The Origin of Life

December-23-10
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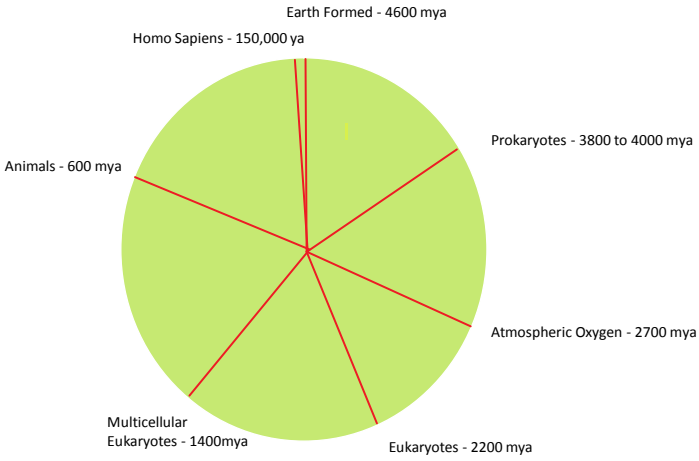
The Origin of Life

Define Life

Formation of Earth	4600mya
Prokaryotes	3800mya
O_2	2700mya
Eukaryotes	2200mya
Multicellular Eukaryotes	1400mya
Animals	600mya
Homo Sapiens	150000ya



Life developed early - evolved within 600my
- Stromatolites - 3500mya
- Caused by cyanobacteria
  - Cyanobacteria increased O_2 levels in atmosphere?

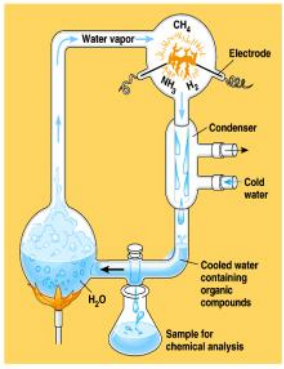
Where did life develop?
- Deep Sea vents
  - Hot, nutrient rich, high pressure
  - However, teeming with life
  - Extremophiles
- Panspermia?
  - Life seeded from outer space.
    - Possible explains speed that life appeared in
  - Extreme conditions - however, extremophiles

Prebiotic Evolution

What do we need?
- Abiotic synthesis
  - Make nucleotides etc.
- Heritable Information
- Formation of Cells
- Three Major Stages
  - Geophysical
    - What was the composition of Earth and the Atmosphere
  - Chemical
    - How could the building blocks be synthesised
  - Biological
    - How do the building blocks organise into cells
- First two stages fairly well understood
- However Biological stage is poorly understood

Geophysical Stage
- What were the condition like on Primordial Earth
  - Shit hot!
  - Took a few hundred million years just to cool down.
- Early Atmosphere
  - H_2O, H_2, CH_4, NH_3, H_2S
  - All of the atoms for biological molecules are present
  - Energy sources for catalysis
    - Ultraviolet light, lightening
  - Reducing atmosphere

Chemical Stage
- Can you make organic molecules from inorganic ones?
- Miller-Urey experiment
  - Important monomers are made (within a week)
    - amino acids
    - Sugars
    - Purines and pyrimidines
- Todays atmosphere has too much oxygen for abiotic synthesis
  - Too oxidising



Miller-Urey Experiment

Chirality
- A chiral molecule is one that is not superimposable on its mirror image
  - Optical isomers (enantiomers)
  - Handed molecules
- Amino acids are handed
  - Same chemical and physical properties
  - Yet different biological properties
- Thalidomide

The Chirality Problem
- Miller-Urey Experiment gave racemic mixture
  - Equal left and right handed molecules
- Biology is homochiral
  - L amino acids, D sugar

Origin of Homochirality
- Homochirality important to the evolution of life
- Specificity is required
  - Enzyme-substrate interactions
- Random Chance?
  - Choice of one over the other
- Extra-terrestrial origin
  - Meteorites containing amino acids
  - However, not racemic
    - Favour L isomer
    - Murchison Meteor

Biologic Stage
- Development of DNA, RNA, Protein triad
- Synthesis of polymers
  - Miller-Urey made monomers

- But how do you get polymers out of those monomers?
- The first cells
- Problem!
  - In order to go from DNA to RNA, and RNA to protein, you need enzymes
  - Enzymes = proteins!
- Solution
  - RNA came first!

RNA

- Can carry information
- Has structural ability
  - Can fold
  - Complementary base pairing
- Can catalyze reactions
- Ribosome
  - Ancient organelle
  - 2/3 RNA, 1/3 Protein

Ribozyme

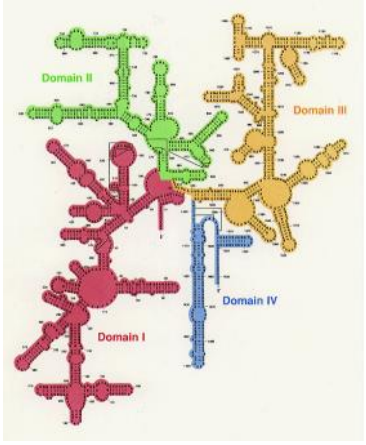
- Discovered by Cech in 1989
- RNA molecules that can catalyze reactions
  - Self-splicing introns
    - Catalyze own excision
  - Ribosome aminotransferase activity

Evolution of Information Transfer

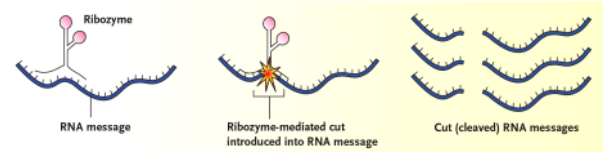
- First cells, just RNA
  - Information, structure and catalysis
- The proteins evolved
  - Took over structure and catalysis
  - More diversity
- Then finally, DNA
  - Took over information
  - More stable than RNA
  - No oxygen (Deoxyribose)
  - Thymine replaces Uracil
    - C to U mutation is very common
    - Enzyme has to be able to differentiate between U that's supposed to be there and one that's not
  - Complimentary strands

The First Cells

- Monomers to Polymers
- Accelerated by clay (montmorillonite) particles
  - High surface area
  - Charged surface
  - Micelle to Vesicle (spontaneous)



RNA Map



Readings

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2.1 What is life

- At a molecular level, living and non living organisms are not that different
  - atoms and molecules are the same for them
- Chemical reactions in living organisms are just modifications of those that take place in the abiotic world
- Living cells obey the same laws of chemistry and physics as the abiotic world
- All forms of live share a set of seven attributes that collectively differentiate them from the abiotic world
  - Display Order
    - all living things are arranged in a highly ordered manner, with cells the fundamental unit of life
  - Harness and Utilise Energy
    - all forms of live acquire energy from their environment and use it to maintain their highly ordered state
  - Reproduce
    - all livings things have the ability to make more of their own kind
  - Respond to Stimuli
    - all living things can make adjustments to their structure, function and behaviour in response to changes in their environment
  - Exhibit Homeostasis
    - all living things are able to regulate their internal environment in order to keep the conditions relatively constant
  - Growth and Development
    - all living things increase their size by increase either the size or number of their cells
  - Evolve
    - populations of living things evolve over generational time to better suit their environment
- Some organisms straddle the definition of life
  - viruses are the best example
  - the characteristics of life that a virus possess (e.g. reproduction) are dependant on it's ability to infect other living cells
  - for this reason, viruses are not considered to be alive by most
- All living things are composed of one or more cell
  - In Prokaryotes - single celled organisms - the one cell is a functionally independent organisms, capable of carrying out all of the characteristics or life
  - In Eukaryotes - multicellular organisms - the characteristics or life are divided amount a number of specialised cells
    - each individual cell in a eukaryote is able to survive alone when placed in a chemical medium that can sustain them
- Cells are the smallest unit that have the properties of life
- Cells arise only from the growth and division of pre-existing cells
  - DNA and RNA, although possessing all the information required to manufacture new cells, cannot organise the assembly of new cells; they need the cellular machinery or a pre-existing cell

2.2 The Chemical Origins or Life

- Early History or Earth marked by bombardment of rocks from the still forming solar system and high volcanic and seismic activity
- The Earth - being or sufficient mass - was able to hold an atmosphere around the plant
  - The atmosphere was derived partially from the original dust cloud that formed the solar system, and partially from gases that were released from the planet's interior as it cooled down
- The Earth took approximately 500 million years to cool to temperatures that could sustain the development of life
  - As the Earth radiated away some of it's heat, it’s surface layers cooled and solidified into the rocks that form the crust
- Conditions on primordial Earth were very different than those found today
  - the primordial atmosphere probably contained high levels of…
    - H_2O vapour from evaporated H_2O at the surface
    - H_2S - Hydrogen Sulphide
    - CO_2 - Carbon Dioxide
    - NH_3 - Ammonia
    - CH_4 - Methane
    - Some of these molecules would have formed spontaneously in the atmosphere, while others would have formed as the result of volcanic eruptions
    - All of these molecules contain the basic building blocks needed to form molecules essential to the formation of life
- The Atmosphere on Primordial Earth was a Reducing Earth
  - The Oparin-Haldane Hypothesis (1920's) states that the organic molecules essential to the formation of life could have been formed abiotically given the conditions present on primordial earth
  - The atmosphere was rich in full reduced molecules - molecules than contain a full compliment of electrons and hydrogen
  - This provided a rich source of electrons that could be easily donated in order to make reactions that lead to the synthesis of large, complex, electron rich organic molecules possible
  - Lack of oxygen in the primordial atmosphere meant that there was an abundance of UV light from the sun - along with lightening - to provide sufficient energy for the build up of complex organic molecules

- Today's atmosphere is an oxidising one - contains 21% oxygen, which is too much for a reducing atmosphere - which prevents the synthesis or complex, electron rich molecule
- The Miller-Urey Experiment (1953) gave experimental proof to the Oparin-Haldane
    - Miller recreated the atmosphere in the laboratory
        - Ran the experiment for a week and found organic compounds such as urea, amino acids and lactic, formic and acetic acids
    - Other molecules thought to have been present in the primordial atmosphere - HCN (Hydrogen Cyanide) and CH_2O (formaldehyde) - have been tested in the Miller-Urey experiment
        - when these were tested, all the building blocks of life were created, including the purine and pyrimidine building blocks or nucleic acids, sugars and phospholipids
- Highly reducing conditions would also have been found near volcanoes and hydrothermal deep sea vents
    - areas around these vents today are teeming with life able to survive despite the extremely harsh conditions present
    - these vents not only release geothermally heated water, but also methane and ammonia
        - building blocks or life could have been formed in these places too
- The key building blocks or life are macromolecules, built up by the synthesis of large numbers of monomers
    - only monomers were produced in the primordial reducing atmosphere
- Today, the synthesis of macromolecules needs enzymes
    - enzymes themselves are macromolecules, so were not present in the primordial atmosphere
    - so how do you make polymers that are required for life without enzymes
- Solid Surfaces - like Clay - provided a catalytic surface for polymerisation to occur on
    - it is doubtful that polymerisation would have occurred in the primordial atmosphere
    - even if it had, the molecules would have quickly hydrolysed and broken down
    - Clay would have been present in evaporating tidal pools
    - Clays consist of thin layers of minerals separated by layers or water
        - this layered structure readily absorbs ions and organic molecules and promotes their interactions; including condensation and other assembly reactions
        - clay can also store potential energy, and could have therefore channelled some of this energy into the reactions taking place
- The first cells to develop would have been Protobionts
    - Protobionts is the term given to a group of abiotically produce molecules surrounded by a membrane like structure
- The Development of protobionts allowed for an internal environment distinctly different from the external one
    - key molecules could be concentrated
    - molecules could attain more order in a closed space
- Experiments show that protobionts could have formed spontaneously given the conditions of primordial earth
    - Lipid molecules - which are hydrophobic - form into selectively permeable lipid bilayers, or liposomes, spontaneously

<u>2.3 The Origins of Information and Metabolism</u>

- Of the critical events needed for the development of life, the development of a system for storage, replication and translation of information - for protein synthesis - and the development of metabolic pathways - to harness energy for metabolism - stand out.
- All Organisms use DNA as their information system
    - DNA functions similarly in all organisms; the information from DNA is copied into RNA which then directs the synthesis of Proteins
    - This flow - DNA to RNA to Protein is common to all forms of life
    - this information pathway is preserved from generation to generation by the ability of DNA to direct it's own replication
    - Changes in DNA contribute to evolutionary changes over generational time
- Enzymes are needed to catalyze the replication of DNA, transcription to RNA and translation to Protein
    - If enzymes are needed, then how did a system in which the products - proteins/enzymes - are required to catalyze each step of the process
- In 1979 Thomas Czech discovered that a group of RNA molecules - know are Ribozymes - can act as catalysts in reactions involving precursor RNA molecules that lead to their own synthesis and also unrelated RNA molecules
    - RNA can do this as it is a single stranded molecule that can fold into specific shapes - much like proteins - giving it specific activity
- Early Life may have existed in an 'RNA World' in which RNA filled the roles of information carriers and catalysts
- Life today is dominated by DNA and proteins because they can do their respective jobs - information and catalysis - far better than RNA can
    - the evolution of these molecules by organisms would have given them a huge advantage over those that had not developed them
- The first cells may have contained only RNA, which then lead to the evolution of a small population of RNA molecules that could catalyze the formation of simple proteins
    - This would give these cells a huge advantage, as proteins are far more versatile than RNA for three reasons
        1) Enzymes have far greater catalytic power than ribozymes
        2) Proteins are far more diverse than RNA; 20 different amino acids make up proteins, opposed to the 4 nucleotide bases that make up RNA
        3) Amino acids can also interact with other amino acids in bonding arrangements not possible between

nucleotides
    - This massive diversity in structure and function of proteins led to their role as dominant structural and functional molecules in the cell
- DNA would then have developed after proteins
- Compered to RNA, DNA is more complex
    - DNA is double stranded and contains the sugar Deoxyribose, which is more difficult to synthesis
    - at first, DNA nucleotides may have been produced by the random removal of oxygen
    - at some point these DNA nucleotides would have paired with RNA nucleotides and assembled into complementary copies of RNA sequences
- Once DNA copies were made, selection may have favoured DNA as it is a better way to store information compared to RNA for three main reasons
    1) DNA is more stable than RNA due to the presence of Deoxyribose instead of Ribose
    2) In DNA the base Uracil has been replaced by Thymine
        - A C to U mutation is very common, therefore in DNA the presence of U is easily recognised as a mutated C and therefore easily repaired.
    3) DNA is double stranded, meaning that in the case of mutation the complimentary strand can be used to repair the damage
- The next development would have been the development of Energy-Harnessing Reaction Pathways
    - in primitive cells, redox reactions were probably among the first energy releasing reactions
    - However the electrons released in oxidation would have been transferred directly to the substances being reduced
    - This single step process would have been very ineffective and wasted a lot of energy, there for the development of a multistep process would have given organisms a huge advantage.
    - The greater efficiency of stepwise energy release would have favoured the development of intermediate carries and paved the way for the evolution of primitive electron transport chains
        - respiration is a good example of this.
- ATP became established as the coupling agent that lings energy releasing - or exogonic - reactions and those requiring energy; otherwise know as endogonic reactions
- ATP probably initially entered the cells as one of many organic molecules and was simply hydrolysed into ADP and Phosphate resulting in the release of energy
    - later, as cells developed, some of the energy release was probably used to synthesis more ATP from ADP and PI
    - Due to efficiency of energy transfer by ATP it gradually became the primary substance connecting energy releasing and requiring reactions

<u>2.4 Early Life</u>

- The earliest conclusive evidence for life comes in structures called Stromatolites - layers of rocked formed by microorganism activity - dated to 3.5 million years ago
- Modern day Stromatolites are formed by the action of cyanobacteria
    - cyanobacteria contain sophisticated metabolism that suggests that earlier life forms must have predated their evolution
- Indirect evidence - in the form of sedimentary rocks depleted of carbon 13; not fixated by organisms - trace life back to 3.9 million years ago
- Panspermia is the hypothesis that very simple life forms present in outer space may have seeded early earth and has two main points of discussion
    - life evolved very quickly.
        - The earth formed 4.6 Billion years ago, with life appearing between 3.9 to 3.5 billion years ago. Given that the earth had to cool own substantially - taking about 500 million years - many argue that this is not a large enough window of time for life to evolve on it's own
    - Life is able to survive in extreme conditions.
        - Extremophiles can thrive under very harsh conditions or temperature, pressure and nutrients and therefore may be able to survive for some time in outer space
        - Prolonged dormancy is a property of spores, which arte highly resistant to changes in the external environment and can restore active growth after exposure to high levels of radiation, water deficiency and extremes of temperature - conditions present in outer space.
- All cells - prokaryotic and eukaryotic alike - share common fundamental features
    - all cells possess selectively permeable membrane to separate the external environment from the cytosol; made up of mostly water, salts and various organic molecules and organelles.
    - all cells possess membrane proteins that control what enters and leaves the cell and are responsible for electron transport chains
    - The DNA of all cells is organised into Chromosomes
    - The processes of translation and transcription are fundamentally similar - relying on ribosomes for the synthesis of proteins from an RNA template.
- Prokaryotes, despite their small size and relative lack of internal membrane organisation are highly diverse
    - prokaryotes display high metabolic flexibility and are able to use a variety of substances as energy and carbon sources and to synthesis all their required organic molecules from simple, raw, inorganic molecules
    - In many ways, prokaryotes are more biochemically diverse than eukaryotes and vastly outnumber all other types of organism and are able to live successfully in almost all of the Earth's surfaces.

The Drake Equation

January-07-11
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Radio Astronomy

- Unable to travel to other planets
- So use Radio Astronomy to detect Radio Waves from other Civilisations
    - Started in the 60's
    - Fuck-shit-all picked up so far

Drake Equation

- Probability that other civilisations exist
- Named after Frank Drake

$N = N_s \times f_p \times n_e \times f_l \times f_i \times f_c \times L$

N = Number of advanced civilisations in our galaxy
N_s = Number of stars
f_p = fraction of stars that have planets
n_e = number of planets that can potentially support life
f_l = number of planets that develop life
f_i = number of planets that develop intelligent life
f_c = number of planets able and willing to communicate
L = average lifetime of a civilisation

N_s

- Roughly 100 billion stars in the Milky Way

f_p

- What fraction of stars have planetary systems?
- How can we detect extra solar planets
    - Transit Method
        - Measure for dimming of stars as planets pass in front of them
    - Astrometry/Doppler spectroscopy
        - Detect "wobble" in the movement of a star
        - Caused by gravitational tug of planet orbiting the star
    - Direct Imaging
        - Not used that often
        - Works for very massive planets

n_e

- how many habitable planets per planetary system
- set n_e as 2 - two habitable planets per system on average
- if only half of all stars have planets
    - still 50 Billion stars with planets!
- Total number of habitable planets
    - $10^{11} \times 0.5 \times 2$ = 100 Billion planets

Water

- habitable zone defined by liquid water
    - has water, habitable
    - no water, inhabitable
    - 0 to 100 degrees = habitable zone
- shouldn't be a liquid at the temperature it is
    - similar molecules turn to gas at -53 degree
    - hydrogen bonding keeps it liquid
- High heat capacity

- minimizes the electrostatic interactions of molecules
    - form hydration shells around molecules
    - protein interactions based on shape, opposed to charge

Life elsewhere in the Solar System?

- may have been life on Mars
- once liquid water
- frozen water and CO_2 at its poles
- Titan and Eropa
    - Titan has dense atmosphere
    - May be life on one or both of the two

f_l

- What fraction of planetary systems develop life
- what do we know of life on Earth?
- If in habitable zone, all planets will develop life eventually
- so f_l is set at 1
- 100 billion planets have life!
    - conservative estimate

f_i and f_c

- proportion of planets with intelligent, communicating life
- set at 10% for each
- 1 billion planets with intelligent, communicating life in our galaxy

L

- Lifetime of a civilisation
- the longer a civilisation is kicking about, the greater the chance that it is kicking about RIGHT NOW!
    - therefore the greater the chance that we can find them
- age of our communicating civilisation = 100 years
- 100 years/10 billion years
- if civilisation lasts 1000 years on average
- L = 1000/10 billion or 1/10000000 then

$N = 10^{11} \times 0.5 \times 2 \times 1 \times 0.1 \times 0.1 \times 0.0000001$

Fermi Paradox

- If so many extra-terrestrial civilisations, where the fuck is everyone?!
- Can't detect them
    - galaxy is too big!
    - distances between them is too big
    - different technologies
        - assume that we all use technology that the other can detect
- or….no others exist

Introductory Biochemistry, Genetics, Molecular Biology

January-11-11
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<u>Transcription and Translation</u>

Gene
- A sequence of DNA that encodes a Protein
  definition for Maxwell's part)

Transcription
- DNA to RNA
- Gene converted in m-RNA
  - Messenger RNA

Translation
- RNA converted into Protein using Ribosome

<u>Expression</u>
- Constitutive
  - Always expressed
- Induced
  - Switch on and off at various times
- Repressed
  - Off.
- Controlled by transcription factors

Transcript abundance
- Amount of Gene present at any one time
  - Amount of transcript present controlled by…
    - Rate of transcription
    - Rate of decay

<u>Measuring Transcript and Protein Abundance</u>
- Northern Blot
  - Transcript abundance
  - Expression measured over time at certain temp.
- Western Blot
  - Protein abundance

<u>Peptides</u>
- Amino acids linked by peptide bonds
  - Hydration reaction
- Amino Acids
  - Amino group
    - NH_2
  - Carboxylic group
    - COOH
  - R-Group
    - Differs from acid to acid
    - Some hydrocarbon group
- Bonds between Amine and Carboxylic form peptide bond
  - Make up peptide backbone

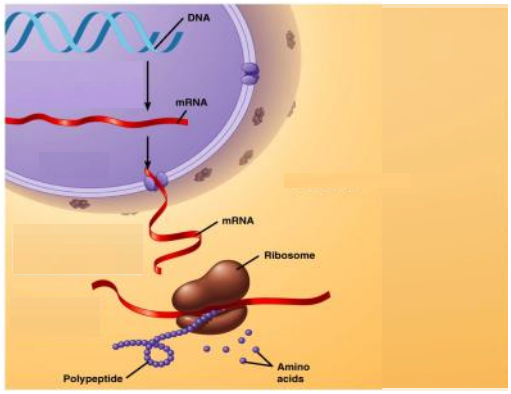
<u>Amino Acids</u>
- Synthesised in Cytosol
  - Polar environment
- Some polar, some non-polar
- Most proteins contain many non-polar amino acids
  - Hydrophobic effect

<u>Protein Structure</u>
- Primary Structure
  - Linear sequence of amino acids
  - Typical protein is about 500 amino acids in lengths
- Secondary Structure
  - Produced by interactions between atoms in the peptide backbone
    - Hydrogen bonding
  - Two major forms
    - α- helix
    - β- sheets
- Tertiary Structure
  - Overall 3-D shape of single chain
  - Formed by folding
  - Bonding between R groups
    - Hydrogen bonding
    - Ionic bonding
    - Van der Waals
    - Disulphide bonding
  - Give functional Protein.
  - Native conformation

❗ To be functional, proteins must be flexible
- Quaternary Structure
  - Two or more native polypeptides
  - e.g. Haemoglobin

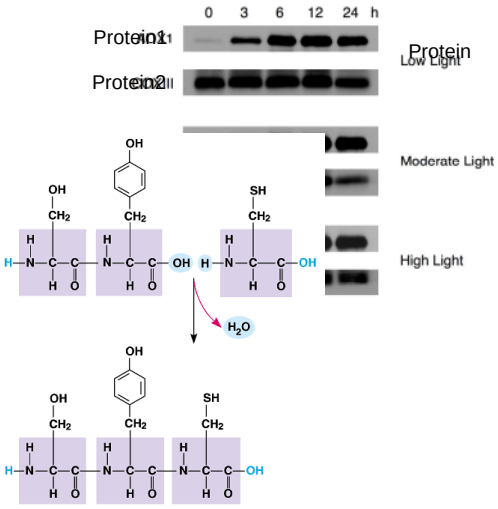
<u>Protein Folding</u>

How do you go from Primary to Tertiary?
- Discovered by Christian Anfinsen

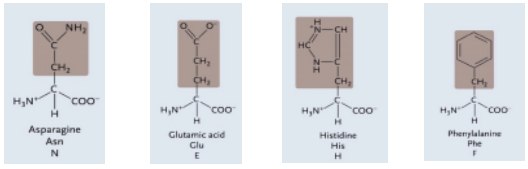


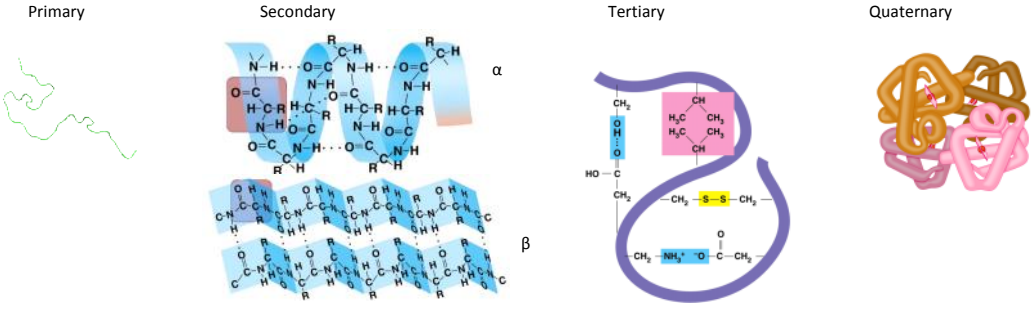
Northern Blot - Transcript abundance

- Western blots - Protein abundance



Amino Acids





Anfinsen's dogma

- e.g. Haemoglobin

Protein Folding

How do you go from Primary to Tertiary?

- Discovered by Christian Anfinsen
- Took a folded protein in vitro
- Unfolded it using Urea (H_2N-CO-NH_2)
- Then removed Urea, refolded it
  - Regains over 90% of original activity
- Protein folding is spontaneous
- Folding dependant solely on primary sequence
- Folding is on a millisecond timescale

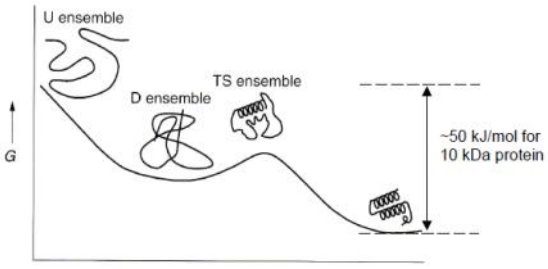
Anfinsen's dogma

Levinthal Paradox

- Assume each amino acid backbone can be in 3 conformational states
- For 101 residues, there as $3^{100} = 5x10^{47}$ conformations
- If the protein can sample a new conformation at a rate of 10^{13} s^{-1} it would take 10^{27} years to try them all
- That's LONGER THAN THE AGE OF THE UNIVERSE!
- Protein must fold in a pre-arranged pathways and in a cooperative manner

What Drives Protein Folding?

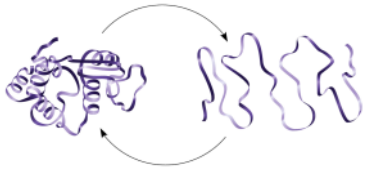
- Native conformation is always lowest energy state
  - Based on Gibbs Free Energy
- Funnel Hypothesis
  - Pathway is not always the same
  - End point is always lowest energy
- Driven by Secondary Structure and Hydrophobic effect
  - Secondary structure comes first
  - Then hydrophobic effect kicks in
  - The more hydrophobic ends exposed, the higher the energy state



Protein Structure Prediction

- Predicting folding is linked to…
  - Many diseases
  - Drug development
- However no existing algorithm for it
- Crystal Structure
  - Crystalized Native conformation
  - X-rays, view shape
  - However expensive
  - Not many crystal structures known

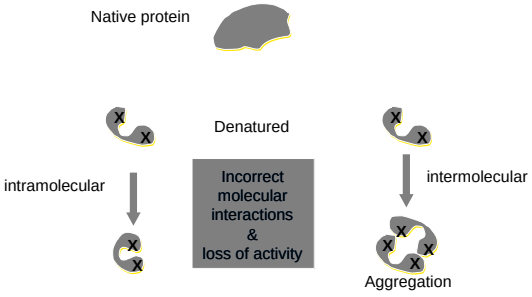
Denaturisation



Protein Denaturation

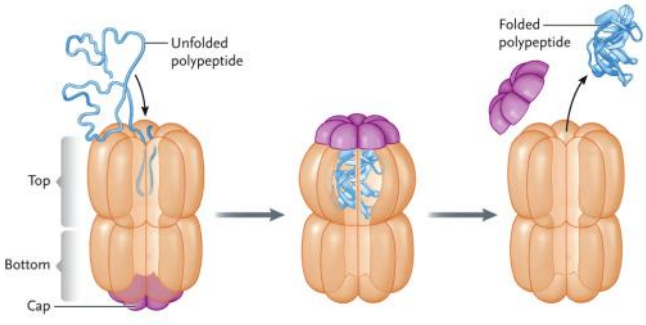
- Loss of native conformation
  - Heating
  - pH
  - Organic Solvents
  - Urea
- Consequences of denaturisation
  - Leads to misfolding
  - Aggregation (interactions between two or more)
  - In general…
    - Incorrect interactions and loss of activity

Consequences of denaturation



Macromolecular Crowding

- At low concentrations folding occurs correctly (.01mgml^{-1})
  - In vitro
- In cells (in vivo) much higher concentrations (<300mgml^{-1})
  - Other interactions to interfere with folding process
- Many proteins helped to acquire correct shape
  - Shielded by molecular Chaperones
- Molecular Chaperones
  - Interact with and stabilise none native forms of proteins
  - Not part of final assembly
  - Assist folding and assembly
  - Modulation of conformation
  - Transport (need to cross membranes)
  - Disaggregation of protein aggregation
  - ATP-dependant
  - Some are constitutively expressed
  - Heat shock proteins
    - Induced under high temps or other stresses
    - Assist folding or prevent denaturisation



Readings

April-09-11
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14.1 The Connection Between DNA, RNA, and Protein

- How do we know that Genes encode Proteins?
  - Archibald Garrod and William Bateson's (1896) work with *Alkaptonuria* - a disease which causes urine to turn black on exposure to air - showed that there is a specific relationship between genes and metabolism
  - George Beadle and Edward Tatum' (1940's) work with *Neurospora crassa* showed the direct relationship between genes and enzymes, and lead to the 'one gene, one enzyme hypothesis' - which was later adjusted to the 'one gene, one polypeptide hypothesis'
- The Pathway from gene to polypeptide has two major steps; transcription and translation
  - Translation is the mechanism by which the information encoded in DNA is made into a complementary RNA copy
  - Transcription is the use of the information encoded in the RNA to assemble amino acids into a polypeptide
  - Francis Crick gave the name Central Dogma to the flow of information from DNA to RNA to Protein in 1956
- In Transcription the enzyme RNA polymerase creates an RNA sequence that is complementary to the DNA sequence for a given gene
  - For each of the thousands of genes in a cell, one strand of the DNA strands is the template strand
  - the template strand is read by the RNA polymerase, transcribing a messenger RNA - or m-RNA.
- In Translation, m-RNA assosciates with a ribosome - a particle on which amino acids are linked into a polypeptide chain
  - as the ribosome moves along the m-RNA, the amino acids specified by the m-RNA are joined together one at a time to from the polypeptide encoded by the gene.
- The processes of transcription and translation are similar in both eukaryotes and prokaryotes with one key difference
  - Prokaryotes transcribe and translate a given gene simultaneously
  - Eukaryotes transcribe a given gene in the Nucleus and then export it to the cytoplasm to be translated
- Both the DNA and RNA alphabet are made up of 4 bases
  - DNA is made up of the bases Adenine (A), Thymine (T), Guanine (G) and Cytosine (C)
  - RNA is made up of the base Adenine, Uracil (U), Guanine and Cytosine
- Despite there being only 4 RNA bases, there are 20 amino acids
  - the nucleotide information that specifies the amino acid sequence of a polypeptide - called the genetic code - uses the RNA bases in groups of at least 3
  - Each sequence of 3 bases is called a codon
- The Codons in DNA are first transcribed into complimentary RNA codons
- The DNA template strand is always read 3' to 5'
  - which means the RNA is made 5' to 3' (polymerases can only add on to the 3' end)
- Of the 64 possible Codons, 61 specify amino acids
  - These are know as sense codons
  - One of these codons - coding for AUG - specifies methionine, which is the first codon translated in any m-RNA and is know as the start or initiator codon
- Three codons - UAA, UAG and UGA - do not specify an amino acid
  - These are know as stop - or nonsense or termination - codons and act as periods indicating the end of the polypeptide encoding sequence
- All but two amino acids are coded for by two or more codons
  - this means that there are many synonyms in the genetic code, leading to degeneracy - or redundancy.
- The genetic code is also commaless

    - the codons are sequential, leading to there being only one correct reading frame for each polypeptide chain marked by the start codon - AUG.
- The genetic code is - save a few exceptions; yeast and protozoans for example - universal; the same codons specify the same amino acids in all organisms
    - this suggests that the genetic code was established in its present form early on in the evolution of life, and has undergone very little change over time

Energy and Thermodynamics

January-13-11
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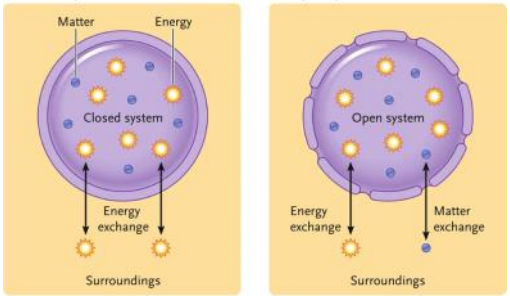
Closed, Open and Isolated Systems



In isolated…

- No exchange of matter or energy

However assume closed = isolated

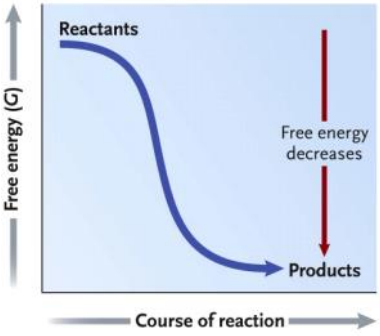
Laws of Thermodynamics

- total energy = constant
- transformations increase entropy
- Spontaneity

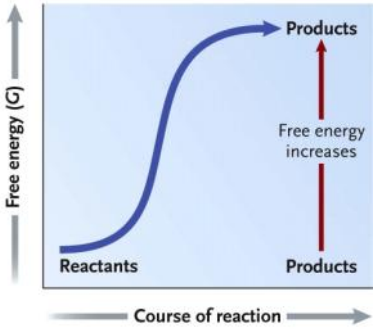
Gibbs Free Energy

- $\Delta G = \Delta H - T\Delta S$
- Exergonic Reaction
  - $\Delta G < 0$
- Endergonic Reaction
  - $\Delta G > 0$

exergonic

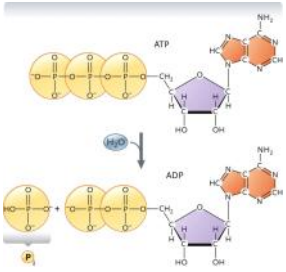


endergonic



<u>Free Energy, Stability, Work Capacity</u>

- More free energy = less stable = greater work capacity
- less free energy = more stable = less work capacity



<u>ATP Hydrolysis</u>

- ATP most dominate energy source
- $\Delta G = -7.3 kcalmol^{-1}$
- Phosphate groups are negatively charge
  - high repulsion
  - bond easily broken

<u>ATP and Energy Coupling</u>

- In body, energy release not lost as heat
  - lost as heat in solution
- using exergonic process to drive an endergonic process
  - e.g. converstion of glutamic acid to glutamine
  - not spontaneous
  - phospahte in ATP used to make glutamic acid unstable and more reactive
  - makes reaction spontaneous
- Not lost as heat for various reason
  - process requires an enzyme
  - ATP breakdown instead of hydrolysis
  - direct transfer of phosphate
    - not released
  - ATP has high Phosphor group transfer potential

ΔG = +3.4 kcalmol-1

glutamic acid + ammonia → glutamine

$\Delta G = -7.3 kcalmol^{-1}$

$\Delta G = 3.9 kcalmol^{-1}$

<u>Equilibrium</u>

- closed systems reach equilibrium
- open systems never do

Readings

April-09-11
4:16 PM

4.1 Energy and the Law of Thermodynamics

- Life is an energy-driven process, however energy can not be measures directly and can only be detected through it ability to do work
    - moving objects against opposing forces; gravity, friction or pressure for example
    - or to push chemicals reactions to completion
- Energy is therefore defined as the capacity to do work
- Energy exists in many different forms
    - heat, chemical, electrical, mechanical, electromagnetic radiation
    - all forms of energy can be converted readily from one form to the other
- All forms of energy can be grouped into two different states - Kinetic and Potential.
    - Kinetic energy is the energy posses by an object when it is in motion
    - Potential energy is energy stored by an object due to its location or chemical structure
- In thermodynamics, the object being studied is the system
    - a system can be anything, from a single molecule to a cell to the planet
- Everything outside of the system is the surroundings
- The universe - within this context - is the total of the system and it's surroundings
- There are three different types of systems; Isolated, closed and open
    - an isolated system does not exchange matter or energy with it's surroundings
        - do not come across them in nature
    - a closed system exchanges energy but no matter with its surroundings
        - the earth can be considered a closed system
    - an open system exchanges both matter and energy with its surroundings
        - all living things are open systems
- There are Two laws of thermodynamics
    - The First Law states: Energy can be transformed from one form into another or transferred from one place to the other, but cannot be created or destroyed
    - The Second Law states: the total disorder - entropy - or a system and its surroundings always increases
        - a system will move spontaneously towards arrangements of greater entropy
        - maintaining low entropy requires the input of work - or energy.
- Life seemingly disobeys the second law of thermodynamics
    - one of the qualities of life is that it is highly ordered; cells make highly ordered molecules from lots of smaller, disordered ones
    - Living cells are open systems, and therefore bring in energy and matter from their surroundings and use them to generate order out of disorder
    - Organisms metabolise food in order to provide the energy needed to maintain this low entropy/high order
- According to the second law, things constantly break down
    - this means that cell structures become damaged and have to be replaced and maintained
    - organisms consume food in order to maintain low entropy
    - however, metabolic by-products released into the surrounds are of high entropy, thus increase the entropy of the surrounds while maintain low entropy of the system
    - the entropy of an organism is allowed to remain low so long as the entropy of it's surrounds continuously increase

4.2 Free Energy and Spontaneous Reactions

- Applying the first and second laws of thermodynamics allows us to determine if a reaction will be spontaneous or not
    - spontaneous reactions may occur at a fast rate; for example combustion, or at a slow

rate - such as the formation of rust on a nail

- Two factors relating to the laws of thermodynamics need to be taken into account to determine if a reaction is spontaneous or not
    - Reactions tend to be spontaneous if the products have less potential energy than the reactants
        - The potential energy of a reaction is called its enthalpy
        - Endothermic reactions absorb energy, thus their products have more energy than the reactants
        - Exothermic reactions give off energy, thus their products have less energy than their reactants
    - Reactions tend to be spontaneous when the products are less ordered than the reactants
- Energy transformations are never 100% effective, therefore the proportion of the systems energy that is available to do work is called the Free Energy - G
- In living organisms, free energy is used in activities such as molecular synthesis, movement and reproduction
- The change in free energy - ΔG - for a reaction can be calculated by the following formula
    - $\Delta G = \Delta H - T\Delta S$
    - ΔH is the change in enthalpy
    - ΔS is the change in entropy
    - T is the absolute temperature in K (°C + 273)
    - The equation says that the free energy change as a systems goes from initial to final states is the sum of the changes in energy content and entropy
- For a reaction to be spontaneous, ΔG must be negative
    - for all chemical and physical processes, there is an interplay of both entropy and enthalpy to determine whether the process will be spontaneous or not
- ΔG represents the difference between the free energy of the final state compared to the initial state
    - a negative ΔG indicates that the products have less free energy than the reactants
    - systems with high free energy are less stable than those with a lower free energy
- Systems can spontaneously change into more stable states, but cannot spontaneously changes into less stable states.
- Another term for maximum stability is equilibrium
    - As system moves towards equilibrium, the free energy of its systems becomes progressively lower, reaching its low point and maximum stability when the reaction system is at equilibrium - at this point, ΔG = 0.
- For each reaction, the point at which is obtains equilibrium is related to the ΔG for the reaction.
    - the more negative the ΔG is, the further towards completion the reaction will proceed before it reaches equilibrium
    - many reactions have a ΔG near to 0, and they may be readily reversed by making slight adjustments to the concentrations of the products and/or reactants
- The ΔG for living organisms is always negative as they are constantly taking in molecules and using them to do work
    - organisms only reach thermodynamic equilibrium upon death
- Reactions can be placed in one of two groups - Exergonic and Endergonic Reactions.
    - an exergonic reaction released free energy - ΔG is negative
    - an endergonic reaction gains free energy - ΔG is positive
- In Metabolism, individual processes are part of metabolic pathways
    - Catabolic pathways involve the breakdown of molecules, and have overall negative ΔG
    - Anabolic pathways involve the building up of complex molecules, are often called biosynthetic pathways, and have overall positive ΔG
    - however, the individual steps do not have to all be the same sign, the overall sign for ΔG just has to be positive or negative

<u>4.3 The Energy Currency of the Cell</u>

- A massive amount of reactions take place within cells that involve the assembly of complex

molecules, and are therefore endergonic
    - cells supply the energy that drives these reactions through the use of ATP, which is highly conserved and used by all forms of life
- ATP contains a large amount of free energy due to its three closely associated phosphate groups
    - phosphate groups are negative, and thus repel each other making ATP unstable and highly reactive
    - the removal of one or two phosphate groups by hydrolysis is a spontaneous process
- When ATP is dissolved in water in a test tube, the hydrolysis reaction releases a large amount of free energy which in turn heats up the surrounding water
    - however this rarely occurs in cells
    - If ATP was hydrolysed in cells then there would be no way to trap the heat produced and use it to do work
    - in fact the build up of heat would often lead to cell death
- The free energy from ATP is harnessed through the use of a process called energy coupling
    - ATP is brought into close contact with a reactant molecule involved in the endergonic reactions by an enzyme
    - The ATP is the hydrolysed and the terminal phosphate group is transferred - again by enzyme action - to the reactant molecule
    - This phosphorylation causes the reactant molecule to become unstable
    - The coupling systems works by joining - or coupling - an exergonic reaction - in this case the hydrolysis of ATP to ADP and Pi - and an endergonic reaction
- Coupling reactions take place continuously throughout all living cells, consuming massive amounts of ATP, so how do cells regenerate ATP?
    - ATP is regenerated from ADP and Pi - an endergonic process - the energy for which comes from the catabolic metabolism of complex, energy rich molecules; such as carbohydrates, fats and proteins.
    - this continuous breakdown and resynthesis of ATP is known as the ATP cycle

Enzymes

January-20-11
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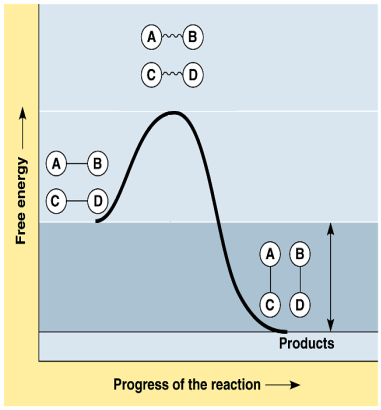
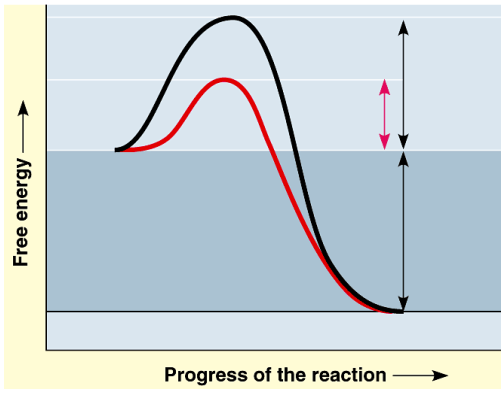
Enzymes

- Enzymes speed up rate of an exergonic reaction
- Biological catalysts
- massive change in rate of reaction
  - 10^12 - 10^20 faster than un-catalysed
- cant do anything to and endergonic reaction
  - to drive endergonic reaction, need energy coupling etc.
- Enzymes do not provide energy!
  - need energy? harvest light, break down ATP etc.



Exergonic Reaction Energy Profile

- for every exergonic reaction, needs to acquire a higher energy state
  - known as transition state
  - energy needed to reach transition state is activation energy
    - E_A
    - very small amount of energy
    - often energy from random collisions
- E_A is a kinetic barrier
  - prevents reaction from occurring quickly
  - e.g. Propane
    - breakdown of propane is exergonic
    - Activation energy is difficult to overcome
    - thermodynamically unstable
    - but activation energy prevents it from just blowing up all the time
    - kinetically stable



Enzymes lower activation energy
(Red line shows reaction in presence of an enzyme)>>>>>>>>>

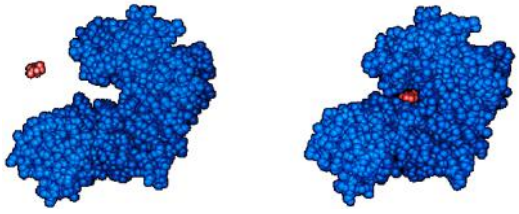
- Rate of reaction is proportional to the number of activated molecules
  - number of molecules that can reach transitional state
- Enzymes lower energy required to reach transitional state
  - more molecules have required energy

Why does life need enzymes?

- Need rates of reaction to be fast
- however cant just give energy to the system (e.g. as heat)
  - Need to remain at temperatures that are compatible with life

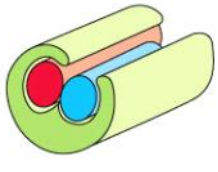
Enzyme Structure

- Enzymes are proteins
  - function is tied to its tertiary conformation
  - flexibility is important - substrate binding changes conformation
    - induced fit model
  - site of catalysis is called active site
    - where substrate binds to enzyme
    - Active site is tiny compared to size of enzyme
- E + S ↔ ES → E + P
  - Substrate binding is an equilibrium
  - Catalysis is a unidirectional reaction
  - Enzyme is not consumed or used up
    - free to react with more substrate
  - Rate of catalysis is stupid fast
    - fastest enzymes can cataylse 500 substrate per second

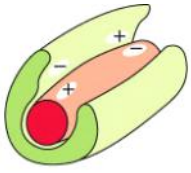


How Do Enzymes Lower E_A

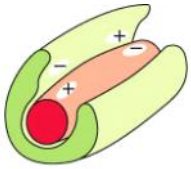
- Precise orientation of two substrates
- Charge interactions
- Conformational Strain
  - substrate not in quite the right shape



orientation



- Charge interactions
- Conformational Strain
  - substrate not in quite the right shape
- Catalytic site mimics the transitional state
  - provides correct orientation, charge etc.

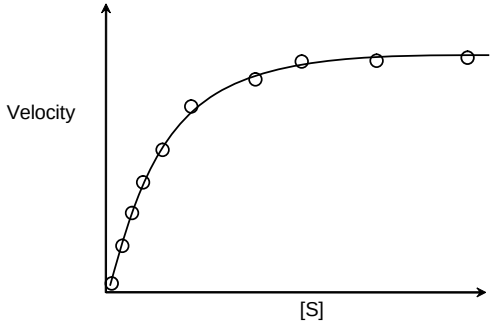


charge

strain

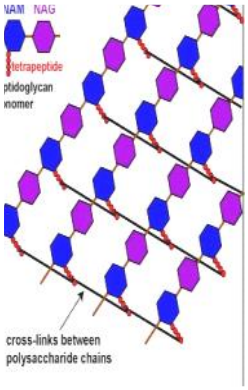
Enzyme Kinetics

Enzyme kinetics



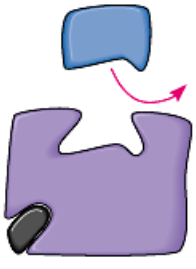
- 10 reaction tubes
  - same [Enzyme]
  - Increasing [Substrate]
- Increasing substrate increases rate of reaction to a point
- Eventually enzymes become saturated
  - all active sites filled at any one time
  - reaction cant proceed any faster
  - V_{max}
    - measures at saturating levels of substrate
- K_m
  - substrate concentration needed to get 1/2 V_{max}
  - measure of affinity
    - attraction between substrate and enzyme

Enzyme Inhibition



Competitive

- Inhibitor directly competes for active site with substrate
- can be overcome by adding more substrate
- can reversible or irreversible
  - inhibitor can bond permanently or temporarily
- inhibition slows down rate of reaction at a given [S]
  - V_{max} is still the same, just at higher [S]
- e.g. Penicillin
  - interferes with synthesis of cell wall in bacteria
  - Peptidoglycan wall
  - Transpeptidase links amino acids to form wall
  - Penicillin mimics amino acids for active site of transpeptidase



Non-Competitive

- inhibitor does not bind to the active site
- binds somewhere else on enzyme
- changes the shape of the enzyme, fucks up active site
- substrate cant bind to active site anymore

Enzyme Prosthetic Groups

- Prosthetic Groups
- e.g. Chlamydononas - Green Alga

- Nitrogen assimilation
  - used to synthesis amino acids
- Mutant cant grow on NO_3^-, only NH_4^+
- No defect in Nitrate reductase apoprotein (protein with out prosthetic group
- Nitrate Reductase needs co-factor, or prosthetic group
  - Molybdenum cofactor needed for functional enzyme
  - cant produce molybdenum cofactor

<u>Enzymes and Growth Rate</u>

Readings

April-10-11
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4.4 The Role of Enzymes in Biological Reaction

- Chemical Reactions require bonds to be broken - either by first being strain or made otherwise unstable - before a reaction can occur
- Getting reactant molecules into this unstable state requires a small input of energy - know as the Activation Energy (E_A)
- Once the reactants gain this necessary activation energy, the enter into a transition state, in which the bonds are unstable and easily broken
- Molecules that take part in chemical reactions are constantly in motion, and occasionally this motion may provide a few of them enough energy to enter into the transition state
- If a significant number of reactant molecules reach the transition state, then the free energy that is released may be enough to get the remaining molecules into the transition state and start the reaction
- While Chemists can use heat to provide the needed activation energy, this is problematic in Biology for two reasons
    - High temperatures destroy the structural components or cells and can result in cell death
    - An increase in temperature would speed up all possible chemical reactions in the cell, not just the ones that are part of metabolism
- So how can you increase the rate of a reaction without raising the temperature?
    - Catalysts are chemical agents that speed up the rate of a reaction without itself taking part in its reaction
    - the most common biological catalysts are a group of proteins known as enzymes
- The activation energy of a reaction represents a kinetic barrier that prevents spontaneous reactions from proceeding quickly
    - the greater the activation barrier, the slower the rate of reaction
- Enzymes increase the rate of the reaction by lowering the activation energy
    - the rate of reaction is proportional to the number of molecules in the transitional state, enzymes increase the rate of reaction by making it possible for more reactants to achieve this transition state
    - although enzymes lower the activation energy of a reaction, the overall free energy change remains the same, the only difference is the path that the reaction takes
- In enzymes reactions, an enzymes combines with reacting molecules and is released unchanged when the reaction is complete
- Each type of enzyme catalyzes the reaction of one molecule or a group of closely related molecules
- The substrate of a reaction only reacts with a small part of the enzyme - the active site.
    - the active site is usually a pocket or groove that is formed when the newly synthesised protein folds into its correct conformational shape
- Lock and Key Fit
    - Enzymes active sites are rigid and only certain substrates or closely related substrates can bind to it
- Induced Fit
    - enzymes are not rigid objects, but are instead flexible
    - prior to substrate binding, the enzyme changes its conformation - or shape - so that the active site becomes an even more precise fit with the substrate
- Enzymes bind to substrates, forming and enzyme-substrate complex
    - Catalysis occurs when the two are joined, with the enzyme converting one or more substrates into one or more products
    - because enzymes are released unchanged after the reaction has occurred, enzymes can rapidly bind more substrate molecules giving the same reaction in what is called the enzymes cycle

- Many enzymes require a cofactor - a nonprotein group such as a metal ion- in order to function properly
    - these cofactors are often essential to active site binding of substrates
- Enzymes can increase the number of reactants that achieve the transitional state by three mechanisms
    - Bringing the reacting molecules closer together
        - many reaction require that the reactants collide with a precise orientation
        - enzymes bring two reactants together in the orientation needed to for the reaction to occur
    - Exposing the reactant molecules to altered charge environments that promote catalysis
        - in some systems, the active site of the enzyme may contain ionic groups whose positive or negative charges alter the substrate in a way that favours catalysis
    - Changing the shape of a substrate molecule
        - the active site may strain or distort a substrate molecule into a conformation that mimics the transitional state
- Several conditions can alter enzyme activity, including changes in substrate concentration, changes in temperature and changes in pH
    - In addition to this, there are also several mechanisms by which enzyme activity can be regulated
- In the presence of excess substrate, the rate of reaction is proportional to the concentration of enzyme in the reaction
    - the enzyme concentration is the limiting factor on the rate of reaction
- In the presence of low concentrations of substrate concentrations the rate of reaction will be very slow due to the infrequency with which the enzyme and substrate molecules collide and react
    - however as the substrate concentration is increased, the rate of reaction will initially increase as the enzyme and substrate molecules collide with increasing frequency
    - However as the enzyme molecules reach the maximum rate at which they can process the substrate molecules, increasing substrate concentration has an increasingly smaller and smaller affect on the rate of reaction
        - at the point that the rate of reaction no longer increases when the concentration of substrate is increased, the enzyme is said to be saturated - that is they are turning over substrate molecules as fast as they can
- The rate at which an enzyme can catalyze a reaction can also be lowered by the action of enzyme inhibitors - molecules that bind to and decrease the activity of an enzyme
    - Inhibitors that bind to the active site of an enzyme display competitive inhibition - they compete with the substrate for the binding of the active site
    - Inhibitors that bind with another site on the enzyme outside of the enzyme display non-competitive inhibition - their binding to the enzyme causes a change in the conformation of the enzyme's active site, meaning that the substrate is no longer able to bind to the active site
- Inhibition an be either reversible, or irreversible
    - In revisable inhibition, the enzyme is readily released and is able to continue binding and processing substrate
    - in non-reversible inhibition, the enzyme is permanently altered - the inhibitors bind strongly through the formation of covalent bonds to the point that the enzymes is completely disabled
        - this type of inhibition can only be undone by the synthesis of more enzymes
- Many cellular metabolites act as reversible inhibitors of enzymes and are part of an important mechanism of metabolic regulation
    - A futile cycle is when two metabolic pathways run simultaneously in opposite directions, having no overall effect other than wasting energy
    - In order to prevent this from occurring, cells are able to regulate enzyme activity in such a way that not all enzymes are active at the same
    - Many enzymes are regulated by natural inhibitors; control by the inhibitors changes enzyme activity to meet the needs of the cell for the products of the reaction catalyzed by the enzyme

- In allosteric regulation, enzyme activity is regulated by the effects non-competitive binding to the allosteric site of an enzyme
    - enzymes controlled by allosteric regulation have two conformation states - a high-affinity (active) and a low-affinity (inactive) state
    - binding of an allosteric activator changes the conformation from low-affinity to high-affinity, conversely the binding of a allosteric inhibitor changes the enzyme from high-affinity to low-affinity
    - allosteric inhibitors are often the product of the metabolic pathway that the regulate
        - this is an example of feedback inhibition
- The activity of most enzymes is strongly altered by changes in pH or temperature
    - enzymes operate within a narrow range of pH and temperature - outside of this range the activity of an enzyme will drop off and eventually reach zero
    - Each enzyme has an optimal range of pH, at which its operates at peak efficiency for increasing the rate of it's biochemical reaction
        - the effects of on the structure and function of the active site become more extreme at pH values farther from the optimum until the rate drops to zero
    - The effect of temperature changes on the activity of an enzyme are controlled by two distinct processes
        - First, temperature has the effect of speeding up all reactions by increasing the kinetic energy and thus the rate of successful collisions
        - Second, temperature has an effect on all proteins - including enzymes.
            - as temperature rises, proteins become denatured as hydrogen bonds and other bonds break
        - the two effects of temperature act in opposition to each other
        - thus the rate of an enzyme-catalyzed reaction peaks at the point that the increase in kinetic energy is the greatest without an significant unfolding of the enzymes structural conformation

Membrane Biology

January-26-11
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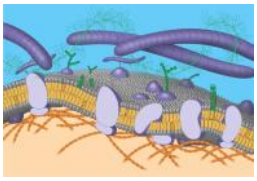

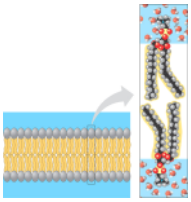

Membranes
- Lipid Bilayer
  - fundamental backbone
  - forms spontaneously
- 50% Protein
  - roles in transport, signal transduction, electron transport

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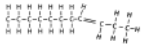

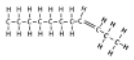

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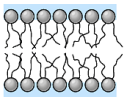
Lipid Molecules
- Amphipathic
  - both hydrophilic and phobic parts
  - Detergents are Amphipathic
- Fatty Acids make up Hydrophobic part
  - saturated - only C-C bonds, full H compliment
  - unsaturated - C=C bonds present
    - Trans-unsaturated
      - Hydrogens across from each other
      - very rare in nature
    - Cis-Unsaturated
      - Hydrogens on same side
      - readily made in nature
- Composition of fatty acids alters shape of bilayer
  - Saturated
    - all straight chains - closely packed
  - Un-saturated
    - more kinks - more space
    - higher viscosity at same temp
  - Viscosity affect by temperature
    - important for organism that change internal temp with ambient temperature, unlike humans
      - Control viscosity by regulating Desaturase enzymes
      - Desaturase introduce C=C bonds
    - normal membrane viscosity = vegetable oil
    - Increase in temperature means decrease in Desaturase levels
      - high [Desaturase] at low temperature



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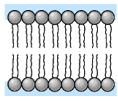

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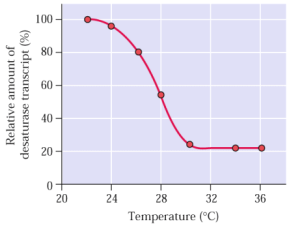
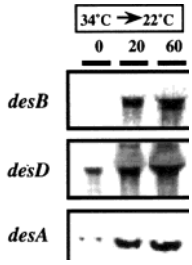
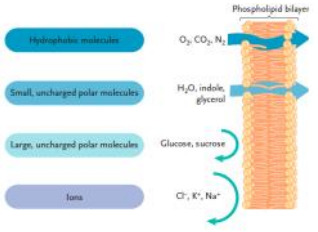
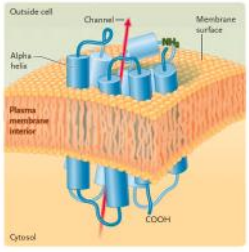


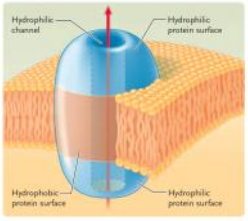


Membrane Permeability
- Permeability of Membranes important for most biological processes
- transport across membranes is dependant on two factors
  - size
  - charge
- Small, Uncharged molecules can diffuse through membrane

So how do you get other stuff across?
- Membrane Proteins



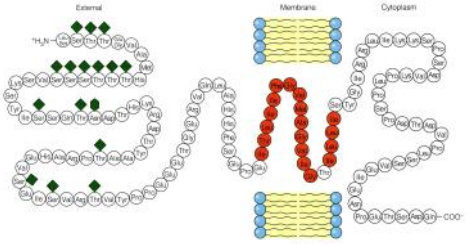




Membrane Proteins
- Proteins which span the lipid bilayer and create a channel which molecules can move through
- Proteins interact with hydrophobic core
  - made up of charge, acidic molecules
  - However, made up of Alpha Helix structures
    - minimizes charges of the backbone
  - Tends to be made up of non-polar amino acids

<u>Transmembrane Protein Prediction</u>

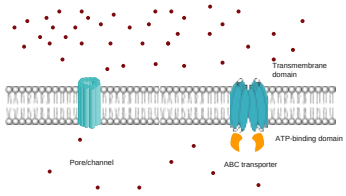
Given the primary sequence of a protein can you predict whether it spans a membrane?
- takes 17 to 22 amino acids to span lipid bilayer
- looking for section dominated by non-polar amino acids
- many proteins loop back across membrane
  - looking for multiple sections of mostly non-polar amino acids



Membrane Transport

Passive Transport
- Simple Diffusion
  - [High] to [Low]
  - for small uncharged molecules
  - driven by entropy
- Faciltated Diffusion
  - diffusion through protein carrier/channel
  - assists diffusion of larger/charged molecules

Membrane transport



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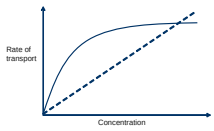
Active Transport
- energy dependant
- going against concentration gradient
- use of transpost proteins
  - eg. ABC Tranporters
    - ATP Binding Cassette Transporters
    - Two components
    - ATP Binding domain
    - Transmembrane domain

Tranport Kinetics

Why Would you want to facilitate diffusion?
- Dotted line = Simple diffusion
  - rate proportional to concentration difference
- Block line = facilitated diffusion
  - at low concentration differences, higher rates of transportion tha simple diffusion alone
  - However can become saturated
    - Only so many transport proteins
  - Past a certain concentration, rate reaches a max.

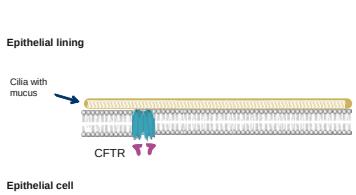
Transport kinetics



Cystic Fibrosis
- most common inherited disease (autosomal recessive)
- average life expectance is around 40 years of age
- impairment of lung and intestinal function
- caused by defect in CFTR gene
  - 6000 bases, 1480 amino acids, 27 exons
  - 400 different mutations can cause Cystic Fibrosis
  - most common is ΔF508
    - 70% of causes
    - deletion of Phenylalanine

Physiology of CFTR function
- In normal lung
  - CFTR pumps Chloride

Physiology of CFTR function

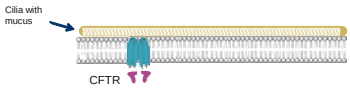


- 70% of causes
- deletion of Phenylalanine

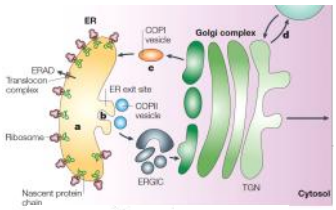
Physiology of CFTR function

- In normal lung
  - CFTR pumps Chloride
  - allows osmosis into Epithelial lining
  - moist Epithelial lining
  - allows for lung clearing
    - expels dust, bacteria etc.
- In Cystic Fibrosis Lung
  - Chloride pump is disrupted
  - Epithelial lining becomes dry
  - become susceptible to bacterial infections
  - breathing is inhibited
- CFTR is found on plasma membrane
- Processes through Secretory Pathway

Epithelial lining

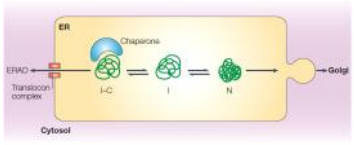


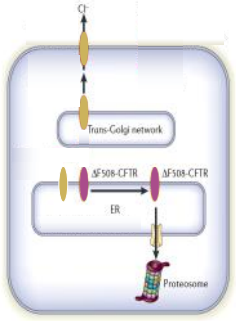
Epithelial cell



Biochemical effects of ΔF508-CFTR

- Mature protein can transport Chloride when made In Vitro
  - 60% of wild type transport
  - more than enough for normal lung function
- Issue is with processing of the protein
- E.R. tags peptide as defective
  - gets degraded
  - gets broken down in Protosome
- Protein Quality Control System in E.R.
  - asses is protein will fold correctly
  - Chaperones and folding sensors detect stability of protein
  - ΔF508-CFTR wont fold correctly, so degraded.





Why is Cystic Fibrosis so common

- 1 in 2,500 Caucasians have CF
- 1 in 22 Canadians carry to Cystic Fibrosis allele
- carriers show no symptoms
- is there a heterozygote advantage?
  - explains high dominance in population
  - Advantage may come from Cholera
  - carriers may lose less water than homozygous dominant
  - more likely to survive Cholera outbreak
  - mass Cholera outbreaks in 19th and 20th century Europe
- Prevalence of Cystic Fibrosis is not present in Asia or Africa
  - Cholera outbreaks a lot less frequent

Readings

April-16-11
6:11 PM

5.1 An Overview of The Structure of Membranes

- Out current view of membrane structure is based on the fluid mosaic model
    - this model proposes that membranes are not rigid, but consist of fluid lipid molecules in which proteins are embedded and float freely
- The lipid molecules of all biological membranes exist in a double layer, called a bilayer
- The lipid molecules of the bilayer vibrate, flex back and forth, spin around their long axis, move sideways and exchange places within the same bilayer half
    - lipids rarely move from one side of the bilayer to the other
    - movement within the bilayer takes place millions of times a second, making the bilayer a hihgly dynamic place
- The mosaic part of the model refers to the fact that membranes contain a massive assortment of different proteins each with a specific function
- Because they are larger tan lipids, the proteins move more slowly in the fluid environment
    - a small number of membrane proteins which anchor the membranes to the cytoskeleton do not move at all
- The relative proportions of lipids and proteins within the bilayer vary depending on the type of membrane
- An important characteristic of membranes is the components of one half of the bilayer differ from those that make up the other - this is know as membrane asymmetry and reflects the different functions performed by each side of the membrane
- The fluid mosaic model is supported by two pieces of experimental evidence
    - Membranes are fluid
        - in 1970 Frye and Edidin grew human and mouse cells separate cultures
        - they marked the human cells with red dye, and the mouse cells with green dye
        - they then fused the human and mouse cells
        - within minutes the two differently coloured proteins had began to mix, and in less than an hour then had completely intermixed
        - based on measured rates, the membrane bilayer appears to have the same fluidity as light machine oil
    - Membrane Asymmetry
        - A block of cells is rapidly frozen using liquid nitrogen
        - the block is then fractured by hitting it with a microscopically sharp knife edge
        - often the fractured bilayer splits along the line of the inner and outer membrane
        - under an electron microscope, the membrane appears as smooth layers with individual proteins embedded
        - it is clear that the particles on either side of the membrane different in size, number and shape

5.2 The Lipid Fabric of a Membrane

- The foundation of all biological membranes is lipid molecules
- Keeping membranes in a fluid states is important to membrane function
    - many organisms can adjust the types of lipids in the membranes such that membranes do not becomes too stiff of fluid
- The Dominant Lipids found in membranes are phospholipids
    - these consist of two fatty acid 'tails' linked to one of several types of alcohols or amino acids by a phosphate group
- A critical property that all phospholipids posses is that the are amphipathic
    - each mo0lecule contains a hydrophobic region - the fatty acid chains - and a hydrophilic region - the phosphate containing head
- When added to an aqueous solution, phospholipids spontaneously associate with each other

and form bilayers
    - these arrangements occur spontaneously because they represent the lowest energy state and are more likely to occur over any other arrangement
- The fluidity of the lipid bilayer is dependant on how densely the individual lipi layers can pack together
    - this is influenced by two major facts
        - the composition of the lipid molecules that make up the membrane
        - the temperature
    - Fatty Acids composed of saturated hydrocarbons tend to have a straight shape, allowing for the lipids to pack more tightly together
    - Lipid molecules with unsaturated fatty acids are less straight as the unsaturation introduces kinks or bends in the fatty acids tail, meaning that the lipids cannot pack as tightly together
- Membranes remain in a fluid state over a relatively wide range of temperatures, however if the temperature drops low enough the phospholipids become more closely packed and the membrane forms a highly viscous semisolid gel
- At a given temperature, the fluidity of the membrane is related to the degree to which the lipids are unsaturated
    - the more unsaturated the membrane, the lower its gelling temperature
    - for most membrane systems, the normal fluid state is achieved by a mix of saturated and unsaturated lipids
- The maintenance of membranes in a fluid state is absolutely essential to cell function
    - exposure to low temperature may result in membrane viscosity increasing to the point that normal membrane permeability is inhibited
    - at high temperatures membranes may become too fluid which may result in membrane leakage
- Most organisms can adjust the fatty acid composition of their membranes so that the proper fluidity is maintained over a relatively broad range of temperatures
    - organisms are able to survive at low temperatures partially because they are able to increase the relative proportion of unsaturated fatty acids in their membrane through the activity of Desaturases
- Desaturases take the saturated fatty acids - all fatty acids are synthesised fully saturated - and remove two hydrogen atoms from neighbouring carbon atoms and introduce a double bond
    - there are a wide range of desaturases that introduce double bonds at different location along the carbon chain
- Changes in transcription of a gene often result in changes in the abundance of its m-RNA transcript and the resulting protein abundance
- The transcript abundance of desaturases increase as temperature decreases
    - this results in an increase in the synthesis and overall activity of the enzyme, which in turn results in higher abundance of unsaturated fatty acids in the membrane
    - by regulating the amount of desaturase present, organisms can maintain the proper fluidity of their membranes
- Besides lipids a group of compounds called sterols also influence membrane fluidity
    - cholesterol - found in animal but not plants of prokaryotes - is the most well known
- sterols act as membrane buffers
    - at high temperatures they help restrain the movement of lip[id molecules - thus reducing the fluidity of membranes - while at low temperatures the disrupt fatty acids from associating by occupying space between lipids - thus slowing the transition to the non-fluid gel state

5.3 Membrane Proteins

- Membrane Proteins can be separated into four major functional categories
    - Transport Proteins either provide hydrophobic channels for substances that cannot freely diffuse across the membrane or change shape and shuffle specific molecules from one side of the membrane to the other
    - A number of membrane proteins are enzymes - such as cytochrome oxidase

    - Receptor proteins involved in signal transduction exist on both sides of the membrane
- All membrane proteins can be classified into one of two distinct categories
    - Integral Membrane proteins are embedded in and span the lipid bilayer, they therefore have polar regions that are exposed to the aqueous environment on either side of the membrane and non-polar regions - made up of sequences of 17 to 20 non-polar amino acids coiled into α-helixes
        - since many Transmembrane proteins span the membrane several times, the non-polar sections are usually linked by polar sections
    - Peripheral Membrane Proteins are positioned on the surface membrane and held in place by non-covalent bonds with exposed portions of Transmembrane proteins
        - most peripheral proteins are on the cytoplasmic side of the membrane

5.4 Passive Membrane Transport

- Membranes control what gets in and out of cells as the hydrophobic nature of a membrane restricts the free movement of many molecule
    - molecules such as oxygen can diffuse easily across the lipid bilayer, while other larger or charged molecules which are also essential cannot
- Passive Transport is the movement of a substance with out the need for ATP - it is driven by diffusion.
    - Simple diffusion is the movement of molecules - small, uncharged or polar - across a selectively permeable membrane from an area of high concentration to one of low concentration
        - large or charged molecules and ions can not move across the membrane by diffusion alone
    - Facilitated Diffusion is the movement of large or charged molecules or ions across the membrane with the help of protein complexes that span the membrane
        - channel proteins simply form hydrophilic channels across the membrane that the molecules or ion can pass
        - carrier proteins also form a passage through the lipid bilayer, however they do so by binding a specific molecule
        - this binding results in a conformational change to the protein, which then carriers the bound molecule across to the other side of the membrane
        - because a single solute is transferred in this manner, it is referred to as uniport transport
    - many transport proteins show a high degree of specificity - much like enzymes - which allows various cells and cellular compartments to tightly control what gets in and out
- Water can also move passively across a membrane by the process of osmosis
    - osmosis is the net movement of water molecules across a selectively permeable membrane by diffusion, from a solution of lesser solute concentration to a solution of greater solute concentration
    - osmosis can occur by simple diffusion or by water-specific transport proteins called aquaporins
    - aquaporins are narrow channels that allow for the single file movement of water and contain a positive charge in the center of the channel that prevents the movement of other molecules through them
    - The movement of water is dictated by solute concentration
        - If the solution surrounding a cell contains dissolved solutes at lower concentration than in the cell - hypotonic - water will diffuse into the cell
        - if the solution surround a cell contains dissolved solutes at higher concentrations than in the cell - hypertonic - water will diffuse out of the cell
        - If the solution surrounding the cell contains dissolved solutes at the same concentration as inside the cell - isotonic - then there will be no net movement of water in or out of the cell

5.5 Active Membrane Transport

- Active Transport concentrates molecules inside cells and moves ions in or out of cells against a concentration gradient - from low concentration to high concentration - through an energy dependant mechanism
- There are two kinds of active transport - primary and secondary
- In primary active transport the same protein that transports a substance also hydrolyzes ATP to power the transport directly
- In secondary active transport, the transport is indirectly driven by ATP
    - the proteins that transport the substance do not hydrolyze Atp, but instead rely on a favourable concentration gradient of ions - built up by primary active transport - as their energy source
- Other features of active transport resemble facilitate diffusion
    - both processes depend on membrane transport proteins
    - both are specific
    - both can be saturated
    - both use carrier proteins that change their conformation as they function

Respiration I

January-27-11
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Metabolic Pathways

Catabolic Pathways
- overall- ΔG
- Breaking something down
- individual steps don't have to all be -ΔG
  - some are exergonic
  - some are endergonic
- However overall, must be -ΔG
- e.g. Cellular Respiration
  - Catabolic Process
  - yet some of the reactions have +ΔG

Anabolic Pathway
- overall +ΔG
- Building something up
- as with Catabolic, not all steps have to be +ΔG
- but must be +ΔG overall.
- e.g. Protein Synthesis
- often called Biosynthetic Pathways

ATP/ADP Cycle
- fundamental to pathways
- cells need free energy
  - comes from ATP
- Energy for ATP synthesis comes from Catabolic Pathways
- ATP Broken down, free energy released used to drive Anabolic Pathways
- cycle runs around 10million times per second.

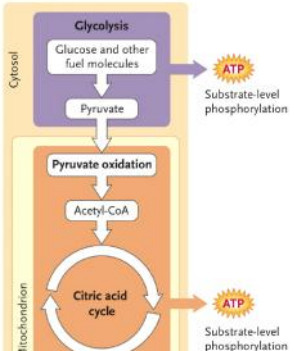
Glucose Breakdown
- glucose broken down to CO_2
- Lots of potential energy in glucose
- No free energy in CO_2
  - Due to C=O bond

Food as Fuel
- Glucose - $O=CH(CHOH)_5$
- Octane - $H_3C(CH_2)_4CH_3$
- Good fuel due to lost of C-H bonds
- C-H bonds are readily oxidised
- $C_6H_{12}O_6 + 6CO_2 \rightarrow 6CO_2 + 6H_2O$
  - oxidation of Carbon

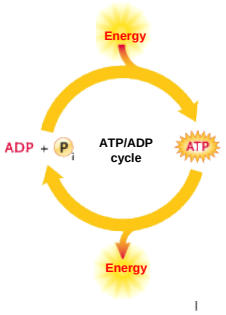
Respiration
- Respiration is controlled combustion
- first process on graph is respiration, second is direct combustion
  - free energy change is the same
  - pathway is different
  - respiration has many small reactions, each with a small E_A
  - Combustion is one large reaction, with large E_A
  - in respiration, heat is bad
    - random, cant be trapped from a biological point of view
    - not efficient for energy transfer between molecules
  - Energy in Respiration is transferred to energy carriers
    - very little heat is lost
  - Both respiration and combustion release 686 $kcalmol^{-1}$

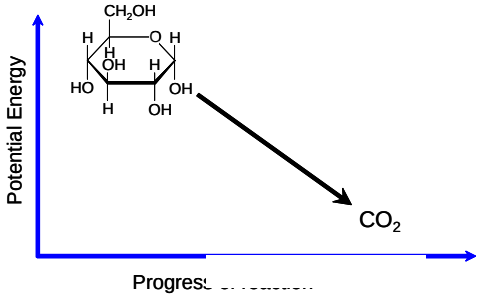
NAD^+
- Electron Carrier
- Nicotinamide adenine dinucleotide
- oxidised form picks up electrons from Glucose
- NAD^+ becomes reduced to NADH
  - $NAD^+ + 2e^- + H^+ \rightarrow$ NADH

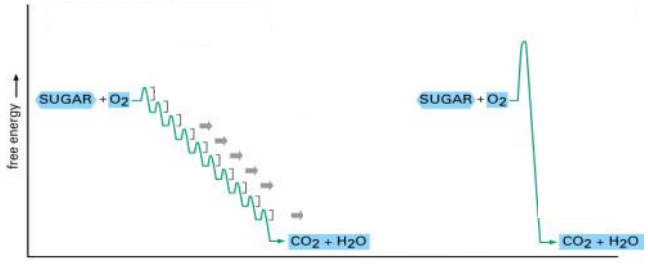
Respiration Overview

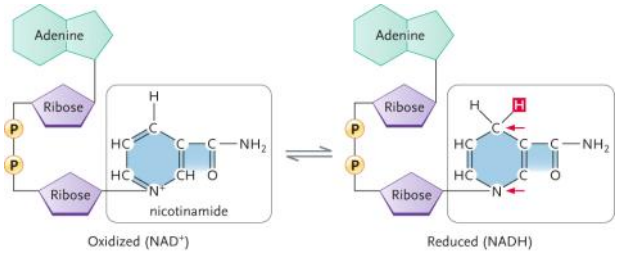


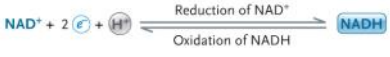
ATP/ADP Cycle

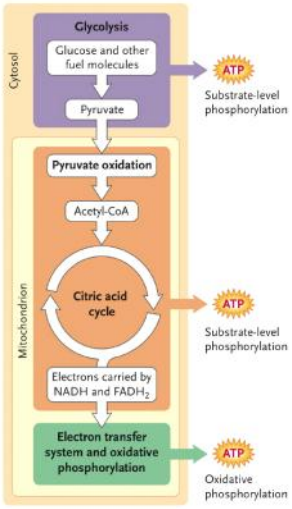


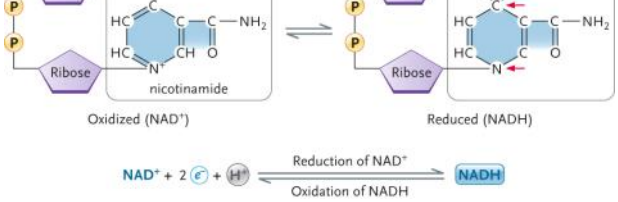








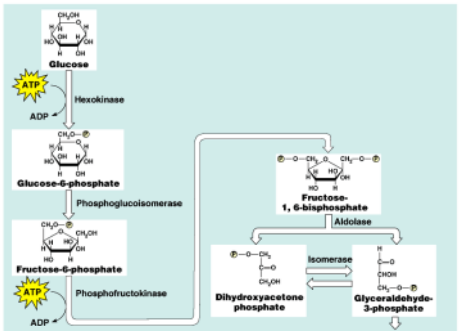




Glycolysis I - Energy Investment

- evolved 3.5billion years ago
- every organism on the planet does it
- anaerobic
- splitting of glucose
- occurs in the cytosol of the cell
- ATP is consumed by enzymes
  - kinases
    - phosphorylates molecules
- Glucose is phosphorylated twice
  - makes molecule charged
  - prevents diffusion out of cell
  - causes instability - more reactive

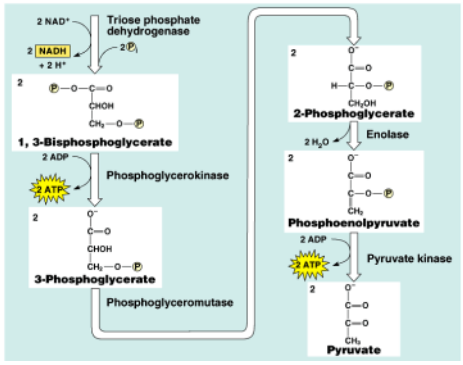
Glycolysis I



Glycolysis II - Energy Payoff

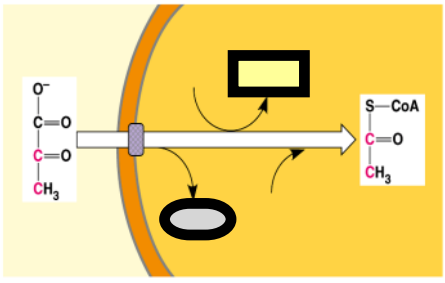
- produces 4 ATP
- Overall 2 ATP produced
- NADH produced
  - gives reducing power
  - electron carriers
  - can reduce other molecules
- End Product is Pyruvate
  - still has plenty of free energy
  - less than glucose though
    - more oxidised
    - two molecules of pyruvate have less free energy than one molecule of glucose - despite same number of carbons
- Reactions involving ATP are substrate level phosphorylation
  - Phosphate groups make substrate unstable
  - removal is facilitated by an enzyme
  - Phosphate combined with ADP to give ATP

Glycolysis II



Linking Glycolysis and the Citric Acid Cylce

- Pryuvate is in the cytosol
- has to move into mitochondrial matrix
- transporter and enzymes involved in transfer
- CO2 removed through Decarboxylation
  - decarboxylase
- Left with Acetyl group
- Dehydrogenase produces NADH through removal of H+
- Acetyl group reacts with Coenzyme-A to produced Acetyl-CoA
- Adding the Coenzyme A makes the molecules more reactive
- Pyruvate dehydrogenase complex is responsible for reaction pathways
  - giant enzyme complex



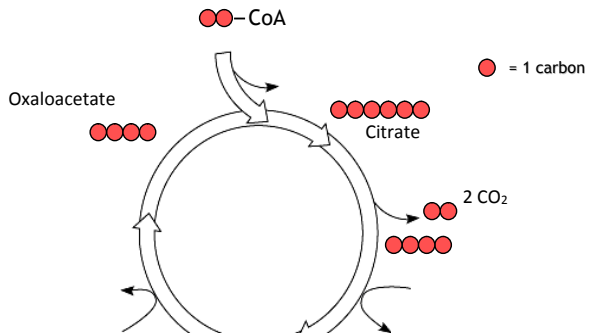
Citric Acid Cycle

- found in mitochondrial matrix
- Oxaloacetate (4 Carbon) is main substrate
  - must be regenerated in order for cycle to work

Oxaloacetate reacts with CoA complex

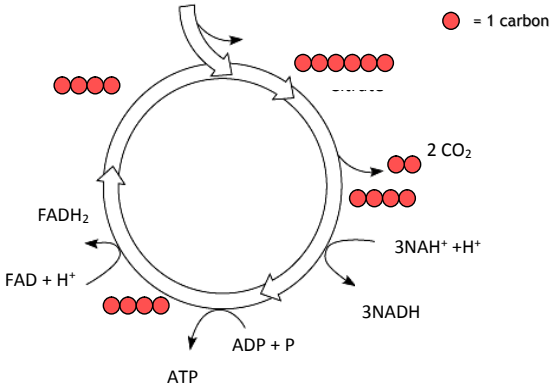
- 6 carbon compound Citrate is produced
- $2CO^2$ removed
- 4 Carbon compound produced
- 3NADH is produced from reduction of 4-C compound
- ATP produced
- Succinate (4-C) is produced.
- Reduced to form $FADH_2$
- Oxaloacetate is produced

The Citric Acid Cycle



Cellular Respiration is not just glucose

- Oxaloacetate is produced

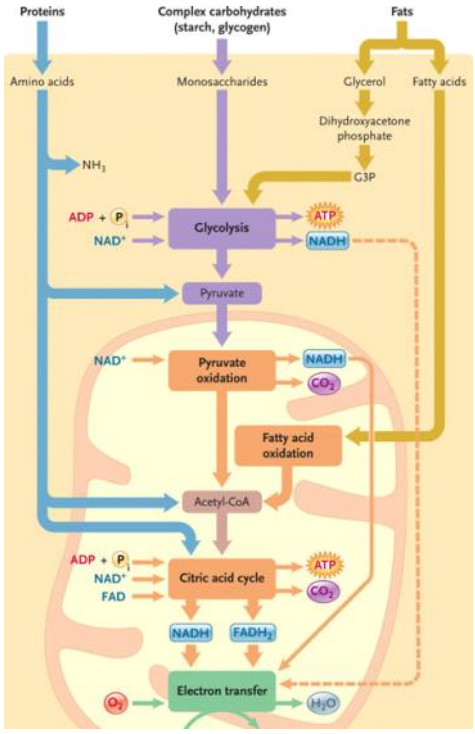


Cellular Respiration is not just glucose

- glucose uses entire pathway
- also used to extract energy from proteins and fats

Disorders of energy metabolism

- inborn errors of metabolism are very rare
- Pyruvate dehydrogenase deficiency is the most common
  - ATP production is impaired
  - causes sever mental retardation
  - brain requires huge amounts of ATP
  - Neurons are not provided with enough ATP
  - can be somewhat overcome by as ketogenic diet
    - 88% fat, 10% protein, 2% carbohydrate
- look for Ratios, not absolute amounts
  - Lactate/Pyruvate ration
  - ATP/ADP Ratio



Readings

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6.1 The Chemical Basis of Cellular Respiration

- By Slowly oxidising energy rich molecules the reactions of cellular respiration are able to extract the potential energy carried by these molecules and convert it into ATP
    - the complete oxidation of food molecules results in the formation of CO_2, which is released into the environment
- What makes a molecule a good food molecule is a high abundance of hydrogen in the form of carbon-hydrogen bonds
    - the electrons in the C-H bond are equidistance from both atoms and this contain a high amount of energy that can be easily liberated
- The potential energy that is contained within fuel molecules is released by their oxidation
    - the term oxidation come from the fact that in many reaction which involve food molecules oxygen is the atom that accepts electrons during oxidation
- The high electron affinity of oxygen makes it ideal as the terminal electron acceptor of cellular respiration
- Cellular respiration is the controlled combustion of fuel molecules
    - in these stepwise processes the trapped potential energy is not liberated in the form of heat - which would be unusable and potentially lethal to a cell - but slowly released and transferred to other molecules
- In the cell the oxidation of glucose occur via a series of enzyme catalyzed reaction each with a small activation energy - the energy from which is transferred to energy carrying molecules
    - the group of enzymes responsible for the transfer of electrons in the stepwise oxidation reaction are called dehydrogenases - these facilitate the transfer of electrons from a fuel molecule to one that acts as an energy carrier or shuttle
- During respiration, the dehydrogenase remove two hydrogens from a substrate molecule, transferring one of them - along with two electrons - to NAD^+ to form NADH
    - the energy transfer between the food molecules and NADH is high with little energy loss - the potential energy carried by NADH is then used to synthesis ATP

6.2 Cellular Respiration - An Overview

- Cellular Respiration can be divided into three stages - Glycolysis, The Citric Acid Cycle and Electron Transport
    - In Glycolysis enzymes break down a molecules of glucose into two molecules of pyruvate - some ATP and NADH is synthesised in these reactions
    - In the citric acid cycle acetyl coenzyme A - formed from the oxidation of pyruvate - enters a metabolic cycle in which it is completely oxidised to CO_2 - again some ATP and NADH are synthesised here
    - In Electron transport the NADH made during the first two steps is oxidised while the liberated electrons are transferred along the electron transport chain to oxygen, producing water
        - the free energy released in this process is used to establish a proton gradient across the membrane, which in turn is responsible for the synthesis of the remaining ATP
- In prokaryotes glycolysis and the citric acid cycle both occur in the cytosol of the cell, whereas electron transport occurs on internal membranes that are derived from the plasma membrane
- In eukaryotic cells the citric acid cycle and electron transport take place in a specialized organelle called the mitochondria - the largest generator of ATP in the cell
- The mitochondria is composed of two membranes - the outer and inner - which together define the two compartments - the intermembrane space and the matrix.

6.3 Glycolysis

- Glycolysis is the first set of reaction that extracts energy from sugar molecules and consists of 10 sequential enzyme catalyzed reaction that lead to the oxidation of glucose to two molecules of pyruvate
    - the potential energy released in these reaction results in the net production of ATP and NADH
- Glycolysis is considered the most fundamental probably one of the most ancient of all metabolic pathways - this is supported by three main points
    - Glycolysis is universal and found in almost all organisms - both pro and eukaryotic
    - Unlike the other stages of cellular respiration glycolysis doe not require O_2, which became abundant around 2.5 billion years ago - almost 1.5 billions after life is thought to have developed
    - Glycolysis occurs in the cytosol of all cells and requires soluble enzymes - it therefore does not require more sophisticated electron transport chains or subcellular compartments to operate
- The are three main points in the reactions of glycolysis to keep in mind
    - An Initial Energy investment is need, followed by an energy payoff
        - glycolysis can be considered as two phases - an initial five step energy investment followed by a five step energy payoff - with two molecules of ATP initially invested in the phosphorylation of glucose and glucose-6-phosphate
        - this then leads to the energy payoff of four ATP and two NADH being produced for every molecule of glucose and every two ATP invested
    - No carbon is lost throughout the process
        - all six carbons from glucose are accounted from in the two molecules of pyruvate
        - the two molecules of pyruvate however - having being oxidised - posses less potential energy that one molecule of glucose
    - ATP is generated by substrate-level phosphorylation
        - this mode of ATP synthesis requires an enzyme to transfer a phosphate group from high-energy substrate molecules to a molecules of ADP - this method of ATP production is also used in the citric acid cycle

<u>6.4 Pyruvate Oxidation and The Citric Acid Cycle</u>

- The two molecules of pyruvate synthesised in glycolysis still contain around 75% of the total potential energy that was originally in the one molecule of glucose
    - the extraction of the remaining free energy by the formation of ATP and NADH is the goal of the Citric Acid Cycle
- The reactions of the citric acid cycle are located in the mitochondrial matrix, so therefore pyruvate must pass through both the inner and outer mitochondrial membrane
    - large pores in the outer membrane allow pyruvate to enter by the process of diffusion
    - however in order to cross the inner membrane a pyruvate specific membrane carrier is needed
- Once in the matrix pyruvate is converted into acetyl-CoA though a multistep process called pyruvate oxidation
    - this process begins with a decarboxylation reaction - the carboxyl group of pyruvate is lost as CO_2
    - this reaction is followed by the oxidation of the remaining two carbon molecules being oxidised to form acetate by a dehydrogenation reaction - which forms NADH from NAD^+
    - The acetyl group then reacts with coenzyme A to form the high energy intermediate Acetyl-CoA
- The Citric Acid Cycle consists of eight enzyme catalyzes reactions - seven of which involve enzyme located in the matrix and one which involves an enzyme bound to the matrix side of the inner membrane - which result in the oxidation of the acetyl groups to CO_2 and the synthesis of NADH, ATP and $FADH_2$
    - for each acetyl-CoA that enter the cycle the following is synthesised
        - 3 NADH
        - 1 $FADH_2$
        - 1 ATP

        - two molecules of CO_2 are also released per one full turn of the cycle
        - the CoA molecule is released and recycled
    - As one molecule of glucose releases two molecules of pyruvate, which in turn each release one molecule of acetyl group, the products of the cycle are doubled when considered as a continuation of glycolysis and pyruvate oxidation

Respiration II

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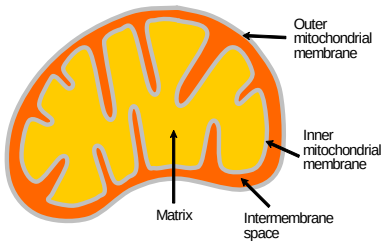
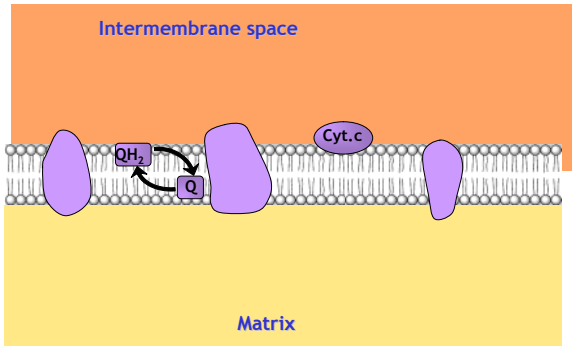
Lecture 8: Respiration II



Citric Acid Cycle
- occurs in matrix of mitochondria
- aqueous environment
  - lots of protons (H^+)

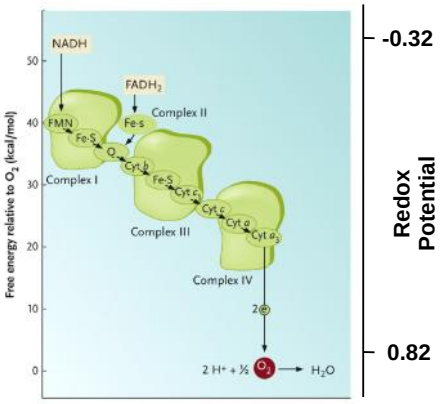
Electron Transport Chain.
- oxidation of NADH
- formation of ATP
- Three major complexes that span membrane
  - major components of respiratory chain
- NADH dehydrogenase
  - 40 protein complex
- Cytochrome Complex
- Cytochrome Oxidase
- Mobile carrier, Ubiquinone
  - shortened to Q
  - diffuses through lipid bi-layer
  - moves electrons
- Cytochrome C
  - shortened to Cyt C
  - found on intermembrane space side of lipid bi-layer

The electron transport chain: A



- NADH → $NAD^+ + H^+ + e^-$
- $NAD^+ + H^+$ stay in the matrix
- e^-
  - transferred to HADN dehydrogenase
  - carried by QH2 ↔Q to Cytochrome complex
  - carried to Cyt C
  - then to Cytochrome oxidase
  - then combines with $2H^+ + 1/2O_2$ to give H_2O
- Electrons move spontaneously down this transport chain
  - carriers ordered to high to low free energy/ low to high redox potential

Why do the electrons move?



Electron Carriers
- Proteins are not oxidised/reduced
- Cofactors that bind to the proteins that are oxidised/reduced
- Electrons transported to due differences in electron affinity between cofactors
  - increasing electronegativity as you move along the chain
- Electrons finally donated to Oxygen
  - oxygen is terminal electron acceptor for mitochondrial respiration

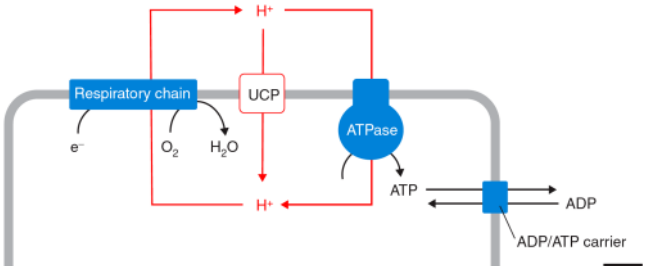
Chemiosmosis

what is the link between electron transport and the synthesis of ATP?
- Electron transport on inner membrane
- Electron transport causes proton pumping
- drops pH of intermembrane space
- the proton gradient across the membrane that is established is used to do work.
- Proton gradient is called Proton-motive force (PMF)
  - measure of proton gradient
  - both concentration and charge difference across the membrane
  - equal to electrical difference established across membrane and difference in concentration
  - PMF = ΔΨ - 59(ΔPh)
  - e.g. pH difference of 1 gives PMF = 200mV

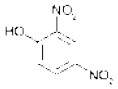
Uses in the Electron Transport Chain
- Oxidative Phosphorylation
- three sites along chain where protons are pumped simultaneously
- free energy from NADH is used by proton pumping
  - takes energy to move protons across membrane
- Changes in charge of Q cycling negated by proton pumping
- as electrons combined with oxygen to give water, protons pumped out of matrix
- Protons pumped back into matrix by ATP synthase
  - free energy in proton-gradient provides energy for ATP synthesis from ADP
  - ATP synthase very similar in all life forms
  - highly conserved complex
  - Electron transport chain and oxidative phosphorylation are coupled processes
  - Oligomycin (antibiotic) inhibits ATP synthases

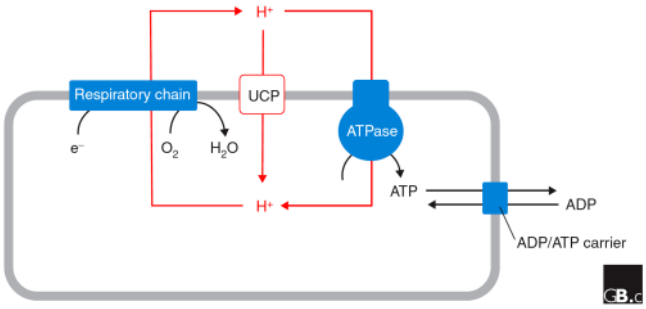
ATP yield
- NADH give 3 ATP
- $FADH_2$ gives 2 ATP

Uncoupling
- Gaps in membrane uncouple Electron transport and ATP synthesis
  - compounds known as uncouplers
- UCP1-proton channel
- regulation of these protein abundance controls rate of uncoupling
- when you uncouple, energy of proton gradient is lost as heat
  - protons can just move through gap in the membrane



- UCP1-proton channel
- regulation of these protein abundance controls rate of uncoupling
- when you uncouple, energy of proton gradient is lost as heat
  - protons can just move through gap in the membrane
  - many conditions when you want this to happen
  - new born children
    - brown adipose tissue
    - contains lots of UCP1
    - non-shivering thermogenesis
      - regulation of heat through uncoupling activity
- There are chemical uncouplers
  - 2,4 - dinitrophenol
  - marketed in 1920's as weight loss drug
  - highly toxic
  - still used today



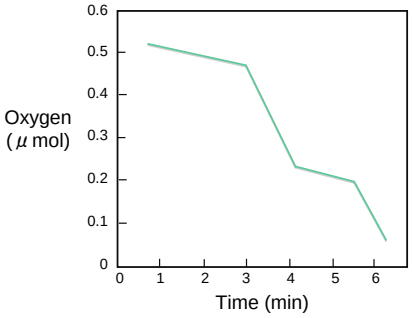


Respiratory Control

- Isolated mitochondria and oxygen electrode in lab
- Glutamate added as substrate for citric acid cycle
  - 1 to 3 minutes is rate of oxygen consumption with just Glutamate
  - low as PMF cannot be dissipated by ATP synthesis
- ADP added
  - 3 to 4 minutes, rate of consumption increases
- Increase in oxygen consumption is due to ADP presence
  - easier to pump protons
  - PMF is being dissipated by ATP synthesis
  - shows that the two processes are coupled
- Oligomycin added
  - 4 to 6 minutes rate slows down again
  - ATP synthesis is inhibited
  - PMF cant be dissipated
- DNP (uncoupler) added
  - PMF drops considerable
  - highest rate of electron transport possible
  - highest oxygen consumption rate

Respiratory control

- Isolated mitochondria

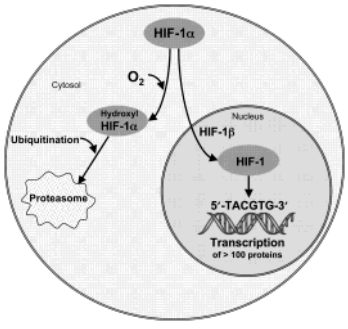


Anaerobic Respiration

- if Oxygen concentrations are low, pyruvate stays in cytosol
- metabolised to either Ethanol or Lactate
- Anaerobic organisms
- must be a sensor for oxygen concentrations within the cell.

Sensing low Oxygen (Hypoxia)

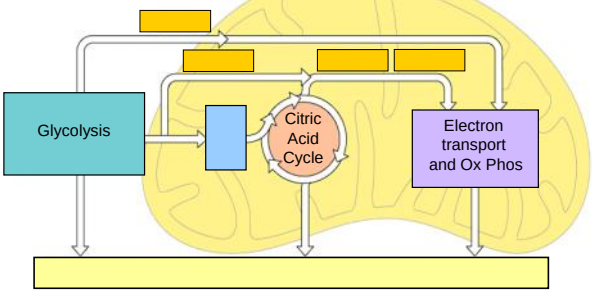
- measure redox homeostasis
  - NAHD/NAD$^+$ ratio is a good marker of oxygen concentrations
  - NADH concentrations too high compared to NAD$^+$ then low oxygen
  - proteins/enzymes sensitive to ratio
- Hypoxia-inducing factor
  - transcription factor (HIF-1)
  - HIF-1 beta is always made
  - HIF-1 alpha is degraded in the presence of high Oxygen concentrations
    - can only be detected in hypoxic conditions
    - stability increases
    - migrates into nucleus, binds with the beta form
    - becomes a functional transcription factor
    - regulates genes involved in glycolysis
    - drives synthesis of specific kinase
    - Kinase phosphorylates Pyruvate dehydrogenase complex
    - inhibits complex, pyruvate cant enter mitochondria
    - remains in cytosol, fermentation occurs
  - example of post-translational regulation



How Efficient is Respiration

- 1 Glucose molecule gives
- 38 ATP IS NOT THE RIGHT ANSWER!!!!!!!

How efficient is respiration ?



Readings

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6.5 Electron Transport and Chemiosmosis

- Following the citric acid cycle all of the carbon present in glucose has been oxidised and released as CO_2
    - the potential energy that had existed in glucose
    - electron transport coupled with the process of chemiosmosis is what extracts the potential energy in these molecules and synthesises additional molecules of ATP
- The electron transport chain is made up of a system of components found on the inner mitochondrial membrane
    - this chain facilitates the transfer of electrons from NAHD and $FADH_2$ to O_2 and is made up of four protein complexes
        - complex I - NADH dehydrogenase
        - complex II - succinate dehydrogenase
        - complex III - cytochrome complex
        - complex IV - cytochrome oxidase
        - while complex II is a single peripheral membrane protein, the rest are multiprotein complexes that span the mitochondrial membrane
- The flow of electrons from one complex to another is facilitated by two mobile electron shuttles - ubiquinone and cytochrome c
    - ubiquinone is a hydrophobic molecules that is found in the core of the membrane and shuttles electrons from complex I and II to III
    - cytochrome c is located on the intermembrane space side of the membrane and moves electrons from complex III to IV
- Each of the complexes is bound to a specific prosthetic group - a redox cofactor - that are responsible for the actual accepting and donating electrons
- The electron carriers in the electron transport chain are organised in a highly specific way - from high to low energy
    - any single part of the chain has a higher electron affinity - and thus reduction potential - that the parts proceeding it
    - therefore the movement of electrons along the chain is spontaneous and releases free energy
- The energy released from electron transport does not in itself produce the synthesis of ATP
    - Instead the released energy is used to do work in transporting protons across the inner mitochondrial membrane into the intermembrane space
    - this creates a higher concentration of protons in the intermembrane space compared to the matrix
- This process of proton translocation occurs at distinct sites along the electron transport chain
    - in complexes I and IV proteins components use the free energy released from electron transport for proton pumping
    - ubiquinone accepts electrons from complexes I and II and at the same time pick up protons from the matrix - they then release these protons into the intermembrane space after migrating through the membrane and donating the electrons to complex III
- The potential energy possessed by a proton gradient is derived from two factors
    - the chemical gradient that exists across the membrane due to the unequal concentration of protons on either side of the membrane
    - the electrical gradient due to the charge of the protons
    - the combination of these two factors produces potential energy know as the proton motive force
- ATP synthesis is linked to the oxidation of energy-rich molecules by electron transport by oxidative phosphorylation, which relies on the action of a large multiprotein complex called ATP synthase
- ATP synthase is a lollipop shaped structure made up of a basal unit - embedded in the inner

membrane - and a head piece - which extends into the mitochondrial matrix - connected by a stalk
    - the basal unit forms a channel through which H^+ can pass freely
    - the proton motive force moves protons in the intermembrane space through the channel in the basal unit down their concentration gradient and into the matrix
- The flow of protons power by ATP synthesis by the binding of the headpiece to individual protons in sites that cause it to rotate in a way that catalyzes the formation of ATP from ADP and Pi
    - active transport pumps are actually ATP synthase working in reverse
- The synthesis of ATP is linked - coupled - to electron transport by the proton gradient that is established across the inner mitochondrial membrane
- however, the two are distinct processes that are not always coupled
    - it is possible to have high rates of electron transport without the synthesis of ATP
- uncoupling of the two can occur by mechanisms that prevent the formation of the proton motive force by making the inner membrane permeable to protons
    - a number of chemical compounds called ionophores act as uncouplers as they form channels across membrane that allow ion - included protons - to move across, thus destroying the proton motive force
    - a group of transmembrane proteins called uncoupling proteins also form channels through which protons can freely move

<u>6.6 The Efficiency and Regulation of Cellular Respiration</u>

- How many molecules of ATP are produced by oxidative phosphorylation as electrons flow through the electron transport chain
- for each NADH oxidise, 10 H^+ are pumped into the inner membrane space
    - 3 to 4 H^+ are also needed to flow back through ATP synthase for the synthesis of one ATP molecule
    - this gives about 3 ATP made for every NADH that is oxidised by electron transport
- The oxidation of $FADH_2$ bypasses electron transport and thus does not lead to as many protons being pumped across the membrane - for each $FADH_2$ oxidised only one molecule of ATP is synthesised
- Total ATP[production for cellular respiration
    - Substrate level phosphorylation during glycolysis produces 2 ATP directly and then 2 molecules of NADH
    - During the oxidation of pyruvate 2 more NADH are produced
    - during the citric acid cycle 2 ATP are produced and a further 6 NADH and 2 $FADH_2$
    - this gives a total of 30 ATP from the oxidation of 10 NADH and 4 from the oxidation of 2 $FADH_2$
    - in total with the 2 from glycolysis and the citric acid cycle this gives 38 molecules of ATP made for each molecule of glucose oxidised
- Assuming the complete oxidation of glucose into ATP, the total energy conserved in cellular respiration is 224kcal mol^{-1}
    - in contrast, glucose contains 686kcal mol^{-1} of energy
    - this give cellular respiration a total efficiency of around 32%
- Cellular respiration includes a large number of enzymes and transport systems with the overall rate of respiration being highly controlled so that ATP production is matched to the demand for it by a cell
    - this is often referred to in general terms of supply and demand - the cell does not waste resources by making excess substrate
- Most metabolic pathways are regulated by the process of feedback inhibition
    - the rate of cellular respiration is also controlled by the feedback of metabolic intermediates
- If excess ATP is present in the cytosol then it binds to phosphofructokinase - and enzyme that catalyzes the reaction of fructose-6-phosphate into fructose-1,6-bisphosphate - and inhibits its action
    - the result is a decrease in the concentration of fructose-1,6-bisphosphate and therefore

as a consequence a drop in the remainder of cellular respiration
    - therefore glycolysis does not oxidise fuel substances when ATP is already in adequate supply
- If energy-requiring activities then take place in the cell the ATP concentration drops and ADP concentration would increase in the cytosol
    - ATP is the released from phosphofructokinase removing the inhibition
    - at the same time ADP activates the enzymes stimulate cellular respiration
    - the rate of glycolysis and ATP production increases proportionally as cellular activities convert ATP to ADP
- The citric acid cycle is at regulated at several steps to match its rate with the cell's energy requirements
    - some enzymes in the cycle are inhibited by elevated ATP concentrations - this automatically slows or stops the cycle when ATP production exceeds the energy demands of the cell in order to conserve fuels
    - phosphofructokinase is also inhibited by NADH and citrate - an intermediate of the citric acid cycle - meaning that the accumulation of either is in indication that the downstream reactions are moving slowly - as would occur under conditions of limited oxygen
- In addition to glucose and other 6 carbon sugars a wide range of carbohydrates, lipids and proteins can enter the cellular respiration pathway at various points
    - Carbohydrates - which are easily broken down into monosaccharaides - enter glycolysis during the early steps
    - Fats - most triglycerides - are another major source of electrons for ATP synthesis and enter the oxidative reactions after being hydrolyzed into glycerol - which enters into glycolysis - and fatty acids - which are split into two carbon fragments and enter the citric acid cycle as acetyl-CoA.
    - Proteins are hydrolyzed to amino acids, have their amino groups removed and then enter the respiratory pathway either as pyruvate, acetyl or intermediates of the citric acid cycle

<u>6.7 Oxygen and Cellular Respiration</u>

- Following glycolysis cellular respiration can continue along one of two different paths depending if oxygen is present or not
- When oxygen is abundant then the pyruvate and NADH are transported into the mitochondria for further oxidation in the citric acid cycle
- If oxygen levels in a cell are low, then the pyruvate and NADH remain in the cytosol where they can be reduced in a series of reactions know as fermentation
- Two types of fermentation reaction exist - lactate and alcohol
    - In lactate fermentation pyruvate is converted into lactate
        - this reaction occurs in the muscle cells of animals whenever strenuous activity results in a demand for ATP that exceeds the rate at which O_2 can be supplied to the electron transport chain
    - Alcohol fermentation occurs in microorganisms such as yeast
        - in this reaction pyruvate is oxidised in two reactions to a molecules of Carbon dioxide and a molecule of ethyl alcohol while NADH is converted to NAD^+
- The reactions of fermentation play a critical role whenever organisms are exposed to conditions in which the oxygen concentration is too low to support oxidative phosphorylation
    - by consuming the NADH generated by glycolysis fermentation reaction keep cytosolic levels of NAD^+ levels high, allowing for glycolysis to continue and produce ATP

Photosynthesis I

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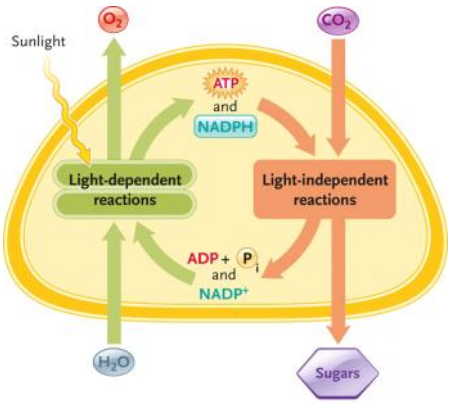
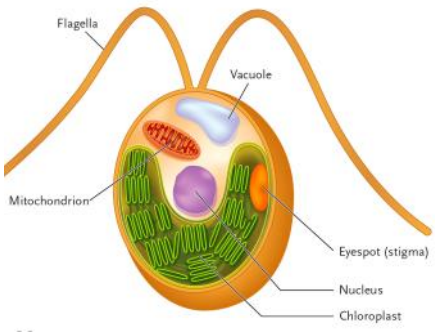




Light Dependant Reaction

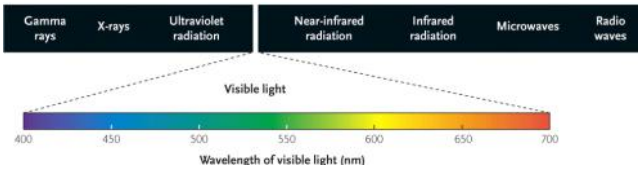
- Free Energy of light used to drive synthesis of ATP
- Endergonic Process
- Oxygen is evolved
- ATP made is used to drive synthesis of sugars
  - in the Light-Independent Reaction
  - Calvin Cycle

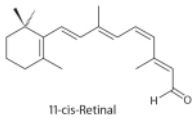
Energy and Information

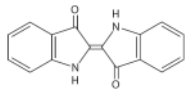
- Chlamydomonas reinhardtii
  - phytoplankton
  - single chloroplast - light used for energy
  - primitive eye - light used for information
  - Use of Phototaxis
    - ability to move towards or away from light
    - sense and then move with Flagella

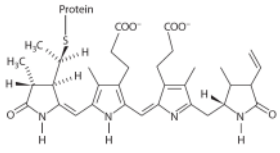
Light

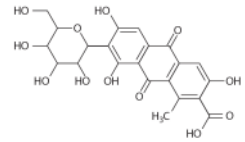
- Electromagnetic Spectrum
- Visible Light is very narrow band
- 400 to 700nm wavelength
- Only Visible part is considered light from biological point of view
- Wavelength - distance between peaks
- Wave-Particle Duality
  - photons of energy
  - discrete particles of energy
- Energy is inversely proportional to wavelength
- $E = hc/\lambda$
  - h = planks constant (6.626×10^{-34} J s)
  - c = speed of light (3×10^{8} ms^{-1})
  - shorter the wavelength, the more energy
  - e.g. photon of blue light has more energy that photon of red light
- Photons can be reflected, transmitted or absorbed
- to be used as energy, the photons must be absorbed.
- molecules in chloroplast that absorbs photon.
- Absorption means the photon reacts with the molecule, exciting an electron - photon absorption
  - energy of photon is transferred to electron, raising it to a higher energy level

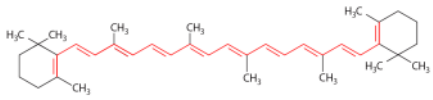


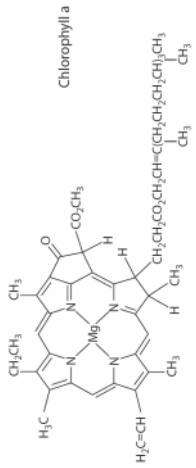












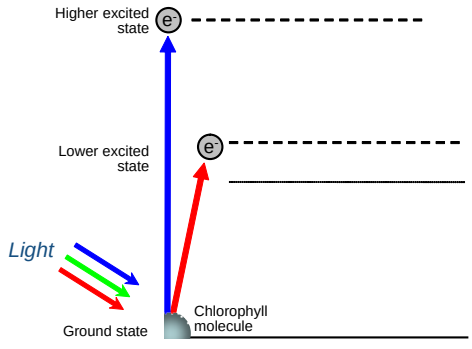
Pigments

- Pigments are organic molecules
- very good at absorbing photons
- Indigo - blue pigment in jeans
- 11-cis-Retinal - main pigment in eyes
- Beta-Keratin and Chlorophyll found in plants
- all have conjugated double bond
- CONJUGATED SYSTEM
  - double, then single bonds
  - Pi-bonding
  - non bonding, low energy electrons
  - available for photon interactions
- Chromophore
  - part of pigment that interacts with photons

Light Absorption and Emission

- photon of white light absorbed by electron
- moved from ground state to higher excited state
- higher excited state is unstable, a lot of energy is lost as heat
- drops back down to the lower excited state
- when blue photon is absorbed, electron jumps to higher state then back down
- when red photon absorbed, can only reach lower excited state
- The lower excited state is the energy level that drives photosynthesis
  - the electron can decay back into ground state
  - or it can lose a small amount of energy, then re-emit a photon
    - Fluorescence
    - energy of the photon released is less than the photon absorbed
    - longer wavelength
  - or photochemistry can occur
    - electron is lost
    - pigment is oxidised, electron removed.
    - drives electron transport
    - electron makes NADH
  - Or energy transfer
    - excited electron can be transferred to another molecule
- One Photon can only excite one electron
- for absorption to take place, the energies must match
  - energy of photon must match energy gap between states

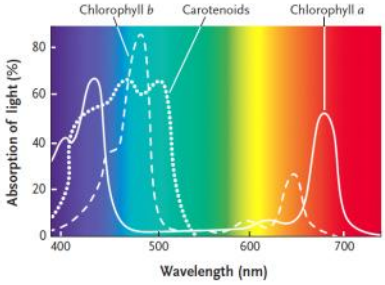
Light absorption and emission



A culture of algal cells does not fluoresce as much as a solution of chlorophyll molecules. Why? (Both contain equal amounts of total chlorophyll)

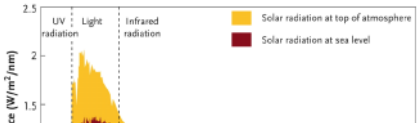
Why is chlorophyll Green

- Colour represents wavelengths that are reflected
- Green photons are reflected by chlorophyll
  - chlorophyll lacks excited state for green wavelength
  - green photons bounced off and hit retina in eye
  - hence why chlorophyll is green

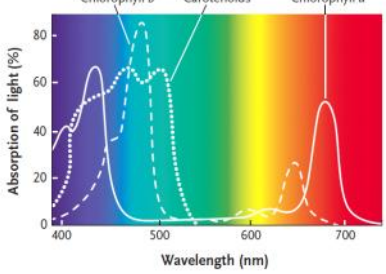


Absroption Spectrum

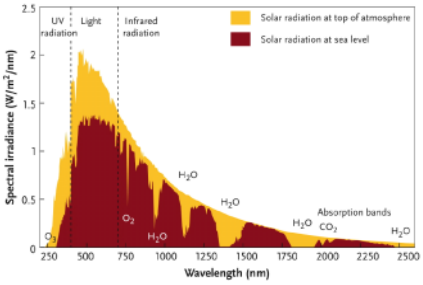
- shows peaks of absorption for blue and red in chlorophyll



- hence why chlorophyll is green



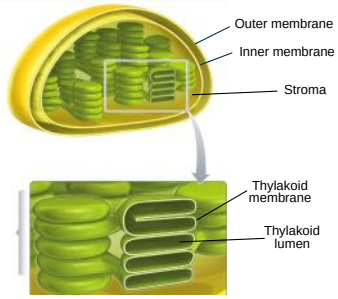
Absorption Spectrum
- shows peaks of absorption for blue and red in chlorophyll
- each molecule has different absorption spectrum



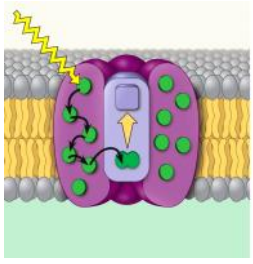
Why Visible Light
- every photosynthetic organism uses visible light
- 99% of all species with eyes use visible light
- Visible light is the dominant wavelengths of electromagnetic spectrum that reaches the earths surface
  - other wavelengths lost on way to earth or in atmosphere
- energy content of visible light is within the correct range
  - Gamma rays and X rays have to much energy
    - ionising energy
  - Radio and Microwaves have too little energy

Chloroplast structure

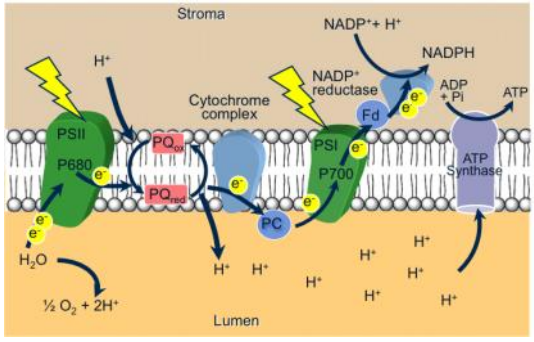
Chloroplast Structure
- where does everything take place? KNOW FOR EXAM!
- Photosynthesis does not only occur in chloroplasts
- Respiration does not only occur in mitochondria
- Prokaryotes can do both, but often do not have Chloroplasts
- Calvin cycle occurs in the stroma (dark reactions)
- Electron Transport occurs on thylakoid membrane (light reactions)



Photosystems and Light Harvesting
- Chloroplasts are not free
- part of a photosystem
- Photosystems are fundamental units of light capture
  - made of two parts
  - Light harvesting complexes
    - giant cross membrane proteins
    - bind chlorophyll and other pigments
    - photons hit, and are transferred to reaction centre
    - pigment molecules are very close together
    - no oxidation/reduction
    - electrons are not transferred, but the energy is.
    - Resonance Energy Transfer
  - Reaction centre
    - surrounded by light harvesting complex
    - had chlorophyll dimer
      - special pair
    - Primary electron acceptor
    - oxidation/reduction occurs here
  - Two types of reaction centre chlorophyll
    - P680 (PSII) - noted asP680* when excited
    - P700 (PSI) - noted as P700* when excited

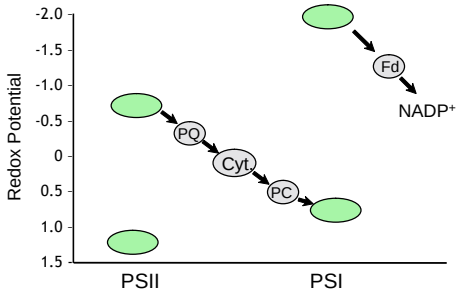


Photosynthetic Electron Transport
- Compare to mitochondrial electron transport
- PQ - plastoquinone
- PC - plastocyanin
- Fd - ferredoxin
- Electron from PSII excited
- travels down chain
- re-excited at PSI
- used to reduced $NADP^+$
- P680 is now oxidised, reduced using electron from splitting of water
- H+ from PQ is used to produce ATP (like mitochondrial electron transport)
- membrane must be imperiable (uncouplers)



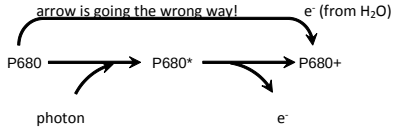
How Do Electrons Flow
- Change in redox potential
- Electrons spontaneously move down the chair
- P680 not easy to oxidase
- photon of light converts it into P680*
- PQ can now easily remove electron, which moves down the chain
- P700 very stable, photon of light converts it to P700*
- electron now easily removed
- Photons convert P680/700 molecules into molecules that an be readily oxidised
- Electrons come from water

Why do the electrons move?



Oxidation-reduction of P680
- P680, in the dark, normal chlorophyll
  - Stable, hard to oxidise
- absorbs a photon, becomes P680* (excited state)
  - easy to oxidise
- Electron transport oxidises P680* to $P680^+$
  - powerful oxidising agent
  - can take an electron from water
  - this electron reduces P680+ back down to the ground state
- P680+
  - Strongest know biological oxidant
  - Redox potential of 1.2V (O_2 is 0.82V)
  - ability to oxidise H_2O
  - Oxygenic photosynthesis

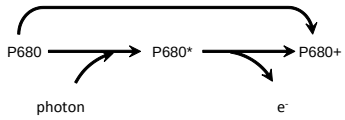
Oxidation-reduction of P680



Anoxygenic Photosynthesis

Anoxygenic photosynthesis

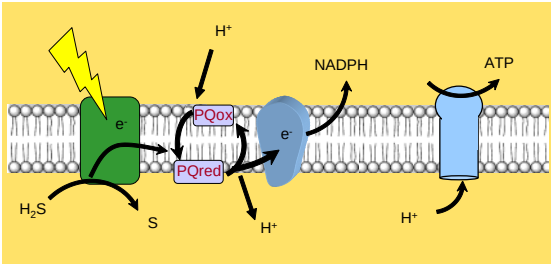
- Oxygenic photosynthesis



Anoxygenic Photosynthesis

- Don't use water
- use H_2S and other molecules instead
- only need for one photosystem
- taken over by oxygenic photosynthesis
- what was the evolutionary advantage gained by development of oxygenic photosynthesis?
  - Water is readily available
  - H_2S is rare
  - organisms can live anywhere that there is water!

Anoxygenic photosynthesis



What was the evolutionary advantage gained by the development oxygenic photosynthesis?

Readings

April-19-11
8:24 PM

7.1 Photosynthesis - An Overview

- Photosynthesis can be divided into two distinct stages - the light dependant reactions and the light independent reactions, or Calvin cycle
    - the light reactions involve the capture of light energy by pigment molecules and the utilization of that energy to form NADPH and ATP through the use of an electron transport chain - which relies on electrons donated from water
    - In the Calvin cycle the electrons carried by NADPH and ATP are used to convert CO_2 into organic sugars by Carbon Fixation - a reduction process that forms mainly three-carbon sugars; which themselves can be combined into glucose
- In photosynthetic eukaryotes both the light reactions and the Calvin cycle take place within the chloroplast - an organelle formed from three membranes that define three distinct compartments
    - between the outer and inner membrane lies the intermembrane compartment - which holds an aqueous environment called the stroma
    - within the stroma is the third membrane system - the thylakoid membranes - which form flattened, closed sacs - the space inside which is called the thylakoid lumen
- The thylakoid membrane houses the molecules that carry out the light reactions - included pigments and electron transfer carriers
- The enzymes that carry out the Calvin cycle are found in the stroma of the chloroplast
- Many photosynthetic prokaryotes also have thylakoid membranes that are formed from infolding of the plasma membrane

7.2 The Photosynthetic Apparatus

- There are two important features of light and pigment molecules
    - the absorption of a photon of light by a pigment molecule excites a single electron from its ground to excited, higher energy state
    - the difference in energy level between the ground and excited state is equivalent to the energy of the photon - if not then the photon is not absorbed by the pigment
- There are three possible fates for an electron which has been excited from it's ground state
    - the excited electron may simply return to its ground state, emitting a lower energy photon that the one it absorbed as it moves down an energy level
    - the energy of the excited electron may be transferred to a neighbouring pigment molecule by the process of inductive resonance
    - the excited electron may be transferred itself to a nearby electron accepting molecule - called the primary acceptor in photosynthesis
- Chlorophylls are the major photosynthetic pigments - the most dominant being chlorophyll a and b, which differ only slightly in structure
    - the second major group of pigments involved is the carotenoids
- During photosynthesis it is only chlorophyll a that is oxidised - donating an electron to the primary electron acceptor
    - chlorophyll b and carotenoids are accessory pigments - they donate excitation energy by inductive resonance
    - photosynthesis is dependant on chlorophylls and carotenoids acting in combination
- Pigment molecules are not free within the thylakoid membrane - they are bound precisely to a number of different proteins
    - these pigment-protein complexes are organised into photosystems
- Photosystems are made up of a light harvesting complex and a reaction centre
    - the light harvesting complex is made up of various pigment-protein complexes
    - the reaction centre is made up of a small number of proteins that bind one of two specialised chlorophyll a molecules in addition to the primary electron acceptor
- There are two different photosystems - PSI and PSII
    - PSI contains a chlorophyll a pigment called P700 that absorbs light of 700nm wavelength
    - PSII contains a similar pigment that absorbs at 680nm - called P680
- The function of the photosystems is to trap photons of light for use as energy to drive the transfer of an electron to the primary electron acceptor - an redox process
    - high rates of redox result from the light harvesting complex absorbing a large range of wavelengths and transferring the energy generated by inductive resonance to the reaction centre

7.3 Photosynthetic Electron Transport

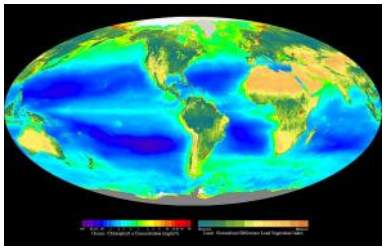
- As with all electron transport chains, the electron carriers of the photosynthetic chain consist of nonprotein organic groups that include the same types that act in the mitochondrial chain - cytochromes, quinones and iron-sulphur centers
- Oxygenic photosynthesis is the result of the development of photosystem II - the sequence of light harvesting and photochemical events within this system are as follows
    - The absorption of photons by the antenna complex followed by the funnelling of energy to the reaction leads to an electron within P680 being excited from its ground state - leading to P680*
    - Once in its oxidised state P680* can be oxidised to $P680^+$ by the primary electron acceptor - pheophytin - which in turn initiates electron transport by donating the electron to plastoquinone - or PQ
    - P680 is them reformed by gaining an electron from water
- P680 is the strongest biological oxidant - the reduction of P680+ to P680 is facilitate by an enzyme subunit of photosystem II called the water splitting complex that is exposed to the thylakoid lumen
- Non Cyclic Electron Transport occurs in 5 steps
    - Oxidation of P680 by the absorption of light energy results in the formation of the excited state of P680 - P680* - which is then rapidly oxidised by pheophytin
    - The Electrons are then transferred to plastoquinone which then migrates through the lipid bilayer acting as an electron transfer link between PSII and the cytochrome complex
        - as plastoquinone gains an electron from PSII it also gains protons from the lumen, which it then releases into the stroma upon donation of its electrons to cytochrome
    - Electron transfer from the cytochrome complex by plastocyanin shuttles electrons between the cytochrome complex and photosystem I
    - The absorption of a photon of light by PSI results in the formation of P700*, which is then oxidised to $P700^+$ by the primary electron acceptor of PSI - ferredoxin - and reduced back to P700 by plastocyanin
    - Electrons are then transferred from PSI to ferredoxin - an iron-sulphur protein - which in turn donates them to the final electron acceptor - $NADP^+$, which is reduced to NADPH by the enzyme $NADP^+$ reductase
- The flow of electrons along the photosynthetic electron transport chain is coupled to ATP synthesis by the build up of a proton gradient -or proto motive force - across the thylakoid membrane - this gradient is derived from three processes
    - protons are translocated into the lumen by cyclic reduction and oxidation of plastoquinone as it moves from PSI to the cytochrome complex
    - The addition of protons from the oxidation of water on the luminal side of PSII
    - The removal of one proton from the stroma for each molecule of NADPH synthesised
- The proto motive force established across the thylakoid membrane is used to synthesise ATP through chemiosmosis and the chloroplast ATP synthase - identical to the ATP synthase used in cellular respiration
- All electron transport chains operate by the spontaneous electron flow downhill from molecules with high energy electrons - or low reduction potential - to molecules of low energy electrons - high reduction potentials
- To get a single electron to move down the electron transport chain it takes two photons of light - one absorbed by each photosystem - therefore it takes eight photons to move the four electrons provided by the oxidation of two molecules of water to one molecule of oxygen
- Photosystem I can function independently of PSII in cyclic electron transport
    - in this process electron transport to NADP+ reductase is not allowed
    - instead ferredoxin donates the electrons back to the plastoquinone pool, which in turn gets continuously oxidised and reduced and continues to move protons across the thylakoid membrane without the involvement of PSII
    - the result of this process is that the energy absorbed from light is converted into the chemical energy of ATP without the reduction of NADP+ to NADPH
    - as the reduction of Carbon Dioxide in the Calvin cycle needs more ATP than it does NADPH these additional ATP molecules are provided by cyclic electron transport - other energy require reactions in the chloroplast also depend on this extra ATP

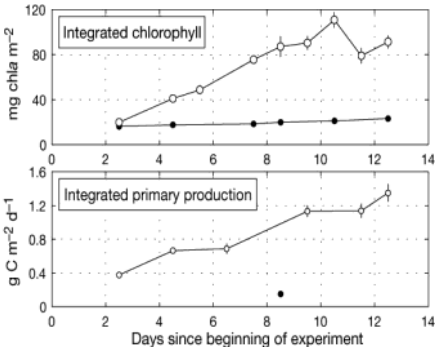
Photosynthesis II

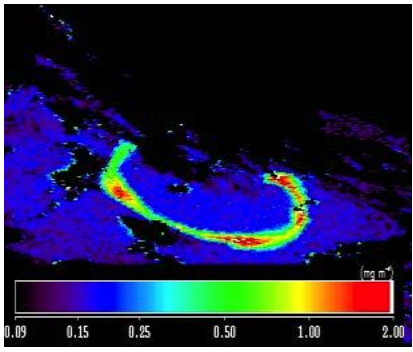
February-08-11
2:27 PM

Global Photosynthesis

- false colour image of global surface chlorophyll
- Global CO_2 fixation 105Gt/year
  - Terrestrial accounts for 56.4 Gt
  - Oceanic accounts for 48.5Gt
    - phytoplankton
- Primary productivity
  - converting CO_2 into sugars
- What controls Phytoplankton distribution
  - blue = low levels
  - green = high levels
  - Sunlight?
    - doesn't explain lower levels near the equator
  - Nutrients in the water
    - blue regions are nutrient poor waters
  - Lots of growth in cold temperatures due to nutrient rich waters
  - Iron is limiting factor in phytoplankton growth







Path of ship dropping Iron

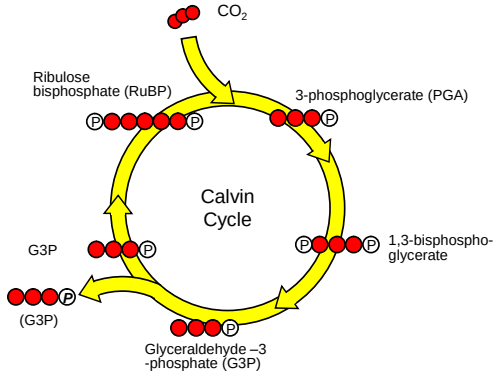
Southern Ocean Iron Release Experiment

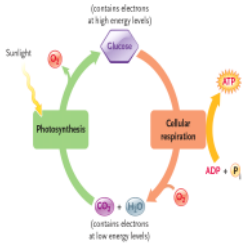
- Dump Iron into nutrient poor waters
- increase phytoplankton growth
- increase levels of CO2 fixation
- lower global CO2 levels
- experiments do work!

The Calvin cycle

Calvin Cycle

- Carbon fixation occurs in Carbon Cycle
- occurs in stroma of chloroplast
- endergonic process
  - need for energy to convert CO2 into sugar
  - energy comes from ATP and NADPH
- For ever one turn of the cycle, one CO2 enters the cycle
- 3CO2 molecules come in
- react with three RuBP (ribulose bisphospahte) - 5 carbon molecules
- form three 6 carbon compounds
  - forms 3-phospho-glycerate (PGA)
  - spontaneous reaction
- 1,3-bisphospho-glycerate is then produced
  - reaction needs energy
  - 2 ATPs needed per molecules, so 6 in total
- Then reduced glyceraldehyde-3-phosphate (G3P)
  - 2 NADPH needed to reduce one molecule, so 6 in total
- G3P is the sugar is released from 3 turns of cycle
  - only one G3P is released
  - the rest are used to regenerate RuBP
  - 3 ATP needed for energy
- ATP energy is used for RuBP regeneration, not Carbon Fixation
- Cycle can be split into three parts
  - Carboxylation
    - when CO2 is added to RuBP
  - Reduction
    - when NADPH is consumed
  - Regeneration
    - regeneration of RuBP

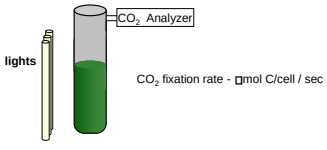




Respiration versus photosynthesis

- How do you measure rates of photosynthesis and respiration if both mitochondria and chloroplasts are in the same cell.
- For every mole of CO_2 fixed, one mole of O_2 is produced
  - 1:1 ratio
- Opposite in respiration
  - still 1:1 ratio of O_2 usage/CO_2 production
- Respiration can be measured by either rate of O_2 consumption, or rate of CO_2 production
- Photosynthesis can be measured by rate CO_2 consumption, or rate of O_2 production

Measuring carbon fixation



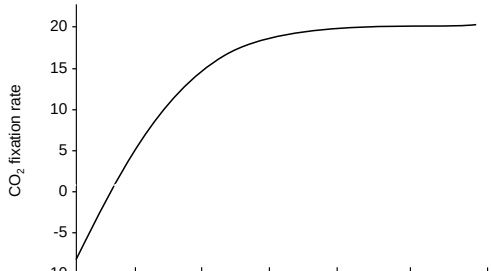
Measuring Carbon Fixation

- Chamber of phytoplankton and light source
- CO_2 analyser to measure CO_2
- as photosynthesis occurs, CO_2 levels decrease
- measure CO_2 fixation rate in µmol cell^{-1} sec^{-1}

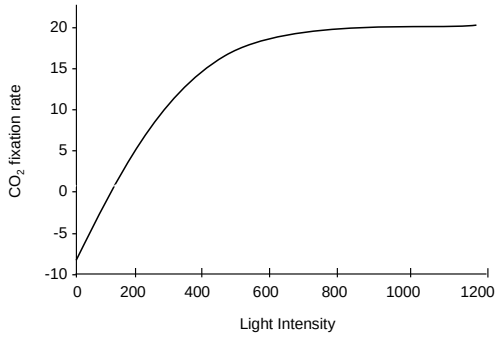
Light Saturation

- CO_2 fixation rate is dependant on light intensity
- in dark, negative rate of CO_2 fixation
  - respiration is occurring
  - photosynthesis is not
- As light intensity increase, so does rate of CO_2 fixation
  - fixation is dependant on light reactions producing ATP, NADPH
- CO_2 fixation has a saturation point
  - enzymes become saturated
  - can no longer regenerate RuBP any faster.
- Light Compensation Point
  - the point at which the rate of respiration is equal to the rate of

Light saturation curve



- CO_2 fixation has a saturation point
  - enzymes become saturated
  - can no longer regenerate RuBP any faster.
- Light Compensation Point
  - the point at which the rate of respiration is equal to the rate of photosynthesis.
  - CO2 production and usage rates are equal.
- Below the light compensation point
  - respiration exceeds photosynthesis
  - plants wont grow for shit!
- for plants to grow/maintain rate of photosynthesis must be greater than the rate of respiration.



Rubisco (RuBP)

- Catalyzes initial carboxylation
- CO2 enters active site
- however O2 can also enter the active site.
  - competitive inhibitor.
- Rubisco is a very ancient enzyme - 3bya
  - predates rise of atmospheric oxygen.
  - natural selection would not have favoured development of enzyme that could not have accepted Oxygen

Photorespiration

- Carboxylation
  - RuBP + $CO_2 \rightarrow$ 2 PGA (3 carbons each)
  - gains carbon
- Oxygenation
  - RuBP + O2 →PGA + Phosphoglycolate - lost as CO2 by respiration
  - loses carbon

Evolution of Rubisco

- Carboxylation versus oxygenation
  - CO_2 (K_m - 10µM)
  - O_2 (K_m - 535µM)
  - Rubisco has a much higher affinity for CO_2
- However
  - atmosphere has so much more O_2 than CO_2
    - 21% O_2
    - 0.05% CO_2
  - 25% of the time, Oxygenation occurs opposed to Carboxylation
- Over evolutionary time, Rubisco Specificity factor has increased
  - active sites have become better at accepting CO_2, less likely to bind O_2

CO2 Concentrating Mechanisms

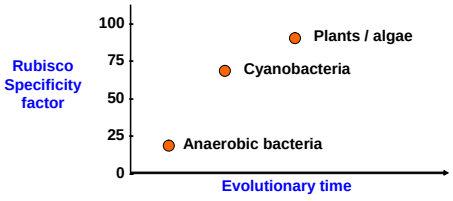
- Methods to lower photorespiration
- e.g. get away from atmospheric levels of CO_2 and O_2
  - Development of pumps etc. to increase levels of CO_2 at the site of Rubisco
- Aquatic photoautotrophs
  - ATP dependant pump moves HCO_3^- into cell
  - Carbonic anhydrase converts the bicarbonate into CO_2
    - can handle 500,000 molecules per second.
  - CO_2 then diffuses into Chloroplast.
- C4 metabolism
  - plants that live in warmer climates
  - two different types of cells in the leaf
  - PEP Carboxylase converts phosphoenol pyruvate in to a four Carbon molecule
  - 4 carbon compound, not 3 carbon PGA.
  - PEP Carboxylase only binds CO_2
  - C4 Compound actively trasnported
  - Releases CO2 in enzyme driven reaction
  - higher levels of CO2 within the plant than the atmosphere
  - Calvin cycle is still the same, just CO2 concentrations are changed prior to the level of the cycle
  - found in Sugarcane, corn.
  - however this process requires more ATP
    - Energetic cost.
    - hence why C4 plants are found in warmer, sunnier areas.

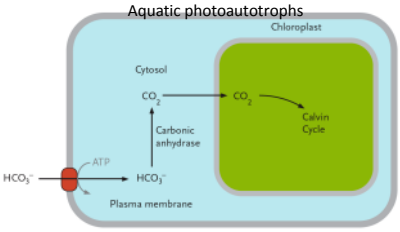
Why are C4 plants more common than C3 plants in climates that are hot an sunny

- higher ATP requirements
- as temperature increases, solubility of gases decreases.
- however, rate of CO_2 solubility decline is greater than the rate for Oxygen.
- therefore, plants that can actively produce CO_2 have an advantage over those that cannot.
  - at higher temperatures, photorespiration is a greater problem than at lower temperatures.
  - C4 plants do not use photorespiration.

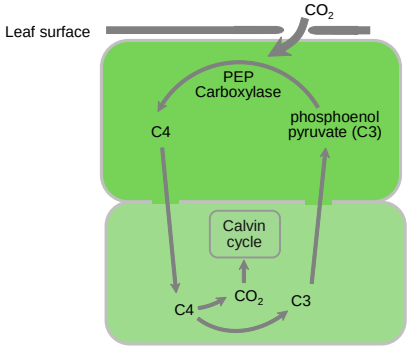
Evolution of Rubisco

- Carboxylation versus oxygenation:
  - CO_2 (K_m ~10 µM), O_2 (K_m ~ 535 µM)
  - But atmosphere is 21% O_2 and 0.05% CO_2.





C4 metabolism



Temperature (°C)	$[CO_2]$ (μM in solution)	$[O_2]$ (μM in solution)	$\frac{[CO_2]}{[O_2]}$
5	21.93	401.2	0.0515
15	15.69	319.8	0.0462
25	11.68	264.6	0.0416
35	9.11	228.2	0.0376

Readings

April-21-11
11:34 AM

7.4 The Calvin Cycle

- In the cytosol of prokaryotic photoautotrophs and in the stroma of the chloroplast a series of 11 enzyme catalyzed reactions use NADPH to reduce CO2 into sugar
    - the process is overall endergonic and thus has energy supplied by the hydrolysis of ATP
    - These reactions make up the Calvin Cycle
- The Calvin Cycle can be divided into three phases - fixation, reduction and regeneration
    - Carbon Fixation involves the incorporation of a carbon atom from CO_2 into a molecule of ribulose-1,5-bisphosphate - or RuBP - in order to produce two molecules of 3-phosphoglycerate - a three carbon sugar.
    - During reduction each of the 3-phopshoglycerate molecules has an additional phosphate added on - from the hydrolysis of ATP - producing 1,3-bisphosphoglycerate, which is reduced further by NADPH to produce glyceraldehyde-3-phosphate - G3P
    - Through regeneration - and for each turn of the Calvin Cycle - two molecules of G3P are produced, from which 5 of the six carbons are rearranged to regenerate a molecule of RuBP - needed to restart the cycle
- For each single turn of the cycle one molecule of CO_2 is converted into one reduced carbon molecule
    - it takes three turns of the cycle to produce one extra molecule of G3P - something that the cell can actually use
    - for the synthesis of this extra molecule of G3P the cycle requires 9 ATP molecules and 6 NADPH - both of which are regenerated from ADP and $NADP^+$ during the light reactions
- Rubulose-1,5-bisphosphate - or Rubisco - is the enzyme that catalyzes the first reaction in the Calvin cycle and is arguable the most important enzyme in the biosphere, providing the source of organic carbon for most of the worlds organisms
    - Rubisco makes up 50% or more of the total protein content of plant leaves and is the worlds most abundant protein
- Rubisco is made up of eight small and eight large subunits
    - the large subunits contain active sites for binding with both CO2 and RuBP
    - The small subunit play a regulatory role - the exact function of which remains unknown
- The synthesis of Rubisco requires the co-ordinated expression of genes from two separate genomes
    - the large subunits are coded for by a gene in the chloroplasts genome, whereas the small subunits are coded fro by a gene in the nuclear genome of the cell
    - after being synthesised the small subunits are transported into the chloroplast to join with the large subunits to produce the functional enzyme

7.5 Photorespiration and CO_2 Concentrating Mechanisms

- Rubisco has two unusual properties
    - it is a very slow enzyme catalyzing the fixation of carbon at only three molecules per second - however this is made up for by its large abundance
    - it is also highly inefficient at fixing carbon due top the fact that it can also bind and incorporate oxygen into RuBP
- The result of this oxygenation reaction results in the formation of a toxic molecule, the metabolism of which results in a loss of CO_2 from the cell
    - the process is named photorespiration due to the fact that CO_2 is released and O_2 is consumed
- O_2 can compete with CO_2 for the active site of Rubisco, thus making it a competitive inhibitor
    - the most likely explanation for this is that Rubisco is a vey ancient enzyme that predates the development of oxygenic photosynthesis and appreciable levels of atmospheric O_2
- When O_2 binds to the active site of Rubisco it acts as an oxygenase rather than a carboxylase

- In the oxygenation reaction the incorporation O_2 leads to the production of one molecule of 3-phosphoglycerate and one molecules of Phosphoglycolate
    - photoautotrophs cannot use Phosphoglycolate and so have to break it down - a process which results in the loss of CO_2
    - the oxygenation reaction results in an overall loss of CO_2 from the cell
- Under equal concentrations of O_2 and CO_2 the carboxylation reaction will dominate due to the fact that Rubiscos active site has a much higher affinity for CO_2 than it does for O_2
    - however under normal atmospheric conditions the carboxylation reaction will occur 75% of the time
- In aquatic environments the concentrations of CO_2 dissolved in water are far below those needed to saturates the active site or Rubisco, therefore aquatic photoautotrophs posses a carbon-concentrating mechanism
    - in this mechanism inorganic carbon is actively pumped into cells to increase the concentration of CO_2 at the active site of Rubisco beyond those possible by diffusion alone
- The dominant form of inorganic carbon in aqueous environments is the bicarbonate anion - HCO_3^- - which is pumped into the cell by an ATP dependant pump where it is converted into CO_2 by the enzyme carbonic anhydrase
    - this system concentrates CO_2 at the active site of Rubisco in high enough concentrations to outcompete any O_2 that may be present
- Terrestrial plants - especially those living in hot and dry climates - face not only the problem of photorespiration but also the issue of water loss - in many species the two problems are linked
    - the surface of leaves is covered by the waxy cuticle that prevents not only water loss, but also the diffusion of CO_2 into the leaves
    - in order to allows high rates of gas exchange plant leaves has stomata
    - plants in hot, dry climates are faced with the problem of needing to open their stomata to allow CO_2 in while also closing the stomata to prevent water loss
- However due to the fact that the solubility of CO_2 decreases more rapidly that O_2 as temperatures rise, the issue of photorespiration becomes a greater problem for plants in warmer climates
- Some plants that have adopted to hot and dry climate have evolved a model of carbon fixation that minimizes photorespiration
    - These plants have developed a second carbon fixation pathway called the C_4 cycle
- In the C_4 cycle CO_2 initially combines with the three carbon molecule phosphoenolpyruvate - PEP - producing a four carbon intermediate - oxaloacetate - which is then reduced to malate by NADPH - malate is then transported to the Calvin cycle and oxidised into pyruvate
    - in order to complete the cycle pyruvate is then converted back into PEP in a reaction that consumes ATP
    - the oxygenation of Rubisco is inhibited by the C_4 cycle because the conversion of malate to pyruvate generates CO_2 - resulting in higher concentrations at the site of Rubisco
- A key distinction between C_4 and C_3 metabolism concerns the carboxylation reactions
    - The C_4 cycle the carboxylation is catalyzed by the enzyme PEP Carboxylase which - unlike Rubisco - does not possess any oxygenase activity
- The C_4 cycle occurs in the mesophyll cells followed by the diffusion of malate into the bundle sheath cells - where the Calvin Cycle occurs
- Why don`t all plants use the C_4 Cycle
    - the cycle has an additional energy requirement - ATP is required to regenerate PEP from pyruvate
    - in hot climates the additional sunshine makes ATP more readily available
    - in more temperate climates the additional ATP requirement is harder to meet given the lack of sunshine
    - C_4 plants also have less need to keep their stomata open and therefore can keep them open for less time than C_3 plants in the same conditions - reducing water loss
- Some plants, instead of running the Calvin and C4 cycles at the same time in different locations simply run them at different times - these plants are known as CAM plants
- CAM plants live in regions that are hot and dry during the day and cool at night
- the Stomata of CAM plants only open at night in order to prevent water loss and allow O2 -

that has accumulated during the day - out and CO2 in

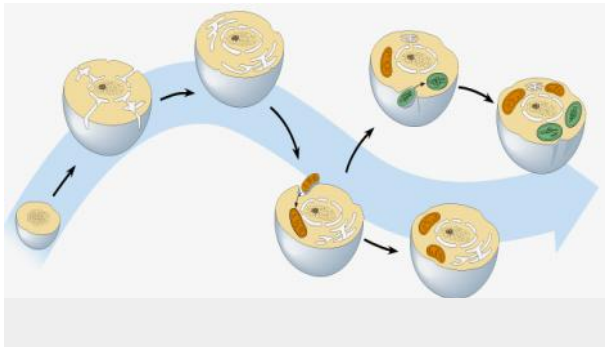
- during the day the stomata close and the Calvin cycle runs of the CO2 generated by the C4 cycle ran during the night

Endosymbiosis

February-18-11
7:29 PM

Endosymbiosis

- Origin of Eukaryotic Cell
- Internal Organelle Organisation that Prokaryotes don't have
- Development of Endomembrane System
  - Nuclear Envelope and ER
  - Evidence that it came from Infolding of Plasma Membrane
- Energy Transducing Organelles
  - Chloroplast and Mitochondria
  - modern day cells descendants of free living prokaryotes
  - Aerobic prokaryotes became Mitochondria
  - Cyanobacteria become Chloroplasts (around 2.5 billion years ago)
- Evidence for Endosymbiosis
  - Morphology
    - they look like bacteria and prokaryotic cells
  - Formation/Division.
    - divide like prokaryotes
    - divide through fission.
  - Electron Transport Chains
    - only organelles that have Electron Transport
    - could provide own energy if free living
  - Genome
    - have own DNA
  - Translation/Translation Machinery
    - have their own Ribosomes etc.



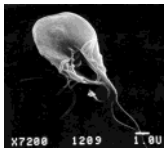
<u>What Drove the Evolution of Eukaryotes</u>

- earliest prokaryotes were anaerobic
- 2.2 bya cyanobacteria appear
  - oxygenic photosynthesis
  - oxygen begins to accumulate
  - terminal acceptor of mitochondrial electron transport
- Prokaryotes that undergo aerobic respiration appear
  - make huge amount of ATP compared to anaerobic.
- Selective advantage for prokaryotic cells engulfing aerobic prokaryotes
  - Increased energy production
  - means that you can get bigger cells
  - greater complexity of cells
  - more specialised cells

<u>Lateral Gene Transfer</u>

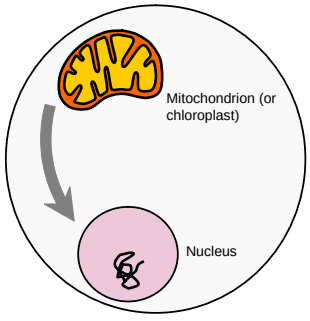
- Early Eukaryotic Cells
  - movement of mitochondrial/chloroplast genes
  - moved to nucleus of cell over millions of years
  - Lateral Gene Transfer.
  - functions of genes remained the same, just location changed
  - Is still occurring today
    - can be shown by detecting were a specific gene is found.
    - look at mitochondrial and nuclear DNA
    - 3 related species
    - Black bar = presence of the gene (Oxidase3)
    - Species A - no lateral gene transfer
    - Species B - it has occurred.
    - Species C - is occurring
      - found in both mitochondria and nucleus.
  - think that the gene is copied, moved to the nucleus, then the original copy is degraded.

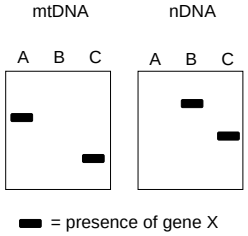
<u>Earliest Eukaryotes</u>



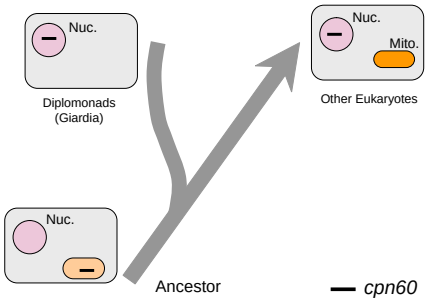
- Giardia
  - belongs to Diplomonads
  - very primitive eukaryote
  - human pathogen
  - grows by fermentation
  - lacks mitochondria
    - never had them, or lost them?
- What is Giardia doing with cpn60
  - cpn60 codes for mitochondrial protein.
  - found in the nucleus
  - Giardia has cpn60
  - suggest ancestor eukaryote with mitochondria
    - lateral gene transfer occurred
    - yet Giardia got rid of mitochondria

Lateral gene transfer



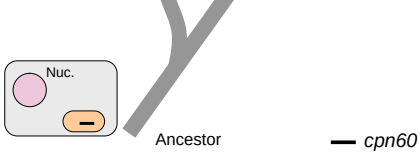


What is Giardia doing with cpn60?



Rubisco assembly requires two genomes

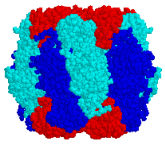
- Giardia has cpn60
- suggest ancestor eukaryote with mitochondria
  - lateral gene transfer occurred
  - yet Giardia got rid of mitochondria
- cpn60 has no known function in Giardia.

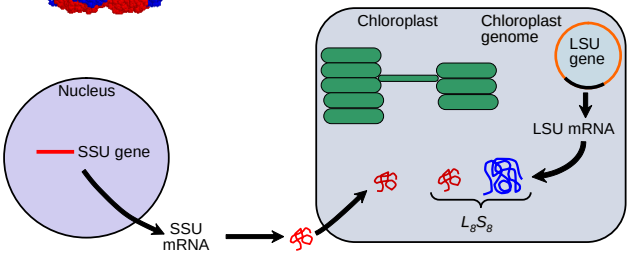


Rubisco Assembly

- Rubisco is the most abundant protein on the planet!
- quaternary structure
  - 8 Large Subunits (LSU)
  - 8 Small Subunits (SSU)
- LSUs and SSUs are not encoded by same genome
  - SSU in nucleus
  - LSU in Chloroplast
  - Coordinative expression
  - Lateral Gene Transfer occurred for one gene but not the other

Rubisco assembly requires two genomes



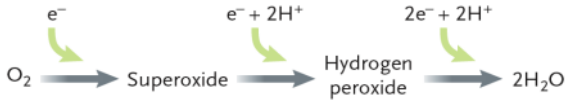


Why does Lateral Gene Transfer occur?

Why move them to the Nucleus

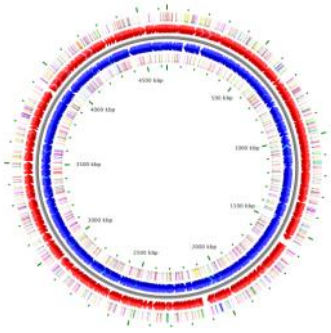
Two hypothesis

- "Who's the Boss"
  - Organelle metabolism must be synced with overall cell metabolism
  - Nucleus takes control for organelles
- Reactive Molecules
  - Organelles home of very reactive molecules
  - Can oxidise DNA - induce mutation
  - Electron Transport produces reactive Oxygen species (ROS)
    - e.g. Superoxide and Hydrogen Peroxide
    - P680 - most powerful biological oxidizer
  - Nucleus is a safer bet.
- Can recombine in the Nucleus
  - can be repaired
  - no other way to fix mutations



Should I Stay or Should I go

- why do some genes move to nucleus and come don't
  - mitochondria still has 37 genes
- Efficiency
  - highly used genes, or need for a lot of product.
  - Reactive Oxygen degrades protein, so new proteins must be made.
  - Local Control for frequently damaged proteins etc.
- Proteins cant always pass through the membranes.
  - too hydrophobic.
- Genes involved in binary fission.
- Haven't moved yet.
- Genetic code is not universal
  - codons mean different things in organelles
  - genes stuck in organelles if it cant get expressed in the nucleus
- However, still not clear as to why some genes remain in organelles and others do not.
- For purpose of notes
  - Haven't gone yet
  - Local Control
  - Transport issues.
  - Folding issues.
  - Expression issues.

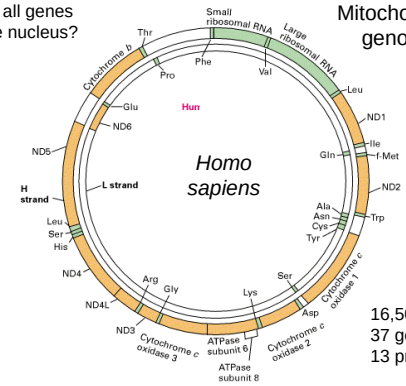


Bacterial Gene

Should I stay or should I go?

- Why haven't all genes moved to the nucleus?

Mitochondrial genome



16,500 bp
37 genes
13 proteins

Readings

April-10-11
6:24 PM

2.5b+c The Theory of Endosymbiosis

- A clear characteristic of eukaryotic cells is the presence of energy-transducing organelles
- A large body of evidence suggests that these energy-transducing organelles - mitochondria and chloroplasts - are the descendants of free living prokaryotes
- The Theory of Endosymbiosis states that the prokaryotic ancestors or mitochondria and chloroplast were engulfed by larger prokaryotic cells, forming a mutually advantageous relations ship and, over time, became inseparable parts of the same organism.
- A Key factor thought to have lead to these events was the rise in atmosphere oxygen
    - as mitochondria carry out aerobic respiration and are thus able to generate far more energy that anaerobes from the same amount of food, the endosymbiosis of these pre-mitochondrial cells by larger ones would have given a huge advantage
    - In a similar manner, modern chloroplast are though to have developed from cyanobacteria - which are photosynthetic
        - As cyanobacteria carry out oxygenic photosynthesis, the host cell would have been able to easily supply the water needed to drive the process
- While all plants contain Mitochondria only plants and algae contain chloroplasts
    - this suggests that the evolution of mitochondria and chloroplasts occurred in two distinct events, with the mitochondrial one occurring first.
- There are several lines of evidence that support the theory of endosymbiosis, all of which highlight the expected similarities between mitochondria and chloroplasts and their prokaryotic ancestors
    - Morphology
        - Mitochondria resemble aerobic prokaryotes and chloroplasts resemble cyanobacteria
    - Reproduction
        - Cells can not make more mitochondria or chloroplasts, which divide by binary fission - as do prokaryotes
    - Genetic Information
        - Both Mitochondria and Chloroplasts have their own genetic information which code for proteins essential to their function
    - Transcription and Translation
        - Both Mitochondria and Chloroplasts both have their own translational and transcriptional machinery
    - Electron Transport
        - both can generate their own ATP through the presence of their own electron transport chains

Gene Structure and Expression.

February-19-11
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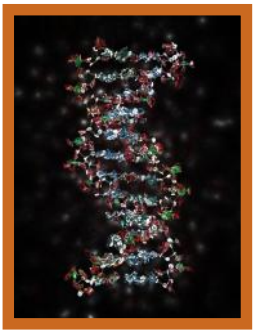
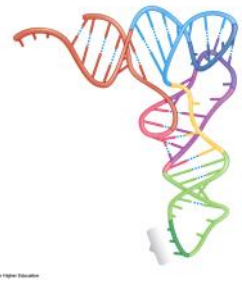
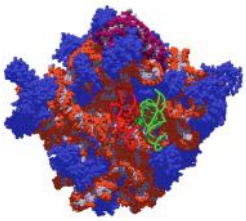
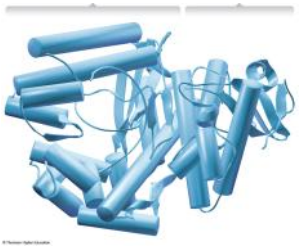








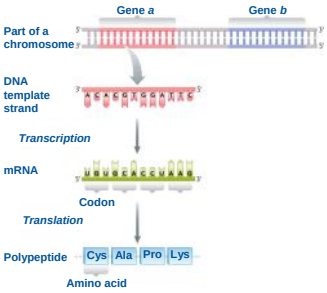
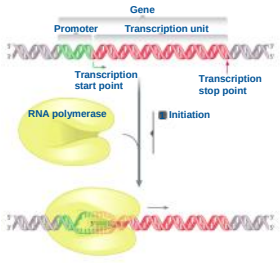
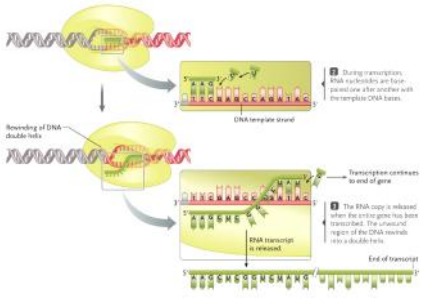
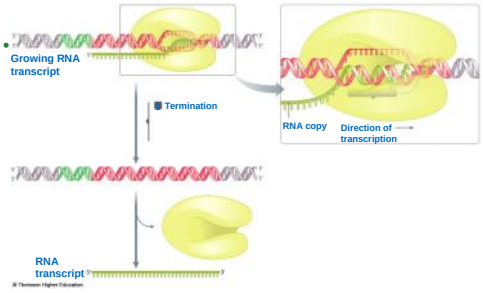


Fig. 14-4







Gene Expression

- DNA
  - complimentary base pairing
- m RNA
  - has a great deal of secondary structure
    - complimentary base pairing
    - pairs with itself whenever it can
    - most thermodynamically stable state.
  - carriers codons to ribosomes
  - can influence translation of codons
  - can influence its own expression
  - riboswitch
    - influence translation of m-RNA
- t-RNA
  - complimentary base pairing
    - pairs with itself
    - gives it its distinct L-shape
- Ribosomes
  - Ribosomal RNA pairs with itself
    - complimentary base pairing
  - catalytic RNA.
- Protein
  - string of amino acids
    - folded to give catalytic shape

Expression

- transcription creates m-RNA template
- gets translated into protein

Promoter Region

- part of DNA sequence
- understood in the DNA
- attract the binding of polymerase
  - locally melts helix
  - adds RNA bases complimentary to DNA
  - A with T
  - C with G
  - all polymerases read 3' to 5'
  - must be antiparallel
  - new strand is made 5' to 3'

Terminator

- part of DNA sequence
- not understood in the DNA
  - Polymerase transcribes right through the terminator
- Terminator is an inverted repeat sequence
  - base pairs with itself
  - makes a "hairpin loop"
  - another example of complimentary base pairing
  - peculiar to prokaryotes

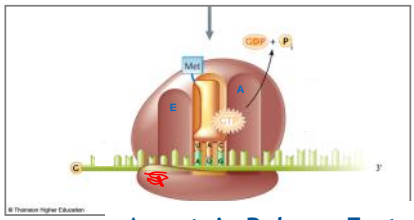
Which DNA strand will be the template for a Gene

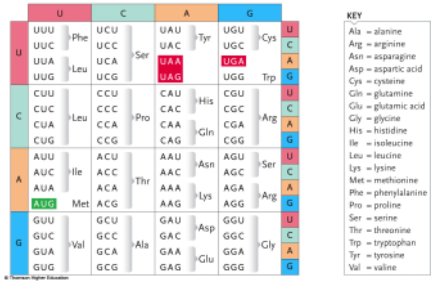
- if bottom strand is template for gene a, which strand will be template for gene b
- need to know which direction polymerase is travelling
- Promoters start transcription
  - polymerase must read 3' to 5'
  - with the promoter on the 3' end
- Template strand for given gene is only for that one gene
  - gene is coded either top or bottom
  - dependant on their promoter

Translation

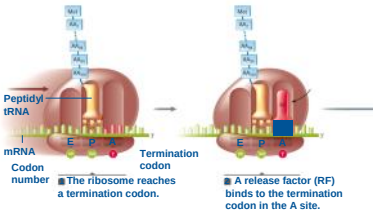
- Initiation
  - caused by initiator t-RNA
    - bound to start codon
  - Ribosomal RNA (red squiggle)
    - base pairing with m-RNA
    - stabilises the initiation process
    - binds at docking sequence
  - Start codons are not at the beginning of the m-RNA
    - 5' UTR (un-translated region)
- Release Factors
  - Terminates Translation
  - no t-RNA recognise stop codons
    - some mutants do
  - Protein release factors
    - recognise the stop codon
- m-RNA is read 5' to 3' end
  - read by ribosomes

Translation initiation is stabilized by mRNA/rRNA base pairing



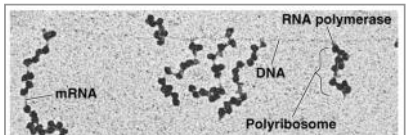


A protein Release Factor terminates translation.

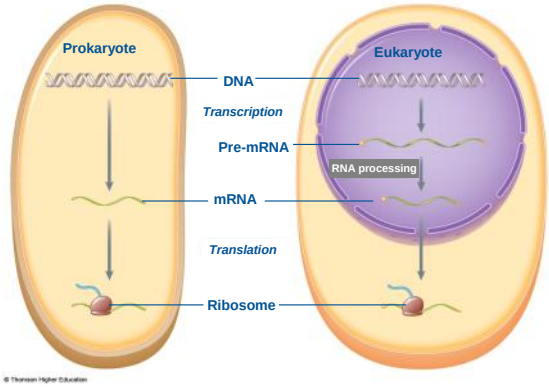


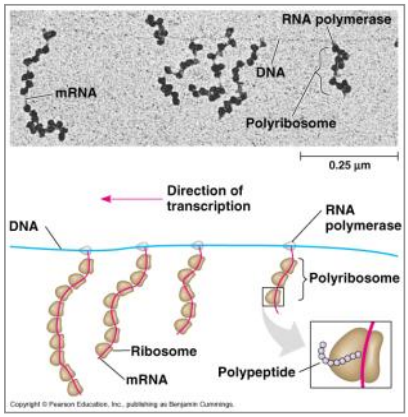
Prokaryotes vs. Eukaryotes

- No nucleus in prokaryotes
  - transcription and translation are coincident
- In eukaryotes, transcription and translation separated by space and time



- In eukaryotes, transcription and translation separated by space and time





Lactose and Lies

February-19-11
9:59 AM

Lac Operon

- Lactose metabolism in E.coli
- Galactosidease
  - Converts Lactose to Galactose and Glucose
    - Glucose fed into Glycolysis
  - Also converts to Allolactose
    - Isomer of Lactose
- Glycolytic enzymes are constitutive
- Glycolysis can be fed into Glycolysis
  - prefered substrate for Krebs Cycle
  - other sugars must be converted to glucose first
    - Maltose
    - Galactose
    - conversion costs energy - ATP
    - therefore net yield of ATP is lower than that of glucose
    - therefore cells prefer to use glucose

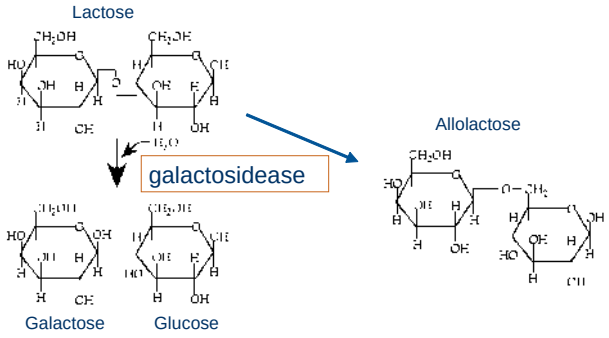
Galactosidease

- Expressed at low levels all of the time
  - gene expression is never really off
  - always available in very low levels
  - effectively off
  - 'Off' just means low probability of expression
  - 'On' means high probability of expression
- Wild type phenotype is "Inducible"
  - Expression increases when cells in counter lactose
  - Induced expression
- Mutant phenotypes
  - $LacI^-$
    - Lacks 'inducibility'
    - constantly 'on'
  - $LacZ^-$
    - mutation in gene that codes of galactosidease
    - always fully off
      - one exception
    - never expression
- Lac Repressor
  - DNA binding protein
  - two binding sites
    - one for allolactose/lactose
    - once of lac operator
  - coded from LacI gene
    - binds to Lac Operator
  - made from LacI
- LacZ and Lac Y
  - both under control of the same promoter
  - means transcript is for two genes at once
  - Lac Repressor prevents polymerase from transcribing
    - sometimes repressor comes off
  - LacZ produces Galactosidease
  - LacY produces Permease
    - transporters that brings lactose into the cell
- Lactose binds to the Lac Repressor
  - prevents the Lac repressor from binding to operator
  - lactose converted to allolactose in cell
- Equilibrium between operator/repressor maintains lactose levels in cell

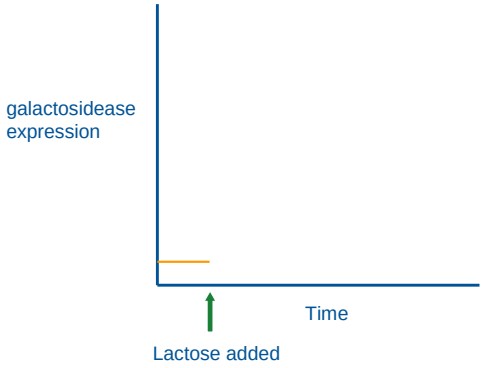
What if it breaks, what if there's a mutation

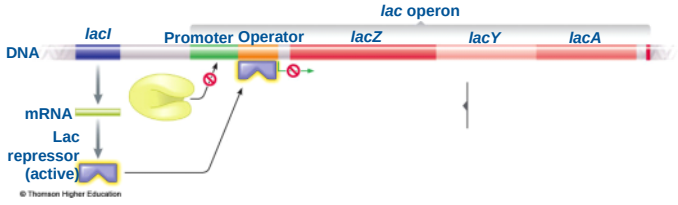
- Mutation in LacZ or Lac Promoter
  - expression of repressor continues
  - expression of Permease continues
  - however no galactosidase expression
    - Lactose is not degraded

Lactose metabolism is regulated by the lac Operon in *E. coli*



Wild type phenotype is "inducible"





        - cell fills up with lactose
- Mutation in LacY or Lac Promoter
    - expression of repressor continues
    - expression of galactosidease continues
    - however no Permease expression
        - Lactose can't enter the cell
        - build up of Lactose outside of the cell
        - eventually no lactose in the cell
        - Lac repressor no longer removed from Lac operator
        - eventually no galactosidease expression
- Mutation in LacI gene or LacI promoter
    - No lac repressor expressed
    - galactosidease and permease expression continues
    - Lactose levels remain low
- Mutation to Lac Operator
    - No inhibition of LacY or LacZ expression can occur
    - lactose levels remain low in cell

<u>Picture</u>

- Don't need to worry about LacA
- Green = lac repressor on diagram
- Red and Blue = part of operator
- Repressor is a diamer
    - bind DNA in to places
    - pull them together
    - causes a loop
        - prevents polymerase from transcribing
        - NOT a hairpin loop
    - Negative control

<u>Negative Control</u>

- When a protein binds with DNA and causes gene expression to DECREASE

<u>Positive Control</u>

- when a protein binds with as DNA and causes gene expression to INCREASE

Both Positive and Negative are examples of how DNA learns and responds to its environment

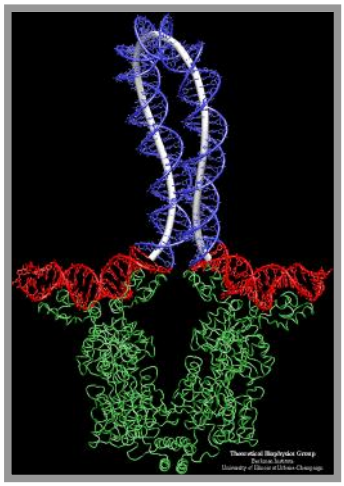
- Proteins are used by DNA to interpret its environment

<u>CAP/c-AMP</u>

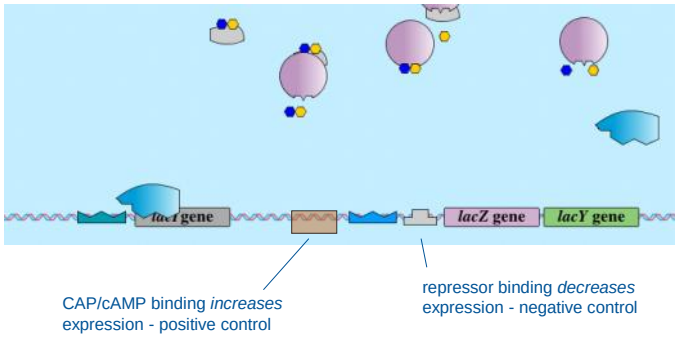
- Cells prefer to use Glucose
- so DNA needs to be able to detect glucose
- uses CAP-binding site to detect
    - attracts the binding of CAP
        - Catabolyte-Activator-Protein
- Binding of CAP increases the expression of the Lac Operon
    - example of positive control
- As Glucose enters the cell levels of Cyclic AMP (c-AMP) decrease
    - Inverse relationship
- c-AMP is needed for CAP binding
    - CAP is made in an inactive state
    - can only bind if it is bound to c-AMP
    - CAP can only bind if Glucose levels are low
- The more Glucose, the less expression of Lac Operon
    - Glucose levels increase
    - c-AMP levels decrease
    - CAP/c-AMP complex levels decrease
    - decrease in binding onto Lac Operon
    - decrease in transcription from Lac Operon
- CAP/c-AMP complex bends DNA
    - makes it more available to RNA polymerase
    - stimulates transcription

<u>Mutation that causes slight drop in galactosidease expression</u>

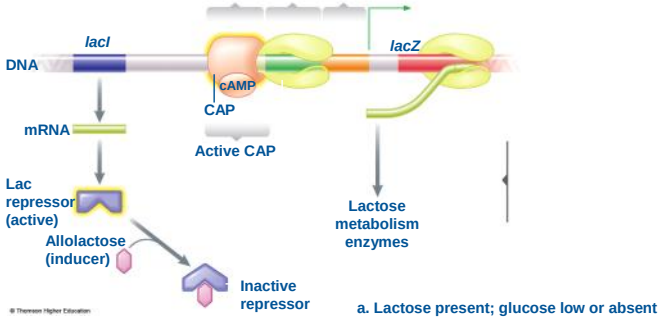
- Less expression that Normal type
    - slight increase in expression when Lactose added

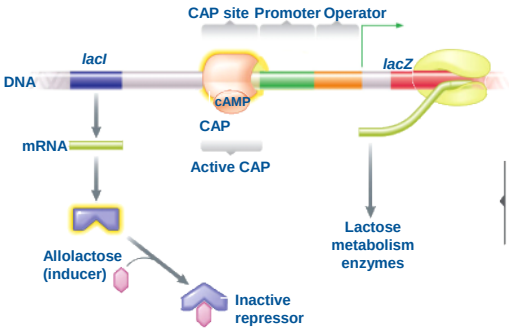


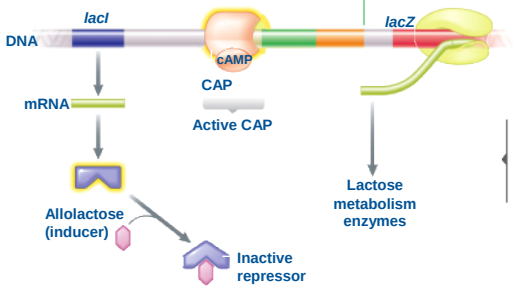
lac expression is also under positive control by CAP/cAMP



CAP binding requires cAMP (only available at low [glucose])







a. Lactose present; glucose low or absent

expression

- Less expression that Normal type
  - slight increase in expression when Lactose added
- no change in expression when Glucose added
  - in normal type, expression drops off when Glucose is added
- Not all mutations are loss of function
  - not all destructive
- Possibly caused by loss of function mutation in CAP coding sequence
  - CAP no longer binds the DNA
  - Operon is still induced
    - less than usual due to lack of positive control from CAP/c-AMP complex
  - when Glucose is added no decrease in expression
- other possible mutations
  - LacL gene to make repressor insensitive to allolactose
  - promote of CAP that stops expression
  - in CAP gene to decrease c-AMP binding
  - LacY gene to decrease activity
  - Lac Promoter to increase affinity for RNA polymerase

Lactose Lie

- lac operon is not for lac
- it works with lactose
- but it did not evolve to digest lactose
- just happens to digest lactose as well

Readings

April-19-11
12:13 PM

15.1 Regulation of Gene Expression in Prokaryotes

- Transcription and translation are closely regulated in prokaryotes in a manner that reflects their life histories
    - prokaryotes have a generational time of minutes, therefore - rather than having complex patterns of long-term cell differentiation and development typical of multicellular eukaryotes - prokaryotic cells undergo rapid and reversible alterations in biochemical pathways that allow them to adapt quickly to changes in their environment
    - this versatile and responsive control system allows the bacterium to make the most efficient use of the particular array of nutrient available at any given time
- When the environment in which a bacterium lives changes, some metabolic processes are stopped and others are started
    - this involves turning off the genes for the processes not needed and turning on the genes for those that are needed
    - for each metabolic processes, several genes are involved - the regulation of which must be co-ordinated
- The co-ordination of the three genes controlling the metabolism of lactose in E. coli are a good example
    - when lactose is absent from the cells, the genes are not expressed
    - when lactose is present, the genes are expressed
    - the control of the genes is at a transcriptional level
- In 1961 Jacob and Monod proposed the operon model for the regulation of lactose metabolism in E. coli
    - an operon is a cluster of genes in a prokaryote and the DNA sequences involved in their regulation
    - each operon is transcribed as a unit from the promoter into a single mRNA
    - this mRNA is then transcribed by to produce a sequence of proteins
    - typically the proteins encoded by an operon catalyze steps in the same function - such as enzymes acting in sequence in a biochemical pathway
    - The other regulatory sequence in the operon is the operator - a short segment to which the regulatory protein binds
    - this regulatory protein is encoded by a gene separate from the operon
- Three genes are involved in the metabolism of lactose - *LacZ*, *LacY* and *LacA*
    - the three genes are adjacent to each other in the order Z, Y, A
    - the genes are transcribed as a unit into a single mRNA by a promoter which resides upstream of the *LacZ* gene
    - *LacZ* codes for the enzyme β-galactosidase, which catalyzes the conversion of lactose into glucose and galactose
    - *LacY* codes fro a permease enzyme that actively transports lactose into the cell
- The Cluster of genes and adjacent sequences that control their expression are called the Lac Operon
    - the operon derives its name from a key DNA sequence regulating its transcription - the operator
    - for the lac operon the operator is a short sequence between the promoter and the *LacZ* gene
- The lac operon is controlled by a regulatory protein named the lac repressor, which is encoded by the gene *LacI* - which lies nearby but separate from the rest of the operon and is synthesised in its active form
- when lactose is absent from the cell the lac repressor binds to the operator and thus blocks RNA polymerase from binding to the promoter and therefore preventing transcription from occurring

    - the lac repressor occasionally falls off, allowing for the transcription of a few molecules of each encoded enzyme in the c ell at all times
- When lactose is present in the cell the lac operon is turned on
    - as lactose enters the cell the β-galactosidease molecules already present convert some of the lactose into allolactose - an isomer of lactose
    - allolactose induces the lac operon by binding to the lac repressor, thus altering its shape so that it can no longer bind to the operator
    - with the repressor removed, RNA polymerase can now bind to the promoter and transcribe the various gene products
- Once the lactose in the cell is used up there is no longer any allolactose to inhibit the action of the lac repressor, which can once again bind to the operator and inhibit the activity of the lac operon
- these controls are aided by the fact that bacterial mRNA is very short lived - three minutes on average
    - this quick turn over permits the cytoplasm to be quickly cleared of the mRNA transcribed from an operon - the enzymes themselves also have a short life span and are quickly degraded
- There is also a positive gene regulation system which regulation the lac operon
    - this system ensures that the lac operon is transcribed if only lactose is provided as an energy source
    - when both lactose and glucose are present in the cell the operator is switched off
    - lactose takes more energy to metabolise than glucose, so glucose is preferred over all other sugars
- The positive gene regulation system works when lactose is present and glucose is absent from the cell
    - lactose is metabolised to allolactose which in turn binds to and inactivates the lac repressor
    - RNA polymerase is recruited to the promoter by active CAP - Catabolyte activator protein - at the cap site - a DNA sequence immediately upstream of the promoter
    - CAP is an activator that is synthesised in its inactive form - it is activated when cyclic adenine monophosphate - cAMP - binds to it
    - When glucose is absent from the cell, cAMP is abundant so therefore CAP is active and able to bind to the CAP site
- If both glucose and lactose are present in the cell then the lac operon is not transcribed
    - metabolism of the incoming glucose triggers a series of events that lead to the inactivation of adenylyl cyclase - the enzyme that catalyzes the synthesis of cAMP from ATP
    - the levels of cAMP drop to the point that it is too low to activate CAP
    - without active CAP in the cell, RNA polymerase is unable to bind to the promoter and thus the operon is not transcribed
- Once glucose is depleted the bacteria shifts to metabolize lactose
    - the inactivation if adenylyl cyclase is reversed, cAMP levels rise and CAP is activated, allowing RNA polymerase to bind to the promoter and transcribe the lac genes
- The positive gene regulation system using CAP and cAMP regulates a large number of other operons that control the metabolism of many sugars
    - this type of regulation system - in which several operons are under the control of a common regulator - is called a regulon
- There are also example of regulation at the translation level
    - some proteins can bind to their mRNAs and modulate their own translation - producing a feedback loop to fine-tune the amounts of proteins in the cell

Eukaryotic Regulation

March-07-11
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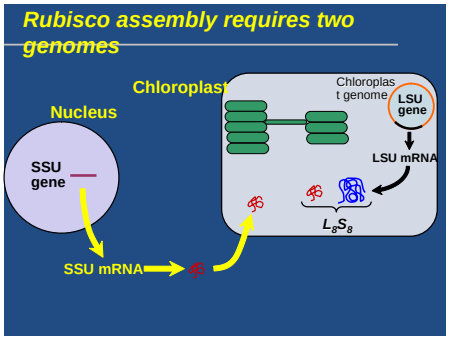


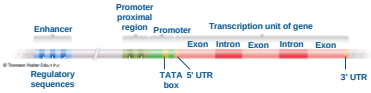
Quorum Sensing in Bacteria.

- Lux operon of Vibrio codes for bioluminescence
- individual bacteria do not express the bioluminescence gene alone
  - don't bio luminesce by themselves
- Able to detect each other
  - able to sense when there are enough around to give of detectable light
  - once there are enough (quorum) the bioluminescence genes are expressed
- Also in virulence genes.

Rubisco Assembly Requires Two Genomes

- some genes coded in chloroplast
- some genes coded in the nucleus
  - lateral gene transfer over evolutionary time.
- How does a gene have to be different when it moves from an organelle into the nucleus
  - organelle is a prokaryotic environment
  - nucleus is a eukaryotic environment

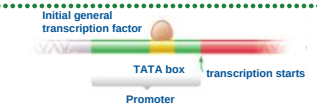
Eukaryotic gene structure is more complicated than prokaryotes

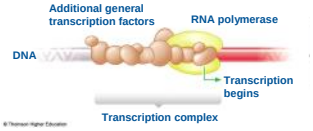


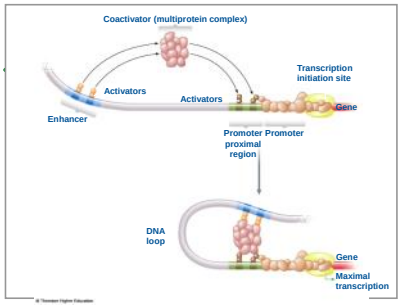
Eukaryotic Gene Structure

- Similar to prokaryotic
  - Promoter regions
  - UTR's
    - Un-translated regions
      - transcribed but not translated
  - start stop codons
- more complicated than prokaryotic
  - more concerned with regulation of transcription initiation
    - more complicated promoters
    - further regulation
      - enhancers
- Eukaryotic promoters attract protein transcription factors
  - initial general transcription factor binds to TATA box (DNA sequence found commonly in eukaryotic promoters) - TATA Binding Protein
  - further transcription factors bind to the TATA binding protein
  - all of these factors influence the 'attractiveness' of the promoter
    - increases likely hood that the promoter will be transcribed
- Enhancers
  - are DNA, understood by cells as DNA
  - promoters have direction
    - location and direction matters
  - Enhancers can be moved around
    - not positional
  - Can also be inverted
    - not directional
  - Due to DNA loop
  - Enhancers are sequences for DNA binding proteins
    - stabilise transcription factors in order to maximise transcription from a particular promoter
    - fold over and make a DNA sandwich
    - by Coactivator DNA binding
      - multiprotein complex
  - Also 'silencers' - interfere with transcription factors
  - Both typically eukaryotic
    - however some of the most powerful promoters are found in viruses.

Eukaryotic Promoters attract protein "transcription factors"



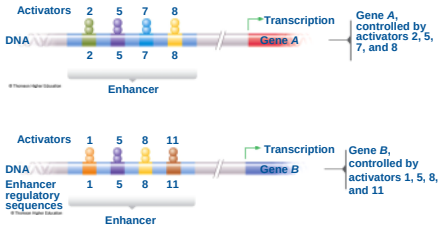


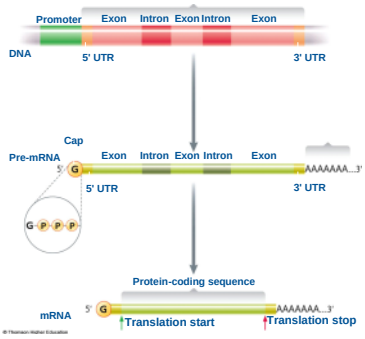


Different Gene Regulation Results from Combinations of Activators

- Different factors bound to enhancers causes different genes to be expressed in different tissues at the same time
- One of the way that one genome can code for all different types of tissues

Differential gene regulation results from combinations of activators



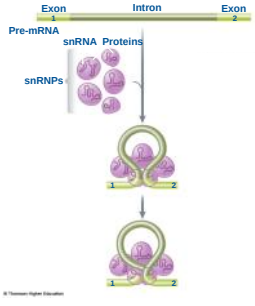


Primary Transcript

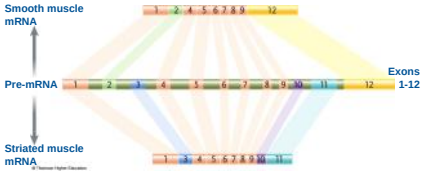
- 3' poly-'A' tail
  - poly-A clipping sequence in DNA
  - downstream of the stop codon.
  - transcribed into m-RNA
  - becomes a site that cuts m-RNA off
    - left with RNA with a free 3' end
    - Poly A polymerase adds poly-A onto the end
    - Poly A is not transcribed from DNA to RNA
      - no "poly-T"
- 5' cap (Gyanine-triphosphate
  - seals the 5' end
  - same as with Poly-A tail
- Introns are spliced out

Splicing

- Ends of Exons do not base pair
- ends of the intron forms covalent bond with itself
- Base pairing occurs between the intron and the snRNPs
- snRNPs
  - small nuclear RNA proteins
- Several snRNPs aggregate around the splice signals on the introns
  - splice signals on either end of the intron.
  - these base pair with the RNA in the snRNP
- snRNAs also base pair with themselves.
  - like t-RNA and m-RNA
  - form structure which gives them their function
- Prokaryotes don't do this.

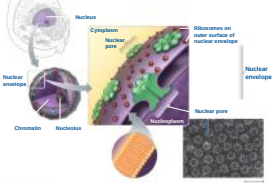


Alternative Splicing: when is an exon an intron?



Alternative Splicing: When is an Exon an Intron

- Striated muscle vs Smooth muscle
- if a region of DNA is an intron or an exon depends on the tissue.
- one of the possible reasons why humans don't have many more genes than a fruit fly or worm or whatever
- one gene can code for different proteins
- e.g. DSCAM
  - codes for 40,000 different proteins



Translation initiation "scans" from 5' cap

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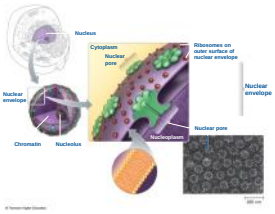
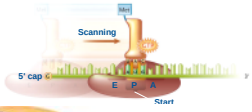
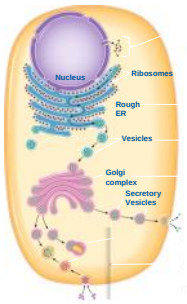


Nuclear Membrane and The Stuff That Moves Through It

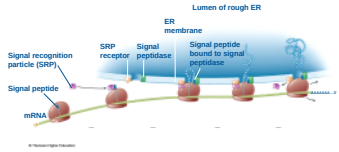
- Fundamental difference between pro and eukaryotes
- Continuous with E.R.
  - ribosomes on the outside
  - pores in it that stuff can be transported through
- m-RNA produced inside the membrane and then transported outside
- all translation begins in the cytoplasm
  - free ribosomes in the cytoplasm
  - small sub-unit finds 5' cap and then slides along scanning until it finds the start codon
  - No docking sequence in Eukaryotes
  - several ribosomes can translate each m-RNA at a time in the cytoplasm
- Only some proteins are functional in the cytoplasm
  - some proteins need to be transported outside of the sell
  - Transport occurs in vesicle, which come from Golgi, which comes from E.R.
- Proteins that are transported out of the cell are translated on the E.R.
- Some proteins are targeted to the E.R. by a signal peptide
  - first few amino acids (signal peptide) are recognised by a Signal Recognition Particle (SRP)
  - the SPR docks the ribosomes onto the E.R.
  - the ribosomes on the E.R. are actively translating
  - the proteins gets translated through the E.R. membrane from where it can be transported through and o of the cell.

Translation initiation "scans" from 5' cap





Some proteins are targeted to the ER by a signal peptide sequence



Readings

April-18-11
11:01 AM

14.2 Transcription -DNA Directed RNA Synthesis

- Transcription is the process by which information coded in sequential DNA bases is transferred to a complimentary RNA Strand
- In Transcription…..
    - In a give gene, only one of the DNA strands acts as a template for the synthesis of the complimentary RNA strand
    - Only a relatively small part of the DNA molecule - the coding sequence of the single gene - serves as a template
    - RNA polymerase catalyzes the assembly of the RNA strand
    - the RNA strand is a single stranded molecule
- Transcription begins when RNA polymerase binds to the DNA, unwinding it near the beginning of a gene
    - RNA polymerase can start the complimentary cops with no need from a primer already in place
    - RNA is made in the 5' to 3' direction, with the 3' to 5' DNA strand being read as a template
- Transcription is the first step in a process whereby particular gene are expressed in a given cell at a given time
    - some of the genes are protein-coding genes that encode m-RNAs for translation
    - others are non-protein-coding genes that encode other types of RNA - such as r-RNA, t-RNA or sn-RNA - that are never translated
- At one end of a gene is a control sequence called a promoter
    - the promoter specifies where on the DNA transcription begins
- The part of the gene that is to be transcribed into RNA is called the transcription unit
- In order to initiate transcription RNA polymerase binds to the promoter, unwinds the DNA in that region, and then begins to start synthesising and RNA molecule at the transcription start point
    - as the RNA polymerase moves along and unwinds the DNA the newly synthesised RNA molecules elongates as nucleotides are added one by one
- The new RNA molecule temporarily winds with the template DNA strand - forming a RNA-DNA double helix, but soon unwinds allowing the DNA double helix to form once the RNA polymerase passes
- Elongation of RNA chain continues until the end of the transcription unit
    - at this point, RNA synthesis terminated and the RNA transcript and the RNAS polymerase are released from the DNA
- Further molecules of RNA polymerase can begin transcribing further RNAs as soon as there is room at the promoter
    - in most genes there are many RNA polymerases spaced along a gene transcribing multiple RNAs at the same time
- In Eukaryotes different RNA polymerases transcribe different types of genes
- In eukaryotes the promoters for protein-coding genes are immediately upstream of the transcription start point
    - further sequences further upstream of the gene regulate the rate of transcription
- a key element of the promoter in most eukaryotic protein-coding genes is the TATA box
    - RNA polymerase cannot recognise the promoter sequence itself
    - instead proteins called transcription factors recognise and bind to the TATA box and then recruit the polymerase
    - One the RNAS polymerase-Transcription factor complex forms, the polymerase unwinds the DNA and transcription can begin
- While in prokaryotes there are two types of specific DNA sequences that signal the end of transcription called terminators there are no equivalent sequences in eukaryotes - instead

the 3' end of the m-RNA is specified by a different process

14.3 Processing m-RNAs In Eukaryotes

- Both pro and eukaryotic m-RNAs contain coding and non-coding regions that play key roles in the process of protein synthesis
    - in prokaryotic mRNAs the coding region is planked by untranslated ends - the 5' and 3' untranslated regions
    - the same elements are present in eukaryotic m-RNAs along with additional non-coding elements
- A eukaryotic protein-coding gene is typical transcribed into a pre-mRNA that must be processed in the nucleus to produce translatable mRNA
    - the nature mRNA then exits the nucleus to be transcribed in the cytoplasm
- At the 5' end of the pre-mRNA is the 5' cap - an inverted guanine containing nucleotide with its 3'-OH group facing the beginning of the molecule rather than the end
    - a capping enzyme adds the 5' cap as soon as RNA polymerase begins transcription without the need for complimentary base pairing
    - the cap remains when the pre-mRNA is processed to mRNA
    - the cap functions as the initial attachment site for the mRNA to ribosomes to allows translation to occur
- The termination of transcription in eukaryotes is different than in prokaryotes
    - there is no terminator sequence at the end of the gene within the DNA
    - At the 3' prime end of the gene is a sequence that is transcribed into the pre-mRNA
    - proteins then bind to this polyadenylation site and cleave the pre-mRNA at this point
    - this signals the RNA polymerase to stop transcribing
    - The enzyme Poly(A) polymerase the adds a chain of 50 to 250 adenine nucleotides
    - the string of nucleotides - called the poly A tail - enables the mRNA produced from the pre-mRNA to be translated efficiently and protects it from being degraded by RNA digesting enzymes in the cytoplasm
- The transcription of unit of protein coding genes also contain non-protein-coding sequences called introns
    - these are spaced out inside of and interrupt the protein-coding sequence
    - these introns are transcribed into the pre-mRNA but are removed during processing in the nucleus
    - the amino acid sequences that are retained in the finished mRNA are called exons
- The process of mRNAs splicing - which occurs in the nucleus - removes introns from the pre-mRNA and then joins the remaining exons together
    - mRNA splicing occurs in a spliceosome - a complex formed between the pre-mRNA and a handful of snRNPs
    - located in the nucleus, each type of snRNP contains a short RNA - a small nuclear, or snRNA -bound to a number of proteins
- The snRNPs bind in a particular order to an intron in the pre-mRNA
    - the first snRNPs to bind recognise and form complimentary base pairs with the mRNA sequences at the junctions of the introns and adjacent exons
    - other snRNPs are then recruited, looping out the intron and then bringing the exon ends together
    - the spliceosome then cleaves the pre-mRNA at the junction between the 5' end of the intron and the adjacent exon -the intron loops back and bonds with itself near the introns 3' end
    - the spliceosome then cleaves the pre-mRNA at the junction between the 3' end of the intron and the second exon and joins the two exons together
    - enzymes then degrade the intron loop
    - the catalytic activity in splicing resides in the RNA component of the spliceosome
    - some introns can also self-splice
    - RNA molecules that catalyze a reaction like a protein are called ribozymes
- The presence of introns in the mRNA-encoding genes many provide a selective advantage to organisms by increasing the coding capacity of existing genes by a process called

alternative splicing - a process that generates new proteins by exon shuffling

- The removal of introns is not absolute and in certain tissues or under certain conditions different exons may joined in different combinations to produce different mRNAs from a single DNA gene sequence
    - This process - known as alternative splicing - greatly increases the number and variety of proteins encoded in the cell nucleus with increasing the size of the genome
    - the different mRNAs produced from the parent pre-mRNA are tanslated to produce a family of related proteins with various combinations of amino acids derived from the same exons
- Different proteins are made in different tissues from the same DNA gene
    - alternative splicing means that the 'one gene-one polypeptide' idea needs to be further refined to 'one gene-one particular polypeptide under particular conditions'
- Another advantage provided by exons is that intron-exon junctions often fall at points dividing major functional regions in encoded proteins
    - these functional divisions may have allowed new proteins to evolve by exon shuffling - the mixing of existing protein regions or domains into novel combinations to create new proteins
    - this process would produce changes much more quickly than by changes individual amino acids at random points

14.3 Translation - mRNA Directed Polypeptide Synthesis

- Translation is the assembly of amino acids into polypeptides
    - in prokaryotes translation takes place throughout the cell
        - the mRNA produced by transcription is not confined within a nucleus and is therefore available immediately for translation
    - in eukaryotes it occurs mostly in the cytoplasm with a few specialised genes being transcribed and translated in the mitochondria and chloroplast
        - the mRNA produced by splicing of the pre-mRNA first exits the nucleus and then is transferred to the cytoplasm
- In translation the mRNA associates with a ribosome while another type of RNA - tRNA - brings amino acids to the complex to be joined onto the polypeptide chain one by one
- tRNAs are small RNA about 75 to 90 nucleotides long with a highly distinct structure that accomplishes there role in translation
    - all tRNAs can base pair with themselves to wind into four double helical segments forming a cloverleaf pattern in 2D
    - At the tip of one of the double helical segments is the anticodon - the three nucleotide segment that pairs with the codon in the mRNA
        - the anticodon and codon pair in an antiparallel manner as with strands in DNA
    - at the other end of the cloverleaf is a double helical segment that links to the amino acid corresponding to the anticodon
    - the cloverleaf folds in three dimensions into an L shaped structure with the anticodon and amino acid binding segment at opposite tips of the L
- The Wobble Hypothesis - Francis Crick - proposes that the complete set of 61 sense codons can be read by fewer than 61 tRNAs due to the pairing properties of the bases in the anticodons - the pairing of the first two nucleotides of the codon is always precise while the pairing of the third base is more flexible
    - the tRNAs anticodon can read codon that have either U or C in the third position, or similarly with an A or G in the third position
- The process of adding an amino acid to a tRNA is called aminoacylation or charging
    - the process adds free energy as the amino acid-tRNA combinations are formed
    - the finished product of charging, a tRNA linked to its correct amino acids is called an aminoacyl-tRNA
    - 20 differed enzymes called aminoacyl-tRNA synthetase - once for each of the 20 amino acids - catalyze the aminoacylation
    - the free energy in the aminoacyl-tRNA eventually drives the formation of the

peptide bonding linking amino acids during translation

- Ribosomes are ribonucleoprotein particles that carry out protein synthesis by translation mRNA into chains of amino acids
    - ribosomes use an information tape in the form of an mRNA molecule as the directions required to accomplish the task of joining amino acids in the correctly ordered sequence
- In eukaryotes ribosomes function in the cytoplasm, either suspended freely or attached to the membranes of the endoplasmic reticulum - opposed to in prokaryotes where they carry out their functions through out the whole cell
    - Chloroplasts and Mitochondria have their own ribosomes in addition to those in the cytoplasm
- A finished ribosome is made up of two parts - the large and small subunits
    - each subunit is made up of a combination of ribosomal RNA and ribosomal proteins
- The Ribosome has special binding sites that bring together the mRNA and aminoacyl-tRNAs
    - The A site is where the incoming aminoacyl-tRNA - carrying the nest amino acid in the polypeptide chain - binds to the mRNA
    - The P site is where the tRNA carrying the growing polypeptide chain is bound
    - The E site is where an exiting tRNA binds as it leaves the Ribosome
- There are three major stages in translation - initiation, elongation and termination
    - Initiation involves the assembly of all the translational components on the start codon of the mRNA
    - Elongation involves reading the string of codons in the mRNA one at a time while assembling the specified amino acids into a polypeptide
    - Termination completes the translation process when the last amino acids has been added to the polypeptide
- In translation initiation a large and small ribosomal subunit associate with an mRNA molecule while the first aminoacyl-tRNA of the new protein chain becomes bound to the AUG start codon
    - the aminoacyl-tRNA used in initiation is a specialised initiator tRNA with an anticodon to the methionine specifying AUG start codon
    - each step in the initiation process is aided by proteins called initiation factors
- In the first step of the initiation process the initiator methionine tRNA forms a complex with the small ribosomal subunit
    - the complex binds to the mRNA at the 5' cap and then moves along the mRNA - scanning - until it reaches the first AUG codon
    - this is the start codon and it is recognised by the met-tRNA's anticodon
    - the large ribosomal subunit the binds completing the ribosome
    - at the end of initiation the met-tRNA is in the P site
    - in prokaryotes translation initiation is different - the rRNA of the ribosomal subunit finds the region with the start codon by direct base pairing with a specific ribosomal binding site on the mRNA just upstream of the start codon
    - after the initiator tRNA pairs with the AUG codon the subsequent stages of translation simply read the nucleotide bases three at a time on the mRNA - the initiator tRNA-AUG base pairing establishes the correct reading frame
- The Central reactions of translation take place in the elongation stage
    - the individual steps of elongation depend on the binding properties of the P, A and E site of the ribosome
    - protein elongation factors aid the elongation events
- The P site can only bind a tRNA linked to a growing polypeptide chain containing two or more amino acids with one exception - the initiator tRNA
- The A site can bind only to an aminoacyl-tRNA
- The tRNA previously in the P site binds to the E site and then leaves the ribosome
- P, A and E sites operate throughout the elongation cycle
- The cycle beings at the point when an initiator tRNA with its attached methionine is bound to the P site - at this point the A site is empty
    - the aminoacyl-tRNA with an appropriate anticodon binds to the codon in the A site

of the ribosome - GTP is hydrolyzed to provide energy for this step
    - Next the amino acid is cleaved from the tRNA in the P site and forms a peptide bond with the amino acid on the tRNA in the A site - peptidyl transferase catalyzes this reaction
    - at the end of this reaction the polypeptide chain is attached to the tRNA in the A site while an 'empty' tRNA remains in the P site
    - The ribosome now translocates along the mRNA to the next codon - again using the energy from GTP hydrolysis
    - the two tRNAs remain bound to their respective codons - this step position the just formed peptidyl-tRNA in the P site while generating an empty A site
    - the empty tRNA that was in the P site is now moved the E site, where it is released from the ribosome
    - the ribosome then repeats the elongation cycle
    - The growing polypeptide chain extends from the ribosome through the exit tunnel as elongation continues
    - elongation is similar in pro and eukaryotes with no major differences beyond being faster in prokaryotes
- Translation switches from the elongation to termination stage when the A site of a ribosome arrives at one of the stop codons - UAA, UAG or UGA - on the mRNA
    - when a stop codon appears at the A site a protein release factor - or termination factor - binds a this site instead of a tRNA
    - The polypeptide chain is released from the tRNA at the P site as usual, however because no amino acid is present at the A site the freed polypeptide chain is released from the ribosome
    - at the same time the ribosome subunits separate and detach from the mRNA - the empty tRNA and protein release factor are also released
    - termination is the same in pro and eukaryotes
- Once the first ribosome has begun translation another one can assemble as soon as there is room on the mRNA
    - the entire structure of an mRNA with multiple ribosomes on it is called a polysome
    - multiple ribosome greatly increase the rate of translation from a single mRNA
- In prokaryotes the absence of the nuclear envelope means that transcription and translation can be tightly coupled
    - as soon as the 5' end of a new mRNA emerges from the RNA polymerase translation can begin
    - this allows prokaryotes to regulate the production of proteins very quickly in response to changing environmental conditions
- Most eukaryotic proteins are in an inactive, unfinished form when they are released from the ribosome
    - processing reactions - including the removal of certain amino acids - convert the protein to its finished form
    - For many proteins - which fold into the final three dimensional shape as the processing reactions take place - helper proteins called chaperones assist in the folding process by promoting the correct 3D shape and inhibiting incorrect ones
    - In some cases the same initial polypeptide may be processed by different pathways to produce different functional proteins
    - this alternative processing is another mechanism that increases the number of proteins encoded from by a single gene - one separate from alternate splicing
    - other proteins are processed into an inactive form that is later activated at a particular time by the removal of covering a segment of the amino acid chain
- As proteins as found in all parts of the eukaryotic cell, the question is how are newly synthesised proteins directed to these locations
    - 'Address codes' written in the form of amino acid sequences serve as sorting signals that direct the proteins to their cellular location or outside of the cell
    - the signals are coded in the DNA, transcribed into the mRNA and the 'printed' in proteins as they are made
    - The signals are recognised and bound by receptors in the location to which the

proteins are addressed

- One major signal pathway sends proteins to the ER
    - in these proteins a short segment of amino acids called the signal peptide - or signal sequence - is in the first part of the polypeptide chain
    - when the signal peptide emerges from the ribosome a protein-RNA complex called the signal recognition particle -SRP - binds to it and temporarily blocks further translation
    - the SRP then binds to a protein in the ER membrane called the SPR Receptor - this docks the ribosome onto the ER membrane
    - the ribosome can now continue protein synthesis and the growing polypeptide chaing being pushed through the ER membrane into the ER lumen
    - here and enzyme - signal peptidase - removes the signal sequence and protein synthesis is completed
    - other signals may cause the protein to be moved to other parts of the ER system or to be excreted out of the cell by vesicles
- Nuclear proteins include a signal bound by receptors in the pore complexes of the nuclear envelope
    - once bound they are pushed through the pore complex into the nuclear interior - a process that requires energy from ATP
    - these proteins retain their signal because they need to re-enter the nucleus every time it is broken down and reforms during the cell division cycle
- Many proteins that become part of organelles are also made on free ribosomes
    - however these proteins have signals that are bound by receptors in the organelle membranes - targeting them for entry into the organelle
- The same basic system of sorting protein signals also distributes proteins in prokaryotic cells
    - the routing signals are also interchangeable - indication that the protein sorting mechanisms evolved early on in the cells

<u>15.2 Regulation of Transcription in Eukaryotes</u>

- There are two general categories of eukaryotic gene regulation - short term and long term
    - short term regulation involves regulatory events in which gene sets are quickly turned on or off in response to changes in environmental or physiological conditions in the cell's or organism's environment
    - Long term gene regulation involves regulatory events requires from an organism to develop and differentiate
        - long term regulation occurs in multicellular but not unicellular eukaryotes
- The regulation of gene expression is more complicated in eukaryotes than it is in prokaryotes
    - there are four different stages of gene expression regulation
        - transcriptional regulation
        - post-transcription regulation
        - translational regulation
        - post-translational regulation
        - the most important of these is transcriptional regulation
- Chromatin remodelling is a crucial event in the facilitating of gene expression - it allows for transcription regulation to occur
- Immediately upstream of the transcription unit is the promoter -
    - a short region often contain the TATA box; which plays an important role in transcription initiation
    - adjacent to the promoter - further upstream - is the promoter proximal region which contains regulatory sequences called promoter proximal elements
    - promoter proximal elements are part of a regulatory system for increasing the rate of transcription
    - more distant from the beginning of the gene is the enhancer - which contains regulatory sequences that determine whether the gene is transcribed at its

maximum possible rate

- To initiate transcription proteins called general transcription facts bind to the promoter in the area of the TATA box which then recruit RNA polymerase and orient the enzyme to start transcription at the correct place
    - the combination of general transcription factors with RNA polymerase is the transcription initiation complex which - on its own - brings about a low rate of transcription
- Activators - regulatory proteins that control the expression of one or more genes - bind to the promoter proximal elements to increase the rate of transcription
    - when bound the activators interact directly with the general transcription factors to stimulate transcription initiation so that many more transcripts are synthesised in a given time
    - Housekeeper genes have promoter proximal elements that are recognised by activators present in all types of cells
    - genes expressed only in particular cells at particular time have promoter proximal elements that are recognised by activators found only in those cell types or at those time
    - the particular set of activators present within a cell at a given time is responsible for determining which genes in that ell are expressed
- Events at the enhancer determine whether a gene is transcribed at its maximal rate
    - particular activators bind to the regulatory sequences within the enhancer
    - a Coactivator - a large multiprotein complex - forms a bridge between activators at the enhancer and the proteins at the promoter and promoter proximal region that causes the DNA to loop around on itself
    - the interactions between the Coactivator, the proteins at the promoter and the RNA polymerase stimulate transcription to its maximal rate
- In some genes, repressors oppose the effects of activators, thus blocking or reducing the rate of transcription
    - the final rate of transcription depends on the balance between the activation signal and the repression signal
- Repressors in eukaryotes work in various ways
    - some bind to the same regulatory sequence as activators
    - some bind at their own specific site in the DNA near where the activator binds and interact with the activator so that it cannot interact with the coactivator
    - others recruit histone deacetylation enzymes that modify histones leading to chromatin compaction making a genes promoter inaccessible to the transcription machinery
- A Characteristic of any gene is the number and types of promoter proximal elements - there may be one or many elements depend on the complexity of the regulation involved
- Both promoter proximal regions and enhancers paly an important role in the regulation of gene transcription
    - each regulatory sequence in the two regions binds a specific regulatory protein
    - since some regulatory proteins are activators and others a repressors the overall effect of the regulatory sequences depends on the particular proteins that bind to them
    - if activators bind to both the regulatory sequences then transcription will be at a maximum
    - however if a repressor binds to one sequence and an activator binds to the other then the amount of gene expression depends on the relative strengths of these two regulatory proteins
- A relatively small number of regulatory proteins control transcription of all protein-coding genes
    - by combining a few regulatory proteins in particular ways, the transcription of an array of genes can be controlled and a large number of cell types can be controlled
    - this process is called combinatorial gene regulation
- Combinatorial gene regulation solves a basic dilemma of gene regulation - if each gene were regulated by a single distinct protein then the number of gene encoding regulatory

proteins would have to equal the number of proteins and so on and so on until the coding capacity of any chromosome - no matter how big - would soon be exhausted
    - because different genes different combinations of regulatory proteins, the number of genes encoding regulatory proteins can be much lower than the number of genes that they control
- Genes that are co-ordinately regulated have the same regulatory sequences associated with them, therefore with one signal the transcription of all the genes can be controlled simultaneously - for example the control of gene expression by steroid hormones
    - a steroid hormone acts on specific target tissues in the body - only cells in those tissues have the steroid hormone in receptors in their cytoplasm that recognise and bind the hormone
    - the hormone moves through the plasma membrane into the cytoplasm where the receptor binds it
    - the hormone-receptor complex then enters the nucleus and binds to specific regulatory sequences adjacent to the genes who's expression is controlled by the hormone
    - this binding activates transcription and proteins encoded by the genes are made rapidly
    - all genes regulated by a specific steroid hormone have the same DNA sequence to which the hormone-receptor complex binds - this sequence is called a steroid hormone response element

<u>15.3 Post-transcriptional, Translational and Post-translational Regulation</u>

- Post-transcriptional regulation directs translation by controlling the availability of mRNAs to ribosomes
- Variations in the pre-mRNA processing can regulate which proteins are made in cells by alternative splicing
    - alternative splicing itself is under regulatory control
    - regulatory proteins specific to the type of cell control which exons are removed from pre-mRNA molecules by binding to regulatory sequences within those molecules
    - the outcome of alternative splicing is that appropriate proteins within a family are synthesised in cell types or tissues in which they function optimally
- Some post-transcriptional controls operate by means of 'masking' proteins that bind to mRNAs and make them unavailable for protein synthesis
    - when an mRNA is to become active other factors - other proteins that are made as part of the developmental pathway - remove the masking proteins and allow the mRNA to enter into protein synthesis
- The rate at which eukaryotic mRNAs break down can also be controlled post-transcriptionally in a mechanism that involves a regulatory molecules either directly or indirectly affecting the rate of mRNA breakdown
- Nucleotide sequences in the 5' UTR also appear to place an important role in the determination of mRNA half-life
    - if the 5' UTR is transferred experimentally from one mRNA to another, then the half-life of the receiving mRNA becomes the same as the host mRNA
    - the controlling sequences of the 5' UTR of an mRNA might be recognised my proteins that regulate its stability
- Gene expression can also be regulated by miRNAs - small single stranded RNAs
    - miRNAs are encoded by a non-protein-coding gene - transcription of the gene produces an RNA precursor to the miRNA
    - the precursor the folds and base pairs with itself forming a loop structure
    - an enzyme - Dicer - then cuts the loop to produce a double stranded RNA of about 21 to 22 base pairs long
    - A protein the binds to and degrades one of the strands leaving a small, single stranded RNA - miRNA
    - Still bout to the protein complex the miRNA can bind to any complimentary mRNA and silence its gene expression in one of two ways
        - either the protein in the complex cleaves the mRNA where the miRNA is

bound to it ot the double stranded segment formed between the mRNA and miRNA blocks the action of ribosomes trying to translate the mRNA

- The process of silencing a gene post-transcriptionally by a small single stranded RNA is termed RNA interference - or RNAi
    - miRNA are one class of RNA that can cause RNAi
    - the other is called small interfering RNA - or siRNA
    - siRNA is produced from a double stranded RNA that is not encoded by nuclear genes - the life cycle and replication of many viruses involves a double-stranded RNA stage
    - siRNA acts exactly like miRNA - mRNA complimentary to the siRNA are targeted and either degraded or have their translation blocked
- Translational regulation controls the rate at which mRNAs are used in protein synthesis
    - translational regulation occurs in essentially all types of cells and species
- During early development of most animals little transcription occurs
    - the changes in protein synthesis patterns seen in developing cell types and tissues instead derive from activation, repression or degradation of maternal mRNA that were present in the mothers egg before fertilization
- One important mechanism for translational regulation involves adjusting the length of the poly A tail of the mRNA
    - enzymes can change the length of the ply A tail on an mRNA in the cytoplasm
    - increasing the length of the tail results in increased translation
    - decreasing the length of the tail results in decreased translation
- Post-translational regulation controls the availability of functional proteins primarily in three ways - chemical modification, processing and degradation
    - chemical modification involves the addition or removal of chemical groups which reversibly alter the activity of the proteins
    - In processing, proteins are synthesised as inactive precursors which are converted to an active form under regulatory control
- The rate of degradation of proteins is also under regulatory control
    - some proteins in eukaryotic cells last for the life time of the individual whereas others last only for a few minutes
    - proteins with a relatively short lifespan include many of the proteins regulating transcription
    - these short lived proteins are tagged by ubiquitin which causes the proteins to be recognised and broken down by a proteasome - a large complex that unfolds the protein and then digests it with protein digesting enzymes
    - cytosolic enzymes then further break down the digesting proteins and recycle the amino acids for use in proteins synthesis or oxidised as an energy source

Epigenetics

March-08-11
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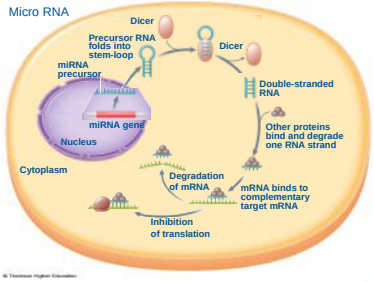
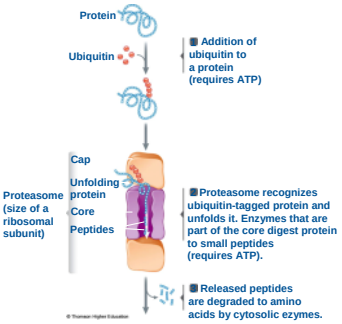


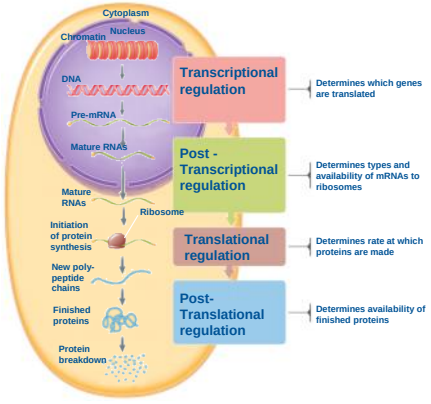
Micro RNA

- transcribed from a gene
- base pairs with itself
- attracts protein called dicer
- Dicer metabolises the mi-RNA to form a small, single stranded RNA
- mi-RNA binds onto m-RNA by complimentary base pairing
- causes either the degradation of m-RNA or the inhibition of translation
  - ability to block expression of a specific genes
  - gene expression regulation



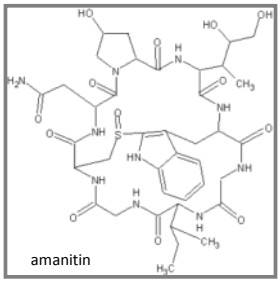
Regulation of Protein Activity

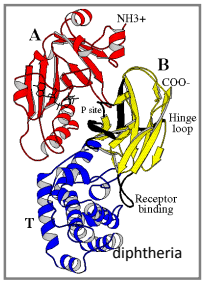
- competitive inhibition
- non-competitive inhibition
- degradation of protein
- acetylation
- methylation
- phosphorylation
- cutting pieces off
- can just get rid of them
  - highly regulated process
  - use of 'tags' such as ubiquitin
  - ubiquitin
    - protein
    - "death tag"
    - proteins tagged with ubiquitin are degraded by the proteasome
    - also used to recover amino acids from un-needed proteins



Metabolic Poisons

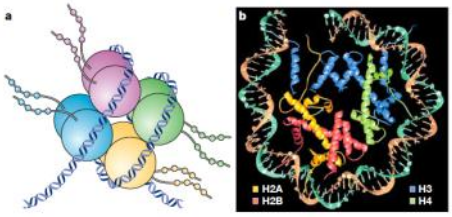
- Amanitin
  - circular molecule
  - made from amino acid backbone but NOT a protein
  - found in amanita mushroom
  - inhibits RNA polymerase
  - useful in labs as a method to block transcription
    - block it, see what happens
- Diphtheria Toxin
  - one of the most potent toxins in the universe
    - from Corynebacterium
  - a single molecule can kill a cell
  - is an enzyme that destroys a translation factor
    - stops translation
    - the cell dies
  - used in labs to strategically stop translation





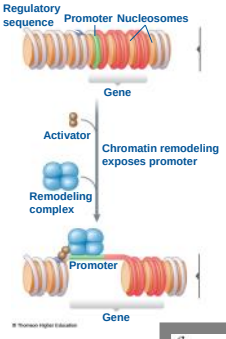
Epigenetics

- Refers to stable changes in gene expression
- do not change the DNA sequence



Histone Acetylation

- proteins in a nucleosome
  - two copies of four different histone proteins
  - two turns of DNA wrapped around the histones
  - help in DNA packaging
  - histones have tails that come off them
  - the tails interact with the DNA
    - histones have a +ve charge
    - DNA has a -ve charge
- can be modified to affect availability of DNA
- the tails get acetylated, reducing their positive charge
  - opens up chromatin structure
  - DNA not as tightly packed
  - increases gene expression
  - DNA more available



Chromatin Remodelling

- Remodelling complexes more nucleosomes out of the way
- opens up the DNA, makes it more available for transcription

DNA Methylation

Changes by Diet

- Think of a mouse, dusty black mouse, agouti coloured mouse
- agouti colour due to yellow band on the black hairs of the mouse
- agouti gene normally expressed in skin to make hair
- mutant agouti gene causes huge, yellow mice with tumours
- Mutant allele is a mutant due to IAP
- IAP
  - has very powerful enhancers
  - drives the expression of agouti gene in ever tissue of the mice, not just the skin
  - mobile element
  - mutant gene cause by presence of IAP
- Methylated IAP
  - DNA of IAP is methylated
  - Cytosine gets methylated
  - methylation turns off IAP
- Methylation primarily shuts genes off
- Heterozygous mouse
  - IAP allele is dominant
  - however if you feed the mother methyl donors, then the babies are not yellow
    - IAP is shut off
- Epigenetic marks of babies can be altered in-utero by the mothers diet
  - marks are stable yet reversible
  - methyl groups can be added on or taken off

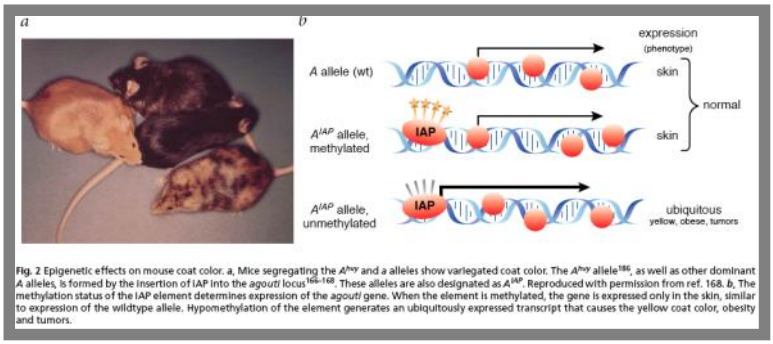
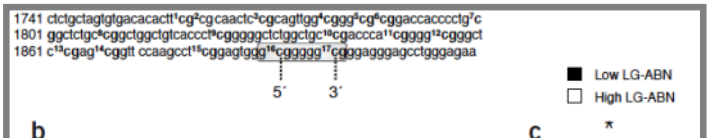
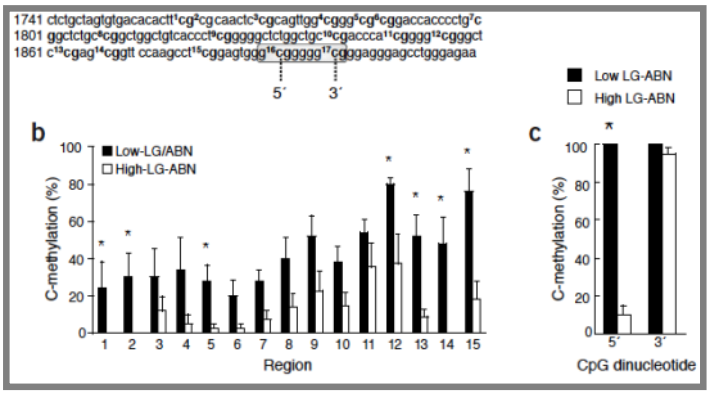


Fig. 2 Epigenetic effects on mouse coat color. a, Mice segregating the A^{hy} and a alleles show variegated coat color. The A^{hy} allele[186], as well as other dominant *A* alleles, is formed by the insertion of IAP into the *agouti* locus[166–168]. These alleles are also designated as A^{IAP}. Reproduced with permission from ref. 168. *b*, The methylation status of the IAP element determines expression of the *agouti* gene. When the element is methylated, the gene is expressed only in the skin, similar to expression of the wildtype allele. Hypomethylation of the element generates an ubiquitously expressed transcript that causes the yellow coat color, obesity and tumors.



- however if you feed the mother methyl donors, then the babies are not yellow
  - IAP is shut off
- Epigenetic marks of babies can be altered in-utero by the mothers diet
  - marks are stable yet reversible
  - methyl groups can be added on or taken off



black bar = methylation in infants who received low maternal care
white bar = methylation in infants who received lots of maternal care

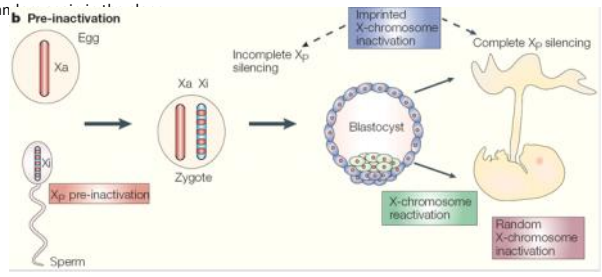
Changes by Maternal Care

- maternal care has an effect on DNA methylation
- methylation is inversely related to maternal care
  - more maternal care = less methylation
  - less maternal care = more methylation
- e.g. Stress responses
  - Methylation of Brain Glucocorticoid Receptor (GR) Promoter
  - infants who receive a lot of maternal care respond better to stress
    - 'chill' mothers leads to 'chill' babies
  - Heritable
    - maternal care produces infants who respond better to stress
    - those infants themselves then give infants a lot of maternal care
    - maternal care = chill infants = maternal care in next generation.
- Maternal care changes the epigenetic marks in infants
  - heritable, stable and reversible
  - persists into adult-hood
- Inheritance of acquired characteristics
- Tutorial
  - Suicide risks
  - methylation as a prevention

X Chromosome Inactivation

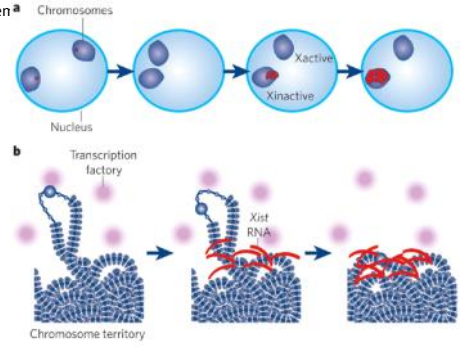
- Rainbow the cat
  - first cat cloned for money
    - owner wanted another one
  - Calico coloured cat
    - all females
  - Clone did not look like the original
    - due to epigenetics
    - x chromosome inactivation

- X chromosome inactivation causes mosaics
  - two separate genetic cell lines
  - one fertilization event
  - gives rise to tissues of two different types
  - some tissues have inactivated mothers chromosome
  - some tissues inactivated fathers chromosomes
  - inactivation of chromosomes is epigenetic.
- X inactivation acts as epigenetic markers over the entire chromosome
  - at beginning, zygote has two active x chromosomes
    - both maternal and paternal x chromosomes are active
  - process of inactivation occurs at random
    - end up with roughly 50/50 split of maternal/paternal
    - tissues are therefore 50/50
  - however, with the clones of rainbow the cat, x inactivation is ran
    - why the clone does not look like the original
    - different inactivation
- How does X Inactivation occur
  - most of what we know comes from mice
    - may be a little different in humans
- Mouse system (In picture to right) (explained moving left to right)
  - Gametes - male and female
    - Xa = active
    - Xi = inactive
    - Xi pre inactivation
    - male x pre inactivation
  - Zygote forms
    - Xa and Xi still the same
  - Blastocyst forms
    - two colours of cells
    - blue/purple cells around the outside go on to form extra-embryonic tissues such as the placenta
    - green cells - E.S. - Embryonic Stem Cells
    - in the purple cells, the paternal X is inactivated ALL of the time
      - no random inactivation
  - Imprinting
    - Mother and Fathers put epigenetic marks on there DNA in their Gametes
      - primarily methylation
      - sex specific epigenetic marks
      - zygotes know which DNA came from mother, which came from Farther
    - X chromosome from the father remains shut off in the placenta
      - why? don't want foreign DNA coding for foreign bodies inside yourself?
    - Also shows up in parent of origin effects in genetic disease
      - some genetic diseases are more sever if you inherit then
  - Embryonic Stem Cells divide and give rise to baby
    - where random X inactivation occurs



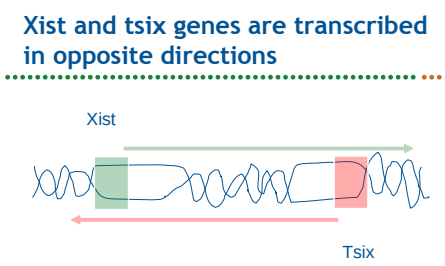
Xist RNA

- X inactive specific transcript
- Inactive X in the nucleus has red dots on it
- Red dots are RNA
- Xist RNA coats the chromosome and silences gene expression
  - RNA binding to DNA to shut off the DNA
  - Possible method of epigenetic regulation
- Inactive X is not entirely shut off though
  - several hundred genes are still expressed
  - how you get women with only one X
- X inactive specific transcript
  - coded on the top strand, reading 3' to 5' left to right
  - looks like any other RNA
  - however never translated
  - does not function as RNA
  - function is to bind onto DNA in an area and begin X inactivation
  - agent of epigenetic control in the X chromosome
  - eventually coats the whole chromosome
  - eventually coats the whole chromosome
- Tsix



Xist and tsix genes are transcribed in opposite directions

    - does not function as RNA
    - function is to bind onto DNA in an area and begin X inactivation
    - agent of epigenetic control in the X chromosome
    - eventually coats the whole chromosome
- Tsix
    - Coded on the bottom strand, reading 3' to 5' right to left
- Competition between Xist and Tsix
    - One or the other may get expressed enough to shut off or antagonise the other
    - not sure exactly how it works
    - example of how genes can overlap
- Both Tsix and Xist do not code for proteins
    - but code in same region, just opposite strands of the DNA helix



Epigenetic Mechanisms

- One way to shut off chromosomes is to pack them tightly
    - during metaphase, chromosomes are not transcribed that much, barely at all
- X inactivation does all of the following
    - RNA binds on to DNA
        - Packs it down, shuts it odd
    - Chromatin packing
        - DNA packed tightly, not transcribed
    - Tight packing attracts methylases, acetylases
    - Cell throws everything it has to ensure that the X chromosome is not expressed
- Inactive X chromosomes replicate late in the cell cycle
    - inactivation is heritable over generation of cells
    - during replication however, inactive X has to be unwound

Readings

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15.2b Chromatin Remodelling

- DNA is organised into chromatin by combination with histone proteins
    - The DNA is wrapped around a core of two molecules, each made up of two molecules made of histones to form a nucleosome
- Genes in regions of DNA tightly wound around Histones are inactive because their promoters are not accessible by the proteins that initiate transcription
- Activating a gene involves changing the state of the chromatin so that the proteins can initiate transcription and bind to their promoters by a process called Chromatin Remodelling, which can occur in one of two ways
    - An activator binds to a regulatory sequence upstream of the genes promoter and recruits a remodelling complex
        - this complex is a protein that displaces the nucleosome from the chromatin thus exposing the promoter
    - An activator binds to a regulator sequence upstream of the genes promoter and recruits an enzyme with acetylates the histone in the nucleosome
        - this loosens the histones association with the DNA and the promoter becomes accessible
        - acetylation is reversible by deacetylation enzymes, which remove the acetyl group from the histones

15.2d Methylation of DNA can control Gene Expression

- DNA Methylation - in which a methyl group is added to cytosine bases in DNA by an enzyme - can regulation transcription
    - the methylation of cytosine in promoters inhibits transcription and turns genes off in a phenomenon knows as gene silencing
- DNA methylation can silence large blocks or genes, or even whole chromosome
    - Barr bodies are created through methylation, turning off all the genes in one of a females X chromosome
- DNA methylation underlines genomic imprinting, a process by which methylation permanently silence transcription of either inherited maternal or paternal alleles of a particular gene
    - this methylation occurs during gametogenesis in a parent
    - An inherited methylated allele is never expressed, it is silenced and know as the imprinted allele
    - therefore the expression of that gene depends solely on the expression of the other, non-imprinted allele
    - The methylation of the parental allele is maintained as the DNA is replicated, therefore a silenced gene remains silenced and inactive in progeny cells

DNA Structure and Replication

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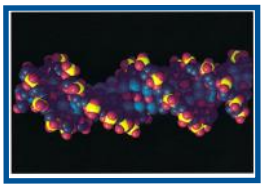
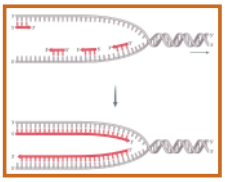
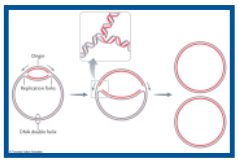
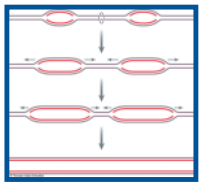


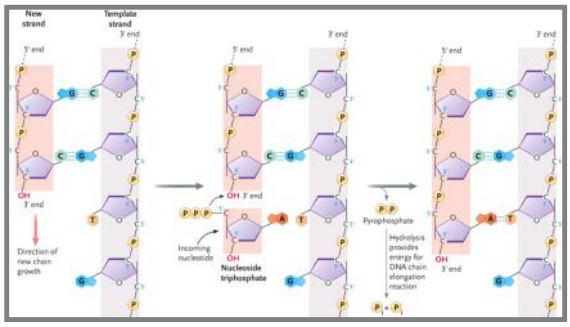






DNA

- DNA information is relatively stable and readily replicated
- Anti-Parallel complimentary base pairing
  - allows for acurate replication
- Replication is semi discontinuous and semi conservative
  - replication forks
    - contain both strands
    - replication of both strands happens at the fork
  - collective proteins know as a replisome
- Begins at an origin
  - specific DNA sequence recognised by replisomes
  - synthesis in both directions
    - bubbles in prokaryotes
    - e.g. Mitochondrial DNA
  - Many origins in Eukaryotes



Elongation by DNA Polymerases

- All DNA polymerases can do only one thing
  - can only extend the 3' end of a properly paired base
  - need for a template

Replication Forks

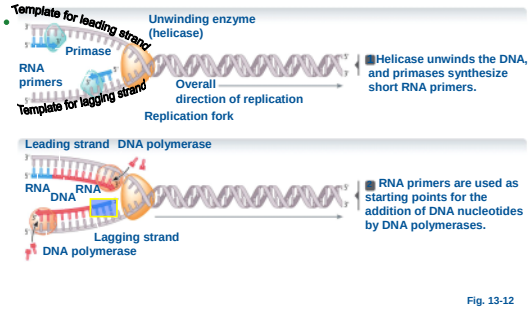
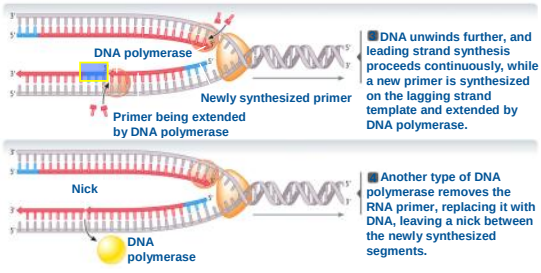


Fig. 13-12

Replication Forks

- DNA polymerase needs a properly paired base
- RNA primase
  - makes RNA
  - insert RNA primers
  - provide first 3' OH that the DNA polymerase can then extend
  - can be done continuously on leading strand
  - must be discontinuous on the lagging strand
    - new primer then must be placed
    - DNA polymerase I then replace this new primer with DNA
    - leaves a nick between newly synthesised segments
    - segments are joined together by Ligase
      - seals the nick

Replication Forks



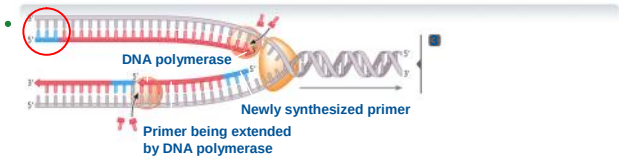
Replisomes

- During DNA replication both strands act as the template
- Replisomes are multi enzyme complexes that replicate both strands of the DNA at the same time.
- DNA on one strand loops in order to be synthesised discontinuously
- Both continuous and discontinuous occur at the same fork at the same time

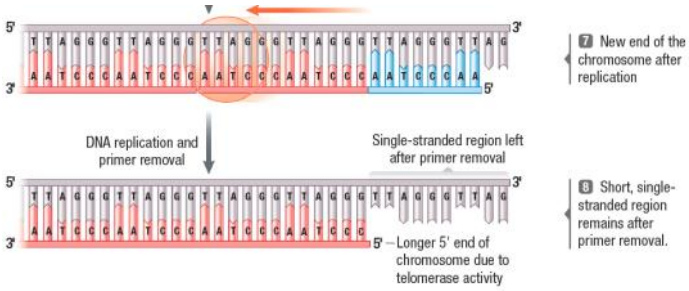
End of the Chromosome

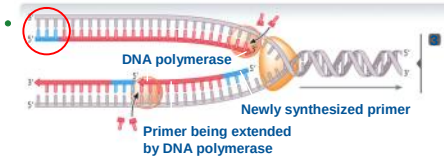
- at the very end of the chromosome there is a primer on the 5` prime end of the leading strand
- this primer gets removed, but it cannot be replaced with more DNA
- leads to chromosome shortening
  - every replication, chromosome gets shorter
  - cant be stopped
- Enzyme Telomerase makes sure that this does not matter
  - extends the 3`prime end
  - brings its own template - piece of RNA
  - makes DNA from an RNA template

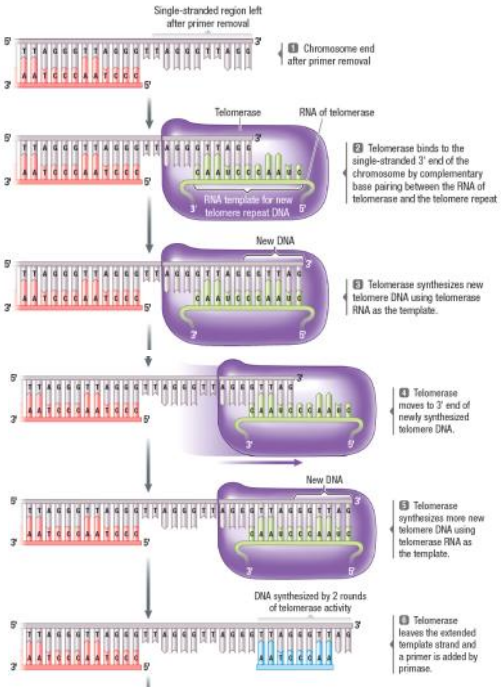
What about the leading primer?



- cant be stopped
- Enzyme Telomerase makes sure that this does not matter
  - extends the 3`prime end
  - brings its own template - piece of RNA
  - makes DNA from an RNA template
  - does this over and over again
  - keeps adding the same sequence
  - leads to repeats on the ends of the chromosome
    - telomeres
  - happens at both 3`ends of the chromosome
  - Means that when the chromosome shortens, you only lose the telomeres, not important coding regions.
  - Telomerase is off in almost all of your cells
    - want chromosome to get shorter
    - want cells to know how old they are
    - so they can die - otherwise may become cancer cells







Readings

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13.3 DNA Replication

- During Replication, the hydrogen bonds between the two DNA strands break and the two strands unwind and separate. Each of these strands then acts as a template for the synthesis or a new strand. This means that each new DNA double helix has one new strand, and one old strand - hence the term Semi Conservative
- During Replication, the complimentary nucleotide chains are assembled from individual nucleotides by enzymes known as DNA polymerases
    - DNA Polymerases catalyze the assembly of a new DNA strand that is complimentary to the template strand
- Each DNA strand has two distinct ends - the 5' end with an exposed phosphate group, and the 3' end with an exposed -OH group - which lie anti-parallel to each other
- One of the characteristics of DNA polymerase is that it can only extend the 3' end of an existing nucleotide chain
    - the polymerase can only add on the complimentary base of the next base on the template strand and catalyze the bonding of the 3' OH the 5' Phosphate group
    - The energy release from the hydrolysis of the 5' phosphate group is used for the formation of the new bond - think energy coupling
- As the new DNA strand is assembled, the 3' -OH is always exposed at it's newest end, while the oldest end of the chain has an exposed 5' phosphate group
    - DNA polymerase is therefore said to assemble nucleotide chains in the 5' to 3' end
    - Due to the anti-parallel nature of DNA, Polymerases read the template strand in the 3' to 5' direction
- The Key Molecular Events of DNA replication are as follows…….
    - The two strand of the DNA unwind in order for replication to occur
    - Nucleotides are added only to an existing chain
    - The overall direction of new synthesis is in the 5' to 3' direction - anti-parallel to that of the template strand
    - Nucleotides enter into the newly synthesised chain according to the A-T, C-G pairing rules
- In order for replication to be semi-conservative, the two strands of parental DNA must unwind and separate - exposing the template strands for DNA synthesis
    - This unwinding process creates a Y-shaped structure - a replication fork - consisting if the two unwound template strands
- The enzyme helicase catalyzes the unwinding
    - Helicase uses the energy of ATP hydrolysis to unwind the DNA helix
- The exposed single-stranded segments are then coated with single-stranded binding proteins which stabilize the DNA and are then displaced as the replication enzymes make the new nucleotide chain from the template strand
- If DNA Polymerase can only add nucleotides onto the 3' end of an existing strand, then how can a new strand be made - there are no pre-existing nucleotides in place
    - Primers - made up of RNA nucleotides - are lain down by the enzyme primase and then removed and replace with DNA later on in the replication process.
- As polymerases can only assemble in the 5' to 3' direction, and because DNA molecules run anti-parallel, only one of the template strands lies in a direction which allows for the new strand to me synthesised in a direction complimentary to the direction of unwinding
    - the other template strands runs in the opposite direction - away from the site of unwinding - meaning that DNA polymerase has to copy in the opposite direction also
    - the polymerases make this strand in short lengths - Okazaki fragments - which are then covalently linked together
    - this kind of replication is known as dis-continuous replication
- The new strand assembled in the direction of the unwinding is called the leading strand, while

the other is called the lagging strand

- Helicase, Primase, DNA Polymerases and other enzymes all coordinate in order to replicate DNA
    - Helicase unwinds the DNA to produce the replication fork
    - Just behind the site of the replication fork, Primase lay down the RNA primes - about 10 nucleotides in length each - which are assembled in the 5' to 3' direction on both of the template chains
    - DNA polymerase then adds DNA nucleotides onto these RNA primers
    - On the leading strand, DNA polymerase continues to add on nucleotides off of one prime
    - However on the lagging strand, the DNA polymerase eventually reaches the RNA primer placed before it
        - when this occurs, a different DNA polymerase binds, replacing the RNA nucleotides in the primer with DNA ones, the enzyme ligase then seals the nick left between the old primer and the last DNA nucleotide added on to the chain
    - This continues on in the same way until the entire DNA molecule has been copied
- This method leads to an issue of chromosome shortening, which is solved by the addition of telomere repeats by the enzyme telomerase
    - When the last primer is removed from the end of the newly synthesised strand, there is no longer a 3' end for DNA polymerase to add on nucleotides and complete the strand
    - this means that every round of replication, the chromosome is shorted - each strand of DNA being used as a template gets progressively shorter
    - In most chromosome however, the functional coding genes are protected by a region of non-coding, repeating sequences of DNA - telomeres
    - These telomeres are what gets shorted during each round of replication, instead of important, functional coding DNA
- The enzyme telomerase maintains this buffer region by added telomere repeats onto the end of chromosomes
    - After the last primer is removed from the template strand, there is a gap at the end of the chain left
    - Telomerase adds its own primer, which then extends from the 3' end, filling the gap
    - when this primer is removed, a gap appears, but it is only in non-coding DNA
    - Telomerase contains its own RNA template, which it uses to make telomere primers
- Telomere shortening has been linked to the aging process, with old/dying cells generally showing greater telomere shortening
- 90% of cancer cells show overly active telomerase
    - this presents a potential cure, if telomerase activity in these cells can be shut off, leading to cell/tumour death
- The site of replication are called replication origins
    - these origins are recognised by proteins which bind to the DNA, stimulating helicase
    - replication proceeds from both side of these origins, and eventually meet up and complete the replication of the chromosome

Gene Mutation

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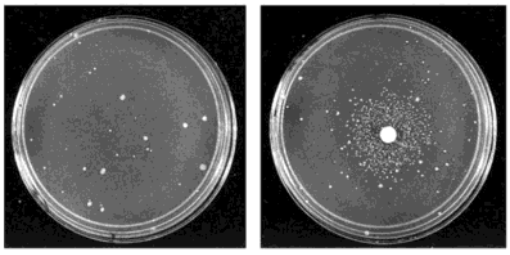
Mutation

- A Mutation is a heritable change in the double-stranded DNA sequence
- common types of change in the DNA sequence
  - base substitutions
  - Insertion
  - deletion
  - reorganisation

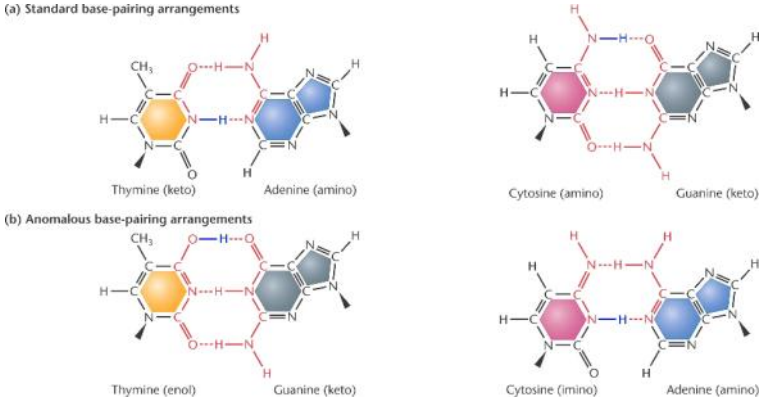
Ames Test

- Preliminary screen for chemical mutagens
- Bacterial Test
- his$^-$ Salmonella
  - dies on minimal medium
  - cant make histodine
  - his$^+$ mutations can grow
  - reversion - when a mutant goes back to normal wild type
    - mutation back to normal
  - spontaneous mutations that restore his$^-$ back to his$^+$
- Use of known mutagen as a control
  - for example cigarette smoke
  - increased the frequency of reversion mutations



What mechanism explains the his- to his+ reversion

- most mutagens simply enhance natural mechanisms for mutations
- Spontaneous tautomeric shifts change base pairing rules
  - Thymine changes from a Ketone to enol form
  - means that it can form 3 Hydrogen bonds
  - therefore calls for G opposed to A
- Some mutagens are tautomerically unstable base analogues
  - Thymine and 5-Bromouracil
  - cells cant tell the difference
  - get incorporated into DNA in exactly the same way
  - however analogues are tautomerically unstable
    - means that they keep switching
    - causes damage leading to mutation



Mutagenesis

- Mechanism based in base pairing
- DNA Polymerase
  - extends 3'prime end
  - need for a template
  - whatever is on the template calls for its pairing partner

Substitution Mutation Mechanism

- Replication Bubble
- Top strand
  - leading for the replisome on the right
  - lagging for the replisome on the left
  - the same DNA strand can be both leading and lagging
- Replication error
  - for example C instead of T
  - leads to an A - C mismatch
    - not a mutation
    - damage
    - only a change in one side of the sequence
  - after the next round of replication, the A will call a T, but the C will call a G
    - that is mutation
    - change occurs in both strands of the double helix
- Basic mechanism for most mutation
  - replication errors

Insertion/Deletion Mechanism

- String of As
- each calls for a T
- Ts can loop out
- means that previous Ts have to get replicated again
- replication error resulting in slipage of one strand against the other
  - means that extra or less bases have to be added in
  - becomes a mutation after the next round of replication

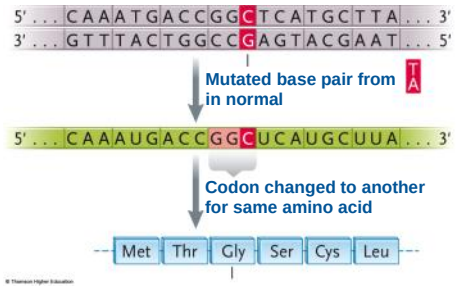
Codon Sequence Corresponds to Amino Acid Sequence

- Silent Mutations
  - the changed codon specifies the same amino acid
  - genetic code is redundant
  - many codons that code for the same amino acids

Silent mutations: changed codon specifies the same amino acid

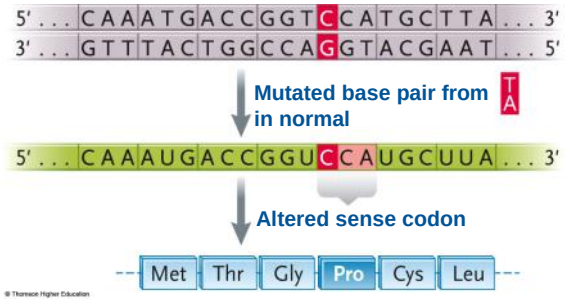
- many codons that code for the same amino acids

Silent mutations: changed codon specifies the same amino acid



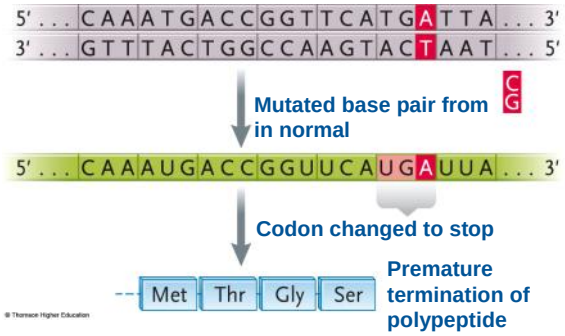
- Missense Mutations
  - Changed codon specifies a different amino acid
  - varying effects depending on where the base is in the codon, where the amino acid is in the protein etc.

Missense mutations: changed codon specifies changed amino acid



- Nonsense Mutations
  - changed codons specifies `stop`
  - leads to premature termination of the polypeptide
  - unlikely to give a functional protein

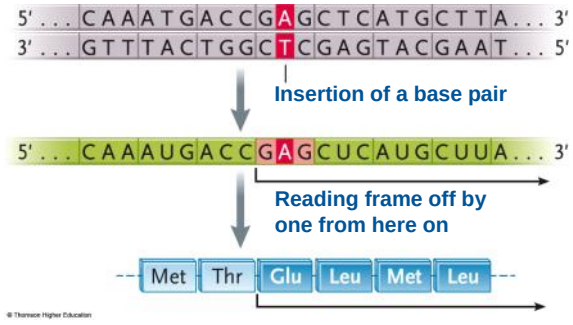
Nonsense mutations: changed codon specifies "stop"



- Indel Mutation
  - shifts the reading frame downstream
  - start codon sets the frame
    - read from here in 3s
  - leads to change in amino acids all the way downstream of the insertion or deletion
  - means that `stop` codons cant be read properly
    - it will read through the stop codon
    - will translate the UTR and keep reading and translating until it hits a new stop codon that it can read.

Indel mutations: shifts the reading frame downstream

Indel mutations: shifts the reading frame downstream



What about mutation that affect DNA outside of coding regions

- Mutations in promoters
- in t-RNA genes
- in Lac Operon

Biological Mutagens

- Biological agent that causes a mutation to the double stranded helix
- Retroviruses are biological mutagens
- e.g. HIV
    - Viruses get inserted into cells
    - reverse transcriptase
        - turns RNA into a single strand of DNA
        - DNA from an RNA template
        - error prone
        - poor proof reading ability
        - throws mutations into the Viral Chromosome as it replicates
        - the single strand of DNA is then reserve transcribed into Double Stranded DNA
        - Intergrase (from the virus) takes the double stranded DNA into the nucleus of the host cell
        - Finds the host chromosome
        - Intergrase makes a nick in host chromosome and inserts the HIV DNA

Chromosome Mutations

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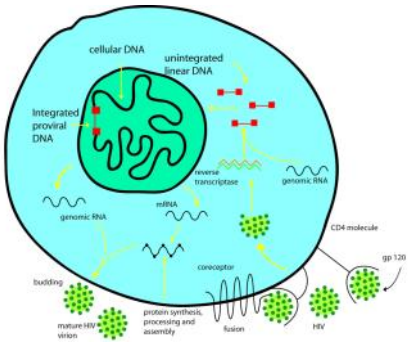
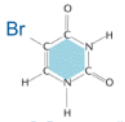
Substitution/Deletions/Insertions
- Small Mutations
- Point Mutations

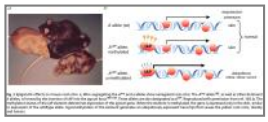


Biological Mutagens
- Retroviruses are biological mutagens
- HIV
  - Retrovirus binds to CD4 receptor molecules on cell membrane
  - Enters cell (internalised)
  - dumps RNA genome into cytoplasm
  - RNA gets reverse transcribed into double stranded DNA
  - DNA crosses the nuclear membrane
  - gets integrated into the host chromosome
  - causes double stranded change in DNA sequence
- Not all viruses are mutagenic
  - Only viruses that enter chromosome directly are
  - retroviruses are
  - viruses such as measles, mumps are NOT mutagenic

Mutagens can be chemical, biological or physical.



5- Bromouracil

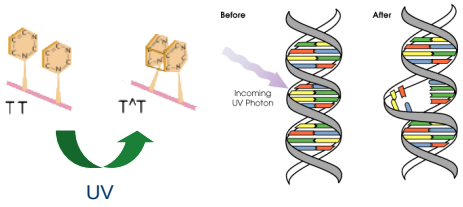


Mobile Elements
- IAP
  - IAP IS DNA
- mutagenic mobile element
- retro-transposon (10.4a)
- mobile elements make up are large amount of genomes
  - e.g. corn genome is 50% mobile elements
  - hop in and out of genes - for example for pigments
    - cause striped phenotypes in corn kernels for example

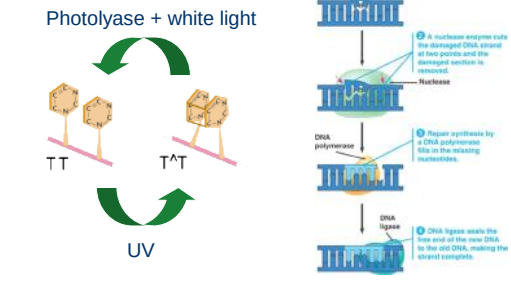
Mutagens can be Biological, Chemical or Physical
- Any chemical that modifies a base in any way, causing it to call the wrong pairing partner is mutagenic
- Biological elements
  - Retro-viruses
  - Retro-transposons
  - F-Plasmid is a mutagen
- Physical Mutagens
  - U.V. Radiation

UV radiation causes thymine dimers that distort the helix.

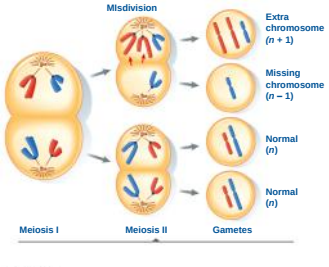
U.V. Radiation
- Photons of U.V. light are absorbed by DNA
- Energy results in a reorganisation of covalent bonds, particular in adjacent Thymine
- results in Thymine dimer
  - two thymine covalent bonded together
- Distorts the DNA double helix
- Dimers are fatal to a cell
  - can not replicate or transcribe DNA if it has a DNA
- Dimers cause damage, not double stranded mutations
- Dimers can be repaired by photolyase or excision
  - Photolyase
    - breaks the covalent bonds in the dimers, restores Thymines
    - photolyase is driven by white light - visible spectrum
    - Tanning beds
      - U.V. light, but no white light
      - prevents ability to repair damage
  - Excision
    - removal of dimers
    - distortion of helix is so dramatic, easily detected by endonucleases
    - make a nick on both sides of the dimer
    - remove the dimer and replaced
    - ligase then seals up the nicks
  - However, the repair is very error prone
    - this can lead to mistakes
    - leads to mutations
    - the more you have to repair, the more chance for mistakes, the more chance for mutations to occur

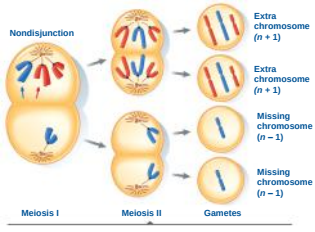


Dimers can be repaired by photolyase or excision.



Radiation
- Chernobyl accident
  - spewed radioactive fallout over most of Europe
- Radioactivity
  - characterised by unstable isotopes
  - unstable nuclei decay
    - produce a variety of products
    - danger depends on product and ionising ability of those products
    - Iodine and Caesium decay creates ionizing radiation
    - create reactive oxygen species in cells
    - these oxygen species cause damage in cells
      - oxidise bases
      - breaking DNA backbones
  - thyroid glands concentrate iodine
    - if exposed to radioactive form, they concentrate that
- Effects of radiation exposure on a large population
  - what to study?
  - how to study?
  - epidemiology
  - What are the effects?
    - Down Syndrome incidence spiked in Belarus 9 months after exposure from Chernobyl accident
    - Exposure to radiation may mess up meiotic segregation
      - Misdivision
      - Nondisjunction
      - affecting the cytoskeleton may be a mechanism for large scale chromosomal mutations
    - Thyroid Cancer rates increased
      - all populations
      - particularly adolescents
      - may be caused be increased exposure to radioactive iodine
  - what if exposure had no effects?
    - accident lead to increase in public health screening programs
      - rates of cancer don't actually increase
      - however detection rates do
  - difficult to establish what the actual effect actually is
  -





        - rates of cancer don't actually increase
        - however detection rates do
    - difficult to establish what the actual effect actually is
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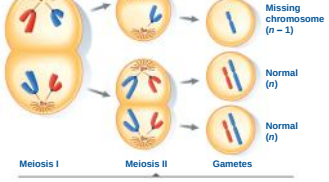
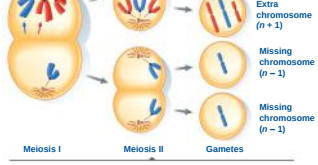
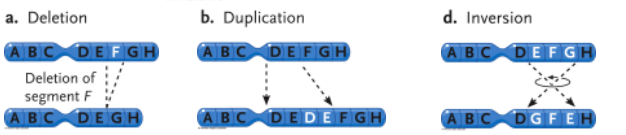


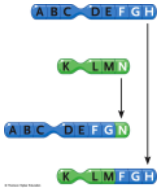


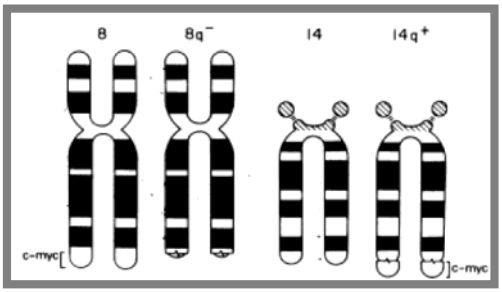
<u>ROS can cause breaks in DNA helix, resulting in rearrangements</u>

- Ionising radiations break chromosomes
- cells attempts to repair damage is imperfect
    - fragments can be lost, duplicated, inverted
- Chromosomal level mutations
- Translocation
    - swapping of chromosome pieces between two chromosome
    - don't lose DNA, just move its location from one chromosome to the other
    - often don't have much effect
- Translocation of chromosome 8 and 14
    - antibodies are proteins
    - when you need antibodies, you need a lot of them
    - antibody genes are under powerful expression control
        - powerful promoters etc.
    - gene products drive cell division
        - Myc
    - Chromosome 14 carriers antibody genes
        - with powerful promoter and enhancer
    - Chromosome 8 carriers Myc gene
    - Translocation leads to mix of antibody genes and Myc
        - leads to antibody promoters and enhancers affecting the expression of myc gene
        - leads to massive expression of gene that drive cell division
        - leads to lymphoma
        - myc is an oncogene
            - oncogenes are genes that when deregulated can result in cancer
            - when regulated perfectly normal part of genome
        - Chromosome level mutation that creates a new gene that affects cell division dramatically



translocation





Cancer

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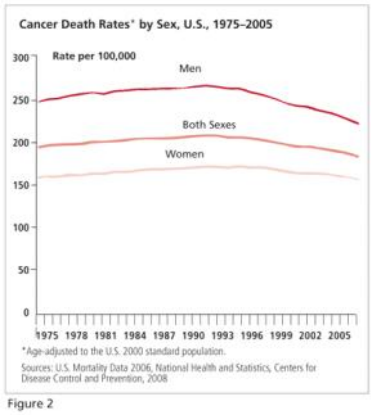


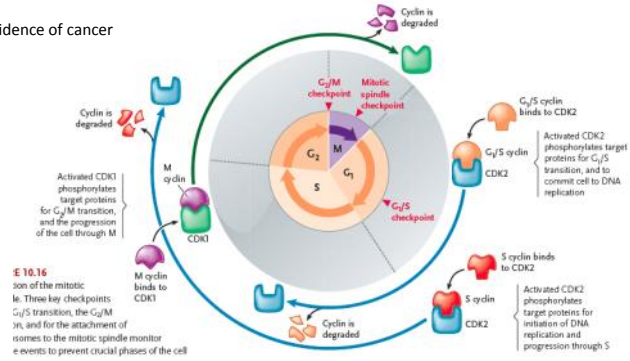
Figure 2

War on Cancer
- Nixon - 1971
- some battles won
  - headway made in some kinds of cancer sometimes
- but overall death rates haven't changed all that much

In Canada, men are at a higher risk of cancer than women
- 40% of women can expect to contract some kind of cancer in their lives
- just <25% of women can expect to die from cancer and related complications
- 45% of men can expect to contract some form of cancer
- just >25% of men can expect to die from cancer
- older you are, the more likely you are at developing cancer
- second most likely non-accidental cause of death
  - heart disease is first

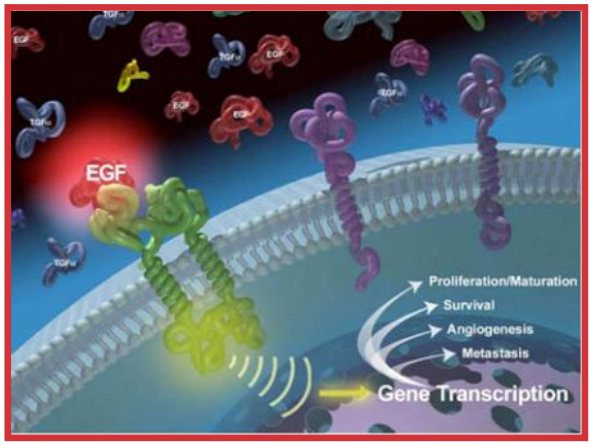
Top Four Cancers
- Most frequent for men is prostate
- most frequent for women is breast cancer
- other two are Lung and Colon
- risk factors
  - exposure to dietary risk factors
  - exposure to environment
    - e.g. smoking for lung cancer
- Lung and colon suggest environmental risk factors
- Breast and Prostate suggest internal risk factors
- balance of and interactions of internal and external factors that contribute to the incidence of cancer

CDK2 (Cyclin Dependant Kinase)
- Cells may actively cycle, sit in G_0 or undergo apoptosis
- CDK2 (Blue Protein)
  - CDK2 is 'Cyclin Dependant Kinase'
  - phosphorylates
  - only active when it is bound to cyclin
  - post-translational regulation example
  - constitutive expression
  - however production of cyclin is cyclic
  - CDK2 binds to G_1/S cyclin
  - CDK2-Cyclin complex phosphorylates its target proteins
  - leads to release of the G_1/S checkpoint
  - cells proceed into S phase
  - Another CDK2-Cyclin complex regulates movement from S phase into M phase

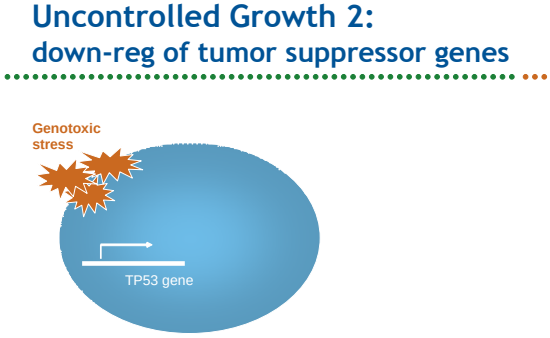


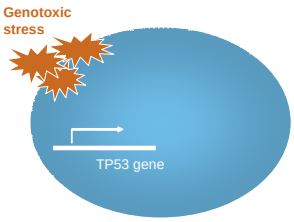
Connection between DNA and Cancer
- Mutations can increase risk of cancer
  - many mutagens are also carcinogens
- Cancer cells tend to expressing Telomerase when they shouldn't be
- Twin Studies
  - twins raised apart have a higher concordance rate for cancer
- Some families have a history of cancer
  - Breast cancer seems to affect some families more than others
- Mobile elements, translocation
  - under control of inappropriate expression

Uncontrolled Growth 1: Up-Regulation and Proto-Oncogenes
- Proto-Oncogenes are essential for normal growth and development
  - EGFR
    - Epidermal Growth Factor Receptor
    - typical Transmembrane protein
    - active site outside the cell that binds protein hormone EGF
    - EGF stimulates cells to divide
    - EGFR signals on the inside of the cell
      - phosphorylation cascade signals presence of EGF on the outside of the cell
      - causes the expression or not of certain genes in the nucleus
    - any step along the pathway could be expressed inappropriately, causing it to become cancerous
- When deregulated proto-oncogenes become cancerous
- Mutation in EGFR, causing it to signal even when EGF is not present, will lead to the cell dividing all the time
  - Up-regulation
- How Might Proto-Oncogenes Be 'Activated'
  - Translocation issues - as with MYC
  - Mutation in promoter
    - causes the promoter to be more attractive to polymerase, resulting in more expression
  - Regulation problem with Enhancers or Repressors
    - Enhancers become more effective than usual



Uncontrolled Growth 2: Down-Regulation and Tumour Suppressor Genes
- Suppressor Genes work against Proto-Oncogenes to ensure that cell division occurs at a regular pace
- Suppressor Genes can under go Genotoxic Stress
  - damage of any kind
  - shortening of telomers to critial length
  - induce the expression of TP53
- TP53 is a transcription factor that binds the promoters of many target genes
  - P53 transcription factor
  - results in.....
    - DNA repair
    - G_1 arrest
    - Apoptosis
- How might a TS (Tumour Suppressor) gene be inactivated
  - mutation in the gene itself
  - High Methylation, shutting off promoter
  - Mutation in microRNAs
    - mistakenly shuts off tumour suppressor gene





- How might a TS (Tumour Suppressor) gene be inactivated
  - mutation in the gene itself
  - High Methylation, shutting off promoter
  - Mutation in microRNAs
    - mistakenly shuts off tumour suppressor gene
    - miRNAs highly important in gene expression, particularly in cancer
    - Oncomirs
      - Onco-microRNAs

Why is Some Cancer 'Sporadic' and Some 'Familial'

- most cancer in this country is sporadic
  - not risk due to family
- However for families that are at higher risk
  - some sort of gene that's damaged or possess certain epigenetic marks that is being inherited through the family
  - people DO NOT inherit activated oncogene
  - more likely that they are inheriting an inactivated TS gene
    - mutation, epigenetic marking
    - inherit one defective allele for the gene
    - at some point in their life, the other allele in one of their cells becomes inactivated too, leading to development of tumours
    - case with most familial breast cancers

Human Papilloma Virus (HPV) is a Carcinogenic DNA Virus

- DNA Virus
  - NOT a retro virus
  - NOT an RNA virus
  - DOES NOT insert into chromosome as part of usual life cycle
- Many different strains cause increased growth (warts) in different tissues in both men and women
- some strains are oncogenic; particularly strong association of strains 16 and 18 with cervical cancer
- HPV is a sexually transmitted virus
- Total HPV prevalence - 27% (women 14 to 59)
- 45% in women ages 20-2Total incidence of 'high risk' type 16 and 18 was 2.3%
- 10 fold increase in risk with more than 1 partner in last year
- highest risk is if partner sometimes uses a condom
  - higher than most of them time, higher than little of the time
- biggest risk factor is previous partners of sex partners

Readings

April-16-11
12:46 PM

9.4 Cell Cycle Regulation

- A number of internal and external regulatory mechanisms control the mitotic cell cycle
    - the cell cycle has built in check-points that prevent critical phases from beginning until the previous phase has been completed
    - Hormones, growth factors and other external controls coordinate the cell cycle with the needs of an organism by stimulating of inhibiting division
- Cyclin-Dependant Kinases (CDK) are major contributors to the regulation of cell division and directly affect the progression of the cell cycle
    - CDKs are protein kinases; enzymes that add phosphate groups to target proteins
    - the are switched on only when combined with the protein cyclin - hence the name Cyclin-Dependant
    - The concentration of cyclin rises and falls with the cell cycle, so therefore so does the activity of CDKs
- Several different cyclin:CDK complexes regulate cell cycle transition at different checkpoints
    - for example at the G_1 to S checkpoint cyclin E reaches a concentration high enough to form a complex with CDK2, thus activating it
    - The CDK2 complex then phosphorylates a number of cell cycle control proteins triggering the progression into S phase
    - after the transition into S phase is made, the Cyclin is degraded and the CDK2 complex becomes inactive again
    - Similar events occur with different cyclin:CDK complexes at the G_2 to M checkpoint
- While the cyclin:CDK complexes directly control the cell cycle, other factors within the cell act as indirect controls by affecting the activity of the cyclin:CDK complexes
    - at each checkpoint, the activity of each CDK complex is blocked from triggering the associated cell cycle transition until the actions of the previous phase are completed
    - for example the CDK1 complex - which stimulates the cell to enter the M phase out of G2 - is kept inactive by phosphorylation until the cell is ready, at which point phosphatase removes the inhibitory phosphate
- Checkpoint control are exerted in many circumstances
    - if the cells DNA has not been fully replicated, then cell division is postponed until the replication cycle has been completed
    - if the DNA suffers damage, the cell cycle is postponed until the damage can be prepared
- The internal control that regulate the cell cycle are modified by signal molecules from outside of the dividing cell
    - in animals these molecules include peptide hormones and growth and death factors
- Many of the external factors bind to receptors at the cells surface, which results in a reaction inside the cell
    - these reaction often include the addition of inhibiting or stimulating phosphate groups to the cyclin:CDK complexes
    - they may also affect the target proteins of the CDK complexes
    - the overall effect is to speed, slow or stop the process of cell division
- Some growth factors can reverse cell arrest and move a cell shunted into G_0 phase back into active cell division
- Cell surface receptors in animals are also able to recognise contact with other cells of with the molecules of the extracellular matrix
    - This contact triggers internal reaction pathways that inhibit cell division by arresting the cell cycle - usually in G_1 phase
- This response is call contact inhibition and stabilises cell growth in fully developed organs and tissues
    - so long as the cells of most tissues are in contract with one another - or with the

extracellular matrix - they are shunted into G_0 phase and prevented from dividing
    - if these contacts are broken, then the freed cells often enter into further rounds of division
- Cells cannot divide indefinitely -eventually the stop dividing.
    - this loss of proliferative ability over time is called cellular senescence
- Hayflick factors are responsible for this senescence - with DNA damage and Telomere shortening thought to be the two main candidates
    - Accumulated DNA damage to cells would result in reduced function as the grow older - especially if the mutations were in genes coding for vital function proteins
    - Once telomeres shorten past a critical length, further rounds of division would result in the shortening of coding regions of the chromosome - once telomeres are reduced apst this length, cells stop dividing
- Cellular Senescence is an important anti-tumour mechanism
    - cells with stimulated telomeres often divide out of control
    - mice engineered to have no telomeres are significantly immune to cancer
    - by the time a cell is short on telomeres, it is already a long way towards cancerous growth
- Cancer occurs when cells lose the normal controls that determine when and how often they will divide
    - cancer cells divide uncontrollably, produced a rapidly growing mass called a tumour
    - cancer cells also typically lose their adhesions to other cells and often become actively mobile, this results in metastasis in which they break lose from the original tumour and spread throughout the body and produce new tumour growth
    - metastasis is promoted by changes that defeat contact inhibition and alter cell surface molecules that bind cells together
- Growing tumours damage surround tissues by compressing them and interfering with bloody supply and nerve function
    - tumours may also break barriers such as the outer skin, internal cell layers or the gut wall - often destroying the separation of body compartments needed for normal function
- Cancer cells have typically accumulated mutations in a variety of different genes that promote uncontrolled cell division or metastasis

    Jack at 16/04/2011 2:33 PM
    - before undergoing mutation, many of these genes code for components of the cyclin:CDK system that controls cell growth or proteins that regulate gene expression, form cell surface receptors or make up[elements of the signalling pathways controlled by receptors
    - when mutations these genes - or oncogenes - encode altered versions of these products
- Some cells are in fact programmed to die by apoptosis - programmed cell death
    - normal development of multicellular organisms is a highly regulated balance between cell proliferation and death
    - the mechanisms for such is an ancient one common to all multicellular eukaryotes
    - initiation of cell death can result from either internal or external signals
- The apoptosis machinery in *C. elegans* is available in all of it's cells
    - the main 'executioner' enzyme is from the family of normally inactive proteases called caspases and is coded fro by the 'cell death abnormal' gene - *ced-3*
    - if a cell is destined to die by apoptosis, the cascade begins when internal development cues stimulate expression of a gene called 'egg laying deficient' -*egl-1*
    - EGL-1 protein then binds to CED-9 protein resulting in the release of bound CED-4 protein and the formation of an active apoptosome
    - CED-3 caspase is then activated and cell death ensues
- Removing cells that are surplus for development is one function of apoptosis, but why else would a cell be programmed to die?
    - it would be beneficial for an organism to provoke apoptosis in cells suffering severe DNA damage, infection or mutation leading to uncontrolled division
    - sometime healthy cells have to die in order to be functional - xylem in plants

15.4 The Loss of Regulatory Controls in Cancer

- The Cell Division of all eukaryotic cells is controlled by genes
- the types of genes exerting this control are basically the same in terms of functions in all eukaryotes
- mutations in these genes can disrupt normal cell growth and division
- occasionally dividing and differentiating cells deviate from their normal genetic program and give rise to tissue masses called tumours
    - the cells lose their normal regulatory controls and revert - either partially or completely - to an embryonic developmental state by the process of dedifferentiation
    - if the cells stay together then the tumour is benign
    - however if the tumour invades or disrupts surrounding tissues then it is said to be malignant and is called cancer
- All the characteristic of cancer cells reflect changes in gene activity
    - many of the genes that become altered encode proteins that control the cell division cycle of normal cells
- A cancer cell does not respond properly to the usual signals and divides without the usual constraints
- Two main types of genes commonly show altered activities as cells become cancerous
- One class is the proto-oncogenes
    - these genes normally encode various kinds of proteins that stimulate cell division
    - In cancer cells the proto-oncogenes are altered to become oncogenes - genes that stimulate the cell to progress to the cancerous state
- Several mechanisms can convert proto-oncogenes to oncogenes
    - Mutation in a genes promoter or other control sequences may disrupt normal regulatory controls, making the gene abnormally active
    - Mutations in the coding segment of the gene may produce an altered forms of the protein that is abnormally active
    - Translocation may move a gene that controls cell division to a new location near a promoter or enhancer sequence of a highly active gene, making the cell division gene overly active
    - Infect viruses may introduce genes to regions in the chromosome where the expression of the gene disrupts cell cycle control or alters regulatory proteins to turn genes on
- Translocation - for example - may affect MYC, a proto-oncogene controlling cell division
    - the activity of MYC is normally highly regulated
    - however MYC lies in a chromosome region that often breaks off causing a translocation that places MYX near the enhancer and promoter of a highly active antibody gene
    - this placement makes MYC continuously active, converting it into an oncogene that triggers rapid and uncontrolled cell division
- Several proto-oncogenes encode for cell surface receptors that bind extracellular signal molecules
    - in general, the oncogene forms of these receptors are continuously activated resulting in the internal pathways they trigger to be continuously active - including those that cause cells to divide
- Another key group of proto-oncogenes encode enzymes forming parts of the internal reaction pathways triggered by surface receptors
    - the most important are the genes encoding for protein kinases
    - some of the proteins phosphorylated by the protein kinases take direct part in the gene regulation of initiation of control proteins which cause cell division to continue at high and uncontrolled rates
- Tumour-Suppressor genes encode proteins that inhibit cell division
    - Both alleles of a tumour-suppressor gene must be inactivated for inhibitory activity to be lost in cancer cells
    - the best know of these genes is TP53

    - normal P53 stops cell division by combining with and inhibiting CDKs that trigger entry into critical stages of DNA replication and mitosis
    - without the normal form of the P53 protein, the CDKs are continuously active and triggering cell division. Inactive TP53 genes are found in many types of cancers
- Cancer rarely develops by the alteration of a single proto-oncogene to an oncogene or inactivation of a single tumour-suppressor gene
    - in almost all cancers, successive alteration in many gene gradually accumulate to change normal cells into cancer cells
    - this is the multi-step progression of cancewr

DNA Technologies

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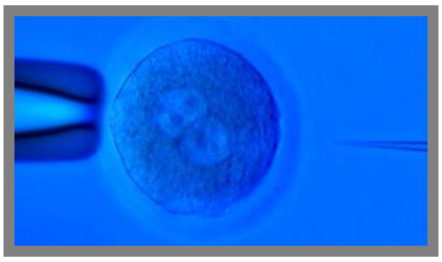
micro-injection



Gene Therapy

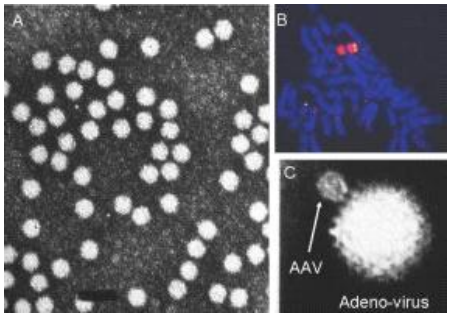
- Many disease caused by a single gene defect
  - opposed to life style diseases such as type II diabetes
    - many gene, environmental factors etc.
  - Single gene disease simple - understood
- Fix them?
- Somatic Gene Therapy and Germ-Line Gene Delivery
- Germ-Lime
  - changing the genes in the egg or sperm
  - need to know before hand that there are gene defects
  - comes along with ethical and technical issues
- Somatic Gene Therapy
  - much easier to do at the moment
  - introduction of the wild type directly into the cells
  - number of gene transfer techniques
    - Electroporation
      - use of electrical current to cause DNA to enter the cell
      - useful for bacterial transformation - insertion of foreign DNA
    - Microinjection
      - directly inject cells with the DNA
    - Biolistics
      - Gene gun
      - shoot DNA into cell
      - coat particles (usually Gold) with dehydrated DNA
      - shoot the particles at the cells
    - Ti Plasmid
      - plant specific
    - Viral Transformation
      - used in humans
- Terms for Transformation (Inserting DNA into cell)
  - Transduction
    - viral transfer
  - Transfection
    - inserting DNA into animal cells
  - Transformation
    - insertion DNA into plant cell
  - However all called transformation (will NOT get tested on the difference)
- In humans, Viruses are used
  - infections
  - easy method of transformation
  - inject foreign DNA into cells

TABLE 2. Clinical trials involving AAV vectors

Condition	Gene product(s)	Phase
CF	CFTR	I/II
Canavan's disease	Aspartoacylase	I
Parkinson's disease	GAD65, GAD65, AADC, neurturin	I
Alzheimer's disease	Beta nerve growth factor	I
Alpha-1-antitrypsin deficiency	AAT	I
Arthritis	TNFR:Fc	I
Leber congenital amaurosis	RPE65	I
Hemophilia B	Factor IX	I
Late infantile neuronal lipofuscinosis	CLN2	I
Muscular dystrophy	Minidystrophin, sarcoglycan	I
Heart failure	SERCA-2a	I
Prostate cancer	Granulocyte-macrophage colony-stimulating factory	I/II/III
Epilepsy	Neuropeptide Y	I

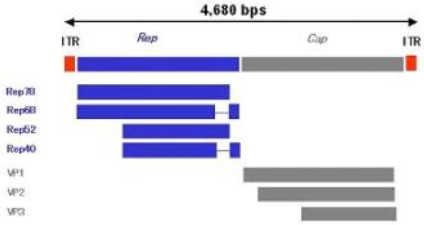
Viruses as Vectors of Gene Transfer

- Factors to consider
  - Pathogenicity
    - important to choose a virus that does not make you sick
    - patient may die from viral infection
    - want a virus that does not cause disease
  - Immune response
    - want a virus that does not cause a significant immune response
    - early trials of gene therapy, 17 year old patient died due to sever immune response
  - Random viral DNA insertion
    - DNA is inserted randomly into chromosome
    - may be inserted into other important genes
    - may damage other important genes
- Trials today use Adeno-Associated Viruses (AAV)



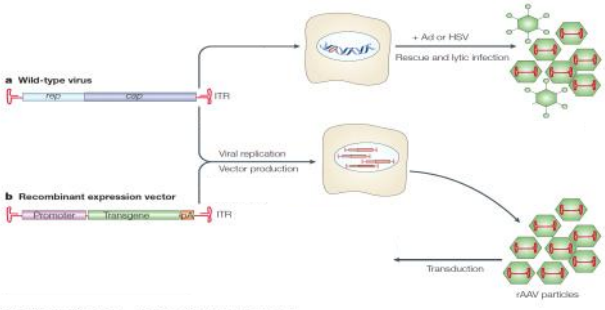
Adeno-Associated Viruses (AAV)

- Humans are primary host
- no know pathology
- very mild immune response
- very prevalent in human population
  - many people have AAV and do not know
  - very innocuous
- AAV genome integrates at a unique site on Chromosome 19
  - NOT random insertion
- Virus by itself does not replicate
  - Requires co-infection to propagate
  - by itself only integrates into Chromosome
  - usually uses factors from Adenovirus or Herpes



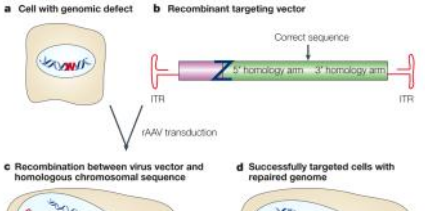
AAV Genome

- ssDNA (Single Stranded DNA)
- 7 genes
  - very small genome
  - 4600 base pairs
- inverted terminal repeats (ITRs)
  - play a role in genome replication and insertion at specific locus of chromosome 19



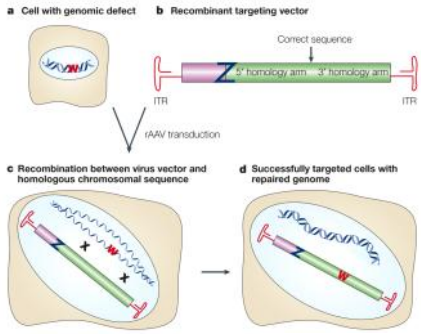
Formation of rAAV Particles

- For gene therapy, want to replace AAV genome
- want to make recombinant genome (rAAV)
- want to swap out rep and cap insert a promoter for needed gene between the UTRs
  - done in laboratory in packaging cells
  - specially cultured human cells that can take up recombinant expression and wild type together
  - leads to functional, mature viral particles being made



Homologue Recombination

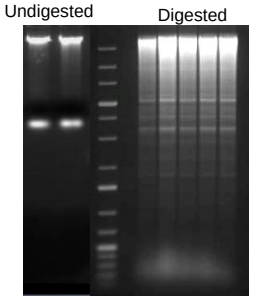
- Ideally, you want the wild type to be integrated into the same locus as the defective mutant gene
- want the wild type to swap out the mutant gene
- AAV allows this to occur
- replace the mutant with the wild type using homologous recombination within the cells



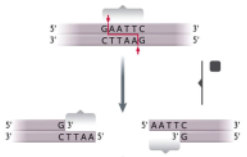
Gene Therapy and CFTR

- How do you get the CFTR gene fragment
- the human genome is sequence
- need the actual DNA to insert
- Isolated genomic DNA
  - use of restriction endonuclease to digest genomic DNA
- Polymerase Chain Reaction (PCR)

Isolated genomic DNA

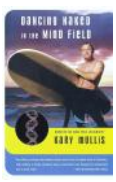


Restriction endonuclease

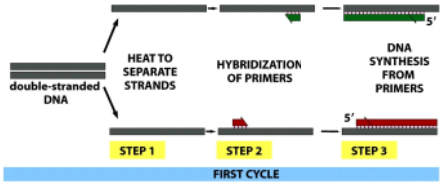


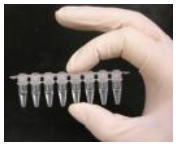
Polymerase Chain Reaction (PCR)

- Kary Mullis (Nobel Prize in 1993)
- In vetro amplification of DNA
- Used in…
  - Forensics
  - Phylogenetic studies
  - Disease testing
  - Gene Studies
- Genes are in very low abundance, not enough to see
- PCR allows you to get gene to high enough levels to see, and manipulate in laboratories
- PCR
  - Double stranded DNA (any form)
  - heat to break Hydrogen bonding (Denaturation)
    - 94°C for a minute or two
  - single stranded DNA primers are then annealed to the single strands
    - 45°C - 65°C for 15 seconds or so
  - Extension of the primers using DNA polymerase
    - 72°C
    - Enzyme is thermostable
    - adds about 1000 bases every 30 seconds
  - after 30 cycles you have a bout a 1 billion fold amplification of DNA
    - 30 Runs takes about 3 hours
  - now have enough DNA to manipulate
    - clone
    - digest
    - look at etc.



PCR cycling



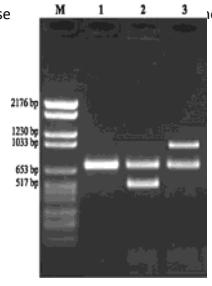


- Components for PCR
  - Set up a reaction
    - DNA template
    - Two oligonucleotide primers
    - Deoxynucleotides
      - d-ATP
      - d-TTP
      - d-CTP
      - d-GTP
    - Buffer components and water
    - Thermostable DNA polymerase
      - isolated from a thermophile
      - stable at 94°C
    - bung it all into PCR tubes and insert into PCR machine
    - PCR machines only heat up and cool down
      - $10,000 water bath

- Power of PCR is based on specificity
  - 4 billion bases in human genome
  - how can it amplify the correct bit of DNA from a few base sequences
  - PCR is based on extreme specifity
    - example 16 base primer for CFTR
    - GCGTGAATGCTAGCTA
    - In any DNA sequence….
      - 1/4 chance of finding an A, T, C or G
      - 1/16 chance of finding an di-nucleotide sequence
      - 1/256 chance of finding a given 4-base sequence
      - chance of finding a specific 16 base sequence is ONE IN 4 BILLION
      - these numbers allow you to amplify a specific se…e entire human genome

Agarose Gel Electrophoresis

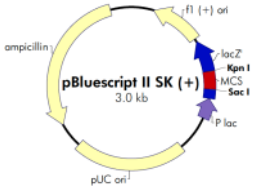
- start with 1-5ng of DNA
  - template for PCR
- cut out wanted band of DNA after electrophoresis
- digest away the agarose
- left with pure DNA
  - important to check that it is indeed the right band of DNA
  - use of cloning



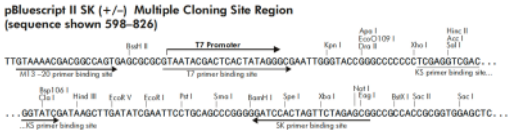
p-Bluescript; a cloning vector

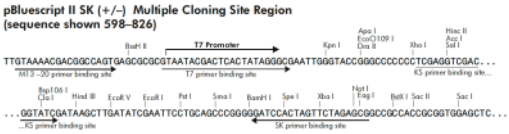
- Most common cloning vector
- selectable markers
  - ampicillin resistance gene
  - origin of replication
  - lac Z
    - required for synthesis of β-Galactosidease
  - lac Z is interrupted by multi-cloning site
- Multi-cloning site
  - enzymes can be used to clone things into that site
  - can only occur within the multi-cloning site

f1 (+) origin 135–441
β-galactosidase α-fragment 460–816
multiple cloning site 653–760
***lac* promoter** 817–938
pUC origin 1158–1825
ampicillin resistance (*bla*) ORF 1976–2833



pBluescript II SK (+/–) Multiple Cloning Site Region (sequence shown 598–826)



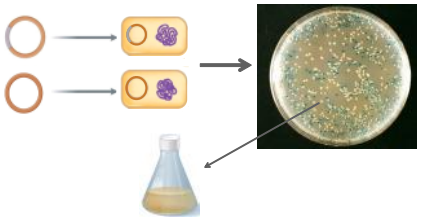


- required for synthesis of β-Galactosidease
  - lac Z is interrupted by multi-cloning site
- Multi-cloning site
  - enzymes can be used to clone things into that site
  - can only occur within the multi-cloning site
  - if something is cloning into multi-cloning site, it renders the lac Z gene non-functional

Bacterial transformation

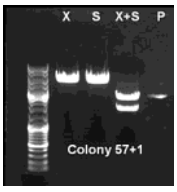
<u>Bacterial Transformation</u>

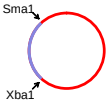
- Easy to get the plasmid into the bacteria
  - special kind of bacterial
- Once plasmid inserted into bacteria, the bacteria is placed on agar plate
- The agar contains ampicillin
  - only bacteria with plasmid will grow on the plate
- The agar also contains X-gal
  - X-gal is a sugar
  - turns blue in the presence of β-Galactosidease
  - if cloned gene is present in the plasmid, then the bacteria will grow white
  - no β-Galactosidease to break down X-gal and turn it blue
- Can then take the bacteria that contain both the plasmid and the cloned gene
- Place it in a broth containing ampicillin
  - grow bucket load of bacteria
- Bacterial DNA can then be isolated from Chromosomal DNA
- Cut with Sma1 and Xba1 enzymes liberates the Bacterial DNA from the Chromosomal DNA
  - then sequence to confirm that the bacterial DNA is actually the gene that you are after



Plasmid digest

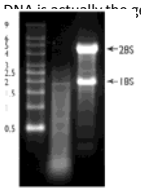
- Isolate plasmid DNA





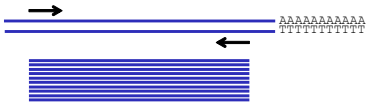
Reverse transcription (RT)-PCR

<u>Reverse Transcription-PCR (RT-PCR)</u>



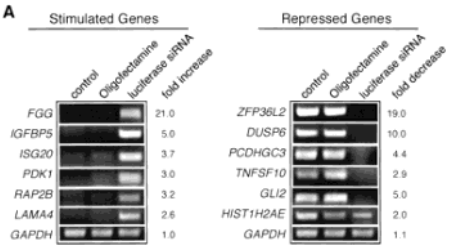
- Measure gene expression
- Clone exreβ-Galactosideasessed gene
- evaluate expression (transcript abundance)
- want m-RNA
- How is m-RNA different than r-RNA or DNA
  - rough 75% of RNA is ribosomal
  - m-RNA is polyadenalated
  - presence of poly-A tail

5'-GCGUGAAUGCUAGCUCCAGUAGAACUGACUGACUAAAAAAAAAAAA-3'
3'-TTTTTTTTTTTT-5'



- aligo d-T primer can be used to bind to the poly-A tail, and extend in the 3' direction using reverse transcription
- formation of c-DNA (complimentary DNA
- can then amplify the c-DNA
- the more m-RNA in cells, the more amplification products
- products are 99% RNA
- DNase 1 used to destroy remaining 1% DNA
  - confirm that you are amplifying c-DNA and not just DNA

RT-PCR assesses transcript abundance



Readings

April-16-11
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16.1 DNA Cloning

- A Clone is a line of genetically identical cells or individuals derived from a single ancestor. DNA cloning is a method for producing many copies of a piece of DNA - the piece of DNA is referred to as a 'gene of interest' - that a researcher wants to study or manipulate
- Each cell contains only two copies of most genes, amounting to a very small fraction of the total amount of DNA in a diploid cell
    - in its natural state in the genome a gene is very difficult to study
    - DNA cloning can produce a large enough sample for experimentation
- Cloned genes are used in basic research to find out about their biological functions
    - they can be used to determine the DNA sequence of a cloned gene, giving ultimate information about its structure
    - manipulating the gene and inducing mutation in it, information about its function and how it is expressed and regulated
- Cloned genes can be expressed in bacteria, and the proteins encoded by the cloned genes can be produced in quantity and then purified
    - these proteins can be used in basic research or - as with the case of proteins of clinical or pharmaceutical importance - the can be used in applied research
- One common method for gene cloning uses bacteria and plasmids
    - DNA that contains the gene of interest is extracted from cells and cut into fragments, which are then inserted into the plasmids producing recombinant DNA molecules
    - the recombinant plasmids are then introduced into bacteria - with each bacteria receiving a different plasmid.
    - As the bacterium grow and divide so does the plasmid, thus amplifying the piece of DNA inserted into the plasmid
- The key to DNA cloning is the specific joining of two DNA molecules from different sources
    - this specific joining of DNA is made possible by restriction endonucleases - or restriction enzymes
- Restriction Enzymes recognise short specific DNA sequences called restriction sites - typically four to eight base pairs long - and cut the DNA at specific locations within those sequences
    - The DNA fragments produced are known as restriction fragments
    - Hundreds of different restriction enzymes have been identified, each one cutting DNA at a specific restriction site
- Most restriction sites are symmetrical - the sequence of nucleotides read in the 5' to 3;' direction on one strand is the same read in the 5' to 3' direction of the other strand
- The restriction enzymes most used in cloning cleave sugar-phosphate backbones to produce DNA fragments with single-stranded ends
    - these ends are called sticky ends as the short single stranded region can form hydrogen bonds with complimentary sticky ends on another DNA molecule cut with the same enzyme
    - this pairing leaves nicks in the DNA BACKBONES, WHICH ARE SEALED BY dna LIGASE
    - The result is recombinant DNA molecule
- Bacterial Plasmids used for Cloning are Examples of Cloning Vectors
    - Bacterial Plasmid Cloning vectors do not occur naturally in bacteria
    - they are plasmids modified to contain two genes that are useful in the final steps of cloning as a method of identifying which bacteria contain the recombinant plasmids needed
    - the amp^R gene encodes an enzyme which makes the bacteria resistant to the antibiotic ampicillin
    - the $LacZ^+$ gene encodes from β-galactosidease which hydrolyzes the sugar lactose and a number of synthetic substrates
- Genomic DNA isolated from the organism in which the gene is found is cut with a restriction

enzyme, while a plasmid cloning vector is cut within the *LacZ* gene with the same restriction enzyme
    - Mixing the DNA fragments and cut plasmid together with ligase produces various joined molecules
    - some of these molecules are recombinant plasmids, while some are nonrecombinat plasmids and others are just two pieces of joined Genomic DNA
- The DNA molecules are the introduced into ampicillins-sensitive-, *LacZ⁻ E.coli*
    - the transformed bacteria are spread onto an agar plate with growth medium containing ampicillin and β-galactosidase substrate Z-gal
        - only bacteria with a plasmid can grow due to the presence of ampicillin
        - The X-gal in the medium distinguishes between bacteria that have been transformed with the recombinant plasmids and those that have not by blue white-screening
            - if the colony produces β-galactosidase, then the X-gal turns blue
            - colonies containing an intact *LacZ* gene - thus not possessing the recombinant plasmid needed - produce the enzyme
            - colonies with the wanted recombinant plasmid can not produce the enzyme due to the DNA fragment inserted into the *LacZ* gene
- How is a clone containing the gene of interest identified among the population of clones?
    - the gene of interest has a unique DNA sequence, which forms the basis of a commonly used identification technique called DNA Hybridisation
- In DNA Hybridisation the gene of interest is identified in the set of clones when it base-pairs with a short, single-stranded complimentary DNA or RNA molecule called a Nucleic Acid Probe
    - the probe is typically labeled with a tag so that investigators can detect it
    - if the sequence of the gene of interest is known, then the information to synthesis the probe
- The starting point for cloning the gene of interest is a large set of plasmid clone carrying fragments which represent all of the DNA of an organisms genome
- a collection of clones that contain a copy of every DNA sequence is called a genomic library
    - a genomic library can be made using plasmid cloning vectors or any other kind of cloning vector
    - the number of clones in the library increases with genomic size
- Another kind of DNA library that is made starting with m-RNA molecules isolated from a cell can also be used
    - to convert the single-stranded m-RNA to double-stranded DNA the enzyme reverse transcriptase is used to make a single stranded DNA complimentary to the m-RNA
    - the m-RNA is then degraded using DNA polymerase to make the second DNA strand, complimentary to the first
        - this results in the creation of Complimentary DNA - c-DNA.
    - after adding restriction sites to each end, the c-DNA is inserted into a cloning vector
    - the entire collection of cloned c-DNA made from the m-RNA isolated from a cell is a c-DNA library
- However a c-DNA library is limited as not all genes are active in every cell
    - the c-DNA library only contains the genes that were active in the cell used as a starting point for the library
    - this make c-DNA libraries useful in identifying changes in gene activity that are responsible for cell differentiation and specilization
- Polymerase Chain Reaction (PCR) is a much more rapid process for producing large quantities of a specific DNA sequence from a mixture of DNA without having to clone the sequence in a host organism - this process is amplification
- PCR is essentially DNA replication where DNA polymerase replicates only a portion of the DNA molecule
    - PCR uses primers for specific sequences to replicate only that specific region of DNA
- PCR is limited by the fact that in order to make the correct primer, you have to know the sequence of DNA that you want to amplify
    - by contrast, cloning can be used to amplify DNA of unknown sequence

Genetically Modified Plants

March-28-11
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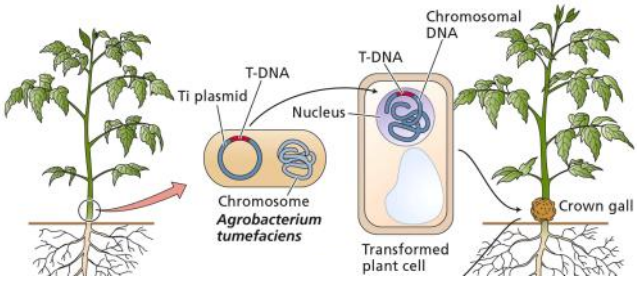
Genetically Modified Organisms

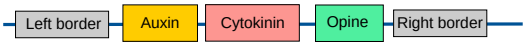
- GMO's
- Both foreign and endogenous genes
  - expressing pig insulin in plants
  - elevate expression of Rubisco
- GMO = transgenic = transformed gene
  - inserting foreign DNA
  - altering expression of a gene
- Why Plants
  - very easy to transform
  - increased crop yield
  - enhanced traits
    - pest resistance
    - stress tolerance
  - Bioreactors
    - synthesis of drugs, plastics etc.
- How Do I transform a plant
  - Biolistic
  - Injection
  - Agrobacterium

Agrobacterium Infection

- Natural way to transform a plant
  - agrobacterium have been around for a few hundred million years
- Agrobacterium will naturally transform a plant
- Agrobacterial contains Ti plasmid
  - tumour inducing plasmid
- Agrobacterium good at infecting plants
  - through damage/wound site
- Upon infection, part of Ti plasmid relocates to nucleus of plant cell
  - incorporated into nuclear genome
  - plant cell set to be transformed
  - NOTE - not whole of the plasmid, just the T-DNA
  - T-DNA is insert randomly
- Genes in the T-DNA that leads to cancer like growth
  - T-DNA encodes proteins that cause cancer like growth
  - gives rise to Crown Galls
- T-DNA
  - insertion defined by two specific sequences
    - Left Boarder and Right Boarder
    - play a role in random insertion
  - between the two border are genes that cause the proliferation of growth
    - Opine - synthesis of amino acid agrobacterium use for food
  - Can remove the genes between the two borders and insert wanted ones
    - use it to transfer wanted genes into plants, cause change in expression etc.

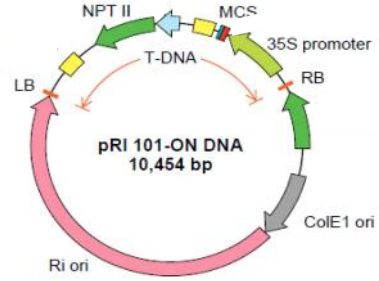
Agrobacterium infection

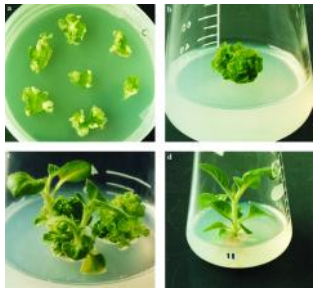




Engineering the T-DNA

- Express foreign genes or overexpress endogenous gene
  - can also shut off expression of a gene
- Can isolate the Ti-plasmid
- Remove pre-existing genes
- inserted wanted genes into the Ti-plasmid within the T-DNA
- insert genes into the multi-cloning site
- just want c-DNA, not promoter, introns etc.
  - just want open reading frame
- want gene to expressed all of the time with high level of transcription
  - high level, constitutive expression
- use of built in 35s promoter
  - from Cauliflower mosaic virus 35s promoter
  - high level, constitutive expression



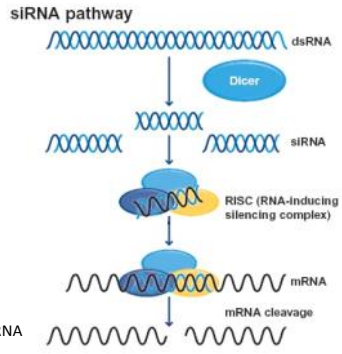


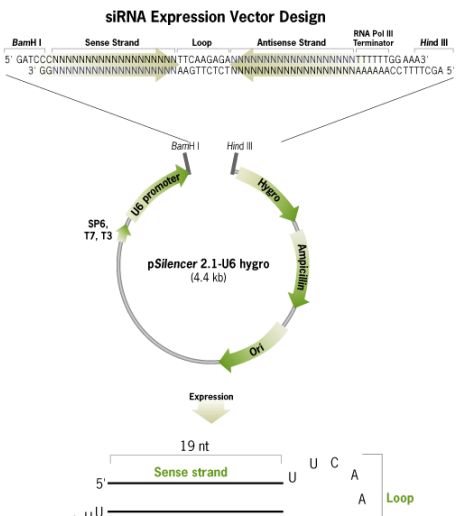
Making a Transgenic Plant

- Co-culture
  - culture of modified agrobacterium and chopped up leaves
    - agrobacteria can infect the plant cells
    - takes no more than a few hours
- Plate dry leaf pieces on a petri plate with antibiotics and plant hormones
- Results in callus tissue
  - rapidly dividing, undifferentiated plant cells
- Single transformed plant cell results in a fully grown transgenic plant with antibiotic resistance
  - ever cell in the transformed plant carries the same inserted gene at the same locus
- Easy to do in plant cells because they are Totipotent
  - from a single cell, you can regrow the entire organism
  - animal cells can not do this

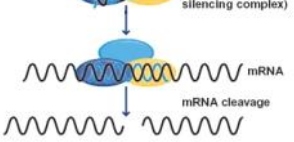
si-RNA Silencing of Gene Expression.

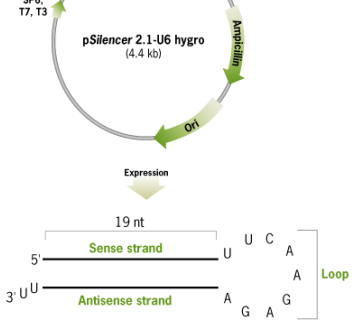
- Small Interfering RNA
  - discovered in 1992
  - RNA interference/si-RNA pathway
- comes from Double stranded RNA
- Dicers break it up in small 19-22 base pair long double stranded RNA
- Incorporated and unwound by RSC Complex
  - opens them up so they become single stranded
- One of the strands is anti-sense
  - anneals and binds to the wild type m-RNA
  - causes m-RNA to be degraded or leads to translational arrest
    - no longer translated
- To shut off wild-type expression, need to synthesis si-RNA
  - can make in laboratory a construct that leads to double stranded RNA
  - insert sense-strand into multi-cloning site
  - clone it in in reverse orientation
  - when both are transcribed, they are compliments of each other and form double stranded RNA





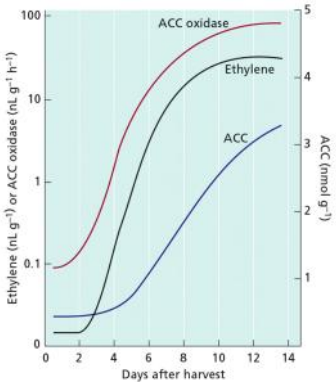
- To shut off wild-type expression, need to synthesis si-RNA
  - can make in laboratory a construct that leads to double stranded RNA
  - insert sense-strand into multi-cloning site
  - clone it in in reverse orientation
  - when both are transcribed, they are compliments of each other and form double stranded RNA with hairpin loop
  - will accumulate in the cytosol of a plant
  - same as si-RNA
    - recognised by RSC complexes
    - break hairpin loop and unwind doubler stranded RNA
  - example of post-transcriptional gene silencing





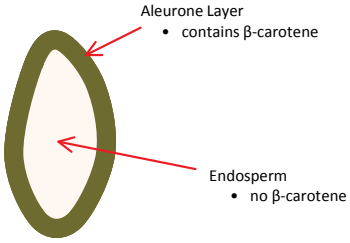
Ethylene and Fruit Ripening

- Ethylene is involved in fruit ripening
- 2 Carbon compound in gaseous form
- produced by metabolic process within the plant
  - Methionine converted to ACC
  - ACC converted to Ethylene by ACC oxidase
- When fruit ripen, a lot of ethylene is produced
- Ethylene is produced right after harvest
  - risk of over ripening
  - race against time to get produce from place of growth to place of sale
- Shutting off the producing of ACC oxidase prevents the production of Ethylene
- Example of transgenic plants
- For example in tomatoes
  - si-RNA line has 5% WT levels of ACC oxidase



Golden Rice

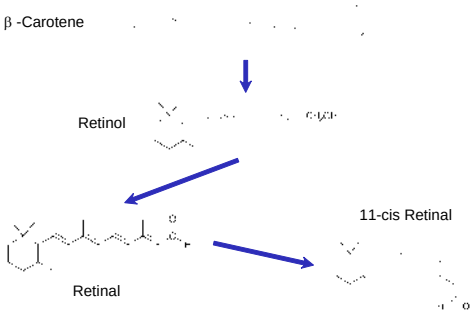
- Large proportion of the world survive on rice diets
  - cheap and grows easily
  - however 250,000 children go blind ever year from rice diets
    - Vitamin A deficiency
  - 124 million children lack enough Vitamin A
    - not actually Vitamin-A, but β-Carotene
    - can synthesis Vitamin-A from β-Carotene
- Aleurone layer of rice contains Beta-Carotene
  - however leaving aleurone layer on highly reduces shelf-life
  - brown rice still has aleurone layer left on
- Golden Rice is a form of transgenic rice in which the endosperm contains β-Carotene



β-carotene, vitamin A and vision

β-Carotene, Vitamin A and Visions

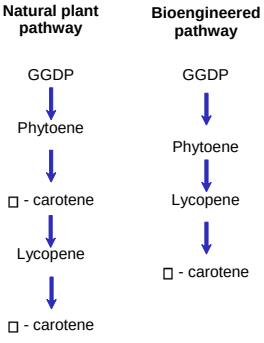
- β-Carotene is converted to Retinol
- Retinol is converted to Retinal
- Retinal is converted to 11-cis Retinal (photoreceptor in eye)



Engineering a biosynthetic pathway

Engineering a Biosynthetic Pathway

- Natural Plant Pathway
  - 4 enzymes needed to convert GGDP to β-Carotene
    - GGDP converted to Phytoene
    - Phytoene converted to ζ-Carotene
    - ζ-Carotene converted to Lycopene
    - Lycopene converted to β-carotene
- Endosperm of rice contains GGDP
- Insertion of 3 genes into the t-DNA in series to make Bioengineered Pathway
  - use of 3 enzymes coded for by DNA and a bacterial enzyme
  - GGDP converted to Phytoene
  - Phytoene converted to Lycopene
  - Lycopene converted to β-Carotene
- Leads to rice that contains β-Carotene within the endosperm



Bt Corn

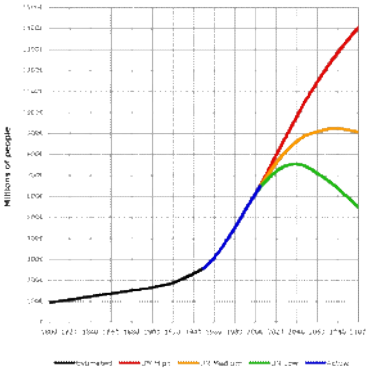
- 95% of corn plant in North America
  - not consumed by humans
- European corn borer
  - insect that destroys corn crops

Bt corn

- 95% of corn plant in North America
  - not consumed by humans
- European corn borer
  - insect that destroys corn crops
  - $1-2billion per annum crop losses
  - use of pesticides
  - also killed by toxin produced by Bacillus thuringiensis
- Companies have developed corn with gene for the expression of the Bt toxin
  - has no effect on humans
  - the toxin is broken down in the intestine

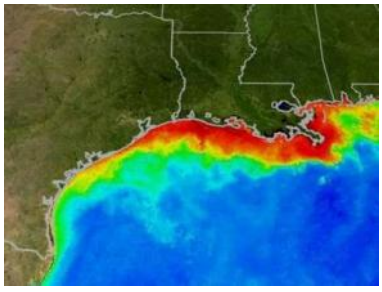
Feeding 10 Billion People

- By 2050, earths pop. will reach 10billion
- need a 120-170% increase in food production in order to feed this population
- Arable land versus crop yield
  - arable land is decreasing
  - but crop yield needs to go up
  - Argument for transgenic plants
    - increased yield
- Sustainable agriculture



Reducing Pesticide and Fertiliser Usage

- Transgenic plants are a method of controlling disease with genetics instead of chemistry
  - 115million kg $year^{-1}$
  - 110,000 non-fatal pesticide poisonings
  - 10,000 cases of cancer and other health problems
  - 35% of supermarket foods have pesticide residues
  - 70 million bird deaths
  - billions of insect deaths
  - 100million tones of Nitrogen fertilizer per year
  - Eutrophication - 'dead zone'
    - higher amounts of nutrients in water than is normal
    - leads to huge amounts of algae growth
    - algae die, bacteria eat algae, consume oxygen
    - leads to water with very low levels of oxygen



Teosinte

Arguments against GM food

- GM technology is 'unnatural
  - molecular breeding is no different than traditional breeding
  - more precise and faster to develop new varieties
  - e.g. Teosinte
    - Mexican grain that over a few thousand years of selective breeding lead to corn
- GM foos are harmful to human health
  - no evidence to suggest such
  - Royal Society of Medicine 2008 review noted that GM foods have been eaten by millions of people for over 15 years, with no reports of ill effects
  - US National Academies of Sciences 2004 report stated that "to date, no adverse health effects attributed to genetic engineering have been documented in the human population"
- Transgenic crops threaten the ecology of natural populations
  - very little evidence to support this
  - paper stating that transgenic pollen from Bt corn harms monarch lavae
    - again very little evidence
- Takes a lot of money and time
  - benefit large corporations at the expense of the small farmer
  - intellectual property rights

Readings

April-09-11
6:27 PM

16.2c Genetic Engineering of Plants

- Genetically Engineered plants have been used to develop plants with resistance to pests and disease, greater tolerance to heat, drought and salinity, increased crop yields and faster growth
- Another aim is the production of seeds with increased levels of amino acids
    - increasing the levels of amino acids deficient in plant seeds can greatly increase the diet of populations who rely mainly on seeds for nutrient
- A further possibility is the development of plants to produce pharmaceutical products
- Wanted genes are inserted into plants through several methods
    - A commonly used method uses agrobacterium, which infect plants at wound sites and produce galls - basically tumours
        - The bacteria contains a large circular plasmid called a Ti-Plasmid - a tumour inducing plasmid.
        - Interactions between the bacterium and the plant activate transfer DNA -or T-DNA - in the Ti-Plasmid, which then integrates into the plant's own genome
        - the genes in the T-DNA are expressed, the products of which cause the plants cells to grow and divide to produce a tumour
        - The Ti-plasmid can be used as a vector to insert wanted genes into a plant
- The most common usage of genetic engineering in plants is the production of transgenic crops
    - these crops are modified to possess pest, virus or herbicide resistance
    - the most common approach to producing pest resistant plants is to introduce the Bt toxin encoding gene from the bacterium *Bacillus thuringiensis*
        - this toxin has been used in powder form in agribusiness for many years to kill insects
    - Transgenic plants that have been modified to express certain viral proteins are resistant to infect by the same virus - although the exact mechanism for how this occurs is unknown. As virus infects cause massive crop losses each year, these transgenic plants are of massive value to the market worldwide
    - Many crops have been engineered to become resistant to herbicide
        - the herbicide glyphosate - or Roundup - is widely used in weed control, and inhibits a certain enzyme in the chloroplast; a process which unfortunately kills crops too
        - Crops engineered to contain a bacterial form of the same enzyme - which is not affected by round up - are resistant to it's effects
    - Crop plants are also modified to alter their nutritional properties
        - crops with increase levels of vitamins and minerals may improve the diets of billions of people who live on mostly rice diets, for example.
    - Plant pharming is the process of engineering transgenic plants to produce medically viable pharmaceutical products
        - the gene coding for the product is cloned into a cloning vector adjacent to the promoter - in this case one that is active in plants - and this recombinant DNA is then introduced into plants
- A key concern is that organisms - such as bacteria - modified in labs during this process may escape and produce new and potentially harmful strains
    - Guidelines list the precautions to be used by laboratories when constructing recombinant DNA molecules, and state that only host organisms which need a growth medium in which to survive may be used
    - However, experiments have shown that recombinant DNA manipulation may be done safely
- While the public does not seem overly concerned with Genetically engineered microorganisms, the use of genetically modified organisms - GMOs - as food does raise

concern

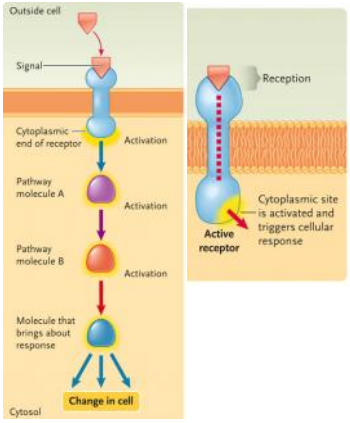
- potential adverse affects of GMOs include the interbreeding with natural species, damage to the environment or harming beneficial insect species
- Different countries have reacted differently to the use of GMOs
    - In Canada, GMOs are widely used
    - the EU however has given greater political opposition to GMOs

Signal Transduction

April-02-11
10:25 AM

Figure Illustrates Signal Transduction
- no chlorophyll in the seedling
- how does it sense the light?
- what kind of signalling process takes place in order for it to grow towards the light?

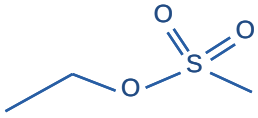
Signal Transduction Pathways
- Easiest way to think of signal transduction is chemicals
  - hormones, growth factors binding to receptors on the plasma membrane
    - hormones and growth factors do not enter the cell, simple bind to the external domain of the receptor protein
    - binding on external side causes conformational change
  - bind to a receptor and causes a cascade of responses through a number of gene products
  - proteins signalling all the way down through the cell
  - many signal pathways lead to a change in the expression of transcription factors
  - causes alterations in nuclear gene expression
- Signal transduction can also be through non-chemical methods
  - for example light (as with plant in figure above)
  - physical things can cause signal transduction
    - when bacteria are moved into low temperature they alter their expression of Desaturase
    - how do their systems sense a change in temperature
- Binding to a receptor can also shut off a receptor
  - binding does not only have to turn on a pathway
- How to elucidate the pathway?
  - what gene encodes what component
  - genome wide analysis to see which genes respond to which signals
    - transcriptions that increase or decrease
  - easy to find what responds to a signal
  - but not so easy to figure out how the signal gets transduced



Genetic Approach
- Goal to identify the genetic basis of a specific phenotype or trait by analysis of mutants
  - components of pathway usually proteins, therefore encoded by genes
  - defective pathway
    - lots of signal, but no response
- Problem is that mutants are very rare
  - thousands of genes, what are the changes that you would ever come across in nature a mutation in the desire pathway
- Therefore have to make your own mutants through Mutagenesis

Mutagens
- have an organism that you want to study a certain pathway in
- you mutate a population through the use of mutagens

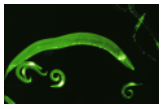
1. irradiation
   - UV
   - X-Ray
2. inspectional elements
   - Transposons
   - T-DNA
     - T-DNA itself causes mutations, do not have to insert anything between the left and right border
     - T-DNA inserts randomly
     - may knock out an essential gene within a signal transduction pathway
     - if T-DNA inserts into the gene for cytochrome oxidase, you've effectively shut off the production of cytochrome oxidase
3. Chemicals
   - Ethyl Methyl Sulfonate (EMS)
     - $C_2H_5OSO_2CH_3$
     - Causes damage to Guanine
     - alkylating agent, adds a ethyl group
     - upon exposure to EMS, Guanine is converted to 0-6-ethylguanine
     - in the next round of DNA replication, the 0-6-ethylguanine calls for a T, not a C
     - causes a G:C to A:T conversion
     - example of a point mutation
     - mutation occurs at the next round of replication, damage is in initial stage



Model Genetic Systems
- Very easy to make mutants
  - however most organisms have thousands and thousands of genes
  - so need an organism with a small genome size
- Use of three common organisms for genetic analysis
  - Drosophila melanogaster (fly)
    - 180 Mb- 13,600 genes
  - Caenorhabditis elegans
    - 97 Mb - 19,099 genes
  - Arabidopsis thaliana (plant)
    - 125 Mb- 25,498 genes
  - all have small genomes for eukaryotic organisms
  - these organisms were also picked because they have short life spans
- Genome size is a weird thing
  - many organisms have genomes 3 to 5 thousand times larger, but look no different
- Generation time is an important factor
  - three organisms are picked because they have a very short generation life time
  - need to mutate a population and analyse the offspring

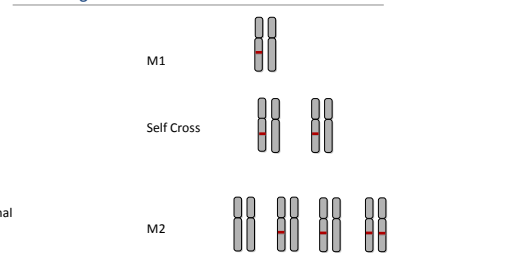
Drosophila melanogaster

Caenorhabditis elegans



Arabidopsis thaliana

Mutagenesis (in Arabidopsis)
- have you population with short generational time and small genome size (Arabidopsis)
- treat seeds with mutagen
- causes single mutation to one chromosome
  - Arabidopsis is a diploid
  - single mutation to one of the homologous chromosomes
- the M1 generation plants grow up, and then you 'self' them
  - cross them with themselves
- why cross them
  - most mutations, most defects are recessive
  - only appear if in homozygous recessive form
  - phenotype of the mutation is not apparent in the M1 generation
- M2 generation has individuals in homozygous recessive genotype
  - expect them to show defects in signal transduction or anything else
- Have to screen the resulting M2 individuals for a phenotype that shows that they are defective in the signal transduction pathway you are looking for
  - has to be an easy, visual screen
  - easy way to asses many plants, like a shit tonne of plants



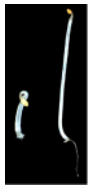
Screening Arabidopsis
- Arabidopsis research facilities contain hundreds of thousands of plants
  - may have to screen 150,000 plants in order to find the specific mutation
- What are you looking for when screening?
  - if not easy to see visual, then you have to run RNA blots

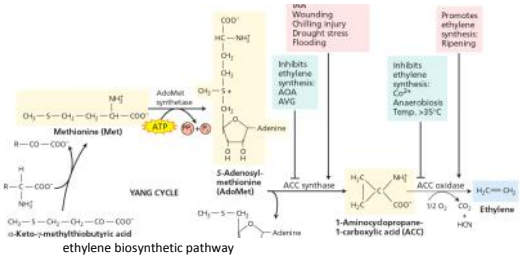
Screening Arabidopsis

- Arabidopsis research facilities contain hundreds of thousands of plants
  - may have to screen 150,000 plants in order to find the specific mutation
- What are you looking for when screening?
  - if not easy to see visual, then you have to run RNA blots
  - so want an easy, visual, powerful screen
  - Ethylene

Ethylene Plant Hormone

- Plays a role in fruit ripening (lecture 21)
- Fruit is a good screen, however fruit is not a good model system
  - takes up too much room
- Screen in Arabidopsis is the Triple Response
  - small seedlings in the presence of ethylene have short, thick and hooked stems (see image to right)
  - in absence of ethylene you get tall, thin, straight seedlings
  - easy screen to detect visually



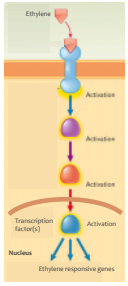


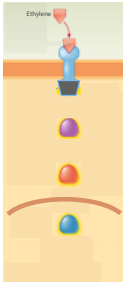
ethylene biosynthetic pathway

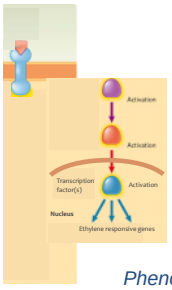
Classes of Mutants

- What kinds of mutants can be expected if you mutate a population
- Biosynthesis
  - defects in ethylene biosynthesis pathway
  - 3 enzyme catalyzed steps
    - defect in any one of the genes that code for these enzymes would cause a defective mutation in the plant
  - Defect could be in the regulation of the pathway
    - too much of the gas being made all of the time
    - pathway is always on all the time and at levels higher than normal
- Signal Transduction
  - assume that the plant is making ethylene perfectly fine
  - problem is in the plants cells recognising the ethylene
    - perception, not synthesis of the signal is at fault for the defect
- How do you distinguish between defects in Biosynthetic pathway and Signal Transduction Pathway
  - give the plant all of the ethylene that it need exogenously
  - don't rely on the plant producing the hormone

Recessive mutation



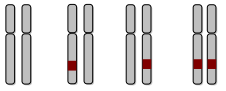




Phenotype vs. Genotype

heterozygous

- show wild type phenotype



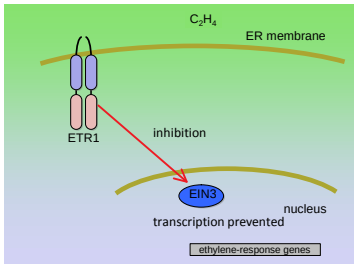
homozygous recessive

- shows mutant phenotype

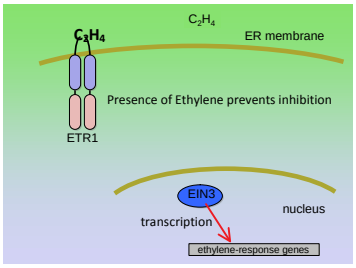
Ethylene Signalling Mutant 1 - etr1 mutant

- Mutagenize the population,
- looking for no triple response with ethylene added exogenously
- etr1 mutant (non recessive mutation)
  - Ethylene response 1 (ETR1)
- Is the mutation recessive?
  - if it is a recessive mutation, then in heterozygous form you should see the wild phenotype plant
  - as long as it has one function chromosome, its fine
- Phenotype vs. Genotype
  - If the mutation is recessive (most are) then only 75% of the population (3/4) should show the wild type phenotype
  - however that's not the case
  - only 20 show the wild type
  - 57 show that they are ethylene insensitive
    - mutant phenotype
  - therefore not recessive
- etr1 is not a recessive mutation

Current model



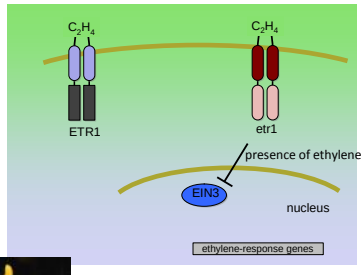
Current model



Current Model for Ethylene Signal Transduction

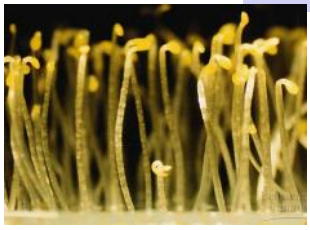
- ETR1 is a ER membrane receptor
  - not known when mutant initially isolated
  - further biochemical analysis shows that it is located on the Endoplasmic Reticulum
  - not a plasma membrane receptor
- ETR1 is active when ethylene is not present
  - domains are functional in absence on ethylene
  - function by the inhibition of EIN3
  - negative regulation of EIN3
- Ehtylene binds to the external domain of ETR1
  - changes the conformation of interior domains, shutting them off
  - repression of EIN3 is lost
  - EIN3 is now free to transcribe ethylene response genes
  - Triple-Response Phenotype is downstream of the EIN inhibition loss

etr1 is a dominant mutation



etr1 is a Dominant Mutation

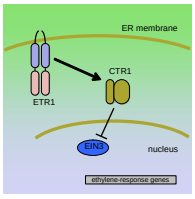
- heterozygous wild type
  - in the absence of ethylene, still inhibit the expression of EIN3
- Heterozygous mutation
  - defective in ethylene binding
  - ethylene does not shut off inhibition of EIN3
  - mutant type is always repressing the function of EIN3
  - triple response does not occur
  - explains the dominance of the mutation



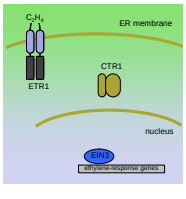
Ethylene Signalling Mutant 2 ctr1

- ctr1
  - Constitutive Triple-Response (CTR1)
  - show triple-response even in the absence of ethylene
  - caused by a defect in the signal transduction pathway

Absence of ethylene



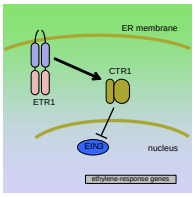
Presence of ethylene



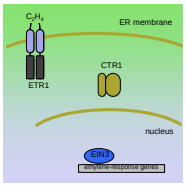
Ethylene Signalling Mutant 2 ctr1

- ctr1
  - Constitutive Triple-Response (CTR1)
  - show triple-response even in the absence of ethylene
  - caused by a defect in the signal transduction pathway
- ETR1 does not directly inhibit EIN3
  - ETR1 interacts with and activate CTR1
  - CTR1 actually inhibits EIN3
  - CTR1 is a repressor
- if ethylene binds to ETR1, then CTR1 is shut off, causing triple response
- pathway is active in the absence on ethylene
  - both ETR1 and CTR1 active and functional in the absence of Ethylene
- Presence of Ethylene shuts off the entire pathway, allowing the activation of EIN3
- ctr1 Mutant
  - in absence of ethylene, ETR1 is functional
  - however CTR1 is defective and unable to function as a repressor or EIN3
    - EIN3 then able to drive expression of triple-response genes
    - if CTR1 cant function, then EIN3 is always one, leading to constituative triple -response
  - if CTR1 is defective, then doesn't matter what happens upstream
    - you'll always get the triple response.

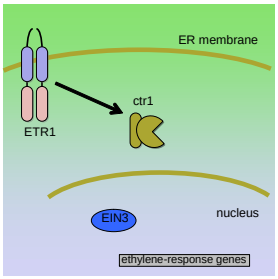
Absence of ethylene



Presence of ethylene



ctr1 mutant



Readings

April-12-11
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8.1 Cell Communication - An Overview

- Communication is critical for the function and survival of the cells that make up a multicellular organism
    - cells need to be able to communicate with one another in a regulated way that is responsible for growth and development and the integrated activities of various organs and tissues within the organism
- Adjacent Cells communicated with each other through direct means of communication
    - this this direct method, small molecules and ions are exchanged between the cytoplasms of the cells
    - Gap Junctions are responsible for this transfer in animal cells
        - the main function of gap junctions is o synchronize the metabolic activities between cells in a tissue
    - Plasmodesmata serve this process in plant cells
- Cells can also communicate through specific contact
    - Certain cells have molecules on their surfaces, which allows them to directly interact with other cells
        - an example of this is the immune system, which recognises pathogens and viruses as being foreign by the detection of the surface molecules
- Cells also posses surface adhesion molecules on their surface
    - these molecules are integral membrane proteins which allow cells to bind to other cells, or to the extracellular matrix
    - these surface adhesion molecules play important roles in cell movement and the coordination of tissue and organ formation in developing embryos
- Cells can also communicate by intercellular chemical messengers
    - in this method, a controlling cell synthesises and releases a specific molecule that acts as a signalling molecule, inducing a response in a target cell - which is not in contact with the control cell
- Target cells process the signal from the controlling cell by the following process
    - Reception - the binding of the signal molecule to the target cell, which has receptors specific for the signal molecule distinguish them from other cells
        - signal molecules are often peptides or steroids, but other molecules such as amines can also act as signal molecules
        - Membrane receptors are usually embedded within the plasma membrane of the cell, with the binding site for the signal molecules on the outside of the cell surface
        - Some receptors are also located within the cell - in this case the signal molecules pass freely through the plasma membrane
        - Receptors at the cell surface usually involve short-lived events, where are internal receptors often act directly on the genome and occur of a longer time
    - Transduction - the process of changing the signal into the form necessary to be understood by the cell and provoke the wanted cellular response
        - The binding of a signal molecule to a receptor is not responsible for the response
        - while transduction may occur in a single step, it often involves a multistep process - or signalling cascade
    - Response - the transduced signal causes a specific cellular response, which depends on the signal and receptors of the target cell

8.2 Characteristic of Cell Communication Systems with Surface Receptors

- Cell communication systems based on surface receptors have three components
    - the extracellular signal molecules released by controlling cells

    - the surface receptors on target cells that receive the signals
    - the internal response pathways triggered when receptors bind a signal
- Surface receptors in mammals and other vertebrates recognise and bind two major types of signal molecules - hormones and neurotransmitters - that are released by control cells and enter the fluids surrounding the cells
    - Hormones are molecules that are released by glands, nerve cells or cells distributed in organs
    - Neurotransmitters are molecules released by neurons that trigger activity in other neurons or other cells in the body
        - some neurotransmitters are released into the blood stream and act basically as hormones
- Once signal molecules are released into the body's circulation they remain for only a certain time - they are either broken down at a steady rate by enzymes in their target cells or excreted by the kidneys
    - this removal process ensures that the signal molecules are active only as long as the controlling cells are secreting them
- The surface receptors that recognise and bind signal molecules are all glycoproteins - integral membrane proteins that extend entirely through the plasma membrane
    - the signal-binding site of the receptor extends from the outer membrane and is folded in a way that closely fits the signal molecules - much like enzymes this fit is specific for each receptor and each signal
- A signal molecule brings about a specific change to the receptor and therefore to the cell that it binds to
    - when a signal molecule binds to a surface receptor the molecular structure of the receptor is changes, causing it to transmit a signal through the plasma membrane and activate the cytoplasmic end of the receptor
    - this activated receptor then initiates the first step in the signal transduction cascade that triggers the cellular response
- Different cell types contain distinct combinations of receptors
    - the combination of surface receptors on a particular cell is not fixed - it changes as the cell develops
    - changes also occur as normal cells are transformed into cancer cells
- Signal molecules binding to a surface receptor trigger a cellular response without entering the cells
    - this is shown by experiments that show that
        - signal molecules have no effect if they are injected into the cytoplasm of the cell
        - unrelated molecules that mimic the structure of the signal molecule can trigger a full response so long as they bind to the recognition site of the receptor
- A second typical characteristic of signal transduction is that the signal is relayed inside the cell by protein kinases
    - the phosphorylated target proteins are modified by the signalling pathway
    - the added phosphate groups either stimulate or inhibit the target proteins activity, which leads to the cellular response either directly or indirectly
- The effects of protein kinases in transduction pathways are balanced or reversed by protein phosphatases - which remove phosphate groups from target proteins - which, unlike protein kinases, are constantly active with in the cell
    - this ensures that the signal cascade is only active as long as the signal molecule is present and inducing
- Signal transduction pathways are also subjected to amplification
    - as many of the proteins that carry out the individual steps are enzymes, once activated each enzyme can activate hundred of proteins or other enzymes further down the pathway
    - the more enzymes catalyzed steps in the pathway, the greater the amplification
- As signal transduction runs its course the receptors and their bound signal molecules are removed from the cell surface by endocytosis
    - both the receptor and the signal molecule may be degraded in lysosomes after entering the cell

- alternatively the receptor may be separated from the signal molecule and recycled to the cell surface while the signal molecules are degraded

Oxygen and Ageing

April-02-11
5:14 PM

Ageing

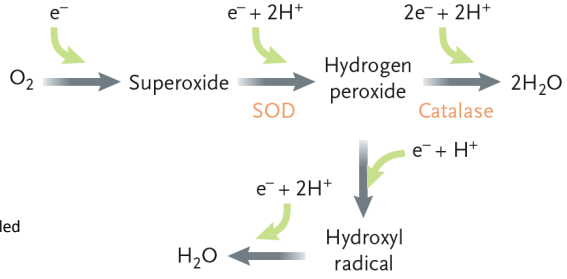
- progressive impairment of function with an increasing probability of death
- as you get older, more age related disease become more prevalent
  - cancer, Parkinson's
- why do we age at all?
- what's the underlying biochemistry
- can split it up into three categories
  - genetic component
    - genes involved in life span control
    - many, many, many genes involved
  - Environmental
    - where you live etc.
  - Metabolic
    - overall metabolic reactions influence aging
    - what you eat etc.
- Average North American Life Spans
  - 1800 - 28 years
  - 1900 - 47 years
  - 2000 - 70 years
  - increase in life span due primarily due to increased health conditions, better sanitation etc.
  - not due to some fundamentally intrinsic change
  - just taking better care of ourselves
  - research suggests that we have reached theoretical maximum of our life spans

The Paradox of Aerobic Life

- Why do many anaerobic organisms die upon exposure to oxygen
  - not that they cant use oxygen
  - they just die upon exposure
- Hyperoxia Treatments
  - treatments in hospitals in which you reach higher levels of oxygen than normal
    - used to give pure O2 during newborn resuscitation, not any more
  - Ischemia
    - tissues can survive without oxygen for longer than they should be able to
    - tissues completely lacking in oxygen become Ischemic (often happens during surgery)
    - reperfusion reintroduces oxygen into the tissues
    - used to be done with pure air, however not anymore
    - now done with 20% oxygen
- The paradox of aerobic life is that…
  - we have a high, constant demand for oxygen
  - however, oxygen is also toxic!

Reactive Oxygen Species - ROS

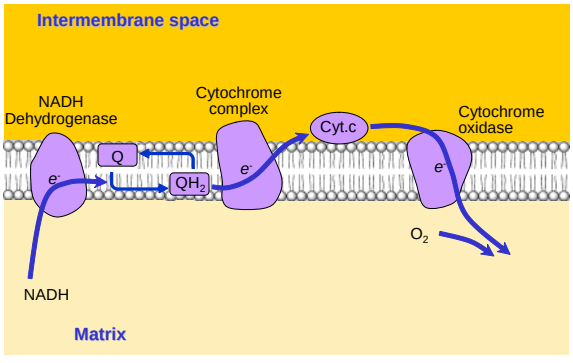
- **$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$**
- Complete reduction of oxygen (to water) takes four electrons
- however can occur stepwise (accepts one electron at a time)
- leads to the formation of partially reduced forms of oxygen
  - highly reactive
  - accepted 1, 2 or 3 electrons out of the 4 to become fully reduced
  - very strong oxidising agents
  - can easily damage biological molecules and can cause cell death if not controlled
- Superoxide -**O_2^-**
  - oxygen accepts 1 electron
- Hyrdogen peroxide -**H_2O_2**
  - superoxide accepts 1 electron and $2H^+$
- Hydroxyl Radical -**OH**
  - Hydrogen peroxide accepts 1 electron and $1H^+$
- Water -**H_2O**
  - hydroxyl radical accepts 1 electron and $2H^+$
- Production of H_2O and ROS is potentially toxic and fatal
  - highly reactive intermediates
  - how do you protect your cells from these intermediates
- Evolution of ROS Scavenging Enzymes
  - Superoxide dismutase (SOD)
    - rapidly converts superoxide into Hydrogen peroxide
  - Catalase
    - rapidly converts Hydrogen peroxide into Water
  - the early development of these enzymes was essential to the evolution aerobic life
- Enzymes explains why some anaerobic organisms die on contact with oxygen
  - do not posses the enzymes needed to deal with ROS

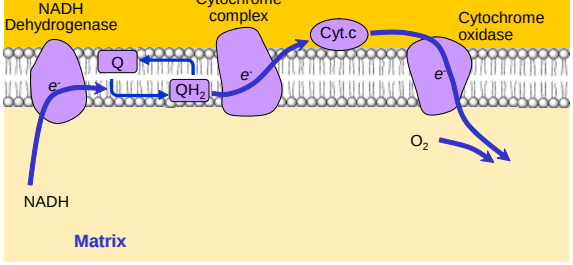


Mitochondria - Major Source of ROS

- Major source of ROS formation in eukaryotic cells is mitochondria
- Cytochrome oxidase
  - terminal electron acceptor
  - where electrons are donated to oxygen
  - would think that this is a site of massive production of ROS
    - if this was true, aerobic life would never have developed
  - cytochrome oxidase holds onto electrons until it has four of them, then donates them all at once
    - does not donate electrons one at a time
    - complete reduction of oxygen to water
    - no ROS formed at all!
- ROS get formed at the level of Ubiquinone
  - Q pool
  - gets reduced and oxidised
  - lots of oxygen diffusing across the membrane

Mitochondria: major source of ROS

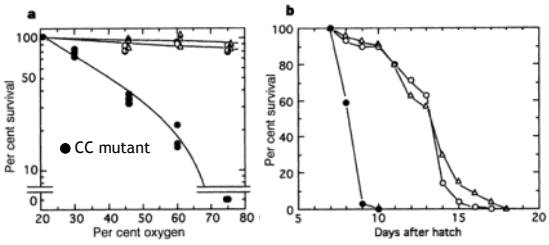




- no ROS formed at all!
- ROS get formed at the level of Ubiquinone
  - Q pool
  - gets reduced and oxidised
  - lots of oxygen diffusing across the membrane
  - sometimes an electron wont get passed down the electron transport chain, but will get diverted by an oxygen diffusing across the mitochondrial membrane
    - less than 1% of the time
  - SOD1 is essential in human mitochondria to remove ROS that are created
- Formation of ROS by electron transport is unavoidable
  - need SOD1 otherwise you'll die
- ROS are an unavoidable consequence of have oxygen as the terminal electron acceptor
- maybe as you age, mitochondria don't work so well
  - many diseases linked to deficiencies in the electron transport chain
  - Parkinson's is linked to a deficiencies in NADH Dehydrogenase
  - deficiencies in SOD1 linked to ALS (amyotrophic lateral sclerosis)
  - lead to elevated levels of Mitochondrial ROS
- anything with affects the efficiency of electron flow down the chain will affect the formation of ROS

Cytochrome complex mutation

- *Caenorhabditis elegans* (C. elegans) - worm
- Mutation - Cytochrome complex

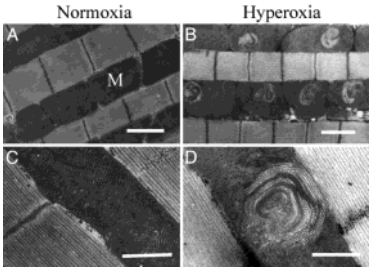


Cytochrome Complex Mutation

- Caenorhabditis elegans (*C. elegans*) - a worm
- cause and isolate a mutation in cytochrome complex
  - mutation causes the complex to not work as it should
  - mutant is much more susceptible to oxygen toxicity
  - mutant dies more easily in higher levels of oxygen
- Under normal oxygen conditions, the mutant does not live as long as the wild type
- pre-treat mutants with anti-oxidants
  - anti-oxidants remove reactive oxygen
  - if you pre-treat the mutants with anti-oxidants, then their life spans are just as long as the wild type
- All of this suggests that Mitochondria are involved in the ageing process

The Mitochondrial ROS Theory of Ageing

- ageing is linked to decrease in mitochondrial function
  - Electron Transport causes ROS
  - ROS cause DNA damage
  - DNA damage causes mitochondrial dysfunction
  - Mitochondrial dysfunction causes more ROS
- The older you get, the more dysfunctional your mitochondria become

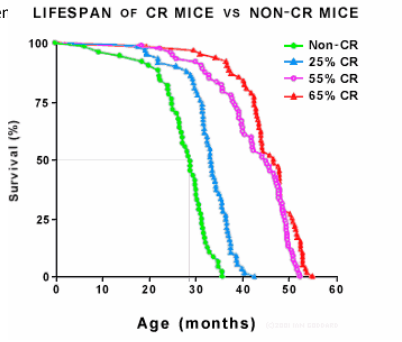


Effects of Hyperoxia

- What happens if you raise flies)drosophila) in 100% oxygen
  - at high levels of oxygen (hyperoxia) you get tell-tale swirls within the mitochondria
  - tell-tale signs of oxidative stress
    - ROS are damaging biological molecules
  - tell-tale swirls are also found in older flies raised in normal levels of oxygen
- Last thing you want to do is breath 100% oxygen
- Oxygen bars
  - fucking stupid idea

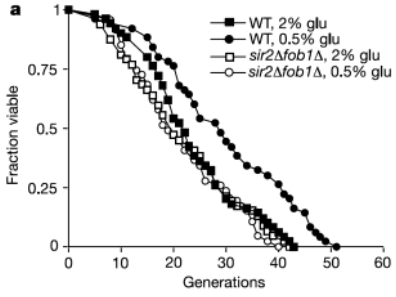
Caloric Restriction

- only thing that extends life that we know that transcends phyloger
  - 50-70% of calories of normal diet (without malnutrition)
  - decreased rate of aging by 30-50%
- Not that this is a good idea, but why does it increase life-span



Why Does CR Increase Lifespan

- Has the same affect across the board
  - very rare
- Reduction in metabolic rate? No
  - No, CR does not lead to a decrease in metabolic rate
  - in fact often leads to an increased rate
- Reduction in Oxidative Damage? No
  - No, correlated but not causal
  - Mice with SOD knockouts
    - more damage but live as long
- Hormesis Hypothesis?
  - CR acts as a little stress
  - Body always under a little stress
  - Stress leads to arguable beneficial adjustment
    - beneficial genes and pathways switched on
  - May be true, not sure
    - difficult to test, others easy to test and prove wrong
- Lifespan seems to be very big picture
  - Genome, Environment etc
  - but CR is metabolic
  - how do you link the two?
  - Sir2
    - required for CR phenotype



Sir2

- Biggest finding to date is Sir2
- Gene that is found in yeast
- seems to be linked to CR
  - data found in yeast, but implications for animals
- Yeast easy to see how long it lives, how many generations between it dies
- in Yeast, CR seems to increase life-span
- If Sir2 is knocked out then the life-span extension brought about by CR is lost
  - Sir2 is essential for CR phenotype to take hold in yeast
- If Sir2 is overexpressed, then life-span is increased yet again

Linking metabolism to lifespan

- Yeast easy to see how long it lives, how many generations between it dies
- in Yeast, CR seems to increase life-span
- If Sir2 is knocked out then the life-span extension brought about by CR is lost
  - Sir2 is essential for CR phenotype to take hold in yeast
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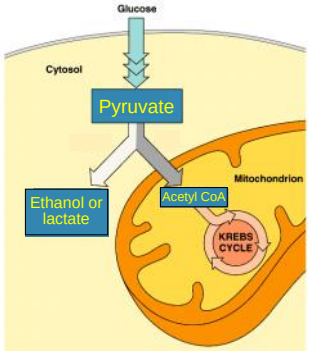
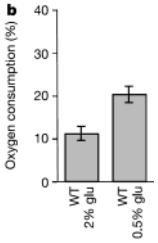
Linking metabolism to lifespan



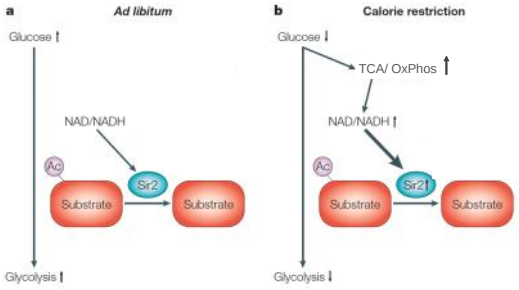


Linking Metabolism to Lifespan

- Respiration
  - Glucose converted to Pyruvate
  - then depending on oxygen levels either aerobic respiration in the mitochondria or anaerobic in the cytosol
- What kind of respiration used is not just dependant on the levels of oxygen, levels of glucose available are affect
- High levels of glucose (in yeast) then you get high levels of fermentation
  - doesn't matter if you have high levels of glucose or not
  - leads to low levels of oxygen consumption and electron transport
- Low levels of glucose give high levels of electron transport
  - CR leads to high levels of oxygen consumption
- In animal cell, high levels of glucose also cause diversion to production of carbohydrates and fatty acids
- NADH made from carbon metabolism can be easily oxidised by electron transport
  - anaerobic respiration doesn't oxidise NADH as fast of effectively

Sir2 is NAD dependent

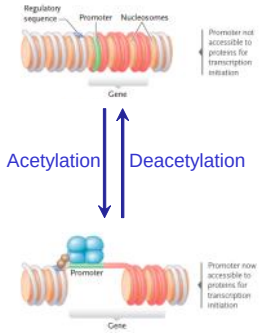
Sir2 requires NAD^+ to function



Sir2 is NAD dependant

- Sir2 requires NAD as a cofactor in order to be activated
  - very strange
- NAD/NADH ratio energy status of the cell
  - if the cell is very oxidised - lots of NAD
  - cell is very reduced - lost of NADH
  - easy to measure in the blood
- Ad libitum
  - Sir2 is inhibited
  - high rates of glycolysis leads to high levels of NADH
  - low NAD/NADH ratio
  - when the ratio is low, Sir2 is not active
  - Sir2 needs NAD in order to be active and functional
- Caloric Restriction
  - increased TCA and Oxidative Phosphrylation
  - NAD/NADH ratio is high
  - Sir2 is active
  - Caloric restriction is linked to NAD by Sir2
- Sir2 must have a major role in yeast if it can affect lifespan

Sir2 is a histone deacetylase

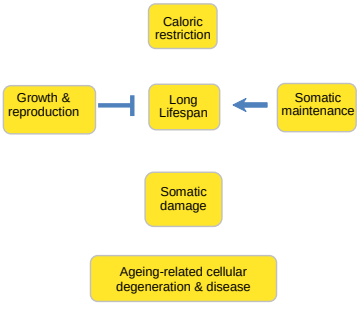


Sir2 is a Histone Deacetylase

- removes acetyl groups from Histones
  - makes the genes less accessible by promoters
- Sir2 keeps part of the yeast chromosome inaccessible
  - Chromosome silencing
  - in particular its r-DNA
  - if those r-DNA genes are expressed, then lifespan is shortened
  - exactly what is in the r-DNA that is linked to lifespan is not entirely understood yet
- Sir2 is a Sirtuin
  - highly conserved form of Deacetylases
  - major regulators of large biological processes

Evolutionary basis of caloric restriction

- Disposable soma hypothesis



Evolutionary Basis of Caloric Restriction

- Disposable soma hypothesis
  - if you put lots of energy into reproduction (fundamental of evolution), then you limit your own long lifespan
  - cant invest resources in maintaining yourself
    - limit resources for repair etc.
  - Somatic maintainece if disposed of
    - all energy goes into reproduction
  - However if you invest heavily in Somatic maintenance then you limit somatic damage
- Somatic Damage leads to ageing related cellular degeneration and disease
- During conditions of starvation and famine, you don't have the resources to put into reproduction
  - all of the resources you do have go into somatic maintenance
  - forgo reproduction

Readings

April-09-11
6:13 PM

6.7d The Paradox of Aerobic Life

- Strictly anaerobic organisms die in the presence of oxygen, why?
- The reason is linked the paradox of aerobic life
    - Although many organisms require oxygen as the terminal electron acceptor in electron transport and thus can not live without it, oxygen is inherently dangerous to all forms of life
- Four electrons are needed to reduce one molecule of oxygen to water
    - if oxygen only accepts 1, 2 or 3 electrons, highly reactive oxygen species - or ROS - are formed as intermediates
- These ROS are strong oxidising agents, and are highly dangerous to biological molecules
- If ROS levels within a cell become too excessive, then it can lead to the destruction of important molecules and cell death
- As most cells contain both an abundance of oxygen and electron rich biological molecules, the formation of ROS is an unavoidable consequence of aerobic life
- Therefore, aerobic organisms have developed an antioxidant defence system - made up of both enzyme and non-enzyme molecules - to intercept and inactivate ROS as they accumulate within cells
    - two major ROS scavenging enzymes are superoxide and catalase
    - the absence of one or both of these enzymes in anaerobic organisms causes a build up of high levels of intercellular ROS, resulting in damage and cell death
    - Antioxidants such as Vitamin C and E act as reducing agents, which rapidly reduce ROS to water before they can do any damage
- However this antioxidant defence system is not 100% effective, and so ROS damage still occurs
    - to deal with this damage, cells have an elaborate system of removal/repair enzymes for proteins, lipids and DNA
- Excessive levels of ROS have been implicated in variety of degenerative processes, and the build up of ROS is thought to underline the aging process itself
- The main advantage of using oxygen as the terminal electron acceptor is that cells can extract more energy from food this way
- In order to further prevent ROS related damage, cells have evolved Cytochrome Oxidase
    - this is the last enzyme in mitochondrial electron transport
    - it contains four redox site - two hemes and two copper ions - each of which can store a single electron
    - However, the enzyme only transfers its electrons to oxygen when all of its redox sites are full
    - this means that it transfers electrons to oxygen without the formation of ROS, and given that 98% of all oxygen metabolized is done so by this enzyme, with out this process aerobic life would never have been able to develop.

Gas Exchange

April-04-11
2:17 PM

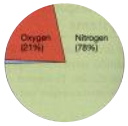
What are the evolutionary developments that have allowed organisms to get big

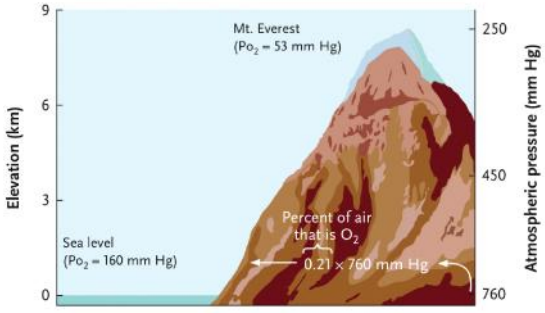
- Problem of size
  - gases move through diffusions
  - how do you diffuse enough gas to get a whale, or a human
  - must have been evolutionary changes to structure and function to allow for increased size
- Surface Area to Volume problem
  - as you get bigger, S.A. to Vol. ratio gets smaller

Organism	Length	SA (m^2)	Vol. (m^3)	S/A:Vol of organism
bacterium	1 mm	6×10^{-12}	10^{-18}	6,000,000:1
amoeba	100 mm	6×10^{-8}	10^{-12}	60,000:1
fly	10 mm	6×10^{-4}	10^{-6}	600:1
dog	1 m	6×10^{0}	10^{0}	6:1
whale	100 m	6×10^{4}	10^{6}	0.06:1

Oxygen Availability - Partial Pressure

- Concentration of air
  - 21% Oxygen
  - 78% Nitrogen
  - constant concentration
    - doesn't matter at what altitude your at
  - concentration is therefore not a good measure of Oxygen availability
- Use Partial Pressure instead (measure in mmHg)
  - measured by change in pressure of air as you move above sea level
  - (Proportion of the total gas)(atmospheric pressure)
  - partial pressure of O_2 at sea level
    - $P(O_2) = (0.21)(760) = 160mmHg$
  - Partial pressure represents a force
- A gas will move spontaneously down a partial pressure gradient





Everest Who's Who

- Hillary and Norgay (1953)
  - first people to reach top
- Reinhold Messner (1977)
  - first one to reach top unassisted (no supplemental oxygen)

Gas Exchange Occurs by Diffusion

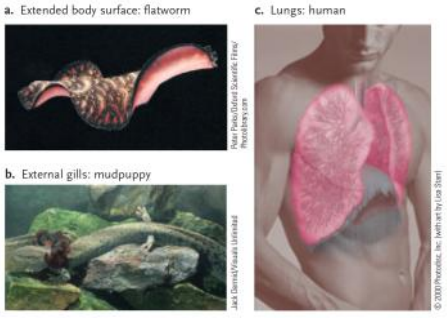
- No active transport, simple diffusion
  - no O_2, CO_2 transporters
- If diffusion distance is very short, no issue with diffusion time
  - e.g. crossing mitochondrial membrane
- However once you reach size of humans, diffusions times make no sense
  - diffusion times are far too long
- How has life evolved to deal with the fact that diffusion of longer distances does not occur fast enough to sustain life

Distance	Diffusion Time	Example
100 angstroms	0.0000001 sec	Cell membrane thickness
1 micron	0.001 sec	Size of most bacteria or mitochondria
10 microns	0.1 sec	Diameter of small eukaryotic cells
2 millimeters	1 hr	Thickness of lens of eye
10 centimeters	120 days	Diameter of sea urchins & other small animals
1 meter	32 yrs	Half height of human

Fick's Law of Diffusion

- $Q = DA\,\frac{P1 - P2}{L}$
- D = Diffusion coefficient
  - different for every gas
  - gases in aqueous environment move slower than they do in air
- A = Area which the diffusions takes place across
- P1 and P2 = the partial pressures in the two compartments
  - Partial Pressure difference across the membrane of whatever
- L = Path Length
- How have organisms over evolutionary time maximised Q

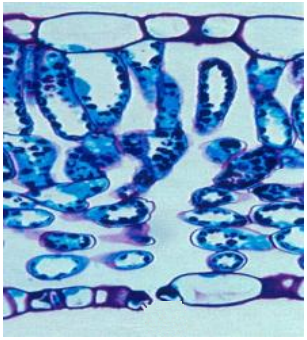
Respiratory surfaces



Respiratory Surfaces

- An organism exchanges gases with the environment across its respiratory surface
  - respiratory surfaces vary with organism
    - e.g. Flatworms - whole organism is respiratory surface
      - Flat surface means higher S.A. to Vol. ratio
- All respiratory surfaces must be wet
  - gases are in solution when they pass into or out of respiratory surfaces
  - problem with CF patients
    - not enough moisture
    - gas exchange limited

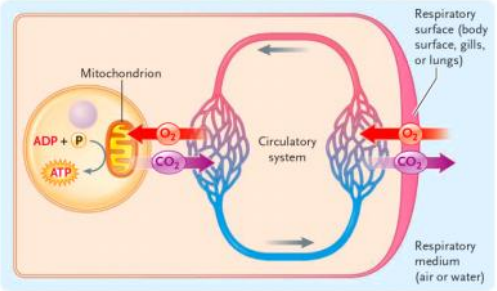
Fick's Law and a Leaf



- Most leaves covered by a waxy cuticle
  - gases cant just diffusion in and out anywhere on a leaf
  - have to diffuse in and out of stomata
- H_2O will diffuse out and CO_2 will diffuse in to a leaf through stomata
- What drive CO_2 uptake into a leaf
  - partial pressure of CO_2 is very low within the leaf
  - CO_2 within the leaf is being consumed by the Calvin cycle
    - Rubisco is Carboxylation the CO_2
- What drives the escape of H_2O
  - Water always escapes a leaf as there is a lot more water inside a leaf than outside
  - Lower Partial Pressure outside of the leaf
- Why is leaf gas exchange so fast
  - leaf has a massive surface area
  - surface are available is actually internal to the leaf structure
    - means massive surface are
  - very short diffusion distances
    - Gases can get right to the very outside of the cell
    - means only a couple of membranes between location of oxygen and the chloroplast

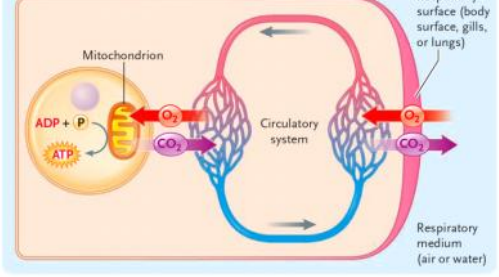
Animal Gas Exchange

- Animals can grow big because of the evolution of circulatory systems
- Circulatory systems link the respiratory surfaces to the interior of the cells
  - still occurs all by diffusion and partial pressures
  - oxygen diffuses down a partial pressure gradient from the lungs into the circulatory system
    - little O_2 in the blood
  - CO_2 diffuses out at the same time
    - high CO_2 in the blood
  - Mitochondria are consuming O_2 and producing CO_2



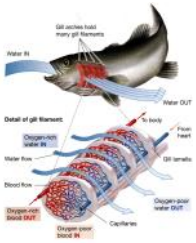
- oxygen diffuses down a partial pressure gradient from the lungs into the circulatory system
  - little O_2 in the blood
- CO_2 diffuses out at the same time
  - high CO_2 in the blood
- Mitochondria are consuming O_2 and producing CO_2
  - O_2 diffuses across partial pressure gradient into Mitochondria
  - CO_2 diffuses out of the mitochondria

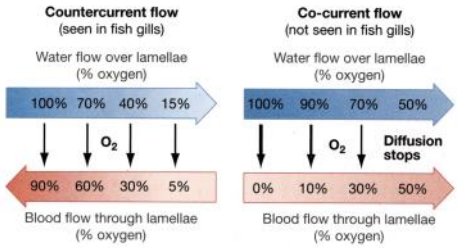
- Haemoglobin increases the carrying capacity
  - primitive circulatory systems rely on aqueous environment
  - O_2 carrying capacity of haemoglobin increases carrying capacity of the circulatory system ~60 times



<u>The Fish Gill</u>

- Water flows through the mouth and through the gills
- O_2 in the water diffuses into the circulatory system that passes through the gills
- Fish have to keep moving
  - diffusion of gas in water is slow than it is in air
  - keep moving so they can find regions with more oxygen once they consume all the oxygen in their area
- Counter Current exchange of oxygen in the gills
  - blood and water flow through gills in the opposite direction
  - diffusions of O_2 is always from water into blood
  - partial pressure gradient is always in the same direction



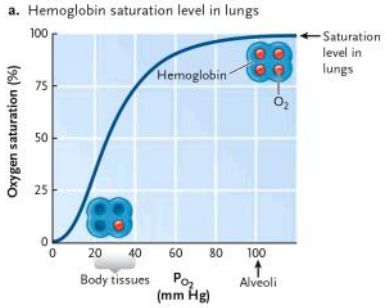


<u>Haemoglobin</u>

- Quaternary Structure
  - four individual proteins and heme groups
- Each heme group has an Iron molecule bound in its center
  - Iron is the binding site of oxygen
  - Respiratory pigment
    - heme is a pigment because it has a conjugated double bond ring system

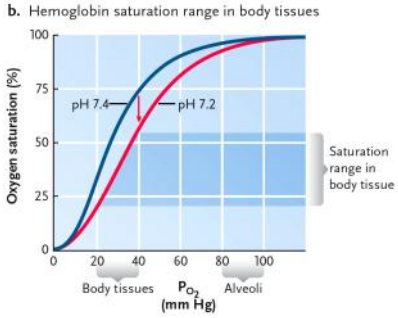
<u>Oxygen Dissociation Curves 1.</u>

- binding of oxygen and heme group is dictated solely by partial pressure of the environment
  - high partial pressure causes binding
  - low partial pressure causes release
- Oxygen bonding has an S shaped curve
- caused by a cooperativity between the subunits
  - under low partial pressure, one oxygen will bind to one subunit
  - this binding changes the conformation of the other subunits, increasing their affinity for oxygen
  - same occurs as further binding occurs
- Partial pressure in tissues whilst resting at about 40
  - this means a 75% oxygen saturation levels of haemoglobin
  - low partial pressure levels can support resting tissues
- In exercising tissues, partial pressure in tissues is lower
  - this means that more oxygen is released more readily
  - only 25% oxygen saturation levels of haemoglobin
- Small change in partial pressure leads to a large change in oxygen saturation levels



<u>Oxygen Dissociation Curves 2.</u>

- Bohr Shift
  - when the pH of the blood is lower, the affinity for oxygen of the haemoglobin is lower
  - Lower pH = lower affinity
- this shift often occurs in blood vessels that are close to heavily active cells
  - for example muscles
- $CO_2 + H_2O \rightarrow H_2CO_3$
  - heavily active cells spew out high levels of CO_2
  - caueses an increase in levels of H_2CO_3, thus causing a drop in pH
- This affect allows haemoglobin to deliver slightly more oxygen than normal to heavily active cells



Readings

April-17-11
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42.1 General Principals of Gas Exchange

- The percentage composition of air does not change with the total amount of air
    - therefore the higher up you go, the less total air there is and the less total oxygen there is, but the percentage composition is still the same
- The density of the air in the atmosphere is measured as atmospheric pressure
    - this decrease with altitude and increases the closer you get to sea level
    - The unit of measure is mm Hg
    - at sea level, the atmospheric pressure is 760 mm Hg
- This atmospheric pressure is a combination of the pressures of all the individual gases in the mixture
    - the individual pressures exerted by each gas is defined as its partial pressure
- Partial pressure is calculated by multiplying the fractional composition of that gas by the atmospheric pressure
- The partial pressure of a gas is the key factors in determining the direction in which a gas will move
    - just as a solute will diffuse from an area of high concentration to one of low concentration, a gas will move from an area of high partial pressure to one of low partial pressure
- The rate at which a gas will diffuse is dependant on a set of factors, which are represented by Fick's Equation of Diffusion
    - $Q = \frac{DA\ (P1\ - P2)}{L}$
    - Q is the rate of diffusion between the two side of the membrane
    - D is the diffusion constant for the particular gas involved
    - A is the area across which diffusion takes places
    - P1 and P2 are the partial pressures of the gas at the two locations
    - L is the path length between the two locations
- Relying on diffusion alone for gas exchange limits both the size and shape of an organisms
    - the importance of these factors is made obvious by the surface area to volume ratio
    - Organisms with a high surface area to volume ration - such as bacteria - can rely on diffusion for gas exchange as the diffusion distance is very small
    - Among multicellular organisms however, an increase in size can be accommodated only if the distance of which diffusion has to take place is minimized
        - flat worms for example are long a thin, which minimizes the distance over which diffusions has to take place
        - Plants also minimise the distance over which diffusion has to take place by allowing gas to enter their leaves through stomata where it is then inside a large internal space with a large surface area in close proximity to the cells
        - In animals gas exchange occurs across a respiratory surface
- The evolution of larger, specialized respiratory surfaces or some means of transporting gases to and from the surface of cells within the organisms has allowed for the development of larger and more complex organisms
    - the respiratory surfaces are often very large, increasing the total area
    - the cells that make up the surface are also squamous epithelium cells, which minimises the diffusion length
    - circulatory systems also often maintain a high difference between P2 and P1
    - all of these factors contribute to large Q value in Fick's equation
- For gases to diffusion across the respiratory surface they must be in solution
    - for aquatic and marine animals that is already the case with gills - outwards extensions of the body surface - acting as the site of gas exchange
    - For terrestrial animals the respiratory medium is air, therefore the respiratory surface is

covered with a thin film of liquid - loss of water is minimised by the location of the respiratory surfaces residing within lungs, deep inside the body of the animal

- While some of the CO_2 produced by the cells remains in solution as a gas significant amounts may combine with water to produce carbonic acid - H_2CO_3 - which dissociates into bicarbonate - HCO_3^+ - and H^+ ions.
    - this mechanism maintains a maximal concentration gradient of carbon dioxide between the cells and the blood
    - the capacity of the blood or bodily fluids for Carbon Dioxide is limited - it is a means of storing the gas in a harmless way until it can be transported to the respiratory surface to be released as a gas
    - most of the dissociated H^+ ions combine with haemoglobin or proteins in the blood - this acts as a buffer, removing excess ions and maintaining the blood at an appropriate pH for the organism
- While all gas exchange occurs by diffusion, there are two adaptions that help to maintain the difference in concentration between inside and outside the respiratory surfaces the most - ventilation and perfusion
    - As animals respire the remove oxygen from the respiratory medium and replace it with carbon dioxide, without ventilation the concentration of oxygen in the respiratory medium would fall and the concentration of carbon dioxide would rise greatly reducing the value of P1 - P2
    - Perfusion is the rate at which blood or other fluids are replace on the internal side of the respiratory surface also keeps p1 -p2 at an acceptable level - in animals with a circulatory system, this system brings blood to the internal side of the respiratory surface, releases carbon dioxide transported from the cells and picks up oxygen from the external side of the respiratory surface
- While aquatic and marine animals have no problem with keeping their respiratory surfaces wet, they do have an issue in obtaining oxygen from water compared to terrestrial animals obtaining it from air
    - the difference in the diffusion constant in gas and water is larger - the ratre of diffusion is about 10000 times faster in air than it is in water
    - for the same volume, there is alos about 30 times less oxygen in water compared to air
    - these two factors require animals that rely on water for gas exchange to pass massive volumes of water over their respiratory surfaces in order to be exposed to the same volume as terrestrial animals
    - the density of water is also about 1000 times greater than air, with its viscosity about 50 times that of air - this means that a great deal of energy has to be expended to move water over the respiratory surface
    - to compensate for these factors, ventilation in most aquatic animals takes place in one direction
    - in addition, temperature and solutes affect the oxygen content of water - as temperature or solute concentration increase the oxygen levels in water decrease.
- The major disadvantage of air as a respiratory medium is that unless it is saturated with water vapour it will constantly evaporate and lose water from the respiratory surface
    - except in an environment with 100% humidity animals lose water by evaporation and have to expend energy to replace it in order to keep the respiratory surfaces from drying

42.2 Adaption For Gas Exchange

- Gills are respiratory surfaces that are branched and fold evaginations of the body which increase the are over which diffusion can take place
    - External gills extend out from the body and do not have protective coverings
    - Internal gills are located within chambers of the body which provides protection for the structure and also allows currents of water to be direct over the gills
- In adult bony fishes the gills extend into a chamber covered by gill flaps - or opercula - one either side of the head that serve as part of a one way pumping system that ventilates the gills
- Sharks, Fishes and some Crustacea takes advantage of one way flow of waster over the gills to maximise the amounts of oxygen and carbon dioxide exchanged with water - this mechanisms

is known as a counter current exchange

- In Counter current Exchange the water flowing over the gills moves in the opposite direction of the flow of blood under the respiratory surface
    - this systems maximises the exchange of gases and prevents the partial pressure difference between the internal side and water side of the respiratory surfaces from reaching 0
    - the over effect of counter current exchange is the removal of 80 to 90% of oxygen content from water - which is important due to the lower oxygen content of water in comparison to air
- Insects breath by a respiratory system consisting of air conducting tubes called tracheae
    - the tracheae are invaginations of the outer epidermis of the animal that consist of epithelial cell lined and reinforced with rings of cuticle - the same as the exoskeleton.

Nitrogen

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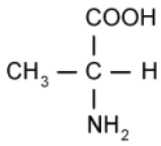


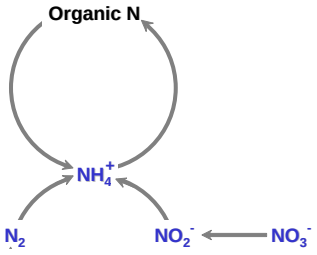
Biological Nitrogen Cycling

- In order to assimilate Nitrogen into biological important molecules it has to be in the form of Ammonia/Ammonium
  - to get nitrogen in to amino acids, heme etc. bio synthesis
  - doesn't matter if bacteria or human
- Ammonia is highly toxic
  - don't accumulate in cells
  - make it, and then move it into some other molecule
  - toxic to almost every living thing
- In the case of humans, we get nitrogen by eating organic nitrogen
  - nitrogen rich foods
    - break down and release the ammonia
  - no capacity to get nitrogen from the environment apart from consuming nitrogen that is already linked to some organic compound
    - nitrogen found in a protein, amino acid etc.
- Where does this organic Nitrogen Come from

Biological nitrogen cycling

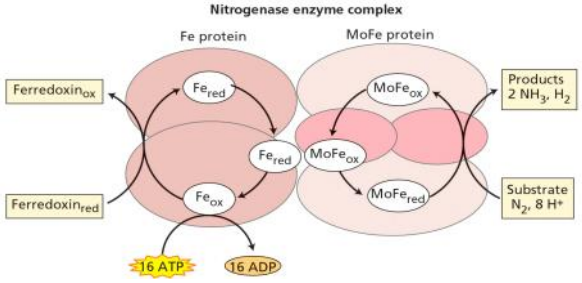
Oxidation State of Nitrogen

- Ammonia is a reduced from of nitrogen, with a oxidation state of -3 from Nitrogen
- Nitrate assimilation is a common pathway for the uptake of nitrogen
  - Nitrate found in soils
    - fertilizers, organic matter
  - Nitrate has an oxidation state of +5
  - therefore the pathway is a reduction process
    - series of enzyme driven reduction reactions
  - Many plants have this pathway, however animals - and humans - do not
- Nitrogen Fixation
  - taking atmospheric atmosphere and reducing it into ammonia
  - N2 has a oxidation state of 0
    - therefore also a reduction reaction to produce ammonia/ammonium



Nitrogen Fixation

- Nitrogen fixation is taking atmospheric nitrogen and reducing that into ammonia
  - $N_2 \rightarrow NH_3$ or NH_4^+
- Very important process
  - massive source of nitrogen that biological organisms should be able to access
  - however it is very hard to access due to N triple bond
- N triple bond is very hard to break - makes atmospheric nitrogen very inaccessible
- Therefore selective advantage to organisms who evolved method for breaking that triple bond
- Industrial Nitrogen Fixation
  - $80x10^9$ kg NH_3 per yr.
  - Takes high pressure and temperature
  - 700C + high pressure
- Natural Nitrogen Fixation
  - $250x10^9$ kg NH_3 per yr.
  - some of this natural fixation comes from lightening - however not very much
  - largest proportion comes from Biological Nitrogen Fixation
  - there is an enzyme that can break N_2 into ammonia
    - Nitrogenase
- There are two groups of organisms that can fix Nitrogen through the use of the enzyme Nitrogenase
  - free-living nitrogen fixers
  - Symbiotic nitrogen fixers
- Only Prokaryotes can fix nitrogen
  - not a single eukaryote on the planet can do it



Nitrogenase Enzyme Complex

- $N_2 + 16ATP + 8H^+ + 8e^- \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi$
- high energy and reducing power investment in order to convert nitrogen into ammonia
  - where does the energy come from to drive this reaction?
- Nitrogenase is an enzyme exclusive to prokaryotes
  - dominant pathway for nitrogen entering the biosphere on the planet
- it has four major protein components
  - Iron and Molybdenum Iron proteins
  - Co-factors for both parts of the protein
- The reducing power comes from ferredoxin
  - reduces the iron
  - ATP is needed
- This causes the molybdenum Iron portion to be reduced, which then in turn reduces the nitrogen producing ammonia
- The enzyme is irreversibly inactivated by Oxygen
  - becomes totally inactive in the present of oxygen
  - suggests that it is a very ancient enzyme that predates the rise of atmospheric oxygen levels\
- so how do you maintain Nitrogenase activity on an oxygen-rich planet

Strategy 1 - Spatial Separation

- Filamentous cyanobacteria
  - can undergo O.P - oxygenic photosynthesis
  - also has nitrogenase
  - separates the two processes
- Nitrogenase is found in the Heterocyst
- Photosynthetic cells are contained in the vegetative cells
- Formation of a Heterocyst is a developmentally controlled process
  - If the cyanobacteria is growing in a solution rich in ammonia, then it does not need the heterocyst
  - however if you then move them into a solution that has no ammonia, the cyanobacteria will the develop

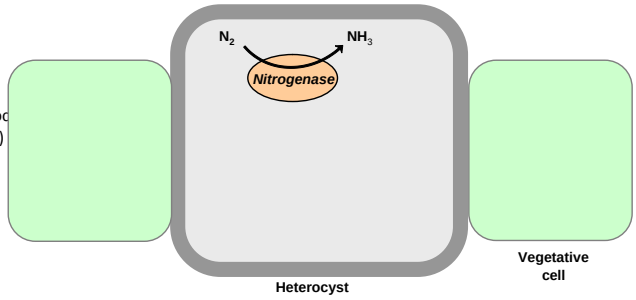
Heterocyst function

- Nitrogenase is found in the Heterocyst
- Photosynthetic cells are contained in the vegetative cells
- Formation of a Heterocyst is a developmentally controlled process
  - If the cyanobacteria is growing in a solution rich in ammonia, then it does not need the heterocyst
  - however if you then move them into a solution that has no ammonia, the cyanobacteria will the develop the heterocysts

Heterocyst function

Heterocyst Function

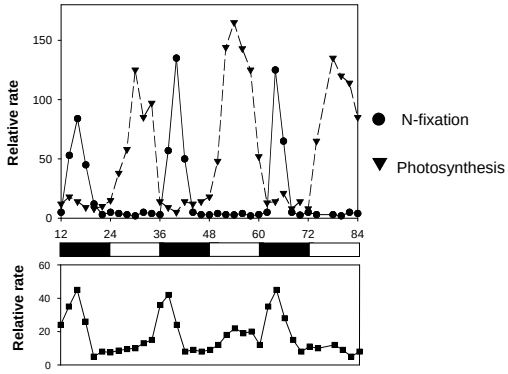
- Nitrogenase needs a lot of energy - needs reducing power and ATP
- Vegetative cells produce a lot of glucose
  - through Rubisco, carbon fixation, Calvin cycle
- Glucose is then imported into the heterocyst
- Respiratory Electron Transport chain would be intact within the Heteroc
  - normal respiration and also PS1 activity (cyclic electron transport)
  - no PS2, so no oxygen produced
- Ferredoxin produced by PS1
- NH3 is produced and quickly incorporated into glutamine



Strategy II - Temporal separation

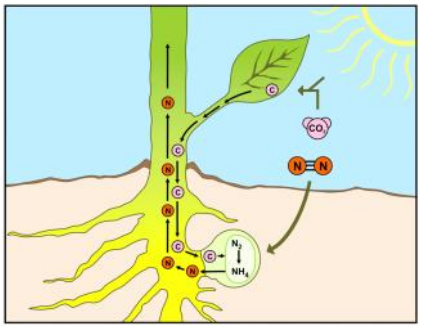
Strategy 2 - Temporal Separation

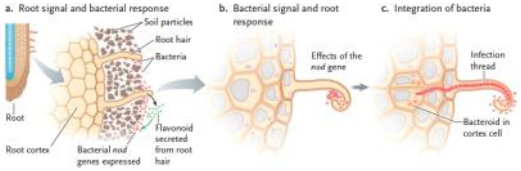
- What if you're a single celled organism
  - cant use spatial separation
  - have to use temporal separation
- Activity of Nitrogenase is highest during the night
  - out of phase with photosynthesis, which occurs during the day
  - two can occur in the same cell if the occur at different time
- Why does nitrogenase activity decline
  - protein degradation
  - protein synthesis
  - protein inactivation
  - all of them can determine the peaks in nitrogenase activity
- High rates of respiration also occur at night
  - coincides with nitrogenase activity
  - respiration makes ATP needed by Nitrogenase
  - respiration also consumes oxygen so that any residual oxygen that may inhibit nitrogenase is being removed

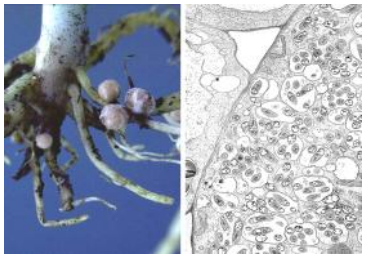


Symbiotic Nitrogen Fixation

- Nitrogen fixing bacteria that infect cells of the plant root
- a symbiotic relationship
  - non obligatory
  - if nitrate or ammonia are present then there is no need for the relationship
  - plants only enter into this relationship if there is no useable nitrogen around
- Not all plants can do this
  - only legumes with Rhizobium
- Bacterium infects the plant root and produces a nodule
  - nodule is filled with bacteria that can fix nitrogen
- Nitrogen is exported from the nodule to the plant
  - used in bio synthesis of everything
- Bacteria gets a reduced form of carbon provided by the plant that it can use in respiration
  - carbon comes from photosynthesis in the plant
- The Nodule is not just an empty bag filled with bacteria, it's a proliferation of plant root cells
  - some of those cells have the bacteria within them
    - bacteriods when they are in the nodule
- The Bacteriods are not really free-living bacteria anymore
  - they are converted into N-fixing organelle
  - no longer divides - suspended life cycle







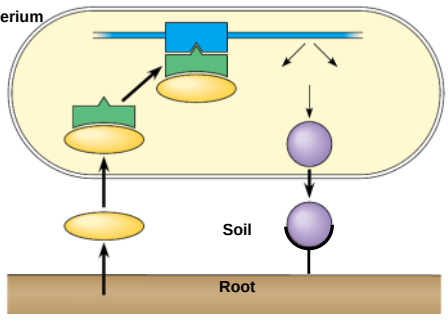
Root Hair Infection Process

- need to differentiate the root cortex and epidermis
- Root hairs are single cells with an elongated surface area
  - helps with absorption of important molecules
- The bacteria interact with the root hair, causing it to curl
- The bacteria breach the cell wall and produce an infection thread
  - the infolding of the plasma membrane is what develops the infection thread
  - the infection thread is a pathway through which the bacteria can access the cortex of the root

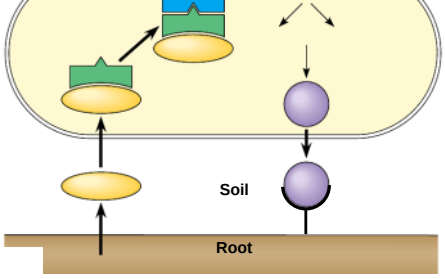
Molecular biology of nodule formation

Molecular Biology of Nodule Formation

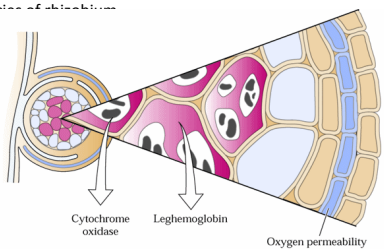
- Why is it so species specific?
- Root senses if there is not enough ammonia, nitrate around
  - releases flavonoid
- the Flavonoid is taken up by the bacterium and interacts with the transcriptional regulator Nod D
  - Nod D is constitutively expressed but not always function
  - Function when it binds to the flavonoid
- Nod D binds to the Nod box (promoter/enhancer sequence) once activated
  - activates the expression of Nod genes
- Nod genes code form Nod enzymes, which then produce Nod factors
  - the Nod factors are released out of the bacteria



- Function when it binds to the flavonoid
- Nod D binds to the Nod box (promoter/enhancer sequence) once activated
  - activates the expression of Nod genes
- Nod genes code form Nod enzymes, which then produce Nod factors
  - the Nod factors are released out of the bacteria
- There are receptors on the root cells that recognise and bind to the Nod factors
  - host specificity comes into play here
  - specific species binds and recognise specific species of rhizobium

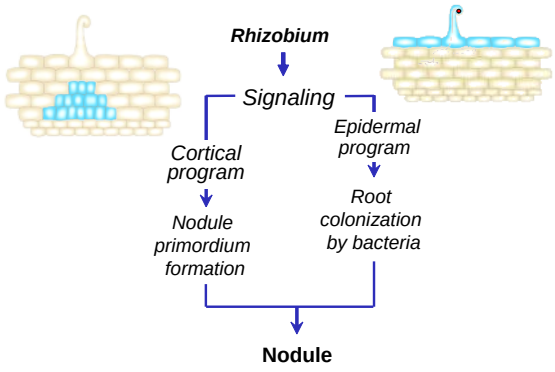


<u>Nodules - How To Control The Oxygen</u>



- Bacteriods have respiration
  - producing ATP by O.P
  - means that the require oxygen
- Bacteriods have a special cytochrome oxidase
  - has a very high affinity for oxygen
  - has a very low K
    - Mitochondria has a K_m of about 100nM
    - Bacteriod has a K_m of about 8nM
- Also have Legemoglobin
  - much like normal haemoglobin
  - binds oxygen
- Oxygen Permeability Barrier means that oxygen cannot easily diffuse into and out of the nodule

Nodule formation requires two programs

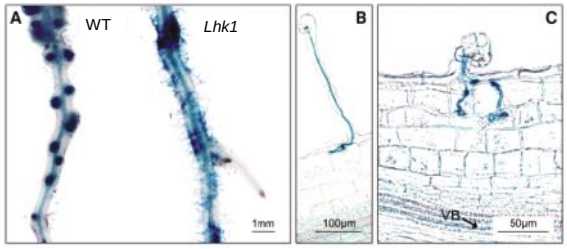


<u>Nodule Formation Requires Two Programs</u>

- two pathways that have to act in concert with each other
  - Epidermal and Cortical Program
  - Both have to synchronised with each other
  - both are activated by Rhizobium interactions at the epidermis
- Epidermal Program responsible for Root Colonization by Bacteria
- Cortical Program responsible for Nodule Primordial Formation
- Signals from the Epidermal move down to the Cortex independent of bateria
  - infection causes signal to travel down to the cortex, thus causing changes in the plant
- Need both programs to work in order to obtain fully developed nodules

Lhk1 mutation aborts cortical program

- Lotus Histidine kinase 1 (*Lhk1*)



<u>Lhk1 Mutation Aborts The Cortical Program</u>

- Lotus Histidine Kinase 1
- If you knock out this gene you get lots of infection thread formation but no cortical program
  - Lhk1 is required for the signal to move from the epidermis and activate the cortical program
- Lhk1 is found in many plant species
  - plant species that cannot form nitrogen fixing root nodules
  - if they could, then fertilizer usage would plummet
- What specifies the ability of legumes to from nitrogen-fixing nodule symbiosis

Readings

April-17-11
12:27 PM

41.3c Plants Can Be Limited By The Availability Of Nitrogen

- A Lack of Nitrogen is the single most common limitation to plant growth
    - Despite the air contain amply gaseous nitrogen, plants lack to the enzyme - nitrogenase - needed to break the triple covalent bond in each molecule of atmospheric Nitrogen
- Some Nitrogen reaches the soil in the forms of nitrate - NO_3^- - and Ammonium - NH_4^+ - ion
    - while plants can absorb both of these inorganic nitrogen compounds, there is usually not nearly enough of them to meet plants' Nitrogen needs
- While organic Nitrogen enters the soil through the decomposition of dead organisms and animal waste, this Nitrogen is bound up in complex organic molecule - such as proteins - that are unavailable to plants
- The actions of bacteria are the main process by which soil Nitrogen is replenished and converted into the absorbable forms
    - these bacteria are all part of the Nitrogen Cycle
- Nitrogen fixation is the incorporation of atmospheric Nitrogen into compounds accessible to plants
    - nitrogen-fixing bacteria that live either in the soil or in symbiotic relationships with plant roots reduce atmospheric nitrogen, producing NH_3 and H_2 - the reaction is catalyzed by the enzyme nitrogenase
    - The Process requires a large investment of energy in the form of ATP
    - In a final step, the HN_3 reacts with water to form NH_4^+ and OH^-
- Ammonification is another process that produces NH4+ through the break down of decaying organic matter
    - this process recycles nitrogen previously incorporated into plants and other organisms
- The NH4+ produced by both processes can be used by plants to synthesis organic compounds
- Most plants absorb Nitrogen as Nitrate - produced in the soil by nitrification of NH4+ through a series of oxidation reactions
    - soils are therefore generally teeming with nitrifying bacteria that carry out this process
    - due to ongoing nitrification, nitrate is far more abundant in most soils than ammonium
    - plants usually take up ammonium directly only in highly acidic soils - low pH is toxic to the nitrifying bacteria
- Nitrate is converted back to ammonium within the root cells by a multistep process
    - the ammonium is then rapidly used to synthesise organic molecules - mainly amino acids - which then ass through the xylem and are transported throughout the plant
- Although nitrogen fixing bacteria live free in the soil, the largest proportion of nitrogen is fixed by Rhizobium
    - these bacteria form symbiotic relationships with the roots of legumes
    - the host plant supplies organic molecules - mostly reduced forms of carbon - to the bacteria to use in respiration
    - the bacteria supply the host plant with reduced forms of nitrogen that the plant uses to produce proteins, amino acids and other nitrogenous molecules
- Normally, a single species of Nitrogen-fixing bacteria colonizes a single legume species
    - The nitrogen fixing bacteria are drawn to the plants roots by flavenoids - chemical attractants secreted by the roots when they sense low levels of ammonium or nitrate in the soil
    - In a response to the flavenoids, the bacterial Nod genes begin to be expressed, the products of which cause the root hairs of the plant to curl towards the bacteria
    - this then triggers more Nod products, which cause the bacteria to release enzymes that break down the cell wall of the plant root cells
    - as the bacteria enter the cells, the plasma membrane forms an infection thread - a tube that extends down to the root cortex - that allows the bacteria to enter the cortex o and become bacteriods - large immobile versions of the bacteria

- Inside of the bacteriods, nitrogenase catalyzes the reduction of Nitrogen to ammonium using ATP provided by cellular respiration
    - Ammonium is highly toxic to cells if it is allowed to accumulate, so it is immediately moved out of the bacteroids into the surrounding nodule cells and converted into organic molecules
- The protein Leghaemoglobin - legume haemoglobin - is also produced by the stimulation of plant nodule cells by the bacterial nod gene products
    - Leghaemoglobin picks up oxygen at the cell surface and moves it inward to the bacteroids
        - this method of oxygen delivery is vital as nitrogenase is irreversibly inhibited by oxygen
        - the Leghaemoglobin provide enough oxygen for respiration without provided excess that could inhibit the nitrogenase

Biological Time Keeping

April-17-11
4:38 PM

How Do Biological Systems Keep Track of Time

- Important as the earth spins on its axis every 24 hours
    - rhythmic nature of day and night (diurnal) that has existed for forever
- organisms have evolved in the presence of this fluctuation of day and night
- many behaviour/physiological/biochemical processes have a rhytmic nature which coincides with this day-night pattern

Diurnal Phenomena

- Foraging/predatory behaviour
- Sleep-wake cycle
- Body Temperature
- Hormone Secretion
- Photosynthesis
- Nitrogen Fixation
- all example of phenomena that obey a cycle
- The period length of these cycles are often 24 hours

The Value Of Keeping Track Of Time

- Although evolved under the conditions of day and night, is there any benefit to actually keeping track of time
    - not just responding to changes in night and day, but actually knowing and anticipate the change
    - what if organisms could anticipate environmental change
- Photosynthetic Gene (to the left)
    - black bar at the top represents night, white represents day
    - m-RNA is expressed during the day - makes sense, photosynthetic proteins needed during the day
    - however gene is turned on before dawn
        - not responding to change in light but anticipating it
- why would you want to do this from an evolutionary stand point
    - you want your photosynthetic machinery to be ready to go before the light comes on
    - not wasting time turning the stuff on during the light
    - maximise energy gained from light period
- How do you explain this
    - not just a simple response to light
    - suggests that there is an endogenous biological clock
    - the organism knows what time it is relative to day/night cycle
    - if you put a plant under constant conditions of light or darkness, the cycle of transcript abundance follows the same pattern
    - Circadian rhythms

Circadian Rhythms

- A Biological rhythm with a period of around 24 hours
    - roughly coincides with the rotation of the earth
- Two major criteria that define a circadian rhythm
    - the rhythm is tuned to the external light environment, but is not a direct response to
        - once the rhythm is set, it will keep going in the absence of the normal diurnal cycle
        - does not need constant stimulus from the external environment
        - they are free running

        - however will not continue for ever - eventually will become messed up
    - There must be a connection between the rhythm and the external environment - the rhythms can be reset and entrained to a new environment
        - for example the effects of Jet Lag

Circadian Phenomena

- A process can be diurnal but non-circadian
    - sleep-wake cycles are an example of a diurnal and circadian control
        - under constant conditions they will slowly move out of whack
        - the same with body temp and hormone levels
    - Photosynthesis is a diurnal but non circadian process
        - while some of the genes may be under circadian control, the actual process needs light, so is diurnal
            - it wont happen under constant dark
- Many hundreds of human genes are under circadian control

Questions About The Clock

- suggests that there is a endogenous biological clock that keeps time for your body
- We know that the clock is not independent - it can be adjusted by the external environment
    - how is the clock synchronized with astronomical time (day and night)
- The clock itself influences changes in hormones and gene expression and body temperature and all these other things
    - how is molecular time linked to changes in physiology/behaviour
- What is the biochemical basis of the clock?

What Do We Mean By Clock

- Every clock has something that oscillates
    - be it pendulum or crystal or whatever

Circadian Rhythms Have A Genetic Basis

- Mutant Drosophila were isolated with mutations whos circadian rhytms were out of whack
- Eclosion Mutants
    - eclosion is when the fly comes out of the larva case
    - rhythmic mutants were isolated when they did not obey the usual circadian cycles
    - eclosion occurred with shorter/longer/random periods that usual

A Biological Clock - Neurospa

- What oscillates in a biological clock is transcription/translation
- in Neurospa the gene is frq
- WC1 and WC2 (transcription factors) activate frq around dawn, and the m-RNA abundance increases and then decreases over a 24-hour period
- following the frq m-RNA, frq protein follows the same pattern but a bit later on
- When the protein starts to accumulate it begins to inhibit its own gene expression by negative feedback
    - this is what causes the system to oscillate over a 24 time period
- This clock can be entrained to changes in the external environment
- Means that there is a photoreceptor that can control the activity of the two transcription factors that activate frq

FRQ Function and Regulation

- Transcription factors are active and cause FRQ expression

- FRQ m-RNA accumulates and makes FRQ protein in the cytosol
- FRQ protein is then phosphorylated by a kinase
- the phosphorylated FRQ then inhibits the activity of its transcription factors in the nucleus
- What switches of the repression so that the cycle can start up again at the start of the next period is that the phosphorylated FRQ protein is degraded in the cytosol
- This is the basis of biological clocks in cells

Clock Controlled Genes

- Positive elements (such as transcription factors) switch on the clock genes
- Negative elements (such as the FRQ protein) accumulate and then inhibit their won transcription
- this oscillates every day
- Positive Elements also control Clock Control Genes - these are the genes that produce the circadian rhythm metabolic and behaviour responses
    - not part of the clock, but linked to it

Circadian in Cyanobacteria

- in every organism - even bacteria - there are circadian rhythms
- this suggests that there is an advantage to having a clock opposed to not having a clock
- Want to screen for mutants in cyanobacteria
- fuse a know circadian photosynthetic gene with a luminescence gene
    - take the promoter and fuse it with the gene lux
        - codes from luciferase - a luminescent protein
    - When psbA is active, the cells make light
    - therefore can easily look for mutant
    - two mutants isolated
        - SP22 - period shorted that 24 hours
        - P28 - period longer than 24 hours

Advantage To Telling Time

- there must be a selective advantage to now having a clock
- the problem is that this is a difficult experiment to do
- Is there a selective advantage to having a clock that is closer to the actual change in light and dark in the environment
- take the three cyanobacteria - wild type and two mutants - and grow them separately
    - grow some of them in constant light and some 12 hour light cycle
        - no advantage of the wild type over the mutants when grown separately
- therefore have to do competition experiments
    - let them compete for resources over 27 days, see which one survives the best
    - start with 50/50 composition of mutant/wild type
    - leave them in a environment with either a short light-dark period (22 hours) or a long light-dark period (30 hours)
    - in the short light-dark period is the mutant type, in the long period the wild type dominates
    - the strain the dominates is the one whose biological clock most closely matches the day-night cycle
        - happens in all experimental combinations of mutant/wild type or mutant/mutant
- do not know the underlying basis of the advantage, just that it does exist

Mammalian Pacemaker Organisation

- Lots of clocks in lots of different tissue, but the main clock is the SCN
    - every other clock listens to this clock
    - SCN is the Superchiasmatic Nucleus
- The SCN can continue under continuous conditions of light and dark, but can be reset by input

of light through the retina and optic nerve
- the hormone melatonin has a role to play
    - melatonin is produced during by the pineal gland
    - during the day the SCN shuts of the production by the pineal gland
    - during the night the inhibition is shit off and melatonin is produced
- melatonin is a major hormone that links all the other clocks in other tissues to the SCN
- the SCN is the only clock that can be influenced by the external environment

Peripheral Clocks And Melatonin

- SCN is the central pacemaker
- a change in the SCN is communicated through the endocrine system - usually by melatonin - to peripheral clocks
- Melatonin levels begin to pick up at around 8pm
- Melatonin keeps the other peripheral clocks up to date with the SCN

Messing Up The Clock

- Jetlag
    - desynchronisation of your SCN
    - the SCN is out of synch with the external light environment
    - leads to lack of appetite, fatigue, insomnia and mild depression
    - all suggest that biological clocks all play an important role
- Shift Workers
    - constant desynchronisation with the SCN and external environment
    - health consequences to this
    - constant symptoms of jetlag
- Two ways that both of these are helped
    - exposure to really bright light when you want to be up - forces SNC to thinking that its day time
    - melatonin consumption when you want to go to sleep
        - forces clock to think that its night time
    - Both help synchronise the SCN with the external

Readings

April-17-11
4:38 PM

15.1a Using Light to Tell Time - Circadian Rhythms

- due to the earths rotation around its axis once every 24 hours, life has evolved under a constant rhythmic cycle of light and dark
    - many physiological and behavioural phenomena posses 24-hour rhythmicity
- Many physiological and behavioural response geared to Earth's day-night cycle are called Circadian Rhythms due to the fact that they oscillate with a period of approximately 24 hours
    - a defining characteristic of circadian rhythms is that they are not direct responses to changes in the external light environment but instead are controlled by an internal organism-based clock
- This biological clock is set by the external light environment but can run a long time without any input from outside the organism
    - in humans for example, daily fluctuations in hormone levels are controlled by a circadian clock and will occur even if a subject is placed in conditions of constant light or darkness
- The fact that all forms of life possess circadian rhythms shows the importance of being able to predict daily fluctuations of light - being able to keep track of day and night allows organisms to anticipate when a process occurs most efficiently during the day and prepare accordingly
    - for example many proteins used in photosynthetic organisms for photosynthesis are synthesised before dawn, thus allowing photosynthesis to occur at maximum efficiency during the day
    - it is thought that circadian rhythms originated to protect replicating DNA from damaging U.V. radiation - the process of DNA replication is under circadian control and in many organisms occurs only at night
- In most animals the central biological clock that controls circadian rhythms is found within the superchiasmatic nucleus - a region of the brain within the hypothalamus
    - the SCN receive light inputs directly from the eye via the optic nerve - which it uses to set the biological clock
    - This clock in turn regulates a wide range of bodily functions - including secretion of melatonin
    - Melatonin is though to have a role in controlling out sleep-wake cycles as its synthesis is active at night time but inhibited during the day
- Several Conditions can interfere with normal circadian cycling - the best example being Jet Lag
    - symptoms related to jet lag include lack of appetite, fatigue, insomnia and mild depression
    - the physiological consequences of jet lack clearly indicate the number of processes that are linked to circadian time keeping
    - they also show that the circadian clock cannot be automatically reset to the new light conditions but may take a few days to become readjusted or entrained
- Many Plants and Animals show cycles of seasonal activities as well as daily cycles
    - in plants this includes the timing of flowering and dormancy
    - in animals this includes migration or hibernation
    - in some parts of the worls changes in day length herald changes in season - plants and animals in these regions mainly depend on the photoperiod to prepare for changes in their seasonal activies