Topic 2

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Mucus

- A thin layer coats the airways
- This captures pathogens
- And is shifted out of the lungs by the movement of cilia on epithelial cells
- CF mucus is drier, and so more sticky and thick
    - This increases the likelihood of lung infection
    - And reduces the efficiency of gas exchange

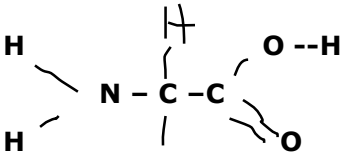
Gas exchange

- Features of the alveoli that make them brill for gas exchange:
    - Large surface area
    - High vascularisation
    - Vey thin
    - Slightly moist
- Fick's Law:
    - Rate of diffusion $\propto$ $\frac{\text{surface area x difference in concentration}}{\text{Thickness of gas exchange surface}}$
- People with CF
    - Have thicker mucus
        - This blocks the tiny bronchioles
        - Preventing efficient gas exchange

Pathogens in lungs

- Usually the mucus is shifted out by the cilia and coughed out or swallowed, removing pathogens from the airways
- CF mucus cannot be shifted so easily
    - The mucus is also anaerobic, breeding dangerous microbes
    - And white blood cells also contribute to the sticky mess

Amino acids & Polypeptides: 20 amino acids



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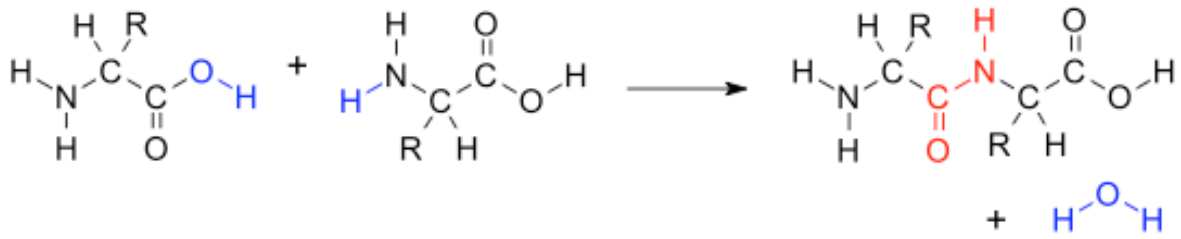
Joined in condensation reactions



Proteins

- Multiple amino acids
- Joined by condensation reactions to form peptide bonds
- Broken by hydrolysis, often with the aid of enzymes
- Structure
    - Primary: Order of amino acids
    - Secondary: ∝-helices or ß-pleated sheets, held by H-Bonds
    - Tertiary: Folding due to disulphide bridges, H-bonds and ionic bonds.
    - Quaternary: Multiple proteins in one, e.g. haemoglobin
- Haem is a prosthetic group (non protein), in a conjugated protein- one that has another chemical group associated with the polypeptide chains
- Fibrous
    - Long chains, often with cross linked multiple polypeptide chains. Insoluble and important structurally; keratin, collagen.
- Globular
    - Compact spherical shape achieved due to hydrophobic side chains
    - Important for metabolic reactions; examples such as enzymes.
    - The 3D shape of these proteins are very important; antibodies, enzymes and transport proteins all rely upon active sites with a specific shape.

Cell membrane structure

- The main structure is a phospholipids bilayer (phosphate group replacing one of the fatty acids in a triglyceride)
    - These have hydrophilic heads (rendered so by the phosphate group)
    - And hydrophobic tails

    - And so form with heads outwards, either in a bilayer or a globe called a micelle.
    - They allow small lipid molecules to pass through
- Fluid mosaic
    - The structure of the cell membrane is often described as a fluid mosaic; it is a dynamic environment chock a block with other molecules. These include
        - Glycoproteins
            - Proteins + A polysaccharide
        - Glycolipids
            - Lipids + A polysaccharide
        - Cholesterol
        - Transport proteins
            - These are specific fit, and can be active or passive transport
        - Channel Proteins
            - Larger molecules and charged ions pass through these. They are often in tandem with a receptor protein, which opens or closes the channel depending upon the nature of the substrate to be transported
    - The fluidity of the membrane depends upon the % composition of unsaturated fatty acids; the more there are, the more fluid it is. This is because the kinks in the unsaturated tails prevent them from packing together. Cholesterol also affects fluidity, reducing it by preventing phospholipids movement.
- Temperature dependent
    - Test with chunks of beetroot, examine speed of departure of purple dye at different temperatures. Test resulting solution with colorimeter.

Transport

- Passive
    - Diffusion
        - Down a conc gradient
            - Osmosis is a special case, involving water and a semi-permeable membrane, which doesn't allow the solute through

        - In the phospholipids bilayer, only small, uncharged molecules can pass through by diffusion
    - Facilitated diffusion
        - Similarly, down a conc gradient, but aided by channel proteins (gated or not) or carrier proteins (still down a conc gradient due to increased likelihood of binding in increased conc)
        - This is for charged molecules or those too large to pass through the bilayer
- Active
    - Specific carrier proteins, aided by ATP, carry substrates AGAINST a concentration gradient
- Bulk
    - Achieved by endo & exocytosis
    - Part of the cell membrane engulfs the substrate (endocytosis) and travels through the membrane, releasing the substrate and reuniting with the other layer of the bilayer on the other side (exocytosis).

CF sticky mucus

- Caused by a fault in the CFTR protein, which leads to water being continually removed from the mucus by osmosis, as Na^+ and Cl^- are also continually removed into the tissue fluid
- This impacts:
    - Gas transfer (see above)
    - Digestive system
        - The mucus blocks enzymatic ducts in the pancreas and liver (the pancreatic duct), preventing full digestion of food
        - Therefore, you get highly nutritious stool
        - And CF sufferers find it hard to gain and sustain weight (malabsorption)
        - There may also be a damaging build up of enzymes in the pancreas, inhibiting insulin regulation and leading to diabetes
    - Reproductive system
        - Sperm ducts are blocked by mucus, reducing fertility, or even absent
        - Cervix is blocked with a thick plug of mucus

- Salty sweat is a sign of CF, as the CFTR protein works in the opposite way in sweat ducts, preventing salt from being pumped back into the tissue fluid

Enzymes

- Specific fit
    - Due to active site shape
    - Specific substrate causes an enzyme-substrate complex to be formed
- Lock and key theory
    - Absolutely specific fit
- Induced fit theory
    - Mostly specific, slight adaptation on the part of the enzyme to fit the substrate
- Provide an alternative reaction pathway with a lower activation energy
- Remain unchanged at the end of the process
- Rate testing
    - Done by testing the time taken for a certain substrate to be metabolised
        - E.G H_2O_2 with catalase (from peas or potatoes), test breakdown into constituent gases at different temperatures

DNA

- Consists of multiple mononucleotides, linked by condensation reactions to form a polynucleotide
- A nucleotide unit consists of a phosphate molecule, a deoxyribose sugar and a nitrogenous base.
- Purine (2 ring)
    - Adenine
    - Guanine
- Pyrimidine (1 ring)
    - Thymine/ Uracil
    - Cytosine
- Arranged in triplet code
- Degenerate
    - Multiple codes per amino acids
- Semi-conservative replication
    - In mitosis, the new cell has one old and one new strand of DNA
- It is non overlapping

    - The codons are distinct from one another, read in non-overlapping blocks of 3

Protein synthesis

- Transcription
    - mRNA in the nucleus creates the sense strand from the antisense template strand of DNA
    - RNA polymerase helps create the complementary strand
    - mRNA chain leaves via nuclear pores
- Translation
    - mRNA attaches to a ribosome, free or on the rough ER
    - tRNA anticodons with attached amino acids join them, starting with the start codon AUG and ending with stop codon UAA, UAC or UGA
    - The amino acids are joined via condensation reactions, forming peptide bonds

DNA synthesis

- Helicase enzyme unzips the DNA
- DNA polymerase builds a new strand, building 5' to 3'; one strand is therefore built completely, the other in small chunks.
- These chunks are later joined by ligase enzymes

Mutations and sickle cell anaemia

- Most mutations occur in non-coding DNA, and so have no effect
- Sickle cell anaemia sees glutamic acid replacing valine
- Rendering the haemoglobin less soluble, and distorting the red blood cells into sickle moon shapes which carry less oxygen and can block blood vessels, causing bad joints.

CF mutation

- There are many different mutations that can give rise to a faulty CFTR protein, and hence CF
- The most common one is a deletion of three nucleotides, causing the loss of phenylalanine and destroying the 3D structure.
- Other problems include
    - ATP not being able to bind and open the ion channel

- Blocked channel

Mendelian inheritance

- Genotype
  - The combination of alleles inherited
- Phenotype
  - The physical expression of these genes; the interaction between the genotype and the environment
- Recessive allele
  - Will not be expressed if a dominant allele is present
- Dominant allele
  - Will always be expressed if present
- Homozygote
  - Containing two copies of the same allele
- Heterozygote
  - Containing two different alleles
- Locus
  - The same position on a pair of homologous chromosomes (1 paternal, 1 maternal)
- Monohybrid inheritance
  - Where the inheritance of a characteristic depends only upon a single gene. Most are controlled in more complicated ways.
    - E.G. Thalassaemia is caused by recessive alleles of a gene on chromosome 11, and affects the manufacture of haemoglobin. However, heterozygous people are somewhat resistant to malaria, giving a slight advantage to heterozygotes.
    - Other recessive illnesses
      - Albinism
      - Phenlyketonuria
      - Sickle cell anaemia
    - Dominant illnesses
      - Dwarfism
      - Huntingdon's
- Mendel
  - Worked with peas

- Particularly height and texture of pea
- Discovered monohybrid inheritance

Gene therapy

1.1. Desired allele is inserted into a target cell via a viral vector or liposomes
1.2. The normal form of the gene is therefore transcribed, translated and expressed
1.3. The functioning protein is provided in target cells

Viral insertion

- Replication sequence removed to prevent widespread infection
- Replaced with desired gene and promotor region
- With CFTR, the virus used has independent DNA, non incorporated into our cells
- But is still transcribed

Liposome insertion

- Desired DNA in plasmid
- Plasmid combined with liposome micelles
- The positive phosphate heads join to the DNA, which is negatively charged
- These complexes are breathed in with a nebulizer
- The liposomes fuse with epithelial cell membranes and carry the DNA into the cell

These are all varieties of somatic cell treatment in that they effect existing body cells.
Ethical objections prevent germ line therapy.

Testing for CF

- Test for salty sweat
- Test for the protein trypsinogen
- Genetic screening
  - Gel electrophoreisis

1. Restriction enymes are used to separate the DNA into fragments
   - These are usually found in bacterium to cut up viral DNA
   - They cut at specific base sequences
2. Gel electrophoreisis is used to separate the fragments according to their size
   - Occurs on an agarose gel layer
3. Southern blotting sees the fragments transferred to nitrocellulose or nylon paper
   - An alklaline buffer solution is used to separate the DNA strands

4. This allows a DNA probe of the desired sequence to be added, binding to any complementary sequences (hybirdization)

- Usually incorporates a radioactive tracer such as ^{32}P

5. The unbound sequences are washed away, and then the paper is viewed under an X-Ray to reveal the presence, or not, of the desired sequence

The uses of genetic screening

- To identify a carrier
- To confirm a diagnosis
- To test an embryo
    - Using amniosentesis to obtain embyrotic fluid
    - Or Chorionic villus sampling (CVS), taking a placental sample through the vagina or wall of the abdomen
- Testing pre implantation (PIGD)
    - Before in vitro fertilisation (IVF)

Four ethical frameworks

1. Rights & Duties: We have rights, and our duty is to satisfy ours and those of others
2. Utilitarianism: Maximise the amount of good in the world; no moral absolutes.
3. Informed consent: Allow people to make their own choices.
4. Leading a virtuous life: Being just, prudent, moderate , brave, charitable etc.