Biology Lecture 1 - Chlamydomonas

Roles of light as used by life

- Light is used as a source of energy and as a source of information about the environment

Characteristics of Chlamydomonas that make it a useful model system

- Genome sequence is useful to study because it has attributes of both animal and plant cells.
- Some human diseases are caused by mutations that make the cilia in humans develop poorly -- Chlamy is helpful for looking at flagella and cilia structure and function because it is a eukaryote and the flagella is identical in chlamy as it is in humans.
- Good for looking at light energy and information because it has an **eyespot** that detects light and then orients chlamy in relation to the light (can either swim towards light or away from it)
- Has a good genetic system that has mutants in different pathways, mutants enable you to elucidate the pathway.
    - We want to elucidate the pathway between the eyespot and the **photoaxis**- (cells movement in relation to where the light comes from)**,** to find out which genes are involved in light reception, and controlling the flagella so that the cell moves.
    - It is homologous with the human eye (this is just a hypothesis)

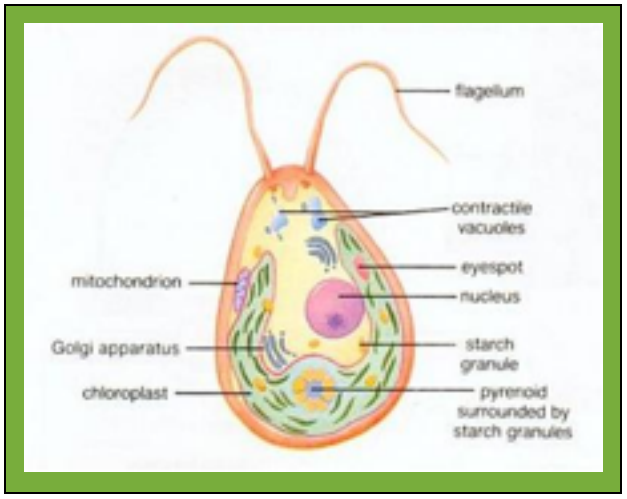
Function of basic components of Chlamydomonas cells

- Nucleus - gene expression/transcription
- Basal Body - organelle found at the base of any flagellum
    - Where the microtubules develop to produce the flagella
- Ribosome - site of protein synthesis
- Mitochondria - ATP factories of the cell (has more than one mitochondria)
- Chloroplast - energy transducing factory of the cell (just 1 cholorplast)
    - Within cholorplast is **pyrenoid** - where carbon fixation takes place

Occurs in Calvin Cycle

Carbon fixation - process by which photosynthetic organisms such as plants turn inorganic carbon (i.e - CO_2) into organic compounds (i.e - carbs)

- Also within the chloroplast is the **eyespot -** enables single chlamy cell to orient itself in relation to light



<u>Relative usefulness of various biological characteristics as measures of complexity</u>

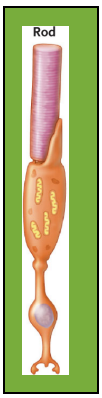
- Cell size - Human cells > Chlamy > E.cole , the bigger the cell, the more space it needs to carry out complex processes and compartmentalize.
- Genome size - can be useful sometimes although some similar organisms have very different genome sizes (some inconsistencies)
- Protein coding genes (PCG) - How many proteins does your genome code for
    - Both genome size and PCG can be misleading (due to junk DNA , etc)

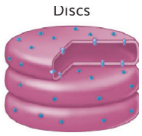
<u>Advantages to Chlamydomonas in being phototactic</u>

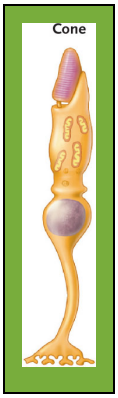
- Eyespot is used for **photoaxis -** movement towards or away from light
- Chlamy can thus move toward light because they want to harvest photons for photosynthesis

<u>Reasons why Chlamydomonas might move away from a light source</u>

- If too much light is absorbed, too much product is formed and as a result too much oxygen is formed and this can lead to **reactive oxygen species** which can destroy the cell if there's too many of them.







<u>Basic structure of rods and cone as photoreceptor cells</u>

- Photoreceptor cells (rods and cones) have photoreceptors which are the 'blue' dots found on the disk which harvest the light(their is a stack of disks making up rods and cones) and each disk has many photoreceptors and these individual photoreceptors trap the light.

<u>Major components involved in phototransduction and their role</u>

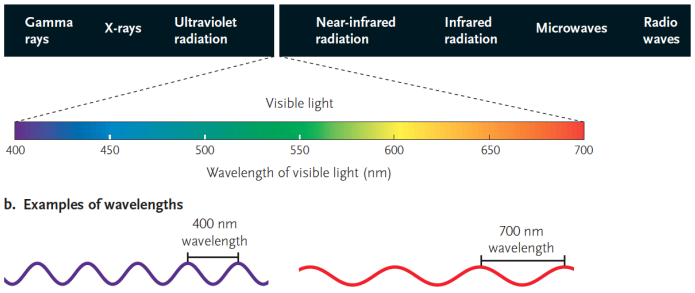
- Pigment found within the discs 'change' when light is harvested, this change activates a pathway called **phototransduction -** when this pathway is active, it activates another protein - **transducin.**
  - SO the membrane complex grabs the photon of light which causes a change(in the discs) that then activates transducin which in turn activates the enzyme **phosphodiesterase**
    - The sodium pump located on the membrane is regulated by **cyclic GMP**, So when cyclic GMP is bound, sodium is transported into the cell and there is a sodium influx (excess)
      - The phosphate group in cyclic GMP is bound to the ribose of GMP at the 5' and 3' position. SO when phosphodiesterase cleaves the 3' bond, a 5'GMP is generated.
        - This results in the cyclic GMP detaching from the transporter and as a result the transporter SHUTS OFF and sodium cannot enter the cell.
          - Light through the processes mentioned above, shuts down the sodium pump which **hyper polarizes** the membrane which leads to an electrical signal being sent down the membrane surrounding the rod or cone
            - The signal moves along the optic nerve and reaches your brain at an incredibly fast speed (as a result you see)

Lecture 2 - Light

Relationship between excited states of a pigment and its absorption fluorescence emission spectrum

- The energy of the fluorescence emission spectrum is always less in energy and longer than the excited state due to the heat loss that occurs from the lower energy level to the sub-lover energy level.

Region of the electromagnetic spectrum known as “visible light”



- Ranges from 400nm wavelength (most energy) to 700nm (least energy)
- Only area of the spectrum visible to the human eye

Relationship between wavelength and energy content of a photon

- They are inversely proportional - as wavelength increases, energy decreases. As energy increases, wavelength decreases.
- Most energy - Gamma rays (shortest) / Least energy - Radio waves (longest)

Molecular characteristic of visible pigments that make them absorb light.

- Pigments have a conjugated system:
    - alternates b/w double bonds and single bonds
    - has many non bonding pi-orbital electrons
        - these electrons are not required for bonding so they’re readily accessible to trap energy (interact with photons of light)

- MOST pigments absorb light and have no role in bonding
  - exceptions apply - retinal (involves bonding electrons)

<u>Relationship between pigments and associated protein</u>

- Pigments (such as chlorophyl or retinal) are bound to proteins
  - If you isolate the protein and are careful enough you can keep the pigment attached - causing the isolated protein to have color
- When a pigment is bound non covalently to the protein, it is called a **pigment protein complex**
- If detachment of the pigment occurs - there will be *free pigment* floating around *(*at the top of the test-tube)
- If the protein does not have a pigment attached, you must stain in with color to see the bands of the protein (in an electrophoresis)

<u>Four “fates: of the excited state of chlorophyll resulting from absorption of photons</u>

- Absorbed photon by an electron will either reach higher excited state or lower excited state depending on the energy of the photon
- Regardless, the HES decays to the LES after 10^-12 seconds due to heat loss
- Once in LES one of 4 fates occurs:

1. Energy is lost as **heat** resulting in the electron going back to ground state
2. Little energy is lost as heat and energy goes to sub excited state - remainder of energy is lost as **fluorescence** which has less energy and longer wave length than original absorbed light (darker color)
3. Using the light to do work - and the work is **photochemistry** -- light is used to change a molecule / change the structure of a pigment
4. Transferring the energy of the excited state pigment to a neighboring pigment.

<u>Reasons why relative fluorescence is different in isolated cholorophyll vs intact cells when exposed to light</u>

- There is less fluorescence in an intact cell when exposed to light because the energy absorbed from exciting the electrons is used to power photosynthesis - thus leaving little to no energy for fluorescence to be released.

- In an isolated chlorophyl the excited electrons have nothing to contribute there energy towards and thus release it as fluorescence.
  - Thus there is more fluorescence released in an isolated chlorophyl

What accounts for the fact that chlorophyll is green in color

- It is green in color because it does not have a green excited state
  - Green photon has more energy than red and less than blue and there is no excited state in chlorophyl at that point so the photon is either reflected or transmitted through the pigment

Quantitative relationship between photons and excited electrons

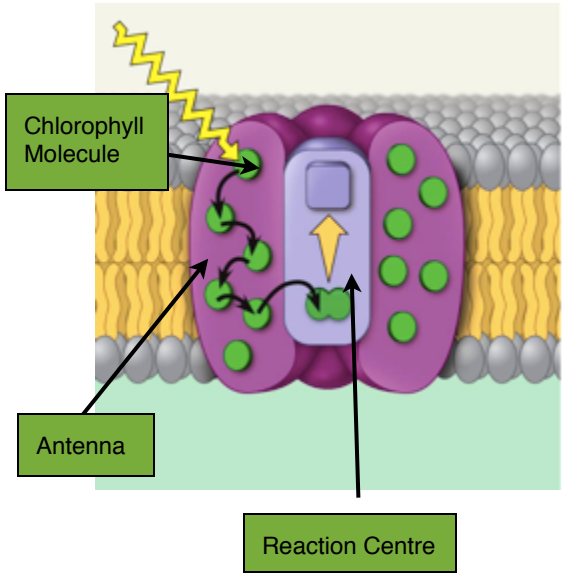
- Only 1 photon can excite 1 electron - photon cannot excite more than one electron and many photons cannot excite one electron. **MUST BE 1:1**

Relationship between photon and energy required to excite electrons in order for photons to be absorbed.

- For the energy to be absorbed, the energy that's in the photon **must match** the amount of energy required to get from the ground state to one of the excited states.

General structure of photosystem

- When chlorophyll in antenna absorbs photon of light - electron gets excited to a higher energy state - the excited state then transfers to a neighboring chlorophyll molecule
- The excited state then moves through the antenna (no photochemistry occurs yet) until the photon reaches the reaction centre where the cholorophyll molecule is excited and then oxidized
- The electron released from the reaction is then used to drive electron transport in the thylakoid membranes of chloroplast (and bacteria)



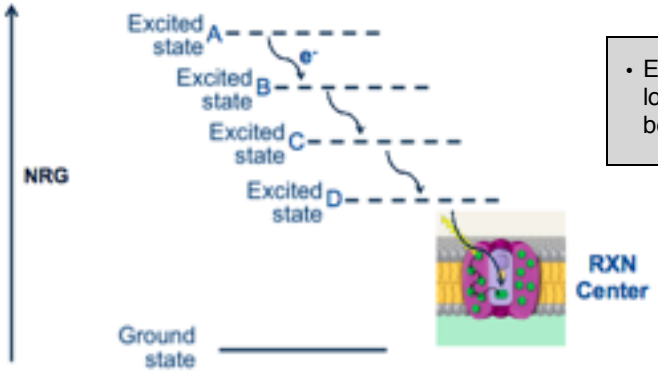
Similarities and differences of the light capturing and photochemistry of phototransduction (retinal) vs. photosynthesis (chlorophyll)

Similarities	Differences

How are excited states of antennae pigments organized to provide for energy transfer to reaction center

- The pigments are very close to one another (6 angstroms - atomic distances) and they are organized as so :

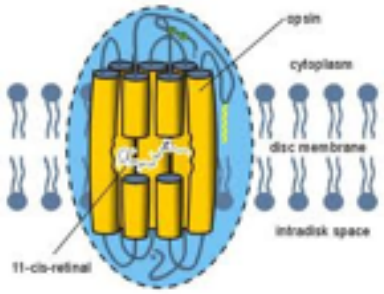
Draw the ground state and lowest excited state of four pigment molecules A,B,C,D that allows for stepwise energy transfer from A to D.



- Excited states get progressively lower to account for heat loss between each shift.

Structure of rhodopsin

- Rhodopsin = retinal + opsin
- Opsin surrounds the retinal



Effect of photon absorption by 11-cis retinal on retinal structure followed by association with opsin protein followed by interaction of transducing with opsin

- Retinal found in its 11-cis configuration (cis means hydrogens are on the same side of double bond) and can be found in all-trans configuration (hydrogens on opposite sides)

- Structurally different molecules but have the same molecular weight
  - To go from cis to trans - the double bond must be broken
    - When retinal absorbs a photon of light you go from 11-cis to all-trans retinal because the photon of light excited one the pi electrons in the double bond - the energy breaks the bond , the molecule swivels and then the double bond is reformed and it is in its all-trans configuration (photochemical event occurring in our eyes)

- Once in trans configuration the retinal becomes linear molecule and no longer fits the bonding site within the opsin - and so it detaches from the opsin.
- This now causes the shape of the protein to change (changes the shape of opsin) - creating a little cleft so that transducin can interact

- Once transducin interacts it activates phosphodiesterase and through a series of processes an electrical signal is sent along the optic nerve to the brain.
  - Opsin can no longer absorb light so it must be recycled so that a new cis-retinal can be incorporated inside the opsin.

Reasons why life has evolved to detect the narrow band of energy represented by “visible light”

- It is the most abundant and dominant light at the top of the atmosphere and on the surface of the earth and is energetically perfect to interact with biological molecules
- Ozone sucks in a lot of the ultraviolet light at the surface of the atmosphere and and at the surface of the earth (so theres very little UV on/around earth)
- Gamma Rays have too much energy so they would obliterate/ionize pigments and molecules. They would also disassociate all the bonds in molecules.
- Radio waves/ micro waves have too little energy and would simple warm up the molecule (cause slight vibration) rather than exciting the electrons

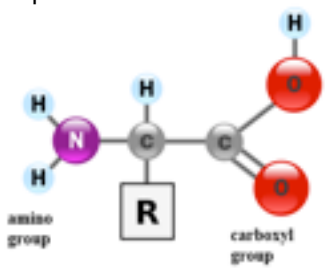
Side note

- In order to absorb a photon of light, white light must be shined on a molecule (chlorophyl for example) and one of the non bonding pi-electrons absorbs the photon.

Lecture 3 - Protein Structure and Function (ISO)

Basic structure of an amino acid and what are the different classes of amino acids.

- Generalized structure of an amino acid has a central carbon atom attached to an amino group (--NH2) , a carboxyl group (--COOH) , and a hydrogen atom
  - The remaining bond of the central carbon is 1 of 20 different side groups represented by the R. R group is called the side chain.



General structure of an α - amino acid

- Differences in side group give the amino acids their individual properties

Different classes of amino acids : non-polar , uncharged polar, negatively charged(acidic) polar, and positively charged (basic) polar amino acids.

Chemistry of the peptide bond and how it is formed

- **Peptide bond -** link b/w each pair of amino acids in a polypeptide.
  - It is formed by a dehydration synthesis reaction between the - NH2 group of one amino acid and the --COOH group of a second.

The four levels of protein structure

1. **Primary structure:** the proteins complete amino acid sequence (determined by nucleotide sequence in coding region of the proteins corresponding gene)

2. **Secondary structure:** regions of alpha helixes (formed by hydrogen bonds), beta sheet, or random coils, in a polypeptide chain.

Hydrogen bonds forms between N--H group of the backbone and the C=O group of the amino acid

The sheet is formed by hydrogen bonds between atoms of each strand. (O - - - H--N)

3. **Tertiary Structure:** overall three-dimensional folding of a polypeptide chain due to ionic bonds, hydrogen bonds, hydrophobic interactions and disulfide bridges.
4. **Quaternary structure:** the arrangement of polypeptide chains in a protein that contains more than one chain. (Two or more polypeptides coming together to form a functional protein)

Bonds in:
Primary - peptide bonds
Secondary - Hydrogen Bonds
Tertiary - ionic, hydrogen, hydrophobic interaction and disulfide bridges
Quaternary - N/A

How are alpha helices and beta sheets formed

- Alpha is formed when hydrogen bonds form between every N--H group of the back-bone and the C=O group of the amino acid
- Beta sheet is formed by side-by-side alignment of Beta strands. Sheet is formed by hydrogen bonds between atoms of each strand.

Lecture 3: Protein Structure & Function

INFORMATION VS ENERGY

Reasons why photosystems have antenna proteins while they eye doesn't

- A photosystem wants to harvest as much light as possible so that it can use it as **energy**
    - Photosynthetic systems don't care about information, just light harvesting
- A photoreceptors arrangement of rods and cones is attempting to harvest light as information - because where there photons come from conveys **information**.

Points of control for regulation of protein abundance

1. Controlling transcription - the conversion of DNA into mRNA. Transcription makes the proteins mRNA and is thus vital
2. Translation is also a regulated process and so if it is halted or controlled

conversion of mRNA to protein

    - There's a level of control at the level of transcription and translation that can be altered to impact protein abundance.

Factors affecting mRNA transcript abundance.

Amount of (specific) mRNA you have for a certain protein is affected by:

Transcription Rate: If transcription rate is high you should make lots of transcript, however this is not always true due to:

- **mRNA decay -** when mRNA is made - they float around for a varying time (20 minutes to hours) before they start to break down .

- **Transcript abundance** - the balance between mRNA decay and Transcription rate (both controlled processes that compete)
    - mRNA decay is a very important *control point*

Steps in making a Northern Blot for measuring mRNA transcript abundance.

1. Isolate the total RNA (from tissue samples for example)
2. Quantify how much total RNA you have and load the same amount of micrograms of RNA into every lane and then run on gel electrophoresis
3. Transfer the RNA to a (nylon) membrane (gel breaks too easy)

4. Incubate the membrane with a solution of probe that washes over the membrane. The probe is a single stranded molecule of DNA that is complementary in sequence to the sequence you are interested in detecting that is stuck on the (nylon) membrane.
5. Once incubated, the probe will hybridize with the complimentary sequence on the membrane
6. Each DNA probe has a radioactive group attached to it so you can detect its location and abundance on the membrane by exposing the membrane to a piece of x-ray photographic film.

Relative abundance of various types of RNA in typical cells.

- 97% of total RNA is ribosome RNA
- 3% of total RNA is mRNA -- of that 3% there is an EXTREMELY small percentage of mRNA that expresses a certain protein (e.g - hexokinase - there would be 100-1000 copies of the specific mRNA that codes for hexokinase)

Steps in making a Western Blot for measuring protein abundance

1. Stain gel if they aren't pigmented protein complexes.
2. Insert equal amount of protein (maybe its from a tissue sample) into 5 lanes of gel electrophoresis (and one marker lane) & run gel.
3. Afterwards gel is transferred to a membrane
4. An anti-body raised in a rabbit/chicken..etc that is specific to a protein (hexokinase) and that antibody then sticks to the corresponding protein(hexokinase)
5. Easy to detect the hexokinase in the lab and thus you know where that specific protein is.

An anti body to hexokinase is made. (It tracks it down)

Usually done by attaching a reporter enzyme to the antibody so that when the antibody encounters the protein, the enzyme releases color.

Characteristics of constitutive vs. induced vs. repressed gene expression kinetics.

- **Constitutive expression ->** when the abundance of protein or transcript doesn't respond to heat.
  - Eg. Actin shows constitutive protein abundance

- Because it's a house keeping protein, it does not respond to change in temperature (many genes show constitutive expression)

Induced expression -> transcript or protein abundance increases as heat increases

Repressed expression -> transcript or protein abundance decreases with exposure to heat shock over time. (As Temp increases, abundance decreases)

Varieties of defects that might account for lower levels of functional photoreceptors

- There could be a defect in transcription and/or translation - there are alot of enzymes required to make opsin and thus trans.c and trans.l are important.
- mRNA decay could be 'going nuts' and thus barely any transcript is accumulated
- Recycling of opsin needs a transcription factor so there could be a mutation in the transcription factor.
- mutation to the opsin gene as a result of poor folding (which is due to mutations)
- Maybe too much light is absorbed due to a mutation and light is damaging the photoreceptor
- Defect in retinal(NOT a protein)-- without retinal you cannot produce rhodopsin
  - **retinal not coded for by a gene - it is the product of a biosynthetic pathway** - E.G: no gene that makes beta-carotene BUT genes code for the enzymes which regulate the biosynthetic pathway and change the chemical structure of beta-carotene.
- Defect in retinal biosynthesis (due to malfunctioning enzymes)

Relationship among polypeptide, apoprotein, cofactor and functional protein

- Retinal is a **cofactor** not a protein - it is required for rhodopsin to work and thus it binds to opsin
- Opsin is the **apoprotein (**protein before it accepts the cofactor) - together they come together to produce a functional rhodopsin (or a functional protein)

A **cofactor** is a non-protein chemical compound that is bound to a protein and is required for the protein's biological activity.

- If any one of the two is malfunctioning or defected then rhodopsin as a whole is defected.
- Apoproteins (opsin) synthesized on the ribosome are not functional until they accept their corresponding co-factor (retinal), when they do accept it, it is known as **post translational modification**

PTM doesn't happen all the time – HOWEVER, all protein pigment protein complexes would have to go through this BUT not all proteins

Post-translational modification (PTM) is the chemical modification of a protein after its translation.

<u>Relationship between protein folding and function.</u>

- A protein must fold into its correct 3-D(a.k.a **conformation)** shape to function properly
- Polypeptide is string of a.a's and once that 'string' folds, you have a protein.
- Enzymes 3-D shape must be very exact to be functional.

MUST FOLD THE SAME WAY EVERY TIME

- To get the exact fold the polypeptide must go from primary to tertiary (a.k.a - **native confirmation of protein)**

<u>Factors affecting proper protein folding (Anfensen's dogma)</u>

- Got a simple enzyme to catalyze a simple reaction (measured the product by observing color change)
  - A low amount of enzyme produced a lot of color which means the enzyme is catalyzing the reaction the way he expected it to.
  - Once he added urea - it disrupted the bonding arrangements that contribute to the tertiary structure of the protein
    - As a result the protein falls apart and so if you add urea and do the same experiment (reaction) you get **NO** color. (enzymes messed up)
- He then got rid of urea thought a series of processes (centrifuge/pour it off/rinse it, etc) and then put the enzyme back into a buffer that doesn't have urea and that **reconstituted** the enzyme (it works again)(refolded in the perfect orientation - it fold back correctly 90% of the time.)

- He showed that folding is VERY FAST and is spontaneous - you don't need any energy for folding to occur.
  - Also showed that the ONLY thing that dictates folding is the primary sequence of the polypeptide (the order of the amino acids)

Even before translation is finished, the amino end of the polypeptide starts to fold.

Lecture 4 : Energy & Enzymes (ISO)

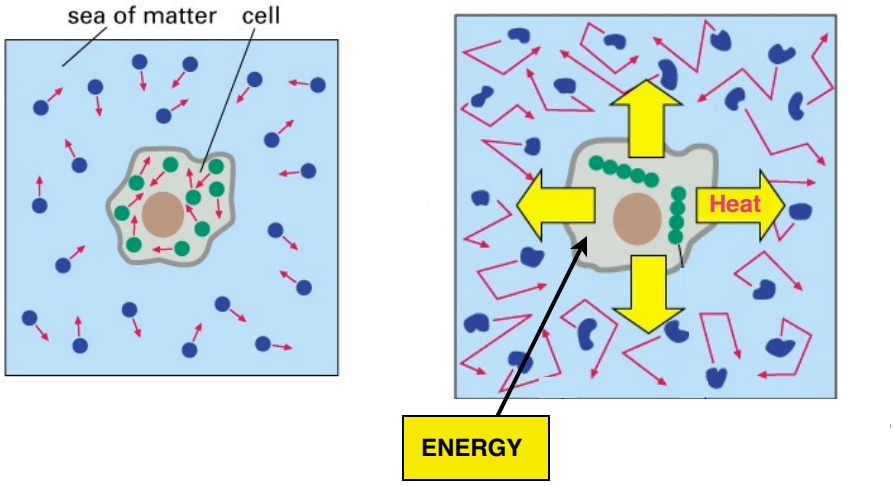
Isolated system - does not exchange matter or energy with its surroundings
Closed system - exchanges only energy with its surroundings
Open system - exchanges both energy and matter with its surroundings.

First law of thermodynamics - energy can be transformed from one form into another or transferred from one place to another, but it cannot be created or destroyed.

Second law of thermodynamics: the total disorder of a system and its surroundings *always* increases.

What is meant by the phrase "it takes energy to maintain low entropy"

- In the course of the thousands of chemical reactions that take place to generate order, living things give off heat and by-products of metabolism such as carbon dioxide that are much less ordered and increase the disorder or entropy of the surroundings.
    - Thus without energy, we cannot undergo processes/reactions that produce order in our cells and as a result we'd die.



Lecture 4 : Energy & Enzymes

Definitions

Potential Energy - A form of energy that has potential for a reaction, though at present is in a stored form.

Chemical Energy - Energy released in a chemical reaction (often in the form of heat)

Kinetic Energy - Energy released due to motion.

Entropy (S) - The amount of disorder in a system

Spontaneous Reaction - A reaction that occurs spontaneously without the input of energy.

Enthalpy (H) - Measure of the total energy of the system

Delta H - The change in Internal energy of a system

Exothermic - energy is released A-->B (A has more energy than B)

Endothermic - energy is absorbed , B has more energy than A

Gibbs Free Energy - Indicates whether a process will occur spontaneously

Exergonic - reaction occurs spontaneously

Endergonic - reaction does not occur spontaneously

Delta G - change in Gibbs free energy

Catalyst - A substance that increases the rate of a chemical reaction

Rate of reaction - the speed at which a reaction occurs

Energy of Activation - minimum amount of energy required to initiate a reaction (energy needed to reach transition state)

Transition state - bonds are strained and ready to break. Point at which reactant molecules will form products.

Kinetic Stability - without external energy applied, reaction occurs EXTREMELY SLOWLY or not at all

Active Site - part of the enzyme where substrates bind

Catalytic Cycle - Process of a substrate binding to an enzyme and forming an enzyme substrate complex, then catalysis occurs - substrate is converted to product after the reaction and produce is released and you are left with an enzyme.

Why life does not go against the second law of thermodynamics

- Cells maintain low levels of entropy due to the huge input of energy they receive but as a result, CO2/other molecules are given off which in turn increase the disorder of the surroundings.

Heat produced and motion of molecules also increases entropy of surroundings.

Why life needs to consume energy

- Life needs to consume energy so that it can stay ordered. If living things stopped brining in energy and as a result became disordered - they would die.

Components of Gibbs Free Energy equation

$$\Delta G = \Delta H - T\Delta S$$

- Delta G - Change in free energy
- Delta H - Change in enthalpy
- Delta S - change in entropy (multiplied by temperature)

Whether or not a given reaction will be spontaneous given Delta G

- If DG is + , the reaction is endergonic and will **NOT** proceed spontaneously
- If DG is - , reaction is exergonic and **will** proceed spontaneously

If entropy is + , more disorder
If entropy is −, less disorder

If enthalpy is positive - endothermic
If enthalpy is negative - exothermic

Role of enzymes in endergonic vs exergonic reactions

- Spontaneity indicates nothing about the rate of a reaction!
- Some reactions can take millions of years to occur spontaneously but with enzymes a spontaneous reaction that can take 78 million years occurs in 20ms
- Thus, enzymes speed up exergonic reactions and have **NO** effect on endergonic reactions -- enzyme cannot change the sign in free energy or the value of Delta G

Relationship between activation energy and rate of reaction

- The amount of energy needed to get to the transition state determines how fast spontaneous reactions happen. Amount of energy needed to get to transition state is energy of activation (or Activation energy)
- The lower the activation energy the higher the rate of reaction (the faster)

How enzymes increase rate of chemical systems

- Enzymes lower the energy required to get to the transition state. Once the activation energy is lowered, more molecules can acquire the energy to get to the transition state.
- Starting and ending free energy does not change - only the path the reaction takes changes (SAME DELTA G)
- Enzymes increase the rate of a reaction by increasing the number of substrate molecules that attain the transition state conformation, done by:

- **Precise orientation of two substrates -** two molecules will rarely come together in the correct orientation to get to the transition state.
    - Binding to the active site actually forces the molecules into the orientation they need thereby mimicking the transition state. This speeds up the reaction drastically
- **Charge interactions -** sometimes the substrate needs a certain charge across them but if you add an enzyme - the amino acids that make the active site of the enzyme provide a charge - it is MUCH easier for the energy of activation to be lowered
- **Conformational strain -** substrate may need to be strained, which happens more frequently in the presence of an enzyme which uses its active site to distort the substrate molecule into a conformation that mimics the active site.

Why biological systems need enzymes

- Reactions need to proceed relatively quickly. This is usually done (by chemists for e.g) by raising the temperature. However, biological molecules cannot handle high temperature or high pressure so enzymes increase the rate of a reaction without increasing the temperature

Importance of tertiary structure to enzyme function

- Enzyme must have correct 3-D structure and the structure must have some *flex*. This is because when a substrate molecules get close to the enzyme it causes the shape of the enzyme to change - **induced fit**

Molecule upon which an enzyme acts

- Correct tertiary structure also ensures that the active site is functioning and in the correct place. (POSITION OF ACTIVE SITE CANNOT BE DETERMINED BY THE PRIMARY SEQUENCE)

Link between enzyme function and growth rate

- Growth rate is a function of enzyme activity
- Enzymes need to come in contact with substrates in order for catalysis to occur.
    - The rate of molecular motion increases with temperature so then catalysis occurs at a faster rate

Thus, enzymes are more active the higher the temp so they can process more substrate and the cell can grow faster (divide more)

At a high temperature (optimum), the catalytic cycle is operating the fastest.

- HOWEVER, if the temperature gets too high (beyond 40 degrees for bacteria) - the enzymes will **denature**

How tertiary structure bonding arrangements are different depending upon the temperature habitat of the organism.

- Hyperthermophiles live at high temps, and so they have stronger/more intramolecular forces between the bond of the protein and as a result have a higher disassociation temperature
- Takes longer for these enzymes to denature
    - Psychrophiles live at low temps and have weaker 'bonds and such' and so they dissociate at much lower temperatures

- There are bond energies associated with the bonds in the tertiary bonding arrangements of enzymes/proteins and if that bond energy is exceeded, the bond will break
    - Heat can denature proteins, PH can affect ionic interactions and urea detergents interfere with the ability of the groups to form bonds.

Lecture 5 - Membrane Structure & Transport

Role of fatty acids in membrane structure

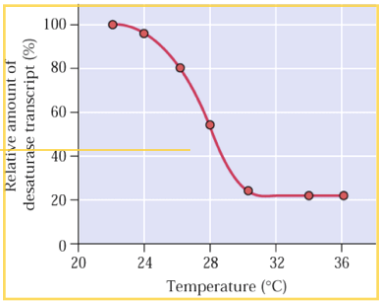
- They are hydrophobic and therefore position themselves away from aqueous environments in cell - as a result can form lipid membrane spontaneously

Relationship of fatty acid saturation levels on membrane fluidity

- If the fatty acids are fully saturated (no double bonds) , they align very close and pack close together and as a result membrane is less fluid
- If fatty acid tails is unsaturated,the tail then has a kink in it and this gives a more fluid membrane
    - The more unsaturation - the more fluid the membrane will be at any given temperature

Relationship of temperature on membrane fluidity

Graph for bacteria grown at different temperatures



- At a low temperature desaturase transcript abundance increases because at low temperatures the membrane is not fluid enough (too cold and rigid - gel like) and thus desaturase introduces double bonds in the fatty acid tails (causing kinks) which increases the fluidity of the membrane.

- If temperature is raised, membrane is fluid enough as is and so transcript abundance of desaturase decreases, if at extremely high temperature, there should be no unsaturated fatty acids at al !!

Relationship of fluidity to membrane functions such as transport

- Fluidity of membrane must maintain itself within a certain range - if it gets too hard, things can't move between the membrane. If it's too lose, ions will leak from one side to the other.

Properties of saturated vs. unsaturated fats

- Saturated : every Carbon has 4 Hydrogen bonds (no double bonds)
  - Very linear
- Unsaturated : Have double bonds causing a kink in the tail. There can be many sites of unsaturation (many double bonds)

Role of desaturases in fatty acid biosynthesis

- Fatty acids are synthesized through a biosynthetic pathway in a totally saturated form (linear) and then to make it unsaturated the enzyme **desaturase** introduces double bonds

Relationship of bacterial desaturase expression vs. temperature

- Bacteria could have 3 desaturase genes that code for different desaturase enzymes. The reason they have different desaturase enzymes is because they differ in where they incorporate/introduce the double bond.
- At high temperature - low desaturase transcript abundance. At low temperature - increase in temperature abundance.

Cells that don't maintain a constant body temperature can modulate fatty acid abundance and fatty acid unsaturation.

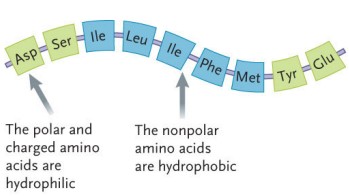
Role of size and charge in movement of molecules across biological membranes

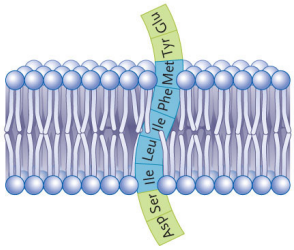
- Small and uncharged molecules get right through the semi permeable membrane
- However a bigger size and a charge are two things that impede movement across a membrane
- To get certain molecules across (glucose for e.g) , proteins are needed.

Characteristics of transmembrane proteins that enable them to interact with hydrophobic core of membrane

1) They could have many alpha helices - the hydrogen bonding that gives rise to the alpha helical structure minimizes the charges of the protein backbone. Thus the alpha helical structure can interact with the hydrophobic tails.

2) Membrane proteins - they have a tail-tail signature.





•Part of the protein that interacts with the membrane tends to be made up of **non-**polar (NOT CHARGED) amino acids.
•Usually 17-20 a.a traverse the lipid bilayer
•A transmembrane protein can be detected based on the primary sequence of a protein (when you see 17-20 non polar amino acids in a row - you know it traverses the bilayer) 3 stretches of it partially spaced out means it crosses 3 times.

<u>Factors influencing simple and facilitated diffusion</u>

- Simple diffusion is influenced by concentration gradient. Oxygen/CO2 diffuse DOWN a concentration gradient [high] --> [low]
- *Free Energy Change* drives diffusion - the more F.E the more ability there is to do work.
    - More molecules on one side than other --> High free energy
    - Equal amounts of molecule on both sides --> lower free energy

Diffusion then decreases the free energy by balancing it out.

Facilitate diffusion operates under the same principles, but it cannot get through the membrane on its own and thus needs a channel to facilitate the diffusion.

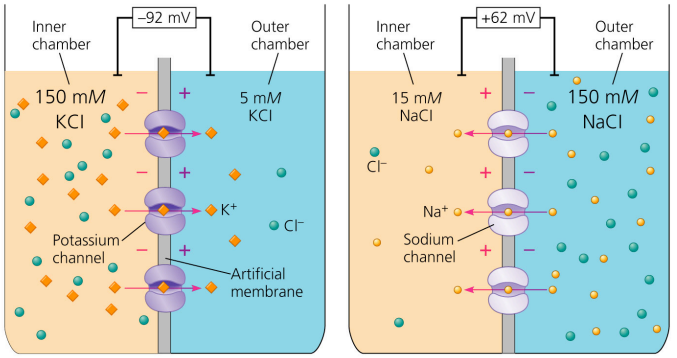
- Many pores (pores are inside channel) are very specific to certain molecules and may only let one molecule through at a time.

Example : Sodium Ion (one will go through its specific channel one by one)

<u>Transport against a concentration gradient (active transport)</u>

- Molecule is moved from a region of low concentration to a region of higher - because this is against free energy change , it requires energy

Role of electrochemical gradient in determining equilibrium concentration of ions



(a) Membrane selectively permeable to K^+ (b) Membrane selectively permeable to Na^+

- In left chamber the membrane is only permeable to potassium. Potassium wants to move from high concentration (150mM) to low concentration (5mM) - chemical aspect
  - However, potassiums movement is retarded by the electrical gradient -- Strong negative charge due to Cl- attracts the K+ molecules and prevents them from going to the right side - electricity aspect
    - This causes a charge difference of -92mV

**** Charge is always mentioned inside in relation to the outside - orange on left side is more negatively charged than blue.

On right side orange is more positively charged (+62mV)

Basis for electrical gradient across photoreceptor cell

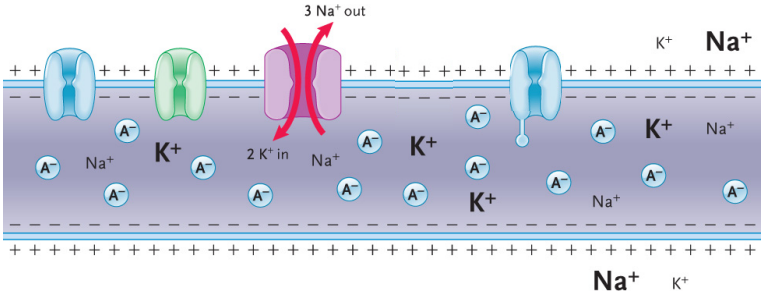
IN DARK

- Charge exists due to all the anions in the cell (proteins, amino acids, etc) There is also lots of sodium outside the cell and lots of potassium inside the cell and they both leak down an electrochemical gradient
  - K leaks OUT and Na leaks IN -- NaK pump then moved them back to there original place (pumps 3 Na out and 2 K in)
- Cyclic GMP gated channels on the plasma membrane are active when cGMP is bound to and this causes a huge influx of sodium which keeps the difference across the membrane smaller than it would otherwise be.

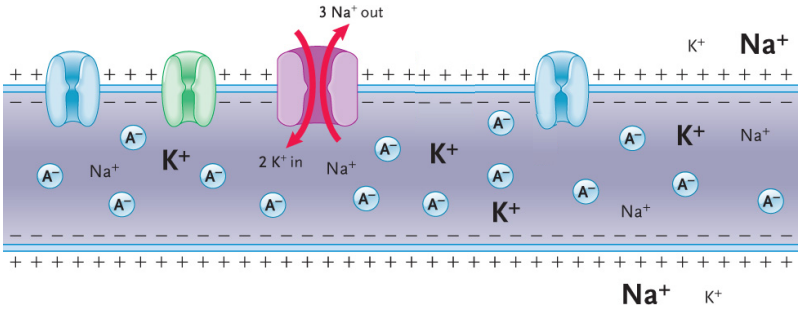
IN LIGHT

- In light, cyclic gmp gated channels are shut off once they are metabolized by phosphodiesterase. As a result, positively charged sodium cannot get into the cell to minimize the negative charge (as it usually would in the dark)
- Potential difference across membrane becomes even greater as positive potassium leaves the cell. This causes **hyper-polarization** of the cell and causes a trigger that blocks the release of glutamate and triggers the electrical impulse.

Usually (in the dark) sodium is coming in (+) and potassium is leaking out (+) so the negative state of -30mV of the photoreceptor cell is maintained. However in the light hyper-polarization occurs.



DARK ^^^



LIGHT ^^^

ABC Transporters are one group of molecules of active transporters

Basic structure of ABC transporter

- Used in active transport and human genome codes for 100's of different ABC transporters
- Composed of two parts : 1) *Transmembrane domain* - part that transports the molecule - different depending on which molecule its designed to transport

2) *ATP binding cassette* - binds ATP to it and uses energy of ATP breakdown (hydrolysis) to fuel the transport

Genetics underlying cystic fibrosis

- Is a homozygous recessive disease (if both parents are heterozygous for it - child has a 1/4 chance of getting two mutant copies of CFTR
- 1 wild type and 1 mutant CFTR does not lead to CF
- Cystic fibrosis is a defect in CFTR (an ABC transporter)
- CFTR is 6000 bases long and is composed of 1480 a.a's
- Most common mutation to CFTR is the ΔF508 (70% of cases)
    - ΔF508 mutation is when CFTR lacks a phenylaneline at the position 508

Cystic Fibrosis Phenotype

- CF leads to problems with lungs and gastrointestinal tract
- CFTR is located on the membrane of epithelial cells that are lined with cilia with mucus.
- Cilia and mucus must be kept wet to get good clearance (be able to cough out any bacteria or dirt that gets in the lungs)
- Cilia and mucus stay wet due to CFTR - it pumps chloride out into the epithelial space and in response there is an osmotic movement of water - water will move from the epithelial cell to the epithelial lining and keep the mucus wet.
- With CF, CFTR cannot pump chloride out and as a result lung tissue sticks together, gas exchange is inhibited, etc.

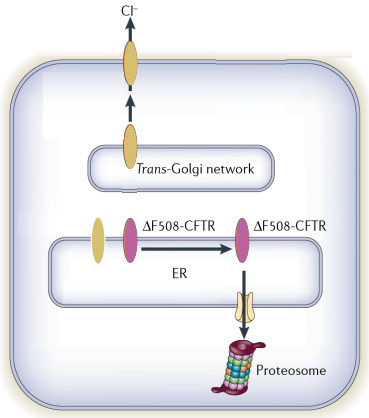
Relationship of CFTR synthesis and folding in the intra-cellular secretory system

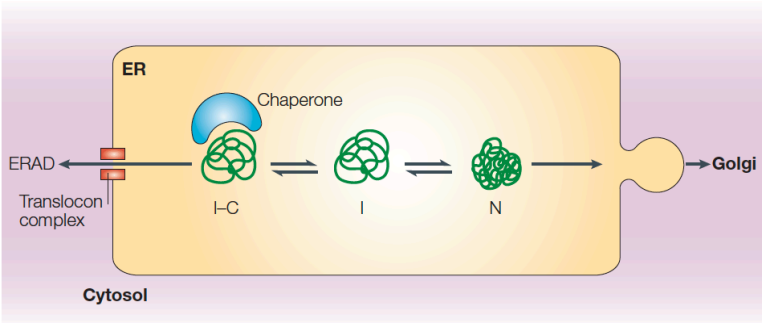
- For CFTR to get to the plasma membrane it must be moved through the secretory pathway
- Wild-type CFTR moves perfectly well through the secretory pathway

What happens to the ΔF508 form of CFTR

- Mutant form gets to the E.R where it is detected by the quality control system - it is detected by the quality control system in the E.R because due to the ΔF508 mutation the protein doesn't fold perfectly and the native conformation of the protein is not perfect
- Thus, **chaperone proteins** in the E.R detect the faulty folding of the protein and tag it
- The tagged CFTR is then sent to the **proteosome -** a giant complex of proteases (enzymes that break down proteins) - broken/malfunction proteins go there to be degraded

- **ΔF508 mutant is **PERFECTLY FUNCTIONAL** (functions 50% as well as the wild type, but it would still be good enough to not cause CF. The only problem is the defective protein never gets a chance to get to the plasma membrane because it is tagged in the E.R and consequently; degraded.





Lecture 6 - Energy Transformation I - ISO

Catabolic Pathway - A metabolic pathway where energy is released by the breakdown of complex molecules to simple compounds

- An example is cellular respiration whereby energy is extracted from the breakdown of food such as glucose (Glucose --> ATP,H20,CO2)
- Overall Delta G is **negative** ,, but both types of pathways can be made of a mixture of both endergonic and exergonic reactions

Anabolic Pathways - consume energy to build complicated molecules from simpler ones; often called biosynthetic pathways

- An example is photosynthesis (CO2 --> glucose) as well as the synthesis of macromolecules such as proteins and nucleic acids
- Overall Delta G is **positive**

<u>How is the structure of ATP linked to the fact that its hydrolysis is strongly exergonic</u>

The components of a water molecule are added as molecules are split into smaller subunits

P_i - Orthophosphate ion or inorganic phosphate

- Both products of the hydrolysis reaction (ADP and P_i) carry a negative charge, and the repulsion between these ionic products favors hydrolysis

- Release of the terminal phosphate allows greater opportunity for hydration , and this an energetically favored state

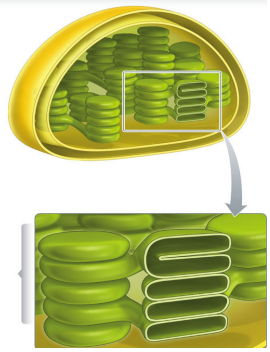
- The orthophosphate group (P_i) can exist in a wide variety of resonance forms, not all of which are available when it is bonded. Thus release of the orthophosphate increases the disorder of the system

Lecture 6 - Energy Transformation 1

Space around thylakoids

Structure of Chloroplast

- Outer membrane covers entire surface of the chlorplast
- Inner membrane lies just inside the outer membrane.
- Between the inner and outer membrane is the is the *inter-membrane compartment*
- Stroma is the aqueous component filled with enzymes (where Calvin Cycle takes place)
- **Thylakoid membranes -** form flattened closed sacs. Space enclosed by a thylakoid is called the *thylakoid lumen.*
    - Thylakoid membrane is where light reactions take place.



Source of electrons and products of electron transport

- There is an electron within P680 that gets excited when the energy from the photon of light is funneled to it. P680* is oxidized into P680+ , P680+ is reduced back to P680 by the donation of an electron from water.
    - H20 is split into 2H+ and 1/2 O2 --- two electrons released from the hydrogens and one of them is used to reduce P680+

As a result of water splitting 1 molecule of molecular oxygen is formed, released into the environment and this accounts for 21% of oxygen in the atmosphere.

PQ accepts an electron from primary acceptor in ps2 (PQ is reduced) and also picks up an H+ (oxidized) so that it has no charge and it moves across the membrane where it dumps the H+ , and it is then oxidized by the cytochrome complex.

Cytochrome complex reduces plastocyanin which then reduces P700+ back to P700 (P700+ oxidizes plastocyanin)

Ferredoxin is oxidized twice by NADP+ reductase , and using two electrons and one proton, NADP+ is reduced to NADPH and the bi product is 2 protons and NADP+

ATP synthase makes ATP using ADP and Pi

Structure of photosynthetic electron transport (see diagram)

Light reaction summarized

- Photons are absorbed by the antenna complex on PS2 and the energy is 'funneled' to the reaction centre to the special chlorophyl ***a*** (Called **P680**)**.**
- As a result an electron in **P680** gets excited (now P680*)
- In its excited state, **P680*** can easily be oxidized and as a result the primary electron acceptor oxidizes P680* making it P680+ -- This oxidation-reduction reaction initiates electron transport.
- P680+ is reduced back to P680 by donation of an electron from water. This donation comes from the **oxygen evolving complex**
- Primary acceptor passes the electron to the **PQ.** As PQ accepts the electron from PS2, it also picks up a proton from the stroma - picking up a proton neutralizes it and makes it easier to move through the membrane

Oxidation Reaction - LOSS OF AN ELECTRON

Reduction Reaction - GAINING AN ELECTRON

OiL RiG (oxygen is losing an e- // reduction is gaining an e-)

Thylakoid lumen is inside membrane of Thylakoid.
Outside the membrane is the stroma

- PQ diffuses through the membrane and then binds to the cytochrome complex. It then donates its electron to a cofactor in the cytochrome complex and releases a proton (H+) into the thylakoid lumen

- From the cytochrome complex, the electron is donated to **plastocyanin**
- Electron goes from plastocyanin to PHOTOSYSTEM 1.

P680+ Is the strongest reducing agent on the planet

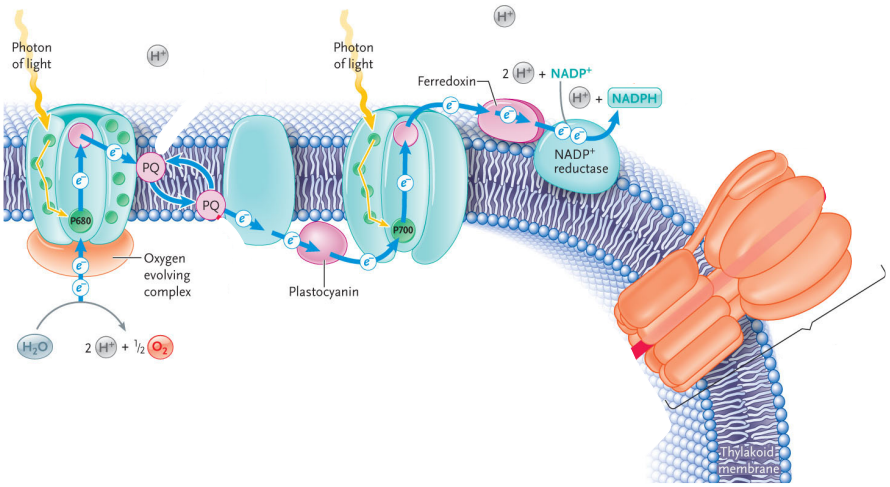
- Simultaneously, A photon of light hits PS1 (usually less energy than the photon that hits PS2) and the energy is funneled to **P700 --> P700***, P700* is then oxidized by the primary acceptor (which is reduced) and then the primary acceptor passes the electron onto **Ferredoxin**

- P700+ is reduced back to p700 by the electron that is coming from plastocyanin. (electron came all the way from ps2)
- Ferredoxin passes the electron to **NADP+** which stores the electron until a SECOND electron is sent (from a photon of light --> p700 --> primary acceptor --> ferredoxin) and then once the second electron reaches the **NADP+ reductase complex**, NADP+ is reduced to **NADPH** (takes two electrons and one proton from the stromas aqueous environment for this reduction)

- Because PQ drops off a proton every time it travels to the cytochrome complex and water is splitting (so theres a lot of H+ in thylakoid membrane) and so there ends up being an **electrochemical** gradient - pH of thylakoid lumen is much lower (more acidic) than that of the stroma and there is a charge difference (more positive on the side of the membrane)
- The electrochemical gradient can be harnessed to do work and this harnessing of the electrochemical gradients energy is **chemiosmosis**

- Protons then want to get out of the membrane (to the more negative stroma because opposites attract) and so they get through the membrane the only way they can which is through the protein complex **ATP SYNTHASE** - this enzyme uses the energy produced from chemiosmosis in the thylakoid membrane to pack an inorganic phosphate (P_i) onto an ADP creating; ATP

- ATP's energy along with the NADPH that was produced is used to drive the **CALVIN CYCLE**



Mechanisms of Electron Flow

- Movement is driven by redox potential difference among the electron carriers
- If you have very positive **redox potential** - you're a very strong oxidizing molecule (Can make another molecule lose an electron easily)
- So more positive redox potential = stronger oxidizing molecule = ability to pull electrons readily away from other molecules

POSITIVE WANTS ELECTRON

NEGATIVE GIVES UP ELECTRON

- A more negative redox potential means you don't hold onto your electrons very strongly and so your readily able to **reduce** other molecules (make them gain an electron)
- For e.g - electrons move fom the cytochrome complex because plastocyanin has greater affinity (more positive redox potential) for those electrons than the

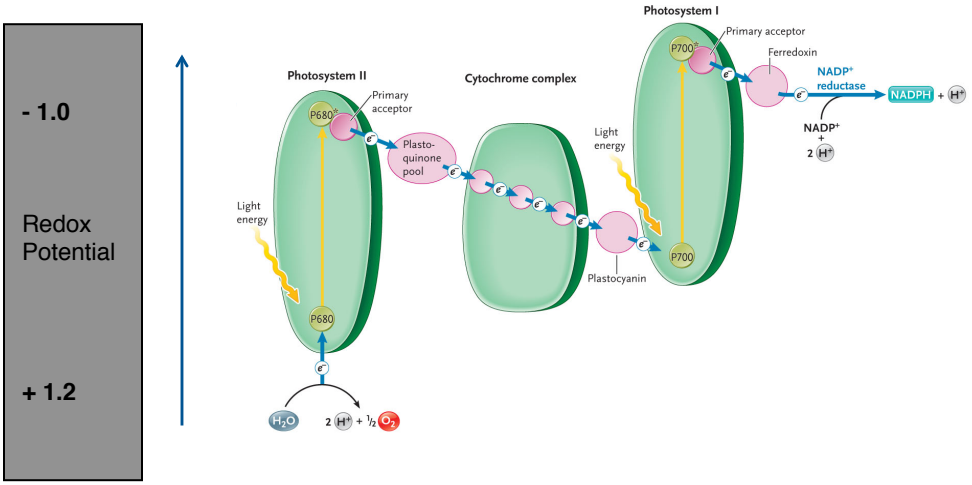
CC does and so its a stronger oxidizing molecule that rips the electron away from the cofactor in the cytochrome complex.

- ESSENTIALLY, the only thing light does in photosynthesis is change the redox potential of the chlorophyl's in the reaction center - because when the chlorophyl is excited - the primary electron acceptor steals the electron MUCH easier.
- If the electron was in its ground state, it would be impossible for the p.e.a to steal an electron from P680/P700
- Once P680 electron gets excited --> P680* **-** It enables electron transport to be spontaneous (moves downhill to PS1)

- The electron decays in energy once it has reached P700 and so it needs another photon of light (second photon of light turns P700 into P700* --> DECREASES ITS REDOX POTENTIAL

- Another photon of light is used to get P700 to the energy level of NADP+
    - Two photons of light required to overcome the energy difference between H20 and NADP+.
- NADP+ oxidized P700* twice before it is reduced to NADPH

- P680* needs a VERY high redox potential to rip an electron away from water, because water is VERY stable. So P680+ is the strongest known oxidant in biology and is responsible for oxygen in the atmosphere.



Phases of Calvin Cycle

- In the stroma of the chlorplast, 11 enzyme catalyzed reactions use NADPH to reduce CO2 into sugar

Phase 1: Fixation.

Fixing CO2 **into** one molecule of five-carbon sugar ribulose (1,5-biphosphate) aka RuBP to produce **two** molecules of the three-carbon compound 3-phosphoglycerate. (catalyzed by Rubisco)

CO2 and RuBP fit into the active site of Rubisco and are 'fixed'

- SO if this is done three times , **six** molecules of 3-phosphoglycerate are formed

Recap of Fixation : 3CO2 + 3(1,5-bisphosphate) -> 6PGA

RuBP – ribulose bisphosphate

Phase 2: Reduction.

Each molecule of 3-phosphoglycerate gets an additional phosphate added from the break down of ATP (In 3 turns you would need 6 ATPS) --> 6 ADP formed

PGA

- This produces 6 (1,3-bisphosphoglycerate)

- 6 NADPH's are used to reduce each 1,3-bisphosphoglycerate forming 6 Glyceraldehyde-3-phosphate (G3P)

G3P

Phase 3 Regeneration

Three turns of the Calvin cycle produces two 6 of G3P (18 Carbon atoms)

- 15 of these carbons are rearranged to regenerate the 3 molecules of RuBP required for the next round of carbon fixation. (5 G3P + 3ATP --> 3RuBP)

IN THREE TURNS

3CO2 (3 carbons) ---(incorporated into)--> 3 molecules of RuBp (15 carbons)

C5H12O11P2

----(producing)---> 6 molecules of 3-phosphoglycerate (18 Carbons)

$C_3H_7O_7P$

6 molecules of ATP are consumed to phosphorylate the 6 molecules of 3-phosphoglycerate , making 6 molecules of 1,3-bisphosphoglycerate (18 C)

6 molecules of NADPH are consumed in converting the 6 molecules into 6 molecules of **G3P (**18 Carbons)

15 Carbons

5 molecules of G3P (15 Carbons) are used to regenerate 3 RuBP molecules (the regeneration requires 3 Molecules of ATP

SO, the cycle makes one extra G3P (3 carbons) after every 3 turns.

- Calvin cycle requires 9 molecules of ATP and 6 molecules of NADPH to make 1 G3P
  - ATP and NADP+ are regenerated from ADP and NADP+ by the light reactions.

Reaction Catalyzed by Rubisco

- Rubisco is the major carbon fixating enzyme - every carbon on the planet has passed through the active site of rubisco at one time or another.

- Catalyzes the following reaction : RuBP + CO2 --> 2 phosphoglycerate OR

- **3RUBP + 3 CO2 --> 6 phosphoglycerate (PGA)**

Difference between carboxylation reaction and oxygenation (photorespitory) reaction

- Carboxylation reaction occurs when CO2 gets into the active site of Rubisco :

$3C_5H_{12}O_{11}P_2 + 3CO_2 \rightarrow 6\ C_3H_7O_7P$

- This reaction ^ leads to a NET INCREASE in carbon and :

CARBON GAIN = GROWTH (had 15 carbon, brought some in ,now 18)

In a photorespitory reaction, oxygen gets into the active site of Rubisco and binds with RuBP. Reaction is

$C_5H_{12}O_{11}P_2$ + O2 --> PGA + Phosphoglycolate (5 Carbons in, 5 Carbons out)

Has 3 Carbons

Has 2 Carbons

- Phosphoglycolate cannot be used so it is broken down into glycolate (which is toxic) --> it is then oxidized USING ATP and released as carbon dioxide
- NET LOSS OF CARBON DIOXIDE. (5 IN , 3 out) and it wastes ATP
    - Also this does the opposite of what plants should do, it releases CO2 instead of O2

Implication of photorespiration of Growth

- Carbon gain = growth , so if photorespiration occurs their is a net loss of Carbon and so the plant DOES NOT grow

Mechanism by which Chlamydomonas concentrates Carbon dioxide

- Chlamy is aquatic - so when CO2 dissolves in an aqueous environment (sea water for e.g) it becomes bicarbonate (**HCO_3-**)
- So in chlamy there is an ATP driven pump that pumps bicarbonate into the cytosol (because it can't diffuse in with its charge)
- Once inside, **Carbonic Anhydrase** (enzyme with the greatest catalytic turn over rate - 200k molecules a second) converts carbonate into CO2, and so once you have a lot of CO2 in the cytosol of chlamy, it diffuses down a concentration gradient into the chlorplast.

- So using this process Chlamy keeps much higher levels of CO2 in the chloroplast and reduces the chances of photorespiration occurring.

SKIP _____

SKIP

Cellular Respiration

Cellular Respiration - collection of metabolic reactions within the cells that breaks down food molecules to produce ATP.

1. *Glycolysis:* Enzymes break down a molecule of glucose into two molecules of pyruvate. 4 ATP and 2 NADH is synthesize,.
2. *Pyruvate oxidation and the citric acid cycle :* Acetyl coenzyme A (acetyl-CoA) which is formed from the oxidation of pyruvate, enters a metabolic cycle, where it is completely oxidized to carbon to Carbon dioxide. Some ATP and NADH is synthesized
3. *Oxidative phosphorylation:* The NADH synthesized by both glycolysis and citric acid cycle is oxidized, with the liberated electrons being passed along an electron transport chain until they are transferred to oxygen, producing water. The free energy released during electron transport is used to generate a proton gradient across a membrane, which in turn, is used to synthesize ATP

GLYCOLYSIS

- Occurs in the cytosol of the cell

1) Glucose receives a phosphate group from ATP, producing glucose-6-phosphate (this is a phosphorylation reaction catalyzed by **hexokinase**

2) Glucose-6-phosphate is rearranged into its isomer - fructose-6-phosphate (isomerization reaction)

3) Another phosphate group from ATP is attached to fructose-6-phosphate, producing fructose-1,6-bisphosphate (second phosphorylation reaction)

4) Fructose is split into G3P and DAP (through a hydrolysis reaction)
5) The DAP produced in **4)** is converted into G3P, giving a total of 2 G3P's (isomerization reaction)

- The first 5 stages are known as energy investment stages, because 2 ATP molecules were used (forming 2 ADP and 2 Pi) to do the two phosphorylation reactions.

6) Two electrons and two protons are removed from each G3P - the energy released is trapped by the addition of a Pi (inorganic phosphate group) to each G3P molecule forming two 1,3-bisphosphoglycerate

The electrons are then accepted by each NAD+ , along with one of the protons. (***Two NADH*** *molecules synthesized)* Other two protons are released from the cytosol.

7) Both phosphate groups of the two 1,3-bisphosphoglycerate are transferred to 2 ADP's to produce 2ATP's (substrate-level phosphorylation reaction)
8) This yields 2 molecules of 3-phosphoglycerate which are then rearranged (by an enzyme) to form two 2-phosphoglycerate (phosphate group is shifted from the 3 carbon to the 2 carbon position)
9) Electrons are removed from both 2-phosphoglycerate and delivered to another part of the molecule. Most of the energy lost by the electrons is retained in the product - two phosphoenol-pyruvate (PEP)[redox rxn]
10) Remaining phosphate group on each PEP are removed and transferred to ADP. This forms **2 ATP** (1 per molecule of PEP) AND two **pyruvate**

- Last 5 stages are the *payoff* stages - *4 molecules of ATP* formed and *2 molecules of NADH*
  - NOTE: no carbon is lost (from 6 to 6) , however potential energy in the two molecules of pyruvate is LESS than that of one molecule of glucose.
- ATP is generated by *substrate-level phosphorylation* - this involves the transfer of a phosphate group from a high-energy substrate molecule to ADP producing ATP

Molecule upon which an enzyme acts

PYRUVATE OXIDATION

- The pyruvate synthesized during glycolysis must past through the outer and inner mitochondrial membrane to begin the citric acid cycle.
- Pyruvate simply diffuses through the large pores in the outer membrane
- Pyruvate then crosses inner membrane by going through a pyruvate-specific membrane carrier.
- Once in the matrix, the conversion of pyruvate into acetyl-CoA begins.
  - A decarboxylation reaction takes place whereby the carboxyl (-COO) group of pyruvate is lost as CO_2.
  - The remaining two carbons are oxidized, producing **acetate**
  - This dehydrogenation reaction leads to the transfer of two electrons and a proton to NAD+, forming NADH
  - The acetyl group then reacts with coenzyme A (CoA) forming acetyl CoA
    - Acetyl CoA still has three C-H bonds which can be used for energy and that is the energy the citric acid cycle looks to extract

A cofactor molecule that helps an enzyme catalyze a particular reaction by binding with it.
- It is NOT a protein

CITRIC ACID CYCLE

- Consists of eight enzyme-catalyzed reactions

1) two-carbon acetyl group is carried by coenzyme A is transferred to oxaloacetate (4 carbons) , forming a 6 carbon compound : **Citrate**

2-8)

- Per turn of the citric acid cycle, **three** NADH, one **FADH2**, and a single molecule of **ATP** are formed.
- However since two molecules of pyruvate enter the mitochondria and turn into TWO molecules of Acetyl-CoA, technically 6 NADH , 2 FADH2 and 2 ATP are formed (along with two oxaloacetates)

SKIP

Lecture 7: Energy Transformation II

Characteristics of ATP

- The hydrolysis of ATP is an exergonic reaction (-7.3kcal/mol)
    - H20 is used to remove the terminal phosphate on ATP (exergonic)
    - ATP hydrolysis is thermodynamically unstable but is kinetically stable.
        - Hydrolysis of ATP is not too fast kinetically, because if it was, hydrolysis would occur too quickly in the cell and the cell would heat up.
- Therefore, hydrolysis reaction does not happen relatively fast in an intact cell but it is spontaneous
- However it is not really ATP hydrolysis, it is ATP breakdown - in ATP breakdown there is a direct transfer of the phosphate group to the substrate (glucose) -- this is done because water cannot get into the active site

Role of C-H bond in bioenergetics

- The free-energy in C-H bonds is converted into ATP.
- The more C-H bonds in food, the more potential energy.
- The cell cannot use C-H's energy directly so it uses the energy of the electrons removed from C-H bonds to do work (Turns the energy from the C-H bond into ATP)

Role of Redox potential in bioenergetics

- The greater the redox potential, the greater the electronegativity.
- Molecules with greater electronegativity hold onto their electrons tightly and thus it takes more energy to remove their electrons (oxidize them)
- The more negative the redox potential the more potential energy a molecule has (can be oxidized easily OR readily reduce other molecules)

Role of FAD, NAD+ as electron carriers

- FAD and NAD+ are reduced during the Krebs cycle to FADH2 and NADH. They then release their free energy when they are oxidized by subsequent cofactors. Energy is ultimately used to pump protons and produce ATP.... FAD and NAD+ are then sent back to the Krebs cycle to be reduced.

Location, products, distribution in nature and purpose of pathways such as glycolysis, CA cycle, respiratory electron transfer, etc..

- **Glycolysis** takes place in the cytosol. Its purpose is to take glucose and turn it into two molecules of pyruvate.
- In the investment stages of glycolysis, 2 molecules of ATP are used to help two phosphorylation reactions occur. (Two ADP's and two inorganic phosphates are formed)
- The payoff stages lead to the product of four ATP and two NADH molecules
- Glucose(6 carbons) has more free energy than the two molecules of pyruvate formed.(3 carbons each - 6 carbons)

Purpose: To break down glucose into 2 pyruvate molecules with free energy that can enter the matrix

Pyruvate Oxidation

takes place in the mitochondrial matrix, Pyruvate loses a carboxyl group (CO_2) , forms NADH and a proton, and then through the addition of coenzyme A becomes acetyl CoA (2 carbons). But since two pyruvates are turning into two acetyl CoA (4 carbons)

Purpose: To turn the pyruvate molecule into Acetyl-CoA which has more free energy (more reactive) than pyruvate

Citric Acid Cycle

- Through a series of 8 enzyme-catalyzed reactions, Acetyl CoA (2C) joins with Oxaloacetate(4C) to make Citrate (6) ,
- Citrate is oxidized and NAD^+ is reduced to NADH. 2 CO_2's are also released
- ATP and $FADH_2$ are also made

- The Reactants used are
  Acetyl-CoA, 3NAD^+ , FAD , ADP, Pi, and 2 H_2O
- Products are
  2 CO_2, 3 NADH, $FADH_2$, ATP, 3H^+ , and CoA

Purpose: Pulls the electrons from the remaining C-H bonds and stores the energy in NADH and FADH2

Oxidative phosphorylation

- Occurs **ON** the inner mitochondrial membrane.
- Facilitates the transfer of electrons from NADH and FADH2 to oxygen
- There are 4 complexes and electron flow from one complex to another is facilitated by two mobile electron shuttles (UQ and cyt C)
- Complex I and IV use the energy released from NADH and FADH2 to pump protons and establish an electrochemical gradient that can be used to make ATP.
- NADH is oxidized at complex 1, its electron is taken by UQ and the energy released is used by complex 1 is used to pump protons into the inter membrane compartment
- FADH2 is oxidized by a cofactor in complex II, electron is also given to UQ at a later time.
- Cofactors in complex IV use the electrons to reduce O2 to water
- Complex I and IV pump protons into the inter-membrane compartment, and UQ drops off a proton in the I.M compartment every trip it makes as well, causing the EC gradient.

Purpose: Extract the potential energy of NADH and FADH2 to synthesize additional ATP

<u>Role of energy coupling in early steps of glycolysis</u>

- Glucose and the enzyme hexokinase come together with a phosphate group to make glucose-6-phosphate
  - The addition of orthophosphate (P_i) onto glucose is an *endergonic reaction (3.3kJ/mol)* - this will not occur spontaneously
    - Therefore, this endergonic reaction must be coupled with an exergonic reaction to make the overall reaction exergonic (spontaneous)
- It is coupled with the hydrolysis of ATP (-7.3cal/mol) -- H20 removes the terminal phosphate of ATP (exergonic)

Its not really hydrolysis, it is actually ATP breakdown (direct transfer of the phosphate from ATP to the substrate.

Relative potential energy of various intermediate compounds

- Potential energy of phosphoralated glucose/substrate > Potential energy of glucose > potential energy of 2 pyruvate molecules > potential energy of CO_2.

Reasons why catabolic intermediates are phosphorylated

1) Catabolic intermediates such as glucose and fructose-6-phosphate are phosphorylated, it makes them more reactive - more readily wanting to breakdown. Glucose-6-phosphate is more reactive and has more free energy associated with it than does glucose.

2) Also phosphorylating glucose gives the molecule a negative charge and by doing so, it cannot easily diffuse through the membrane. By charging it , you make it stay in the compartment it should be in.

Link between glycolysis and Citric Acid Cycle

- The energy in the C-H bonds of pyruvate formed in glycolysis are eventually converted into ATP and electron carriers such as NADH.
- Another link between them is the pyruvate dehydrogenate complex. The pyruvate formed in glycolysis is catalyzed by the PDC to turn into acetyl CoA; which is then used for the citric acid cycle

CITRIC ACID CYCLE = KREBS CYCLE

- Pyruvate oxidation

Role of pyruvate dehydrogenase complex

- PDC is a big enzyme complex that transforms pyruvate into Acetyl CoA - remaining energy in Acetyl CoA is extracted by the citric acid cycle.

Diagnostic value of relative rations of bioenergetic intermediates (ATP,pyruvate,NAD etc)

Relative location of electron transport chain components relative to mitochondrial membrane, matrix, inter membrane space

- The components of the electron transport chain are located on the inner mitochondrial membrane. A majority of the reactions take place in the mitochondrial matrix and the proton byproducts are shuttled to the inter-membrane compartment

{Insert picture}

Role of oxygen in electron transport

- Process of redox reactions occur as electrons are passed along a chain of electron carriers until they are passed to oxygen - the final electron acceptor. Oxygen has the most positive redox potential and thus can be easily reduced. So when the electrons finally reach oxygen, oxygen is reduced by two electrons and turns into water.
    - Oxygen is responsible for removing electrons from the system. If there was no oxygen, electrons could not be passed among the cofactors and the energy in the electrons could not be released to do work
        - Oxygen is necessary to “drain” electrons from the system, otherwise all of the carriers would remain reduced and electron transport would have to stop.

Role of NADH in electron transport

- The electron donation of NADH (negative redox potential) to FMN is what starts the electron transport chain.
- The free energy of NADH that is released during electron transport is used to do work -- pump protons.

Role of cofactros in ETC

- Cofactors are bound to protein subunits in complexes I,III, and IV.
- cofactors are redox-active cofactors that alternate between reduced and oxidative states as they accept electrons from upstream molecules and then donate electrons downstream to molecules

An inorganic or organic nonprotein group that is necessary for catalysis to take place

- Cofactors essentially carry the electron donated from NADH all the way to oxygen
- Each successive cofactor has a more positive redox potential so that it can oxidize the cofactor before it.

Relationship between redox potential of ETC intermediates and “flow” of electrons

- Electrons flow from NADH - the lowest (most negative) redox potential all the way to oxygen - the highest redox potential. Thus, electron movement is thermodynamically spontaneous and moves down a free energy gradient.

Link between ETC and synthesis of ATP

The energy of the electrons released during ETC is used to do work - the work of transporting protons across the inner mitochondrial membrane from the matrix to the inter-membrane space. The electro-chemical gradient formed can be used to do work (chemiosmosis)

- ATP synthase then catalyzes ATP synthesis using energy from the H+ gradient across the membrane

Effect of uncoupling agents on ATP synthesis

- Uncoupling agents inhibit the production of ATP
- Uncouplers give protons an alternative route of getting back into the mitochondrial matrix.
- A proton is much more likely to enter through the uncoupler than through ATP synthase, and without protons entering ATP synthase, no ATP is synthesized
- Thus, uncouplers can allow for high rates of electro transport but prevent chemiosmotic ATP synthesis .
- If this happens, the energy released during electron transport is not used to do work, it is released as heat instead
- For that reason, newborn kids and hibernating animals can use uncouplers to generate heat and regulate body temperature. (Less energy, more heat)
- If there is low expression of uncouplers, metabolic rate would go up (catabolism of food stores) and this accounts for a higher rate of oxygen consumption -

oxygen is continually reduced and turned into water and no of ATP is made --> leads to obesity (Body continually needs more energy and so it eats more -- eat a lot of sugars that supply quick energy.

Goal of making lactate under conditions of hypoxia

- Hypoxia = deficient oxygen
- When oxygen is absent or in short supply, the pyruvate molecule does not enter the mitochondrion (stays in the cytosol) and as a result, NADH is not oxidized (no NAD is regenerated)
- With no oxygen, NADH cannot be oxidized and ETC will stop. If you cant do that, glycolysis will stop working because glycolysis needs NAD+ (which is usually restored by ETC)
- In **lactate fermentation** pyruvate (3 carbons) is converted into the three carbon compound; lactate.
- This conversion takes in NADH (and one proton) but replenishes NAD+ so that glycolysis can occur under low oxygen.

Role of NAD+/NADH in sensing hypoxia

- Ratio of NAD+ / NADH is known as **redox homeostasis**
- If ratio is too low, too much NADH in relation to NAD+ , which means you have no problem making NADH (glycolysis and CAC are making NADH just fine) BUT you have a problem oxidizing NADH (which ETC and ox-phos should be doing)
- So if you have too much NADH, the most obvious problem is you don't have enough terminal electron acceptors (oxygen) --> could result in going into lactate fermentation/ HIF1 activity

Role of HIF1 activity on pyruvate metabolism

- HIF-1alpha is synthesized and located in the cytosol. If theres a lot of oxygen in the cell, HIF-1alpha never makes it to the nucleus; it gets degraded

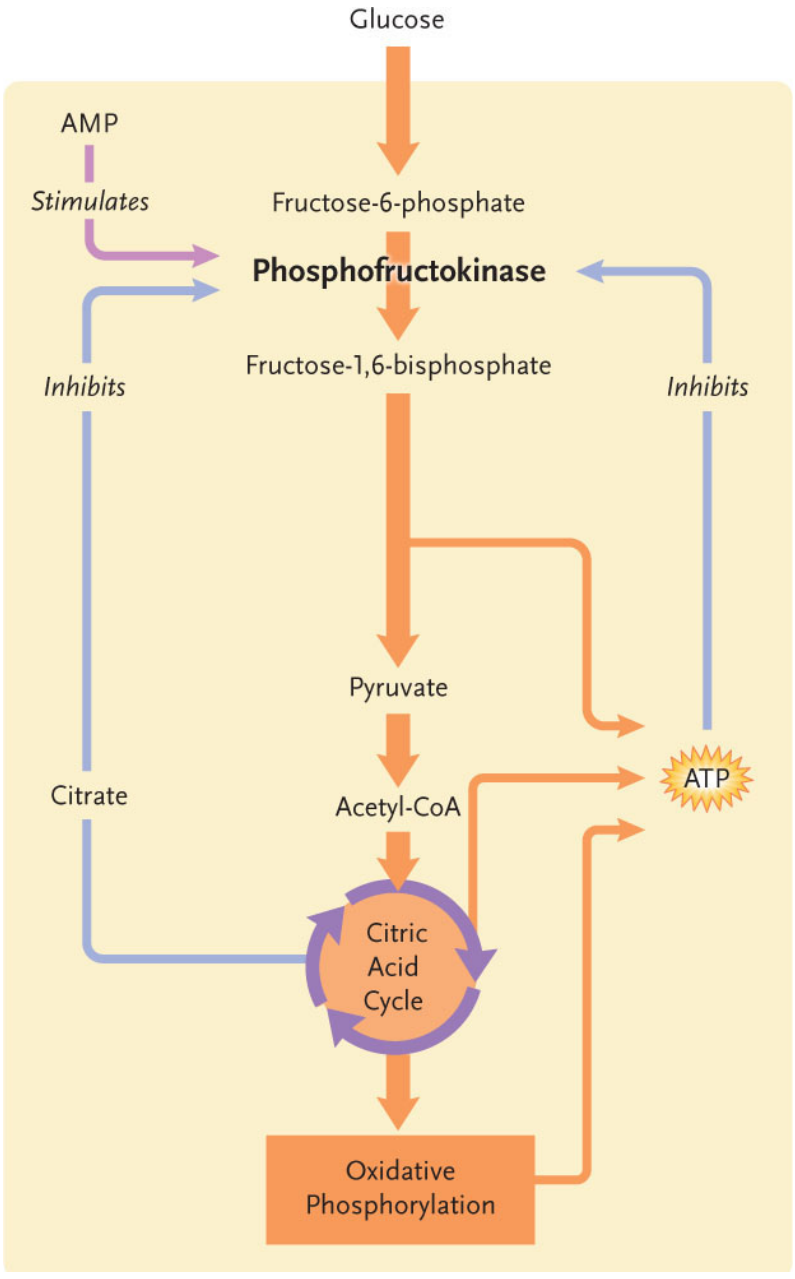
A protein

- It gets hydroxylated and then it is tagged by a molecule called ubiquitin - and then it is sent to the proteosome to be degraded.
- Under low levels of oxygen, it is NOT degraded, so it migrated to the nucleus, binds to the HIF-1beta transcription factor where it makes a functional dimer called HIF-1 transcription factor.
- HIF-1 transcription factor activates a gene which encodes for the enzyme **pyruvate dehydrogenase kinase -** this kinase is synthesized, and then goes to block the *pyruvate dehydrogenase complex* (the cluster of enzymes in the mitochondria)
- This inhibits the metabolism of pyruvate and so pyruvate does not enter the mitochondria - lactate fermentation occurs instead.

Feedback regulation of glycolysis, CA cycle and ETS

- Cellular respiration is controlled by supply and demand - it does this through feedback inhibition : the end products of the pathway (ATP) inhibit the enzyme early in the pathway.
- Phosphofructokinase catalyzes the conversion of fructose 6 phosphate to fructose 1,6-biphosphate.
- Phosphofructokinase is an allosteric enzyme - its activity can be adjusted by the binding of certain metabolic activators and inhibitors
- PFK is regulated by ATP and AMP
- when ATP levels in cell are low, ADP and AMP levels are higher, and vice versa
- AMP is a known allosteric inhibitor of PFK - so if excess ATP is present in the cytosol it binds to the PFK inhibiting its action.
- This decreases the concentration of fructose 1,6-biphosphate and so less pyruvate is made --> less acetyl -CoA made --> less NADH and FADH2 made --> less ATP made , and eventually cellular respiration slows down, resuming ATP levels to normal.
- When ATP is used up, the inhibition of PFK is released.
    - As ATP is used up, AMP is accumulated and acts as an allosteric activator of the enzyme PFK --> more ATP is made and faster
- PFK also sensitive to levels of citrate (produced by CAC

- Increased citrate concentrations means the demand for ATP is low which could imply low levels of oxygen because citrate is not generating NADPH since NADPH will not be oxidized by oxygen if there's too little of it
    - Could also mean citrate is not needed as a carbon skeleton for anabolic reactions.


Glucose
AMP
Stimulates
Fructose-6-phosphate
Phosphofructokinase
Fructose-1,6-bisphosphate
Inhibits
Inhibits
Pyruvate
ATP
Citrate
Acetyl-CoA
Citric
Acid
Cycle
Oxidative
Phosphorylation

PHOTOTRANSDUCTION REVISITED

- Retinal found in its 11-cis configuration (cis means hydrogens are on the same side of double bond) and can be found in all-trans configuration (hydrogens on opposite sides)
- Structurally different molecules but have the same molecular weight
    - To go from cis to trans - the double bond must be broken
        - When retinal absorbs a photon of light you go from 11-cis to all-trans retinal because the photon of light excited one the pi electrons in the double bond - the energy breaks the bond , the molecule swivels and then the double bond is reformed and it is in its all-trans configuration
- Once in trans configuration the retinal becomes linear molecule and no longer fits the bonding site within the opsin - and so it detaches from the opsin.
    - Opsin can no longer absorb light so it must be recycled so that a new cis-retinal can be incorporated inside the opsin.

- This now causes the shape of the protein to change (changes the shape of opsin) - creating a little cleft so that transducin can interact
- Once transducin interacts it activates the enzyme phosphodiesterase

    - The sodium pump located on the membrane is regulated by **cyclic GMP**, So when cyclic GMP is bound, sodium is transported into the cell and there is a sodium influx (excess)
        - The phosphate group in cyclic GMP is bound to the ribose of GMP at the 5' and 3' position. SO when phosphodiesterase cleaves the 3' bond, a 5'GMP is generated.
            - This results in the cyclic GMP detaching from the transporter and as a result the transporter SHUTS OFF and sodium cannot enter the cell.
                - Light through the processes mentioned above, shuts down the sodium pump which **hyper polarizes** the membrane which leads to an electrical signal being sent down the membrane surrounding the rod or cone
                    - The signal moves along the optic nerve and reaches your brain at an incredibly fast speed (as a result you see)

Lecture 8 : Integrated Metabolism

Change in respiration rate (oxygen consumption) in isolated mitochondria by addition of NADH, ATP, uncoupler, etc.

Mitochondria: If mitochondria is added to an oxygen electrode chamber at 2 minutes before anything else : Oxygen is consumed , this causes a drop in the line (very slight slope). Mitochondria consumes oxygen so that it may run mitochondrial electron transport

NADH: Added at 4 minutes : Oxygen consumption increases even further (greater slop/more steep than mitochondria) , by adding NADH, glycolysis' NAD+ stores can be replenished and make more NADH which eventually leads to more electron transport and a greater need for oxygen.

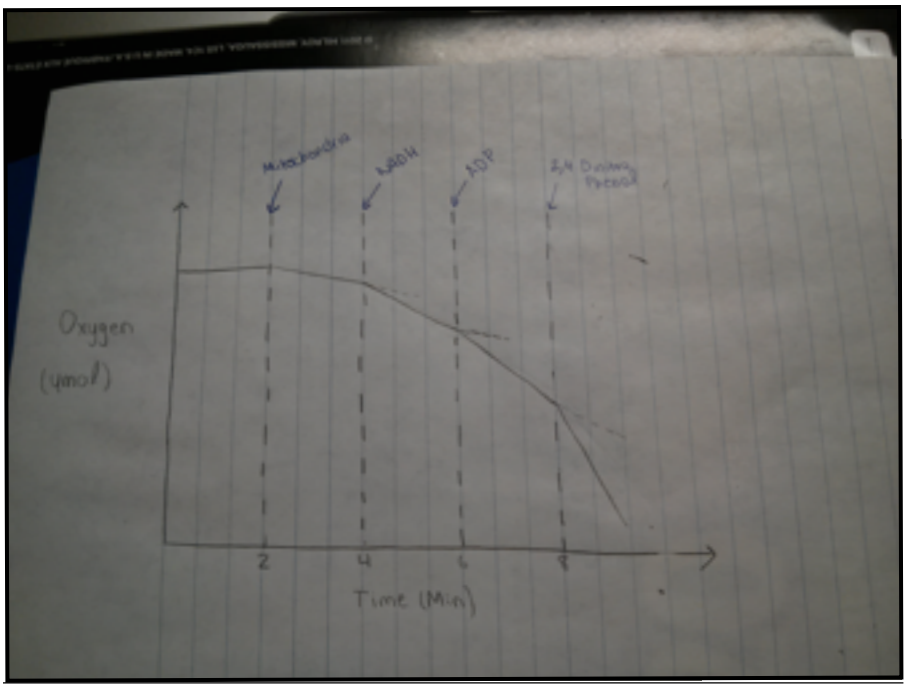
NADH is substrate that can keep cellular respiration running and so oxygen consumption goes up.

ADP: Added at 6 minutes: Even greater increase in slope than NADH's addition

- Without ADP , ATP cannot be synthesized and thus the proton gradient becomes VERY high , and the pH in the inter-membrane compartment becomes very low. It then becomes harder and harder to pump protons into a space that already has a lot of protons and this limits rate of ET.
    - So once ADP is added (the substrate that dives the phosphorylation of ATP), ATP can be synthesized and ET can resume to an enhanced efficiency. This is known as respiratory control

Uncoupler (2,4 DiNitro Phenol)**:** Added at 8 minutes - An uncoupler completely gets rid of the concentration gradient. Without the concentration gradient, protons are pumped/flow MUCH quicker and electron transport as a whole operates much faster. This increase in ET leads to an increase in oxygen consumption (but no ATP is made)

Slope uncoupler > slope ADP > slope NADH > slope Mitochondria



Definition of respiratory control and how it is accomplished (proton gradient)

Respiratory control - rate of electron transport will be limited or dependent upon the availability of ADP (substrate)

Oxidative phosphorylation is also controlled by substrates such as Pi, O2, NADH, FADH2

- When there is no ADP , ATP cannot be synthesized. As a result, the concentration gradient continues to build making it harder and harder for protons to be pumped into the inter-membrane compartment. This slows electron transport, inhibits ATP synthesis and slows down oxygen consumption.
    - This ensures that substrates will not be oxidized wastefully.
- When ADP is aded, oxygen uptake proceeds at an enhanced rate until all of the added ADP has been converted to ATP.

Metabolic links between chloroplast and mitochondria

The metabolic link between chloroplast and mitochondria in a chlamydomonas cell is **REDUCED CARBON**

- Light reactions use ADP to make ATP..... ATP is fed into the calvin cycle and comes out as ADP (which is then used in light reactions)
    - So basically, ATP stays within the chloroplast it is not exported

So then how does mitochondria get carbon to make ATP in chlamy if it does not eat?

- Mitochondria gets its reduced carbon from the calvin cycle - Calvin cycle makes G3P, which exits through a transporter on the chloroplast into the cytosol
- This G3P (reduced carbon) can either be turned into Glucose/A.A/F.A/Pigments (Heme) / Chlorophyll ... etc
    - OR the G3P into pyruvate which then enters the mitochondria of Chlamy and then pyruvate oxidation occurs, making *Acetyl CoA* which is fed into the Citric acid cycle (CO_2 is released) which then eventually makes ATP and water.

- Reduced carbons produced in both Calvin Cycle, CAC, and glycolysis are constantly fed into amino acid and fatty acid biosynthesis.

Reasons why Chlamydomonas can grow as a heterotroph on certain reduced carbon compounds but not others

- Chlamy cannot grow as a heterotroph on glucose because it does not have a glucose transporter. Glucose cannot pass Chlamy's membrane

- However, Chlamy can grow heterotrophically (it can live in the dark) - the molecule it can grow on in the dark is *acetate* (it has 3 C-H bonds which it can use for energy) and sure enough there is an acetate transporter on chalmys membrane that can bring acetate into the cell (there is acetate in chlamys

environment). Once in the cytosol, acetate can enter the mitochondria and there acetate can be converted into Acetyl-CoA

- So chlamy can grow heterotrophically on certain carbon compounds.

Lecture 9 - Saturation Curves

How to measure carbon fixation in Chlamydomonas

- Put chlamy cells in a CO2 analyzer

How one can distinguish between gas exchange in mitochondria from that taking place in the chloroplast of a Chlamydomonas cell

- Measure the CO2 fixation rate of chlamy when the lights are off - This ensures that not carbon dioxide is being produced by the chloroplast and thus an gas exchange or consumption of carbon dioxide is due to cellular respiration (we can assume the rate in the dark is constant)

Identify major parts of a light response curve of carbon fixation

The CO2 fixation rate will increase linearly as the light intensity increases - the rate is linear because the rate of the calvin cycle is directly proportional to the products of the light reactions. It then begins to

What metabolic processes and external factors may influence the change in rate as a function of light

- The linear portion of the curve is limited by NADPH and ATP supplies(products of the light reactions) - needed to work the calvin cycle

However, as light intensity increases, the following limit/change the rate of carbon fixation:

- The speed at which the enzymes in the Calvin cycle can work
    - The speed of Rubisco
- Light saturation is dependent on turnover rate of the enzymes
    - The speed at which RuBP can be regenerated
- **If** not enough CO2 is available, the speed at which CO2 can be fixed is obviously hindered.
- Once the curve begins to bend (plateau) - any light supplied past that point is considered excess light that can be damaging - proteins that p680 and p700 are attached to could break down.

Light compensation point

- The point at which the rate of CO_2 being brought in by the chlorplast matches perfectly with the amount of CO_2 being released by the mitochondria is known as the **LIGHT COMPENSATION POINT** (when CO_2 fixation rate = 0)
  - In order to have a healthy plant, you must be above the light compensation point - many organisms have different LCP's but the light intensity must result in a CO_2 fixation rate over 0 (over the LCP) in order for there to be a net gain in carbon and ultimately growth

Principle of measuring enzyme kinetics as a function of substrate concentrations

- To measure the rate at which enzymes make product, the enzyme concentration MUST be kept constant. Thus you can have different tubes with different substrate concentrations and see how fast the enzymes work. (can be observed through color change)
- As you add more substrate, the enzyme can process more and thus the velocity of the reaction goes up
- At a certain amount of substrate added, the velocity plateaus (the curve plateaus) - this is because the enzyme has reached its maximum speed for turnover rate.

The relation between Km and Vmax

- The measure of the interaction between the substrate and the enzyme is called Km
- Km is the substrate concentration that gets 1/2 Vmax
- Km is a measure of affinity - the attractiveness between substrate and the enzyme complex
- If you have a high Km, you have a LOW affinity (you need more substrate to reach the same speed that an enzyme would reach with less substrate)
- If you are effecient at getting substrate and binging it, you have a very low Km
- The greater the Km the less the affinity between the enzyme and the substrate.

Effect of a competitive inhibitor on enzyme kinetics (Vmax,Km)

- When an inhibitor is added, the Vmax **DOES NOT** change
- The Vmax does not change because so much substrate can be added to the point where the fact that there is an inhibitor there is irrelevant.
- BUT, if an inhibitor is added, the Km becomes larger
  - If 3 inhibitors are added to 4 molecules of substrate - this will affect the 1/2Vmax due to the high ratio of inhibitor to substrate - this decreases the rate and so you need more substrate to get 1/2Vmax , because at lower levels the inhibitor makes a difference
    - As a result the Km shifts right

BECAUSE THE INHIBITOR AFFECTS IT

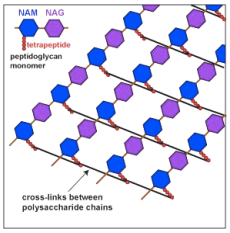
- At high levels of substrate (20 molecules of substrate and 3 inhibitors) Vmax stays the same because there is so little inhibitor compared to substrate that there is no difference

LOW Km = HIGH AFFINITY

- So Vmax and 1/2 Vmax is the same for normal catalysis and for catalysis with an inhibitor
  - However Km is greater when there is an inhibitor (in respectable amounts)
    - Because a competitive inhibitor competes with a substrate for an enzyme's active site, it lowers the enzyme's likelihood of binding substrate (less affinity) and slows the reaction velocity, but leaves the actual amount of end product unchanged

Structure of peptidoglycan

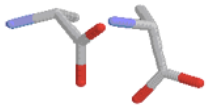
Bacterias cell wall is made out of **peptidoglycan -** a combination of glycan (complex carbs - the blue and purple hexagons) and peptide chains that link the whole system together.



- So when bacteria replicates and needs a new cell wall, **transpeptidase** is the enzyme that fuses this cell wall together -- without transpeptidase you cannot make peptidoglycan.

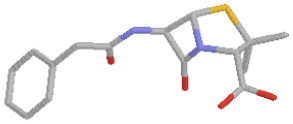
- Transpeptidase is a bacterial enzyme that brings two amino acid groups(carbs) at the ends of two peptides together and links them up. (The two amino acids are shown in the diagram below)

amino acids



NAM and NAG (usually go into active site of transpeptidase)

<u>How penicillin mimics structure of peptidoglycan to inhibit transpeptidase function</u>



• Penicillin looks identical to the two amino acids used by transpeptidase at the bottom (both have 3 red strands oriented the same)

• So, penicillin tricks transpeptidase and gets into its active site and thus can competitively inhibit the enzyme -

- Thats why when you're on penicillin you need LOTS of it so that it can outcompete the regular substrates and so it binds to transpeptidase and 'destroys' it.