Lecture #9

Lecture 9
2/23/04

Reference- Fidelity in PKS
PNAS **97** 14188 (2000)

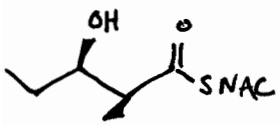
Specific Examples (cont)

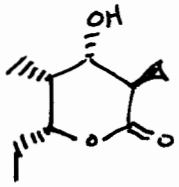
1) Erythromycin

We can predict biosynthetic pathway from gene sequence

p15 Handout 2a (review of last lecture)

Make precursor to feed into PKS module (w/ N- acetyl cysteamine-(NAC) tail instead of CoA)
Tried Modules 2, 5, and 6 with TE domain pasted onto the end of the module
Each of these modules has the same domain organization
In all cases they were able to load precursor and make the same product, with similar kinetics





Precursor **Domain Order** **Product**

They also compared loading the chemically synthesized precursor to making the starting material with module 1
Again, they found similar results and kinetics

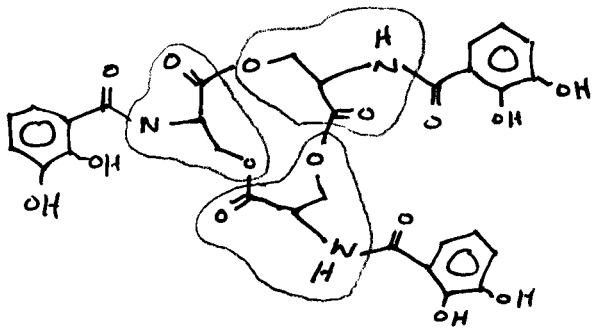
These machines are very forgiving, bodes well for our ability to manipulate them and make combinatorial libraries of compounds

They also varied the linker regions- swapped polypeptide linker regions
Complementary linkers vs. non-complimentary linkers
They got the same product in both cases, with rates constants for non-complementary linkers a factor of ten lower
Is this significant? Suggests importance of linkers

% yield? Are there any other side products in these reactions? We need to look at all of these experiments in more detail

Erythromycin is probably the best understood natural product, and we still have a lot to learn

2. Enterobactin- NRPS
Siderophore- binds Fe^{3+}
$K_D = 10^{-52}$ M Wow! How do you measure something like that?!



Enterobactin structure with three serines circled

Iterative "waiting room" model

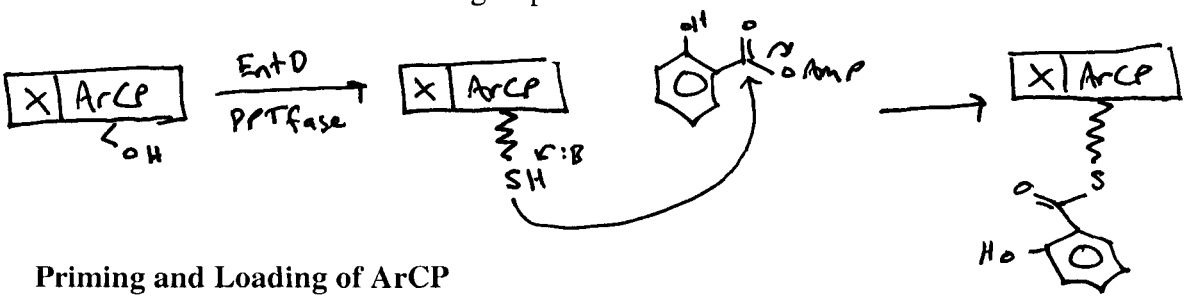
Genes Ent E,D,B,F
E,D,B are small, F is 140kDa

-discovered the importance of PPTfase (EntD) because these proteins were inactive before modification with phosphopantethiene swinging arm

EntE is the activating domain- adenylates salicylate, aromatic compound

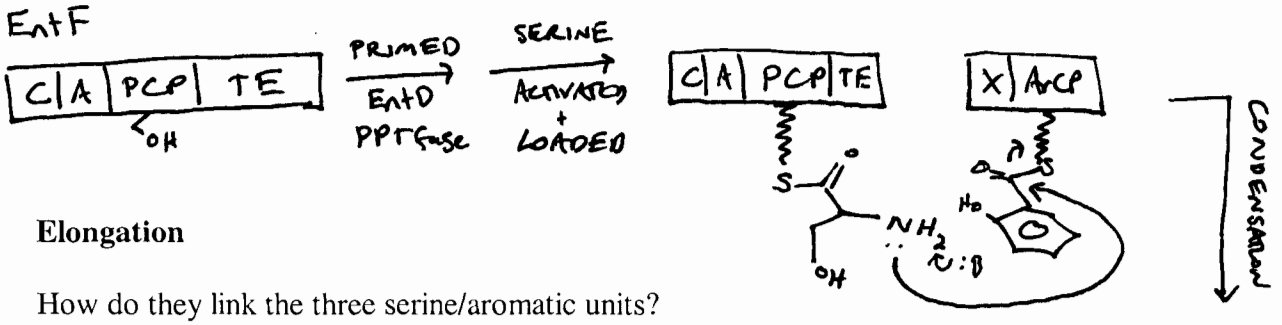
Activation
(To study this pathway they used a substrate analog with only one hydroxyl group)

EntB has two domains
X is involved in aromatic amino acid biosynthesis, we won't discuss here
ArCP domain (or T domain) is modified with swinging arm by EntD (PPTfase)
Then loads activated aromatic group



Priming and Loading of ArCP

-EntF is also primed with a swinging arm by EntD (PPTfase)
-Serine is then activated by the A domain (adenylated by ATP)
-activated amino acid loaded onto PCP domain
-Condensation catalyzed by C domain

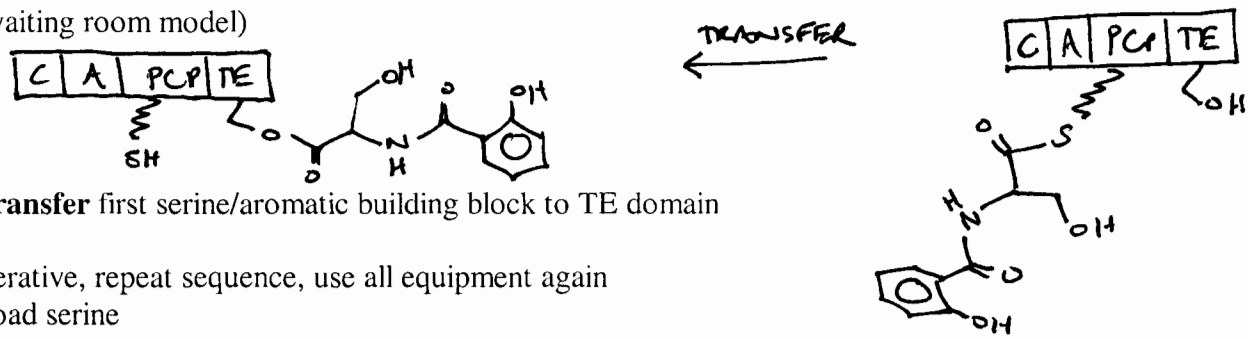


Elongation

How do they link the three serine/aromatic units?

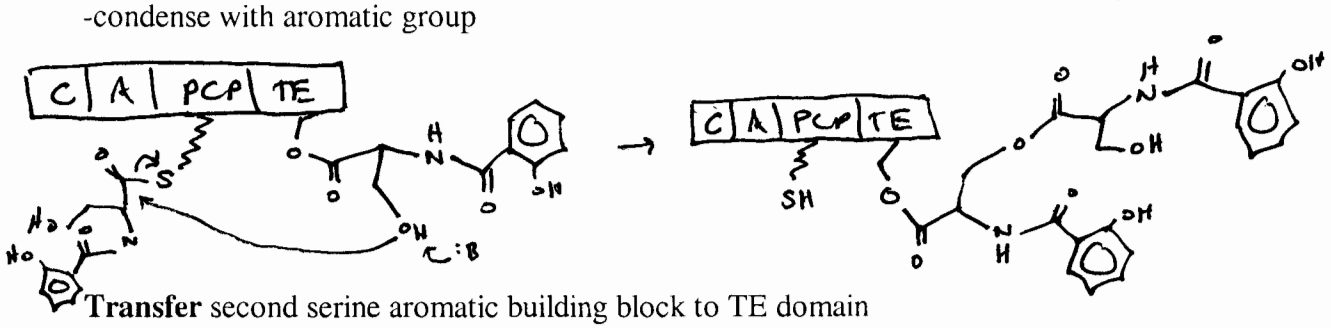
2 Store each unit on TE domain!

(waiting room model)



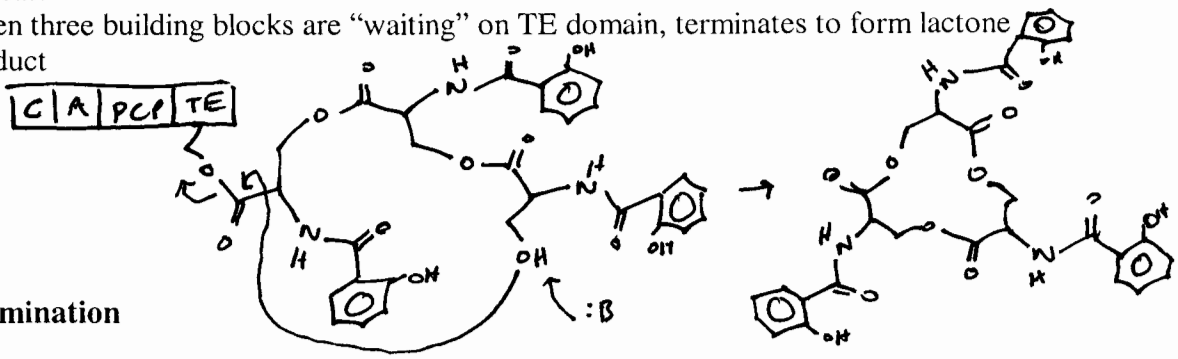
Transfer first serine/aromatic building block to TE domain

Iterative, repeat sequence, use all equipment again
-load serine
-condense with aromatic group



Transfer second serine aromatic building block to TE domain
(Form ester linkage with first serine)

Repeat!
When three building blocks are "waiting" on TE domain, terminates to form lactone product

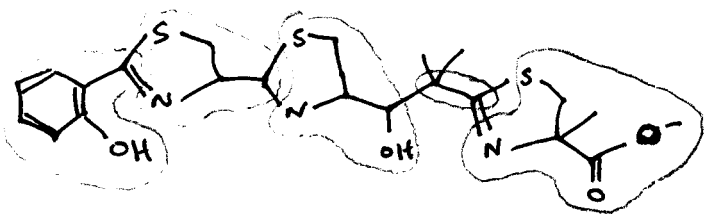


Termination

Experiment- to test iterative "waiting room" model

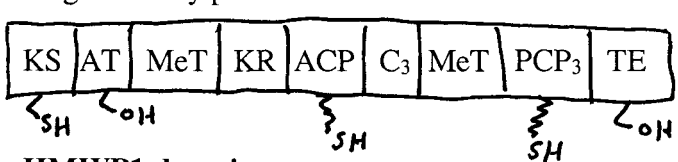
Site-directed Mutagenesis
Mutate the ser nucleophile of TE to cys (better nucleophile) S->C
Elongation rate was selectively increased relative to hydrolysis rate
Intermediates built up, and they were able to isolate TE domain with 1,2, and 3 serine/aromatic building blocks attached
-Thioesters are acid stable, so stop reaction with acid
-Limited proteolysis to isolate TE domain
-Use more extensive proteolysis and then identify/sequence peptides and sequence with mass spec
Found dimers, trimers, and monomers attached specifically at nucleophilic cysteine of TE domain
With normal serine nucleophile, there is no barrier to the last step (hydrolysis is fast) so intermediates don't build up

2) Yersiniabactin (see reference at beginning of notes) mixed PKS-NRPS Siderophore-
Found in bacteria that causes bubonic plague



Yersinabactin structure with building blocks circled
Building blocks- Aromatic group, 2 serines, malonyl or methyl malonyl CoA, serine
Look at the biosynthetic pathway on your own, in class we will focus on fidelity and editing
Cyclization requires ATP

3 subunits
HMWP1 9 domains (our focus)
HMWP2 7 domains
Single solitary protein



HMWP1 domains
Question: Acetyl CoA is prevalent in the cell (FA synthesis, degradation, amino acid building block, etc...), can it load in the middle of this pathway?
Can AcetylCoA be loaded? Yes
Can it be edited? Yes
Hydrolyzed rapidly, but requires entire "bucket brigade" -we'll discuss this further next time