Chapter 21
Homework Assignment

- The following problems will be due once we finish the chapter:

2, 3, 6, 7, 8, 9

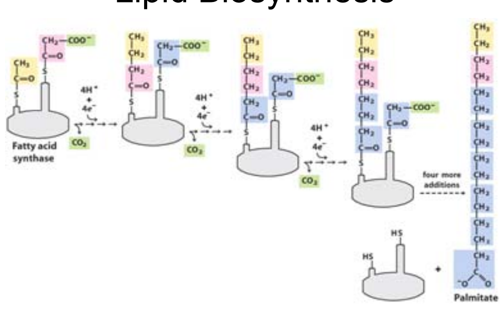
- Additional Problem:
  - Using structures, write out the reaction steps of fatty acid synthesis beginning with Malonyl-CoA and Acetyl-CoA already attached to the FA Synthase Complex. Identify the enzyme and any required cofactors for each step. Use arrows to show which reactions are irreversible and which are reversible.

No Sections 21.3 or 21.4

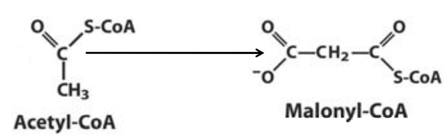
Biosynthesis of Fatty Acids
Breaking vs. Making Fatty Acids: It's Just Not the Same…

- They occur by different processes
- They utilize different enzymes
- They occur in different cellular compartments
  - Cytosol for Biosynthesis, MT for breakdown
- They exploit different size "unit blocks":
  - 2-carbon for breakdown (Acetyl-CoA)
  - 3-carbon for building (Malonyl-CoA)
- Luckily for us, these differences ensure that they happen at different times…

Chapter 21
Lipid Biosynthesis



Biosynthesis of Fatty Acids
So, let's make some Malonyl-CoA

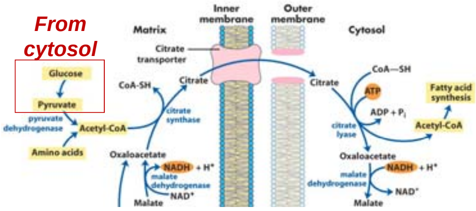


- What do we need to convert between Acetyl-CoA and Malonyl-CoA?
- Is the Acetyl-CoA where we need it to be?
- Do we know any cofactors that can help us out?
- Do you think this is going to cost us?

Biosynthesis of Fatty Acids

Where Does Cytosolic Acetyl-CoA Come From?

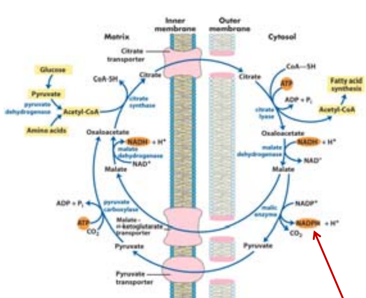
From cytosol



- Acetyl-CoA comes from citrate, which can come out of the TCA cycle (under what conditions?)
- But there's a "location" problem, and a problem of reducing power…

Biosynthesis of Fatty Acids

One Transporter is Not Enough!



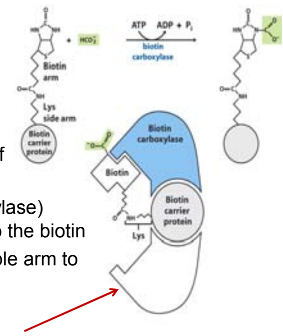
- And malic enzyme can also be part of the solution
- Under what conditions would the cell want to have abundant acetyl-CoA in the cytosol?

Do you think this might be useful later?

Biosynthesis of Fatty Acids

OK. Now, we need some CO_2…

- ***Acetyl-CoA Carboxylase (ACC)*** catalyzes this addition
- ACC is a <u>trifunctional</u> enzyme:
  - One subunit (aka. Biotin Carrier Protein) carries the biotin, attached via the ε-amino group of a lysine residue
  - One subunit (aka. Biotin Carboxylase) activates CO_2 by transferring it to the biotin
  - The BCP then uses its long flexible arm to carry the CO_2 to the third subunit

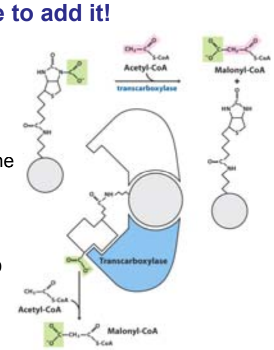


What do you think the 3rd subunit does?

Biosynthesis of Fatty Acids

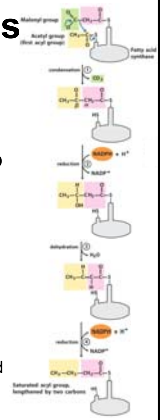
….and someone to add it!

- ACC is a <u>trifunctional</u> enzyme:
  - This third subunit (aka. Transcarboxylase) transfers the CO_2 to acetyl-CoA, converting it into malonyl-CoA, to be used in the next step of the biosynthesis reaction
- Great! The beginning of our storage of fats **<u>and</u>** you have to pay for it. Yippee…



Biosynthesis of Fatty Acids

After Activation, Biosynthesis!

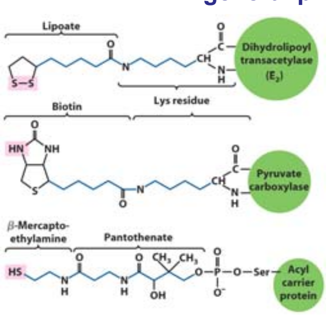


- To make a fatty acid, first a 2-carbon unit is activated, becoming malonyl-CoA
- Conceptually mirroring β-oxidation, a four-step process then lengthens the nascent fatty acid chain by 2 carbons per cycle
  - Condensation, Reduction, Dehydration then 2nd Reduction
- Employing a remarkable enzyme complex called the ***Fatty Acid Synthase Complex***
  - This complex contains 7 different activities
  - And contains a long flexible prosthetic tether derived from pantothenate (where else is this used?) to hold the growing chain

Biosynthesis of Fatty Acids

Cofactors as Biological Tethers

- A general principle -

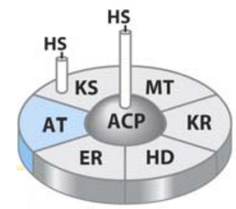


- Lipoate – “swinging arm” of pyruvate dehydrogenase
- Biotin – carries CO_2 in an important anaplerotic reaction
- Pantothenate (Vit B_5) – tethers the growing chain in fatty acid synthetase

Biosynthesis of Fatty Acids

First, a Close Up of Fatty Acid Synthase

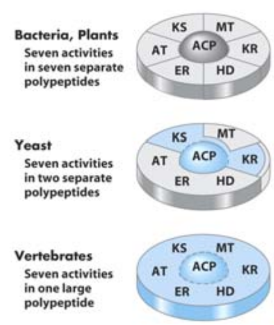
- The core of the bacterial ***Fatty Acid Synthase (FAS)*** system contains seven separate polypeptides and at least three others that act during biosynthesis
- Throughout the process, intermediates remain covalently attached as thioesters to one of two thiol groups of the FAS complex
- The activities include:
  - Acyl Carrier Protein (ACP)
  - Acetyl-CoA-ACP Transacetylase (AT)
  - Malonyl-CoA-ACP Transferase (MT)
  - β-Ketoacyl-ACP Synthase (KS)
  - β-Ketoacyl-ACP Reductase (KR)
  - β-Hydroxyacyl-ACP Hydratase (HD)
  - Enoyl-ACP Reductase (ER)



Can you guess what these do?

Biosynthesis of Fatty Acids

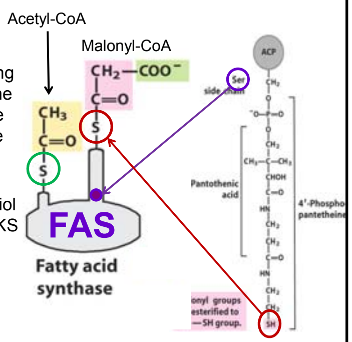
FAS: From Subunits to Domains



- Remarkably, although each of the activities arose separately at the bacterial level, by the time vertebrates finished evolving, a single very large protein was enough to encompass all of the activities of the fatty acid synthase.

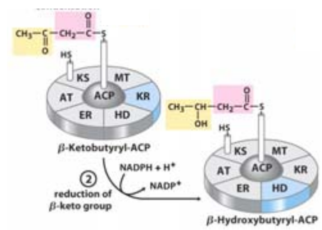
Biosynthesis of Fatty Acids
Who's Holding Whom? And How?

- The ACP prosthetic group (4'-phosphopantetheine; 4'-PPT) serves as a flexible arm, tethering the growing fatty acyl chain to the surface of FAS while moving the substrate to each enzyme active site. Cool.
- The acetyl- and malonyl-CoA thioesters can "load" onto the thiol groups of a cysteine residue in KS and ACP-4'PPT respectively
  - Loading is catalyzed by AT and MT
- This primes the system for the subsequent reactions



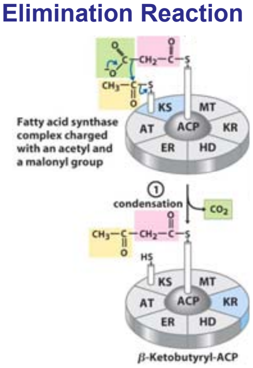
Biosynthesis of Fatty Acids
Step 2: A Reduction Reaction

- The acetoacetyl group now undergoes a reduction to convert the β-ketone into an alcohol
- Reducing equivalents are provided in the form of NADPH (Why not NADH??)
- Note that the D isomer is formed



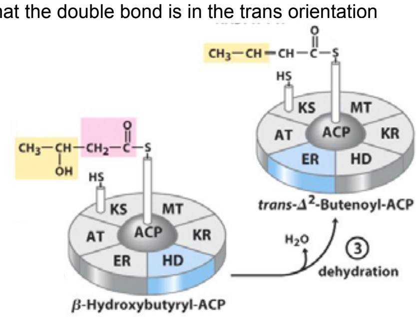
Biosynthesis of Fatty Acids
Step 1: A Condensation & Elimination Reaction

- Once the Malonyl (ACP) and Acetyl (KS) groups are in place, the two acyl groups are condensed by KS
- CO_2 is release (eliminated!) producing a four carbon acyl chain (butyryl) with a ketone off the β-carbon
- The two-carbon unit of Acetyl-CoA is now the terminal unit of the new acetoacetyl group
- The CO_2 released here is the same carbon group added from HCO_3^- by Acetyl-CoA carboxylase



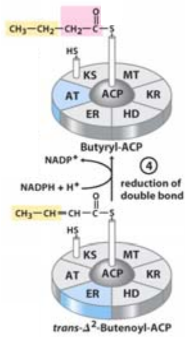
Biosynthesis of Fatty Acids
Step 3: A Dehydration Reaction

- The alcohol is now dehydrated, producing the alkene between the original α and β carbons
- Note that the double bond is in the trans orientation



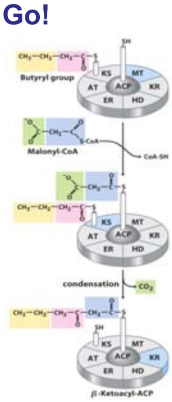
Biosynthesis of Fatty Acids
Step 4: Another Reduction Reaction

- The trans alkene is now hydrogenated to produce the alkane
- Again, the reducing equivalents are provided in the form of NADPH
- Observe that all of the previous four reactions have been carried out tethered to the 4'PPT of ACP
- Also, the original acetyl group attached to KS is at the terminal end of the chain
- Starting to look like a FA, but need more carbon!



Biosynthesis of Fatty Acids
So Around We Go!

- After the first complete cycle, the fully reduced butyryl group (4 Carbons) is now transferred back to the Cys residue of KS (by AT)
- Thus freeing up the 4'PPT tether of ACP to accept another moiety of malonyl-CoA and the cycle can continue until the FA is completed.



Biosynthesis of Fatty Acids
The Final Tally for Seven Additions

- The overall reaction for the synthesis of Palmitate (16:0) from acetyl-CoA occurs in two parts:
  - The formation of Malonyl-CoA
  - The cyclic addition of Acetyl-CoA to the end of the growing FA chain

7 Acetyl-CoA + ~~7 CO_2~~ + 7 ATP → ~~7 Malonyl-CoA~~ + 7 ADP + 7 P_i

A-CoA + ~~7 M-CoA~~ + 14 NADPH + 14 H^+ → Palmitate + 8 CoA + ~~7 CO_2~~ + 14 $NADP^+$ + 6 H_2O

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8 A-CoA + 7 ATP + 14 NADPH + 14 H^+ → Palmitate + 8 CoA + 7 ADP + 7 P_i + 14 $NADP^+$ + 6 H_2O

So, the ATP cost is basically: $$\#\ \text{ATP Required} = \frac{[\#\ \text{of Carbons}]}{2} - 1$$

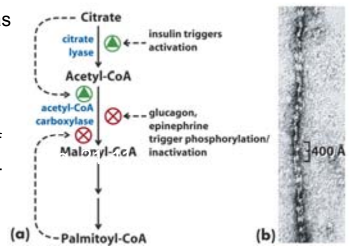
Biosynthesis of Fatty Acids
Characteristics of Fatty Acid Biosynthesis

- As is typical for biosynthetic pathways, the reaction sequences are:
  - Endergonic
  - Reductive
- And they employ:
  - ATP as the metabolic energy source
  - The electron carrier NADPH as reductant
  - Large and sophisticated enzyme complexes

Biosynthesis of Fatty Acids

Think about the regulation…

- Why should feedback be as shown, and why should citrate, especially, play such a central role?
- Both citrate and malonyl-CoA regulate the choice of oxidizing metabolic fuel vs. its storage as fatty acids, and involves allosteric signals

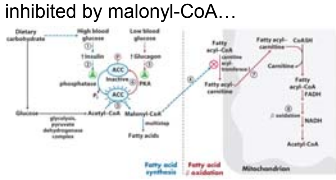


- Acetyl-CoA carboxylase is also regulated by phosphorylation, which causes depolymerization of its filaments and thus inactivation

Biosynthesis of Fatty Acids

How Are Choices About Fatty Acid Metabolism Made?

- Fatty acids are a valuable fuel, and are burned only when their energy is needed
- In the cytosol of liver cells, fatty acyl-CoA's are:
  - Either taken into mitochondria for β-oxidation
  - Or converted into TAGs and phospholipids by cytosolic enzymes
- This metabolic fork is governed by the rate of uptake of fatty acyl-CoA's into mitochondria
- Which can be inhibited by malonyl-CoA…



Biosynthesis of Fatty Acids

Recall the Acyl-Carnitine/ Carnitine Transporter

- Shuttle responsible for the magic trick of supplying fatty acyl-CoA's to the mitochondrial matrix, where β-oxidation takes place
- Remember, transport is the ***rate-limiting step*** in fatty acid oxidation
- Therefore, this is the point of regulation by malonyl-CoA, which inhibits acyl-carnitine transferase I

Why Malonyl-CoA?

Biosynthesis of Fatty Acids

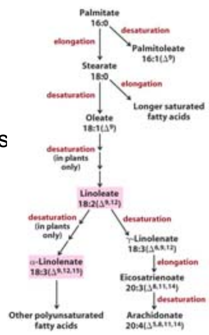
The Crosstalk Between Two Pathways

- If there is plentiful energy from carbohydrates, not all the glucose can be oxidized or stored as glycogen
- The excess carbs are channeled into biosynthesis of fatty acids (for storage as TAGs)
- As often, this is not simply the reverse of β-oxidation, but entails as its first step
- Carboxylation of acetyl-CoA to produce **malonyl-CoA**
- Rather than reversing ***thiolase***, which has other consequences (discussed later)

Biosynthesis of Fatty Acids

What about the BIG FAs?

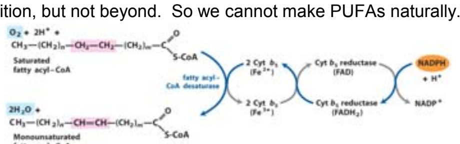
- Palmitate (16:0) is the principle product of Fatty Acid synthesis in animal cells
- This FA can then serve as the precursor of other, longer chain FAs via ***fatty acid elongation systems*** present in the smooth ER and the MT



Biosynthesis of Fatty Acids

What about the MUFAs and PUFAs?

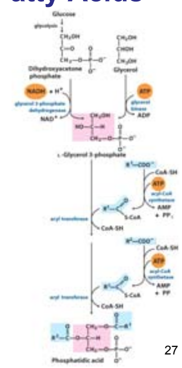
- Palmitate and Stearate (18:0) can also serve as precursors to unsaturated FAs (mono- and poly-)
- The double bond is introduced by an oxidative reaction catalyzed by ***fatty acyl-CoA desaturase***
- This enzyme is a ***mixed-function oxidase***
- Two different substrates, the FA and NAD(P)H undergo two electron oxidations to produce the unsaturated FA and water (from O_2)
- Mammalian hepatocytes can introduce a double bond at the Δ^9 position, but not beyond. So we cannot make PUFAs naturally.



Biosynthesis of TAGs

But we don't store *FREE* Fatty Acids

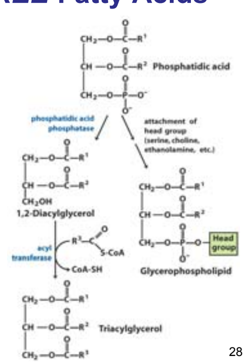
- The storage form for fats in mammals is TAGs.
- That means there must be a process to:
  - Produce glycerol; and
  - To attach three free FAs to it to produce the TAGs
- **<u>Glycerol-3-phosphate</u>** serves as the precursor for glycerol.
  - Formed from DHAP **<u>or</u>** Glycerol (Huh?)
- This compound is acylated at the first two hydroxyl groups to produce **<u>Phosphatidic acid</u>**



Biosynthesis of TAGs

But we don't store *FREE* Fatty Acids

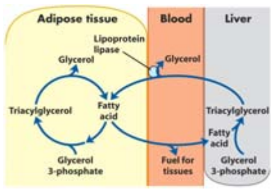
- Phosphatidic acid can then go in two directions
  - TAG formation
  - Phospholipid formation
- Final destination of the phosphatidic acid is (of course!) a point of regulation.
- Of course, formation of TAGs is all hormone driven…



Biosynthesis of TAGs

The Triacylglycerol Cycle

- Approximately 75% of all FAs released by lipolysis are reesterified to form new TAGs rather than run through oxidation pathways.
  - This ratio holds even under starvation conditions
- Some of this recycling occurs in the adipose tissue, prior to the FAs ever being released into the blood stream

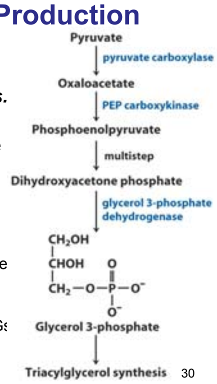


- Some takes place in the liver, where free FAs are repackaged as TAGs and sent back to the adipose tissue.
- The function of this "apparently" futile cycle is not well understood, but there are some ideas:
  - Constant flow between the two tissues means a certain level of TAGs are always available in the bloodstream. Great if need energy suddenly….

Biosynthesis of TAGs

Glycerol-3-Phosphate Production

- Adipose tissue produces Glycerol-3-Phosphate in a shortened version of gluconeogenesis called ***Glyceroneogenesis.***
- Conversion of pyruvate to DHAP is followed by conversion to Gly3P by a Dehydrogenase (NAD^+)
- Process has multiple roles:
  - Controls rate of FA release into the blood (Adipocytes)
  - May control rate of free FA delivery to MT for use in thermogenesis (Brown Fat!)
  - Supports the synthesis of enough Gly3P to account for ~65% of FAs converted back to TAGs (hepatocytes)



Biosynthesis of TAGs

Regulation of Glyceroneogenesis

- A main regulation point for this process occurs with ***PEP carboxykinase (PEPC)***
- ***Glucocorticoid hormones*** (such as ***cortisol***) regulate the expression levels of PEP carboxykinase reciprocally in the liver and the adipose tissues.