Lecture #7

Lecture 7
2/18/04

March 3&4 Peter Leadley (discovered erythromycin genes) will be giving organic chemistry seminars

Protein organization

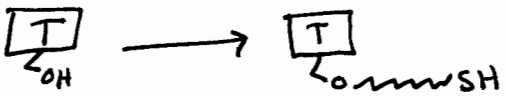
Type I PKS- non-iterative
The polypeptide sequence is the template for the biosynthetic pathway

Type II PKS iterative (like FAS)
same module used over again for next reaction

Handout 2c – We know a huge amount of structural information about the individual domains, but we still have no intact structure of the complete system.
Recently, a docking model was done of ACP to MAT. They proposed that a negatively charged surface of ACP interacts with positively charged surface on MAT (charge complementarity).
Important question: How do these proteins link and communicate with each other? Domains are sometimes all on one polypeptide, sometimes separate.

B. CHEMISTRY (post-translational modification, initiation, elongation, decoration, termination, fidelity)

I. priming:



The discovery of the enzyme that attaches the swinging phosphopantethiene arm was a major breakthrough (Phosphopantethiene transferase – PPTfase)

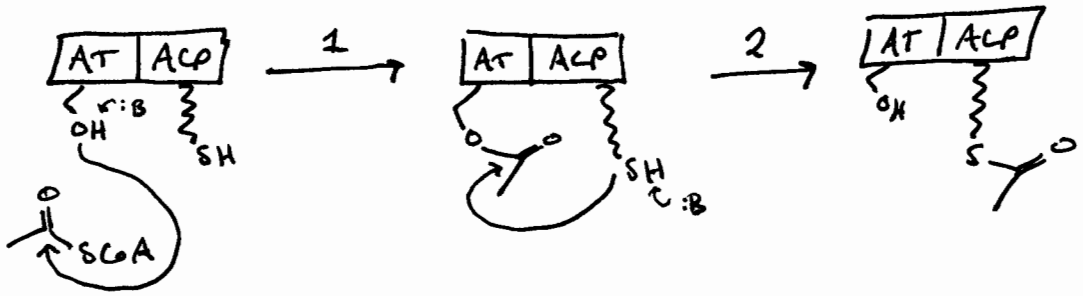
ACP, PCP, and Aromatic carrier protein (ArCP) (now all these proteins are referred to as "T" domains) have conserved active site structure with a serine that is modified with a swinging arm. The proteins are inactive until post-translational modification with the swinging arm.

Serine modified with phosphopantethiene arm (which will be represented from here on as a squiggle).

II. Initiation or Loading:

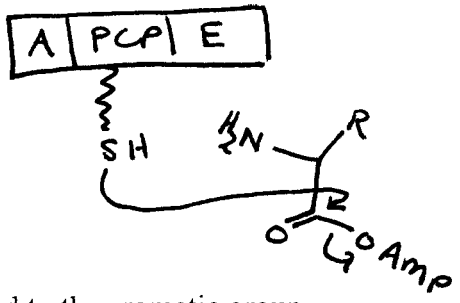
PKS (i.e. erythromycin -> Debs1)

1) Acyl group attaches to serine of AT
2) Acyl group is loaded onto the swinging arm of ACP



NRPS (i.e. gramicidin- cyclized decapeptide)

1) [A] domain makes adenylated amino acid
2) aa is then transferred to swinging arm of PCP
   [E]= epimerization domain

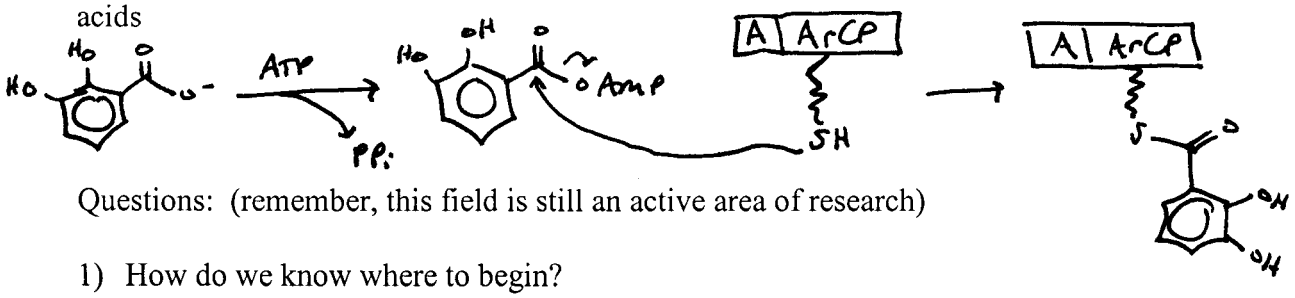


In addition to ACP and PCP, there are ArCPs

1) [A] domain activates the carboxylic acid attached to the aromatic group
2) Activated aromatic group is transferred to the swinging arm of ArCP

(completely analogous to NRPS)

-aromatic groups come from the biosynthetic pathway that makes aromatic amino acids



Questions: (remember, this field is still an active area of research)

1) How do we know where to begin?

Believed for Type I PKS: start at N-terminus of polypeptide and move along like an assembly line

2) Can you begin in the middle of the polypeptide?

We think yes

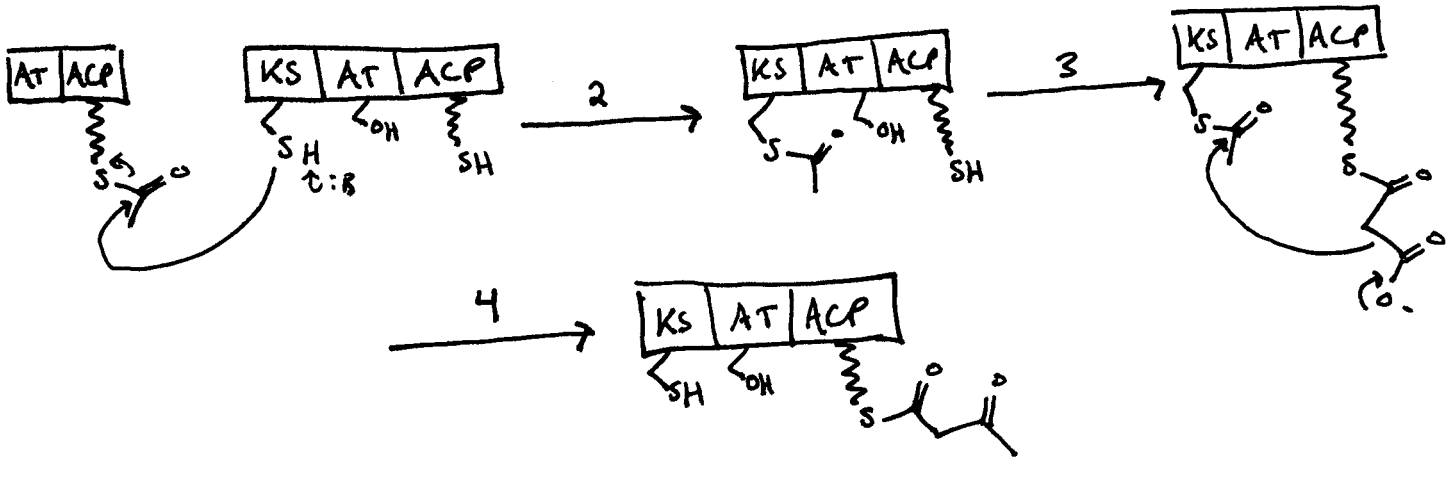
3) What causes the sequence of events without any mistakes? How is linearity enforced? (or is it?)

4) How do you make the steps in the pathway irreversible?

II. Elongation (translocation and condensation)

PKS

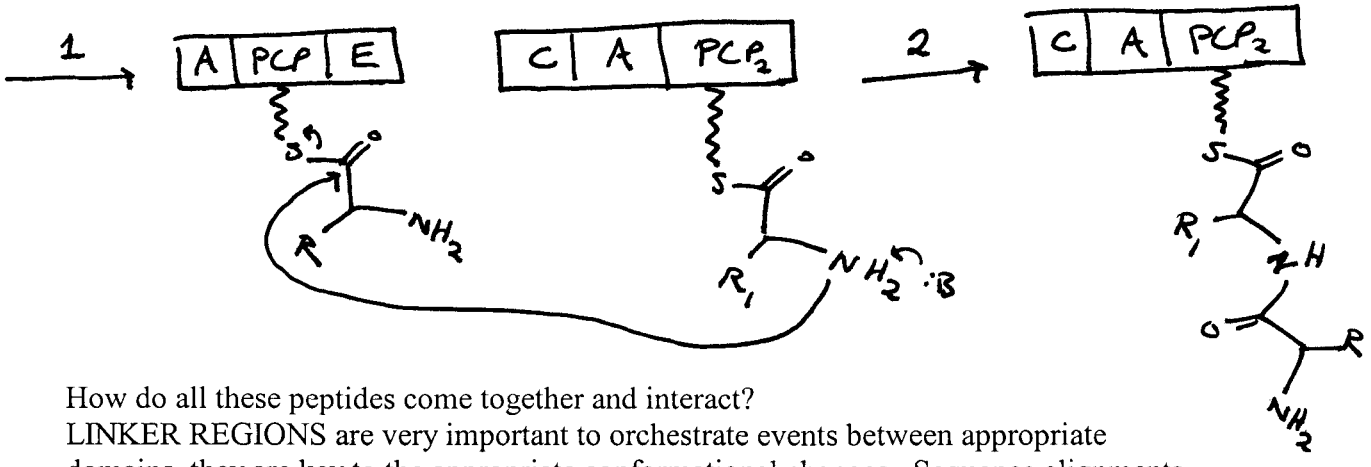
1) acyl group on ACP
2) translocation of acyl group to KS
3) malonyl CoA loaded on next ACP by AT
4) condensation reaction to extend chain (like FAS)



NRPS

1) [A] domain activates amino acid and loads it on PCP
2) Translocation and condensation in one step

Order of reactions is defined by the gene sequence



How do all these peptides come together and interact?
LINKER REGIONS are very important to orchestrate events between appropriate domains, they are key to the appropriate conformational changes. Sequence alignments suggest that the linker regions are 20 to 30 amino acids

The linkers may be important between modules (**inter**molecular) or between domains within a module (**intra**molecular)

Play a key role in allowing the chemistry to happen

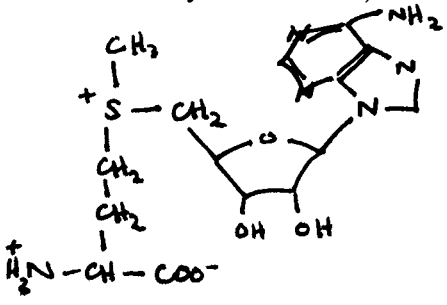
Question: When can you load again? An you have two or more natural products made at the same time? After the first module is finished and the product has moved on to the second module, can the first module be reloaded?
We think you can have more than one domain loaded at a time like an assembly line. Think about polysomes in poly peptide sythesis.
How do you tell whether module 1 and 2 can be carrying out chemisty simultaneously? - Use of mass spectroscopy. You will have a methods paper by Walsh/Kelleher groups that address this issue.

III. Decoration, Auxiliary Domains

PKS (like FAS)

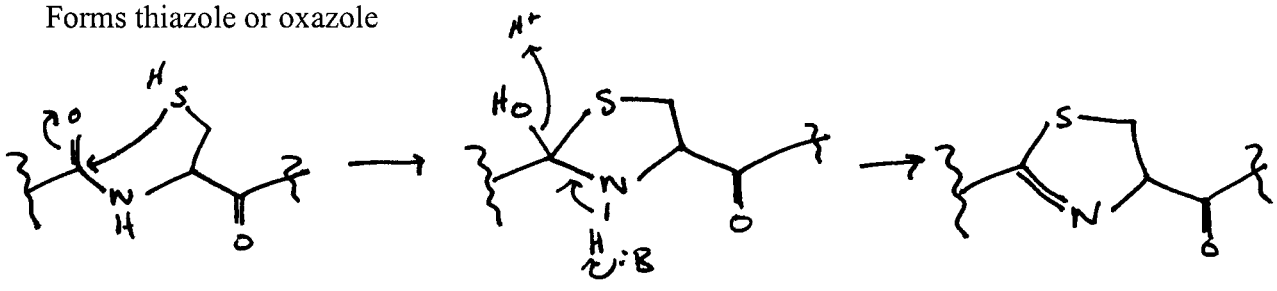
ER- enoyl reductase
KR- ketoreductase
DH- dehydratase
MeT – methyl transferase
(major methylating agent is SAM- s-adenosyl-methionine)

SAM structure



NRPS

E- epimerization
MeT- methyl transferase (SAM)
H- Hydroxylation (add OH group) (either heme Fe, O_2, like cytochrome p450 or non-heme Fe^{2+} alpha-ketoglutarate)
G- glycoslyation (add sugars)
Cy- cyclization domains
Forms thiazole or oxazole



Reaction of Cy domains on serine or cysteine

ATP DEPENDENT

↑ SHOWN

Next time: termination
Specific examples of PKS and NRPS