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CELLULAR ENERGETICS

REVIEW THE CONCEPTS

1. The proton motive force (pmf) is generated by a voltage and chemical (proton) gradient across the inner membrane of mitochondria and the thylakoid membrane of chloroplasts. Like ATP, the pmf is a form of stored energy, and the energy stored in the pmf may be converted to ATP by the action of ATP synthase. Proton diffusion will decrease the proton gradi-ent across the mitochondrial inner membrane, thus decreasing the pmf and therefore the formation of ATP from ADP, leaving the cell with less energy to carry out its energy-dependent processes. At high doses, DNP can be fatal.

2. Similarities between bacteria, mitochondria, and chloroplasts reflect the proposed endosymbiotic origin of mitochondria and chloroplasts. Mitochondrial and bacterial ribosomes resemble each other and differ from eukaryotic cytosolic ribosomes in their RNA and protein compositions, their size, and their sensitivity to antibiotics. Bacterial and mitochondrial ribosomes are sensitive to chloramphenicol but resistant to cycloheximide. Eukaryotic cytosolic ribosomes are sensitive to cycloheximide and resistant to chloramphenicol. Also, comparing the mitochondrial DNA of multiple classes of eukaryotes, both unicellular and multicellular, all can be seen to derive from a common ancestor with a genome similar to contemporary symbiotic bacteria that invade host eukaryotic cells. Mitochondrial DNA in different contemporary eukaryotes can be derived from this common ancestor by deletion of different sets of genes in the mitochondria of different eukaryotes and the transfer of genes essential for mitochondrial function to the nucleus.

3. The unique properties of the mitochondrial inner membrane include the presence of membrane invaginations (termed cristae), a higher than normal protein concentration, and an abundance of the lipid cardiolipin. The cristae increase the surface area of the inner membrane, thereby increasing the total amount of membrane and hence electron-transport chain components: ATP synthase molecules and transporters of reagents and products of the citric acid cycle and oxidative phosphorylation are all increased. The higher concentration of proteins involved in electron transport and ATP synthesis further increases the capacity of mitochondria to synthesize ATP. Finally, cardiolipin enhances the barrier properties of the inner membrane by reducing the membrane's permeability to protons.

4. Glycolysis does not require oxygen, but the citric acid cycle and the electron-transport chain do require oxygen to function. In the case of the citric acid cycle, oxygen is not directly involved in any reaction, but the cycle will come to a halt as NAD^+ and FAD levels drop in the absence of oxygen. For electron transport, oxygen is required as an electron acceptor. In the absence of oxygen, certain eukaryotic organisms (facultative anaerobes) as well as certain cells (mammalian skeletal muscles during prolonged contraction) can produce limited amounts of ATP by glycolysis (a process known as fermentation).

5. In glycolysis, NAD^+ is reduced to NADH. When oxygen is present, the electrons of this electron carrier are eventually donated to the electron-transport chain in oxidative phosphorylation. In the absence of oxygen, oxidative phosphorylation does not occur, and thus NADH is not oxidized, eventually leading to a shortage of NAD^+. Fermentation reactions oxidize NADH, replenish the store of NAD^+, and thus allow glycolysis to continue.

6. Electrons are passed to the electron carrier NADH in the cytoplasm. The NADH must then travel to the inner mitochondrial membrane, where the electrons are utilized by the electron-transport chain. NADH molecules can freely pass through the outer mitochondrial membrane through the channel protein, porin, which allows free passage of small molecules. As the mitochondrial inner membrane is impermeable to NADH, electrons cannot be passed directly from this electron carrier to the electron-transport chain. Instead, electron shuttles, such as the malate-aspartate shuttle, indirectly transfer electrons via intermediates that shuttle back and forth across the inner membrane.

7. Fatty acids are oxidized in the mitochondria and the persoxisome, but unlike the mitochondria, oxidation in the peroxisome does not generate ATP.

8. Prosthetic groups are small nonpeptide organic molecules or metal ions that are tightly associated with a protein or protein complex. Several types of heme, an iron-containing prosthetic group, are associated with the cytochromes. The various cytochromes in the electron-transport chain contain heme prosthetic groups with different axial ligands, and as a result, each cytochrome has a different reduction potential so that electrons can move only in sequential order through the electron carriers.

9. The multiprotein complexes in the electron-transport chain pass electrons between proteins within a single complex and from one complex to the next. As this occurs, the electrons undergo a drop in electrical potential. The released energy is used to transport protons across the inner membrane, generating a proton gradient that is later used by ATP synthase. Respiration supercomplexes would be an efficient way to quickly pass electrons from one complex to the next, increasing the speed at which the process could occur. They have been demonstrated using native PAGE and electron microscopy. CoQ functions within the inner membrane, bringing electrons to complex III. In the process, it picks up protons on the matrix side of the inner membrane, later depositing them into the inner membrane space. This is only possible because CoQ is soluble within the membrane bilayer.

10. The underlying reason for the difference in ATP yield for electrons donated by $FADH_2$ and NADH is that the electrons carried in $FADH_2$ have less potential energy (43.4 kcal/mol) than the electrons carried in NADH (52.6 kcal/mol). Thus, $FADH_2$ transfers electrons to the respiratory chain at a later point than does NADH, resulting in the translocation of fewer protons, a smaller change in pH, and fewer synthesized ATP molecules.

11. ATP synthase is comprised of the F_0 and F_1 components. F_0 is embedded in the mitochondrial inner membrane, and it is through this component that protons travel from one side of the inner membrane to the other, as they move down their electrochemical gradient. F_1 is connected to F_0 and possesses the ATPase catalytic activity. One of its subunits is responsible for nucleotide binding, inter-acting with ADP and P_i molecules, and releasing ATP following its formation. This process is mirrored in vesicle acidification,althoughratherthangenerating ATP, the ATPase portion of the enzyme hydrolyzes ATP, which is coupled to proton pumping against a gradient. This build-up of protons results in a pH decrease in the vesicle lumen. The overall process can be thought of as ATP synthesis in reverse.

12. Aerobic bacteria carry out oxidative phosphorylation by the same processes that occur in mitochondria (and are simpler and easier to work with than mitochondria). Glycolysis and the citric acid cycle take place in the bacterial cell cytosol, while electron-transport components are localized to the bacterial plasma membrane. Since electron transport takes place at the plasma membrane, the pmf is generated across the plasma membrane. In addition to using the pmf to synthesize ATP, aerobic bacteria also use the pmf to power uptake of certain nutrients and cell swimming.

13. In addition to providing energy to power ATP synthesis, the pmf also provides the energy used by several active transport proteins to move substrates into the mitochondria and products out of the mitochondria. The OH− gradient, which results from generation of the pmf by electron transport, is used to move HPO42 − into the matrix, and the voltage gradient contribution of the pmf drives exchange of ADP for ATP.

14. The Q cycle functions to double the number of protons transported per electron pair moving through a specificcomplexoftheelectron-transportchainand thereby maximizes the pmf across a membrane. In mitochondria, the specifi c complex is the $CoQH_2$-cytochrome *c* reductase complex, while in chloroplasts it is the cytochrome *bf* complex, and in purple bacteria it is the cytochrome bc_1 complex. Using mitochondria as an example, the Q cycle is believed to function as follows: $CoQH_2$ arrives at the Q_o site on the intermembrane space side of the $CoQH_2$-cytochrome *c* reductase complex; it delivers two electrons to the complex, and releases two protons into the intermembrane space. Next, one electron is transported directly to cytochrome *c* while the other partially reduces a CoQ molecule bound to the Q_i site on the inner side of the complex, forming a CoQ semiquinone anion. CoQ dissociates from the Q_o site and is replaced by another $CoQH_2$, which delivers two more electrons and releases two protons to the intermembrane space. As before, one electron is transferred to cytochrome *c*, but the other combines with the CoQ semiquinone anion at the Q_i site to produce $CoQH_2$, thus regenerating one $CoQH_2$. In sum, the net result of the Q cycle is that four protons are transported to the intermembrane space for every two electrons moving through the $CoQH_2$-cytochrome *c* reductase complex.

15. False. While ATP is generated in photosynthesis, this energy is used to create sugars, which the cells use in a variety of different processes (including respiration). The endosymbiont hypothesis explains that primitive prokaryotes that were capable of ATP generation or sugar production were engulfed by eukaryotic cells through phagocytosis, producing the double membrane seen in these organelles. The prokaryote and eukaryote developed a symbiotic relationship and eventually the prokaryote lost its independence.

16. $6CO_2 + 6H_2O \longrightarrow 6O_2 + C_6H_{12}O_6$ O_2-generating photosynthesis uses the energy of absorbed light to create, via electron donation to quinone, the powerful oxidant a$^+$ form of the reaction center chlorophyll. This, in turn, acts to remove electrons from H_2O, a poor electron donor. The electrons are then passed along an electron-transport chain, and the stored energy is converted to other forms for subsequent use in ATP synthesis and carbon fixation.The O_2 is not used in subsequent reactions in this pathway and thus is a by-product of the removal of electrons from H_2O.

17. Photosynthesis consists of four stages. During stage 1, which occurs in the thylakoid membrane, light is absorbed by the reaction center chlorophyll, a charge separation is generated, and electrons are removed from water, forming oxygen. During stage 2, electrons are transported via carriers in the thylakoid membrane to the ultimate electron donor, $NADP^+$, reducing it to NADPH, and protons are pumped from the stroma into the thylakoid lumen, producing a proton gradient across the thylakoid membrane. During stage 3, protons move down their electrochemical gradient across the thylakoid membrane through F_0F_1 complexes and power ATP synthesis. Finally, during stage 4, the ATP and NADPH generated in the earlier stages are used to drive CO2 fixation and carbohydrate synthe-sis. CO2 fixation occurs in the stroma and carbohydrate (sucrose) synthesis occurs in the cytosol.

18. Chlorophyll *a* is present in both reaction centers and antenna. Additionally, antennas contain either chlorophyll *b* (vascular plants) or carotenoids (plants and photosynthetic bacteria). Antennas capture light energy and transmit it to the reaction center, where the primary reactions of photosynthesis occur. The primary evidence that these pigments are involved in photosynthesis is that the absorption spectrum of these pigments is similar to the action spectra of photosynthesis.

19. Photosynthesis in green and purple bacteria does not generate oxygen because these bacteria have only one photosystem, which cannot produce oxygen. These organisms still utilize photosynthesis to produce ATP by utilizing cyclic electron flow to produce a pmf (but no oxygen or reduced coenzymes), which can be utilized by F_0F_1 complexes. Alternatively, this photosystem can exhibit linear, noncyclic electron flow, which will generate both a pmf and NADH. For linear electron flow, hydrogen gas (H_2) or hydrogen sulfide (H_2S) rather than H_2O donates electrons, so no oxygen is formed.

20. PSI is driven by light of 700 nm or less and its primary function is to transfer electrons to the final electron acceptor, $NADP^+$. PSII is driven by light of 680 nm or less, and its primary function is to split water to yield electrons, as well as protons and oxygen. During linear electron flow, electrons move as follows: PSII (water split to produce electrons) $\longrightarrow$ plastoquinone (Q) $\longrightarrow$ cytochrome *bf* complex $\longrightarrow$ Plastocyanin $\longrightarrow$ PSI $\longrightarrow$ $NADP^+$. The energy stored as NADPH is used to fix CO_2 and ultimately synthesize carbohydrates.

21. The Calvin cycle reactions are inactivated in the dark to conserve ATP for the synthesis of other cell molecules. The mechanism of inactivation depends on the enzyme; examples include pH-dependent and Mg^{2+}-dependent enzyme regulation, as well as reversible reduction-oxidation of disulfide bonds within certain Calvin cycle enzymes.

22. Rubisco (ribulose 1,5-bisphosphate carboxylase) is a large enzyme present in the stromal space of the chloroplast. Rubisco is the enzyme responsible for adding (fixing) inorganic carbon in the form of CO_2 to the five-carbon sugar ribulose 1,5 bisphosphate, which is rapidly cleaved into two molecules of 3-phosphoglycerate that can be converted into starch and sugars.