

**MMBB 300/380 Fall 2007**  
**Exam 4 Concepts and Problems**

**Regarding the different processes that we will have covered (gluconeogenesis, PPP, glycogen metabolism, TCA cycle, ETS, Ox Phos, etc), you should know the following basics:**

- 1) What is the function or functions of a specific pathway? (Likely energy or biosynthetic precursors; what form [ATP; NADH, NADPH, ribose-5-P, etc]?)
- 2) What metabolite(s) feeds into the pathway/what metabolite(s) is/are generated?
- 3) Where does the pathway occur (plant/animal/bacteria; which tissues (if relevant); does it occur in different compartments within a given cell)?
- 4) When does each process/pathway occur (under what conditions; this is linked to item #1)?
- 5) How is each process or pathway regulated?
- 6) Recognize the various prosthetic groups (biotin, NAD, FAD, FMN, TPP, lipoamide, etc, etc) & know their primary function(s). (i.e. biotin is used to add a carboxylate group to a carbon)
- 7) For the TCA cycle you will have to reproduce part or all of the pathway on the exam.

**Gluconeogenesis**

1. Gluconeogenesis occurs in which organs and which subcellular compartments?
2. What precursors feed into gluconeogenesis?
3. Compare the net cost or gain of glycolysis vs gluconeogenesis (ATP, NADH, etc).
4. Gluconeogenesis shares some of the glycolytic enzymes. Which are bypassed & why?
5. Know the details of how pyruvate (cytosolic) is converted to PEP (cytosolic). What organelle(s) are involved? How is malate involved? Is malate involved when the precursor is primarily lactate? Why?
6. What does it mean that glycolysis & gluconeogenesis are reciprocally regulated?

**Pentose Phosphate Pathway**

1. What metabolite feeds into this pathway?
2. What two important molecules are formed by the oxidative branch?
3. How is excess ribose recycled?
4. How would this pathway be used to synthesize ribose if NADPH production was not needed?

**Glycogen degradation & synthesis**

1. Where is glycogen found & what is its primary function?
2. Know the enzymes and the metabolites (phosphorylase & glycogen synthase are the key enzymes).
3. What is an important advantage to extensive branching in glycogen?
4. How many high energy phosphate bonds does it cost to add glucose to a glycogen granule?
5. What is UDP-glucose? How is it formed and used?
6. What is glycogenin & how does it function to regulate the size of the glycogen granule?
7. How are glycogen degradation & synthesis reciprocally regulated? (i.e. adrenaline increases [cAMP] which activates protein kinase A which acts on phosphorylase kinase & glycogen synthase simultaneously).

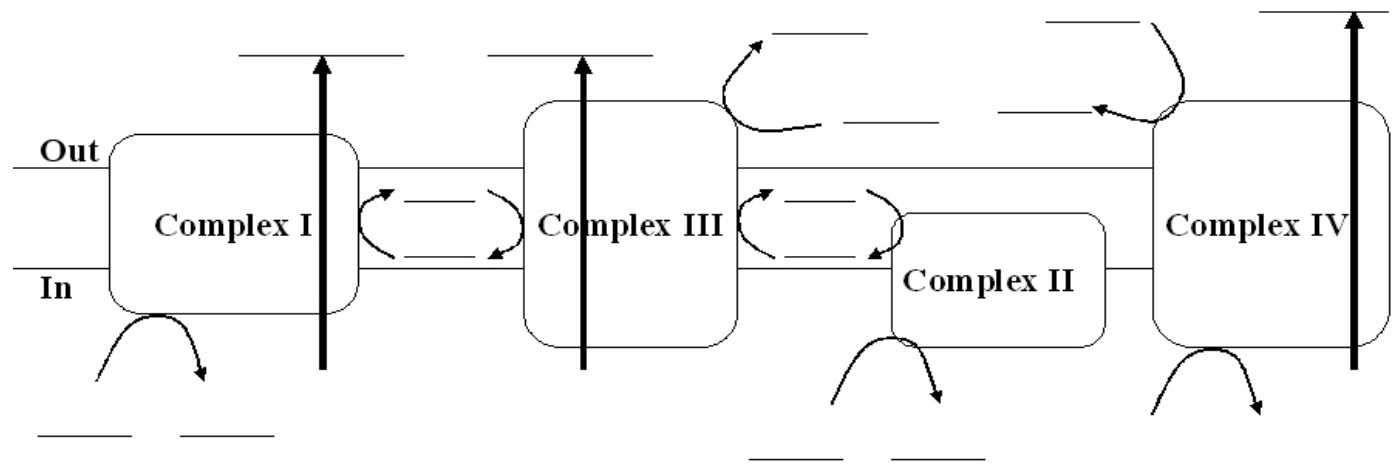
**Citric Acid Cycle (Krebs Cycle; TCA Cycle)**

1. 1 complete cycle with Acetyl-CoA yields  $2\text{CO}_2 + \text{CoA} + 3\text{NADH} + \text{FADH}_2 + \text{GTP}$
2. Know the participants (molecular structure of the metabolic intermediates; also know where  $\text{CO}_2$ , GTP, NADH &  $\text{FADH}_2$  are generated).
3. Pyruvate DH Complex:
  - A. How big is it?
  - B. What are the different enzymatic properties of this complex?
  - C. What are the (biochemical) advantages to having these enzymes in a single complex?
  - D. What Coenzymes are involved (there are at least 5)? Which are directly bound to the acetyl group?
  - E. Know that this complex is inhibited by high [ATP] & [NADH].

- Which enzymes in the cycle are regulated? Which effectors activate? Which inactivate?
- Know that the  $\alpha$ -Ketoglutarate DH Complex is extremely similar to the Pyruvate DH Complex. Both complexes make an acyl CoA product.
- Know that Succinate DH (contains a tightly bound FAD) is embedded in the inner mitochondrial membrane & is directly involved with the ETS.
- A symmetrical molecule (citrate) can bind asymmetrically to an asymmetric protein (binding site of aconitase for example).
- $\alpha$ -KG & OAA give rise to 10 of the 20 common amino acids (though not all 10 are necessarily made in all organisms; ie, humans can't make the essential aa's); these 2 Citric acid cycle intermediates also give rise to the purine and pyrimidine bases of the nucleotides.

### Electron Transport System (ETS)

- What does it mean for one atom to be more "electronegative" than another? Who coined this term & the concept?
- What is an oxidation-reduction reaction?
- If given the reduction potential ( $E^\circ$ ) for 2 half-reactions, be able to determine the  $\Delta E^\circ$  for the whole oxidation-reduction reaction & identify if the rxn is thermodynamically favorable.
- What is the difference between respiration and fermentation?
- How do porins differ from specific membrane transporters
- What are the 4 different complexes in ETS? How are they related?
- What are the electron donors and acceptors for each of the ETS complexes? What is the terminal electron donor for mitochondrial ETS?



- How are FMN and FAD related?
- How many protons get pumped (& where) for each set of 2 electrons that flow thru each complex?
- What are the soluble (can freely diffuse) carriers of electrons? In what medium do they diffuse? How many electrons can each carry?
- How much energy would be liberated if NADH gave 2 e-'s to  $O_2$  under standard conditions?
- When cytochrome c oxidase (complex IV) poisons such as  $NaN_3$ ,  $CN^-$  &  $CO$  are given to a cell, the ETS shuts down completely. Why is this?

### Oxidative Phosphorylation

- What is the typical difference in pH ( $\Delta pH$ ) on either side of the inner mitochondrial membrane?
- What is a typical membrane potential for the same membrane?
- The proton-motive force is a combination of what 2 potential energies?
- How much energy is available if a proton ( $H^+$ ) moves across the inner mitochondrial membrane to enter the mitochondria?

5. What is the primary function of the ATP synthase (F1F0 ATPase)?
6. What portion of the ATP synthase is stationary & what portion spins?
7. What powers the spinning?
8. How does the spinning relate to ATP synthesis?
9. In what organelle or compartment is this ATP made? Where is most of the ATP used? How does it get from one place to the other?
10. When ATP & ADP are exchanged for one another at the inner membrane, why does this result in a net loss of the membrane potential?
11.  $\text{Glucose} + 6 \text{O}_2 + \sim 30 \text{ADP} + \sim 30 \text{P}_i \rightarrow 6 \text{CO}_2 + \sim 30 \text{ATP} + \sim 36 \text{H}_2\text{O} \quad \Delta G = \sim -3000 \text{kJ/mol}$
12. The efficiency of this process (assuming the cellular  $\Delta G$  for ATP hydrolysis is  $-50 \text{kJ/mol}$ ) is  $\sim 50\%$ .
13. Proton gradients are a form of free energy currency in all living cells.

## Partial Key

**Regarding the different processes that we have covered (PPP, glycogen metabolism, TCA cycle, ETS, Ox Phos, etc), you should know the following basics:**

- 1) What is the function or functions of the pathway? (Likely energy or biosynthetic precursors; what form [ATP; NADH, etc]?) **Example: PPP: oxidative branch yields 2NADPH & 1 Ribose-5-P for every Glc-6-P; NADPH is used for reductive biosynthesis & Ribose-5-P is used to synthesize nucleotides.**
- 2) What metabolite(s) feeds into the pathway/what metabolite(s) is/are generated? **Example: PPP: Glc-6-P feeds into the oxidative branch; this branch yields NADPH & Ribose-5-P; The nonoxidative branch can convert excess Ribose-5-P and Ribulose-5-P into Frc-6-P and Gly-3-P. The nonoxidative branch can also convert Frc-6-P and Gly-3-P into Ribose-5-P in times when NADPH production is not needed but the production of ribose is needed.**
- 3) Where does the pathway occur (plant/animal/bacteria; which tissues; does it occur in different compartments within a given cell)? **Example: PPP: Although we didn't discuss it, the Pentose Phosphate Pathway exists in most all organisms & cells; PPP operates in the cytosol.**
- 4) When does each process/pathway occur (under what conditions; this is tied into item #1)? **PPP: When NADPH (reductive biosynthesis such as fatty acid synthesis) and/or Ribose-5-P is needed (nucleotide biosynthesis for RNA (transcription) or DNA (mitosis)).**

### Gluconeogenesis

3. Compare the net cost or gain of glycolysis vs gluconeogenesis (ATP, NADH, etc). **Glycolysis (Glucose => 2 pyruvate) generates 2 ATP & 2 NADH (these are the high energy compounds). Gluconeogenesis costs 4 ATP, 2 GTP & 2 NADH.**
4. Gluconeogenesis shares some of the glycolytic enzymes. Which are bypassed & why? **The three steps catalyzed by hexokinase, PFK-1 & pyruvate kinase; all 3 of the large negative  $\Delta G$  steps. The large negative  $\Delta G$  steps can not be overcome due to the thermodynamics. Thus, new reactions are used to bypass these steps.**
6. What does it mean that glycolysis & gluconeogenesis are reciprocally regulated? **Some of the same effectors activate an enzyme in one direction but inhibit the corresponding reaction in the other direction. Example: Frc-2,6-BP & AMP both activate PFK-1 (glycolytic direction) but both inactivate Frc-1,6-bisphosphatase (gluconeogenic direction). This helps to keep competing pathways from operating in the same cell at the same time.**

### Pentose Phosphate Pathway

1. What metabolite feeds into this pathway? **Usually Glucose-6-P which normally comes from glucose but can arise from other sugars. In addition, this pathway can run backwards, so it is possible that Fructose-6-P & Glyceraldehyde-3-P can feed into the pathway through the nonoxidative branch**
2. What two important molecules are formed by the oxidative branch? **Two NADPH & one ribose-5-P per glc-6-P**
3. How is excess ribose recycled? **Through the nonoxidative branch to form Frc-6-P & Gly-3-P**
4. How would this pathway be used to synthesize ribose if NADPH production was not needed? **Use Frc-6-P and Gly-3-P and the nonoxidative branch of the PPP.**

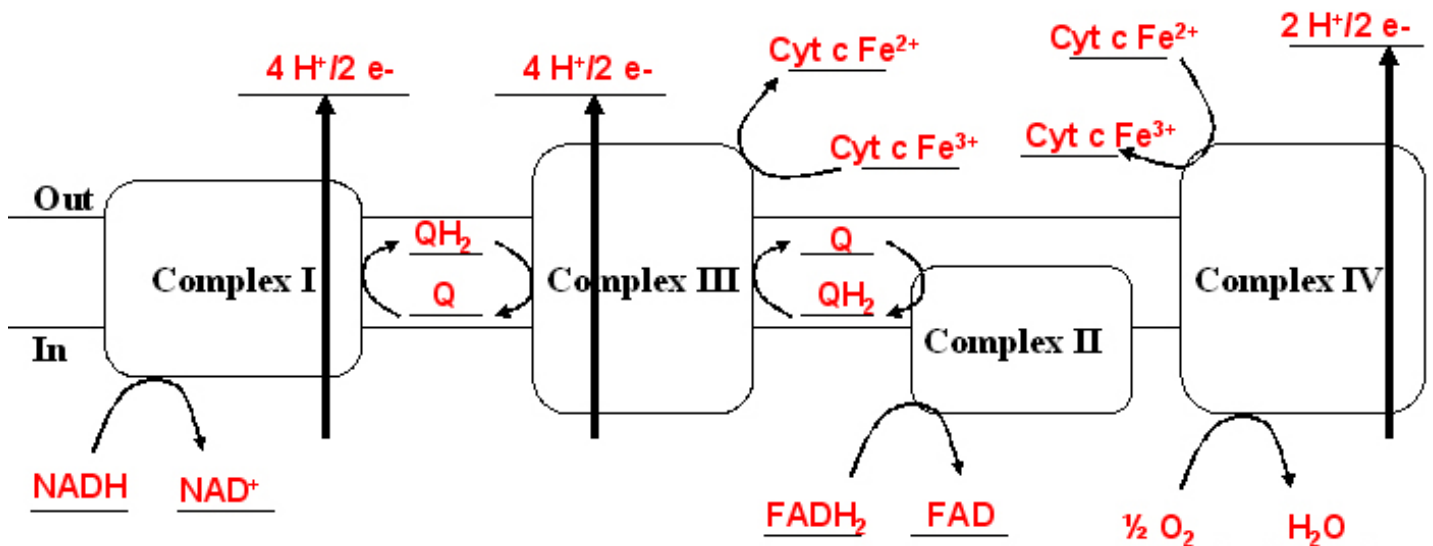
### Glycogen degradation & synthesis

1. Where is glycogen found & what is its primary function? **Cytoplasm of liver & muscle of higher animals; serves as a glucose storage device.**
2. Know the enzymes and the metabolites (phosphorylase & glycogen synthase are the most important of the enzymes).
3. What is an important advantage to extensive branching in glycogen? **Each branch can be degraded simultaneously by different phosphorylase enzymes; provides quick release of a lot of glucose.**

- How many high energy phosphate bonds does it cost to add glucose to a glycogen granule? **If you start with glucose, it takes 3 high energy phosphate bonds; one to make Glc-6-P and two to make UDP-Glucose which produces  $PP_i$  which is quickly lost as  $2 P_i$ . If you start with Glc-6-P, then it only costs two high energy phosphate bonds (the two to make UDP-Glucose).**
- What is glycogenin & how does it function to regulate the size of the glycogen granule? **The glycogen branched polymer of glucose is initially anchored on a tyrosine residue of the glycogenin protein. Glycogen synthase, the primary enzyme responsible for extending the glucose polymers must be bound to glycogenin in order to add more glucose to the nonreducing ends. Once the particle is too big for the synthase to stay bound and still reach the ends of the polymer, further synthesis stops.**

### Electron Transport System (ETS)

- What does it mean for one atom to be more “electronegative” than another? Who coined this term & the concept? **When electrons are passed from one atom to an atom with a higher electronegativity, energy is released. Linus Pauling introduced the concept of electronegativity.**
- What is the difference between respiration and fermentation? **In respiration, the terminal electron acceptor is an inorganic molecule, such as  $O_2$ . In fermentation, the terminal electron acceptor is an organic molecule.**
- How do porins differ from specific membrane transporters? **Porins are simple channels that allow all molecules smaller than 1,000 Da to pass freely across the membrane.**
- What are the electron donors and acceptors for each of the ETS complexes? What is the terminal electron donor for mitochondrial ETS?  **$O_2$  is the terminal electron acceptor.**



- How are FMN and FAD related? **FMN is a single nucleotide while FAD contains one FMN and one AMP as a dinucleotide. Both can take on a single electron to form a stable semiquinone intermediate and both can take on 2 electrons to form  $FADH_2$  or  $FMNH_2$ .**
- How many protons get pumped (& where) for each set of 2 electrons that flow thru each complex? **Complex I & III:  $4H^+ / 2 e^-$ ; Complex II: no protons pumped; Complex IV:  $2H^+ / 2e^-$ .**
- What are the soluble (can freely diffuse) carriers of electrons? In what medium do they diffuse? How many electrons can each carry? **Coenzyme Q (Q) is lipid soluble and can diffuse freely within the membrane. Some Q molecules are essentially permanently bound to a particular protein and are not free to diffuse. The oxidized state is ubiquinone and is normally symbolized by “Q” while the fully reduced state is ubiquinol and is normally symbolized by  $QH_2$ . A semi-quinone state is also possible and is**

usually written as  $\text{QH}^\cdot$ . Thus Q is capable of carrying or transferring 1 or 2 electrons at a time.

Coenzyme Q is responsible for carrying electrons from Complexes I and II to Complex III. Cytochrome c ( $\text{Cyt}_c$ ) is a water-soluble electron carrier. It is a small protein (12.4 kDa) and is capable of carrying only one electron at a time.  $\text{Cyt}_c$  is responsible for carrying electrons from complex III to complex IV in the mitochondria.

11. How much energy would be liberated if NADH gave 2 e<sup>-</sup>'s to O<sub>2</sub> under standard conditions? **220 kJ/mol**
12. When cytochrome c oxidase (complex IV) poisons such as NaN<sub>3</sub>, CN<sup>-</sup> & CO are given to a cell, the ETS shuts down completely. Why is this? **Blocking complex IV prevents  $\text{Cyt}_{c_{\text{red}}}$  from donating e<sup>-</sup> to complex IV. Without  $\text{Cyt}_{c_{\text{ox}}}$  to pick e<sup>-</sup> from Complex III,  $\text{QH}_2$  can't dump off electrons and so on.**

### **Oxidative Phosphorylation**

1. What is the typical difference in pH ( $\Delta\text{pH}$ ) on either side of the inner mitochondrial membrane?  **$\Delta\text{pH} = 1-1.5$  pH units**
3. The proton-motive force is a combination of what 2 potential energies? **Chemical gradient ( $[\text{H}^+]$ ) and charge gradient (electric potential).**
4. How much energy is available if a proton ( $\text{H}^+$ ) moves across the inner mitochondrial membrane to enter the mitochondria?  **$\sim 23$  kJ/mol**
5. What is the primary function of the ATP synthase ( $\text{F}_1\text{F}_0$  ATPase)? **Make ATP from ADP &  $\text{P}_i$ ; uses a proton gradient as fuel.**
6. What portion of the ATP synthase is stationary & what portion spins? **The  $\epsilon$  &  $\gamma$ -subunits are the only subunits that we know for certain spin. Part of the  $\text{F}_0$  transmembrane protein may also spin.**
7. What powers the spinning? **Protons flowing down the gradient (from the intermembrane space back into the mitochondrial matrix).**
8. How does the spinning relate to ATP synthesis? **Briefly, the spinning  $\gamma$ -subunit turns inside of the  $\text{F}_1$  sphere which does not spin. As the  $\gamma$ -subunit turns it changes the 3-D structure of the  $\text{F}_1$  sphere so that ATP can be released from one of the  $\alpha$ - $\beta$  dimers.**
9. In what organelle or compartment is this ATP made? **Mitochondrial matrix.** Where is most of the ATP used? **Cytoplasm** How does it get from one place to the other? **ATP/ADP translocase.**