BI/CH 422/622

OUTLINE:

Introduction and review
Transport
Glycogenolysis
Glycolysis
Other sugars
Pasteur: Anaerobic vs Aerobic

Exam-1 material

Fermentations
Pyruvate
Krebs' Cycle
Oxidative Phosphorylation
- Electron transport
- Chemiosmotic theory: Phosphorylation

Exam-2 material

Fat Catabolism
Fatty acid Catabolism
- Mobilization from tissues (mostly adipose)
- Activation of fatty acids
- Transport; carnitine
- Oxidation: β-oxidation, 4 steps:

Protein Catabolism
Amino-Acid Degradation
- Dealing with the nitrogen; Urea Cycle
- Dealing with the carbon; Seven Families

Nucleic Acid & Nucleotide Degradation

Exam-3 material

PHOTOSYNTHESIS:
Overview of Photosynthesis
Key experiments:
Light Reactions
- energy in a photon
- pigments
- HOW
- Light absorbing complexes-"red-drop experiment"
  - Reaction center
  - Photosystems (PS)
    - PSII – oxygen from water splitting
    - PSI – NADPH

Proton Motive Force – ATP
Overview of light reactions

ANABOLISM I: Carbohydrates

Carbon Assimilation – Calvin Cycle
- Stage One – **Rubisco**
  - Carboxylase
  - Oxygenase
  - Glycolate cycle
- Stage Two – making sugar
- Stage Three – remaking Ru 1,5P2

Know mechanism

Overview and regulation
Calvin cycle connections to biosynthesis
C4 versus C3 plants
Kornberg cycle – glyoxylate

Carbohydrate Biosynthesis in Animals
- precursors
- Cori cycle

Gluconeogenesis
- reversible steps
- irreversible steps – four
  - energetics
  - 2-steps to PEP in mitochondria: Pyr carboxylase-biotin & PEPCK
  - FBPase
  - G6Pase

Glycogen Synthesis
- UDP-Glc
- Glycogen synthase
- branching

Pentose-Phosphate Pathway
- oxidative-NADPH
- non-oxidative-Ribose 5-P

Regulation of Carbohydrate Metabolism
- Acetyl-CoA/Pyruvate
- Pyruvate/PEP
- F6P/FBP: Fru 2,6P2
- Glc/Glc6P: sequestration
- Glycogen: PKA/PP1
- Insulin signaling

Anaplerotic reactions

End of Exam-4 material

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ANABOLISM II OUTLINE:

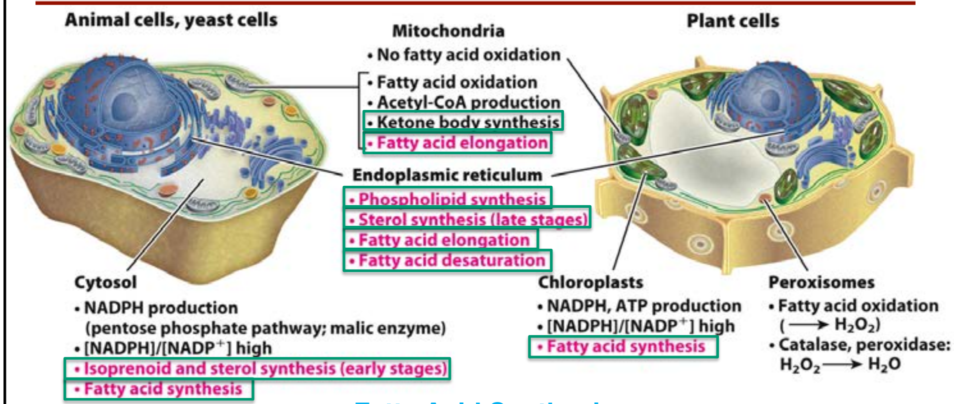
Biosynthesis of Fatty Acids and Lipids

Fatty Acids
- contrasts
- location & transport
- Synthesis
  - acetyl-CoA carboxylase
  - fatty acid synthase
    - ACP priming
    - 4 steps
- Control of fatty acid metabolism
- Diversification of fatty acids
  - elongation
  - desaturation
  - Eicosanoids
    - Prostaglandins and Thromboxane

Triacylglycerides

Membrane lipids
- Glycerophospholipids
- Sphingolipids
- Isoprene lipids: **Cholesterol**
  - Ketone body synthesis
  - Mevalonate
  - **Cholesterol**
    - bile acids
    - steroids
    - metabolism
    - control of cholesterol biosynthesis

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

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**

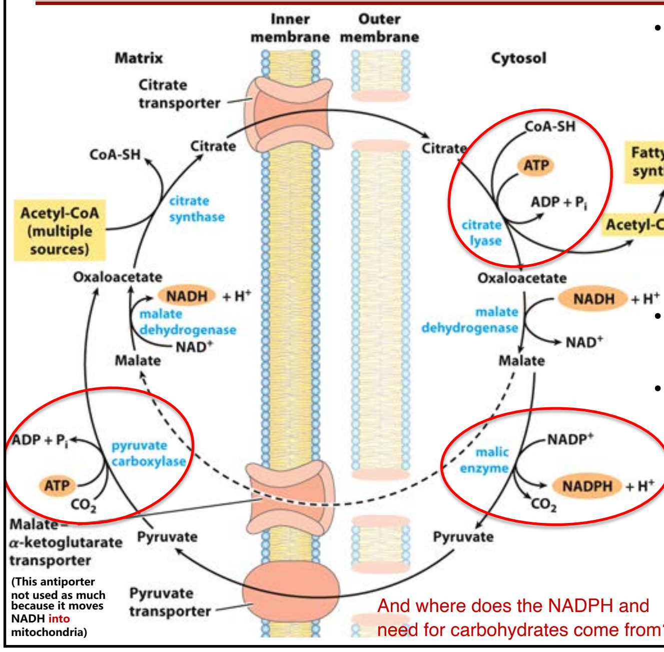
7 acetyl-CoA + 7 CO_2 + 7 ATP → 7 malonyl-CoA + 7 ADP + 7 P_i ….more later

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

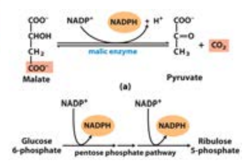
How is the acetyl-CoA transported out of mitochondria?

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*

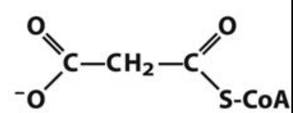


- But fatty acids are made in the *cytosol*
- So acetyl-CoA is smuggled (**transported)** into the cytosol
  - cost of 2 ATPs
  - Converted back to acetyl-CoA by citrate lyase
  - Therefore, cost of FA synthesis is 3 ATPs per 2-C unit ….. Where is third?

Fatty Acid Biosynthesis

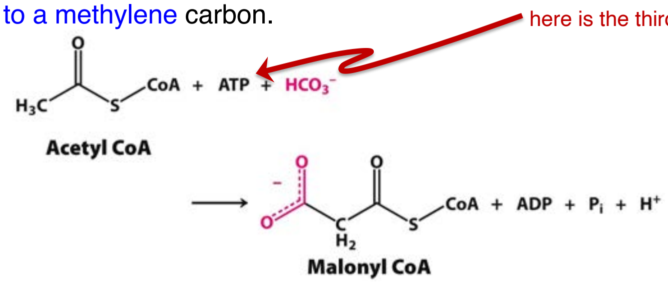
OMSGAP

- 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

here is the third ATP!



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

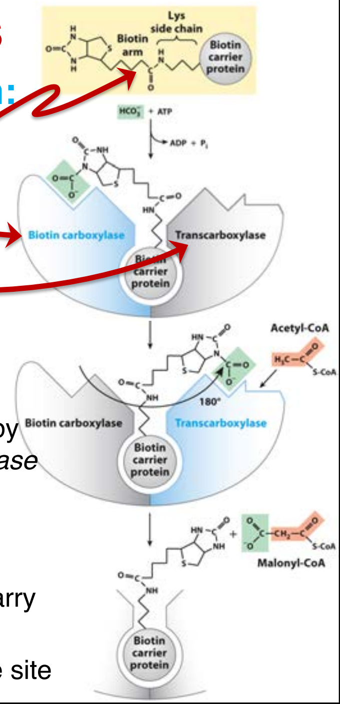
Fatty Acid Biosynthesis

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.

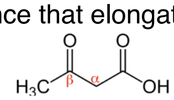
– Two-step Rxn similar to carboxylations catalyzed by *pyruvate carboxylase* and *propionyl-CoA carboxylase*
  - CO_2 binds to biotin.
    - Reaction with ATP produces carboxy-phosphate.
    - Activated CO_2 is attached to N in ring of biotin.
  - Enzyme undergoes conformational change to carry carbamoyl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



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



- 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
  - 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.

- 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)

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

Fatty Acid Biosynthesis

FAS I vs. FAS II

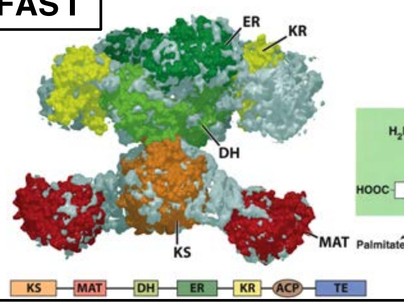
FAS I

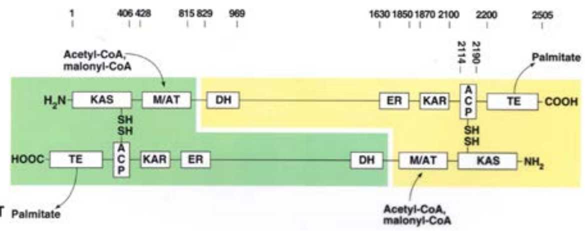
- Single polypeptide chain in vertebrates
- Leads to single product: palmitate 16:0
- C-15 and C-16 are from the acetyl-CoA used to prime the Rxn
- FAS I in vertebrates and fungi

FAS II

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

FAS I

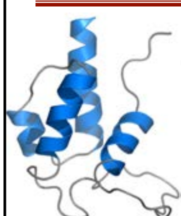




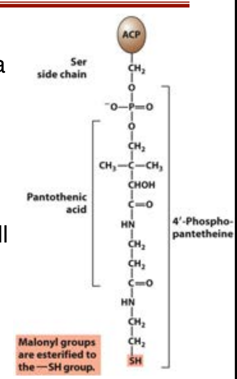
What you can't see is the ACP……

Fatty Acid Biosynthesis

Acyl Carrier Protein (ACP)

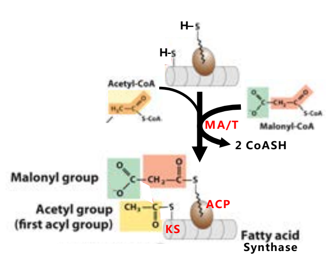


- The *E. coli* ACP is a small 77-residue protein with a a covalently attached prosthetic group, 4'-phosphopantetheine, at a Ser residue.
  - In vertebrate FAS, it's a domain with a flexible arm to tether the growing acyl chain
- Delivers acetate (in the first step) or malonate (in all the next steps)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



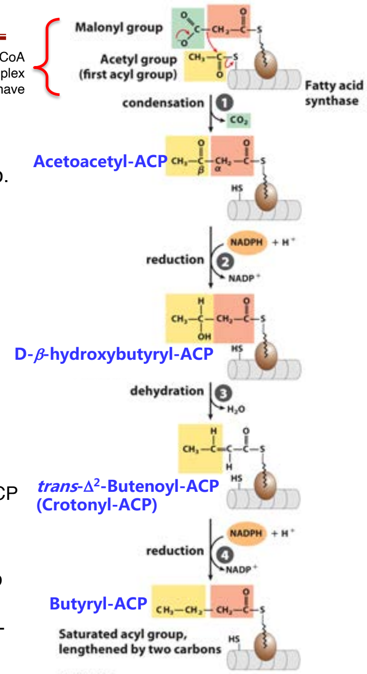
Priming FAS

- 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 (MAT)
  - 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 MAT



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)

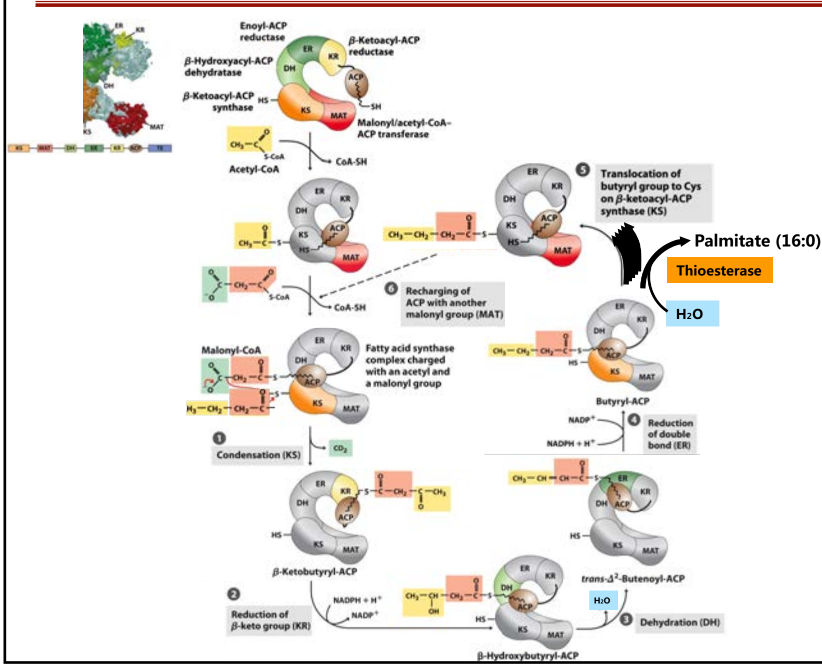
Step 3: Dehydration: OH group from C-2 and H from neighboring CH_2 are eliminated, creating double bond (trans-alkene).

- 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.

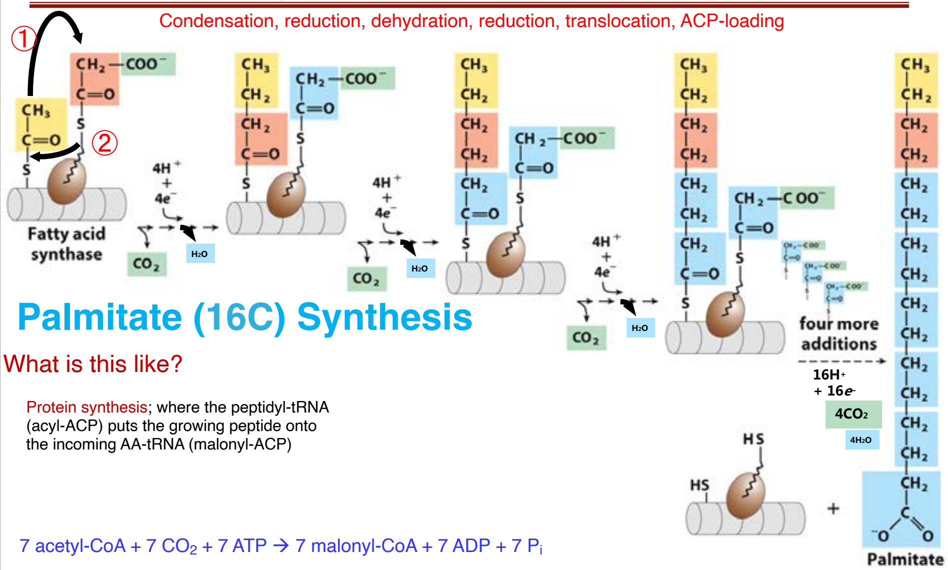
- NADPH is the electron donor to reduce double bond of *trans*-Δ^2-butenoyl-ACP to form butyryl-ACP.
- catalyzed by enoyl-ACP reductase (ER)

Fatty Acid Biosynthesis



Fatty Acid Biosynthesis

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



Palmitate (16C) Synthesis

What is this like?

Protein synthesis; where the peptidyl-tRNA (acyl-ACP) puts the growing peptide onto the incoming AA-tRNA (malonyl-ACP)

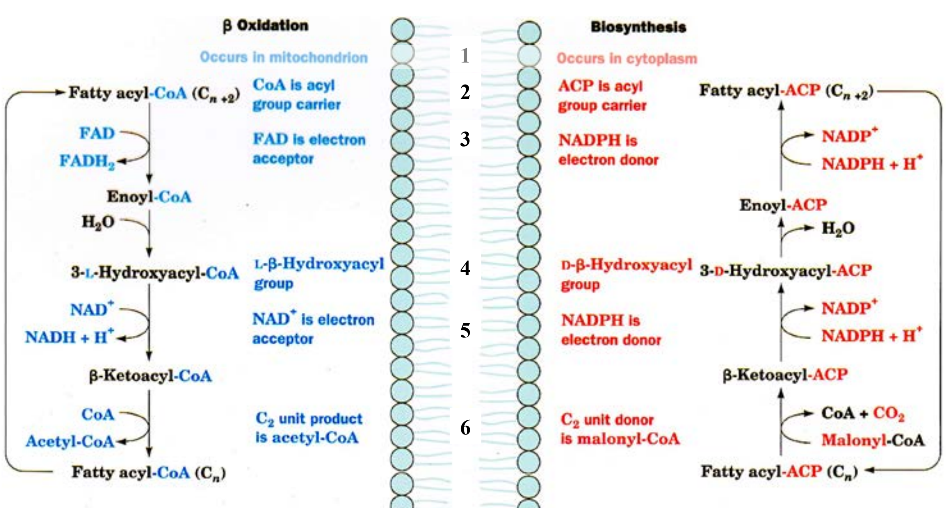
7 acetyl-CoA + 7 CO_2 + 7 ATP → 7 malonyl-CoA + 7 ADP + 7 P_i

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

Fatty Acid Biosynthesis



Comparison of Fatty-Acid Degradation

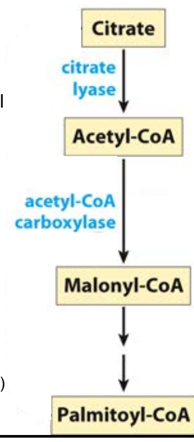
versus

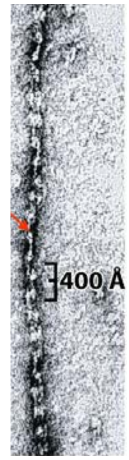
Fatty-Acid Synthesis

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}).
    - High [Citrate] signals excess energy to be converted to fat.
  - When $[acetyl\text{-}CoA]^{mt}$ ↑, $[citrate]^{mt}$ ↑ ….. 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)





Fatty Acid Biosynthesis

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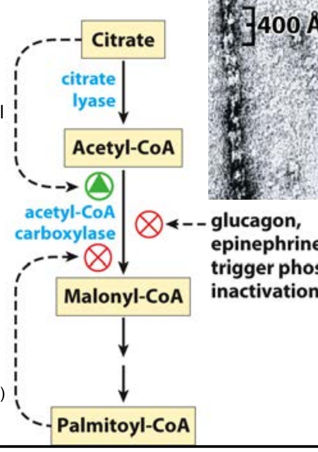
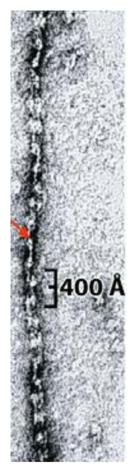
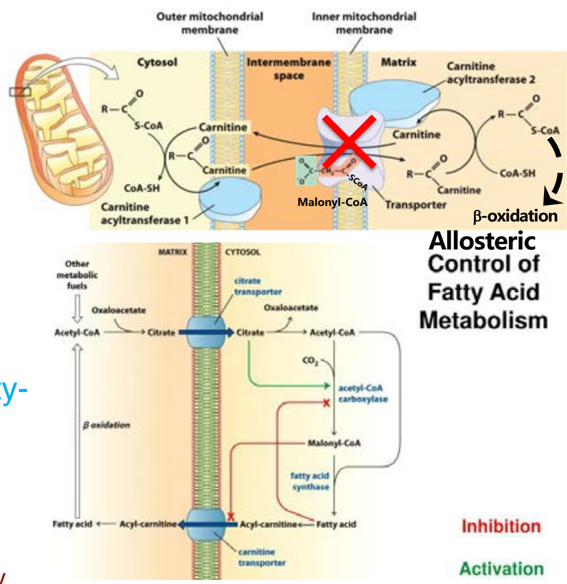




Fatty Acid Biosynthesis

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

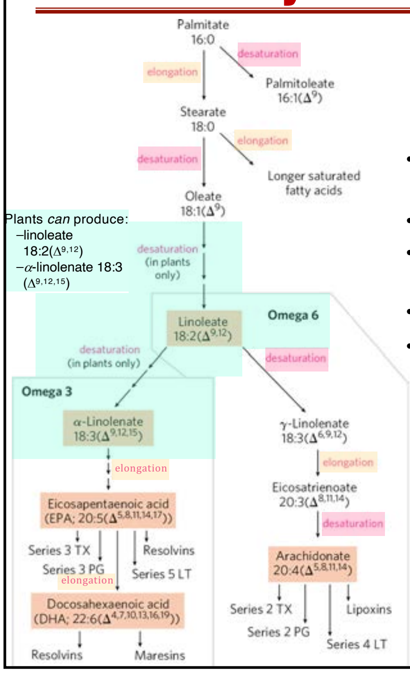
- β oxidation of fatty acids occurs in mitochondria.
- If fatty acyl-CoAs are not transported in, they cannot be degraded
- Transport is via carnitine transporter.
- Blocking β oxidation with initial committed product of fatty-acid synthesis: malonyl-CoA



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

ensures that fat synthesis and oxidation don't occur simultaneously

Fatty Acid Biosynthesis



Diversification of Palmitate:

Elongation

Fatty Acid Elongation Systems

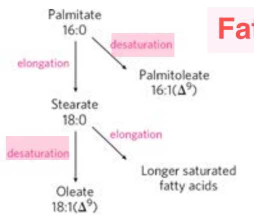
- Elongation systems in the endoplasmic reticulum and mitochondria create longer fatty acids.
- Use CoA derivatives
- As in palmitate synthesis, each step adds units of 2 C. Use malonyl-CoA.
- Stearate (18:0) is the most common product.
- Plants make linoleate (Ω6) and α-linolenate (Ω3), animals can't; But they can elongate these ingested essential FA
  - These fatty acids are "essential" to humans.
    - Polyunsaturated fatty acids (PUFAs) help control membrane fluidity.
    - PUFAs are precursors to eicosanoids
  - The ratio of omega-6 to omega-3 fatty acids in the diet, if too high, can lead to cardiovascular disease

Eicosanoids

PG = Prostaglandins
TX = Thromboxanes
LT = Leukotrienes

Fatty Acid Biosynthesis

Diversification of Palmitate: Desaturation



Fatty Acyl-CoA Desaturase

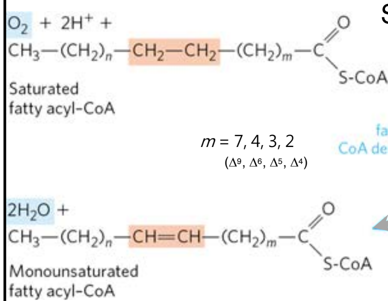
- Looks like a mixed-function oxidase: OXIDASE
- Humans have Δ^4, Δ^5, Δ^6, and Δ^9 desaturases but *cannot* desaturate beyond Δ^9.

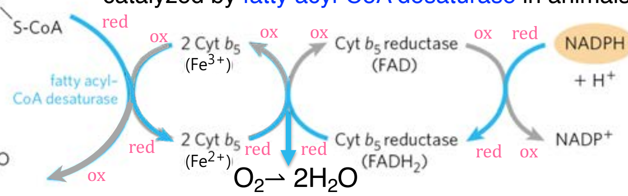
FOR EXAMPLE: for a *Δ^9-desaturation*

Palmitate(16:0) → palmitoleate(16:1, Δ^9)

Stearate (18:0) → oleate (18:1, Δ^9)

catalyzed by fatty acyl-CoA desaturase in animals





requires NADPH; enzyme uses cytochrome b_5 and cytochrome b_5 reductase

Both donate electrons to O_2 (total of 4)

In plants & bacteria the desaturases work on PL, not fatty-acyl CoA; needed for rapid changes in fluidity