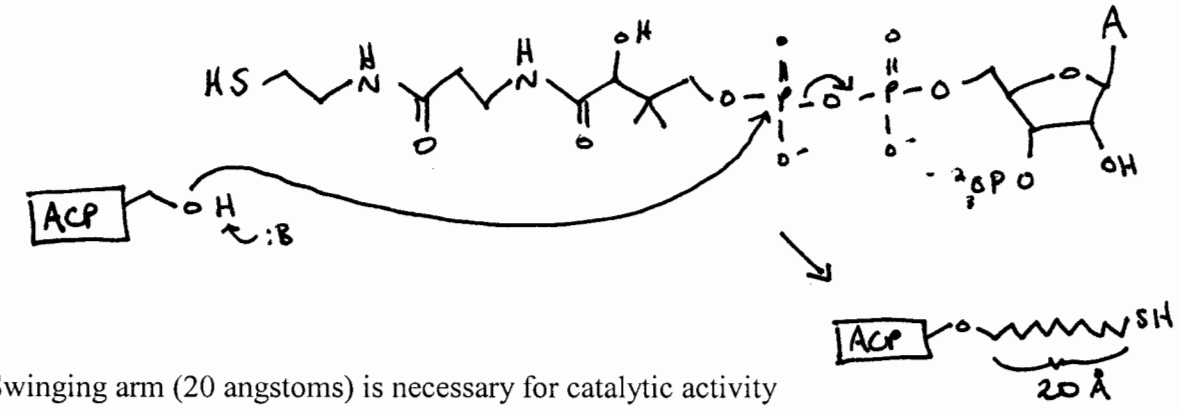
Lecture #5

2/13/04 Lecture 5

4) CHEMISTRY of FAS as paradigm for other molecular machines
(continued from lecture 4)

Post-translational modification –pantethiene arm.



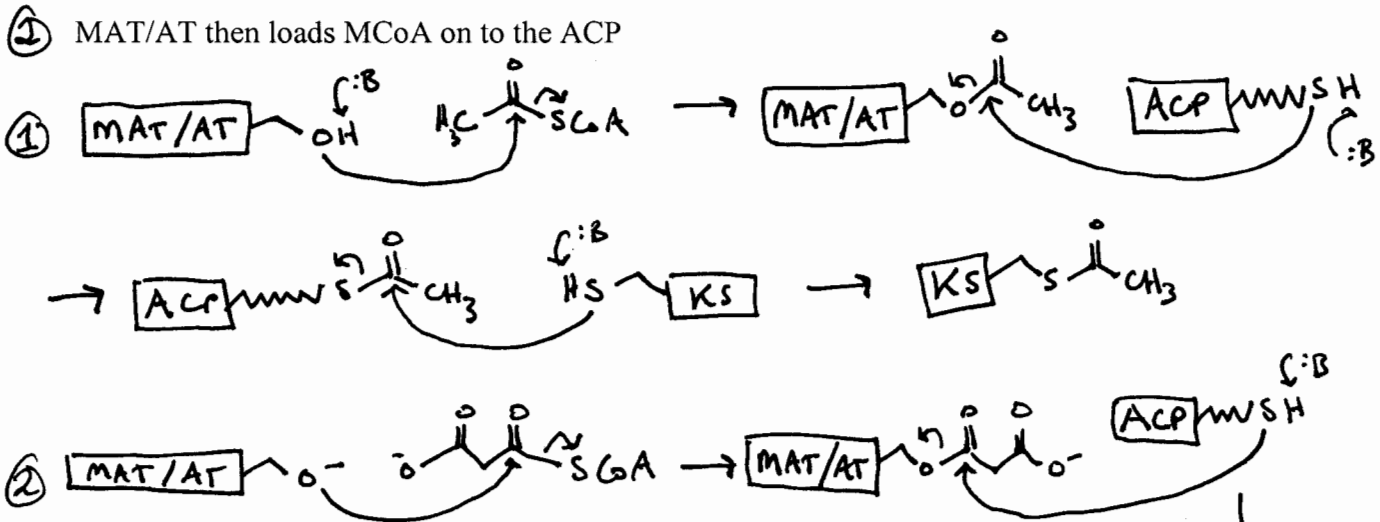
Swinging arm (20 angstoms) is necessary for catalytic activity

A. Loading, Initiation

In human FAS, the malonylCoA acyl carrier protein transferase (MAT)
Resides on the same domain as the AceylCoA acyl carrier protein transferase (AT).
-Serine is involved in covalent catalysis

① serine of MAT/AT attacks acetyl CoA, transfers to swinging arm of ACP, then transferred to serine of KS

② MAT/AT then loads MCoA on to the ACP



In the Bacterial system, the AT is loaded with ACoA, and the acetyl group is then transferred directly to the KS
This is an example of the subtle variation in the mechanism depending on the organism/system

What is going on in ACP in the mammalian FA?

How can the single swinging arm interact with all these domains?

Hypothesis: a little patch of conserved negative charge (see handout, Es and Ds on the same helix where Ser that is pantetheinylated is found) near the swinging arm may interact with positive charge in each of the other domains

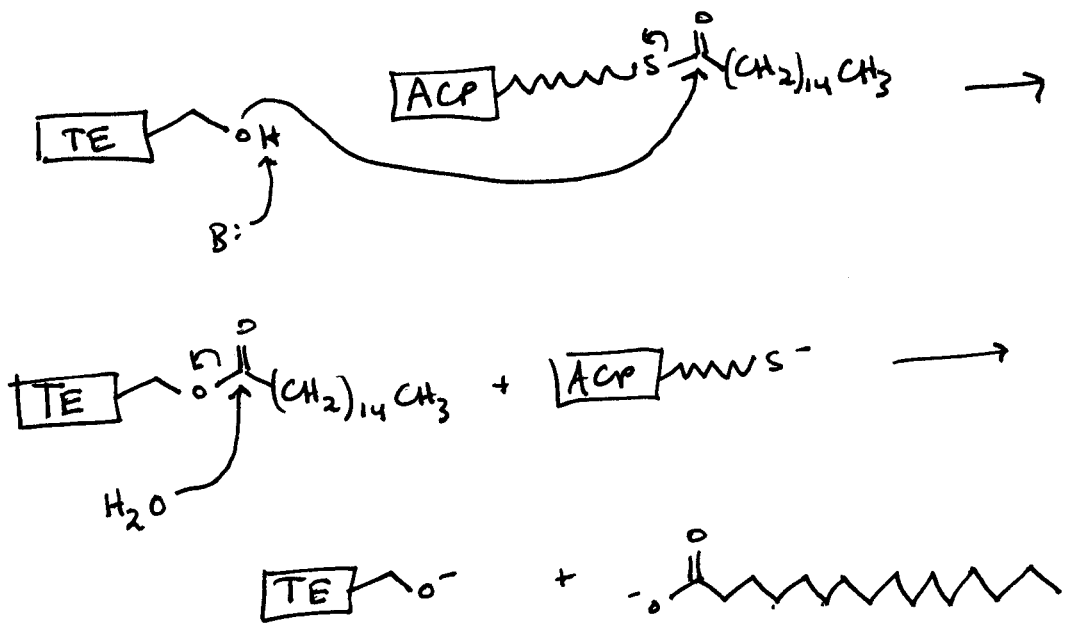
But, how does it perform each reaction in order with perfect fidelity?

In bacterial systems, where everything is on separate proteins, how do the domains interact, order their reactions, and faithfully produce the correct product?

B. Chemistry of Auxiliary domains (KR, DH, ER) – 2 reductions with NADPH
We will not discuss this chemistry in detail. The ER is on problem set one and the chemistry of the ER and KR is similar.

C. Chain termination- How does the FAS know when to stop? Why does it always faithfully cleave at C_{16} (in humans)

Serine of TE activated for nucleophilic attack by His-Asp-Ser catalytic triad
-tetrahedral intermediate that collapses to form an acyl enzyme. The acyl enzyme is then hydrolyzed to give palmitate and to regenerate ACP and TE.



Other endings are possible- a nucleophile other than H_20 could come in and attack, Coenzyme A could be one nucleophile. An intramolecular nucleophile could generate a lactone (erythromycin next section)

5) MEDICAL INTERLUDE
-obesity
-tuberculosis

Obesity is a major medical problem, and the problem is increasing

Leads to Type II diabetes and heart disease

Causes:
1) genetic predisposition
Hypothesis: at one point in evolution, humans lived in hunter/gatherer societies, and spent all their time looking for food. They needed to evolve a way to store energy in between finding meals
2) increased high energy food
3) decreased physical activity

epidemiological data
Definitions: Body Mass index (BMI)

Mass (kg)/ (height (m))^2

BMI <18 =thin
18.5-24.5= healthy
25-29=overweight
30-39.9=obese
>40- dead!

See Nature article in Handout 2a
Increasing BMI= increasing incidence of type II diabetes

PIMA Indians- same genetic background, separated to two locations (Arizona and Mexico) Different environments led to different diets. The group whose diet included a larger fat intake, also had dramatic increase in type II diabetes

Lifestyle: McDonalds and other fast food has a HUGE amount of fat

Relationship of obesity to FAS

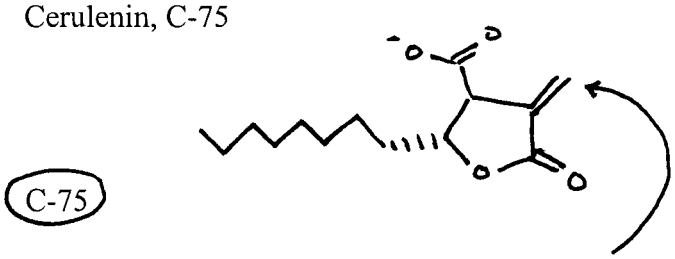
In humans, FA biosynthesis occurs during periods of energy surplus
Fuel-> channeled into energy storage

Gene knockouts or specific inhibitors give insight into metabolic pathways and regulation of these pathways

Inhibitors = "chemical genetics"

If specific and tight binding, the inhibitors are equivalent to a gene knockout, except that the inhibition, if non-covalent,can be reversible.

Example of FAS inhibitors
Cerulenin, C-75



C-75

Probably inhibits through 1,4 Michael addition the alpha, beta unsaturated lactone

Experiments in mice:

1) C-75 inihibits FA biosynthesis
Experiment: - mouse was fed ^{14}C labeled acetate, look for incorporation to FA

2)mice injected with C-75 have dramatic weight loss (compared with control mice and fasting mice)

3)inhibition of FA biosynthesis, the [MCoA] increases (hard to measure this because MCoA is unstable)

Hypothesis: MCoA is the sensor of the "fed state" (although it may not be a direct sensor, probably involves a variety of neuropeptides found in the hypothalmous)