BB 422/622

ANABOLISM I: Carbohydrates

Overview of Photosynthesis
Key experiments:
- Light causes oxygen, which is from water splitting (Hill)
- NADPH made (Ochoa)
- Separate from carbohydrate biosynthesis (Rubin & Kamen)

Light Reactions
- energy in a photon
- pigments
- HOW
- Light absorbing complexes
  - Reaction center
  - Photosystems (PS)
    - PSI – oxygen from water splitting
    - PSII – NADPH

Proton Motive Force – ATP
Overview of light reactions
Carbon Assimilation – Calvin Cycle
- Stage One – Rubisco
  - Carboxylase (Know Mechanism)
  - Oxygenase
  - Glycolate cycle
- Stage Two – making sugar
- Stage Three – remaking Ru 1,5P_2

Overview and regulation
Calvin cycle connections to biosyn. (Know Pathway)
C3 versus C4 plants
Kornberg cycle – glyoxylate

Carbohydrate Biosynthesis in Animals
- precursors
- Cori cycle

Gluconeogenesis (Know Pathway)
- reversible steps
- irreversible steps – four
  - energetics
  - 2-steps to PEP
    - mitochondria
    - Pyr carboxylase-biotin
    - PEPCK
  - FBPase
  - G6Pase

Glycogen Synthesis
- UDP-Glc
- Glycogen synthase
- branching

Pentose-Phosphate Pathway (Know Pathway)
- Oxidative phase
- Non-oxidative/recycling phase
- ROS
- NADH/NADPH shuttles

Regulation of Carbohydrate Metabolism
- Acetyl-CoA/Pyruvate
- Pyruvate/PEP
- F6P/FBP: Fru 2,6P_2
- Glc/Glc6P
- Glycogen

Anaplerotic reactions

ANABOLISM II: Lipids

Biosynthesis of Fatty Acids and Lipids
- Fatty Acids
  - contrasts
  - location & transport
  - Synthesis
    - acetyl-CoA carboxylase (ACC)
    - fatty acid synthase (FAS)
      - ACP priming
      - 4 steps
  - Control of fatty acid metabolism
- Diversification of fatty acids
  - elongation
  - desaturation
- Eicosanoids
- Prostaglandins and Thromboxane
- Triacylglycerides
- Membrane lipids
  - Glycerophospholipids
  - Sphingolipids

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ANABOLISM II:
Biosynthesis of Fatty Acids & Lipids

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ANABOLISM II: Biosynthesis of Fatty Acids & Lipids

1. Biosynthesis of fatty acids
2. Regulation of fatty acid degradation and synthesis
3. Assembly of fatty acids into triacylglycerol and phospholipids

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4. Metabolism of isoprenes
   a. Ketone bodies and Isoprene biosynthesis
   b. Isoprene polymerization
      i. Cholesterol
      ii. Steroids & other molecules
      iii. Regulation
      iv. Role of cholesterol in human disease

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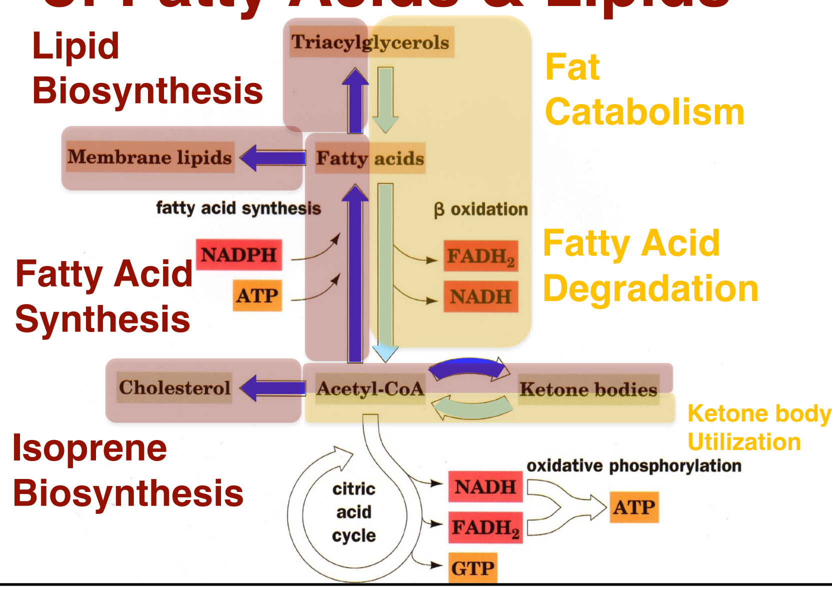
ANABOLISM II: Biosynthesis of Fatty Acids & Lipids



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Fatty Acid Biosynthesis

Catabolism
Anabolism

- Contrast with Sugars
  - Lipids have hydro-carbons not carbo-hydrates
  - more reduced=more energy
  - Long-term storage vs short-term storage
  - Lipids are essential for structure in ALL organisms: membrane phospholipids
- **Catabolism** of fatty acids
  - *produces* acetyl-CoA
  - *produces* reducing power (NADH, $FADH_2$)
  - takes place in the *mitochondria*
- **Anabolism** of fatty acids
  - *requires* acetyl-CoA and sufficient carbohydrates
  - *requires* reducing power from NADPH
  - takes place in *cytosol* in animals, *chloroplast* in plants

Anabolism of fat will occur only when there is excess, carbon, electrons, and ΔG

Acetyl-CoA & carbohydrates → carbon; NADPH → electrons; ATP → ΔG

How is this monitored in the cell?

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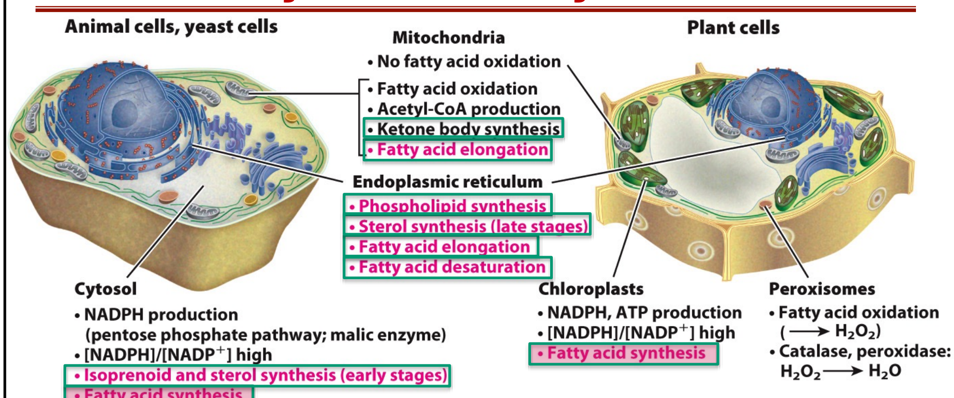
Fatty Acid Biosynthesis



Fatty Acid Synthesis

Cytosol for animals | *Chloroplast* for plants

Both of these compartments are where there are ample Sources of NADPH:

in adipocytes: pentose phosphate pathway and malic enzyme:

NADPH is made as malate converts to pyruvate + CO_2.

in hepatocytes and mammary gland: pentose phosphate pathway

NADPH is made as glucose 6-phosphate converts to ribulose 6-phosphate.

in plants: photosynthesis

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Fatty Acid Biosynthesis

EXAMPLE: Synthesis of Palmitate (16:0)

Where do the carbons come from? **Acetyl-CoA**

1. 8 acetyl-CoA x 2 carbons = 16 carbons (palmityl-CoA)
2. Longer fatty acids and desaturases use palmityl-CoA
3. 1 acetyl-CoA ”primes” the enzyme
   a. other “acetyl-CoA-derived units” are ACTIVATED by carboxylation (recall gluconeogenesis)
   b. Used to make 7 malonyl-CoAs… using? **ATP & CO_2**

7 acetyl-CoA + 7 CO_2 + 7 ATP → 7 malonyl-CoA + 7 ADP + 7 P_i ….more later

2. Seven cycles of condensation, reduction, dehydration, and reduction… using **NADPH** to reduce the β-keto group and trans-double bond

acetyl-CoA + 7 malonyl-CoA + 14 NADPH + 14 H^+ → palmitate (16:0) + 7 CO_2 + 8 CoA + 14 $NADP^+$ + 6 H_2O

How is the acetyl-CoA transported out of mitochondria?

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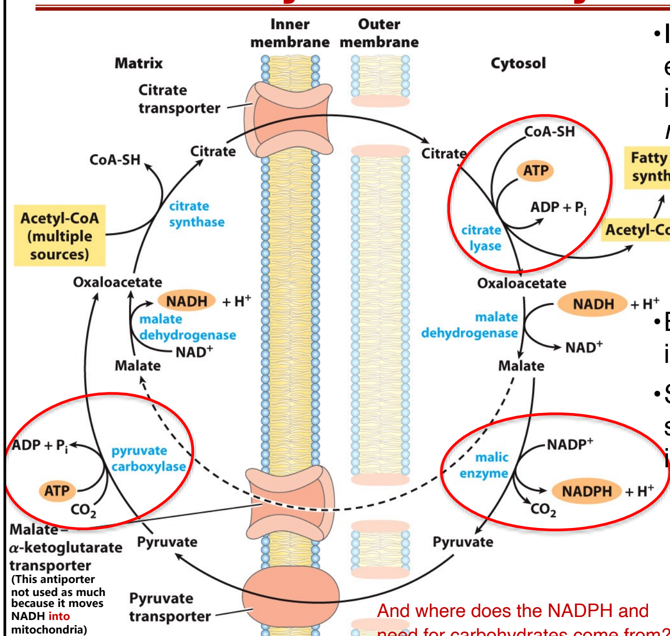
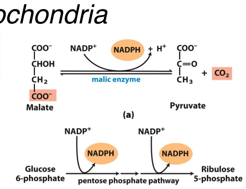
Fatty Acid Biosynthesis



And where does the NADPH and need for carbohydrates come from?

- In non-photosynthetic eukaryotes…Acetyl-CoA is made in the *mitochondria*



- But fatty acids are made in the *cytosol*
- So acetyl-CoA is smuggled (**transported**) into the cytosol
  - cost of 2 ATPs
  - Converted back to acetyl-CoA by citrate lyase
  - Therefore, cost of FA synthesis is 3 ATPs per 2-C unit ….. Where is third?

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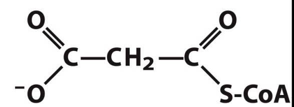
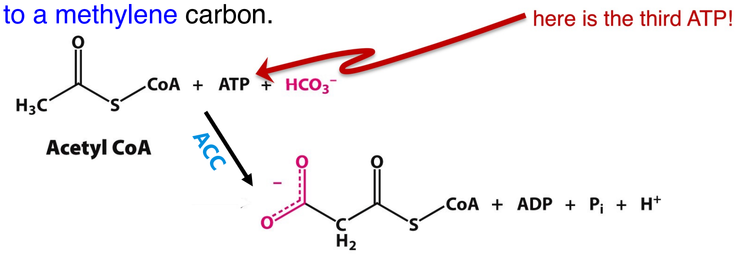
Fatty Acid Biosynthesis

- Fatty acids are built in several passes, processing one acetate unit at a time.
- The acetate is coming from activated malonate in the form of malonyl-CoA.
- Each pass involves reduction of a carbonyl carbon to a methylene carbon.



Malonyl-CoA



- Making the Malonyl-CoA:
  - Reaction carboxylates acetyl-CoA
  - Catalyzed by *acetyl-CoA carboxylase (ACC)*

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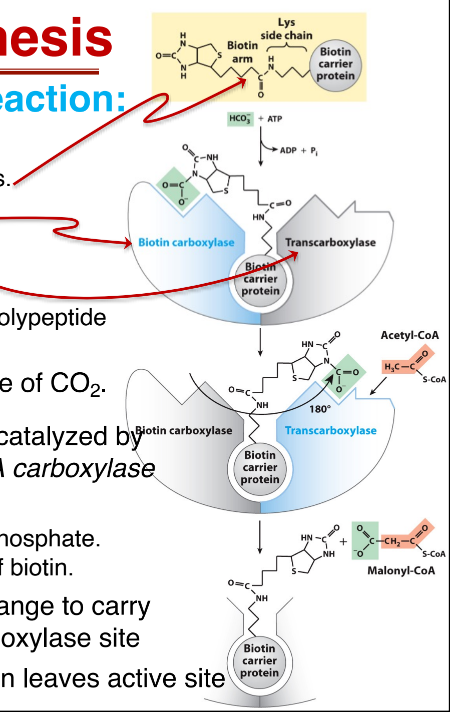
Fatty Acid Biosynthesis

Acetyl-CoA Carboxylase Reaction:

–The enzyme has three subunits:
- One unit has biotin covalently linked to Lys.
- Another subunit is biotin carboxylase
- The third subunit is a transcarboxylase
- Biotin carries CO_2.
- In animals, all three subunits are on one polypeptide chain.

–HCO_3^- (bicarbonate) is the soluble source of CO_2.

– Two-step Rxn; similar to carboxylations catalyzed by *pyruvate carboxylase* and *propionyl-CoA carboxylase*
- CO_2 binds to biotin.
  - Reaction with ATP produces carboxy-phosphate.
  - Activated CO_2 is attached to N in ring of biotin.
- Enzyme undergoes conformational change to carry carbamoyl (carboxybiotin) to transcarboxylase site
- CO_2 attaches to acetyl-CoA, which then leaves active site



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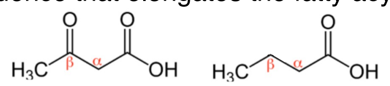
Fatty Acid Biosynthesis

Fatty Acid Synthase (FAS)

- Catalyzes a repeating four-step sequence that elongates the fatty acyl chain by two carbons at each step
- NADPH as as the electron donor



- Condensation with acetate
  - β-ketoacyl-ACP synthase (KS)
- Reduction of carbonyl to hydroxyl
  - β-ketoacyl-ACP reductase (KR)
- Dehydration of alcohol to alkene
  - β-hydroxyacyl-ACP dehydratase (DH)
- Reduction of alkene to alkane
  - enoyl-ACP reductase (ER)
- Chain transfer/charging
  - malonyl/acetyl-CoA ACP transferase (M/AT)

- Overall goal: attach acetate unit (2-carbon) from malonyl-CoA to a growing chain and then reduce it.
- Reaction involves cycles of four enzyme-catalyzed steps:
  - condensation of the growing chain with activated acetate (malonyl-CoA)
  - reduction of carbonyl to hydroxyl
  - dehydration of alcohol to trans-alkene
  - reduction of alkene to alkane
- The growing chain is initially attached to the enzyme via a thioester linkage on the acyl-carrier protein (ACP).

What is this "ACP"?
...let's look at the structure.

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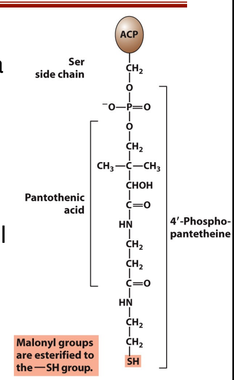
Fatty Acid Biosynthesis

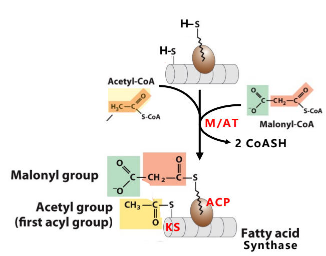
Acyl Carrier Protein (ACP)

- The *E. coli* ACP is a small 77-residue protein with a covalently attached prosthetic group, 4'-phosphopantetheine, at a Ser residue.
  - In vertebrate FAS, it's a domain with a flexible arm to tether the growing acyl chain
- Delivers acetate (in the first step) or malonate (in all the next steps) to the fatty acid synthase enzymes
- Shuttles the growing chain from one active site to another during the four-step reaction



Priming FAS

- Two thiols must be **charged with the correct acyl groups** before the condensation reaction can begin.
  - thiol from 4-phosphopantethine in ACP
  - thiol from Cys β-ketoacyl-ACP synthase (KS)
- The acetyl group of acetyl-CoA is transferred to ACP.
  - catalyzed by malonyl/acetyl-CoA transferase (M/AT)
  - ACP passes this acetate to the Cys of the KS domain of FAS 1.
  - ACP –SH group is recharged with malonate from malonyl-CoA again catalyzed by M/AT



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Fatty Acid Biosynthesis

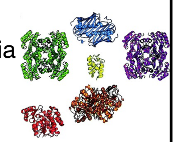
FAS I vs. FAS II

FAS I

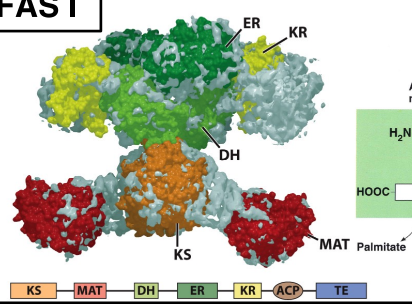
- Single polypeptide chain in vertebrates
- Leads to single product: palmitate 16:0
- C-15 and C-16 are from the acetyl-CoA used to prime the Rxn
- FAS I in vertebrates and fungi

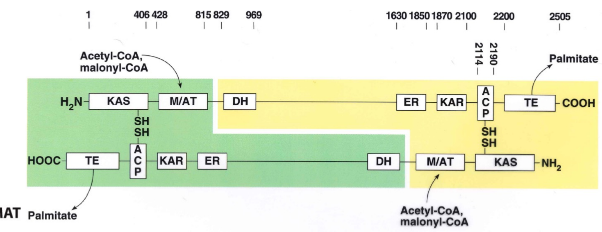
FAS II

- Made of separate, diffusible enzymes
- Makes many products (saturated, unsaturated, branched, many lengths, etc.)
- Mostly in plants and bacteria



FAS I





What you can't see is the ACP……

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Fatty Acid Biosynthesis

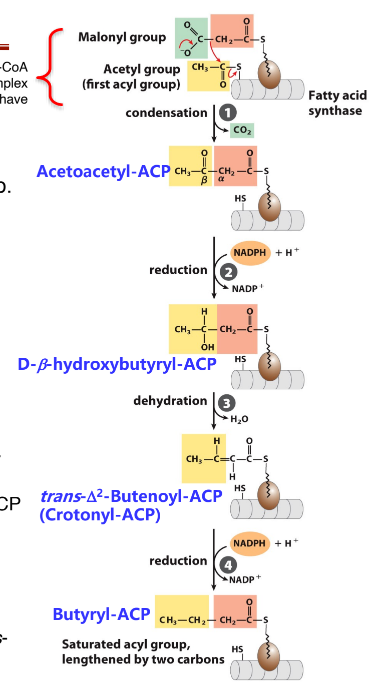
Note that malonyl-CoA and acetyl-CoA have already been attached to complex via thioester linkages to enzyme and have shed their CoA attachments.

Step 1: Condensation reaction attaches two C from acetyl group (or longer fatty acyl chain) to two C from malonyl group.
- release of CO_2 activates malonyl group for attachment
- creates β-keto intermediate (acetoacetyl-ACP)
- Catalyzed by β-ketoacyl-ACP synthase (KS)

Step 2: First reduction: NADPH reduces the β-keto intermediate to an alcohol.
- carbonyl at C-3 reduced to form D-β-hydroxybutyryl-ACP
- NADPH is 2e^- donor
- catalyzed by β-ketoacyl-ACP reductase (KR)

Step 3: Dehydration: OH group from C-2 and H from neighboring CH_2 are eliminated, creating double bond (trans-alkene).
- OH and H removed from C-2 and C-3 of β-hydroxybutyryl-ACP to form *trans*-Δ^2-butenoyl-ACP
- catalyzed by β-hydroxyacyl-ACP dehydratase (DH)

Step 4: Second reduction: NADPH reduces double bond to yield saturated alkane.
- NADPH is the electron donor to reduce double bond of *trans*-Δ^2-butenoyl-ACP to form butyryl-ACP.
- catalyzed by enoyl-ACP reductase (ER)



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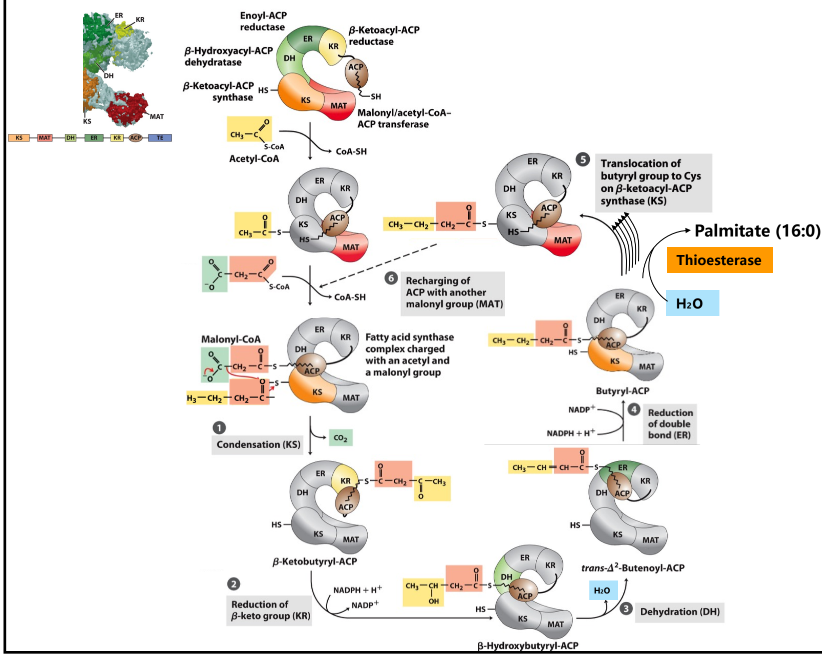
Fatty Acid Biosynthesis



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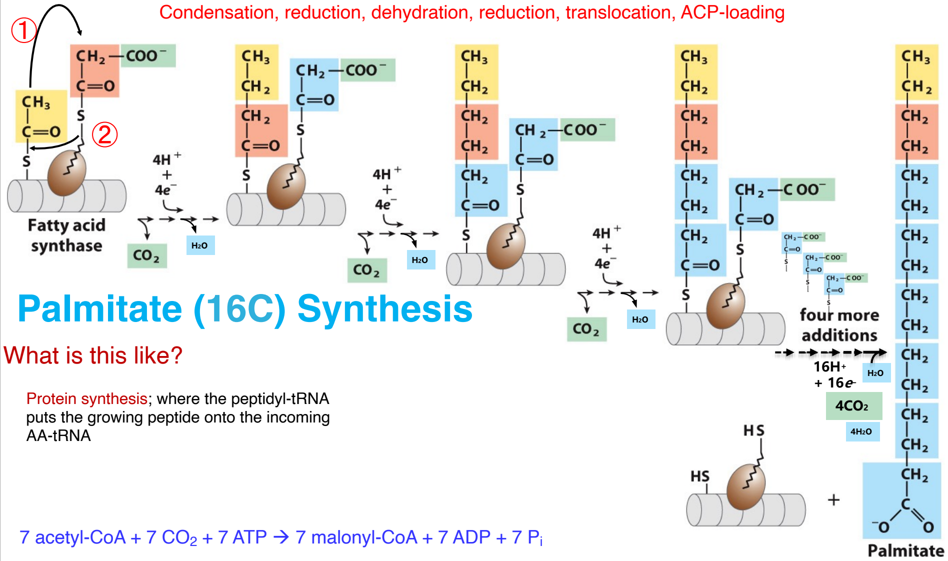
Fatty Acid Biosynthesis

Condensation, reduction, dehydration, reduction, translocation, ACP-loading



Palmitate (16C) Synthesis

What is this like?

Protein synthesis; where the peptidyl-tRNA puts the growing peptide onto the incoming AA-tRNA

7 acetyl-CoA + 7 CO_2 + 7 ATP → 7 malonyl-CoA + 7 ADP + 7 P_i

acetyl-CoA + 7 malonyl-CoA + 14 NADPH + 14 H^+ → palmitate (16:0) + 7 CO_2 + 8 CoA + 14 $NADP^+$ + 6 H_2O

8 acetyl-CoA + 14 NADPH + 14 H^+ + 7 ATP → palmitate (16:0) + 8 CoA + 14 $NADP^+$ + 7 ADP + P_i + 6 H_2O

If you add in the transport (8x2=16): 23 ATP

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1. Biosynthesis of fatty acids
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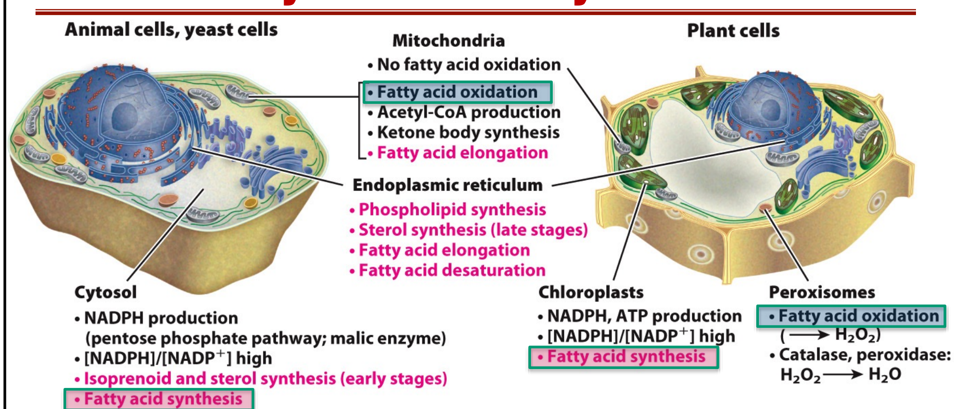
Fatty Acid Biosynthesis



Fatty Acid Synthesis

Cytosol for animals — *Chloroplast* for plants

Both of these compartments are where there are ample Sources of NADPH:
pentose phosphate pathway, malic enzyme, and photosynthesis

Fatty Acid Oxidation

Mitochondria

How does cell regulate which pathway is working?

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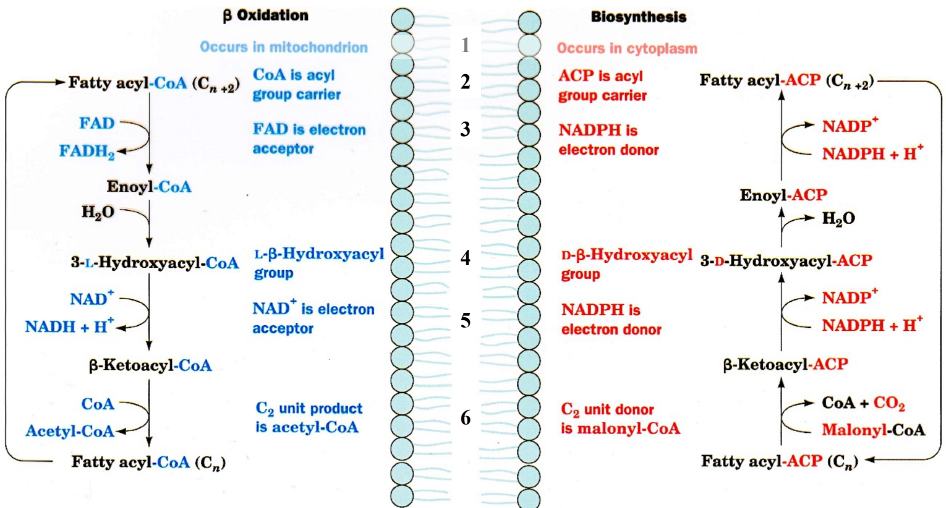
Fatty Acid Biosynthesis



Comparison of Fatty-Acid Degradation

versus

Fatty-Acid Synthesis

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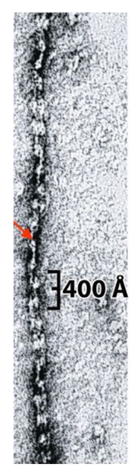
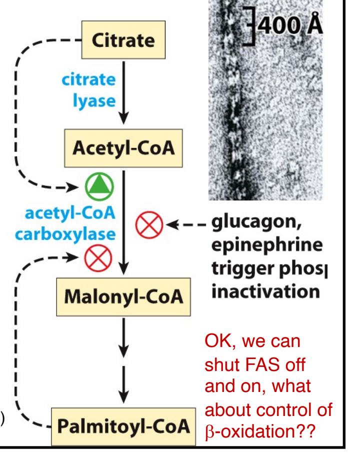
Fatty Acid Biosynthesis

Control of Fatty-Acid Synthesis

- **Acetyl CoA carboxylase (ACC) catalyzes the committal step.**
- **Allosteric Control**
  - Inhibited when energy is needed, fatty acids are acylated for degradation, and inhibit ACC.
    - ACC is feedback-inhibited by **palmitoyl-CoA**.
  - ACC is *activated* by **citrate**.
    - Citrate is made from acetyl-CoA in mitochondria (acetyl-CoA^{mt}).
    - Citrate signals excess energy to be converted to fat.
  - When [acetyl-CoA]^{mt} ↑ it is converted to citrate… citrate is exported to cytosol.
- **Hormonal Control**
  - Glucagon and epinephrine: leads to activation of AMP-dependent Protein Kinase (AMPK)
    - Phosphorylation inactivates ACC
    - Phosphorylation reverses the polymerization → monomers (inactive)
  - Insulin: leads to activation of Protein Phosphatase 2A
    - Dephosphorylation activates ACC
    - When dephosphorylated, ACC polymerizes into long filaments (active)
- **Changes in gene expression**
  - example: excess of certain polyunsaturated fatty acids (eicosanoids) bind to transcription factors called peroxisome proliferator-activated receptors (PPARS)





OK, we can shut FAS off and on, what about control of β-oxidation??

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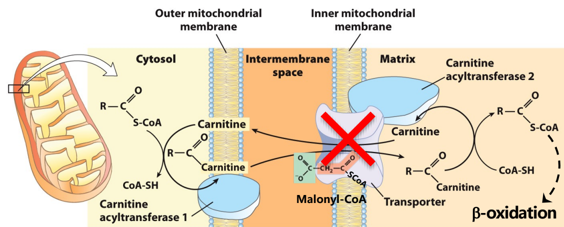
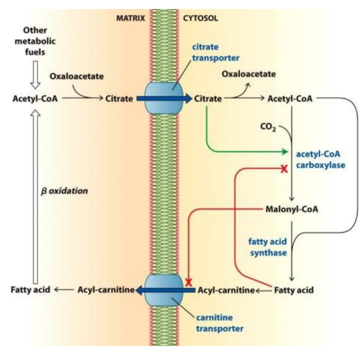
Fatty Acid Biosynthesis

Recall how β-oxidation starts: Acyl-Carnitine/Carnitine Transport:

- β oxidation of fatty acids occurs in mitochondria.
- If fatty acyl-CoAs are not transported in, they cannot be degraded
- Transport is via carnitine transporter.
- The cell blocks β-oxidation at transport using the initial committed product of fatty-acid synthesis: malonyl-CoA



Allosteric Control of Fatty Acid Metabolism



Inhibition

Activation

Reciprocal Control of Fatty-Acid Degradation *versus* Fatty-Acid Synthesis

ensures that fat synthesis and oxidation don't occur simultaneously

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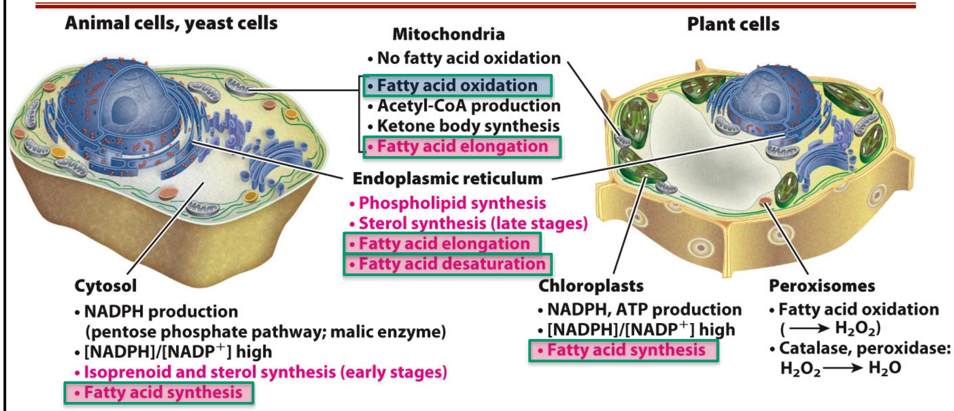
Fatty Acid Biosynthesis



Fatty Acid Desaturation

Endoplasmic reticulum

Fatty Acid Elongation

Endoplasmic reticulum and *Mitochondria* (animals)

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