SECTION 2: CARBOHYDRATES

INTRODUCTION

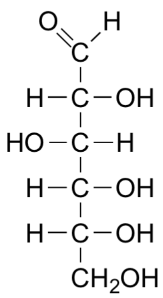
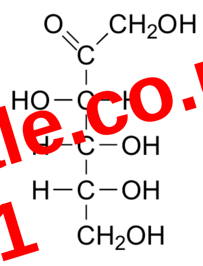
Carbohydrates essentially contain Carbon, Hydrogen and Oxygen

- Condensation – loss of water molecule
- Hydrolysis – addition of water molecule

Uses of monosaccharide:

1. Energy sources to produce ATP
2. Building blocks to synthesize polysaccharides
3. Raw material for synthesis of other organic molecules

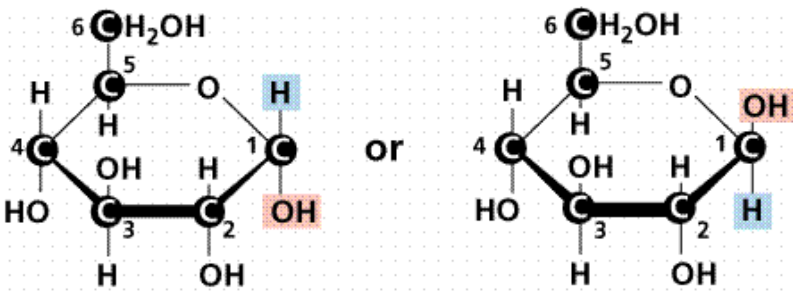
Aldo and Keto sugars:

Aldo	**Keto**
Contains carbonyl group which is an aldehyde	Contains carbonyl group which is a ketone
 glucose	 fructose
Found at the terminal end	Found anywhere else

Structures of monomers:

May exist as linear or ring structure

- Ring structure is predominant as it is energetically more stable
- In ring structure, anomeric carbon (carbon bonded to TWO oxygen atoms) is formed
  - Hydroxyl group attached to anomeric carbon is anomeric hydroxyl group
- Alpha and Beta configurations
  - If C_1 and C_6 are on the **same side** – Beta, **different side** – Alpha



(c)α-Glucose (d)β-Glucose

LEVELS OF PROTEIN STRUCTURE

Levels of Protein:
Generally four main levels, Primary, Secondary, Tertiary and Quaternary

Level	Structure
Primary	Refers to specific number and linear sequence of amino acid Peptide bonds at the polypeptide backbone
Secondary	Regular coiling and folding of a polypeptide chain Held together by regularly spaced hydrogen bonds at the polypeptide backbone
Tertiary	Further bending, twisting and folding of the polypeptide of the secondary structure to give a precise 3-dimensional conformation 4 types of bonds that occur are H-bonds, ionic bonds, HI and disulfide bonds
Quaternary	Overall protein structure that results from the association of two or more polypeptide chains to form a functional protein

Secondary structure:
Contains mainly the **α**-helix and the **β**-pleated sheet

α-helix

- Takes the form of an extended spiral spring
- Stabilized by H-bonds between n and n+4th residue of same polypeptide that are parallel to main axis
- All C=O and –NH groups of polypeptide backbone participate in H-bonding
- R groups project perpendicularly outwards, avoiding steric interference

β-pleated sheet

- Takes the form of an extended zigzag, sheet-like conformation
- Also stabilized by H-bonds between adjacent regions of the same polypeptide
- Does not contain bulky R groups as they cause steric interference that project above and below the plane in an alternate manner

Types of bonds:

Type of bond	R groups involved
H-bonds	Polar, uncharged aa
HI	Non-polar aa
Ionic bonds	Polar, charged aa
Disulfide bonds (covalent)	Oxidation of sulfhydryl groups (–SH) – cysteine

QUATERNARY STRUCTURE PROTEINS

Haemoglobin:
Mainly to transport oxygen and is found within the red blood cell

Structure	Function
Contains haem prosthetic group (Fe^{2+}) Contains 4 haem prosthetic group	Binds to O_2 reversibly → take up / release O_2 Increases efficiency in transport
Folding of haemoglobin • Exterior: hydrophilic aa • Interior: hydrophobic aa	Makes it soluble in blood → O_2 transport in blood Formation of the hydrophobic cleft • Allows the HPG to bind
Contains 2**α** and 2**β** chains – 4 sub units	$Fe^{2+} + O_2$ → changes the shape of haemoglobin Binds to O_2 (cooperativity) • Binds in increasing affinity
Globular (spherical) • Making it compact	Able to have many haemoglobin Packing many into single RBC, increasing efficiency of transport

Collagen:
Collagen is a protein made up of amino acids and linked together by peptide bonds

Structure	Function
Primary structure - repeating tripeptide sequence • Glycine – X – Y • Proline, hydroxyproline / hydroxylysine	High Tensile Strength
Secondary structure - α-chain: long, straight chain 3 chains wound together to form triple helix / tropocollagen • Held together by extensive H-bonds between the chains	
Tropocollagen molecules lie side by side to form fibrils • Linked by covalent cross links • Between carboxyl end of one molecule and amino end of another tropocollagen molecule	Higher Tensile Strength
Adjacent tropocollagen molecules stacked in staggered array to from collagen fibers	

SECTION II: TRANSPORT ACROSS MEMBRANE

Active and Passive transport:

Passive	Active
Down concentration gradient	Against concentration gradient
No use of ATP	Use of ATP
Simple diffusion, Facilitated diffusion, Osmosis	Active transport, Endocytosis, Exocytosis

Diffusion: movement of molecules from a region of higher concentration to a region of lower concentration, down a concentration gradient

<u>Simple Diffusion</u>

- For molecules that are small and hydrophobic
- Diffusion gradient provides driving force for movement of molecules
- Ions are charge and hydrophilic and cannot diffuse through the hydrophobic core of the phospholipid bilayer

<u>Facilitated Diffusion</u>

- For larger, hydrophilic substances
- Transport protein is used to enhance / increase the rate of transport

Carrier	Channel
Posses binding site for solute	Posses central water-filled hydrophilic pore due to presence of charged/polar aa residues Surface next to fatty acid tails are hydrophobic
Undergoes conformational changes	Does not undergo conformational changes

<u>Factors affecting rate of diffusion</u>

1. Concentration gradient
2. Distance of diffusion
3. Area of diffusion
4. Structure through which diffusion occurs: Presence of transient gaps
5. Size and type of diffusing molecule: Smaller molecule faster
6. Temperature

Osmosis: net movement of freely moving water molecules from a region of less negative water potential to a region of more negative water potential through a selectively permeable membrane

<u>Water potential</u>

Measures tendency for water to move from one region to another

Water potential [-ve] **= solute potential** [-ve] **+ pressure potential** [+ve]

Solute potential – ability of a solute to make the water potential more negative
Pressure potential – pressure exerted by cell wall on its contents

Results for second generation

- Second generation of bacterial growth in ^{14}N-medium produced one band of low density and one band of intermediate density
- As replication of each hybrid $^{15}N^{14}N$ DNA molecule produces one daughter DNA molecule containing $^{14}N^{14}N$ and another DNA molecule $^{15}N^{14}N$ hybrid
- The dispersive model can be eliminated as only a single DNA band would have been expected

Mechanism of DNA Replication:

Stage	Explanation
Location of Replication origins	• DNA replication is semi-conservative • Begins at the origin of replication
Separation of Parental DNA strands	• 2 parental strands unwind and separate by breaking the H-bonds, facilitated by helicase • Topoisomerase helps to relieve tension ahead of the replication fork by creating transient nicks in DNA • Single-stranded DNA becomes templates for synthesis of new strands • Kept single-stranded by single-stranded DNA-binding proteins
Synthesis of RNA primer	• Primase catalyzes synthesis of RNA primer • In order to provide free 3'OH end for DNA polymerase to initiate synthesis of DNA strand
Synthesis of Daughter DNA strands	• Free deoxyribonucleoside triphosphate (dNTPs) are incorporated via complementary base pairing with parental strands, A to T, G to C • DNA polymerase catalyzes phosphodiester bond formation between free 3'OH end of terminal nucleotide of growing daughter DNA strand and 5' phosphates of incoming dNTPs • The above happens through formation of CBP with the DNA template – bond breakage in pyrophosphate lost from dNTP releases free energy for phosphoester bond formation • DNA synthesis occurs in the 5' → 3' direction • In replication fork, leading strand is synthesized continuously • Lagging strand is discontinuously synthesized as Okazaki fragments • Okazaki fragments are ligated together by DNA ligase

Chapter 7: Eukaryotic Gene Expression

Definition: Gene expression is the process where information within a gene is used, to first synthesize RNA and then to a protein eventually to affect the phenotype of an organism

Introduction:
Transcription (DNA→RNA) – DNA used as template for synthesis of mRNA in nucleus
Translation (RNA→Polypeptide) – mRNA used as template for synthesis of polypeptides in cytoplasm

<u>GENE EXPRESSION THROUGH RNA</u>

Comparison between RNA and DNA:

Characteristics	RNA	DNA
Similarities		
1. Both are made up of polynucleotides – consist of phosphate group, pentose sugar, base 2. Both have sugar-phosphate backbone joined by phosphodiester bond 3. Both are polymerized by condensation synthesized 4. Both are synthesized by complementary base-pairing of nucleotides using a template 5. Both make use of 3 common nitrogenous bases: A, C, G		
Differences		
Molecular mass & size	Smaller molecular mass	Larger molecular mass
No. of units	One polynucleotide chain	Two polynucleotide chain
3D structure	Almost always single stranded • Folded into complex tertiary structure	Always double stranded • Coiled around histone proteins, organized into chromosomes
Ration of bases	A:U =/= G:C =/= 1:1	A:T = G:C = 1:1
Monomers	Ribonucleotides	Deoxyribonucleotides
Pentose sugar	OH group at C2	H group at C2
Chemical stability	Less stable – due to additional reactive 2'OH group	More stable – lacking 2'OH group
Nitrogenous base	A, <u>U</u>, G, C	A, **<u>T</u>**, G, C
Basic forms	mRNA, tRNA, rRNA, snRNA, siRNA	Only one basic form
Location	Throughout the cell	Exclusively in nucleus (as well as mitochondria and chloroplast)
Amount per cell	Varies	Constant

Roles and types of RNA:
1. Messenger RNA (mRNA) – carries information about DNA, codes for aa sequence of proteins
2. Transfer RNA (tRNA) – adaptor molecule in protein synthesis, translates mRNA sequence into aa sequence
3. Ribosomal RNA (rRNA) – plays catalytic and structural roles in ribosomes (peptidyl transferase activity)

TRANSCRIPTION (DNA → RNA):

Definition: Process in which complementary RNA copy is made under the direction of the template strand of a specific region of the DNA molecule, catalyzed by the enzyme RNA polymerase. First part of gene expression

Components required:
1. Gene
2. RNA polymerase
3. General / basal transcription factors
4. Ribonucleotides

Gene
Section of the DNA that contains information in the form of a specific sequence of nucleotides to direct the synthesis of one polypeptide chain or RNA

Promoter
- Contains RNA polymerase binding site and transcription start site
- Also contains TATA box, binding site for general transcription factor called TFIID
- Determines which of two strands used as template

Transcription Unit / coding region
- Segment of DNA transcribed into single-stranded RNA molecule
- Only 1 of 2 strands used for template of transcription
- Read in 3' to 5' direction, synthesis of RNA in 5' to 3' direction
- RNA synthesis occurs in transcription bubble where DNA is transiently separated into 2 strands

Termination Sequence
- Found at end of gene, codes for polyadenylation sequence 5'(AAUAAA)3' in pre-mRNA
- Results in transcription termination

Template strand	Non-template strand
Strand that is transcribed Sequence is complementary to RNA Template to direct synthesis of RNA molecule	Strand not transcribed Sequence is exactly the same as RNA, except that T is replaced by U

RNA Polymerase
- Allows DNA double helix to transiently unwind
- Catalyzes formation of phosphodiester bond between free 5' phosphate group of incoming ribonucleotide and free 3' OH group of growing RNA chain
- Reanneals unwound DNA behind it
- Carries out proofreading functions and removal of incorrectly inserted ribonucleotide

General transcription factors
- Positions RNA polymerase correctly at promoter
- Separate 2 strands of DNA for transcription to begin
- Release RNA polymerase from promoter to begin elongating the RNA

Cystic Fibrosis (CF): Involves mutation in the CFTR (cystic fibrosis transmembrane regulator) gene found on chromosome 7, codes for chloride channel protein found in membranes of cells lining lungs, liver and pancreas

<u>Genetic and molecular basis</u>

- Deletion of triplet base TTT in the template strand of CFTR results in the absence of the 508th aa residue, phenylalanine (Phe)
- Primary sequence of protein is changed, leading to change in 3D conformation and function of chloride channel protein
- Results in synthesis of non-functional CFTR channel, does not allow Cl- to diffuse out of epithelial cells
- Thus water does not leave the epithelial cells due to absence of steep water potential, resulting in production of thick mucus:
    - Narrowing of air passages, reducing air flow and making breathing difficult
    - Blockage of ducts (e.g. pancreatic ducts → affecting digestion)
    - Increased diffusion distance for gas exchange in lungs, insufficient oxygen supplied to CF patient
    - Bacterial infection – thick mucus serves as breeding ground for bacteria
    - Inability of cilia to sweep away bacteria, fungi spores and other particles

<u>CAUSES OF GENE MUTATIONS</u>

Spontaneous mutations: Mutations that occur naturally (without use of chemical/physical mutagenic agents)

DNA Replication and Repair

- DNA polymerase sometimes insert the wrong, too many or too few nucleotide
- Some of the mistakes are corrected immediately through proofreading
    - DNA polymerase recognize mistakes and replace incorrectly inserted nucleotide
- Some are corrected after replication through mismatch repair
    - Enzymes recognize and fix deformities in the secondary structure of the DNA molecule
- Those that fail to be recognized are then passed down from one cellular generation to the other
- Those that occur in germline cells cause it to be transmitted to the next generation
- Purine-purine / pyramidine-pyramidine → transitions
- Else known as transversion

DNA Slippage

- Daughter/parental DNA strand slips during DNA replication, followed by folding back of strand
- Mispairing between daughter DNA strand and parental template strand resulting in gene duplication or deletion

<u>Slippage of daughter strand</u>

- Results in insertion of repeats in daughter strand

<u>Slippage of parental strand</u>

- Results in deletion of repeats in daughter strand

Addition/Loss of One or More Haploid Sets of Chromosomes (Polyploidy):
Refers to the instance where more than two copies of haploid chromosome set are found

- More common in plants
    - Autopolyploidy: addition of sets of chromosomes of same species
    - Allopolyploidy: addition of sets of chromosomes of different species due to interspecific mating

Autopolyploidy	**Allopolyploidy**
Occurs in a few ways: • Failure of all chromosomes to segregate (complete non-disjunction) • Two sperms fertilize an ovum, resulting in triploid zygote • Produced under experimental conditions by crossing diploids with tetraploid	• Haploid ovum fertilized by haploid sperm, resulting diploid hybrid is usually sterile • If new genetic recombinant undergoes natural or induced chromosomal doubling (through non-disjunction), a fertile hybrid can be produced

CHANGES IN CHROMOSOME STURCTURE

Includes deletion and duplications of genes or part of chromosome

- Exchanges and transfers are called translocations

Deletion

- Deletion can occur near one end or from interior of chromosome and causes genotype to be altered
- If deletion affects same gene loci on both homologous chromosomes, effect is lethal
- If only one is affected, alleles on non-deficient homologue will be expressed, even if recessive

Duplication

- Arise as a result of unequal crossing over between synapsed chromosomes during meiosis
- Or through replication error prior to meiosis

Inversion (normally better tolerated)

- Segment of chromosome is turned around 180^o
- Does not involve loss of genetic information, but simply rearranges linear sequence

Translocation (normally better tolerated)

- Movement of a chromosomal segment to a new location in the genome
- Rearrangement of genetic material

~ END ~

Chapter 11: Genetics of Viruses

INTRODUCTION

Viruses are often known as obligate intracellular parasites as:

- **Obligate** – by necessity
- **Intracellular** – within the cell
- **Parasite** – an organism living and feeding on host

Explanation:

(1) Viruses can only survive and reproduce in the host cell using host cell machinery at the expense of the host's life-sustaining functions

(2) Virions are merely packaged sets of genes in transit from one host cell to another, and they lack RNA polymerase to perform transcription, ribosomes to perform translation, thus requiring use of host cells ribosomes to translate viral mRNA into viral proteins

(3) Viruses cannot respeire to generate or store energy in the form of ATP but need to derive it for all metabolic functions from host cell

(4) Viruses cannot obtain nutrition and thus need to parasitize host cell for basic building materials such as amino acids, nucleotides and lipids (fats)

Are viruses living or non-living organisms?

Arguments for LIVING	Arguments for NON-LIVING
Viruses can reproduce within host cell to produce new virions • Replication involves synthesis of large number of viral components in host cell, followed by assembly into virions Viruses contain genetic materials and are able to direct metabolic processes • They posses their own genetic material and carry genes necessary to instruct host cell to synthesize new viruses Viral genomes (RNA/DNA) can evolve • Different viruses vary greatly in structural and genetic complexity and no single gene is shared by all viruses	Viruses are not cells • They do not have protoplasm or organelles, basically containing protein capsid surround a nucleic acid core composed of DNA/RNA Viruses lack characteristics of living organisms • Unable to carry out metabolic processes (nucleic acid and protein synthesis) • Not able to obtain nutrition (aa and nucleotides) • Cannot synthesize their own ATP as energy source • Unable to respond to stimuli • Neither grow nor excrete

Conclusion: Viruses are infectious particles which are active in intracellular virus state, or inactive in extracellular state

4. Enzymes

- Aid in entering cells in the initial stages or in release of virus from host cell
    - **Lysozyme:** makes small in bacterial cell wall for viral nucleic acid to enter
    - **Neuraminidase:** breaks down glycosidic bonds of glycoproteins and glycolipids, thus aiding in liberation of virus
- Many enzymes are also involved in nucleic acid replication / transcription (polymerase)
    - DNA-dependent **RNA polymerase**
    - RNA-dependent **RNA polymerase**
    - DNA-dependent **DNA polymerase**
    - RNA-dependent **DNA polymerase**

GENERAL REPRODUCTIVE CYCLE OF VIRUSES

Each type of virus replicates only in a very limited number of cell types where suitable host cells are known as host range. They identify their host cells by

- A complementary fit between proteins on outside of virus and
- Specific receptor molecules on surface of host cell

Types of viruses:

1. Bacterial viruses / bacteriophages (T4 & lambda phage)
2. Animal viruses
3. Plant viruses

General steps in viral reproduction:

Adsorption, **P**enetration, **S**ynthesis and Replication, **A**ssembly, **R**elease

Bacteriophages:

They are generally viruses that infect bacteria and involved in the transfer of genetic material between bacteria by transduction

T4 Phage – Virulent Phage

Structure

General
1. Genomic – nucleic acid: linear double stranded DNA 2. Capsid – head and tail of the phage
Specific
1. Head contains DNA of the virus 2. A tail: consists a tail sheath, multiple tail fibers and base plate • Tail sheath: surrounds a central tube an contracts during penetration to thrust the central tube through the host cell wall and membrane • Tail fibers: allow the phage to adsorb onto the surface by binding to the specific receptors site found on cell surface

VERTICAL GENE TRANSFER

Occurs through binary fission, a form of asexual reproduction in which two equal-sized genetically identical daughter cells are produced from a single parent cell

- Includes replication of bacterial chromosome
- Unable to give rise to genetic variation

Process:

1	Bacterial chromosome attached to plasma membrane before replication
2	DNA replication begins at single origin of replication • Replication bubble is formed when two DNA separate and each strand is used as template for synthesis of daughter strand in semi-conservative DNA replication
3	Cell growth follows where each circular DNA molecule is attached to the cell membrane
4	Cell elongates and membrane growth causes two chromosomes to be moved apart
5	Cell division controlled by septal ring, directs the assembly of the septum • Septum ring extends as cell membrane invaginates as new cell membrane and cell wall materials are added to it • Separates two daughter cells
6	Invaginating cell membrane and newly formed septum splits cell intro two genetically identical daughter cells by cytokinesis

HORIZONTAL GENE TRANSFER

Transfer of genetic material between bacterial cells allow for genetic recombination and generation variation in bacterial populations – essential for natural selection

Mechanism involves donor and recipient cell, three possible pathways:

1. Transformation
2. Transduction
3. Conjugation

DNA must be integrated into chromosome of recipient cell through **homologous recombination**

Transformation:

Process by which a recipient cell takes up small fragments of naked DNA from the surrounding environment, where DNA originates from:

- Donor bacterial cell which lysed and releases DNA to surrounding environment
- Artificially constructed plasmids

Only competent bacterial cells can undergo transformation, which require presence of competence factors – cell surface proteins that bind to DNA fragments and aid in their uptake

Process

1	Competent recipient cell takes up one or more donor DNA fragments into its cytoplasm via competence factors
2	Homologous recombination of donor DNA fragment with homologous section of recipient cell's chromosome
3	Homologous segment of donor cell's DNA incorporated into recipient cell's chromosome and that of recipient cell is excised and degraded • Recipient cell now known as recombinant cell

*Transformation can be induced through heat shock or electroporation

<u>**CONTROL OF GENE EXPRESSION IN PROKARYOTES**</u>

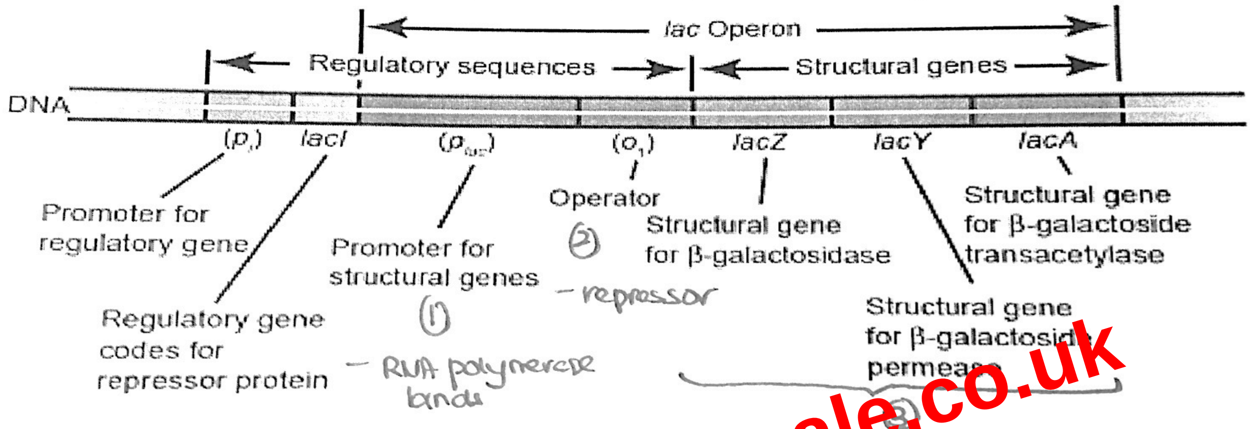
Control in prokaryotes is much less complex and since no post-transcriptional modification is possible, translational regulation is not common utilized

Coordinated control – Operon:

A transcription unit where structural genes with related functions are generally located adjacent to each other and placed under control of the same promoter and regulator regions

- Major mode of control exists at **transcriptional control**
- Controls amount of a protein and regulation of this occurs mainly ar level of transcription (how much mRNA is produced)

An example of a simple operon, the *lac* operon, is shown in **Fig 36**.



An operon typically consists of:

1. Promoter – lies upstream of structural genes, provides binding site for RNA polymerase and initiate transcription
2. Operator – regulates rate of transcription of structural genes by interacting with specific repressor protein
3. Structural genes – code for protein or RNA molecules which form part of cellular structure of have enzymatic function
4. Regulatory genes – code for specific protein that regulates expression of structural gene (repressor proteins)

Structural genes	Regulatory genes
Codes for functional RNA, structural protein, enzyme, or any functional protein within cell	Codes for regulatory proteins that regulate transcription of structural genes
Usually found as part of operon	Lie outside the operon

Why operons are necessary:

- Allows for coordinated regulation of functionally related genes using a single on-off switch
- Only produces enzymes when required
- Prevents inefficient use of resources
- Presents a selective advantage – use variety of sugars