formed by the DH, ER (upper body), and KR domains (arms). (B) Top view of FAS with the ER and KR domains resting on the DH domains. (C) Bottom view showing the arrangement of the KS and MAT domains and the continuous electron density between the KS and MAT domains.
FATTY ACID BIOSYNTHESIS (type II FAS)

NB. the following sequence of slides have been adapted from: http://www.courses.fas.harvard.edu/%7echem27/
• $\text{AT}_1$ loads acetyl group onto $\text{KS}_1$
FATTY ACID BIOSYNTHESIS

\[ \text{ACP}_1 \rightarrow \text{AT}_1 \rightarrow \text{KS}_1 \rightarrow \text{KR}_1 \rightarrow \text{DH}_1 \rightarrow \text{ER}_1 \rightarrow \text{ACP}_2 \rightarrow \text{MT}_2 \]

- Pantetheine
- Cys
- Me
- SH

\[ \text{ACP}_1 \rightarrow \text{AT}_1 \rightarrow \text{KS}_1 \rightarrow \text{KR}_1 \rightarrow \text{DH}_1 \rightarrow \text{ER}_1 \rightarrow \text{ACP}_2 \rightarrow \text{MT}_2 \]
FATTY ACID BIOSYNTHESIS

- AT\textsubscript{1} loads malonyl group onto ACP\textsubscript{1}

Malonyl-CoA

Pantetheine

Cys
FATTY ACID BIOSYNTHESIS

ACP₁ → AT₁ → KS₁ → KR₁ → DH₁ → ER₁ → ACP₂ → MT₂

Pantetheine

Cys

O

Me

O

SH
• KS₁ catalyzes Claisen condensation
FATTY ACID BIOSYNTHESIS

ACP₁  →  AT₁  →  KS₁  →  KR₁  →  DH₁  →  ER₁  →  ACP₂  →  MT₂

Pantetheine

Cys

SH
FATTY ACID BIOSYNTHESIS

- KR$_1$ catalyzes reduction of ketone
FATTY ACID BIOSYNTHESIS

ACP₁ → AT₁ → KS₁ → KR₁ → DH₁ → ER₁ → ACP₂ → MT₂

Pantetheine

Cys → SH

Me

S

SH

O

OH
FATTY ACID BIOSYNTHESIS

- DH₁ catalyzes dehydration of alcohol

Pantetheine

Cys

SH

Me

SH

• DH₁ catalyzes dehydration of alcohol
FATTY ACID BIOSYNTHESIS

ACP₁ → AT₁ → KS₁ → KR₁ → DH₁ → ER₁ → ACP₂ → MT₂

Pantetheine

Cys

SH

S

O

Me
FATTY ACID BIOSYNTHESIS

- ER₁ catalyzes reduction of alkene
FATTY ACID BIOSYNTHESIS

ACP₁ → AT₁ → KS₁ → KR₁ → DH₁ → ER₁ → ACP₂ → MT₂

Pantetheine

Cys → SH

S

Me

SH
FATTY ACID BIOSYNTHESIS

- KS$_2$ catalyzes translocation to module 2
FATTY ACID BIOSYNTHESIS

\[ H_1 \rightarrow ER_1 \rightarrow ACP_2 \rightarrow MT_2 \rightarrow KS_2 \rightarrow KR_2 \rightarrow DH_2 \rightarrow ER_2 \rightarrow TE \]

Pantetheine

\( \text{Cys} \)
\( \text{S} \)
\( \text{Me} \)
\( \text{OH} \)
\( \text{SH} \)
FATTY ACID BIOSYNTHESIS

- MT<sub>2</sub> loads malonyl group onto ACP<sub>2</sub>
FATTY ACID BIOSYNTHESIS

The diagram illustrates the fatty acid biosynthesis pathway, showing the enzymes and intermediates involved. The pathway includes enzymes such as ACP₂, MT₂, KS₂, KR₂, DH₂, ER₂, and TE, with key intermediates like Pantetheine, Cys, Me, OH, and Ser.
• KS<sub>2</sub> catalyzes Claisen condensation
FATTY ACID BIOSYNTHESIS

H₁ → ER₁ → ACP₂ → MT₂ → KS₂ → KR₂ → DH₂ → ER₂ → TE

Pantetheine

Cys SH

Ser OH

Me

S

O

O

O
• KR₂ catalyzes reduction of ketone
FATTY ACID BIOSYNTHESIS

H₁ ER₁ → ACP₂ → MT₂ → KS₂ → KR₂ → DH₂ → ER₂ → TE

- Pantetheine
- Cys
- Ser OH

Chemical structures:
- Serine (Ser)
- SH
- Me
- OH
- COOH
• DH₂ catalyzes dehydration of alcohol
FATTY ACID BIOSYNTHESIS
• ER₂ catalyzes reduction of alkene
FATTY ACID BIOSYNTHESIS

H₁ → ER₁ → ACP₂ → MT₂ → KS₂ → KR₂ → DH₂ → ER₂ → TE

Pantetheine

Cys SH

S

Me

OH

Ser
FATTY ACID BIOSYNTHESIS

- TE catalyzes transesterification
FATTY ACID BIOSYNTHESIS

H₁ → ER₁ → ACP₂ → MT₂ → KS₂ → KR₂ → DH₂ → ER₂ → TE

Pantetheine

Cys → SH

SH → Me

O → Ser
FATTY ACID BIOSYNTHESIS

• TE catalyzes hydrolysis
FATTY ACID BIOSYNTHESIS

Pantetheine

\[
\begin{align*}
H_1 & \quad \text{ER}_1 & \quad \text{ACP}_2 & \quad \text{MT}_2 & \quad \text{KS}_2 & \quad \text{KR}_2 & \quad \text{DH}_2 & \quad \text{ER}_2 & \quad \text{TE} \\
& \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \ quad
Biosynthesis of Unsaturated Fatty Acids

- **two mechanisms** are known for the introduction of double bonds into fatty acids:
  - in **BACTERIA**: anaerobic [O] → monounsaturated FAs (**MUFA**s)
  - in **MAMMALS, INSECTS & PLANTS**: aerobic [O] → **MUFA**s & polyunsaturated FAs (**PUFA**s)
Biosynthesis of Prostaglandins & Thromboxanes

- **prostaglandins** & **thromboxanes** are derived from further oxidative processing of arachidonic acid
- both are important **hormones** which control e.g. smooth **muscle contractility** (blood pressure), **gastric secretion, platelet aggregation & inflammation** (<nM activity)
  - various pharmaceuticals including **corticosteroids** & **aspirin** inhibit biosynthethetic steps in these pathways

![Chemical diagram showing the biosynthesis of prostaglandins and thromboxanes](image-url)
Biosynthesis of Leukotrienes

- **leukotrienes** are the other main class of 2° metabolites derived from **arachidonic acid**
  - they are potent (<nM) **inflammatory substances** released during allergic reactions

![Chemical reactions and structures](image)
The Polyketide Pathway

- **Polyketides** are also sometimes known as acetogenins
- acetyl CoA is also the starting point for the biosynthesis of polyketide secondary metabolites
- these metabolites are topologically very different to the fatty acid metabolites but are in fact synthesised in a very similar fashion. The significant difference is that during the iterative cycle of chain extension **the β-keto group is generally not completely reduced out**. This gives rise to huge structural diversity based around a 1,3-oxygenation pattern & cyclisation to give aromatic compounds

nb. unlike fatty acids. polyketides are NOT biosynthesised by humans – only microorganisms (bacteria) & fungi
Polyketides

- the structural variety of **polyketide secondary metabolites** is very wide:
  - *NB.* starter units marked in red; extender units in bold black; post oligomerisation appended groups in blue

- **6-methylsalicylic acid** (antibiotic)
- **orsellinic acid**
- **citrinin** (kidney toxin ‘yellow rice disease’)
- **Griseofulvin** (treatment for ring worm infections)
- **actinorhodin** (antibiotic)
- **aflatoxin B1** (mycotoxic carcinogen)

- **rapamycin** (immunosuppressant)
  - *NB.* a mixed polypropionate/acetate

- **6-deoxyerythronolide B**
  - *NB.* a polypropionate

- **erythromycin A** (antibiotic)
  - *NB.* a polypropionate

- **mevinolin** (=**lovastatin**)
  - (anti-cholesterol)
Historical Perspective – ‘The Acetate Hypothesis’

- **1907: James Collie** (University of London) effects conversion of *dehydroacetic acid* to *orcinol* by boiling with Ba(OH)$_2$ (while trying to deduce the structure of the former):

  - Collie perceptively postulated the *triketone* as an intermediate & suggested that this might also be an *intermediate* in the *biosynthesis* of *orcinol* (the ‘polyketide hypothesis’)

- **1955: Arthur Birch** used $^{14}$C labelled acetate to show that 6-methylsalicylic acid (ex. *Penicillium patulum*) was biosynthesised by head-to-tail oligomerisation of 4 × *acetate units* and proposed the following biogenesis – proceeding via a *tetraketide intermediate* (cf. Collie!):
Biosynthesis of Polyketides – Oligomerisation Steps

- **polyketides** are biosynthesised by a process very similar to that for **fatty acids**
  - the key **differences** are:
    - greater variety of starter units, extender units & termination processes
    - absent or incomplete reduction of the iteratively introduced $\beta$-carbonyl groups: ie. each cycle may differ in terms of KR, DH & ER modules & stereochemistry

- This leads to **enormous diversity**...
Polyketide Diversity

- **starter units:**
  - acetyl CoA
  - propionyl CoA
  - butyryl CoA
  - isobutyryl CoA
  - \( R = \text{Me, Et, }^{n}\text{Pr, }^{i}\text{Pr} \)

- **extender units:**
  - malonyl CoA (C\(_2\))
  - Me-malonyl CoA (C\(_3\))
  - Et-malonyl CoA (C\(_4\))
  - \( R' = \text{H, Me, Et} \)

- **non-functional or missing KR, DH, ER:**
  - no KR
  - no ER
  - no DH
  - none missing

- **stereochemistry:**
  1) side chain stereochemistry (determined by \(KSn\))
  2) OH stereochemistry (determined by \(KR_{n+1}\))
  3) alkene stereochemistry (determined by \(DH_{n+1}\))

- **termination step:**
  - depends on nucleophile that releases product at TE stage:
    - \( \text{Nu} = \text{H}_2\text{O} \)
    - \( \text{Nu} = \text{OH} \) intramolecular
    - \( \text{Nu} = \text{CoA} \)
    - NADH
Biosynthesis of Polyketides – Overview of PKS

- The *in vivo* process of polyketide synthesis involves *PolyKetide Synthases (PKSs)*:
  - **PKSs** (except Type II, see later) comprise the same 8 components as **FASs**, i.e. (ACP & 7× catalytic activities): ACP, KS, AT, MT, [KR, DH, ER & TE]
  
- **Type I PKSs**: single (or small set of) multifunctional protein complex(es)
  - *modular (microbial)* - each ‘step’ has a dedicated catalytic site (→ macrolides)
  - *iterative (fungal)* – single set of catalytic sites, each of which may operate in each iteration (cf. FASs) (→ aromatics/polyphenols - generally)

- **Type II PKSs**: single set of discrete, dissociable single-function proteins
  - *iterative (microbial)* - each catalytic module may operate in each iteration (cf. FASs) (→ aromatics/polyphenols)

\[ \text{KS = keto synthase; AT = acetyl transferase; MT = malonyl transferase; KR = keto reductase; DH = dehydratase; ER = enoyl reductase; TE = thioesterase; ACP = acyl carrier protein} \]
POLYKETIDE BIOSYNTHESIS [Type I – (modular)]

NB. the following sequence of slides has also been adapted from: http://www.courses.fas.harvard.edu/%7echem27/
• AT₀ loads starting group (propionyl) onto ACP₀
POLYKETIDE BIOSYNTHESIS

ACP₀ → AT₀ → ACP₁ → AT₁ → KS₁ → KR₁ → DH₁ → ER₁ → ACP₂ → A

Pantetheine

O=S
Me
• KS\textsubscript{1} catalyzes translocation to module 1
POLYKETIDE BIOSYNTHESIS
- \textit{AT}_1 \text{ loads methylmalonyl group onto ACP}_1
POLYKETIDE BIOSYNTHESIS

P0 → AT0 → ACP1 → AT1 → KS1 → KR1 → DH1 → ER1 → ACP2 → AT2

Pantetheine

Cys

O

O

Me

O

Me

O

Me
• KS\textsubscript{1} catalyzes Claisen condensation
POLYKETIDE BIOSYNTHESIS

Pantetheine

Stereocenter
• KR$_1$ catalyzes reduction of ketone
POLYKETIDE BIOSYNTHESIS

P0 \longrightarrow AT0 \longrightarrow ACP1 \longrightarrow AT1 \longrightarrow KS1 \longrightarrow KR1 \longrightarrow DH1 \longrightarrow ER1 \longrightarrow ACP2 \longrightarrow AT2

Pantetheine

\text{Cys} \quad \text{SH}

\text{S}

\text{O}

\text{OH}

\text{Me}

\text{Me}

\text{Stereocenter}
• no DH$_1$ activity
POLYKETIDE BIOSYNTHESIS

- no ER$_1$ activity
• KS₂ catalyzes translocation to module 2
POLYKETIDE BIOSYNTHESIS

H₁ → ER₁ → ACP₂ → AT₂ → KS₂ → KR₂ → DH₂ → ER₂ → TE

Pantetheine

Cys

O

* Me

HO

Me

SH

OH

Ser
Biosynthesis of Erythromycin – *Type I*(modular) PKS

- **6-deoxyerthronolide** is a precursor to *erythromycin A* – bacterial antibiotic (*Streptomyces erythreus*):
  - propionate based heptaketide; 3 multifunctional polypeptides (DEBS1, DEBS2 & DEBS3, all ~350 kDa)
Biosynthesis of Mevinolin – *Type I*(iterative) PKS

- **mevinolin (=lovastatin®)** – cholesterol lowering metabolite of filamentous fungus *Aspergillus terreus*
  - inhibits HMG-CoA → mevalonate (see next lecture) – rate-limiting step in biosynthesis of *cholesterol*
  - acetate based polyketide composed of a diketide and nonaketide linked by an ester
  - 2 × Type I (iterative) PKSs: LNKS and LDKS...both contain *MeT (methyl transferase)* activities
  - Hutchinson *et al.* *Science* *1999*, 284, 1368 ([DOI](https://doi.org/10.1126/science.284.5412.1368))

![Chemical Reaction Diagram]

- LNKS
- LDKS
- MeT (methyl transferase)
- HMG-CoA
- Mevinolin
- Lovastatin®
Type II PKSs – *Enzyme Clusters (Microbial)*

- **Type II PKSs**: single set of discrete, dissociable single-function proteins (ACP & 6× catalytic functions): *ACP, KS*$_\alpha$, *KS*$_\beta$, [KR, DH, ER, & TE] [NB. NO acetyl or malonyl transferases (AT, MT)]
  - *iterative* - each catalytic module may operate in each iteration (cf. FASs) (→ aromatics/polyphenols)
- these clusters (generally) use *malonate* as BOTH *starter* & *extender* unit
- their *ACP proteins* are able to load malonate direct from malonyl CoA (no MT required)
  - the *starter malonate* is *decarboxylated* by ‘ketosynthase’ β (*KS*$_\beta$) to give S-acetyl-ACP
  - the *extender malonates* undergo *decarboxylative Claisen condensations* by *ketosynthase α* (*KS*$_\alpha$)
- these clusters rarely utilise *KR, DH* or *ER* activities and produce ‘true’ polyketides:

\[
\begin{align*}
\text{CoAS} & \quad \text{CONH}_2 \\
\text{SH} & \quad \text{SH} \\
\text{ACPs} & \quad \text{ACP}
\end{align*}
\]

\[
\begin{align*}
\text{KS}_\beta & \quad \text{ACP} \\
\text{KS}_\alpha & \quad \text{ACP} \\
\text{KS}_\alpha & \quad \text{ACP} \\
\text{KS}_\alpha & \quad \text{ACP} \\
\text{KS}_\alpha & \quad \text{ACP}
\end{align*}
\]

\[
\begin{align*}
\text{S-acetyl-ACP} & \quad \text{S-acetyl-ACP} \\
\text{S-acetyl-ACP} & \quad \text{S-acetyl-ACP} \\
\text{S-acetyl-ACP} & \quad \text{S-acetyl-ACP} \\
\text{S-acetyl-ACP} & \quad \text{S-acetyl-ACP} \\
\text{S-acetyl-ACP} & \quad \text{S-acetyl-ACP}
\end{align*}
\]

\[
\begin{align*}
\text{KS}_\beta & = \text{‘keto synthase }\beta\text{’ (=decarboxylase!)}; \\
\text{KS}_\alpha & = \text{‘keto synthase }\alpha\text{’ (=ketosynthase!)}; \\
\text{KR} & = \text{ketoreductase;} \\
\text{DH} & = \text{dehydratase; ER} & = \text{enoyl reductase; TE} & = \text{thioesterase; ACP} & = \text{acyl carrier protein}
\end{align*}
\]
Biosynthesis of Actinorhodin – *Type II PKS*

- **actinorhodin** – octaketide **bacterial antibiotic** (*Streptomyces coelicolor*)
  - Hopwood *Chem. Rev.* 1997, 97, 2465 ([DOI](https://doi.org/10.1021/je0654880))

- **octaketide** synthesis then cyclisation? (as shown above)
- **hexaketide** synthesis then cyclisation then two further rounds of extension?
- Indications can sometimes be gleaned from **biomimetic syntheses**...

- **timing** of 1st cyclisation and mechanism of **control of chain length** uncertain
  - **octaketide** synthesis then cyclisation? (as shown above)
  - **hexaketide** synthesis then cyclisation then two further rounds of extension?
Scope of Structures - Type II PKS

- *microbial polyphenolic* metabolites:

  - **pentaketides** (5x C₂)
    - ![Pentaketide](image1)
    - *eugenone*
  
  - **hexaketides** (6x C₂)
    - ![Hexaketide](image2)
    - *plumbagin*
  
  - **heptaketides** (7x C₂)
    - ![Heptaketide](image3)
    - *rubrofusarin*
  
  - **octaketides** (8x C₂)
    - ![Octaketide](image4)
    - *emodin*
  
  - **nonaketides** (9x C₂)
    - ![Nonaketide](image5)
    - *tetracycline*
  
  - **decaketides** (10x C₂)
    - ![Decaketide](image6)
    - *rabelomycine*

- many display interesting biological activities...
Primary Metabolism - Overview

1) 'light reactions': hv -> ATP and NADPH
2) 'dark reactions': CO₂ → sugars (Calvin cycle)

Primary metabolism

**Primary metabolites**
- oligosaccharides
- polysaccharides
- nucleic acids (RNA, DNA)

**Secondary metabolites**
- SHIKIMATE METABOLITES:
  - cinnamic acid derivatives
  - aromatic compounds
  - lignans, flavinoids
- ALKALOIDS:
  - penicillins
  - cephalosporins
  - cyclic peptides
- FATTY ACIDS & POLYKETIDES:
  - prostaglandins
  - polyacetylenes
  - aromatic compounds, polyphenols
  - macrolides
- ISOPRENOIDS:
  - terpenoids
  - steroids
  - carotenoids

**Primary metabolism**
- CO₂ + H₂O
- PHOTOSYNTHESIS
- glycolysis
- glucose & other 4,5,6 & 7 carbon sugars
- phosphoenol pyruvate
- erythrose-4-phosphate
- pyruvate
- acetyl coenzyme A
- acetoacetyl coenzyme A
- malonyl coenzyme A
- mevalonate

**Citric acid cycle (Krebs cycle)**
- SCoA
- CoA