MITOCHONDRIA LECTURES OVERVIEW

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Mitochondrial Structure

The arrangement of membranes: distinct inner and outer membranes, The location of ATPase, DNA and ribosomes

- *The structure of a mitochondrion; properties of the inner versus outer mitochondrial membranes*
- *Outer mitochondrial membrane-highly porous and is permeable to most ions and small molecules.*
- *Inner mitochondrial membrane-highly impermable, transport of molecules requires proteins in membrane. All of the enzymes and proteins required for oxidative phosphorylation are in the inner membrane.*
- *Mitochondrial matix-contains oxidative enzymes, pyruvate dehydrogenase, enzymes of the TCA cycle, and enzymes of fatty acid oxidation.*

Glycolysis

- Glycolysis converts glucose to two pyruvates and two electron pairs on NADH, while 2 ATP (net) are produced
- Fermentation (in the absence of oxygen) transfers those electrons to pyruvate, producing lactate (or ethanol plus carbon dioxide in yeasts)

- *Glycolysis is the anaerobic catabolism of glucose.*
- *It occurs in virtually all cells. In eukaryotes, it occurs in the cytosol.*
- *NET REACTION: Glucose + 2NAD+ → 2 Pyruvate + 2 ATP+2NADH + 2H+ 2H2O*
- *The free energy stored in 2 molecules of pyruvic acid is somewhat less than that in the original glucose molecule.*
- *Some of this difference is captured in 2 molecules of ATP.*
- *Know glycolysis [acerobic and anaerobic].*
- *Know the names and structural formulas of all the metabolites (intermediates) in glycolysis in the proper order; know the names of all the enzymes and coenzymes*

Pyruvate Oxidation

- mechanisms of pyruvate decarboxylation
- This enzyme oxidatively decarboxylates pyruvate, attaching the 2-carbon remnant, acetate, to coenzyme A (CoA); the oxidation transfers electrons on NAD as NADH, which later are used in electron transport.

Overview of the Tricarboxylic Acid (TCA) Cycle

- Acetyl-CoA, though one turn of the cycle, is oxidized by 4 electron pairs to CO_2, which is the ultimate product of glucose oxidation
- The electrons are stored as NADH (or $FADH_2$) and then passed down the electron transport chain to produce ATP
- From glucose, the sum is: 2 ATP (SLP in glycolysis); 2 GTP (SLP in TCA); 10 NADH (2 in glycolysis, 2 in pyruvate decarboxylation, and 6 in TCA); 2 $FADH_2$ (TCA)
- *Summary of Kreb's cycle: 3 NADH + H; 1 FADH2; 1 GTP (ATP)-substrate level phosphorylation; 2 CO2*

Fatty Acid Oxidation

- Acetyl-CoA produced by fatty acid oxidation is then oxidized by the same TCA cycle as is used for the acetyl-CoA from glycolysis [Acetyl-CoA is also an intermediate formed from the breakdown of a number of amino acids]
- *FA OXIDATION, NET REACTION: FA + ATP + CoA → Acyl-CoA + PPi + AMP*
- *FA are activated in cytoplasm to Acyl CoA; Acyl CoA transport through the mitochondrial membranes via acyl carnitine intermediate [CPT I, CPT II]; Fatty acid-CoA in mitochondria is substrate for β-oxidation.*
- *In β-oxidation, the fatty acid broken down to release acetyl-CoA. The process involves 4 main steps (see next slide): dehydrogenation; hydration; oxidation; thiolysis.*

Redox Reactions

- Redox reactions can best be understood as the sum of two half reactions
  - One reaction generates electrons (oxidation)
  - One reaction consumes electrons (reduction)

- $2H^+ + 2e^- = H_2$ standard (0 mV by definition) reaction

Types of Electron Carrier

- Five types of electron carriers: *Flavins* [are present in flavoproteins and in the coenzymes FAD and FMN]; *Cytochromes* are proteins that contain a heme prosthetic group]; *Copper proteins* [Cu alternate between the +1 and

+2 state]; *Ubiquinone* [rem.: ubisemiquinone, ubiquinol]; *Iron-sulfur proteins* [bind iron atoms]

Electron-transport Components (ETC)

- Electron transport extrudes protons at three sites (by complexes I, III, and IV)
- extruding protons, mitochondria and bacteria build up a charge gradient (delta-psi, usually outside positive) and chemical gradient (delta-pH, usually with lower pH outside) that together influence the thermodynamics of proton translocation
- Proton-motive force (PMF) is the sum of delta-psi and delta-pH
- The PMF drives ATP synthesis

ETC - Electron Entry

- High-energy electrons (NADH) reduce complex I
- Lower-energy electrons ($FADH_2$) are generated by succinate dehydrogenase (the only membrane-bound enzyme of the TCA cycle) and passed directly to ubiquinone, bypassing complex I

ETC structure

- Complex I (NADH dehydrogenase) ; Complex III (cytochrome bc1 complex); Q Cycle; Cytochrome [links complex III with complex IV] complex IV (ctochrome oxidase reduce O_2 to $2H_2O$)

Formation of Proton-Motive Force

- Extruding protons leads to an excess of protons on the outside of the cell relative to the inside (delta-pH = $pH_i - pH_o$)
- delta-p = delta-psi - 2.3·(RT/F)·delta-pH
- delta-p = delta-psi - 59 mV·delta-pH

ATPase (ATP Synthase)

- ATPase in mitochondria is the synthesis, not hydrolysis, of ATP
    - $ADP + P_i = ATP$
    - $nH^+_{out} = nH^+_{in}$
    - $ADP + P_i + nH^+_{out} = ATP + nH^+_{in}$

Bacterial ATPase

- Bacterial ATPases often work in the reversed direction

Structure of F-type ATPase

- The head is called the F_1 portion; it includes:
    - Head (alpha, beta, and delta subunits)
    - And neck (gamma, and epsilon subunits)
- The part of the protein imbedded in the membrane is the F_0 portion
- Integral subunits (a and c) and b subunit
- The F_1 portion ($alpha_3beta_3$gamma-delta-epsilon) contains three catalytic sites for ATP synthesis
- The F_0 portion (ab_2c_{12}) is a proton channel
- The enzyme couples proton flow with ATP synthesis

ATP Synthesis

- three catalytic sites of an ATP synthase is in a different configuration:
    - L (loose) conformation binds ADP and P_i loosely
    - T (tight) conformation binds ADP and P_i or ATP very tightly
    - O (open) conformation binds both very loosely, allowing the release of ATP
- Energy must be supplied to loosen the binding (converting it to the O conformation); this energy must be supplied by the delta-p

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Other Uses of proton motive force (PMF, or delta-p)

- Mitochondria:
    - delta-p drives phosphate uptake
    - delta-psi drives ATP uptake and ADP release from the mitochondrion
    - These uses of delta-p may raise the H^+:ATP ratio in mitochondria

- Bacteria:
  - Active transport
  - Motility

B. THERMINOLOGY

Chemiosmosis - the production of ATP by coupling the transfer of H+ across a membrane, down their concentration to the phosphorylation of ADP.

Mitochondrial Structure – (a) Cristae — the folds of the inner mitochondrial membrane. (b) Matrix — the space in the mitochrondria internal to the cristae.

Oxidation - loss of electrons from a substance involved in a redox reaction.

Oxidative phosphorylation - the production of ATP making use of the proton motive force across the inner mitochondrial membrane.

Proton-motive force - the potential energy contained in the electrochemical gradient produced by the vectoral transport of protons across biological membranes.

Reducing agent — a molecule that transfers electrons to reduce another molecule.

Reduction - the gain of electrons by a substance involved in a redox reaction.

Substrate-level phosphorylation - when ATP is made by transferring a phosphate group from another molecule to ADP

ATP is the most readily accessible chemical energy store for use in metabolic reactions; ATP is a high energy molecule because the bonds between the three phosphate groups are unstable, and release a lot of energy when broken; ATP provides energy to a reaction by transferring a phosphate to an intermediate and increasing its energy level.

Glycolysis is the conversion of glucose to pyruvate (plus 2ATP plus 2NADH) in the cytosol of a cell.

NAD+ accepts a pair of electrons to become NADH. NADH is a reducing agent in many reactions.

The Krebs cycle occurs in the matrix of the mitochrondria;The first step of the Krebs cycle adds two carbons from acetyl CoA to oxaloacetate (4C) to make citrate (6C). As the cycle is completed, oxaloacetate is regenerated, 1 ATP, 3 NADH and 1 FADH2 are produced, and two CO2 released per pyruvate molecule.

The electrons from the NADH and FADH2 produced in glycolysis and the Krebs cycle are passed through the electron transport chain in the inner mitochondrial membrane. The vast majority of the ATP produced indirectly, as a result of the electron transport chain.

The electrons are passed to a series of carriers, each one more electronegative than the next.

O2 is the most electronegative, and is the final electron acceptor in the mitochrondrial ETC.

The electrochemical gradient contains potential energy, which is used by ATP synthase to phosphorylate ADP as protons are allowed to move from the intermembrane space to the matrix.

C. QUESTIONS

1. Anoxic glycolysis results in the formation of how many moles of ATP from ADP and Pi (per mole of glucose)

a) 1
b) 2
c) 6
d) 36
e) 38

2. Glycolysis leads to the production of _______________ and two molecules of ATP. In the absence of oxygen, fermentation leads to the production of _________________. Glycolysis plus the citric acid cycle can convert the carbons of glucose to ___________ , storing the energy as ATP, ________________ and ______________.

a. lactic acid, pyruvate, CO2, NAHD, FADH2
b. pyruvate, lactic acid, CO2, NADH, FADH2
c. CO2, lactic acid, pyruvate, FADH2
d. O2, lactic acid, pyruvate, FADH2
e. glucose, lactic acid, CO2, FADH2

3. At the end of glycolysis, each molecule of glucose has yielded 2 molecules of _________, 2 molecules of __________, and a net of 2 molecules of ___________.

a. FAD, NAD+, ATP
b. CO2, NAD+, ADP
c. Lactic acid, ethanol, CO2
d. pyruvate, NADH, ATP
e. H2O, CO2, ATP

4. In the absence of oxygen, the primary purpose of fermentation is to:

a. produce amino acids for protein synthesis
b. generate a proton gradient for ATP synthesis
c. oxidize glucose to generate reduce electron carriers
d. generate alcohol for beverages
e. regenerate NAD+ from NADH allowing glycolysis to continue

5. The inside part (analogues to the cytosol of a bacterium) of a mitochondrion is called:

a) cytosol
b) stroma
c) intermembrane space
d) matrix
e) periplasm

6. Porins may be found:

a) in the outer membrane of gram-negative bacteria
b) in the outer membrane of chloroplasts
c) in the outer membrane of mitochondria
d) in the inner membrane of mitochondria
e) in a), b) and c) but not d)

7. The terminal electron acceptor during mitochondrial respiration:

a. H2O
b. NAD+
c. FAD
d. ATP
e. O2

8. During TCA cycle, the conversion of succinate to fumarate

a) in oxidation that passed electrons fo FAD to make FADH2
b) is a reduction that passes electrons to FAD
c) is a reduction that passes electrons to NAD+
d) ia a isomerization
e) none of the above

9. The major production of ATP during aerobic metabolism occurs when electrons from ____________ and ________________ are transferred to __________________.

a. FADH2, NADH, H20
b. O2, FADH2, NADH
c. FADH2, O2, NADH
d. NADH, O2, FADH2
e. FADH2, NADH, O2

10. Glyoxylate shunt

a) provide extra electrons to make ATP
b) produce additional intermediates in the TCA cycle
c) prepare precursosrs for FA synthesis
d) prepare glucose for fermentation
e) provide signal to neighboring cells

11. In the presence of an uncoupler, one may expect:

a) rate of electron transport to decrease
b) rate of electron transport to increase
c) ATP synthesis to stop
d) Both a and c
e) Both b and c

12. Which of the following statements about mitochondria is false?

a. They contain an inner and an outer membrane.
b. The region enclosed by the inner membrane is termed the matrix.
c. They contain DNA and ribosomes.
d. They are an important site for energy production in cells.
e. They contain stacked internal thylakoid membranes.

13. If you isolate mitochondria and place them in buffer with a low pH they begin to manufacture ATP. Why?

a. Low pH increases the concentration of base causing mitochondria to pump out H+ to the inter membrane space leading to ATP production.
b. The high external acid concentration causes an increase in H+ in the inter membrane space leading to increased ATP production by ATP synthetase.
c. Low pH increases the acid concentration in the mitochondrial matrix, a condition that normally causes ATP production.
d. Low pH increases the OH- concentration in the matrix resulting in ATP production by ATP synthetase.

14. In eukaryotic cells during aerobic respiration, most ATP synthesis occurs associated with

a) at the cell's unit membrane
b) at the endoplasmic reticulum
c) at the mitochondrial outer membrane
d) in the folds of the mitochondrial inner membrane
e) in the thylakoids of the mitochondrion

15. The electron transport chain is located predominantly in the:

a. Outer membrane of the mitochondria.
b. Intermembrane space of the mitochondria.
c. Inner membrane of the mitochondria
d. Matrix of the mitochondria
e. Cytoplasm of the cell

16. What cellular compartment becomes acidic (high concentration of hydrogen ions) during mitochondrial electron transport?

a. Mitochondrial stroma
b. Cytoplasm.
c. Endoplasmic reticulum.
d. Space between inner and outer mitochondrial membranes
e. Thylakoid membranes

17. ATP synthase can produce ATP using as a direct energy source:

a) energy from the conversion of glucose to pyruvate.
b) energy from the oxidation of pyruvate producing CO_2 and H_20
c) energy from a proton gradient established in mitochondria
d) energy derived from the breakdown of NADH and $FADH_2$
e) energy from the metabolism of amino acids

18. In the F-type ATP-ase, the catalyc site(s) at which ATP binds is:

a) in the Fo portion
b) in the F1 portion
c) in the F3 portion
d) in the F3 portion
e) none of the above

19. Synthesis of one ATP by the F-type ATPase is accomplished by:

a) the complete rotation of the gamma subunit
b) 120 degree of rotation of the gamma subunit
c) 180 degree of rotation of the gamma subunit
d) substrate-level phosporylation
e) none of the above

20. ATP is synthesized in the F-type ATPase when the binding site is in:

a) homeostasis
b) the F configuration
c) the T configuration
d) the O configuration

21. Which of the following activities in mitochondria require proton-motive force?

a) ATP synthesis
b) Phosphate transport
c) ATP transport
d) ADP transport
e) all of the above

Correct Answers: 1b, 2b, 3d, 4e, 5d, 6e, 7e, 8a, 9e, 10b, 11e, 12e, 13b, 14d, 15c, 16d, 17c, 18b, 19b, 20, 21e

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