

1. Metabolite in metabolic reactions like condensation / hydrolysis

2. Important solvent for metabolic reactions -> Polar molecules and ionic compounds dissociate in water – are hydrophilic

3. High heat capacity buffers change in temperature – requires more energy for temperature of water to be increased

4. Large latent heat of vaporisation = Cooling effect / can absorb lots of heat energy before evaporating so little water loss

5. Strong cohesion between water molecules due to hydrogen bonding = columns of water in tube-like transport cells of plants, produces surface tension where water meets air, adhesion between water molecules and other molecules

- Molecules spread out when frozen to accommodate more bond formation -> floats = organisms survive below

3.1.8 Inorganic ions

- Found in the solution of the cytoplasm and body fluids of organisms
- Each type has a specific role depending on properties

3.2 Cells

3.2.1 Cell structure

3.2.1.1 Structure of eukaryotic cells

- **Nucleus** – chromosomes consisting of protein-bound linear DNA, central region nucleolus, double membrane with nuclear pores to let molecules in and out
- **Cytoplasm** – where chemical reactions take place
- **Mitochondria** – aerobic respiration, ATP, inner membrane folds into cristae = sites of respiration, small circular DNA not associated w proteins
- **Chloroplast** – short / circular DNA, thylakoid stacks into grana surrounded by stroma / starch, double membrane
- **Golgi apparatus** – modifies protein -> glycoproteins / lipoproteins, stores protein, packages protein into vesicles to transport within and outside of cell, modifies triglycerides
- **Lysosomes** – Golgi vesicle releases lysosomes / lipase – hydrolytic enzymes that break down bacterial cell wall
- **Ribosomes** – site of protein synthesis, not membrane bound, amino acids joined to form polypeptides, 2 subunits
- **Rough endoplasmic reticulum** – ribosomes on surface involved in protein synthesis, proteins folded up inside
- **Smooth endoplasmic reticulum** – synthesis and storage of molecules such as steroids and sterols
- **Cell wall** in plants, algae and fungi – long, straight chains linked by H bonds for strength -> turgid / flaccid
- **Cell vacuole** in plants – temporary food store of sugars and amino acids, some pigment
- Complex multicellular organisms – specialised for specific functions, organised into tissues -> organs -> systems
- Tissue = collection of similar cells that perform specific function, organ = combination of tissues coordinated to perform variety of functions with one predominating vital system = organs working together as single unit

3.2.1.2 Structure of prokaryotic cells and of viruses

- Prokaryotic – flagellum = movement, cytoplasm = reactions, single circular DNA free in cytoplasm / no association to proteins, one + thin cell wall of murein (glycoprotein), ribosomes, cell membrane = transport, capsule -> difference to eukaryotic = flagella, not membrane bound organelles, plasmids, capsule, cell wall, smaller ribosomes
- Viruses – acellular, non-living, capsid, genetic material = RNA/ DNA, attachment proteins to attach to host cell

3.2.1.3 Methods of studying cells

- **Optical microscopes** – cheap, natural colours, small, mobile, living cells, 0.2 micrometers resolution, wavelength of visible light restricts maximum resolution
- **Electron microscopes** – vacuum therefore no living cells, sliced, beam of e- guided by magnets
- **Scanning** = 3d, electrons bounce / **transmission** = 2d, higher resolution, electrons go through
- Magnification = size of image / size of real object

Cell fractionation / ultracentrifugation – to study = break down cells to isolate organelles

- Homogenisation – cells physically broken up by homogeniser, so organelles free from sediment
 - Homogenate in solution – cold to slow down enzyme activity to prevent organelle digestion, buffered = constant pH to stop denaturing of enzymes, isotonic to prevent osmosis
 - Homogenate filtered to remove debris and complete cells = prevent interference to results
 - Ultracentrifugation = slow, heaviest organelles = thin sediment, supernatant removed = spun higher speed
- Most to least dense = nuclei, chloroplast, mitochondria, lysosomes, membranes, ribosomes

3.2.2 All cells arise from other cells

- Multicellular organisms – not all cells retain ability to divide, eukaryotic that retain the ability show a cell cycle:

Interphase – DNA replication

MITOSIS

Prophase – chromosomes condense, nuclear envelope disintegrates, spindle fibres form

Metaphase – spindle fibres attach to centromeres of chromosomes, lines chromosomes in middle

Anaphase – spindle fibres shorten pulling sister chromatids apart to opposite poles of the cell

Telophase - chromosomes decondense, nuclear envelope reforms

Cytokinesis - membrane pinches inwards and cytoplasm and cell divides

- **Populate with aphids, remove aphids, leave stylets, collect exudates, record time taken to show fluorescence, measure distance between injection site and stylet, distance / time = rate**

3.4 Genetic information, variation and relationships between organisms

3.4.1 DNA, chromosomes and genes

- **Prokaryotic DNA = short, circular, unassociated with proteins / Eukaryotic = long, linear, associated to histones**
- Mitochondria and chloroplasts of eukaryotic cells have short, circular DNA that are not associated with proteins
- Gene - base sequence of DNA that codes for amino acid sequence of polypeptide and or functional RNA
- A gene occupies a fixed position, called a locus, on a particular DNA molecule
- A sequence of three DNA bases, called a triplet, codes for a specific amino acid

*Universal code – useful for all life, non-overlapping = separate, degenerate - different triplets = same amino acid

*Non-coding multiple repeats - regions of chromosome where sequence of bases doesn't code for protein

- Exons – region in gene that is expressed as protein / Introns – region in gene that doesn't code for protein

3.4.2 DNA and Protein Synthesis - transcription = mRNA, translation = polypeptides

- Genome = complete set of genes in a cell, proteome = full range of proteins that cell is able to produce
- rRNA – ribosomal RNA, ribosomes are made of proteins and RNA coded for by genes in DNA
- tRNA – transfer RNA, carries amino acids to ribosome to make proteins, single strand folded into clover, shape held by hydrogen bonds between bases, 3 specific bases exposed at one end = anticodon
- mRNA – messenger RNA, single strand complementary to template strand DNA, length depends on gene (exons)
- **DNA uncoils as strands separated by RNA polymerase from ATP, RNA nucleotides attach by complementary base pairing, RNA polymerase joins nucleotides, introns removed, exons joined, mRNA leaves nucleus to ribosome, tRNA attached to amino acid, anticodon binds w codon, amino acids joined by peptide bonds w ATP**
- Prokaryotes = direct mRNA from DNA, eukaryotes = pre-mRNA which is spliced to form mRNA.

DNA	mRNA	tRNA
Double polynucleotide chain Length can vary Pentose sugar = deoxyribose Linear shape ACTG H bonds present between base pairs Triplets Chemically very stable	Single strand Length can vary Pentose sugar is ribose Linear shape ACGU Codon Chemically the bases are	Single strand Pentose sugar is ribose Cloverleaf shape ACGU H bonds present between base pairs Anticodon

3.4.3 Genetic diversity can arise as a result of mutation or during meiosis

- **Gene mutations from changes in chromosome base sequence that arise spontaneously in DNA replication**
- **Gene mutations include base deletion and base substitution**
- **Due to degenerate nature of genetic code, not all base substitutions change sequence of amino acids**
- **Mutagenic agents can increase the rate of gene mutation by high energy / ionising radiation / chemicals**
- **Mutations in number of chromosomes arise spontaneously by chromosome non-disjunction during meiosis**
- Two nuclear divisions for formation of four haploid daughter cells from single diploid parent cell
- **DNA replicates, chromosomes condense, spindle fibres join to centromeres for chromosomes to line up along middle, spindle pull one of each pair to poles, cytokinesis = two daughter cells, chromosomes lined along middle by spindle fibres, sister chromatids are pulled apart, nuclear envelope and nucleoli reform, cytokinesis**
- Genetically different daughter cells from independent segregation of homologous chromosomes – first division
- Crossing over between homologous chromosomes = further genetic variation = exchange of alleles

3.4.4 Genetic diversity and adaptation

- Genetic diversity = the number of different alleles of genes in a population, factor enabling natural selection:

1. Random **mutations** result in new alleles of a gene – therefore there is **variation** between individuals in the population

2. Many mutations are harmful but, in certain environments, new gene allele means individual has **selective** advantage

3. **Survive and reproduce**, advantageous allele is inherited by members of the next **generation**

4. Over many generations, the new allele increases in **frequency** in the population

- Genotype = combination of alleles, phenotype = characteristics expressed
- **Development of antibiotic resistance in bacteria = natural selection – directional selection**
- **Antibiotics = kill bacteria by stopping formation of cell walls / breaking down cell walls / attacking ribosomes**
- **Resistance = enzymes that destroy antibiotics, protein channel = barrier, carrier proteins that pump antibiotics**

3.4.5 Species and taxonomy

- **Two organisms belong to the same species if able to produce fertile offspring**
- Courtship behaviour = necessary for successful mating, prior to mating

*Allows individuals to mate with individuals of the same species

*Pair bond formation = territorial animals - animals enter individual space without triggering aggressiveness for mating

Deeper into medulla $[Na^+]$ increases so w.p. of filtrate is always less negative than the tissue fluid its next to.
Osmosis can occur across whole length of collecting duct as w.p. gradient is maintained.
Water moves out of collecting duct into the tissue fluid and then into vasarecta capillaries.

Counter-current multiplier

Na^+ moves out of ascending limb and water moves out of descending so w.p. in descending limb decreases slowly.
Process repeats down descending limb due to counter current between the ascending and descending limb.

The loop of Henle

Longer loop of Henle increases the concentration gradient of Na^+ which increases the w.p. gradient for longer deeper into the tissue fluid of the medulla so more water is reabsorbed by osmosis.

ADH – produced by the hypothalamus that secretes ADH into the posterior pituitary gland where it is stored.

Osmoreceptor detects water potential so loses / gains water by osmosis

Lack of water triggers hypothalamus to increase frequency of impulses secreted to thirst centre of the brain and specialised nerve cells in the posterior pituitary gland causing more ADH to be released into the blood.

ADH binds to receptors on the CD / DCT causing vesicles to fuse with the membrane so more aqua porins are embedded.

3.7 Genetics, populations, evolution and ecosystems

3.7.1 Inheritance

Alleles of a gene are dominant, recessive or codominant - in humans, alleles at a locus are homozygous or heterozygous

- Chromosome – one long, super-coiled DNA molecule that has several genes along its length
- Homologous pair – matching pair of chromosomes which have the genes in the same order
- Gene – a length of DNA that codes for one polypeptide or protein / Allele – different forms of the same gene
- Locus – the position of a gene on a chromosome
- Diploid – cell with two copies of each chromosome / Haploid – cell with one copy of each chromosome
- Homozygous – two identical alleles of gene / Heterozygous – two different alleles of a gene
- **Genotype – genetic constitution / Phenotype – genotype expression with environmental interaction**
- Dominant – allele always expressed / Recessive – allele expressed only in absence of a dominant allele
- Codominant – when both alleles are expressed in the phenotype of a heterozygote
- Autosome – chromosome that is not a sex chromosomes / Sex-linked – gene located on X or Y chromosome
- Autosomal linkage – genes on same chromosome are linked, alleles of genes are likely to be inherited together
- Epistasis – the interaction of different genes

Monohybrid crosses

1. Phenotype 2. Genotype 3. Gametes 4. Punnett's square 5. Offspring genotype and phenotype 6. Phenotype ratio

- When both parents are heterozygous, phenotype ratio is always 3:1
- Codominant alleles – use superscript capital letter, when both alleles are expressed in heterozygote phenotype

Dihybrid crosses between combination of gametes

- Gametes have one of each letter and body cells have two each of letter
- $SsYy \times SsYy \rightarrow 9 : 3 : 3 : 1$
- $SSyy \times ssYY \rightarrow 1 / SSYY \times ssyy \rightarrow 1$
- $Ssyy \times ssYy \rightarrow 1 : 1 : 1 : 1$

Evidence that the condition is recessive

- Offspring has different phenotype to parents
- Parents must have dominant phenotype and be heterozygous

Sex-linkage and sex determination

- Recessive phenotype is always expressed in the absence of a dominant allele
- Any gene that is on the X or the Y chromosome is called sex-linked
- X chromosomes are larger than Y chromosomes as it as some alleles that the Y chromosome do not
- Males are not heterozygous or homozygous for sex-linked conditions as they have one copy of a gene
- Males suffer from recessively - caused X diseases even if they have one copy of the recessive allele
- **Red-green colour blindness – recessive condition (use a c on the X chromosome to indicate)**
- **Haemophilia is the lack of VIII clotting factor – recessive condition (use a h on the chromosome)**

Sex-linked rule: dominant dads have dominant daughters and recessive mums will have recessive sons

Proving not sex-linked: 1. Find where rule is broken 2. Explain phenotype of parent and offspring if sex-linked

Proving condition is recessive: instance where parents have the dominant phenotype and offspring doesn't indicates the parents are carriers of the recessive allele and offspring inherited recessive allele from each parent

Autosomal linkage

- When there is no linkage, it is equally likely for the four combinations of gametes to occur
- When there is linkage, the most common gametes have chromosomes separated with same genes
- If crossing over occurs, then gametes form that are less common

Genome – the bases of all the DNA in a cell

Proteome – the full range of proteins that a cell can produce

Sequencing projects have read the genomes of a wide range of organisms, including humans.

Determining the genome allows the proteome of an organism to be determined as three bases code for one amino acid.

Eukaryotes have introns and non-coding DNA, so it is more difficult to work out their proteome from their genome.

Applications:

1. Identification of potential antigens for use in vaccine production
2. Can look for disease-causing alleles or alleles that increase the risk of developing a condition
3. Identify alleles that cause drug resistance in pathogens

Sequencing methods are continuously updated and have become automated

3.8.4 Gene technologies allow study of gene function to understand organism function and the design of processes

3.8.4.1 Recombinant technology

Recombinant DNA technology involves the transfer of DNA fragments from one organism, or species, to another

Since the genetic code is universal, the transferred DNA can be translated within cells of the transgenic organism

1. Isolation

There are three ways of obtaining a desired gene which can be amplified by the vivo or vitro method:

Using a gene machine

- Base sequence produced accurately in a short time; no introns so transcribed by prokaryotic cells

Amino acid sequence of gene determines mRNA sequence, DNA sequence found and typed into computer for international standards, automated process creates series of nucleotides that join, PCR makes a complete double stranded DNA

Using reverse transcriptase

- Cells contain two copies of each gene so difficult to obtain DNA fragment, so mRNA obtained instead
- mRNA doesn't have introns in it
- To make insulin for instance obtain mRNA from beta cells in the Islets of Langerhans

mRNA of gene isolated from cell, mixed with reverse transcriptase and free nucleotides, reverse transcriptase uses mRNA as template, complementary base pairing occurs, single stranded cDNA forms and acts as a template so free nucleotides attach, DNA polymerase joins adjacent nucleotides in the new strand together to form phosphodiester bonds

Using restriction endonuclease

- A restriction endonuclease is an enzyme that cuts DNA at a specific base sequence called a recognition site
- Palindromic sequence is a section of DNA which is the same if the 3' to 3' on one strand and 3' to 5' on the other
- Different endonucleases act at different specific recognition sequences (palindromic) because of their different tertiary structures being complementary to the shape of different sequences of bases
- Different enzymes cut DNA in different way like staggered cuts that leave sticky ends and or blunt cuts

In Vivo Cloning – for copies of desired gene, add promoter and terminator sequences of genes transcribed in the host

2. Infection

A vector transfers DNA from one organism to another.

Cut the fragment and plasmid with the same restriction endonuclease so they have the same complementary sticky ends,

DNA ligase is used to form the phosphodiester bonds between the two sections of DNA, so they are joined together

- Hydrogen bonds form between the complementary bases in the sticky ends of the plasmid and the DNA
- Possible for plasmid to close without gene causing a solution of non-recombinant and recombinant plasmids

3. Transformation

Use restriction endonuclease to cut out desired gene, use same enzyme to cut plasmid, insert desired gene into plasmid,

DNA ligase joins desired gene to plasmid forming recombinant plasmid, mix those plasmids with bacterial cells in Ca²⁺ solution, use heat shock to make the cell membrane permeable to take up the recombinant plasmid, let the cells replicate

4. Identification

Insertion and transformation could lead to bacteria with no plasmid, bacteria with the recombinant and bacteria without.

Marker genes help identify cells that have been successfully transformed like GFP or lactase (colourless substrate -> blue)

Marker genes can be used in the following ways:

1. Insert desired gene into the marker gene so marker gene will not be expressed
2. Attach desired gene next to the marker gene, so they are next to each other so marker gene will be expressed

Replica plating - many proteins resistant to different antibiotic

Use plasmid which codes for proteins for varied antibiotic resistance, restriction enzyme used cuts plasmid within gene where desired gene would be put, bacteria placed onto petri dish, kill Type 1 bacteria by treating with specific antibiotic, transfer unkilld bacteria to a new plate, make replica of plate using nylon membrane, treat one plate with the other antibiotic which will kill Type 3 bacteria and compare between the plates to identify desired

In Vitro Cloning referred to as the Polymerase chain reaction

1. Mix together DNA nucleotides, DNA polymerase and primers and the section of DNA you want to amplify
2. Heat DNA to 95 degrees so that the H bonds break, and the strands separate