Aromatic polyketides were at the start of the biosynthetic field
One of the first:

- biomimetic synthesis (Collie, 1907)
- isotopic labeling studies (Birch, 1955)
- gene cluster sequence (Hopwood, 1984)

Figures removed due to copyright reasons.
Please see: Fig.2A-D in *Top Curr Chem* 209 (2000): 1-51.
We now know that there are many variations of the polyketide pathway.

Type I PKS: Catalytic domains are linked together on same polypeptide
Each catalytic domain is used once- "noniterative"
Recognized 1991

Type II PKS: Catalytic domains are individual proteins
A Catalytic domain is used for multiple claisen condensations "iterative"
Recognized 1984

Type III PKS: Similar to Type II
No ACP; all substrates are acyl-CoA
Called chalcone synthases and frequently found in plants
Recognized 1999

Iterative Type I PKS: Catalytic domains are linked together on same polypeptide and used iteratively
Recognized in last 2-3 years
Figure removed due to copyright reasons.
Please see Fig. 1 in *Current Opinion in Chemical Biology* 7 (2003): 285-295.
DNA of Type II PKS pathway Actinorhodin was sequenced in mid 1980’s:

Inspired the search for the genes and was accomplished in 1984 by Hopwood (this in turn inspired the search for the erythromycin genes)

Although the genes are known it is not completely clear how the enzymes work (shown on next pages)- Enzymes observed were:

“Minimal” PKS: ACP, KS, KSβ chain length factor (CLF)
KR (ketoreductase) → reduces carbonyl to hydroxyl
CYC (cyclase) like dehydratases
ARO (aromatase) CYC and ARO very similar → same type of protein

Questions??
No AT domain (loading by either fatty acid AT, by KS or by ACP)
No TE domain
Regioselectivity of reduction/cyclization
Unknown function for second KS domain that lacks active site cysteine (KSβ or CLF (Chain Length Factor))

What controls the chain length?
Polyketide Biosynthesis

Type II Polyketide Biosynthesis: Aromatic Polyketides

malonyl-CoA -> usual substrate

single peptide

initiation

- CO₂
deaketide

Enzymes of aromatic polyketide biosynthesis bacteria separate proteins; fungi single protein

16 carbons

"iterative"

minimal polyketide synthase module

type II ACP domains have AT activity
Figure removed due to copyright reasons.
Please see Fig. 44 in *Nat Prod Rep* 18 (2001): 380-416.
KR / reduction
changes way PK folds on itself
1. Where is AT domain?
ACP has some endogenous AT activity, though perhaps not enough to support in vivo biosynthesis likely that an AT from FAS synthesis or elsewhere is recruited (Biochemistry (1998) 37, 2084-2088)

2. What controls chain length?
KS forms a heterodimer: second protein called KSβ or CLF (Chain Length Factor)
proof that this controls chain length

Crystal structure and mutagenesis studies suggest that a channel formed between the KS and CLF control the length of the polyketide

In vitro: mutate G116T mutation changes from 20 length to >65% C16
Figure removed due to copyright reasons.
Please see Fig. 1 in *JACS* 125 (2003): 12708-12709.
Where is the thioesterase?
self cleavage from the thioester (see novobiocin biosynthesis from NRPS section)

A set of “design rules” were proposed in 1995 (Nature (1995) 375, 549-554).
May be outdated

“Minimal PKS” sets the chain length- can switch out an ACP without affecting length but a KS and KSB from same cluster are required for consistent chain lengths

A KR domain reduces carboxyl to the hydroxyl (act reduces C9)

First ring formation appears to be formed from the minimal PKS (or is not enzymatically catalyzed)

Position of reduction affects regioselectivity of cyclization
  reduction at C9 cyclization at C7/C12; if reduce at C7 cyclize at C5/C10 (regiospecificity less well defined when reduction does not occur)

Aromatization domains will only recognize specific carbon length
  act ARO recognizes only 16 carbon chains

KR --> each KR has own regioselectivity
  --> timing of KR. --> sometimes before ARO/CYC
    sometimes acts after ARO/CYC
Figure removed due to copyright reasons.
Please see Fig. 11 in Top Curr Chem 209 (2000): 1.
Polyketide Biosynthesis

Type II Polyketide Biosynthesis: Aromatic Polyketides

Figure courtesy of:
Tang, Yi, Taek Soon Lee, and Chaitan Khosla.
Birch (1954) recognized that polyketones can be made by condensation of acetates. What happens if *Penicillium patulum* are fed isotopically labeled acetates?

\[ \text{AcOH} \rightarrow \text{HO} \rightarrow \text{6-methyl salicylic acid} \]

\* = $^{14}\text{C}$ (radioisotope)

Support for Collie's 1907 hypothesis that aromatic compounds were made from polyketone structures in the cell.
Labeled acetates can be converted into malonyl-CoA

starting material

acetate thiokinase

acetyl CoA carboxylase

"Primary metabolism"

immediate precursor
Labeling with 13C isotopes facilitated NMR studies

How does labeling work?
- Indicates the starting material
- Provides structural information

Assign unlabeled compound- then see which signals increase
Use non-decoupled 13C spectra to observe splitting from the protons

13C, 2H labeled acetate
upfield shift when a deuterium is attached to a C13 (alpha effect) or is one carbon removed (beta effect)

4 enriched signals
8 = singlet
2 = d
4 = d
6 = d

6-methyl salicylic acid

α field signal increases
more pronounced
less pronounced

A = acid, B = base
Double label enables observation of 13C-13C coupling. Isotopically labeled substrate becomes diluted only one 13C acetate gets incorporated per structure. Therefore, only observe coupling between 2 carbons that belong to the same acetate.

\[
\begin{align*}
\text{Splitting of } C_1 = C_2 \\
\text{Splitting of } C_3 = C_4 \\
J_{1,2} &
eq J_{3,4}
\end{align*}
\]

\[
\text{C2-C3 splitting}
\]

\[\text{doublet} \quad C_8 \sim 160 \text{ ppm} \quad J = 6 \text{Hz}
\]
\[\text{C1} \sim 100 \text{ ppm} \quad J = 6 \text{Hz}
\]

\[\text{13C spectrum}
\]
Figure removed due to copyright reasons.
Please see Fig. 8 in *Nat Prod Rep* 18 (2001): 380-416.
Gene clusters of calicheamycin and C-1027 have been shown to be made up of polyketide synthases. Feeding studies in 1989 established an acetate starting material (Science (2002) 297, 1170, 1173).
Figure removed due to copyright reasons.
Please see Fig. 23 in *Nat Prod Rep* 18 (2001): 380-416.
Polyketide Biosynthesis
Type II Polyketide Biosynthesis: Aromatic Polyketides

Diels Alderase?

Has nature discovered the Diels-Alder reaction??

lovastatin nonaketide synthase

20°C aq. KR DH dienophile

CO-S-CoA diene nonaketide

lovastatin

Rate enhancement without $t_{1/2}$ 60h with $k_{cat}$ ~4s$^{-1}$

after addition of the enzyme lovB (LNKS)

JACS (2000) 122 11519-11520
Figure removed due to copyright reasons.

Figure removed due to copyright reasons.
Figure removed due to copyright reasons.
Please see Fig. 6-13 in Nat Prod Rep 18 (2001): 380-416.