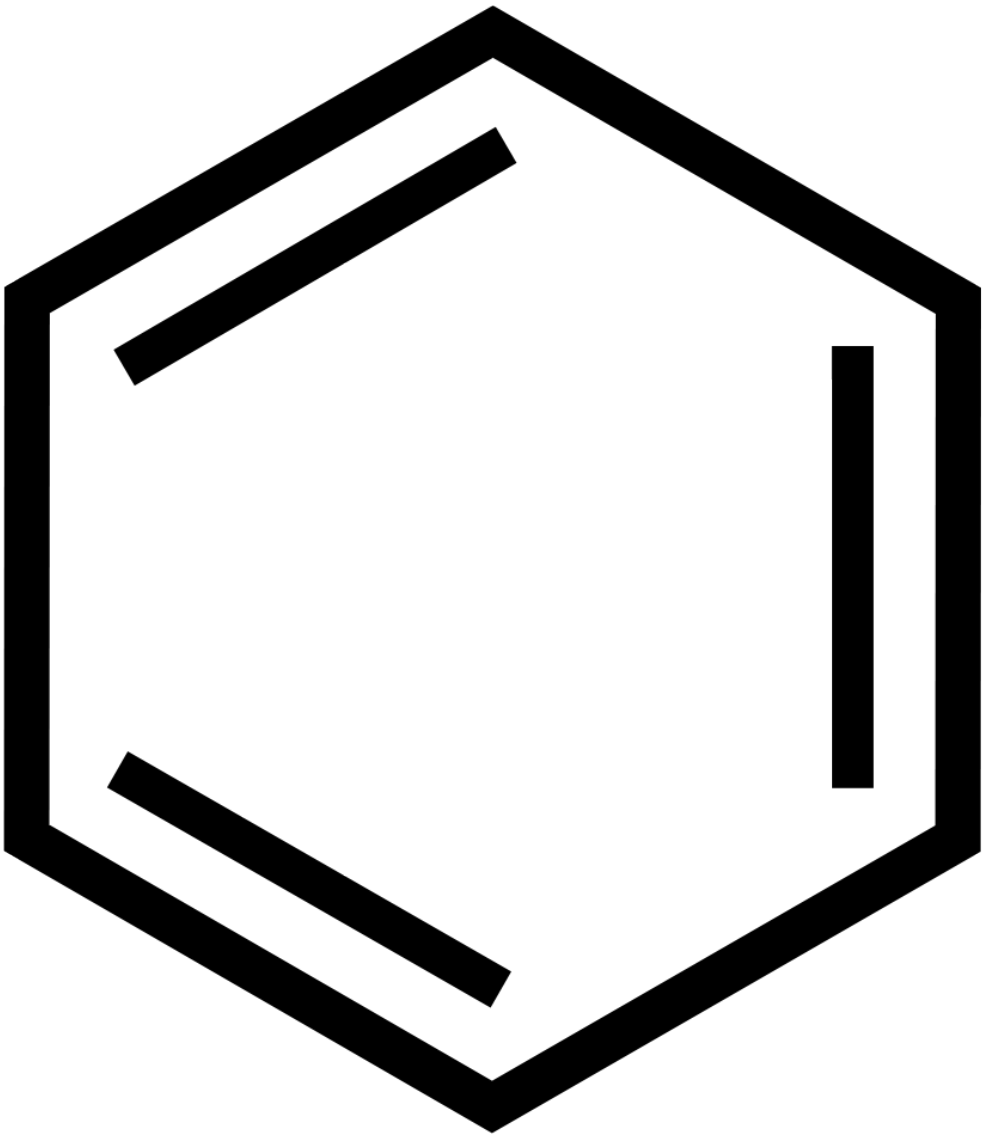

CLUTCH

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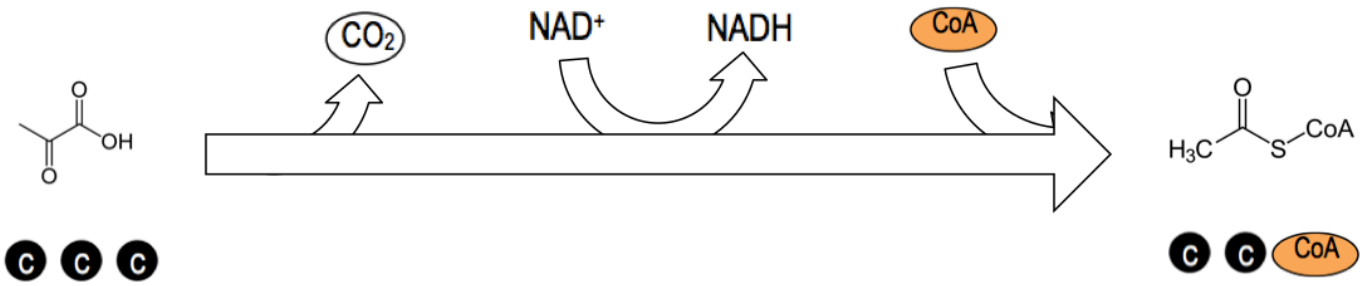
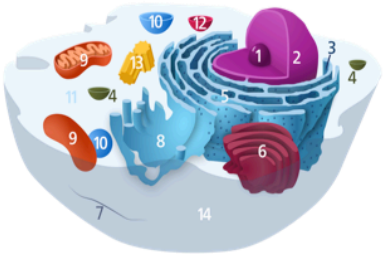
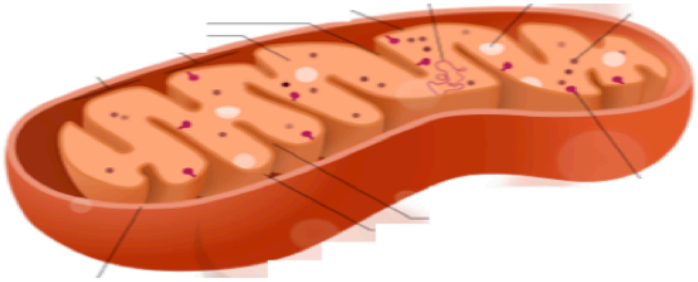
CONCEPT: PYRUVATE OXIDATION

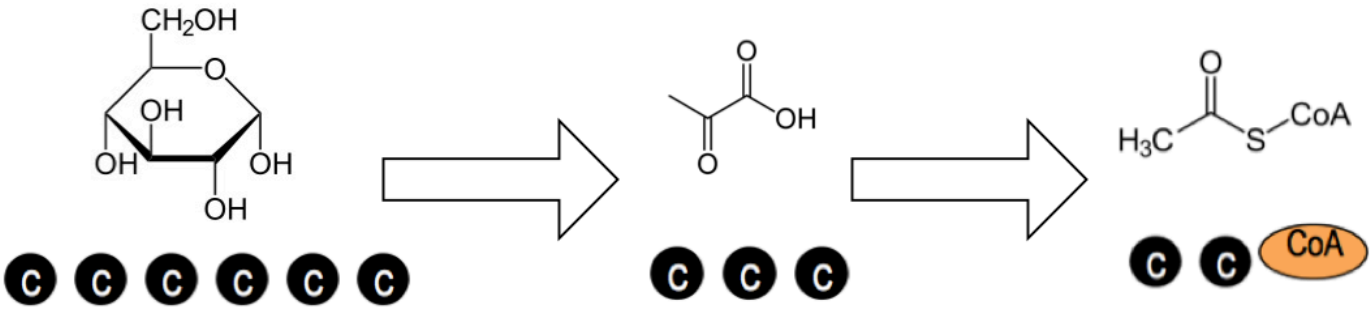
- Pyruvate dehydrogenase ($\Delta G'^{\circ}$ = -33 kJ/mol) – complex of 3 enzymes: E1, E2, and E3
  - □ E1 – pyruvate dehydrogenase, E2 – dihydrolipoyl transacetylase, E3 – dihydrolipoyl dehydrogenase
  - □ Pyruvate dehydrogenase uses 3 substrates: pyruvate, CoA-SH (reduced form), and NAD^+
  - □ Pyruvate dehydrogenase uses 3 cofactors: FAD, lipoate, TPP
  - □ Produces 3 products: Acetyl-CoA, CO_2, NADH
  - □ It is negatively regulated by ATP, acetyl-CoA, NADH, and fatty acids
  - □ It is positively regulated by AMP, CoA, and NAD+





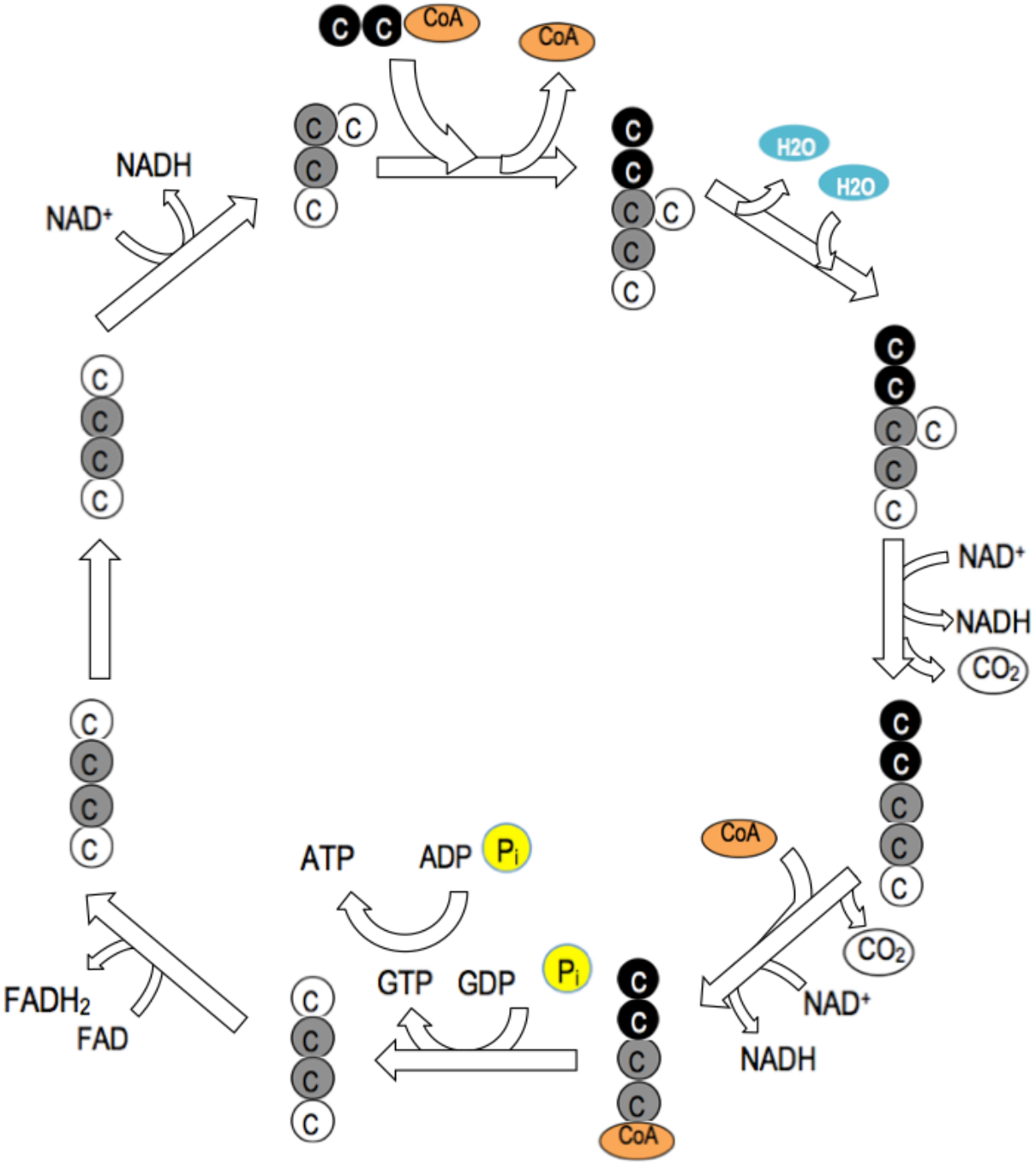


- Acetyl-CoA will contain carbons 1 and 2, or 5 and 6 from glucose
  - □ Acetyl-CoA will contain carbons 2 and 3 of pyruvate



CONCEPT: CITRIC ACID CYCLE

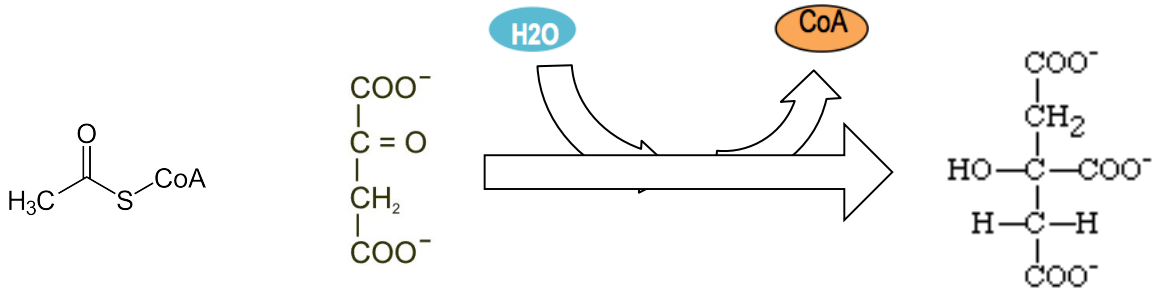
- 1 acetyl- CoA generates 3 NADH, 1 $FADH_2$, and 1 GTP/ATP
- The ΔG in steps 1, 3, and 4 is considerably negative, so these reactions drive the cycle
- The ΔG of steps 2, 5, 6, 7, and 8 is close to 0, so these reactions are readily reversible
- Every turn of cycle, only ½ the labeled carbons come off as CO_2 due to the randomization of succinate's orientation



CONCEPT: CITRIC ACID CYCLE

1. Citrate synthase ($\Delta G'^{\circ}$ = -32 kJ/mol) – acetyl-CoA + oxaloacetate → citrate

□ Water is consumed in the reaction, and CoA is released



2. Aconitase ($\Delta G'^{\circ}$ = 13 kJ/mol) – citrate → *cis*-aconitate (enzymatic intermediate) → isocitrate

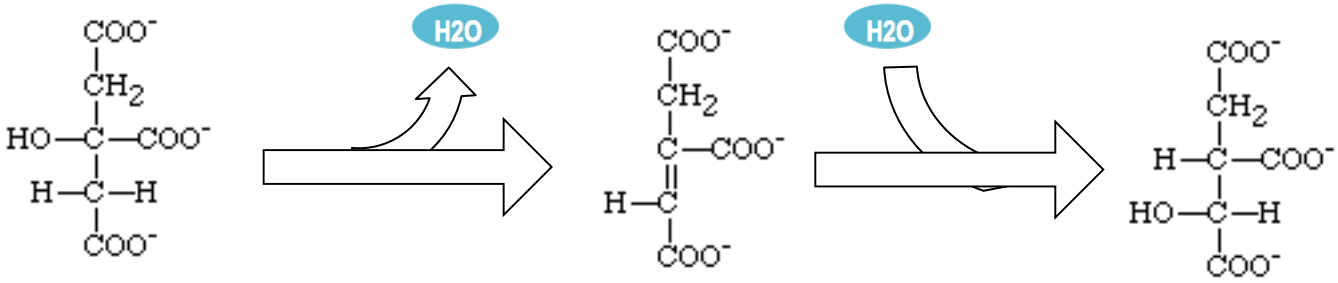
□ Removes water, then adds it back in

□ Citrate is prochiral, meaning it's not chiral, but acts chiral because aconitase binds citrate in one orientation

□ Fe-S complex held in active site by cysteine residues

□ It is an iron response regulatory molecule; changes shape due to lack of iron, and can then bind RNA

- Ferritin is produced when iron is high in blood, transferrin is produced when iron is low in blood

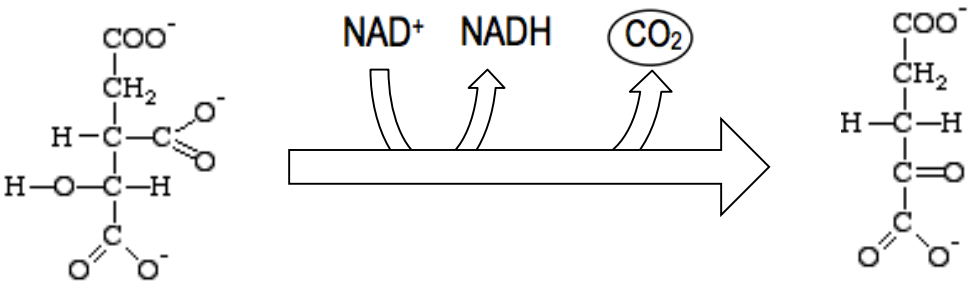


3. Isocitrate dehydrogenase ($\Delta G'^{\circ}$ = -21 kJ/mol) – isocitrate → α-ketoglutarate

□ Generates NADH and releases CO_2

□ Oxidative decarboxylase that uses NAD^+ or $NADP^+$ as the electron acceptor, and Mn^{2+} as a cofactor

- Does not use TPP, lipoate, FAD, CoA like pyruvate dehydrogenase or α-ketoglutarate dehydrogenase

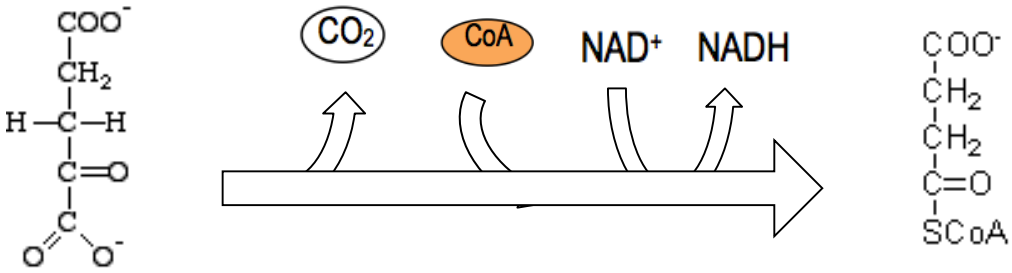


CONCEPT: CITRIC ACID CYCLE

4. α-Ketoglutarate dehydrogenase complex ($\Delta G'^{\circ}$ = -33.5 kJ/mol) – α-ketoglutarate → succinyl-CoA

□ Releases CO_2, adds CoA, and generates NADH

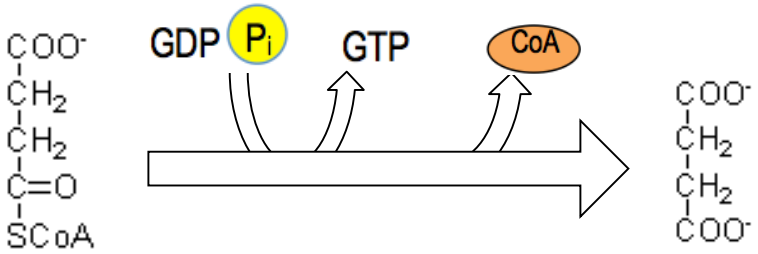
□ Just like pyruvate dehydrogenase, it uses cofactors FAD, lipoate, and TPP; it uses CoA and NAD+ as substrates



5. Succinyl-CoA synthetase ($\Delta G'^{\circ}$ = -3 kJ/mol) – succinyl-CoA → succinate

□ Thioester bond is broken, and GTP is formed from GDP and P_i via substrate-level phosphorylation

- Can form ATP from GTP via nucleoside diphosphate kinase reaction ($\Delta G'^{\circ}$ = 0 kJ/mol)
- GTP is used for protein synthesis; mitochondria have their own DNA, therefore need some GTP



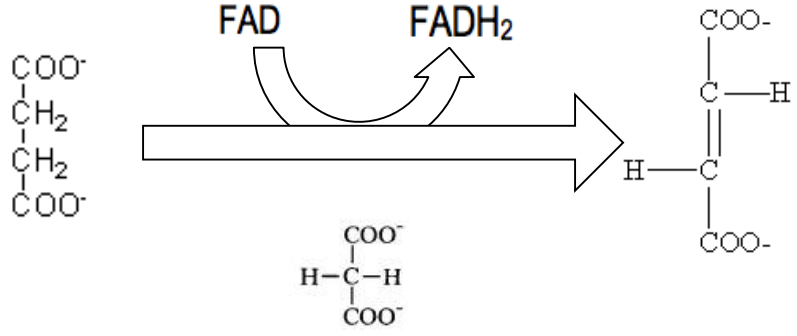
6. Succinate dehydrogenase ($\Delta G'^{\circ}$ = 0 kJ/mol) – succinate → fumarate

□ Converts –ane to –ene (*trans* form) and produces $FADH_2$, actually part of complex II in electron transport chain

□ Malonate is competitive inhibitor of this enzyme because it has similar chemical structure to succinate

□ Succinate is symmetrical, so it's orientation in the active site will be random

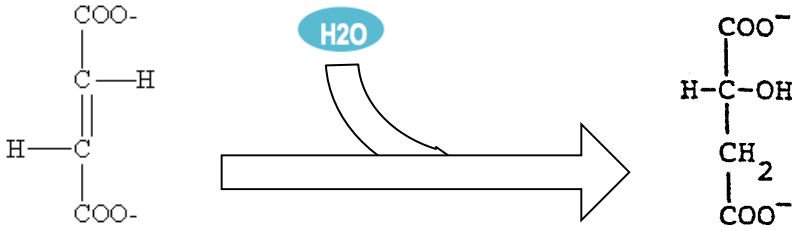
- Every turn of cycle, only 1/2 the labeled carbons come out due to randomization, next turn 1/4, then 1/8



CONCEPT: CITRIC ACID CYCLE

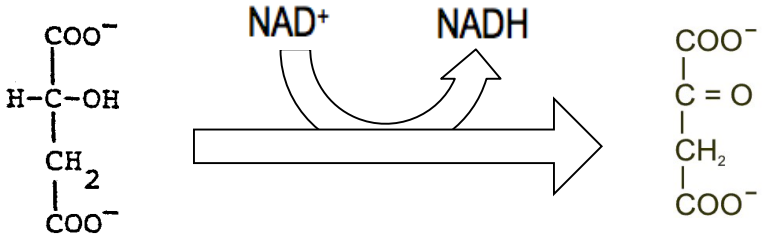
7. Fumarase ($\Delta G'^{\circ}$ = -4 kJ/mol) – fumarate → L-malate

- □ Water added in two parts: first as OH^-, then H^+



8. Malate dehydrogenase ($\Delta G'^{\circ}$ = 30 kJ/mol) – malate → oxaloacetate

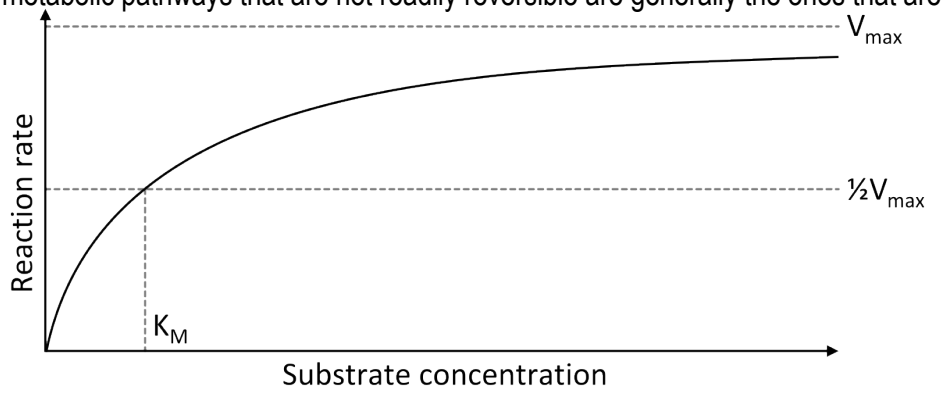
- □ Generates NADH
- □ ΔG close to 0 in biological conditions



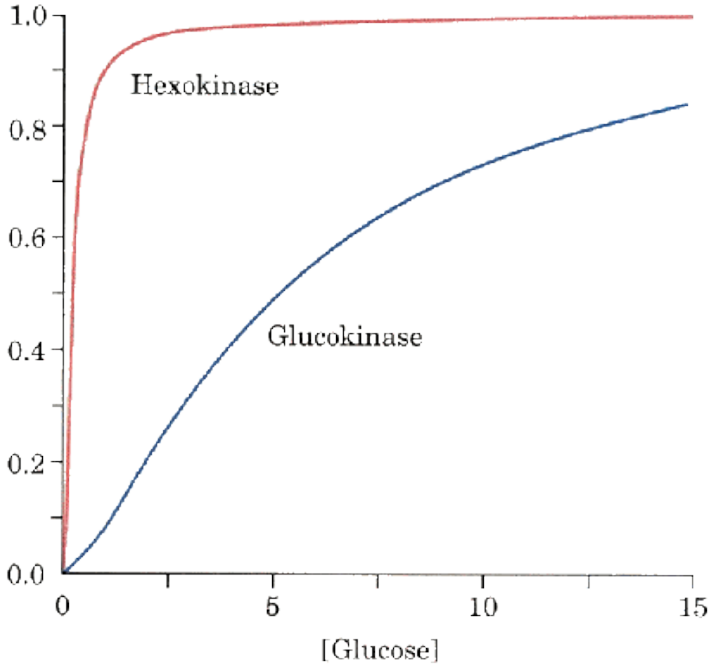
- NADH will ultimately generate 2.5 ATP, and $FADH_2$ will ultimately generate 1.5 ATP
  - □ NADH generated by glyceraldehyde 3-phosphate dehydrogenase will generate 1.5 or 2.5 ATP
- 1 glucose → 30-32 ATP; 5-7 from glycolysis + 5 from pyruvate oxidation + 20 from citric acid cycle
  - □ Glycolysis generates 2 NADH + 2 ATP → 5-7 ATP
  - □ Pyruvate oxidation generates 2 NADH → 5 ATP
  - □ Citric acid cycle generates 6 NADH + 2 $FADH_2$ + 2 ATP/GTP → 20 ATP
- Generally, the citric acid cycle is regulated by the energy poor, and energy rich molecules involved in respiration
  - □ Energy rich molecules inhibit the cycle: ATP and NADH
  - □ Energy poor molecules stimulate the cycle: AMP, ADP, NAD^+
  - □ Pyruvate dehydrogenase is inhibited by ATP, acetyl-CoA, and NADH; stimulated by AMP, CoA, and NAD^+
  - □ Citrate synthase is inhibited by NADH, succinyl-CoA, citrate, and ATP; stimulated by ADP
  - □ Isocitrate dehydrogenase is inhibited by ATP; stimulated by ADP
  - □ α-Ketoglutarate dehydrogenase complex is inhibited by NADH and succinyl-CoA
- Anaplerotic reactions – generate oxaloacetate to replace the loss of acceptor molecules from the cycle
  - □ Many molecules from the citric acid cycle are important biosynthetic precursors for amino acids
  - □ Reactions 8, 7, 6, and 5 can be easily reversed to produce succinyl-CoA, also ß-oxidation of certain fatty acids

CONCEPT: METABOLIC REGULATION

- Substrate concentration in cells is close to K_m of their respective enzymes; ensures enzyme activity fluctuates with Δ[S]
  - □ Elasticity coefficient – slope of line on Michaelis-Menten, most elastic in 0-K_m range
  - □ ADP and ATP concentration don't fluctuate too much in a cell, AMP concentration can fluctuate drastically
    - AMP-activated protein kinase has a wide range of effects on metabolism (fatty acid oxidation in the heart)
  - □ Enzymes of metabolic pathways that are not readily reversible are generally the ones that are regulated



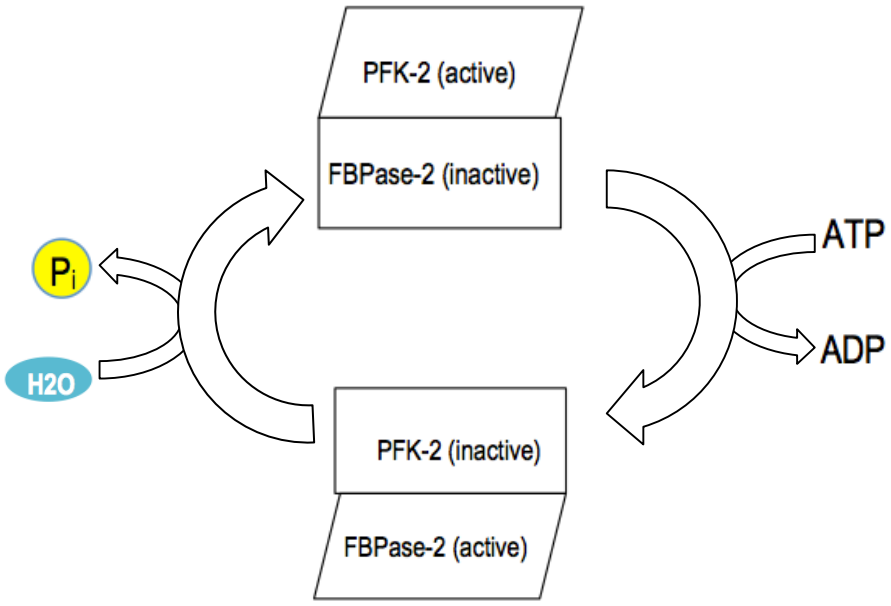
- Glycolysis and gluconeogenesis must be tightly controlled, and regulated in conjunction, to prevent futile cycles
- Hexokinase I is the most influential enzyme on the rate of glycolysis, followed by phosphofructokinase-1
  - □ Glucose 6-phosphate (the product of hexokinase's reaction with glucose) inhibits hexokinase
- Glucokinase (hexokinase IV) – present in liver cells, stored in the nucleus, and has a much higher K_m than hexokinase I
  - □ Glucose causes glucokinase to move from the nucleus to cytoplasm
  - □ Fructose 6-phosphate causes glucokinase to move back into nucleus
  - □ It is NOT inhibited by glucose 6-phosphate, allowing for the supply, not demand, of glucose to gauge rxn rate



CONCEPT: METABOLIC REGULATION

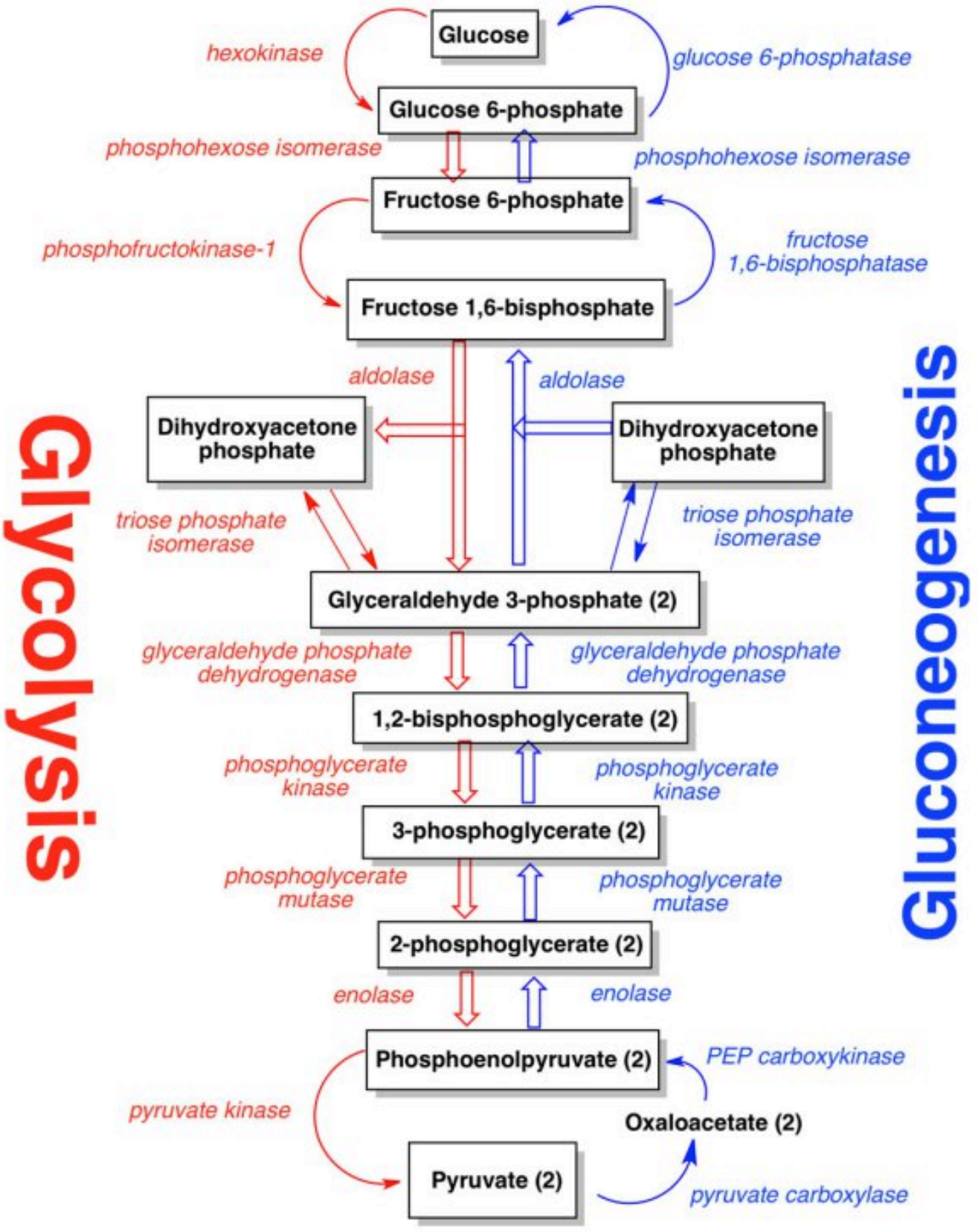
- Phosphofructokinase-1 (PFK-1) – catalyzes the commitment step of glycolysis
  - □ Has allosteric regulatory sites that bind ATP; high [ATP] lowers enzyme activity
  - □ Citrate is transported out into cytoplasm for biosynthesis, and high concentrations will inhibit PFK-1
  - □ ADP and AMP stimulate the enzyme's activity
  - □ Fructose 2,6-bisphosphate is an allosteric activator, and the most important regulator of PFK-1
    - PFK-1 is considerably less active without fructose 2,6-bisphosphate
    - Fructose 2,6-bisphosphate is effective at extremely small concentrations (~1μM)
    - Fructose 2,6-bisphosphate inhibits fructose 1,6-bisphosphatase, PFK-1's gluconeogenic counterpart
    - AMP also inhibits fructose 1,6-bisphosphatase, and therefore inhibits gluconeogenesis
- Phosphofructokinase-2 (PFK-2) – generates fructose 2,6-bisphosphate to regulate the action of PFK-1
  - □ Insulin causes phosphate group to be removed from enzyme, activates PFK2
  - □ Glucagon leads to phosphorylation of enzyme, activating fructose bisphosphatase 2
  - □ Fructose 2,6-bisphosphate is broken down by fructose bisphosphatase 2; part of same protein as PFK-2
  - □ Phosphoprotein phosphatase dephosphorylates PFK-2; stimulated by xylulose 5-phosphate

INSULIN GLUCAGON



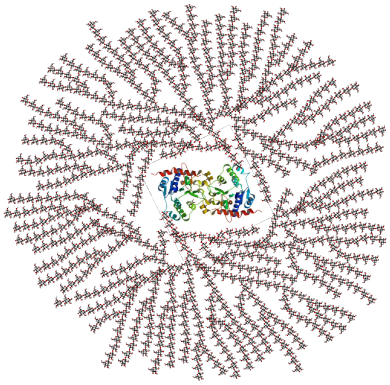
- Pyruvate kinase – last enzyme of glycolysis; only in the liver does glucagon cause PKA to phosphorylate and inactivate it
  - □ Stimulated by fructose 1,6-bisphosphate, an upstream glycolytic substrate
  - □ Inhibited by ATP, acetyl-CoA, and long-chain fatty acids (also alanine, which is derived from pyruvate)
- Pyruvate carboxylase is activated by acetyl-CoA

CONCEPT: METABOLIC REGULATION

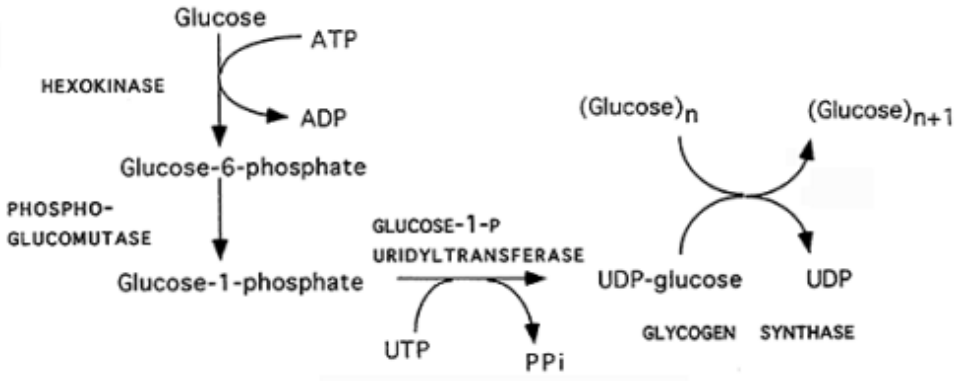


CONCEPT: GLYCOGEN METABOLISM

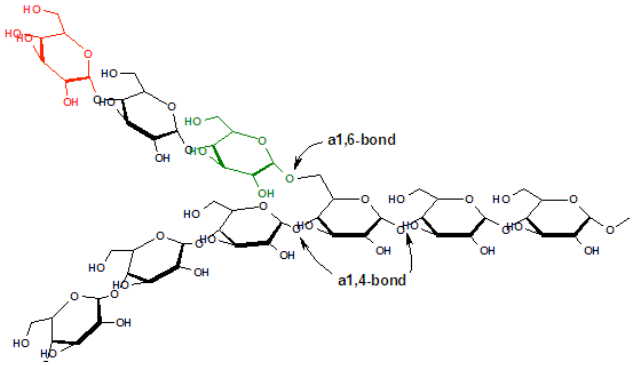
- Glycogen – highly branched glucose polymer that uses α(1→4) glycosidic linkages, with α(1→6) branch points
  - □ Sugar chains tend to be about 12-14 subunits long
  - □ Glycogenin – protein core of glycogen; the first sugars are hooked onto Y residues



- Glycogen synthase – enzyme that catalyzes the synthesis of glycogen
  - □ It's a synthase, so it doesn't use NTPs; instead uses UDP-glucose, and UDP is released from reaction
  - □ Elongates at the nonreducing end of the sugar, ultimately forming chains about 12-14 subunits long
  - □ GSK3 normally phosphorylates, and inactivates, glycogen synthase
    - Insulin inhibits GSK3, thereby activating glycogen synthase
  - □ Protein phosphatase 1 dephosphorylates glycogen synthase, activating it
    - Stimulated by insulin, glucose 6-phosphate, and glucose; inhibited by glucagon and epinephrine

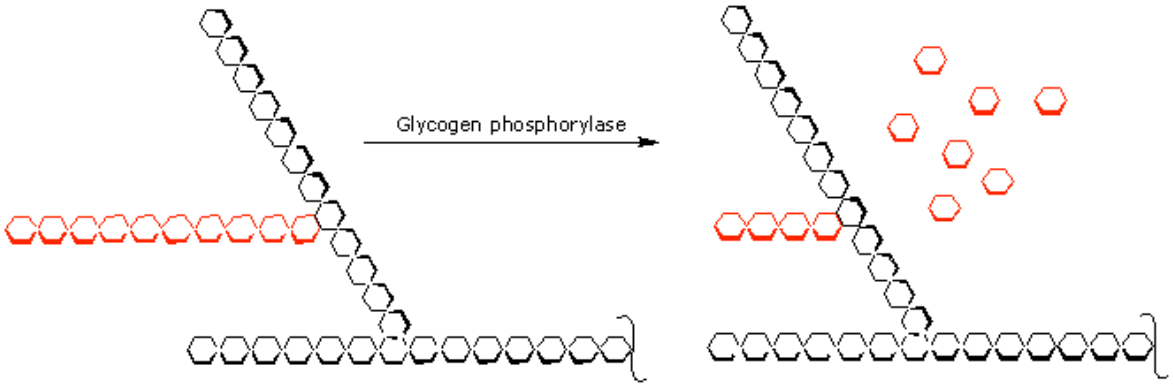


- Branching enzyme transfers 6-10 subunits of the chain formed by glycogen synthase onto the 6 position of glucose

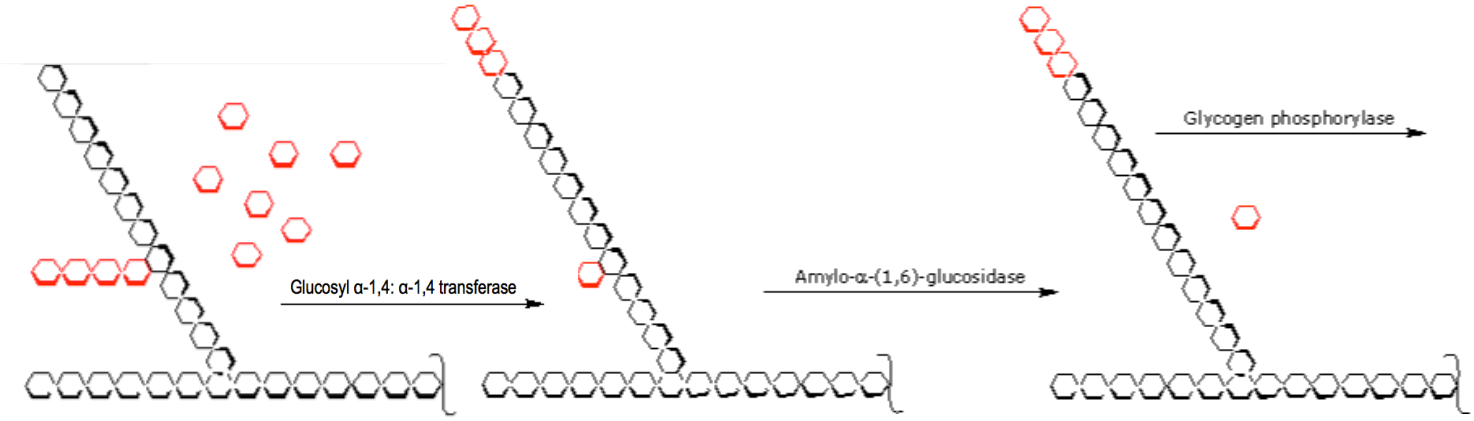


CONCEPT: GLYCOGEN METABOLISM

- Glycogen phosphorylase – breaks down glycogen by removing subunits from nonreducing end via phosphorolysis
    - □ Breaks off glucose subunits as glucose 1-phosphate, which must be converted to glucose 6-phosphate
        - Phosphoglucomutase ($\Delta G \sim 0$) – glucose 1-phosphate → glucose 6-phosphate
    - □ Glucagon, epinephrine, and AMP lead to the phosphorylation and activation of glycogen phosphorylase
        - Phosphorylase kinase b adds 2 phosphate groups when stimulated by glucagon, epinephrine, and AMP
    - □ Glucose allosterically regulates, exposes phosphate groups to make them easier to remove
    - □ Due to extensive branching, many phosphorylases can work simultaneously to rapidly deliver a lot of glucose



- Debranching enzyme – transfers 3 sugars from one branch to another, then removes branch point glucose
    - □ Hydrolyzing the α-1,6 bond produces the only glucose in glycogenolysis

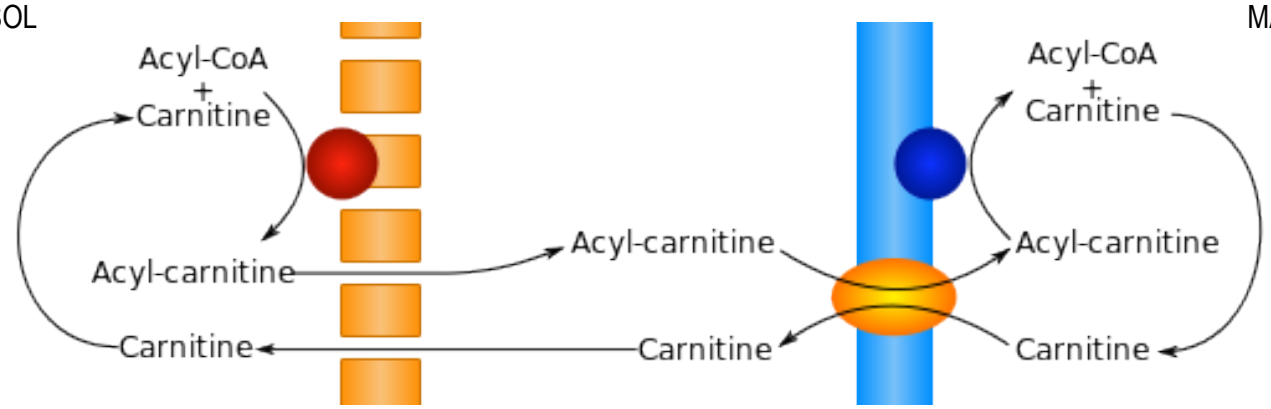


- High blood glucose: insulin up→ glycogen breakdown low, glycogen synthesis up, glycolysis up
- Low blood glucose: glucagon up, glycogen breakdown up, glycolysis and glycogen synthesis down
- Glycogen phosphorylase and glycogen synthase are phosphorylated and dephosphorylated together
    - □ The two enzymes are affected in the opposite ways (activation/deactivation) by these chemical modifications

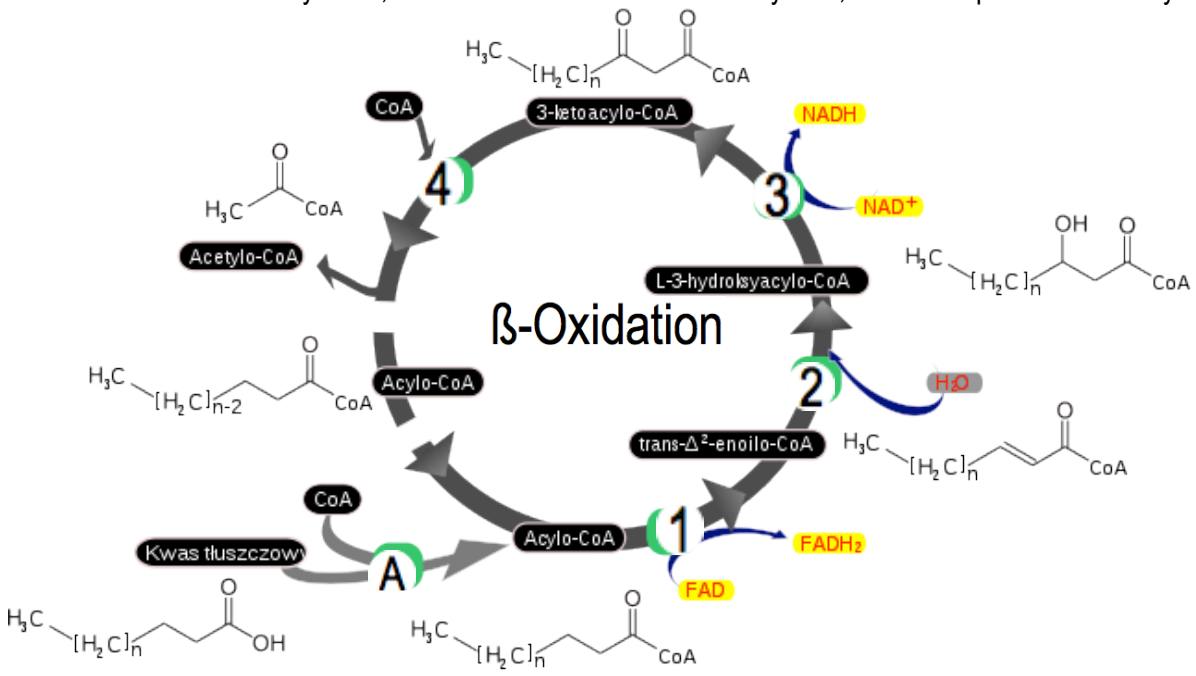
CONCEPT: FATTY ACID OXIDATION

- Fats are used for energy and water storage, and can be broken down into glycerol and fatty acids
  - □ Glycerol can be converted to DHAP (G3P) to enter glycolysis; yields 1 ATP and 2 NADH (nonfermentable sugar)
  - □ Fatty acids undergo ß-oxidation to enter the Citric Acid Cycle as acetyl-CoA (or succinyl-CoA in some cases)
    - Fatty acids are activated by converting them to fatty acyl-CoA (costs "2 ATP", or 2 acid anhydride bonds)
    - Transported into mitochondrial matrix bound to carnitine via antiporter that moves carnitine into cytosol
    - Carnityl acyl transferase I attaches carnitine to fatty acyl-CoA and carnityl acyl transferase II removes it

CYTOSOL MATRIX

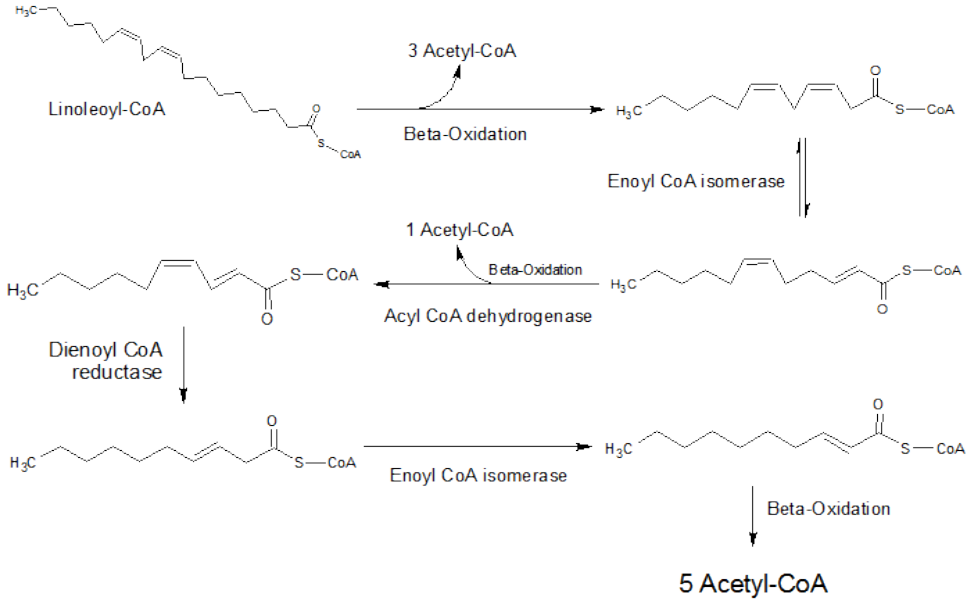


- ß-Oxidation – removes 2 carbon units at a time, as acetyl-CoA, from fatty-acyl-CoA; occurs in mitochondrial matrix
  1. Oxidation of an –ane to –ene (acyl-CoA dehydrogenase); FAD→$FADH_2$, like succinate dehydrogenase
  2. Add water to –ene to form alcohol (enoyl-CoA hydratase); like fumarate to malate
  3. Oxidize alcohol (beta-hydroxyacyl-CoA dehydrogenase); NAD^+→NADH, like malate dehydrogenase
  4. Thiolase cleaves off acetyl-CoA, and adds CoA to new end of fatty acid; last round produces 2 acetyl-CoA*

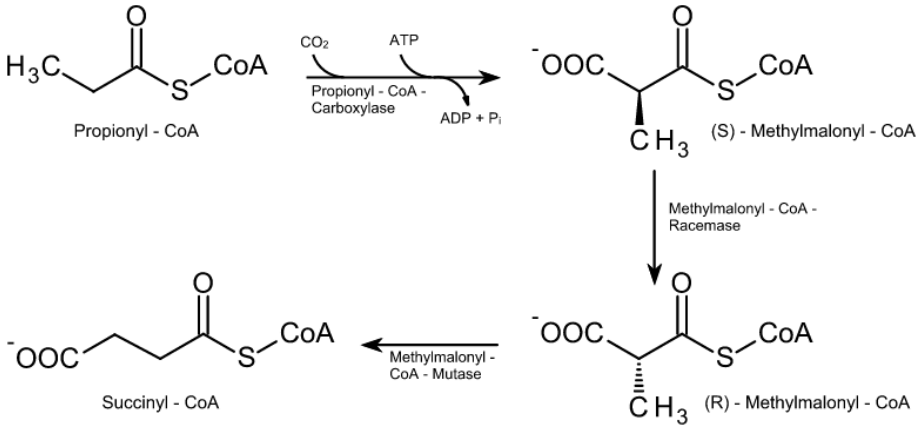


CONCEPT: FATTY ACID OXIDATION

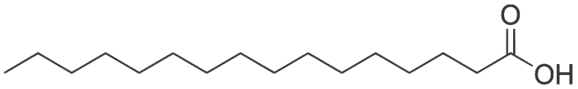
- Unsaturated fatty acids use an isomerase to move the –ene if necessary; *trans* is ok, *cis* must be rearranged
  - □ Will not generate $FADH_2$ from the oxidation of a point of unsaturation
  - □ Multiple points of unsaturation may prevent rearrangement by isomerase
    - NADPH is used to reduce –ene if isomerase can't rearrange the double bond



- Odd numbered fats generate acetyl-CoA and propionyl-CoA (3 carbons) in the last round of ß-oxidation
  - □ Add CO_2 to propionyl-CoA, then isomerase rearranges into succinyl-CoA (used in step 5 of TCA)



PRACTICE: How much ATP will the ß-oxidation of palmitic acid produce?



- ß-Oxidation

$FADH_2$: NADH:

- Citric Acid Cycle

$FADH_2$: NADH:

PRACTICE: CITRIC ACID CYCLE

1. Which of the following *not* true of pyruvate dehydrogenase complex?

a. both NAD+ and FAD participate
b. biotin participates in the decarboxylation
c. the reaction occurs in the mitochondria
d. two different –SH groups participate
e. all of the above

2. Glucose labeled in the third and fourth carbons with ^{14}C is converted to pyruvate by glycolysis and to acetyl-CoA with pyruvate dehydrogenase complex. Where is the label acetyl-CoA?

a. 100% in the thioester
b. 50% in the thioester
c. 100% in the methyl
d. 50% in the methyl
e. it is not there at all

3. Malonate is a competitive inhibitor of succinate dehydrogenase. If malonate is added to respiring mitochondria, which molecule would you expect to decrease in concentration?

a. succinate
b. fumarate
c. α-ketoglutarate
d. isocitrate
e. pyruvate

4. Which of the following is not an intermediate in the Citric Acid Cycle?

a. acetyl-CoA
b. citrate
c. oxaloacetate
d. malate
e. isocitrate

5. Oxaloacetate uniformly labeled with ^{14}C in all carbons (all carbons have the same radioactivity) is added to mitochondria with an adequate supple of acetyl-CoA (unlabeled). In the first cycle, how much radioactivity will remain in oxaloacetate?

a. 100%
b. 50%
c. 25%
d. 12.5%
e. none

PRACTICE: CITRIC ACID CYCLE

6. The conversion of one mole of acetyl-CoA to two moles of CO_2 results in the production of:

a. 1 mole of ATP
b. 1 mole of $FADH_2$
c. 1 mole of NADH
d. 2 moles of GTP
e. 2 moles of citrate

7. The reaction in the Citric Acid Cycle most similar to pyruvate dehydrogenase converts:

a. fumarate to malate
b. isocitrate to α-ketoglutarate
c. succinate to aconitate
d. malate to oxaloacetate
e. α-ketoglutarate to succinyl-CoA

8. The reaction in the Citric Acid Cycle that produces a GTP converts:

a. citrate to isocitrate.
b. malate to oxaloacetate.
c. fumarate to malate.
d. succinyl-CoA to succinate.
e. succinate to fumarate.

9. Which Citric Acid Cycle intermediate is considered prochiral?

a. citrate
b. isocitrate
c. malate
d. oxaloacetate
e. succinate

10. The conversion of one mole of pyruvate to three moles of carbon dioxide by pyruvate dehydrogenase and Citric Acid Cycle produces _____ moles of NADH, _____ moles of $FADH_2$, and _____ moles of ATP (=GTP).

a. 2:2:2
b. 3:3:1
c. 3:2:0
d. 4:1:1
e. 4:2:1

11. The glyoxylate cycle:

a. occurs in muscle, but not the liver.
b. occurs in the liver, but not muscle.
c. produces nucleic acids.
d. uses acetyl-CoA for energy and synthesis of biosynthetic precursors.
e. is an alternative pathway when oxygen is low.

12. Citric Acid Cycle intermediates are used to make _____, and must be replaced via anapleurotic reactions that form _____.

a. fatty acids; succinate
b. glycerol; malate
c. amino acids; acetyl-CoA
d. porphyrins; oxaloacetate
e. glucose; pyruvate

13. Diagram the isocitrate dehydrogenase reaction showing ALL ATOMS of the substrate(s) and product(s), but not of the atoms of recyclable energy compounds such as ATP, GTP, NAD^+, $FADH_2$, CoA, etc.

PRACTICE: GLUCOSE AND GLYCOGEN REGULATION

14. The most sensitive indicator of the energetic status of a cell is:

a. ATP.
b. GDP.
c. ADP.
d. AMP.
e. glucose.

15. The enzyme glycogen phosphorylase:

a. catalyzes cleavage of ß(1-4) bonds.
b. catalyzes cleavage of α(1-4) bonds.
c. hydrolyzes glucose.
d. catalyzes cleavage of α(1-2) bonds.
e. catalyzes cleavage of ß(1-2) bonds.

16. Glycogen branching enzyme catalyzes the formation of:

a. α(1-2) bonds.
b. α(1-3) bonds.
c. α(1-4) bonds.
d. α(1-5) bonds.
e. α(1-6) bonds.

17. Glycogenin is:

a. regulatory of glycogen synthase.
b. catalyzes conversion of starch to glycogen.
c. exceptionally large.
d. the gene for glycogen synthase.
e. the primer for glycogen synthesis

18. Glycogen phosphorylase-a can be allosterically inhibited by:

a. cAMP.
b. AMP.
c. glucose.
d. glucagon.
e. GTP.

19. Phosphofructokinase-2 is inhibited by:

a. glucose.
b. ATP.
c. insulin.
d. cAMP.
e. glucagon.

PRACTICE: GLUCOSE AND GLYCOGEN REGULATION

20. Fructose 2,6-bisphosphate concentration in cells is about:

a. μM.
b. mM.
c. nM.
d. pM.
e. M.

21. Why is the K_m of glucokinase (hexokinase IV) so much higher than that of hexokinase I?

22. The V_{max} of glycogen phosphorylase from muscle needs to be greater than that of the liver. Give two reasons for why this is.

Reason 1:

Reason 2:

PRACTICE: FATTY ACID OXIDATION

23. In the catabolism of fat (triacylglycerols), glycerol enters central metabolism as:

a. glucose.
b. dihydroxyacetone phosphate.
c. acetate.
d. pyruvate.
e. glyceryl-CoA.

24. Carnitine is:

a. a 15 carbon fatty acid.
b. a Citric Acid Cycle cofactor.
c. essential for intracellular transport of fatty acids.
d. a rare amino acid found in proteins.
e. present only in carnivores.

25. What is the correct order of enzymes in β-oxidation?

1. β-hydroxyacyl-CoA dehydrogenase
2. thiolase
3. enoyl-CoA hydratase
4. acyl-CoA dehydrogenase

a. 1, 2, 3, 4,
b. 2, 3, 4, 1
c. 4, 3, 1, 2
d. 2, 4, 3, 1
e. 3, 1, 4, 2

26. The conversion of one palmitic acid (16:0) through β-oxidation and the Citric Acid Cycle and all of the energy intermediates are converted to ATP equivalents that equal:

a. 3
b. 108
c. 32
d. 1000
e. 10

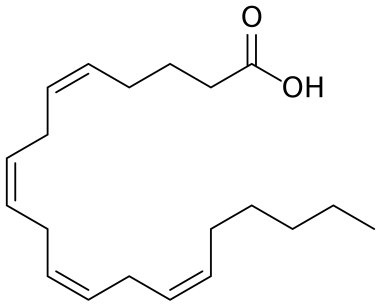
27. The conversion of one palmitoleic acid (16:1) through β-oxidation and the Citric Acid Cycle and all the energy intermediates are converted to ATP equivalents that equal:

a. 4
b. 106
c. 31
d. 1004
e. 12

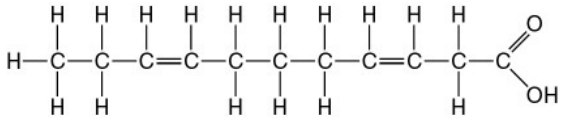
28. Which compound is an intermediate of ß-oxidation?

a. CH_3-$(CH_2)_{18}$-CO-COOH
b. CH_3-CH_2-CO-CH_2-CH_2-O-PO_4^-
c. CH_3-CH_2-CO-CO-O-CoA
d. CH_3-CO-CH_2-CO-CoA
e. CH_3-CH_2-CO-CH_2-OH

29. Why does the molecule below require NADPH to reduce a C=C double bond during ß-oxidation?



30. Consider the β-oxidation of the following fatty acid:



- How many rounds of β-oxidation are necessary to convert it acid to acetyl-CoA?

- What is the output of reduced electron carriers and ATP?