BIOC2000 Summaries

R Lecture 1

What kinds of molecules are biomolecules?

- Biomolecules are made by biological systems and have a function in biological systems
- They are macromolecules such as proteins, DNA, carbohydrates and lipids and their constituents (amino acids, nucleotides, sugars)
- Carbon chains comprising predominantly C, H, O, N and P
    - Because of its bonding versatility
        - 4 electrons to form bonds
    - carbon can produce a broad array of carbon–carbon skeletons with a variety of functional groups; these groups give biomolecules their biological and chemical personalities.

- Minerals are not biomolecules but can be included in the production of a biomolecule (for example iron is a component of haem)

How are biomolecules/macromolecules organised and how does this determine their function?

- Cells build supramolecular structures
    - Macromolecules and their monomeric subunits differ greatly in size. Monomeric subunits are joined together by covalent bonds.
    - Supramolecular structures (the secondary and tertiary structures, shapes) are held together by noncovalent interactions →
        - Hydrogen bonds
            - Between polar groups due to electronegativity disparities between bonded atoms
        - Ionic interactions
            - Between charged groups (typically in salts)
        - Hydrophobic interactions
            - Among nonpolar groups in aqueous solutions (as well as hydrophilic interactions among polar groups in aqueous solution)
        - Van der Waals interactions
            - Random variations in the positions of the electrons around one nucleus may create a transient electric dipole, which induces a transient, opposite electric dipole in the nearby atom. The two dipoles weakly attract each other, bringing the two nuclei closer. These weak attractions are called van der Waals interactions.

- Their function is determined by the atoms, their configuration and thus the molecules resultant shapes and properties

What are the thermodynamics behind macromolecules?

- They have high free energy because they take energy to build
- They can be broken down to yield electrons
- Macromolecular backbones are made of covalent bonds whilst their secondary and tertiary shapes are determined by the intermolecular forces that result

R Lecture 2

Why is solubility an important distinguishing property of biomolecules and how is it determined?

- Solubility determines the function and shape of many biomolecules
    - Lipids are hydrophobic but can have soluble heads which allow them to form micelles or bilayers which result in their use as cell membrane components
    - The insoluble R groups of aminoacids in proteins determine their position and function and shape

- The role of weak interactions (IM forces) in aqueous systems determines solubility
    - The very different electronegativities of H and O make water a highly polar molecule, capable of forming hydrogen bonds with itself and with solutes. Hydrogen bonds are fleeting, primarily electrostatic, and weaker than covalent bonds. Water is a good solvent for polar (hydrophilic) solutes, with which it forms hydrogen bonds, and for charged solutes, with which it interacts electrostatically.

        - POLAR MOLECULES dissolve due to energetically favourable water-solute hydrogen bonds being formed breaking water-water bonds
            - Caused by electronegativity disparities between bonded atoms
                - Electronegativity increases as the atom is closer to filling its outershell and with an increase in size of the nucleus relative to the number of shells/electron repulsion hence why fluorine (top right) is the most electronegative
                - Fluorine will often ionise atoms which is why it is not common in biomolecules
        - CHARGED MOLECULES dissolve due to electrostatic interaction between the polar water molecules and the charge
        - NON-POLAR MOLECULES cannot easily dissolve as they are not able to readily form hydrogen bonds with the water which are more favourable than the water-water bonds
- Hydrogen bonds are broken and reformed constantly in water

Macromolecule	Soluble?
DNA	Yes → The phosphate groups on the backbone of the double helix structure

	are negatively charged and therefore able to form bonds with water
RNA	Yes → Phosphate group and also the single stranded RNA leaves single unpaired nucleotides with the ability to form hydrogen bonds (since in a double stranded DNA/RNA the nucleotides are held together by hydrogen bonds)
Polysaccharides	Yes and No → OH groups on the sugars make hydrogen bonds with water However larger structures which are structured in a way that they can hydrogen bond with each other and form a strong lattice will not hydrogen bond with water (e.g. cellulose)
Proteins	Sometimes → Depends on the R group
Lipid	No → To minimize the surface exposed to water, nonpolar compounds such as lipids form aggregates (micelles) in which the hydrophobic moieties are sequestered in the interior, associating through hydrophobic interactions, and only the more polar moieties interact with water.

R Lectures 3 and 4: Weak Forces

What holds a biomolecule together?

The shape of a biomolecule largely dictates its function and what it is able to interact with. Proteins follow a folding pathway which is due to the weak forces between groups in the protein.

It is important to note, however, that whilst covalent bonds don't drive the folding, the orientation and proximity of atoms that are allowed to interact is defined by the allowable bond angles. Covalent bonds can restrict the space options that the backbone can explore.

How can we quantify the strength with which two things bond?

Affinity – the strength with which two molecules interact/measure of the ΔG associated with the binding of two things. ΔG denotes the strength with which two things interact.

Energetically favourable interactions have a negative ΔG.

Covalent bonds have higher energies released when they are made than weak intermolecular forces. For both, bonds form due to the negative ΔG associated with the process – energy must be released.

Bond	Energy kJ/mol
H-H	436
C-H	414
C-C	343
C-O	351

Weak Force	Energy kJ/mol	Distance (nm)
van der Waals	0.4-4.0 kJ/mole	0.3–0.6
Hydrogen bonds	12-30 kJ/mole	0.3
Ionic inter'ns (attraction of opposite charge)	20 kJ/mole	0.25
Hydrophobic interactions	<40 kJ/mole	-

Factors affecting affinity include:

- Opposing charges
- Both non-polar
- Hydrogen bonding possibility (partial positivity)

What is the difference between degradation and denaturation in proteins?

Degrade: covalent bond breakage

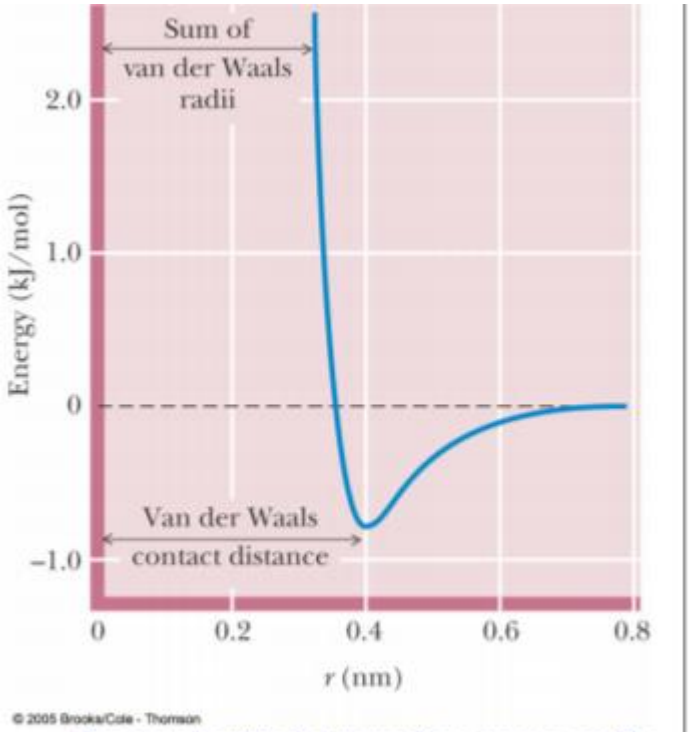
Denature: breaking weak forces between groups in protein resulting in loss of secondary and tertiary structures

Applying heat to a protein will often cause denaturation as weak forces between groups break and the protein loses its shape and thus functional capacity. Once a protein has been denatured it doesn't reform the same shape – it remains permanently denatured. This is mainly attributed to the fact that the groups are not in the same orientation to reform the weak forces.

When a protein is made it folds when half synthesised by the ribosomes – therefore groups are bent and isolated and allowed to make weak forces during synthesis.

Give detail of weak interactions:

- Although weak interactions are easier to break, they are additive.
- Van der Waals forces
    - Interactions occur between the positively charged nuclei and the electrons of nearby atoms through temporary induced dipoles. This is because electrons in atomic orbitals are moving and sometimes they are more heavily concentrated on one side of an atom as opposed to the other.
    - Positively charged nucleus will attract electron density of neighbouring atom on opposite molecule causing its electron density to shift to the other side of the nucleus. Electron repulsion of having all the electrons on only one side of the atom results in a shift of both of these electron clouds to the other side of the atom.
        - TRANSIENT OSSCILATING DIPOLE OR INDUCED DIPOLE
    - Strength depends on relative size of the atoms or molecules and the relative distance between them
    - The greater the area the stronger the interaction
    - Spontaneity of vdw forces dictated by distance between 2 nuclei



The van der Waals interaction energy profile as a function of the distance, *r*, between the centers of two atoms.

- These forces are cumulative. Many molecules mediate packing and interactions via extensive van der waals contacts.

- Electrostatic interactions (charge-charge)
  - Ionized groups are attracted to each other due to their opposite charges and can remain near each other in a stable configuration
    - Salt bridges – in proteins both partners are ionised
    - Also between Mg2+ and the phosphate groups of the nucleotide in the DNA polymerase mechanism (see lecture 8)
- Hydrogen bonding
  - a proton (H) covalently bonded to an electronegative atom → second electronegative atom that serves as the hydrogen bond acceptor.
  - Due to the partial negative charge of the electronegative atom and the partial positive charge of the H bonded to an electronegative atom
  - Relative strength of the hydrogen bond is proportional to the H bond donor and the H bond acceptor (more polar atoms form stronger bonds)
  - Biological molecules contain many groups that can hydrogen bond to each other and to water
  - H bonding drives base pairing DNA, secondary structure in protein formation

What is the importance of hydrogen bonding in water? How does this allow it to act as a solvent?

- The structure of water allows it to have a dipole due to the electronegative nature of oxygen. It takes electrons from the covalently bonded Hydrogens, leaving partial charges on the two ends of the molecule.

- This dipole allows it to hydrogen bond heavily to itself. These bonds are made and broken repeatedly – this is highly thermodynamically favourable because it is chaotic and the bonds are enthalpically favourable.
- In ice, water makes the maximum number of hydrogen bonds (4) which forces it to become static as opposed to water which is considered flickering clusters of bonds.
- Solubility:
    - The solubility of different molecules in biological systems depends on their interactions with water
    - If there are charged regions, hydrogen bonds can be formed and therefore water-water bonds are exchanged with water-solute bonds.

What is the hydrophobic effect?

1. Hydrophobic molecules in water interact thermodynamically unfavourable with water. This may happen due to
    a. Short range ordering of water around the hydrophobic molecule
    b. Reduction of the number of favourable hydrogen bonds
2. Ordered water (or water with reduced hydrogen bonds) is highly energetically unfavourable due to a decrease in ΔS or increase in ΔH
3. It is more energetically favourable for a biological system if the hydrophobic groups cluster together, leaving the water to be disordered and maximise the hydrogen bonding.

R Lecture 5: Free Energy and Basic Thermodynamics

Why is the order in cells and organisms counterintuitive?

A negative Gibbs free energy requires a high entropy which cells do not exhibit. The synthesis of larger biomolecules plus many other processes are highly energetically unfavourable.

How does the cell overcome this?

The cell employs coupling – some sort of energy releasing event such as they hydrolysis of ATP (negative ΔG) with an energetically unfavourable event (positive ΔG).

To take a system such as ATP into a more stable state entails a negative Gibbs free energy change.

What are the laws of thermodynamics?

1st – for any physical or chemical change, the total amount of energy in the universe remains constant; energy may change form or it may be transported from one region to another but I cannot be created or destroyed.

2nd – in all natural processes, the entropy of the universe increases

What is Gibbs Free energy?

- The maximum amount of energy available from a reaction or system to do work under conditions of constant temperature and pressure.
- -ΔG doesn't tell you how fast a reaction will occur/its likelihood
    - It tells you this reaction can occur
- ΔG does not include activation energy – the activation energy determines the likelihood of the event (the larger the activation energy the less likely to occur)
- -ΔG = spontaneous
- +ΔG = nonspontaneous

Formula:

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

What is enthalpy or ΔH?

- Heat content of reacting system
- Relies on bond enthalpies and is calculated as the difference between the heat of formation of the products and the heat of formation of the reactants
- A negative change in enthalpy indicates an exothermic reaction indicating a set of bonds that have a lower bond energy than the set we started with → RELEASES HEAT
- A positive change in enthalpy indicates an endothermic reaction indicating a set of bonds that have a higher bond energy than the set we started with → THESE REQUIRE ENERGY/HEAT TO OCCUR

What is entropy or ΔS?

- The degree of component randomness or disorder in a system
    - The amount of flexion and rotation around bonds contained in the molecules that are in the system
    - The number of atoms or molecules in a system
    - The distance these things are from one another

SUMMARY

	-ΔS	+ΔS
+ΔH	Non-spontaneous	Spontaneous at high temperatures
-ΔH	Spontaneous at low temperatures	Spontaneous at any temperature

Examples of Gibbs Free Energy calculations

Dissolving of Glucose

- Spontaneous reaction

- Glucose crystals broken apart and form hydrogen bonds with water solvent
    - Goes from hydrogen bonding with itself to hydrogen bonding with water → $\Delta H \sim 0$
    - Goes from one ordered molecule to many disordered molecules within water, also means water is less ordered around a single entity → Large $+\Delta S$
    - Therefore $-\Delta G$ and a spontaneous reaction

R Lecture 6: High Energy Molecules

How does the cell overcome the counterintuitive nature of its ordered system in driving reactions?

Altering temperature in biological systems

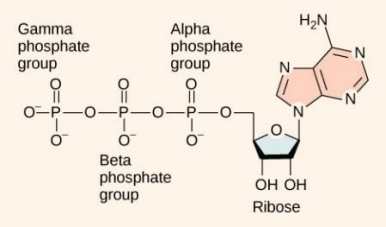
- Dramatic temperature alteration cannot occur in biological systems as it is limited by the temperatures under which a system can exist
- Instead we have to rely on entropy and enthalpy

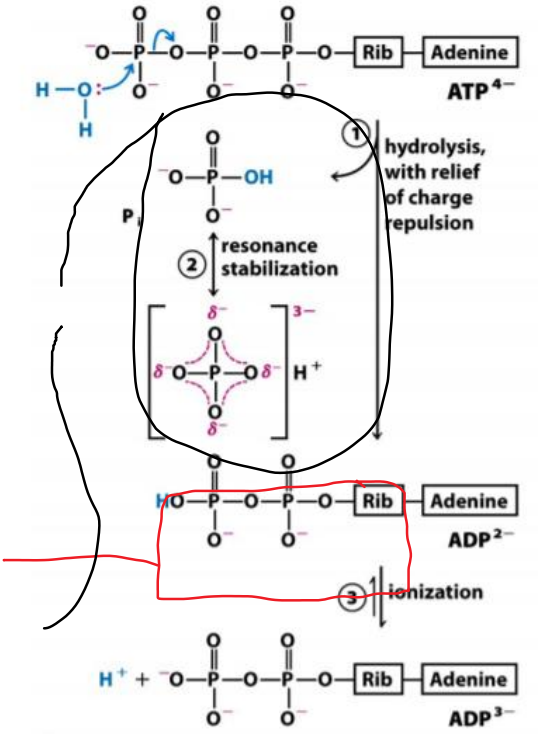
Reaction Coupling

- Coupling of reactions can drive apparently impossible processes. Standard free energy changes are additive -> you can add a positive delta G to a negative delta G of higher magnitude to produce a spontaneous reaction.

ATP hydrolysis

- ATP is the master molecule of energy release and allows the cell to control energy release and reaction coupling
- ATP is a ribose nucleic acid with adenine as the base and a triphosphate group attached to the 6^{th} carbon
- ATP is highly unstable due to the steric interactions of the phosphate groups





- Nucelophillic attack of the gamma phosphate group by the oxygen in water results in the gamma phosphorus giving up its electron (in its bond with the oxygen molecule shared with the beta phosphorus) and produces inorganic phosphate and ADP. This results in

  INCREASE IN ENTROPY
  - More rotation of the remaining phosphate groups in ADP
  - Higher movement of electrons between Os in the inorganic phosphate

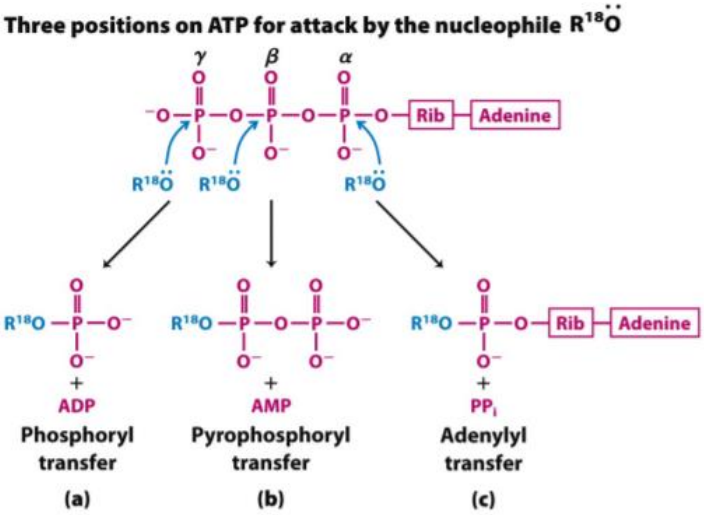
  Energy release due to the These both result in a large, positive entropy change thus causing a spontaneous reaction.

Can you harvest energy again from ADP?

- Yes, ADP → AMP

Can the original hydrolysis reaction occur at the beta phosphate?

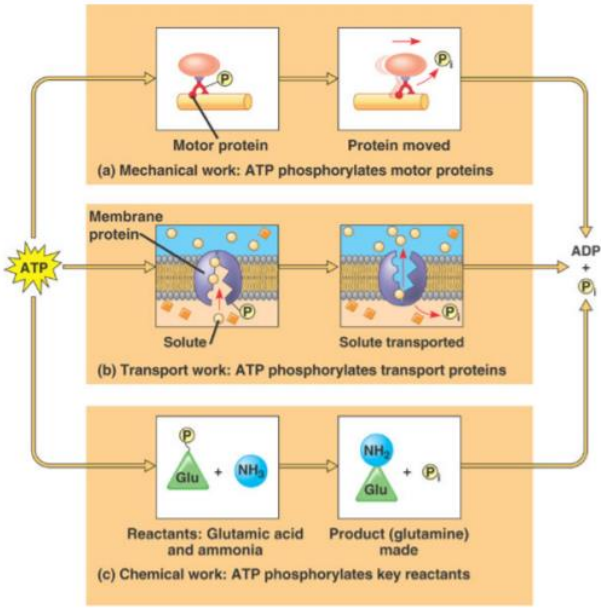
- Yes, all three positions are open for nucelophillic attack



Examples of ATP function in cells

1. ATP breakage can provide the driving force for conformation change such as in Rho protein which sits on RNA and helps to remove it from RNA polymerase. ATP hydrolysis powers movement.

2. DNA helicase seem uses a similar mechanism to unwind DNA at the replication fork.
3. Production of glutamine (addition of glutamic acid and ammonia)
4. Active transport across a membrane against a concentration gradient powered by ATP pumps

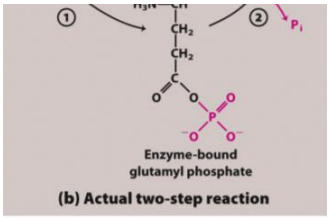


Phosphoryl group transfer mechanism

- The transfer of ATPs gamma phosphate group is a common mechanism of ATP energy donation

  EXAMPLE 1

  In the production of gutamine from glutamic acid and ammonia, ATP initiates a two step process
  - Step 1: ATP dephosphorylation and phosphorylation of glutamate (group transfer)
  - Step 2: substitution reaction of phosphate by NH3 as the phosphate group is a good leaving group
  - This produces a spontaneous reaction due to the high entropy of Pi on its own



EXAMPLE 2

Phosphoryl group transfer also happens to proteins to cause massive conformational changes such as in proton pumps.

EXAMPLE 3

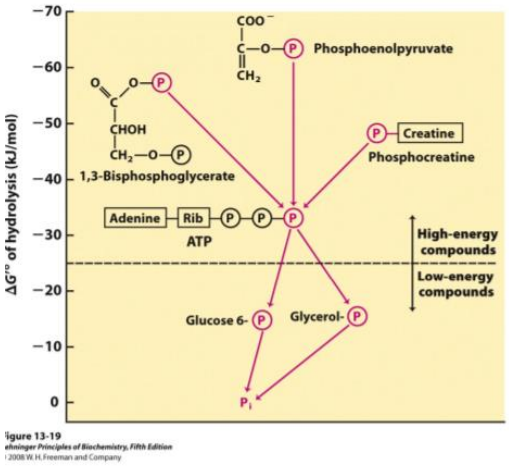
Phosphate groups on ATP are not the only possible transfer group.

For example AMP can be transferred to a molecule leaving PPi.

Or PPi can be transferred leaving AMP.

EXAMPLE 4

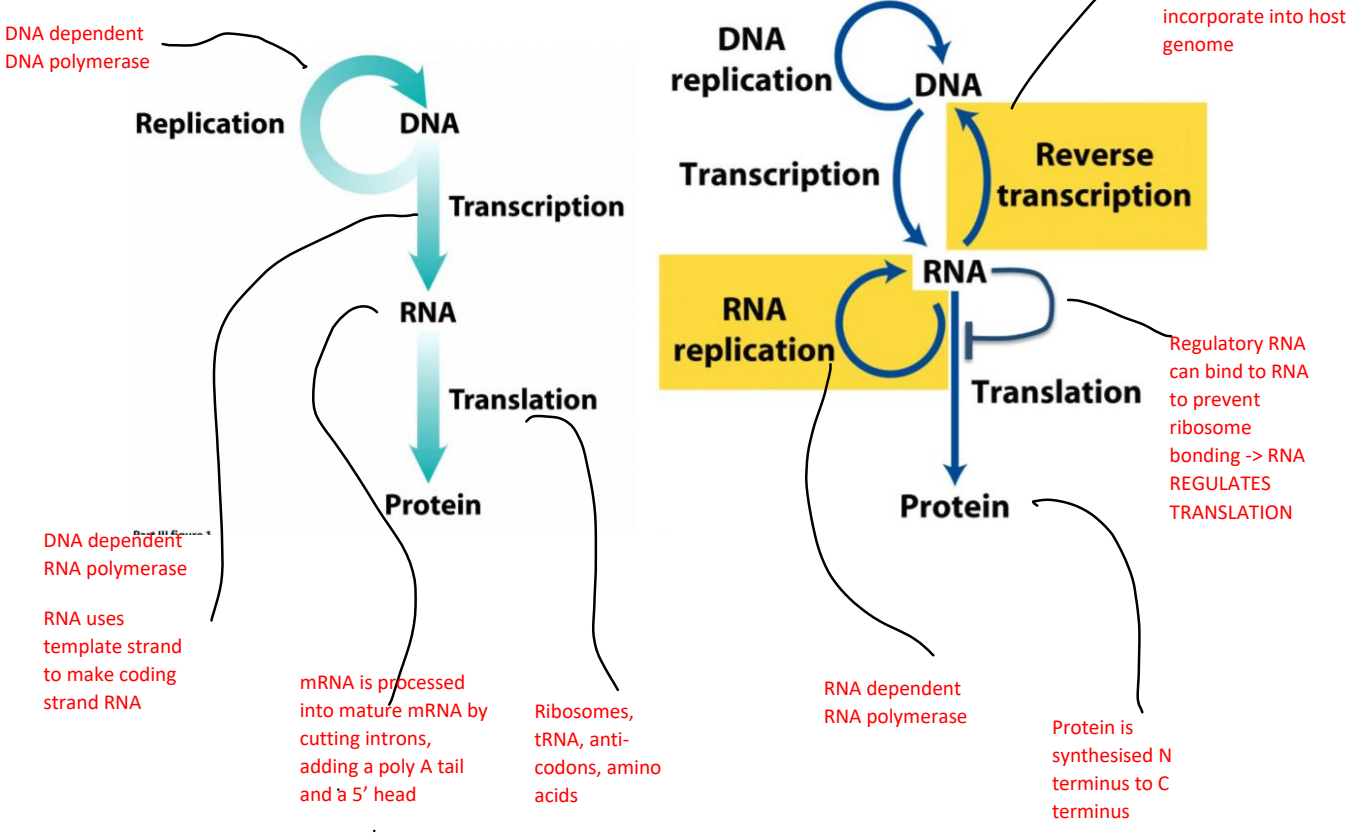
ATP can be **generated** itself from the transfer of phosphate from other high energy molecules.

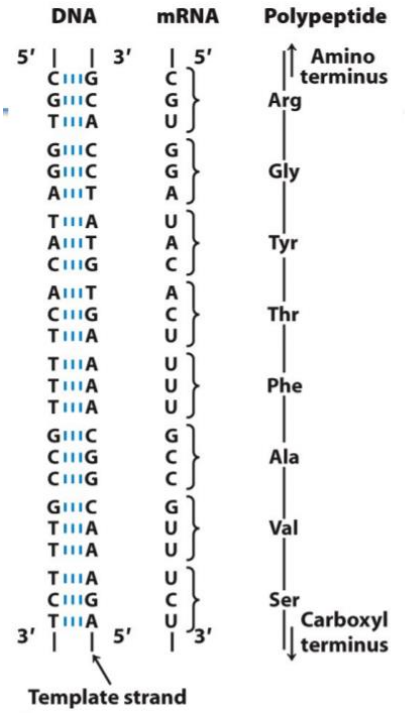


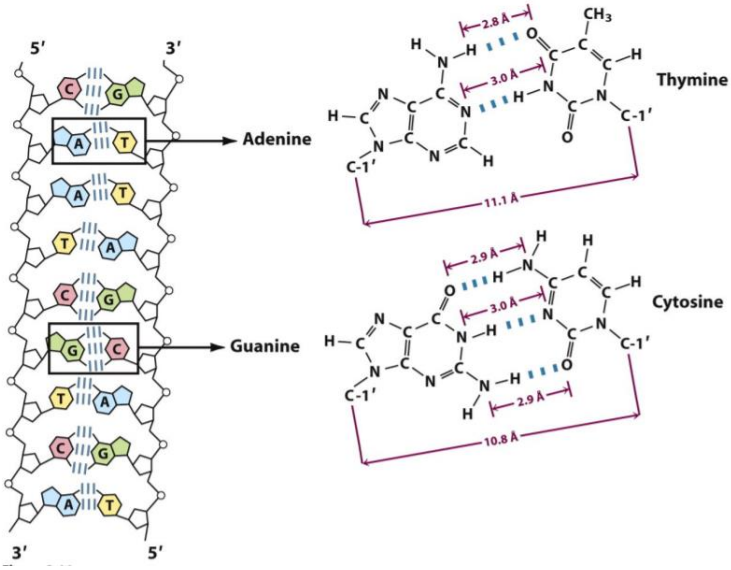
How do non-polar molecules force energetically unfavourable changes in the structure of water?

- It is more favourable for hydrophobic groups to cluster
- When lipids cluster with their hydrophobic tails facing inwards this causes
  - Water to become less ordered (increase in entropy)
  - Maximum number of hydrogen bonds to be made, bonds between waters and hydrophilic heads (decrease in enthalpy)
- This results in a negative Gibbs Free energy change thus explaining why non-polar separation in water is spontaneous.

Lecture 7: The Central Dogma







A – T → Two Hydrogen Bonds

G – C → Three Hydrogen Bonds

H (bound to an electronegative element) is a hydrogen bond donor, N or O are hydrogen bond acceptors (due to their electronegativity)

A and T and G and C do not necessarily have to be exclusive.

Lecture 8: DNA Replication

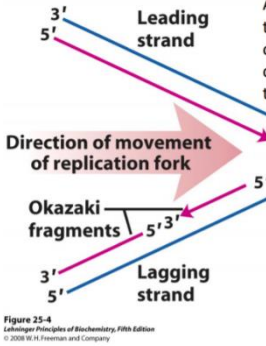


Figure 25-4
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A new DNA strand (red) is always synthesized in the 5′→3′ direction. The template is read in the opposite direction, 3′→5′. The leading strand is continuously synthesized in the direction taken by the replication fork.

The other strand, the lagging strand, is synthesized discontinuously in short pieces (Okazaki fragments) in a direction opposite to that in which the replication fork moves. The Okazaki fragments are spliced together by DNA ligase. Bacterial Okazaki fragments are ~1-2 x 10^3 nucleotides long. In eukaryotic cells, they are 1/10 of that length.

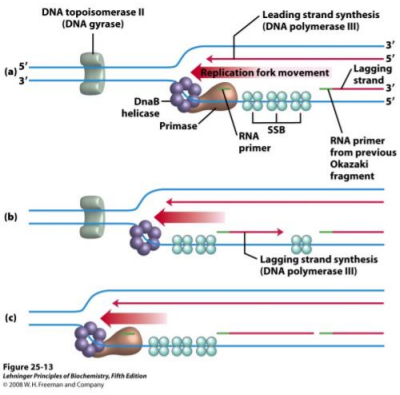

Figure 25-13
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Synthesis of Okazaki fragments.
(a) At intervals, primase synthesizes an RNA primer for a new Okazaki fragment. Note that if we consider the two template strands as lying side by side, lagging strand synthesis formally proceeds in the opposite direction from fork movement.

(b) Each primer is extended by DNA polymerase III.

(c) DNA synthesis continues until the fragment extends as far as the primer of the previously added Okazaki fragment. A new primer is synthesized near the replication fork to begin the process again.

DNA polymerase III

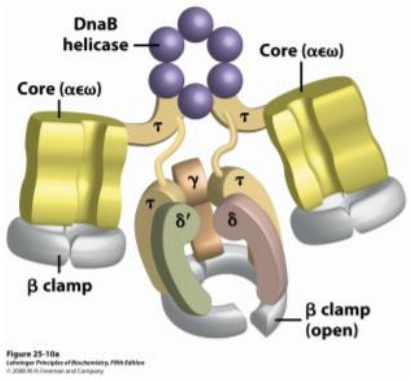
It is important to note that there is a clamp that attaches both polymerases to the DNA helicase which unravels DNA at the replication fork.

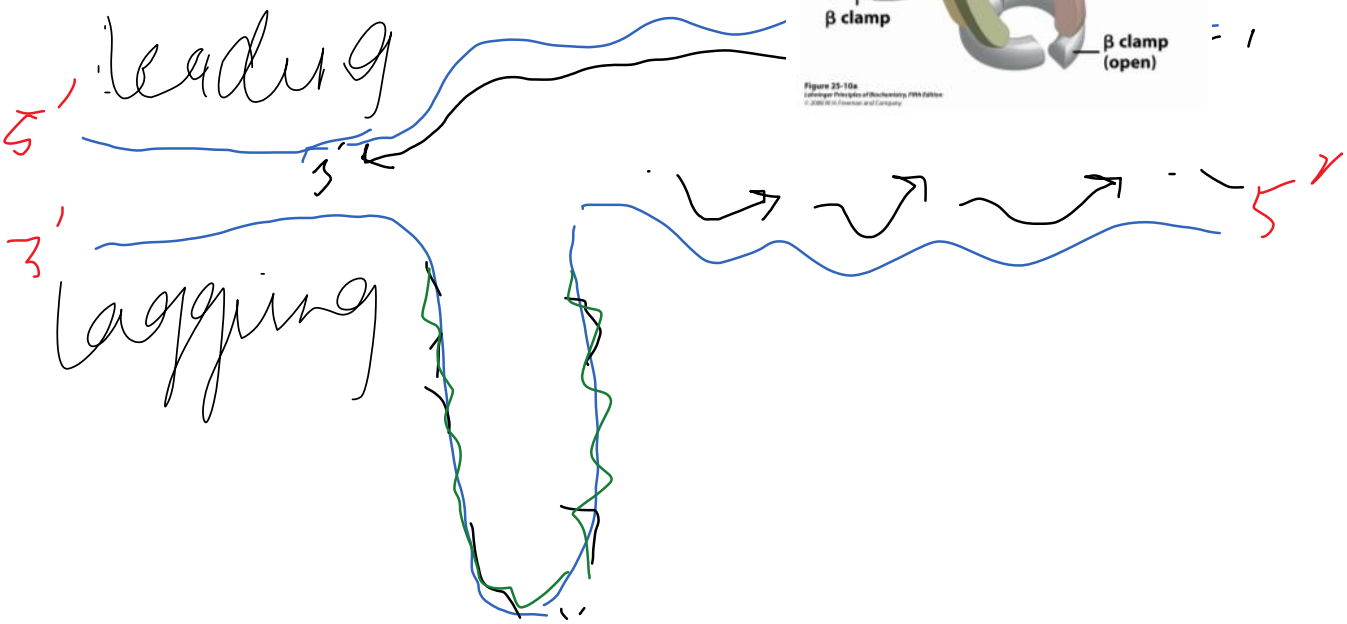
What is the problem of enzyme kinetics in DNA replication?

Vmax = the maximum rate at which an enzyme can operate

Vmax is achieved by having the optimal temperature, surplus of substrate (saturating concentration), optimal pH, product may need to be removed (for enzyme with reversible processes), no inhibitors of enzyme in the system, optimal fold of enzyme and correct charge on the active site

ISSUE – The consideration of On and off rate of DNA polymerase on lagging strand means that lagging strand would lag too far behind the leading strand replication and the cell would run out of single stranded binding protein to keep the lagging ssDNA stable.





The cell needs to make the leading strand and lagging strand go at the same rate.

SOLUTION – The lagging strand polymerase is tethered to increase the on-rate and allow it to keep up with the leading-strand polymerase.

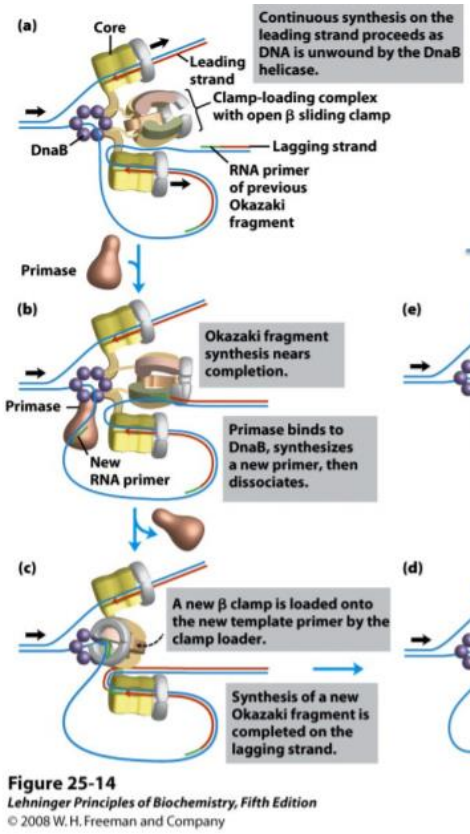


Figure 25-14
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The lagging-strand polymerase is tethered to increase the on-rate and allow it to keep up with the leading-strand polymerase

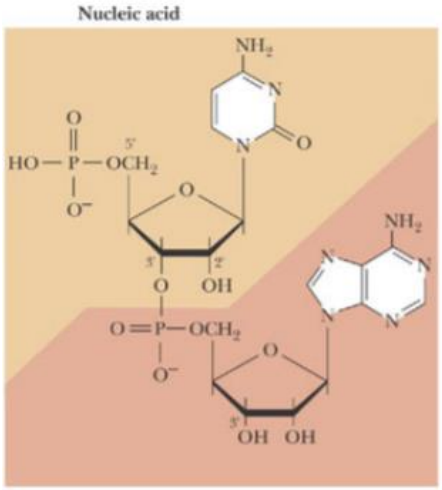
Lehninger Figs 25-10 & 25-15 are also helpful for understanding this.

The level of detail shown on this slide & the previous slide is not examinable.

Both polymerases face the same direction but for the lagging strand DNA is looped and turned through it so synthesis is in the right 5' – 3' direction.

How do enzymes add bases?

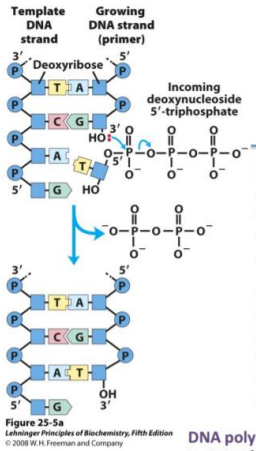
Nucleic acids are polymers of nucleotides linked by bonds between the 3'-OH (hydroxyl) of the ribose ring of one nucleotide to the 5' – PO4 (phosphoryl) of its neighbouring nucleotide. Creates a phosphodiester bond that links successive nucleotide units. The resultant backbone of alternating pentose and phosphate groups is highly polar → 5' end and 3' end.



Note this is not DNA but RNA because the 2' carbon is attached to an OH group rather than an H. The linkage is the same however.

Why is the 3' end necessary?

The addition of nucleotides happens at the 3' OH group on the 3' carbon of the deoxyribose sugar. The DNA polymerases catalyse the nucleophilic attack from oxygen on the 3' end of the deoxy/ribose on the first phosphorus on the triphosphate of the incoming base. This results in the release of PPi (pyrophosphate) which is a good leaving group and has a – delta G associated with it leaving.



Why is the 3' end necessary?

Where does DNA polymerase add the next base?

Nucleophile: A species possessing one or more electron-rich sites, such as an unshared pair of electrons, the negative end of a polar bond, or pi electrons. Also known as electron donor. It "seeks" positive centres of other atoms or molecules.

Nucleophilic substitution or "attack": is a fundamental class of substitution reaction in which an "electron rich" nucleophile selectively bonds with or attacks the positive or partially positive charge of an atom attached to a group or atom called the leaving group; the positive or partially positive atom is referred to as an electrophile.

DNA polymerisation occurs via nucleophilic substitution reactions. Pg 979 of Lehninger has more details.

How does DNA polymerase catalyse addition?

- Two Mg2+ ions coordinated to the phosphate groups of the incoming nucleotide triphosphate
    - Coordination due to the negative charge on he phosphates
- Three Asp residues (at least 2 highly conserved in all DNA polymerases)
- Mg2+ ion nearest to the alpha phosphate facilitates attack of 3' hydroxyl group (of the present DNA chain) on the alpha phosphate of the nucleotide triphosphate. The other Mg2+ facilitates the displacement of the pyrophosphate.
- Both ions stabilise the pentacovalent transition state by holding them in place
- IMPORTANT TO NOTE:
    - Mg2+ is also coordinated by the polymerase itself, as the Mg2+ sits in a negative cloud created by the Asp residues of the polymerase.