Biology Final Review

Phylogeny, Population Genetics and Population Growth

Refer to Study Guides

Macromolecular Self-Assembly

Lipid Bilayers

- Biological Compartments
    - Intermolecular regions
    - A membrane limited space within which molecules are free to diffuse
    - selectively permeable to membrane
- Amphipathic molecules
    - Hydrophobic + Hydrophilic regions
    - Component of biological membranes
    - Distinct, separate, regions with different solubility properties
- Plasma membrane
    - Lipids + Proteins
    - Surrounds a cell
    - Separates the internal cell and the external environment
    - Regulates passage of molecules and ions into/out of the cell
- Features of Membrane Lipids (Phospholipids)
    - Polar + non-polar region (Amphipathic)
        - Polar head groups: interacts strongly with water
        - Acyl tails: do not interact with water
        - Creates a water-free zone in the center of the bilayer

        - Hydrocarbon chain
        - Bonded with oxygen
- Phospholipids form either micelles or bilayers when suspended in water
- Molecular Self-Assembly
    - Molecules/Complexes of molecules form a particular and characteristic 3D conformation or structure
    - Result of
        - Interaction with surrounding water molecules
        - Intermolecular interaction
        - Intramolecular interaction
        - Hydrophobic + Hydrophilic interactions between and within molecules
    - Extends from single molecules to complexes to organelles
- Hydrophobic association
    - Two stages
        - Entropy driven clumping of hydrophobic groups
        - Formation of L-vdW interactions between adjacent hydrophobic groups
    - Hydrogen bonds in adjacent water molecules are reoriented tangentially to the surface
    - Inner layers of water are highly constrained
    - Reduced entropy: more structured than "free" water; reduced hydrogen bonding potential
    - Hydrophobic groups "squeeze" the water out of the region, increasing entropy and hydrogen bonds
    - $\Delta G = \Delta H - T(\Delta S_{water} + \Delta S_{phospholipids})$
- Hydrophilic interactions
    - To water; to polar molecular groups
    - Ionic interaction

Properties of the Membrane

- Which forms more readily?
    - Micelles - Maximizes the area of hydrophilic interactions (large free energy change associated)
        - Intermediate end for detergents (detergent destroys bilayers and breaks into micelles)
    - Bilayer (energy barrier to overcome from hydrophobic tail reentering water)
        - Driving force to form bilayers
            - Entropy
            - L-vdW interactions
            - Brownian motion
- General properties of membrane phospholipid bilayer
    - Selective permeability
        - Small, non-polar molecules move across quickly
        - Charged, large molecules cross slowly
        - Permeability vs. Hydrophobicity
            - High hydrophobicity, high permeability
            - High hydrophilicity, low permeability
        - Diffusion rates
            - Small hydrophobic molecules > Small uncharged polar molecules > Larger uncharged polar molecules > Ions
        - Osmosis
            - Solutions of different concentrations are separated by a membrane that is permeable to water but not to the solutes
            - Water moves to solution with higher solute concentration
            - Hypertonic: $[in] < [out]$; Isotonic: $[in] = [out]$; Hypotonic: $[in] > [out]$
            - Prevention of cell explosion

- Maintain isotonic environment
- Build a cell wall
- "Secrete" water by secreting ions
- Better membrane less permeable to water

- Self-healing/sealing
    - Small hole → Membrane will close it
    - Torn into fragments → Vesicles
- 2D fluidity
    - Weak bonds from L-vdW interactions and hydrogen bonding
    - Individual molecules can move within the bilayer
    - Free to diffuse within the plane of the membrane
    - Hard for phospholipid leaflets to flip
        - Hydrophilic groups breaking hydrogen bonds - high energy barrier
        - Hydrophobic groups prevent charged molecules from passing through
    - Fluorescence recovery after photobleaching (FRAP)
    - Acclimation to temperature change
        - Decreasing temperature, decreased fluidity; Increasing temperature, increased fluidity
    - Fatty acid composition
        - Saturated acid: Lack double bonds, favours tight packing in membrane bilayers
        - Unsaturated acid: has double bonds, reducing the tightness of packing in membrane bilayers
        - Long hydrophobic chain: Increases L-vdW interactions; short hydrophobic chain: decreases L-vdW interactions
- Protein container
- Separate compartments

Proteins and Amino Acids

- Amino Acid Structure
    - Amino group + Carboxyl group + α-Carbon + Side chain
    - Hydrophilic and hydrophobic amino acids
- Protein Function
    - Antibodies and complement proteins: Defense - destruction of foreign bacteria and viruses
    - Contractile and motor proteins - Movement
    - Enzymes - Catalyze chemical reactions
    - Peptide hormones - Act as signals to coordinate activities of cells
    - Receptor proteins - Receive signals from outside the cell and initiate response
    - Structural proteins - Provide support for cells and tissues
    - Transport proteins - Move ions or molecule across membranes
- Proteins = Polymers of AA
    - Polypeptide bonds: consistent internal polarity (N to C)
- Structure of Proteins
    - Primary Structure: Amino acid sequence (Start with a N-terminus, end with a C-terminus)
    - Secondary Structure: Formation of hydrogen bonds between atoms of polypeptide backbone with other AA (α-helix and β-sheet)
        - Proteins typically contain regions that form both structures
        - α-helix: Each carbonyl group forms a hydrogen bond with an amide group 4 residues away
        - β-sheet: Adjacent strands can run in parallel or antiparallel
        - What determines which shape to form?
            - Different side chains
            - Side-chain interactions
            - Side-chain - water interactions
    - Tertiary Structure: 3D shape of a protein
        - Stabilized by interactions of side chains with other side chains or atoms of the polypeptide backbone

                - Hydrophobic/hydrophilic/ionic association
                - Disulfide bonding (formed in endoplasmic reticulum by enzyme)
            - X-ray diffraction
            - In globular proteins
                - Hydrophilic groups located on the surface
                - Hydrophobic groups located inside
        - Quaternary Structure: Interactions of protein subunits
            - More than one subunit
            - Stabilized by all of the forces that stabilize tertiary structure

- Self-Assembly of a Protein
    - Random thermal movements of protein, of water molecules, and by the interaction of different chemical groups along with backbone of protein, with water to achieve their normal minimum energy configuration
    - Configuration is through trial & error
    - Chaperone: chaperon fully synthesized proteins, ensure proper native folding
- Amino acid substitution in proteins (mutations)
    - Hydrophobicity/hydrophilicity of amino acids and the change in conformation of proteins
    - Assume substitution of an amino acid of a particular type by a different amino acid of the same class will have relatively little effect on the structure of the molecule (Hydrophobic replaces hydrophobic)
- Protein denaturation
    - Denaturation agents: heat, molecules that interfere with hydrogen bonds (urea)
    - Often irreversible
    - Can renature if careful
    - Urea is a strong hydrogen bond competitor, so it disrupts hydrogen bond forming intramolecularly
    - Renaturation

- Place a dialysis bag (with urea) in distilled water for hydrogen bonds to reform

Membrane Proteins

- Roles
    - Transporter of molecules and ions
    - Enzymes (electron carriers, ATP synthase)
    - Receptor and Signal transducers
    - Anchors for other proteins (cytoskeleton)
- Types
    - Integral membrane proteins: contain one or more membrane crossing domains (glycophorin)
        - Embedded in the membrane
        - Each membrane crossing domain contains regions of hydrophobic amino acid residues that traverses the hydrophobic core of the membrane
        - Sequences of amino acids with primarily hydrophobic side chains
        - Hydrophobic side chains in hydrophobic regions + Hydrophilic side chains in hydrophilic regions
        - Charged on the outer surface of the bilayer
    - Peripheral membrane proteins: attached to the outside of the membrane by interaction with integral membrane proteins
- Hydropathy plots
    - Shows free energy change associated with immersion sequential groups of 3 amino acids in water (start at the N end)
        - Positive free energy change (hydrophobic)
        - Negative free energy change (hydrophilic)

Processing and Targeting of Proteins

- Proteins must be targeted to their proper destinations
    - Cytosolic proteins
        - Folded, covalent modification, cleavage
    - Proteins of the endomembrane system (ER/Golgi system)
        - Folded, disulfide bond formation, Glycosylation, cleavage
- Targeting (Signal Sequence + Receptor)
    - Signals encoded within proteins
    - Signals direct the protein to a specific organelle
    - Signals must be present for protein to leave the cytosol compartment
    - General Principle
        - Regulatory signals required for the processing or function of macromolecules are generally encoded within the macromolecules themselves
    - Options
        - No targeting signal - remain in cytosol
        - N-terminal signal - targeting to ER or organelles other than nucleus
            - Signal sequence cleaved off
        - Internal targeting signal - targeting to nucleus
            - Signal sequence not cleaved off
    - Requirements
        - Specific signal sequence in the transported protein
        - Specific protein receptor that recognizes that signal sequence
- Targeting to endomembrane system
    - Co-translational insertion into endoplasmic reticulum
    - Proteins then proceed by vesicle transport to Golgi Apparatus and then on to the plasma membrane or other destination
- Rough ER
    - Ribosomes on ER translate protein destined for the ER or membrane proteins

        - Translation of all proteins, regardless of destination, begins in the cytosol
- Co-translational Insertion
        - Signal Recognition Particle binds to a signal sequence and halts translation
        - SRP binds to the SRP receptor on the ER membrane
        - SRP receptor brings the ribosome to a transmembrane channel, SRP dissociates; protein synthesis resumes, and the growing polypeptide chain is threaded through the channel
        - Protein ends up in the lumen of ER for further action
        - Once enter ER, no returning to cytosol
- Targeting of proteins to membranes
        - Membrane proteins are co-translationally inserted
        - Arrangement of the protein in the membrane is controlled by amino acid sequences in the protein (Start and Stop transfer signals)
        - Stop transfer sequence
            - Stops transfer of protein
            - Contain a membrane crossing domain
            - May have same sequence as internal start sequence
            - **IMPORTANT**: order in which these regions are encountered

Regulation of Genetic Expressions

Lac Operon - Regulation of transcription in bacteria

- Trans-acting regulatory factor (external factor that binds to a regulatory DNA sequence) + cis-acting regulatory sequence = gene (in)activity
- Regulatory Region
        - Activator binding site - When protein binds transcription occurs much more frequently
        - Promoter - RNA polymerase binding site
        - Repressor site - When protein binds transcription is blocked

        - Positive regulation
            - Transcription only occurs when an activator protein binds to a site near the promoter and then RNA polymerase can bind to the promoter
        - Negative regulation
            - Transcription can occur when only RNA polymerase binds to the promoter
            - If repressor protein binds to the DNA and inhibits RNA polymerase binding to the promoter, no transcription occurs
- Compared to eukaryotes, prokaryotes
    - DNA is not packaged in chromosomes
    - mRNA is not processed
    - No nuclear membrane that separates transcription and translation
- Lactose catabolism in *E. coli*
    - Can use lactose as sole energy and carbon source
    - β-galactosidase required to break down lactose
    - Cells grown on glucose cannot break down lactose, have very little β-galactosidase
    - In the absence of glucose, β-galactosidase enzyme activity **induced** by lactose (control of expression by environmental factor)
- Constitutive - always being active/produced; Facultative - active/produced only under specific conditions
- Lac Operon
    - *lacI*: structural gene for the repressor protein
    - CRP-cAMP binding site
    - Promoter
    - *lacO*: the binding site for the *lac* repressor protein
        - In the absence of lactose, repressor protein translated by *lacI* binds to *lacO* and prevents transcription
        - In the presence of lactose, repressor protein is induced by lactose (allosteric regulation), unable to bind to *lacO*, promoter recruits RNA polymerase complex and transcription of **polycistronic** mRNA occurs

        - Lac repressor binding sites at each side of *lacO* sequence
        - Each site has two binding sequences in opposite orientation
        - Repressor tetramer complex has two dimers, one of which binds to each binding site
    - *lacZ*: coding sequence for β-galactosidase
    - *lacY*: coding sequence for galactoside permease
- General principle
    - Binding of a ligand to a protein changes the conformation of the protein
    - To get *lac* activity
        - Lactose present to remove *lac* repressor
        - Glucose absent or low to allow activation of CRP activator of Lac Operon by cAMP
- Effect of glucose on Lac Operon
    - Cells growing in presence of glucose use glucose
    - If switched to lactose, will use lactose $\rightarrow$ induction of β-galactosidase activity
    - When both are present, glucose is preferred
    - $Lactose \xrightarrow{\beta-galactosidase} Galactose + Glucose$
    - Glucose inhibits β-galactosidase synthesis
    - CRP protein (binds to CRP binding site - an activator) activated by cAMP
        - $ATP \xrightarrow{Adenyl\ cyclase} cAMP + pyrophosphate$
        - High glucose, low adenyl cyclase activity, low cAMP production, no activation of Lac Operon transcription via activated CRP
- Summary
    - Multiple switches for genetic regulating expression (positive and negative control)
    - Transcription activity is regulated by DNA binding proteins
    - Signals control gene expression by modulating transcription factor activity

        - Transcription factor activity is regulated by ligand binding
        - Affinity of enzymes for substrates can be compared to affinity of transcription factors for DNA binding sites
        - Mutations can define genes and gene regulatory regions

Eukaryotic Regulatory Gene Expression

- Transcription Factor Binding
    - Gel shift analysis to determine which DNA fragments bind to a particular protein
        - Binding of protein reduces DNA mobility in an electrophoretic gel
    - Footprint analysis to determine the location of protein binding sites
        - Tight binding of a protein to DNA prevents nuclease (DNase I) from binding to the DNA and from cutting the molecule
    - Gel shift analysis for target competition experiments to assess affinity of TF / DNA binding
        - Binding of protein to DNA produces a "gel shift" - a change in the position of the DNA band that can be used to estimate the relative amount of bound DNA
    - Quantitative assessment of TF binding
        - $Ligand + Protein \leftrightarrow PLcomplex$
        - $K_a = \frac{[PL]}{[P][L]}$
        - Plot fraction of protein with bound ligand vs. ligand/DNA concentration
            - K_d concentration of substrate at which 50 of the protein molecules are complexed with ligand
            - $Fraction\ bound\ (\%saturation) = \frac{[ligand]}{K_d + [ligand]}$
            - Lowest affinity, largest K_d

- Eukaryotes
    - Similar model to prokaryotic regulation
    - Regulatory Regions

        - Promoter: Has sequence common to most genes
            - Most common sequence: TATA box
            - All eukaryotic promoters are bound by the TATA-binding protein
        - Promoter proximal element: Has sequence that is unique to this gene
            - Coordinately regulated genes often have similar sequenced PPE
            - Binding sites for regulatory transcription factors
            - Located just upstream of the promoter
- Transcriptional Regulation
        - Specific TF bind to
            - PPE
            - Enhancer regions that are at a distance from the promoter
        - All eukaryotes have enhancers
            - May locate upstream, downstream, or within the transcription unit; in introns, in untranscribed 5' or 3' sequences
        - Enhancer - Increasing rate of transcription
            - Regulatory proteins bind to these are activators/co-activators
        - Silencer - Decreasing rate of transcription
            - Regulatory proteins bind to these are repressors/co-repressors
        - Enhancers work through DNA bending to bring the associated TFs into contact with the proteins of the basal transcription complex
- Different cell types express different genes because they have different histone modifications and contain different regulatory proteins
        - Certain proteins produced only in certain types of cells
- Coordinated control of gene expression
        - A single TF may interact with many target genes

- Action can be positive or negative
- TF act in Boolean logic to control transcription
- A single gene can be acted upon by many TFs
- Britten and Davidson model

Making a Fly

- Preformation
    - Important for establishing axial coordinate system of the egg
    - Maternal mRNAs are placed at the poles of the oocyte by the cells of the mother's ovary, defining the anterior-posterior axis of the embryo
    - *bicoid* is localized at the anterior pole of the oocyte
- Events of early development in the fly
    - Cleavage
        - Egg is one big cell
        - After fertilization, nuclei divide without forming cell membranes
        - One cell, many nuclei
        - 13 rounds of DNA replication followed by nuclear division happen in one single giant cell
        - Nuclei arranged around the periphery of the cell $\rightarrow$ Syncytial blastoderm stage
    - Cellularization
        - Cycle 14 - synchronous formation of cells all around the periphery of the egg $\rightarrow$ Cellular blastoderm stage
        - Followed by gastrulation $\rightarrow$ internal cell mass
    - *bicoid* mRNAs are translated and give rise to a gradient of *bcd* protein (diffuses away from the source and establishes a gradient throughout the embryo)
- Loss of *bcd* protein: no anterior development; Loss of *nos* protein: no posterior development
- Morphogens

        - A substance that supplies positional information that governs the formation of a characteristic structure in a developing embryo
            - *bcd* protein: anterior morphogen; *nos* protein: posterior morphogen
            - *bcd* protein gradient is formed after fertilization, mRNA is still localized
            - mRNA is not free to diffuse, protein is free to diffuse
- Modeling the *bcd* gradient
    - SDD model: synthesis, diffusion, destruction
    - Protein produced by mRNA at the anterior continually
    - Diffuses outward
    - Protein has a characteristic life time in which a fixed fraction of the protein is lost
- Big Ideas
    - 1
        - Gene expression in development is hierarchical
        - Genes that are active early in development produce proteins that regulate activities of genes that act later
        - Maternal gradients → Gap genes → Pair-rule genes → Segment polarity genes → Homeotic genes …
    - 2
        - Different genes are expressed in different regions of the embryo
        - Gradients are established in different regions
        - These proteins are morphogens
        - Different target genes are activated or repressed at **different** concentrations of TF protein
        - Translation of *hb* inhibited by *nos*; Translation of *cd* is inhibited by *bcd*; Transcription of *hb* is activated by *bcd*…
- Gap genes
    - Different gap genes active in different regions based on the concentrations of *bcd*, *hb* and other TFs
    - *hb* boundary sharpening

- Posterior boundary of the *hb* expression domain becomes progressively more sharply defined
- Consequence of interaction of *bcd* and *hb* proteins with the *hb* promoter
- Positive feedback: *hb* protein binds to *hb* promoter to activate *hb* expression

- Organization of *hb* promoter region
  - Multiple *hb* and *bcd* binding sites
  - Binding of multiple activating TFs (increased frequency of transcription)
  - Cooperative binding - binding of a TF to a strong site increases probability of binding of the same TF to an adjacent weak site
- Require high concentration (low affinity), require low concentration (high affinity) for activation
- Kruppel promoter organization - presence of multiple *hb* and *bcd* binding sites
  - Cooperative binding from *bcd* and *hb*
  - *bcd* is an activator
  - *hb* is a repressor
- Gap gene expression divides the embryo into zones
  - Gap gene products are TFs that are also morphogens (shorter distance)
  - Regulate expression of segmentation genes
  - Gap genes inhibit each other
- Competitive binding
  - Same binding site is not large enough for more than one TF
  - Either A or B
    - Dependent on concentration and affinity

- Pair rule genes (regulated by gap gene products)
  - Even-skipped - Expressed in odd numbered segments
    - Expressed in 7 bands each corresponding to an odd numbered segment
    - Each stripe controlled by a particular enhancer element

        - Two sets of enhancers (embryonic enhancers and other enhancers that act later after cellularization)
        - Stripe 2 (*eve-2*)
            - Inhibited by *Gt* and *Kr* proteins
            - Activated by *bcd* and *hb* proteins
            - Competitive binding of TFs (needs higher affinity in farther gradients)
    - Odd-skipped - Expressed in even numbered segments
- Homeotic Genes (Segment polarity genes set up cues to example accurate expression of homeotic genes)
    - Specify and identity of a body part or segment during embryonic development
    - *Antennapedia* gene (antenna development)
    - *Bithorax* gene (wing development)
    - *Hox* gene
        - AA sequence of the DNA binding domains (homeodomains) of *Hox* gene products are very similar from one organism to the next
        - Fly: segments
        - Mice: spinal cords
        - Plants: buds, leaves, flowers
- Summary
    - Initial positional cues come from TF gradients
    - With passage of time domains in the embryo become defined with increasing precision
    - Results in a pattern of segments that differentiate in response to their position in the embryo based on the local pattern of gene activity
    - This pattern leads to activation of appropriate homeotic genes that form the definitive positional information system for animals.
    - Position-specific TFs

Biological Energy Transformation

Enzymes and Transport Proteins

- Enzyme
    - Accelerates the reaction by reducing activation energy
    - Change in free energy is the same
    - Fraction of molecules reacting increases if activation energy decreases, increasing the rate of reaction
    - Binds specifically to reactants
    - Catalyzes the reaction in both directions
    - Does not affect equilibrium position
    - How does it work
        - Has high affinity for reactants, binds tightly to them
        - Increase local concentration of reactants by bringing them to close proximity
        - Increases probability of bond breakage and reaction
        - Products have lower affinity for enzyme
    - If protein is unfolded, no enzymatic activity; only has activity when properly folded
- Enzyme-Substrate Complex
    - Non-enzymatic: $S \leftrightarrow P$
    - Enzymatic: $E + S \leftrightarrow ES \leftrightarrow EP \leftrightarrow E + P$
- Enzyme Action
    - Initiation: Reactants bind to the active site in a specific orientation
    - Transition state facilitation: Interactions between enzyme and substrate lower the $E_{activation}$ required
    - Termination: Products have lower affinity are released, enzyme unchanged
- Energy change during enzyme reactions
    - Sub reactions
        - Formation of E-S complex

        - Main reaction - Catalysis (Rate-Limiting Step)
        - Release of products
- Regulation of enzyme function
    - Co-factors: Ions, co-enzymes
    - Inhibition
        - Competitive (Inhibitor and substrate compete for the same site)
        - Non-competitive inhibition or allosteric regulation
    - Consider Threonine dehydratase
        - Activated allosterically by threonine (reactant of the pathway)
        - Inhibited allosterically by isoleucine (product of the pathway)
- Measuring binding of ligands
    - K_d: half-saturation concentration
    - Binding affinity of the protein for the ligand is $\frac{1}{K_d}$, affinity high, K_d is low
    - Michaelis-Menten Kinetics
- Catalyzing endergonic reactions (ΔG is positive)
    - Main energy input: ATP (bonds linking the 3 phosphates has very high potential energy)
    - Hydrolysis of ATP $\rightarrow$ energy to drive endergonic reactions
- Transport Protein
    - Simple diffusion
        - Molecules move down their concentration gradient
    - Facilitated diffusion (Passive transport)
        - Solutes interact with a transmembrane protein
        - Diffuses down its concentration gradient
        - No local application of energy is required
        - Process
            - Protein binds to substrate with moderate to high affinity

        - Configuration of protein is metastable, stochastic movement of protein exposes binding site to one side of the membrane or the other
        - Finite probability of the ligand being "spontaneously" released from the binding site
        - Ligand will flow through the protein, down the ligand concentration gradient
    - Carrier mediated facilitated diffusion - rate of transport
        - Substrate concentration
        - Avidity of substrate binding
        - Max throughput of the carrier
- Active transport
    - Change the conformation of the protein and move the solute across the membrane against its concentration gradient to a higher free energy state (hydrolysis of ATP)
    - Moves Na^+ out of the cell and K^+ into the cell, building up an electrochemical gradient across the membrane (ATPase pump)
    - Cellular electrochemical gradient
        - Transmembrane voltage is $50mV$
        - Hydrophobic core of membrane is $3nm$ thick
        - Voltage gradient is $167kV/cm$
        - Concentration difference in ions
        - Electrical charge difference
        - $\Delta G = RTln(\frac{[in]}{[out]}) + ZF\Delta P$
    - Indirect active transport
        - Transport protein uses the ion electrochemical gradient from above active transport to move a different molecule across the membrane against its gradient
        - $|\Delta G_{ion}| > |\Delta G_{molecule}|$
        - Symport - driving ion and transported molecule in the same direction

        - Antiport - ion and molecule in opposite direction
    - Na^+ glucose transporter
        - Symport system - Drives the uptake of glucose against its concentration gradient by moving down its electrochemical gradient
        - Apical epithelium of small intestine

Glycolysis and Fermentation

- Glycolysis (Sugar $\rightarrow$ Pyruvate)
    - Energy Input and Payout phases
        - Energy input: Glucose $\xrightarrow{2ATP}$ Fructose 1,6 bisphosphate + 2ADP
        - Cleavage: F 1,6 B $\rightarrow$ 2 Glyceraldehyde 3 phosphate
        - Energy Payout: G3P $\rightarrow$ pyruvate + 2ATP + NADH
        - Net yield: 2 ATP, 2 NADH, 2 Pyruvate (per glucose)
    - Limited energy yield
    - Subject to Feedback Regulation
    - Reduction of NAD+
    - Substrate level phosphorylation of ADP
- Regulation of glycolysis
    - High ATP level inhibits phosphofructokinase (feedback inhibition)
    - ATP is both a substrate and a regulator
    - Allosteric regulation
        - Activation: ADP, AMP
        - Inhibition: ATP
    - 2 ATP binding sites on ppfk: substrate site and regulatory site

Fermentation (electron acceptor not present)

- Electrons are not accepted
    - Oxidize NADH by sticking the electrons back to them
- Two variants of the process
    - Lactate production
    - Ethanol production
- No net oxidation
- Energy released is a consequence of molecular rearrangement producing changes in internal energy
- Limited energy yield

Respiration

- Export electrons out of the cell to electron acceptors
- Use high energy electrons that are in the process of being exported to do work in the form of ADP phosphorylation; use potential energy of electrons to drive phosphorylation
- NADH also carries a lot of free energy
- Synthesis of Acetyl Co-A from pyruvate
    - Pyruvate enters the mitochondrial matrix via the transporter in the inner membrane
    - Pyruvate dehydrogenase oxidizes pyruvate, releasing CO_2, reducing NAD+ and attaching the remaining acetyl group to CoA (an acetyl carrier)
    - Acetyl-CoA is the activated from of acetate
    - Regulation
        - Negative: High ATP, Acetyl-CoA or NADH (reduce pyruvate dehydrogenase activity by phosphorylation)
        - Positive: High NAD+, CoA or ADP (increase pyruvate dehydrogenase activity by dephosphorylation)
        - PDK (PD kinase) phosphorylates PD, inhibited by pyruvate
        - PDP (PD phosphatase) dephosphorylates PD, stimulated by PEP and AMP, inhibited by ATP, NADH and Acetyl Co-A

Citric Acid Cycle

- Occurs in mitochondrial matrix
- Cyclical reactions
- Acetate transferred from Acetyl Co-A to citric acid to start
- Eight carboxylic acids become progressively more oxidized
- Oxidation of these substrates drives reduction of electron carriers NAD and FAD
- Acetate oxidized to 2 CO_2 molecules
- Acetate acceptor is regenerated
- 4 reactions in which reduced electron carriers are produced store released energy in electron carriers
- Pyruvate completely oxidized to CO_2
- Regulation
    - $Oxaloacetate \xrightarrow{regulated\ by\ ATP} Citrate$, feedback inhibition
    - Multiple sites of feedback inhibition from ATP and NADH
    - High ATP + NADH = inhibition; High ADP + NAD = activation
- Net yield (substrate level phosphorylation)
    - 2 ATP
- Source of initial carbon skeletons for the synthesis of many biologically important molecules via many different metabolic pathways.

Chemiosmotic Production of ATP (Oxidative Level Phosphorylation)

- Reduced electron carriers
    - Drive the formation of proton gradient
        - Phosphorylation of ADP to ATP
- Net Yield
    - 28 ATP
- Energy in electron to drive ADP phosphorylation
    - Transfer electrons to final electron acceptor
    - Make electrons do as much work as possible to store energy by building up an electrochemical gradient

        - Use the gradient to drive phosphorylation of ADP
        - Active transport run in reverse
- Occurs in mitochondria, chloroplasts and in bacteria
        - Ions moving down a gradient generating ATP rather than ATP being used to drive ions against a gradient
- Early cells
        - Mechanisms to pump protons out of the cell, powered by ATP and electron transport
        - Electron-transport-powered pumps became efficient enough to run the ATP-driven pump in reverse
- Electrochemical gradient
        - During transfer, at each transfer, there is a decrease in free energy that drives transport of H^+ across the membrane
        - Electrons are eventually transferred to a terminal electron acceptor
- Electron transport complexes
        - Four multi-protein electron transport complex that comprise the respiratory electron transport chain
        - They contain both enzymes (NADH dehydrogenase and electron transporter proteins and proton pump proteins)
        - Complex I and II strips electrons from NADH and $FADH_2$
        - Cytochrome C moves electron to Complex IV where oxygen is reduced to form water
- Proton electrochemical gradient - huge potential energy level (160mV), membrane potential ~533kV/cm
- ATP synthase complex - uses potential energy stored in the electrochemical gradient to drive the phosphorylation of ADP to make ATP
        - Reversible enzyme system
        - F_0 unit (drives rotation of the stalk) and F_1 unit (rotation of stalk changes the conformation in the knob portion)
        - 3 consecutive conformations: *open, loose and tight*
        - *Tight* conformation "crushes" ADP and Pi together to form ATP
        - 3 rotations form one ATP

Evolution of Respiration

Light Reactions of Photosynthesis

Dark Reactions of Photosynthesis

Mitochondria and Chloroplasts as Bacterial Cells

Chemoautotrophs

Refer to notes for all above five topics