A-LEVEL BIOLOGY NOTES

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CHAPTER 12 ENERGY AND RESPIRATION

ENERGY

– living organisms require energy continuously to survive

– DNA replication; protein synthesis; active transport; movement; maintain body temperature

ATP

– adenosine triphosphate

– formed by combination of adenine, ribose, phosphate group

ATP as energy currency

– when ATP hydrolysed, energy released

$$\underset{}{ATP} + H_2O \rightarrow \underset{30.5kJ\ mol^{-1}}{ADP} + P_i + H_2O \rightarrow \underset{30.5kJ\ mol^{-1}}{AMP} + P_i + H_2O \rightarrow \underset{14.2kJ\ mol^{-1}}{adenosine} + P_i$$

$$ATP + H_2O \rightarrow ADP + H_3PO_4\ (P_i) \pm 30.5kJ\ mol^{-1}$$

– energy currency: immediate donor of energy for cell's energy-requiring reactions

– ATP highly suitable for this role because of its features:

1. readily-hydrolysed to release energy
2. molecularly small
3. water-soluble
4. easily transported around the cell

synthesis of ATP

– 2 ways:

1. reorganising chemical bonds (chemical potential energy) during glycolysis and Krebs cycle (substrate-linked reactions)
2. electrical potential energy (transfer of electrons in mitochondria)

– chemiosmosis

1. movement of ions across a selectively-permeable membrane, down their electrochemical gradient

2. during chemiosmosis, free energy from series of reactions that make up the electron transport chain is used to pump H^+ across membrane, establishing an electrochemical gradient
3. H^+ can only pass through the inner membrane via membrane protein called ATP synthase
4. as ions move through ATP synthase, ADP turns into ATP
5. production of ATP via chemiosmosis in mitochondria is called oxidative phosphorylation

– ATP synthase

1. has three binding sites and a part of the molecule that rotates as H^+ pass
2. produces structural changes in the binding sites and allows them to pass sequentially through three phases:
   – binding ADP and P_i
   – forming tightly-bound ATP
   – releasing ATP

role of ATP in active transport

– for active transport

– for sodium-potassium pump (protein that acts as an ATPase and catalyses hydrolysis of ATP to ADP + P_i)

RESPIRATION

– process which organic molecules act as fuel and are broken down in a series of stages to release chemical potential energy, which is used to synthesise ATP

glycolysis

– splitting (lysis of glucose)

– net gain 2 ATP, 4 ATP produced, 2 used

– in cytoplasm

– steps:

1. phosphorylation of glucose to fructose phosphate; requires 1 ATP
2. phosphorylation of fructose phosphate to fructose bisphosphate; requires 1 ATP

3. fructose bisphosphate breaks down into 2 triose phosphate molecules
4. hydrogen then removed from each TP and transferred to NAD, forms intermediates; produce 2 ATP
5. intermediates form 2 pyruvate; produces 2 ATP

link reaction

– in mitochondrial matrix

– net gain 0 ATP, 0 ATP used

– steps:

1. pyruvate passes by active transport from cytoplasm, through outer and inner membranes of mitochondrion and into mitochondrial matrix
2. pyruvate decarboxylated and dehydrogenated and combined with coenzyme A to give acetyl coenzyme A
3. H removed transferred to NAD

Krebs cycle

– also known as citric acid cycle or tricarboxylic acid cycle

– in mitochondrial matrix

– net gain 2 ATP, 0 ATP used

– closed pathway of enzyme-controlled reactions

– steps:

1. ACoA combines with 4C compound (oxaloacetate) to form 6C compound (citrate)
2. citrate is decarboxylated and dehydrogenated to yield CO2 and H which are accepted by NAD and FAD
3. oxaloacetate regenerated to combine with another ACoA

– 2 CO_2 produced, 1 FAD and 3 NAD reduced, 1 ATP generated via intermediate compound

oxidative phosphorylation

– takes place in inner mitochondrial membrane

– net gain 28 ATP, 0 ATP used

– energy for phosphorylation of ADP to ATP comes from activity in the electron transport chain

– steps:

1. NADH and FADH passed to electron transport chain
2. here, H are removed from 2 hydrogen carriers and are split to H^+ and e^-
3. electron transferred to first a series of electron transport carriers
4. respiratory complex is one of the membrane proteins involved in transferring the electron
5. as an electron moves from one carrier at high energy level to another at lower energy level, energy is released
6. some of this energy used to move protons from matrix to space between inner and outer membranes of mitochondrial envelope, lowering pH there
7. produces higher concentration of protons in intermembrane space than in the matrix, setting up concentration gradient
8. protons pass back to matrix through protein channels in inner membrane, moving down concentration gradient; ATP synthase associated with each channel
9. as protons pass through channel, their electrical potential energy is used for chemiosmosis
10. oxygen is final electron acceptor; in matrix, electron and proton transferred to oxygen, reducing it to H_2O

ROLES OF COENZYMES

NAD	FAD	CoA
nicotinamide adenine dinucleotide	flavin adenine dinucleotide	coenzyme A
derived from vitamin nicotinamide	derived from vitamin riboflavin (B2)	derived from vitamin pantothenic acid (B5)
accepts 2 H^+ when reduced	accepts 2 electrons and 1 proton when reduced	forms part of acetyl-S-CoA which is part of Krebs

MITOCHONDRIAL STRUCTURE AND FUNCTION

structure

– rod shaped/filamentous

– 0.5-1.0 μm in diameter

– not rigid, can change shape

– surrounded by envelope of 2 phospholipid membranes

1. outer membrane smooth
2. inner membrane folded into cristae, gives large surface membrane
3. mitochondria from active cells have longer/denser cristae
4. outer membrane relatively permeable to small molecules, inner less permeable
5. inner membrane studded with tiny spheres (ATP synthase, 9nm diameter), attached to inner membrane by stalks

function

inner membrane	**matrix**
site of electron transport chain	site of link reaction/Krebs cycle
contains proteins necessary from electron transport	contains enzymes required for link reaction/ Krebs cycle
space between 2 membranes has lower pH than matrix due to protons released into intermembrane space by activity of electron transport chain	contains 70s ribosomes
	activity of ATP synthase forms ATP
	energy for ATP production from proton gradient between intermembrane space and matrix

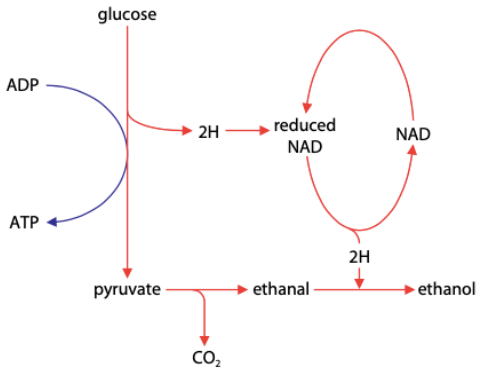
RESPIRATION WITHOUT OXYGEN

– reasons for anaerobic respiration:

1. when free oxygen not present, hydrogen cannot be disposed of by combination with oxygen
2. electron transfer chain then stops working, no ATP formed by oxidative phosphorylation

– 2 anaerobic pathways (both in cytoplasm):

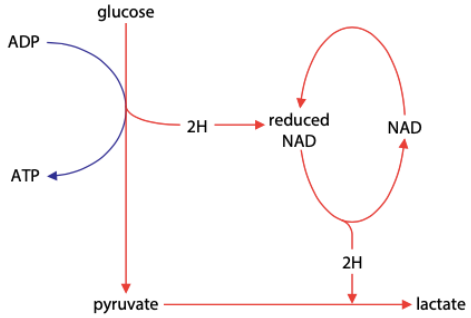
1. alcoholic fermentation (glucose to ethanol)
    – H from NADH passed to ethanol, releases NAD
    – allows glycolysis to continue
    – pyruvate decarboxylated to ethanal
    – ethanal becomes ethanol by alcohol dehydrogenase



2. lactic fermentation:

– with oxygen deprivation, pyruvate acts as H acceptor and converted to lactate by lactate dehydrogenase

– NAD released and allows glycolysis in anaerobic conditions



– lactate and ethanol are toxic

1. ethanol has no reverse reactions
2. lactate becomes pyruvate OR CO_2 + H_2O OR glycogen

OXYGEN DEBT

– 'paying back' oxygen deficit

– when exercise begins, more oxygen is needed to support aerobic respiration in muscles

– oxygen demand increases, but meeting the demand requires time

– lactic fermentation occurs during this time, thus building up oxygen deficit

– when exercise stops, person continues to breathe deeply and absorb oxygen at higher rates than at rest

– oxygen needed for:

1. conversion of lactate to glycogen in liver
2. deoxygenation of haemoglobin in blood
3. high metabolic rate, as many organs are operating at above resting level

RESPIRATORY SUBSTRATES

– when lipids are respired, C atoms are removed in pairs, as acetyl CoA, from fatty acid chains fed to Krebs cycle

– the C—H skeletons of amino acids are converted to pyruvate or acetyl CoA

energy values of respiratory substrates

– most energy generated in aerobic respiration from oxidation of hydrogen to H_2O when NADH/FADH passed to electron transport chain

– greater number of H in structure of substrate, greater energy value

– fatty acids have greater energy value per unit mass, or energy density, than carbohydrates or proteins

– energy value of substrate is determined by burning known mass of substance in calorimeter

carbohydrates	15.8
lipid	39.4
protein	17.0

respiratory quotient (RQ)

– CO_2 given out / O_2 taken in

ADAPTATIONS OF RICE FOR WET ENVIRONMENTS

– often grown in ‘paddies’ (fields where ground intentionally flooded, as this is condition needed for growth

– how rice adapts to flooded conditions:

1. grow taller, so top part of leaves/flower spikes above water to allow O_2/CO_2 exchange through stomata
2. stems contain loosely-packed cells forming tissue called aerenchyma which allows

gases to diffuse through it to other parts of plant, including parts underwater

3. supplemented by air trapped between ridges of underwater leaves
4. lack of O_2 makes ethanol build up in tissues; ethanol is toxic, but cells in rice roots can tolerate high levels
5. produce more alcohol dehydrogenase that breaks down ethanol; this allows plants to grow actively when O_2 is scarce, using ATP from alcoholic fermentation

CHAPTER 13 PHOTOSYNTHESIS

AN ENERGY TRANSFER PROCESS

– trapping/fixation of CO_2 and its subsequent reduction to carbohydrate, using hydrogen and water

– takes place inside chloroplasts

$$nCO_2 + H_2O \xrightarrow{\text{light in pres. of chlorophyll}} (CH_2O)n + nO_2$$

PIGMENTS IN CHLOROPLASTS

– primary & accessory

– arranged in light-harvesting clusters called photosystems

– two types of photosystems: I & II

– in a photosystem, several hundred accessory pigment molecules surround a primary pigment molecule, and energy of light absorbed by different pigments is passed to the primary pigment

– the primary pigments are two forms of chlorophyll and act as reaction centres

LIGHT DEPENDENT REACTIONS

– include: splitting of H_2O via photolysis to give H^+ ions (protons);
synthesis of ATP in phosphorylation

– takes place in presence of suitable pigments that absorb certain wavelengths of light

– H^+ combines with carrier NADP to form NADPH

– ATP and NADPH are passed from light dependent reactions to light independent reactions

– photophosphorylation of ADP to ATP depends on electron flow

cyclic photophosphorylation

– involves only photosystem I

– steps:

1. light absorbed by photosystem I is passed to primary pigment
2. an electron in chlorophyll is excited to higher energy level and emitted from the chlorophyll molecule (photoactivation)
3. excited electron captured by electron acceptor and passed back to chlorophyll via chain of electron carriers
4. enough energy released to synthesise ATP from ADP + P_i by chemiosmosis
5. ATP passes to light independent reactions

non-cyclic photophosphorylation

– involves both photosystems I and II; Z-scheme

– steps:

1. light is absorbed by both photosystems
2. excited electron is emitted from primary pigments of both reaction centres
3. these electrons are absorbed by electron acceptors and pass along chains of electron carriers, leaving photosystems positively charged
4. primary pigment of photosystem I absorbs electron from photosystem II
5. its primary pigment receives replacement electron from photolysis of H_2O
6. ATP synthesised, electron lose energy while passing through carrier chain

photolysis of water

– photosystem II includes H_2O-splitting enzyme that catalyses H2O breakdown

$$H_2O \rightarrow 2H^+ + 2e^- + {}^1/_2O_2$$

– oxygen is waste product

– H^+ combines with electron from photosystem I and NADP to form NADPH

$$2H^+ + 2e^- + NADP \rightarrow NADPH$$

– NADPH passes to the light independent reaction and is used in carbohydrate synthesis

Hill reaction

– redox: involve transfer of electrons from electron donor (reducing agent) to and electron acceptor; sometimes H is transferred so that dehydrogenation is equal to oxidation

– isolated chloroplasts have ‘reducing powers’ and liberates oxygen from H_2O in presence of oxidising agent

– reducing power demonstrated by using redox agent that changes colour on reduction
– technique can be used to investigate effect of light intensity/ wavelength on rate of photosynthesis of a suspension of chloroplasts
– Hill used Fe^{3+} as acceptor, but DCPIP can substitute for NADP
– DCPIP becomes colourless when reduced

LIGHT INDEPENDENT REACTIONS

– Calvin cycle

1. carbon fixation

– CO_2 attaches to 5C sugar, ribulose 1,5-bisphosphate; catalysed by enzyme rubisco
– product is highly unstable 6C intermediate that immediately splits to 3C sugars, 3-phosphoglyceric acid (2 3PGA produced per CO_2)

2. reduction

– phosphate group from ATP incorporated into each molecule of 3PGA to be 1,3-bisphosphoglycerate; it then reduces and phosphate is lost, becoming glyceraldehyde 3-phosphate; e^- pair required for reduction comes from NADPH
– energy provided when ATP forms ADP, and when NADPH forms $NADP^+$
– G3P is 3C, takes 3 rounds of Calvin cycle to get enough carbons to export one G3P; 6 molecules of G3P per CO_2 that enters cycle
– not net production, as 3 molecules of 5C (RuBP) required for each G3P formed
– other 5 recycled

3. regeneration

– RuBP regenerated; through reactions that rearrange 5 G3P into 3 RuBP with 3 ATP
– each turn of Calvin cycle makes 2 G3P, so 3 CO_2 makes 6 G3P
– 9 ATP, 6 NADPH used up

importance of Calvin cycle

– most (5/6) of G3P used to regenerate RuBP, but remainder (1/6) G3P used to produce other molecules needed by plant
– some G3P condenses to 6C phosphate which are used to produce starch for storage, sucrose for translocation, cellulose for cell walls
– other triose phosphates to convert glycerol to fatty acids to produce lipids for cell

membrane or to acetyl CoA for respiration in production of amino acids in protein synthesis

CHLOROPLAST STRUCTURE AND FUNCTION

– photosynthetic organelle

– diameter 3-10 μm

– in leaves, amount is the most in palisade mesophyll cells

– surrounded by an envelope of 2 phospholipid membranes

– filled with substance stroma, site for Calvin cycle

– stroma contains DNA loop which codes some chloroplast proteins

– in stroma, there is a system of membranes, which is a series of flattened fluid-filled sacs called thylakoids

– a stack of thylakoid forms granum, which then collectively forms grana

– membranes of grana provide large surface area for the light dependent reaction, for maximum light absorption

– membranes of grana also hold ATP synthase for ATP production through chemiosmosis

LIMITING FACTORS

– factor that controls a process and will inhibit or slow rate of reaction when in short supply

factor	**description**
light intensity	– lack of light means light dependent stage cannot function, inhibiting photosynthesis – optimum amount of light maximises the rate of production for ATP and NADPH
carbon dioxide concentration	– lack of this substrate means there cannot be carbon fixation in the Calvin cycle, so no GP or TP molecules are made so there are no products – when CO_2 concentration is low, the amount of G3P produced is limited as CO_2 is needed for its production, and therefore the rate of photosynthesis is affected

temperature	– affects the rate of light independent reaction; energy that drives this process is heat – at higher temperatures, molecules have more kinetic energy so collide more often and are more likely to react when they collide – many enzymes are involved during the process of photosynthesis; at low temperatures, these enzymes work slower; at high temperatures enzymes are denatured

GROWING PLANTS IN PROTECTED ENVIRONMENTS

– understanding effect of environmental factors on rate of photosynthesis allows management of crops when grown in protected environments, e.g. glasshouses
– sensors monitor light intensity, humidity and CO_2 concentration
– plants grow hydroponically, with roots in nutrient solution whose nutrient content can be varied at different stages of plant's growth
– all factors managed by computer to maximise yield of crop
– insect pests and fungal diseases are more easily controlled

C4 PLANTS

– first compound produced in light dependent reaction contains 4C atoms instead of 3C
– sorghum and maize

avoiding photorespiration

– photorespiration wasteful pathway; occurs when Calvin cycle enzyme rubisco acts on oxygen rather than CO_2
– when this happens in C3 plants, less photosynthesis takes place, because some RuBP is being wasted and is less available to combine with CO_2
– happens most readily in high temperatures and high light intensity (low altitude, tropics)
– method to keep RuBP and rubisco well away from high oxygen concentrations:
    1. cells that contain RuBP and rubisco are arranged around the vascular bundles, and are called bundle sheath cells
    2. this makes RuBP and rubisco have no direct contact with the air inside the leaf
    3. CO_2 absorbed by mesophyll cells, which are in contact with air

4. mesophyll cells contain enzymes called PEP carboxylase, which catalyses the combination of CO_2 from air with 3C substance called phosphoenolpyruvate (PEP)
5. compound formed in this reaction is oxaloacetate
6. oxaloacetate converted to malate and passed to bundle sheath cells
7. CO_2 removed from malate and delivered to RuBP by rubisco in normal way
8. light independent reaction then proceeds as usual

TRAPPING LIGHT ENERGY

– chloroplasts contain different pigments which absorb different wavelengths of light

– photosynthetic pigments of higher plants form two groups

1. chlorophylls: primary pigments
2. carotenoids: accessory pigments

group	**pigment**	**colour**	**absorbance**
chlorophylls	chlorophyll a chlorophyll b	yellow-green blue-green	absorb mainly in the red and blue-violet regions of light spectrum; they reflect green light, which is why plants look green
carotenoids	ß carotene xanthophyll	orange yellow	absorb mainly in blue-violet region of the spectrum

– **absorption spectrum** is graph of absorbance of different wavelengths of light by pigment

– **action spectrum** is graph of rate of photosynthesis at different wavelengths

– the shorter the wavelength, the greater energy it contains

– different pigments present in chloroplast can be separated by paper chromatography

R_f = distance travelled by pigment spot / distance travelled by solvent

CHAPTER 14 HOMEOSTASIS

HOMEOSTASIS

– maintains constant environment for cells in body

– physiological factors controlled in mammalian homeostasis:

1. core body temperature
2. metabolic wastes (CO_2; urea)
3. blood pH
4. blood glucose concentration
5. water potential of blood
6. gas concentrations in blood (CO_2; O_2)

INTERNAL ENVIRONMENT

– all conditions inside body in which cells function

– cell's immediate environment is tissue fluid

– features of tissue fluid that influence cell activities:

1. temperature: low temp. slows down metabolic reactions; high temp. denatures proteins/enzymes
2. water potential: decrease, water moves out by osmosis, slows met. reactions; increase, water moves into cell, causes bursting
3. conc. of glucose: lack causes slow/stop of respiration, cell deprived of energy source; excess causes water to move out of cell

– homeostatic mechanics work by controlling blood composition, therefore controls tissue fluid composition

HOMEOSTATIC CONTROL

negative feedback control loop

– negative feedback: keeps changes in factor within narrow limits around a set point

– minimises difference between actual value of factor and ideal value

– maintains homeostatic balance

– involves receptor (sensor) and effector (muscles/glands)

– receptors detect stimuli involved with condition/physiological factors being regulated

corrective actions

– effect is to correct

– input: receptors detect internal/external stimuli and send information through nervous system to central control in brain/spinal cord

– output: central control instructs effector to carry out action

– continuous monitoring of factor by receptors produces steady stream of information to control centre that makes continuous adjustments to output

– factor fluctuates around set point

coordination systems in mammals

– transfer information between different parts of body

– nervous system: information comes in electrical impulses; transmitted along nerve cells (neurones)

– endocrine system: use chemical messengers (hormones) that travel in blood; long distance cell signalling

CONTROL OF BODY TEMPERATURE

thermoregulation

– controls body temperature, involves both coordination systems

– mammals generate heat; released during respiration; heat produced by liver cells, absorbed by blood flowing through liver and distributed throughout rest of body

– hypothalamus: central control for body temp. in brain

1. receives constant input of sensory information about blood/surrounding temp.
2. has thermoreceptor cells continually monitoring core temperature (temp. inside body that remains close to set point)
3. keeps temperature within narrow limits of set point
4. receives temp. information from other sources (e.g. skin)
5. skin temp. first to change if there is change in surrounding temp., skin receptor gives early warning about possible change to core temp.

6. if core/surrounding temp. changes, hypothalamus sends impulses to activate changes

decrease	increase
vasoconstriction – muscles in walls of arterioles that supply blood to capillaries near skin surface contract – narrows lumen of arterioles and reduce blood supply to capillaries so less heat lost from blood	**vasodilation** – muscles in arterioles in skin relax – more blood to flow through capillaries, heat lost to surroundings
raising body hairs – muscle at base of hair contract to increase depth of fur – traps air close to skin (air poor heat conductor)	**lowering body hairs** – muscles attached to hairs relax so they lie flat – reduces depth of fur and layer of insulation
decrease sweat production – reduces loss of heat by evaporation from skin surface	**increase sweat production** – sweat glands produce more sweat – can evaporate on surface of skin to remove heat
shivering – involuntary contraction of skeletal muscles generate heat – heat absorbed by blood and carried to rest of body	
increase adrenaline secretion – adrenaline increases rate of heat production in liver	
behavioural responses – curling up to reduce surface area exposed to air – huddling together – finding source of warmth – wear warm clothing	**behavioural responses** – resting/lying down with limbs spread out to increase surface area exposed to air – wear loose fitting clothes – turning on air con – drinking cold drinks

in gradual decrease of temp. – hypothalamus releases hormone to activate anterior pituitary gland – this gland releases thyroid stimulating hormone (TSH) – TSH stimulates thyroid gland to secrete hormone thyroxine into blood – thyroxine increases metabolic rate, which increases heat production in liver	**in gradual increase of temp.** – hypothalamus reduces release of TSH – less thyroxine produced

EXCRETION

– removal of toxins/unwanted products of metabolism

– excretory products: CO_2 and urea

1. CO_2: produced by cells respiring aerobically
2. urea: produced in liver, from excess amino acids; transported from liver to kidney via blood plasma; kidneys excrete it dissolved in water, as urine

deamination

– the removal of amino groups from amino acids

– amino group (—NH_2) is removed along with H atom; they combine to form ammonia (NH_3)

– keto acid that remains may enter Krebs cycle and be respired; or converted to glucose; or converted to glycogen or fat for storage

– ammonia very soluble and toxic, can cause immense damage; this is prevented by converting ammonia immediately to urea

$2NH_3 + CO_2 \rightarrow CO(NH_2)_2$ (urea) + H_2O

– urea

1. urea is main nitrogenous excretory product of humans
2. diffuses from liver cells into blood plasma
3. must be excreted each day to prevent build up of concentration in blood
4. filtered out and excreted as blood passes through kidneys

– other nitrogenous excretory products include:

1. creatinine
   – creatine made in liver from certain amino acids
   – usually used in muscles as creating phosphate

– acts as an energy store

– some converted to creatinine and excreted

2. uric acid

– made from breakdown of purines from nucleotides

STRUCTURE OF KIDNEY

name	**description/function**
renal artery	brings blood to kidney
renal vein	brings blood out of kidney
ureter	narrow tube that carries urine from kidney to bladder
urethra	single tube carrying urine from bladder to outside of body
capsule	covers whole kidney
cortex	lies beneath capsule; contains nephrons and blood vessels
medulla	makes up central area
pelvis	area where the ureter joins with kidney
nephrons	tiny tubes making up the cortex; one end of the tube forms Bowman's Capsule
Bowman's capsule	cup-shaped structure which surrounds glomerulus
glomerulus	tight network of capillaries; each glomerulus supplied with blood by branch of renal artery called **afferent** arteriole; the capillaries of glomerulus rejoin to form **efferent** arteriole; efferent arteriole leads off to form network of capillaries running closely alongside rest of nephron blood enters through renal artery, into afferent arteriole; enters Bowman's capsule; leaves Bowman's capsule; blood runs through capillaries, then out to renal vein
loop of Henle	long hairpin loop in medulla; creates very high concentration of sodium and chloride ions in tissue fluid in medulla; has descending and ascending limbs; descending limb permeable to water; ascending limb impermeable to water
collecting duct	joined with DCT, leads down through medulla and into pelvis

proximal convoluted tubule	twisted region, tubule running towards centre of kidney; lining of PCT nephron made of single layer of cuboidal epithelial cells cuboidal epithelial cells adaptation for function: 1. microvilli to increase surface area of inner surface facing lumen 2. tight junctions holding adjacent cells together so fluid cannot pass between cells 3. many mitochondria to provide energy for Na^+—K^+ pump proteins in outer cell membranes 4. co-transporter proteins in membrane facing lumen
distal convoluted tubule	another twisted region, tubule running from medulla up to cortex; first part of DCT functions similarly to ascending limb, second part same as collecting duct

ULTRAFILTRATION

– blood in glomerular capillaries separated from lumen of Bowman's capsule by two cell layers and basement membrane

1. endothelium: first cell layer; lining of capillary; has more gaps than other capillaries
2. basement membrane: made up of network of collagen and glycoproteins;
3. epithelial cells: second cell layer; make up inner lining of Bowman's capsule; have tiny finger-like projections with gaps between them called podocytes

– holes in capillary endothelium and gaps between podocytes quite large, easy for substances dissolved in blood plasma to enter capsule through blood

– basement membrane stops large molecules from getting through (protein; RBC; WBC)

process of ultrafiltration

1. diameter of lumen of afferent arteriole wider than efferent arteriole
2. leads to high blood or hydrostatic pressure
3. plasma/fluid passes through gaps or fenestrations between endothelial cells
4. basement membrane acts as filter or selective barrier
5. red blood cells, large proteins, molecules greater than 68000(MM) cannot pass through
6. podocytes are qualified to pass through

7. filtrate passes into renal capsule

REABSORPTION

in proximal convoluted tubule (selective reabsorption)

– reabsorption of glucose

– steps:

1. basal membranes of cells lining PCT nearest to capillaries; Na^+—K^+ pumps in in these membranes move Na^+ out of cells (Na^+ carried away in blood)
2. this lowers conc. of Na^+ in cell, so they passively diffuse into it, down conc. gradient, from fluid to tubule lumen
3. passive movement of Na^+ into cell down conc. gradient provides energy to move glucose molecules, even against concentration gradient (this is indirect/secondary active transport, since ATP used for something else
4. in cell, glucose diffuses down conc. gradient through transport protein in basal membrane, into blood
5. all glucose, amino acids, vitamins, some Na^+ and Cl^- reabsorbed out of PCT and into blood
6. removal of these solutes increases filtrate water potential, decreases blood water potential; potential gradient exists between blood and filtrate
7. water moves down this gradient through cells into blood and carried back into circulation

– half of urea in filtrate reabsorbed because: it is a small molecule; conc. in filtrate higher than in capillaries, so diffuses passively through PCT cells and into blood

– uric acid and creatinine not reabsorbed

in loop of Henle and collecting duct

– reabsorption of water

– in ascending limb:

1. cells lining this region actively transport sodium/chloride ions out of fluid, into loop, into tissue fluid
2. this decreases water potential in tissue fluid and increases water potential of fluid inside ascending limb

– in descending limb:

1. cells lining this region are permeable to water and ions
2. as fluid flows down this loop, water from filtrate moves down water pot. gradient into tissue fluid by osmosis
3. at same time, sodium/chloride ions diffuse into loop down their conc. gradient

– fluid becomes more concentrated towards bottom of loop

– fluid continues up ascending limb, loses sodium/chloride ions, becomes less concentrated

– counter-current multiplier: having two limbs side by side with fluid running in opposite directions enables max. conc. of solutes to be built up inside and outside of tube at bottom of loop

– cells of ascending limb and cells lining collecting ducts permeable to urea, which diffuses into tissue fluid; as result, urea is also concentrated in tissue fluid in medulla

– fluid continues to DCT and collecting duct

in distal convoluted tubule and collecting duct

– in DCT and collecting duct, sodium ions actively pumped out of fluid in tubule into tissue fluid, from where they pass into blood

– potassium ions actively transported into tubule

– rate at which these ions are moved in/out can vary, helps regulate conc. of ions in blood

CONTROL OF WATER CONTENT

osmoregulation

– control of water potential of body fluids; involves hypothalamus, posterior pituitary gland and kidneys

– osmoreceptors are specialised sensory neurones in hypothalamus that constantly monitors water potential of blood

– when osmoreceptors detect decrease in blood water pot., nerve impulses sent to where they terminate in posterior pituitary gland, stimulating release of ADH

– effect of ADH is to reduce loss of water in urine by making kidneys absorb it

how ADH affects kidneys

– ADH acts on cell surface membranes of collecting duct, makes them more permeable to water

– aquaporin: water-permeable channels in surface membranes of collecting duct cells

– how ADH increases water reabsorption in collecting duct:

1. ADH binds to receptors in surface membrane of cells lining collecting duct
2. this activates series of enzyme-controlled reactions, ending with the production of active phosphorylase enzyme
3. the phosphorylase causes vesicles, surrounded by membrane containing aquaporins, to move to cell surface membrane
4. the vesicles fuse with cell surface membrane
5. water can now move freely through membrane, down water pot. gradient, into concentrated tissue fluid and blood plasma in medulla

– when there is increase in water potential of blood:

1. osmoreceptors no longer stimulated and neurones in posterior pituitary gland stop secreting ADH
2. aquaporins moved out of surface membrane of collecting duct cells, back into cytoplasm; reduces collecting ducts' permeability
3. produces dilute urine
4. water potential in blood constant

CONTROL OF BLOOD GLUCOSE

– homeostatic control of blood glucose concentration carried out by two hormones secreted by endocrine tissue in pancreas

– this tissue consists of group of cells, known as islets of Langerhans, contains:

1. α cells secrete glucagon
2. ß cells secrete insulin

– α and ß cells act as receptors and central control of this homeostatic mechanism

– glucagon and insulin coordinate actions of effectors

negative feedback control for blood glucose concentration

for high blood glucose concentration	**for low blood glucose concentration**
receptors α and ß cells in islets detect rise in blood glucose	**receptors** α and ß cells in islets detect fall in blood glucose
less glucagon, more insulin produced	more glucagon, less insulin produced

effectors liver cells respond to less glucagon; no glycogen breakdown; liver, muscle, fat cells respond to more insulin; increased uptake and use of glucose	**effectors** liver cells respond to more glucagon by breaking down glycogen into glucose; liver, muscle, fat cells respond to less insulin; reduced uptake of glucose

insulin

– signalling molecule

– protein

– cannot pass through cell membranes to stimulate mechanisms within cell directly

– instead, it binds to receptor in cell surface membrane and affects cell indirectly through mediation of intracellular messengers

– stimulates activation of enzyme glucokinase, which phosphorylates glucose; this traps glucose inside cells, as phosphorylated glucose cannot pass through transporters in cell surface membrane

– stimulates activation of two other enzymes:

1. phosphofructokinase
2. glycogen synthase

– together adds glucose molecules to glycogen; increases size of glycogen granules

glucagon and cell signalling

– decrease in concentration of insulin in blood reduces rates of uptake and use of glucose by liver and muscle cells; uptake continues at slower rate

– glucagon binds to different receptor molecules in liver cells' surface membrane

– binding of glucagon to receptor activates G protein that in turn activates enzyme in membrane that catalyses conversion of ATP to cyclic AMP (second messenger)

– cyclic AMP binds to kinase enzymes within cytoplasm that activate other enzymes

– kinase enzymes activate enzymes by adding phosphate groups to them via phosphorylation

– enzyme cascade amplifies original signal from glucagon

– glycogen phosphorylase is at the end of enzyme cascade; when activated it catalyses breakdown of glycogen to glucose (removes glucose units from 'ends' of glycogen)

– this increases glucose conc. inside cell so it diffuses out through GLUT2 transporter

proteins into blood

– glucose made from amino acids and lipids via gluconeogenesis

– as result of glucagon secretion, liver releases extra glucose to increase conc. in blood

adrenaline and glucose

– adrenaline increases conc. of blood glucose by binding to different receptors on surface of liver cells that activate same enzyme cascade as glucagon and lead to same end result

– also stimulates breakdown of glycogen stores in muscles during exercise

DIABETES MELLITUS

– sugar diabetes; 2 forms

1. type I, insulin-dependent, juvenile-onset diabetes
    – pancreas incapable of secreting sufficient insulin
    – due to deficiency in gene coding for insulin production
    – or due to attack on ß cells by body's immune system
    – usually begins very early in life
    – receive regular injections of insulin, take blood samples to check insulin effectivity, some have mini-pumps to deliver exact volumes of insulin when needed
2. type II, non-insulin-dependent
    – pancreas does secrete insulin, but liver/muscle cells do not respond to it
    – begins relatively late in life, often associated with diet and obesity
    – rarely need injections; diet and regular frequent exercise to keep blood glucose conc. in normal limits

– symptoms of both are similar:

1. after carbohydrate meal, glucose absorbed into the blood, conc. increases and stays high
2. glucose, extra water and salts present in urine
3. person consequently feels extremely hungry/thirsty

– uptake of glucose into cells slow, even when there is plenty of glucose in blood, thus cells lack glucose and metabolise fats and proteins as alternative energy source

– leads to build-up of substances in blood called keto-acids (or ketones); produced when body metabolises fat, decreases blood pH

URINE ANALYSIS

– presence of glucose/ketones in urine: diabetic

– long-term presence of proteins in urine: disease affecting glomeruli; kidney infection; high blood pressure

dip sticks

– also known as test strips

– used to test urine for a range of different factors (pH, glucose, ketones, protein)

– sticks to test glucose:

1. contain immobilised enzymes glucose oxidase and peroxidase on small pad at one end of stick
2. pad immersed in urine
3. if contains glucose, glucose oxidase catalyses its oxidation to gluconolactone
4. hydrogen peroxide also produced; peroxidase catalyses its reaction with colourless chemical in pad to form brown compound
5. resulting colour is matched against colour chart; darker colour, more glucose present

biosensor

– problem with urine test: do not indicate current blood glucose conc., but if it was higher than renal threshold in period of time while urine was collecting in bladder

– biosensor allows diabetics to check blood to see how well they are controlling glucose conc.

– uses a pad containing glucose oxidase

– steps:

1. small sample of blood placed on pad, which is inserted into machine
2. glucose oxidase catalyses reaction to produce gluconolactone
3. at same time, tiny electric current generated
4. current detected by electrode, amplified, and read by meter
5. meter produces reading; greater current means more glucose present

HOMEOSTASIS IN PLANTS

– stoma is hole between guard cells

– guard cells are highly specialised cells that respond to wide range of environmental stimuli and thus control internal atmosphere of leaf

– stomata open in response to:

1. increasing light intensity
2. low CO_2 conc. in air spaces within leaf

– when stomata open, leaves gain CO_2 for photosynthesis but lose water in transpiration

– stomata close in response to:

1. darkness
2. high CO_2 conc. in air spaces in leaf
3. low humidity
4. high temperature
5. water stress (when there are high rates of transpiration; lack of water)

– disadvantage of closing: decrease in CO_2 supply, decrease in photosynthesis

– advantage: water retained inside leaf

opening and closing of stomata

– each stomatal pore surrounded by two guard cells

– guard cells open when they gain water to become turgid; they close when they lose water and become flaccid

– guard cells gain/lose water by osmosis

– closing of stomata reduces CO_2 uptake and rate of transpiration; so only occurs when reducing loss of water is important factor

opening		**closing**	
1	decrease in water potential brought by activities of transport proteins in surface membrane	1	H^+ pump proteins stop
2	ATP-powered proton pumps actively transport H^+ out of guard cells	2	K^+ leave guard cells and enter neighbouring cells
3	decrease in hydrogen ion conc. in cell causes channel proteins to open so K^+ move in	3	water potential gradient obtained in opposite direction

4	this is because removal of H^+ leaves inside of cell negatively charged compared to outside	4	water leaves guard cells, they become flaccid, stoma closes
5	electrochemical gradient: K^+ drawn to electrical gradient and diffuse into cells down conc. gradient		
6	K^+ in cell lower solute and water potential, making water pot. gradient between inside/outside of cell		
7	water moves in by osmosis through aquaporins		
8	increases turgor, stoma opens		

abscisic acid

– in conditions of water stress, abscisic acid (ABA) produced to stimulate stomatal closure

– known as stress hormone; coordinate responses to stress

– if plant subjected to difficult environmental conditions (e.g. high temp./reduced water supply), it responds by secreting ABA

– guard cells have ABA receptors on cell surface membranes; when ABA binds with receptors, it inhibits proton pumps to stop H^+ being pumped out

– stimulates movement of Ca^+ into cytoplasm through cell surface membrane and tonoplast

– Ca^+ acts as second messenger to activate channel proteins to open

– channel proteins allow negatively charged ions to leave guard cell

– this stimulates opening of channel proteins to let K^+ move out of cell, and closure of channel proteins to let K^+ in

– loss of ions raises water potential, water moves out, guard cells flaccid, stomata close

CHAPTER 15 COORDINATION

NERVOUS COMMUNICATION

central nervous system (CNS)	brain and spinal cord
peripheral nervous system (PNS)	cranial nerves (connected to brain) spinal nerves (connected to spinal cord)
neurones	nerve cells carry information directly to target cells coordinate activities of sensory receptors and effectors

motor neurones

– transmit impulses from CNS to effectors (muscles/glands)

– cell body lies within CNS, nucleus always in cell body

– dark specks in cytoplasm are regions of rough ER that synthesises proteins

– dendrites: thin cytoplasmic processes extensions from cell body and often have many branches

– motor neurone has many highly-branched dendrites to give large surface area for endings of other neurones

– axon: very long, conducts impulses over long distances

– within cytoplasm of axon, there are some organelles; ends of axon branches have many mitochondria and vesicles containing transmitter substances

– vesicles involved in passing impulses to an effector cell

sensory neurones

– transmit electrical impulses from receptors to CNS

– have same basic structure as motor neurones

– cell body may be near source of stimuli or in ganglion (swelling of spinal nerve)

relay neurones

– entirely in CNS

myelin

– rings surrounding a neurone

– made by specialised cells, Schwann cells, that surround axon of some neurones

– myelin sheath

1. made when Schwann cells wrap themselves around axon in spirals all along its length
2. made largely of lipid with some protein
3. sheath affects speed of conduction of nerve impulse

– nodes of Ranvier: small uncovered areas of axon between Schwann cells (2-3μm long)

reflex arc

– pathway along which impulses are transmitted from a receptor to an effector without involving 'conscious' regions of brain

– within spinal cord, impulse will also be passed on to other neurones which take it to brain

– happens at same time as impulses travelling along motor neurone to effector

– reflex action: effector responds to stimulus before there is voluntary response from conscious part of brain

– steps:

1. receptor takes up stimuli, sends nerve impulses
2. impulses pass through sensory neurone
3. through dorsal root of spinal nerve, cell body of sensory, cell body of relay, cell body of motor, ventral root of spinal nerve, motor neurone, then output at effector

transmission of nerve impulses

– neurones transmit electrical impulses

– impulses travel very rapidly along cell surface membrane of one cell to another

– not a flow of electrons

– brief changes in distribution of electrical charge across cell surface membrane called action potentials; caused by very rapid movement of Na^+/K^+ ions into and out of axon

resting potential

– when there is a difference in electrical potential of inside axon and outside axon (inside axon often 60-70mV lower than outside)

– produced and maintained by Na^+—K^+ pumps in cell membrane

– pumps constantly move ions in/out of axon (3 Na^+ out, 2 K^+ in)

– more protein channels for K^+ than Na^+, so K^+ diffuses back out faster than Na^+ diffusing back in

– many large negatively charged molecules inside cells that attract K^+ reduces chance that they diffuse out

– result of this is overall excess of negative ions inside membrane

– membrane relatively impermeable to Na^+, but Na^+ can still move in due to:

1. steep concentration gradient
2. inside membrane is negatively charged, attracting positively charged ions
3. 'double' gradient like this called electrochemical gradient

1. action potential

– caused by changes in permeability of cell surface membrane to Na^+ and K^+

– other channels in surface membrane open/close depending on electrical potential voltage across membrane; these are called voltage-gated channels (they close when membrane is at resting potential)

– depolarisation:

1. electric current used to stimulate axon causes opening of voltage-gated channels, allowing Na^+ to enter
2. potential difference changes across membrane, becomes less negative
3. depolarisation triggers positive feedback; more channels open, more Na^+ enter
4. action potentials only generated if potential difference reaches -60mV to -50mV; this is called threshold potential; less than this, action potential does not occur

– repolarisation:

1. after about 1 ms, all Na^+ voltage-gated channels close so Na^+ stop diffusing in; at same time, K^+ channels open, K^+ diffuse out of axon, down conc. gradient
2. K^+ moving out removes positive charge in axon, returning potential difference to normal

2. transmission of action potentials

– action potential triggers production of action potential in membrane on either side of axon
– temporary depolarisation of membrane at site of action potential causes local circuit to be set up between depolarised region and resting regions on either side of it
– local circuits depolarise adjoining regions and generate action potentials in them
– in body, action potentials begin at one end and ‘new’ action potentials generated ahead
– that is because region behind it still recovering from previous action potential, and Na^+ voltage-gated channels shut tight, cannot be stimulated to open
– refractory period: when axon is unresponsive
– consequences of refractory period:
    1. action potentials are discrete events; do not merge
    2. there is minimum time between action potentials occurring at neurone
    3. length of refractory period determines max. frequency at which impulses are transmitted along neurones

3. how action potentials carry information

– action potentials do not change in size; frequency does
– strong stimulus produces rapid succession of action potentials, one after another; likely to stimulate more neurones; produces action potentials in many more neurones
– weak stimulus results in fewer action potentials per second; stimulates less neurones; result in action potentials only passing along one/two neurones
– brain can interpret frequency of action potentials arriving along axon of sensory neurone and no. of neurones carrying it, to get information on:
    1. strength of detected stimulus
    2. nature of stimulus (light, heat, touch)
    3. deduced from position of stimulus (retina, skin)

4. speed of conduction

– slow in unmyelinated neurones
– myelin speeds up travel rate of action potential by insulating axon membrane

– Na^+/K^+ cannot flow through myelin sheath, impossible for depolarisation/action potentials to occur
– action potentials can only occur in nodes of Ranvier, where channel/pump proteins are concentrated
– saltatory conduction: local circuits exist from one node to the next so action potentials jump between them
– in myelinated axon, saltatory conduction can increase transmission speed
– diameter can affect transmission speed; thick axons transmit impulses faster, less resistance

5. what starts action potential

– receptor cell responds to stimulus by initiating action potential
– they are transducers, convert energy in one form into energy in an electrical impulse in a neurone
– some receptors are specialised cells that detect specific type of stimulus
– tongue:
    1. covered in many papillae; each papilla has many taste buds over surface
    2. within each taste buds are chemoreceptors covered in receptor proteins; sensitive to chemicals
    3. chemoreceptors that detect salt directly influenced by Na^+
    4. Na^+ diffuse through selective channel proteins of microvilli membrane, leads to depolarisation; the increase in positive charge inside cell is receptor potential
    5. if stimulation enough by Na^+, receptor potential can stimulate voltage gated Ca^+ channels to open
    6. Ca^+ enter cytoplasm and lead to exocytosis of vesicles containing neurotransmitter
    7. neurotransmitter stimulates action potential in sensory neurone that transmits impulses to taste centre in cerebral cortex of brain
    8. sweet chemoreceptors have protein receptors to stimulate G protein and produce cyclic AMP, which acts as second messenger, leads to closure of K^+ channels, depolarisation

9. weak stimulus, no activation; all-or-nothing law; threshold level constant

synapses

– synaptic cleft: gap between two neurones

– presynaptic/postsynaptic neurones: neurones on either side of cleft

1. mechanism of synaptic transmission

– steps with acetylcholine and cholinergic synapses:

1. arrival of action potential opens Ca^+/Na^+ VGC in part of membrane of presynaptic neurone beside synaptic cleft, Ca^+/Na^+ diffuse into cytoplasm
2. influx of Ca^+ stimulates vesicles containing ACh to move and fuse with presynaptic membrane, emptying contents into synaptic cleft
3. ACh diffuses across synaptic cleft
4. cell surface membranes of postsynaptic neurone contains receptor proteins partially complementary to ACh; ACh binds temporarily with it
5. binding changes shape of protein and opens chemically gated ion channels (stimulated by neurotransmitter) so Na^+ diffuse into cytoplasm of postsynaptic neurone and depolarise membrane
6. ACh prevented from permanently binding by recycling using acetylcholinerase, which catalyses hydrolysis of ACh to acetate and choline
7. choline taken back to presynaptic neurone, combined with ACoA to form ACh; ACh transported into presynaptic vesicles, ready for next action potential
8. entire sequence takes 5-10 ms; depolarisation only occurs when potential difference above threshold for that neurone

2. roles of synapses

– ensure one-way transmission; impulses can only pas in one direction at synapse as neurotransmitter released at one side, receptors at another

– allow integration of impulses; if depolarisation of postsynaptic neurone does not reach threshold, no impulse sent to that neurone; brain not overloaded

– allow interconnection of nerve pathways in two ways:

1. individual sensory/relay neurones have axons that branch to form

synapses with many different neurones

2. there are many neurones that terminate on each relay/motor neurone as they have many dendrites to give a large surface area for many synapses

– synapses are involved in memory and learning; if brain frequently receives information about two things at same time (e.g. face and sound), new synapses form in brain that link neurones involved in passing of information along particular pathways

MUSCLE CONTRACTION

	type of muscle		
	striated	**cardiac**	**smooth**
appearance in light microscope	stripes (striations) at regular intervals	stripes (striations) at regular intervals	no striations
cell structure	multinucleate (syncytium)	uninucleate cells joined by intercalated discs	uninucleate cells
shape of cells	long, unbranched cylinder	shorter with branches that connect to adjacent cells	long, unbranched cells that taper at ends
organisation of contractile proteins inside cell	parallel bundles of myofibrils	parallel bundles of myofibrils	contractile proteins not organised into myofibrils
distribution in body	attached to skeleton	heart	tubular structures
control	neurogenic	myogenic	neurogenic

structure of striated muscle

– sarcolemma: cell surface membrane

– sarcoplasm: cytoplasm

– sarcoplasmic reticulum (SR): ER

– transverse system tubules/T-tubules: deep infoldings of cell surface membrane into the interior of muscle fibre, run close to SR

– membranes of SR have huge numbers of protein pumps that transport Ca^{+} into cisternae of SR

– sarcoplasm contains many mitochondria, often packed tightly between myofibrils; these carry out aerobic respiration to generate ATP for muscle contraction

– striations

1. produced by regular arrangement of myofibrils in sarcoplasm
2. each myofibril striped in exact same way, lined up precisely against next one and produces a pattern
3. with electron microscope, myofibril is made up of smaller components (filaments)
4. parallel groups of filaments lie between groups of thin ones
5. Z line provides attachments for thin filaments, M line for thick ones
6. part of myofibril between two Z lines called sarcomere
7. myofibrils cylindrical, so Z line also called Z disc

structure of thick and thin filaments

thick	**thin**
myosin (fibrous protein with globular head)	actin (globular protein)
fibrous portion helps anchor molecule into thick filament	linked together to form a chain
myosin molecules all lie together in bundle with globular heads pointing away from M line	two of these chains twisted together to form thin filament
	also twisted around actin chains is fibrous protein tropomyosin; troponin attached to actin chain at regular intervals

how muscles contract

1. sarcomeres in each myofibril get shorter as Z disc pulled closer together (sliding filament)
2. when muscle contracts, Ca^{+} released from stores in SR and bind to troponin; this stimulates troponin to change shape
3. troponin and tropomyosin proteins move to different position on thin filaments, exposing parts of actin molecules which acts as binding sites for myosin
4. myosin heads bind with these sites, forming cross-bridges between two types of filament
5. myosin heads tilt, pulling acting filaments along towards centre of sarcomere

6. heads hydrolyse ATP, provide enough energy to force heads to let go of actin
7. heads tip back to previous position and bind again to exposed sites of actin
8. thin filaments moved as a result of power stroke, so myosin heard bind to actin further along, then hydrolyse more ATP to let go again

stimulating muscle to contract

at the neuromuscular junction	**at the muscle fibre**
action potential arrives	depolarisation of sarcolemma spreads down T-tubules
action potential causes diffusion of Ca^+ into neurone	channel proteins for Ca^+ open and Ca^+ diffuse out of SR
Ca^+ cause vesicles containing acetylcholine to fuse with presynaptic membrane	Ca^+ binds to troponin; tropomyosin moves to expose myosin binding-sites on actin filaments; myosin heads form cross-bridges with thin filaments and the sarcomere shortens
acetylcholine released and diffuses across synaptic cleft	
acetylcholine molecules bind with receptors in the sarcolemma, causing them to open channel proteins for Na^+	
Na^+ diffuse in through open channels in sarcolemma; this depolarises membrane and initiates action potential which spreads along membrane	

providing ATP for muscle contraction

– available in sarcoplasm

– produced by respiration (both aerobic respiration in mitochondria and lactic fermentation in sarcoplasm)

– produced by creatine phosphate (stored in sarcoplasm); immediate source of energy when ATP in sarcoplasm used up

1. phosphate group can quickly/easily be removed
2. combined with ADP to produce ATP
3. if energy still required, creatine converted to creatinine and excreted in urine

HORMONAL COMMUNICATION

– control by nervous system ‘expensive in terms of energy; hormones provide alternative

– hormones made in endocrine glands (gland: group of cells secreting substances)

– hormones are cell signalling molecules

hormonal control of human menstrual cycle

– menstrual cycle approximately 28 days

– ovulation steps:

1. potential female gamete starts to develop
2. primary follicle produced by development of tissues surrounding developing gamete
3. primary follicle becomes secondary follicle
4. secondary follicle develops into ovarian or Graafian follicle
5. at ovulation, gamete released
6. remaining tissue forms corpus luteum

– uterine cycle (synchronised with ovarian cycle):

1. most of endometrium lining lost during menstruation if no fertilisation occurred; at same time, follicle develops in ovary
2. endometrium develops; ovarian follicle produced
3. endometrium maintained until corpus luteum degenerates
4. endometrium most developed and contains many blood vessels; ovulation occurs

– coordinated by glycoprotein hormones released by anterior pituitary gland/ovaries

– APG secretes follicle stimulating hormone (FSH) and luteinising hormone (LH); these control ovary activity

– follicles develop which secrete oestrogen

– after female gamete released in ovulation, remains of follicle secretes progesterone

– menstruation is beginning of cycle; lasts 4-8 days; AGP secretes LH/FSH so conc. increases

– in ovary, one follicle becomes dominant one; LH/FSH stimulates secretion of oestrogen from cells surrounding follicle

– oestrogen in blood has negative feedback effect; stimulates endometrium to thicken

– when oestrogen conc. in blood quadruples, it stimulates secretion of LH and some FSH; LH causes dominant follicle to burst and shed its gamete into oviduct

– follicle collapses to form corpus luteum (yellow body) which secretes progesterone and oestrogen; these two maintain lining of uterus so it is ready to receive embryo
– progesterone inhibits APG from secreting FSH so no more follicles develop
– high oestrogen/progesterone levels in second half of cycle inhibit LH/FSH secretion; less stimulation of corpus luteum so it begins to degenerate and secrete less oestrogen progesterone
– decrease in oestrogen/progesterone means endometrium not maintained and menstruation begins; also releases APG from inhibition, so FSH secreted for cycle

BIRTH CONTROL

birth control pill

– contains synthetic steroid hormones to suppress ovulation
– synthetic over natural because not broken down so rapidly, therefore act for longer
– oral contraceptives taken daily for 21 days then stop for a week during menstruation
– oestrogen/progesterone suppress secretion of FSH/LH, thus prevents ovulation
– oestrogen/progesterone can also be given via skin patch/injection/skin implants
– pills containing progesterone only may allow ovulation; they only reduce sperm's ability to fertilise egg and make mucus more viscous so less easily penetrated by sperm

morning-after pill

– taken after unprotected sex; up to 72 hours after
– contains synthetic progesterone-like hormone
– reduces chances of sperm reaching and fertilising egg; in other cases, prevents implantation of embryo into uterus

CONTROL AND COORDINATION IN PLANTS

electrical communication in plants

– action potentials triggered when membrane depolarised
– microelectrodes inserted into leaf cells detect changes in potential difference that are very similar to action potentials in animals
– depolarisation results from outflow of negatively charged Cl^-; repolarisation achieved in same way by outflow of K^+
– plant cells transmit waves of electrical activity; action potentials travel along cell

membranes of plant cells and plasmodesmata

chemical communication in plants

– plant hormones/growth regulators

– produced by variety of tissues; carried in phloem/xylem sap or from cell to cell

– two types of plant growth regulator:

1. auxin — elongation/determines length of roots/shoots
2. gibberellins — seed germination/stem elongation

– plant hormones interact with receptors on surface of cells/cytoplasm/nucleus; receptors initiate series of chemical/ionic signals that amplify/transmit signal within cell

– auxins and elongation growth:

1. principal: IAA (indole 3-acetic acid)
2. auxins synthesised in growing tips (meristems) of shoots/roots
3. transported back down shoot, or up root, by active transport from cell to cell
4. growth in plants occurs at meristems, three stages:
   – cell division (mitosis)
   – cell elongation (absorption of water)
   – cell differentiation
5. auxins stimulates cells to pump H^+ into cell wall; this acidifies it, leading to loosening of bonds between cellulose microfibrils and matrix surrounding them
6. cells absorb water by osmosis and pressure potential stretches/elongates wall
7. auxin binds to receptor protein on membrane; stimulates ATPase proton pumps to move H^+ across membrane from cytoplasm to wall
8. decrease in pH activates expansin proteins; expansins loosen linkages between cellulose microfibrils; microfibrils free to expand

gibberellins

– high conc. in young leaves and stems

1. gibberellins and stem elongation

– dominant allele required to be present for synthesis of enzyme in a pathway that produces active form of gibberellin (GA1)

– active gibberellin stimulates cell division and cell elongation in stem

– substitution mutation in this gene changes Ala to Thr in primary structure of enzyme near active site, produces non-functional enzyme; gives rise to recessive allele

2. gibberellins and seed germination

– when seeds shed from parent plant, it is in state of dormancy (contains little water and metabolically inactive); allows seed to survive in adverse conditions
– seed contains embryo, surrounded by endosperm (energy store of starch); outer edge of endosperm is protein-rich aleurone layer; whole seed covered by tough waterproof protective layer
– absorption of water in start of germination stimulates embryo to produce gibberellins; these diffuse to aleurone layer and stimulate cells to synthesise amylase
– amylase mobilises energy reserves by hydrolysing starch into endosperm, converting them to soluble maltose molecules
– maltose molecules converted to glucose and transported to embryo, providing carbohydrate source that can be repaired to provide energy during embryo growth

CHAPTER 16 INHERITED CHANGE

karyogram	a photograph or diagram of a set of chromosomes from an individual; chromosomes are normally arranged in homologous pairs in order of size; sex chromosomes may be shown separately
homologous chromosomes	pair of chromosomes in a diploid cell that have same structure as each other, with same genes at same loci, and that pair together to form bivalent during first division of meiosis
gene	length of DNA that codes for particular protein or polypeptide
allele	particular variety of gene
locus	position at which particular gene is found on particular chromosome; the same gene is always found at the same locus
diploid cell	possesses two complete sets of chromosomes; abbreviation is 2n
haploid cell	possesses one complete set of chromosomes; abbreviation is n
genotype	alleles possessed by an organism
homozygous	means having two identical alleles of a gene
heterozygous	means having two different alleles of a gene
phenotype	an organism's characteristics, often resulting from an interaction between its genotype and its environment
dominant allele	effect on phenotype of heterozygote identical to effect in homozygote
recessive allele	only expressed when no dominant allele is present
codominant allele	both have effect on phenotype of heterozygous organism
F1 generation	offspring resulting from a cross between an organism with a homozygous dominant genotype, and one with a homozygous recessive genotype
F2 generation	offspring resulting from a cross between two F1 (heterozygous) organisms
test cross	genetic cross in which an organism showing a characteristic caused by a dominant allele is crossed with an organism that is homozygous recessive; the phenotypes of offspring can be a guide to whether the first organism is homozygous or heterozygous

linkage	the presence of two genes on same chromosome, so that they tend to be inherited together and do not assort independently

HOMOLOGOUS CHROMOSOMES

– in a human karyogram

1. 22 matching pairs of homologous chromosomes; each pair given a number
2. non-matching X and Y chromosomes are sex chromosomes that determines sex; other chromosomes called autosomes
3. pairs of chromosomes can be distinguished by shape, size, and distinctive banding pattern when stained with certain stains

– each chromosome has characteristic set of genes coding for different features

– gene for particular characteristic always found at same position (locus) on chromosome

– a gene for a given characteristic may exist in different forms (alleles), expressed differently

haploid and diploid cells

– haploid, one set of chromosomes (n)

– diploid, two sets of chromosomes (2n)

– in humans, normal body cells are diploid (46 chromosomes), gametes are haploid (23 chromosomes)

TWO TYPES OF NUCLEAR DIVISION

– **growth**; when single-celled diploid zygote grows into an adult with millions of cell, new cells must be genetically identical, with same number of chromosomes as cells that divided to produce them; done by **mitosis**

– **sexual reproduction**; number of chromosomes is halved before fertilisation; results in gametes containing only one set of chromosomes; done by **meiosis**; reduction division

– meiosis also introduces genetic variation into gametes and zygotes produced

– genetic variation also arise from mutation

MEIOSIS

– meiosis I and meiosis II

1. meiosis I: reduction division resulting in two daughter nuclei with half the no. of

chromosomes of parent nucleus

2. meiosis II: chromosomes behave as in mitosis, so each of two haploid daughter nuclei divides again; results in 4 haploid nuclei

– two events help produce genetic variation; independent assortment of homologous chromosomes, and crossing over (between chromatids of homologous chromosomes)

meiosis I

1. early prophase I

– as mitosis early prophase

– centromeres move to opposite ends of nucleus

2. middle prophase I

– homologous chromosomes pair up

– process called synapsis

– each pair called bivalent

3. late prophase I

– nuclear envelope breaks up as in mitosis

– crossing over of chromatids may occur

– nucleolus ‘disappears’ as in mitosis

– bivalent crossing over:

1. chromatids break and reconnect to another chromatid
2. chiasma (p. chiasmata): point where crossing over occurs
3. one or more chiasmata may form, anywhere along length

– at the end of prophase I a spindle is formed

4. metaphase I

– bivalents line up across equator of spindle attached by centromeres

– spindle formed, as in mitosis

5. anaphase I

– centromeres do not divide, unlike in mitosis

– whole chromosomes move towards opposite ends of spindle, centromeres first,

pulled by microtubules

6. telophase I

– nuclear envelope re-forming

– nucleolus re-forming

– cytokinesis

– remains of spindle

– chromosomes reached poles of spindle

– animal cells usually divide before entering meiosis II

– many plant cells go straight into meiosis II with no reformation of nuclear envelopes or nucleoli

– during meiosis II, chromatids separate as in mitosis

meiosis II

7. prophase II

– nuclear envelope and nucleolus disperse

– centrosomes and centrioles replicate and move to opposite poles of the cell

8. metaphase II

– chromosomes line up separately across equator of spindle

9. anaphase II

– centrosomes divide and spindle microtubules pull the chromatids to opposite poles

10. telophase II

– similar to telophase of mitosis, but in meiosis telophase II

– 4 haploid daughter cells are formed

gametogenesis in humans

spermatogenesis	**oogenesis**
formation of male gametes	formation of female gametes
takes place inside tubules in testes	takes place in ovaries

diploid cells divide by mitosis to produce diploid spermatogonia	diploid cells divide by mitosis to produce oogonia
spermatogonia grow to form diploid primary spermatocytes	oogonia begin to divide by meiosis but stop when they reach prophase I; primary oocytes at this stage; still diploid
meiosis I takes place forming two haploid secondary spermatocytes	all of this before birth of female; at puberty, some primary oocytes get further in meiosis, forming two haploid cells
meiosis II produces haploid spermatids	division is uneven, produces secondary oocyte with more cytoplasm; other is little more than nucleus, called polar body
spermatids mature to spermatozoa	each month, one secondary oocyte released to oviduct from ovary; if fertilised, continues meiosis and becomes ovum
	chromosomes of spermatozoan and ovum join to form single diploid nucleus (zygote)
	fewer gametes made than spermatogenesis
	longer process with ‘waiting stages’

gametogenesis in flowering plants

– male gametes produced in anthers; female gametes in ovules

– male gamete formation:

1. inside anthers, diploid pollen mother cells divide by meiosis to form 4 haploid cells
2. the nuclei of these cells then divide by mitosis, but cytokinesis does not occur; results in cells with 2 haploid nuclei
3. these cells mature into pollen grains, each surrounded by tough exine and thinner intine
4. one of the haploid nuclei is called tube nucleus, the other generative nucleus

– female gamete formation:

1. in ovule, large diploid spore mother cell develops
2. this divides by meiosis to produce 4 haploid cells
3. all but one degenerates, and surviving haploid cell develops into embryo sac
4. embryo sac grows larger, its haploid nucleus divides by mitosis 3 times, forming 8 haploid nuclei
5. one of these becomes female gamete

– fertilisation

1. occurs when male gamete from pollen grain fuses with female gamete inside ovule
2. this forms diploid zygote, which grows into embryo plant

GENETICS

alleles

– different varieties of same gene

– on chromosome 11; each cell has 2 (maternal/paternal) copies of ß-globin polypeptide gene

– GGACTTCTC normal ß-globin polypeptide; GGACATCTC sickle cell anaemia

genotype

– Hb^A normal; Hb^S sickle cell

– Hb stands for locus of haemoglobin gene

– Hb^AHb^A, Hb^SHb^S both homozygous

– Hb^AHb^S heterozygous

GENOTYPE AFFECTS PHENOTYPE

– Hb^AHb^A completely normal

– Hb^SHb^S all sickle cell haemoglobin

– Hb^AHb^S half normal, half sickle cell; carrier

– phenotype is observable characteristics of an individual

INHERITING GENES

– when both parental gametes carry HbAHbS, offspring's possible genotypes can vary

	Hb^A	Hb^S
Hb^A	Hb^AHb^A	Hb^AHb^S
Hb^S	Hb^AHb^S	Hb^SHb^S

– probability of Hb^AHb^A/Hb^SHb^S 0.25; Hb^AHb^S 0.50

– production of gametes with 2 different genotypes via meiosis of heterozygous cell

1. homologous chromosomes each carry different allele of gene for ß-globin polypeptide

2. during prophase I, homologous chromosomes pair
3. at end of meiosis I, homologous chromosomes separate into different nuclei
4. two haploid cells formed, with different genotypes
5. each haploid cell divides again, form 4 daughter cells
6. each cell develops into gamete, half with Hb^A, half with Hb^S

genetic diagrams

– standard way of showing genotypes of offspring that might be expected from 2 parents

– first, make a key table for the different genotypes and their corresponding phenotype

– next make a second table containing the following information:

1. parental phenotypes
2. parental genotypes
3. gametes
4. offspring genotypes and phenotypes (punnet square)

dominance

– dominant allele affects phenotype, even in heterozygous genotypes

– codominant alleles both have effect on phenotype (red allele and white allele in one genotype produces pink colour)

– recessive allele does not affect phenotype in heterozygous cells, only in homozygous recessive genotypes

test crosses

– do a genetic diagram

– to determine genotype

MULTIPLE ALLELES

– when a gene has more than two alleles

– e.g. human blood groups

– I^A and I^B codominant, I^O recessive to all

SEX INHERITANCE

– sex chromosomes are X and Y

– not always homologous, do not always have genes in the same locus

– Y shorter than X, carries less genes

SEX LINKAGE

– sex-linked gene: gene found in a part of X chromosome, part not present in Y

– factor VIII protein coding for blood clotting

– H normal dominant allele producing factor VIII, h recessive causes haemophilia

– gene for haemophilia present in X chromosome only

– X^H and X^h

– haemophilia only found in males, females may be carriers

DIHYBRID CROSSES

– look at inheritance of two genes at once

– at metaphase of meiosis I, pairs of homologous chromosomes line up on equator independently of one another

– for two pairs of chromosomes, there are two possible orientations

– at end of meiosis II, each orientation gives 2 kinds of gamete; therefore 4 types of gamete altogether

– independent assortment (chromosomes lining up in different ways)

INTERACTIONS BETWEEN LOCI

– there are cases where different loci interact to affect one phenotypic character

– (this is the one with the large 4x4 Punnet square thing)

AUTOSOMAL LINKAGE

– when two or more gene loci are on same chromosome, they do not assort independently in meiosis as they would if on different chromosomes; genes said to be linked

– linkage is presence of two genes on same chromosomes, inherited together, do not assort independently

CROSSING OVER

– during prophase I of meiosis, pair of homologous chromosomes (a bivalent) can be seen to be joined by a chiasmata

– the chromatids of a bivalent may break and reconnect to another, non-sister chromatid

– results in exchange of gene loci between maternal/paternal chromatid

– produces recombinant classes

χ^2 (CHI-SQUARED) TEST

$$\chi^2 = \sum [(E - O)^2/(E)]$$

– E is expected value, O is observed value

– allows comparison for observed results with expected results, and decide whether or not there is significant difference between them

– steps:

1. work out expected results (no. of offspring x probability)
2. record E, O in table
3. calculate difference between each set of results
4. square the differences
5. put it in formula
6. degree of freedom = no. of classes of data, minus 1
7. look into table of chi-squared values

degrees of freedom	probability greater than			
	0.1	0.05	0.01	0.001
1	2.71	3.84	6.64	10.83
2	4.60	5.99	9.21	13.82
3	6.25	7.82	11.34	16.27
4	7.78	9.49	13.28	18.46

8. if value is at 0.05 or larger, no significant difference

MUTATIONS

– an unpredictable change in genetic material of an organism

– gene mutation: change in DNA molecule structure, gives rise to different alleles of a gene

– chromosome mutation: change in structure/no. of whole chromosomes in cell

– mutagen: substance that increases chances of mutation occurring

– 3 ways of gene alteration:

1. base substitution: one base replaces another

2. base addition: extra base added to sequence
3. base deletion: base lost from sequence

– base addition/deletion may cause frame shifts, produce useless protein
– base substitution that does not affect on organism called silent mutation (when sequence still codes for same amino acid)

sickle cell anaemia

– caused by base substitution
– CTT replaced by CAT (Glu to Val)
– when haemoglobin dissociated with oxygen, 'unusual' ß-globin polypeptide makes it less soluble
– haemoglobin molecules the stick together, forming long fibres inside red blood cells
– red cells pulled out of shape, into sickle shape
1. useless in carrying oxygen
2. stuck in capillaries
– suffer severe anaemia

albinism

– dark pigment melanin is totally/partially missing from eyes, skin, hair; pale blue or pink irises, pale skin/hair, pupils appear red
– also poor vision, rapid/jerky movements of eyes, tendency to avoid bright light
– caused by mutation at several loci, as autosomal recessive
– mutation in gene for enzyme tyrosinase results in absence of tyrosinase or presence of inactive tyrosinase in cells responsible for melanin production
– in melanocytes, first two steps of conversion of amino acids, tyrosine into melanin cannot take place; tyrosine cannot be converted to DOPA and dopaquinone
– tyrosinase is an oxidase and has 2 Cu atoms in active site which bind to oxygen molecule

Huntington's disease

– mutation inherited as dominant allele
– neurological disorder
– involuntary movements (chorea) and progressive mental deterioration
– brain cells are lost, ventricles of brain become larger

– mutation is unstable segment in gene on chromosome 4 coding for protein, huntingtin

– normal is small number of repeats for CAG, HD larger number of repeats for CAG (called a stutter)

GENE CONTROL IN PROKARYOTES

– transcription factors:

1. control transcription of gene
2. proteins that bind to specific DNA sequence and control flow of information from DNA to RNA by controlling formation of mRNA

– structural genes and regulatory genes

1. structural genes: code for proteins required by cell (form part of cellular structure; act as enzymes)
2. regulatory genes: code for proteins that regulate expression of other genes

– repressible and inducible enzymes

1. synthesis of repressible enzyme can be prevented by binding a repressor protein to specific site called operator, on bacterium's DNA
2. synthesis of inducible enzyme occurs only when substrate is present; transcription of gene occurs as result of inducer (substrate) interacting with protein produced by regulatory gene

– lac operon controls gene expression in prokaryote

– operon is length of DNA making up a unit of gene expression in bacterium; consists of one or more structural genes and also control regions of DNA recognised by products of regulatory genes

lac operon

– ß-galactosidase hydrolyses disaccharide lactose to monosaccharides glucose and galactose

– consists of cluster of 3 structural genes and length of DNA including operator and promoter regions

– structural genes:

1. lacZ, coding for ß-galactosidase
2. lacY, coding for permease (allows lactose to enter cell)
3. lacA, coding for transacetylase

– regulatory gene close to promoter, not part of operon

– sequence of events when no lactose in medium which bacterium grows:

1. reg. gene codes for protein called repressor
2. repressor binds to operator region, cloys to gene for ß-galactosidase
3. in presence of bound repressor at operator, RNA polymerase cannot bind to DNA at promoter region
4. no transcription, as of 3 struc. genes can take place

– repressor protein is allosteric (has 2 binding sites)

1. when protein binds to a molecule at one site, it affects ability to bind to a different molecule at the other site
2. site that binds to DNA separate from site for lactose
3. when lactose binds to its site, protein shape changes so DNA binding site closed

– when lactose present in medium where bacterium grows

1. lactose taken up by bacterium
2. lactose binds to rep. protein, distorts its shape, prevent binding of DNA at operator
3. transcription no longer inhibited, mRNA produced from 3 struc. genes; genes switched on and transcribed together

– advantages of mechanism:

1. allows bacterium to produce ß-galactosidase, permease and transacetylase only when lactose available, and to produce them in equal amounts
2. avoids waste of energy/materials in producing enzymes for taking up/hydrolysing sugar that bacterium never meets
3. sugar can be hydrolysed when available

– when bacterium in medium with both glucose and lactose, lac operon repressed

GENE CONTROL IN EUKARYOTES

– eukaryotes have more ways to regulate gene expression than prokaryotes

– factors may bind to promoter region of gene; may increase/decrease transcription of gene

– role is to make sure genes are expressed in correct cell at correct time/extent

– effects of transcription factors:

1. general transcription factors necessary for transcription to occur; they form part of protein complex binding to promoter region of gene concerned
2. other factors activate appropriate genes in sequence, allowing correct pattern of

development of body regions

3. transcription factor is responsible for determining sex in animals
4. transcription factors allow responses to environmental stimuli
5. some transcription factors (e.g. products of proto-oncogenes/tumour suppressor genes) regulate cell cycle, growth and apoptosis (programmed cell death)
6. hormones have effect through transcription factors

– gibberellin causes increase in transcription of mRNA coding for amylase

1. gibberellin causes breakdown of DELLA proteins
2. DELLA proteins inhibit binding of transcription factor to gene promoter
3. by causing breakdown of DELLA protein, gibberellin allows factor to bind to target promoter
4. transcription of gene can take place, results in increase of amylase production

CHAPTER 17 SELECTION AND EVOLUTION

VARIATION

– genetic variation caused by:

1. independent assortment of chromosomes and alleles during meiosis
2. crossing over between chromatids of homologous chromosomes during meiosis
3. random mating between organisms within species
4. random fertilisation of gametes
5. mutation

– first four processes reshuffle existing alleles in population

– genetic variation when offspring allele combination differs from parents cause phenotypic variation

– mutation does not reshuffle alleles already present, instead produce new alleles

1. mutation when mistake occurs in DNA replication
2. new base sequence occurs in gene
3. unpredictable change in gene is gene mutation
4. mutation on somatic cells (body cells) no effect on organism
5. mutation in sex cells that divide to form gametes, which may form zygote, affects offspring

– genetic variation provides raw material on which natural selection can act

– phenotypic variation also caused by environmental factors

continuous and discontinuous variation

continuous variation	**discontinuous variation**
quantitative	qualitative
difficult to distinguish	easily distinguishable categories
has intermediates	no intermediates
e.g. height	e.g. four blood groups

genetic basis	
different alleles at single gene locus have small effects on phenotypes	different alleles at single gene locus have large effects on phenotypes
different genes have same, often additive, effect on phenotype	different genes have quite different effects on phenotype
large number of genes may have combined effect on particular phenotypic trait; these genes are polygenes	

– dominance and gene interaction reduce phenotypic variation

environmental effects on phenotype

– environmental effects may either allow full genetic potential height to be reached, or stunt it

– less food, less nutrition, less light intensity, low temperature

NATURAL SELECTION

– as population of organism increases, so do environmental factors that keep down numbers

– types of factors:

1. biotic: caused by other living organisms (e.g. predation, food competition, disease)
2. abiotic: caused by non-living components of environment (e.g. water supply)

– factors aim to slow rate of growth of population

– over period of time, population size will oscillate around a mean value

– variation within population means some organisms may have advantageous features (e.g. coat colour)

– fitness: used to refer to extent of organism's adaptation to environment; capacity of an organism to survive and transmit genotype to offspring

– selection pressure: increases chances of some alleles to be passed on to next generation (e.g. predation)

– natural selection: effects of selection pressures on frequency of alleles in population; raises frequency of alleles conferring an advantage and reduce those with disadvantage

EVOLUTION

– general theory that organisms have changed over time

– stabilising selection: when natural selection keeps things the way they are

– directional selection: if new environmental factor/allele appears, allele frequencies may also change

– disruptive selection: occurs when conditions favour both extremes of population; maintains different phenotypes (polymorphism) in population

new environmental factor

– organisms with features adapted to new environmental factor more likely to survive

– pass genes to offspring

new allele

– mutation may give rise to harmful/neutral/useful alleles

– new allele may give better adaptation to environment, hence giving organism selective advantage

– over more generations, useful allele may become the majority feature

– changes in allele frequency in population are basis of evolution

– evolution occurs because natural selection gives some alleles better chance go survival

1. antibiotic resistance

– antibiotics are chemicals produced by living organisms that inhibit/kill bacteria

– penicillin stops cell wall formation, preventing cell reproduction

– resistance to penicillin starts when bacteria produce enzyme penicillinase, inactivates penicillin

– bacteria only have single loop of DNA, so mutant allele has immediate effect on phenotype

– bacteria with resistance survive and reproduce

– alleles for antibiotic resistance often occur on plasmids

– plasmids can be transferred from one bacterium to another, even cross-species

2. industrial melanism

– greater frequency of dark (melanic) allele appeared peppered moths

– majority of dark moths in industrial areas, pale wings in non-industrial areas

– changes in environmental factors only affect likelihood of allele surviving in population; not likelihood of allele arising by mutation

3. sickle cell anaemia

– people who are heterozygous (HbAHbS) less likely to suffer serious attacks of malaria

– two strong selection pressures acting on HbA HbS:

1. selection against homozygous HbS people very strong, as they become seriously anaemic
2. selection against homozygous HbA also very strong, more likely to die of malaria

– heterozygous have strong selective advantage (not anaemic)

genetic drift

– change in allele frequency that occurs by chance, as only some of organisms of each generation reproduce

– small number of individuals separated from a larger population

– have different allele frequencies from parent population

– founder effect: further genetic drift alters allele frequencies more, causing different direction of evolution from parent population

Hardy-Weinberg principle

– calculation to see proportions of genotypes

– p is frequency of dominant allele, q frequency of recessive allele

– total of whole population is 1, frequencies in decimals

$$p + q = 1$$

– chance of offspring inheriting dominant/recessive allele from both parents

p^2 or q^2

– chance of offspring inheriting dominant allele from one parent and recessive from another

pq

– so altogether

$$p^2 + 2pq + q^2 = 1$$

– do not apply when:

1. population is small
2. significant selective pressure against one of the genotypes

3. migration of individuals carrying one of two alleles in/out of population
4. non-random mating

– when ratios of different genotypes in population determined, predicted ratios in next generation can be compared with observed values
– differences can be tested for significance using chi-squared test
– if differences significant and migration/non-random mating discounted, directional selection occurring in population

ARTIFICIAL SELECTION

– when important selection pressures on organisms applied by humans

selective breeding of dairy cattle

– desired features:
1. docility (easier to control)
2. fast growth rates
3. high milk yields

– individuals showing desired features chosen for breeding, process repeated over generations until ‘disadvantageous’ alleles almost entirely lost
– problems:
1. animals are large and need time to mature
2. gestation period long
3. number of offspring produced is small

– progeny testing to see if male’s female offspring valuable with desired features

crop improvement

– using selective breeding and gene technology; add/alter genes into species to change its characteristics
– selective breeding has produced different varieties of wheat
– breeding also to obtain resistance to fungal diseases
– wheat plants now have much shorter stems:
1. easier to harvest
2. higher yield (more energy to produce seeds instead of growing tall)
3. less susceptible to being knocked flat by heavy rain

4. produce less straw

– dwarf varieties of wheat carry mutant alleles of two reduced height (Rht) genes

1. genes code for DELLA proteins
2. DELLA reduce effect of gibberellin on growth
3. mutant allele produce more of transcription inhibitors
4. 'Tom Thumb'; plant cells do not have receptors for gibberellins, cannot respond

inbreeding and hybridisation in maize

– maize is sturdy, tall grass with broad, strap-shaped leaves
– grows best in climates with long, hot climates
– if maize plants are inbred (crossed with plants with similar genotypes), plants in each generation become progressively smaller/weaker
– inbreeding depression occurs because homozygous plants less vigorous than heterozygous ones
– outbreeding (crossing with other, less closely-related plants), produces heterozygous plants that are healthier, grow taller, and produce higher yields
– if outbreeding done at random, it results in a lot of variation between individual plants
– maize needs to be both heterozygous and uniform
– farmer can buy homozygous seeds from companies using inbreeding, then cross them to produce F1 offspring with same genotypes
– different crosses between them can produce large number of different hybrids
– produce characteristics like high yields, resistance to pests and diseases, good growth in nutrient-poor soils or short water supply

DARWIN-WALLACE THEORY OF EVOLUTION BY NATURAL SELECTION

– observation 1 organisms produce more offspring than are needed to replace parents
– observation 2 natural populations tend to remain stable in size over long periods
– deduction 1 there is competition for survival
– observation 3 there is variation among individuals of a given species
– deduction 2 the best adapted variants will be selected for by the natural conditions operating at the time; in other words, natural selection will occur; best variants will have selective advantage
– natural selection means to select particular alleles or groups of alleles

SPECIES AND SPECIATION

– speciation is the formation of new and distinct species in the course of evolution

– species: group of organisms with similar morphological/physiological/biochemical/ behavioural features; interbreed to produce fertile offspring; reproductively isolated

reproductive isolation

– condition when species are unable to interbreed and produce healthy, fertile offspring due to barriers/isolation

– prezygotic (before zygote is formed) isolating mechanisms:

1. individuals not recognising one another as potential mates or not responding to mating behaviour
2. animals being physically unable to mate
3. incompatibility of pollen and stigma in plants
4. inability of a male gamete to fuse with female gamete

– postzygotic isolating mechanisms

1. failure of cell division in zygote
2. non-viable offspring (offspring that soon die)
3. viable, but sterile offspring

allopatric speciation

– geographical isolation

sympatric speciation

– occurs through polyploidy

– polyploid organism has more than two complete sets of chromosomes in its cells

– can happen if meiosis goes wrong when gametes being formed

– if two such gametes fuse, zygote ends up with 4 sets of chromosomes (tetraploid)

– more common in plants due to asexual reproduction

MOLECULAR COMPARISONS BETWEEN SPECIES

comparing amino acid sequences of proteins

– when amino acid sequence of particular protein is compared in different species, number

of differences gives measure to how closely relation the species are

comparing nucleotide sequences of mitochondrial DNA

– differences in nucleotide sequences of mitochondrial DNA (mtDNA) can be used to study spread and origin of a species

– human mitochondrial DNA inherited from female line (zygote contains ovum mitochondria)

– mitochondrial DNA mutates faster than nuclear DNA

– mitochondrial DNA not protected by histone proteins, and oxidative phosphorylation in mitochondria can produce forms of oxygen that acts as mutagens

EXTINCTIONS

– rhino extinctions due to:

1. lack of political support for conservation
2. increasing demand for rhino horn
3. internationally organised criminal groups targeting rhinos

CHAPTER 18 BIODIVERSITY, CLASSIFICATION, CONSERVATION

species	group of organisms with similar morphology and physiology, which can breed together to produce fertile offspring and are reproductively isolated from other species
ecosystem	a relatively self-contained, interacting community go organisms, and the environment in which they live and with which they interact
habitat	the place where a species lives within an ecosystem
niche	role of organism in an ecosystem

BIODIVERSITY

– degree of variation of life forms in an ecosystem

– tropical forests and coral reefs the most species-rich areas

– three levels:

1. variation in ecosystem or habitats
2. number of different species in the ecosystem and their relative abundance
3. genetic variation within each species

species diversity

– species richness means the number of species in a community

– species diversity takes species richness into account, but also includes measure of evenness of abundance of different species

genetic diversity

– diversity of alleles within genes in the genome of a single species

– can be assessed by finding out what proportion of genes have different alleles and how many alleles there are per gene

– genetic differences between populations of the same species exist because populations may be adapted slightly differently in different parts of their range

– important in providing populations with ability to adapt to changes in biotic and abiotic factors

assessing species diversity

1. collecting organisms and making species lists

– first task when assessing species diversity is to identify and catalogue types of organism and build a species list; use identification keys (dichotomous key)
– do timed search throughout area to see how many species can be collected
– use porter for smaller organisms

2. sampling

– to find out which species are present in an ecosystem, and the size of the population
– take samples from an area and use it to make an estimate of total numbers in area
– can be random or systematic
– if area looks reasonably uniform or has no clear pattern to the way species are distributed, use random sampling

3. random sampling using quadrats

– a quadrat is a square frame that marks off an area to identify different species present and to measure their abundance
– samples must be taken randomly to avoid bias
– mark out an area with measuring tapes and use random number generator for the coordinates of sampling points
– calculate species frequency and species density
– species frequency is measure of chance of particular species being found in any one quadrat
– species density is measure of how many individuals there are per unit area
– percentage cover:
    1. divide the quadrat into smaller quadrats with fair intervals
    2. decide approximately the percentage of area inside the quadrat is occupied by each species

estimating numbers of mobile animals

– mark-release-recapture method
– catch as many individuals as possible using traps and suitable bait
– mark the individual in a way that does not easily disappear, or does not affect future chance of survival
– marked individuals are counted, returned to their habitat, and left to mix randomly with rest of population
– when enough time elapsed for mixing to take place (make sure not too long that migration takes place), another large sample is captured
– the number of marked and unmarked individuals is counted
– proportion of marked to unmarked individuals is then used to calculate an estimate of total number in population

SIMPSON'S INDEX OF DIVERSITY

$$D = 1 - (\sum (n/N)^2)$$

– n is total number of organisms of a particular species
– N is total number of organisms of all species
– value of D ranges from 0 to 1; value near 0 represents very low species diversity
– advantage is that there is no need identify name of organism
– comparisons using this diversity index should be on a 'like for like' basis, so communities and organisms chosen should be similar

SYSTEMATIC SAMPLING

– randomly select a starting point in the field and lay out a measuring tape in a straight line
– sample the organisms that are present along the line, called a transect
– record identity of organisms that touch the line at set distances
– line transect will give qualitative data that can be presented
– belt transect technique is to place a quadrat at regular intervals along the line and recording abundance of species within the quadrat
– data from line transect can be shown as a drawing
– data from belt transect can be shown as a kite diagram or plotted as a set of bar charts

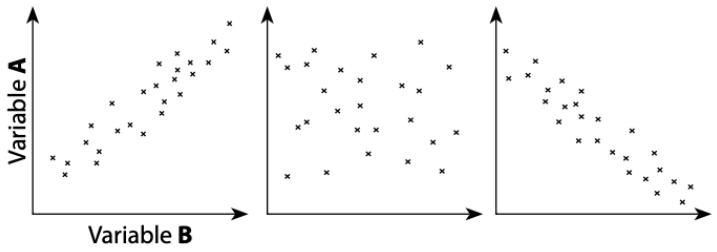
CORRELATION

Pearson's linear correlation

– can only be used when there is linear correlation

– draw scatter graph to check if relationship between 2 continuous variables appears to be linear

– positive linear correlation, no correlation, negative linear correlation



– data must be distributed normally

– linear correlation has coefficient of 1; no correlation is 0

$$r = \frac{\sum xy - n\bar{x}\bar{y}}{ns_x s_y}$$

Spearman's rank correlation

– make a null hypothesis that there is no correlation between percentage cover of 2 species

$$r_s = 1 - (6 \times \sum D^2)/(n^3 - n)$$

– where n is number of pairs of items in sample and D is difference between each pair of ranked measurements

– draw a scatter graph to see if it looks to have correlation between abundance of 2 species

– use spreadsheet

– if correlation coefficient close to +1, can conclude that there is positive correlation between 2 species and that the strength of association very high; reject the null hypothesis

CLASSIFICATION

– taxonomy: study/practice of classification which involves placing organisms into taxonomic units, taxa (s. taxon)

– taxa form hierarchy

– species, genus (p. genera), family, order, class, phylum (p. phyla), kingdom, domain

domain Bacteria

– prokaryotic

– characteristic features:

1. cells with no nucleus
2. DNA exists as a circular ‘chromosome’ with no histone proteins associated with it
3. smaller circular molecules of DNA called plasmids are often present
4. no membrane-bound organelles
5. 70S ribosomes
6. peptidoglycan cell wall always present
7. cells divide by binary fission
8. usually exists as single cells or small groups of cells

domain Archaea

– also prokaryotic

– characteristic features:

1. cells with no membrane-bound organelles
2. DNA exists as circular ‘chromosome’ with histones associated with it
3. smaller circular molecules of DNA called plasmids often present
4. 70S ribosomes, more similar to eukaryotic ribosomes
5. cell wall always present, not peptidoglycan
6. cells divide by binary fission
7. usually exists as single cells or small groups of cells

domain Eukarya

– characteristic features:

1. cells with nucleus and membrane-bound organelles
2. DNA in nucleus arranged as linear chromosomes with histone proteins
3. both 80S and 70S ribosomes; 70S in chloroplasts/mitochondria
4. chloroplast and mitochondrial DNA circular like in prokaryotes
5. unicellular, colonial, multicellular
6. cell division by mitosis

7. sexual and asexual reproduction

kingdom Protoctista

– any eukaryote that is not a fungus, plant, or animal

– characteristic features:

1. eukaryotic
2. single-celled/in groups of similar cells
3. some have animal-like cells (no cell wall) and are sometimes known as protozoa
4. others have plant-like cells with cellulose cell walls and chloroplasts, e.g. algae

kingdom Fungi

– eukaryotic

– no chlorophyll, do not photosynthesise

– heterotrophic nutrition; use organic compounds made by other organisms as source of energy and molecules for metabolism

– reproduce via spores

– simple body form, unicellular or made of long threads called hyphae

– large fungi produce large compacted masses of hyphae aka fruiting bodies to release spores

– cell walls made of chitin

– no cilia/flagella

kingdom Plantae

– multicellular photosynthetic organisms

– characteristic features:

1. multicellular eukaryotes with ells that are differentiated to form tissues and organs
2. few types of specialised cells
3. some cells have chloroplasts and photosynthesise
4. cells have large, often permanent vacuoles for support
5. autotrophic nutrition
6. cell walls always present, made of cellulose
7. cells may occasionally have flagella

kingdom Animalia

– nervous system unique to Animalia kingdom

– characteristic features:

1. multicellular eukaryotes with many different types of specialised cells
2. cells that are differentiated to form tissues and organs
3. cells do not have chloroplasts and cannot photosynthesise
4. cell vacuoles are small and temporary
5. heterotrophic nutrition
6. no cell walls
7. communication via nervous system
8. cells sometimes have cilia or flagella

VIRUSES

– microorganisms whose structure only visible under electron microscope

– acellular

– virus not part of classification system because it has none of the features traditionally used for classification; arguable that they are living organisms at all

– have particles made of proteins and nucleic acids found in cellular organisms

– no metabolism

– when infecting cells, they make use of b biochemical machinery of host cell to copy nucleic acids and to make proteins, often leading to destruction of host cells

– energy required for processes provided by respiration in host cells

– taxonomic system is RNA or DNA

THREATS TO BIODIVERSITY

– five major threats to biodiversity

1. habitat loss and degradation of environment
2. climate change
3. excessive use of fertilisers and industrial and domestic forms of pollution
4. overexploitation and unsustainable use of resources
5. the effects of invasive alien species on native species, endemics

– destruction of natural environment leads to habitat loss

– habitat fragmentation: habitat becomes divided to small areas

– deforestation

– overexploitation of resources

– pollution

– industrialisation, produce greenhouse gases (carbon dioxide and methane)

– buildup of greenhouse gases leads to climate change

– rise in sea levels cause loss of coastal ecosystems

WHY BIODIVERSITY MATTERS

moral and ethical reasons

– we share our planet with huge range of other organisms and have no right to drive them to extinction

ecological reasons

– the higher the diversity of an ecosystem, the less likely it is to be unbalanced by changes in conditions or threats such as pollution

– ecosystems of direct value to humans (e.g. drugs; antibiotics)

aesthetic reasons

– people gain pleasure from studying/appreciating natural world

– provide inspiration for artists

– wildlife is source of income in form of ecotourism

– ecotourism also provides jobs and contributes to economy

social and commercial reasons

– high yielding crops

– provide genetic resources

other services

– forests can absorb carbon dioxide, reducing effect of its increase in atmosphere

– organic tase material added to waters broken down by microorganisms

– transpiration of plants contributes to water cycle

– termites, ants, fungi, bacteria recycle elements

PROTECTING ENDANGERED SPECIES

national parks

– areas of land that are controlled by government, protected by legislation

– agriculture, building, mining, industrial activities strictly controlled

– captive breeding and reintroduction programmes

– restriction on human activity in national parks

– tourism brings in money to pay for maintenance

– inform people and raise awareness

– some designated by international bodies

zoos

– provide protection for endangered and vulnerable species

– captive breeding programmes

– research

– assisted reproduction:

1. solution to inbreeding
2. collect semen and store it frozen in sperm bank
3. semen samples checked for sperm activity, diluted with medium containing buffer solution and albumen; placed in straws, stored in liquid nitrogen at -196ºC
4. artificial insemination
5. females undergo hormonal treatment to stimulate super ovulation
6. excess embryos flushed out and transferred to other surrogate mothers (embryo transfer)
7. in vitro fertilisation, oocytes collected by inserting needle into ovaries to withdraw mature follicles; oocytes then cultured in medium before mixing with semen; zygote cultured to form embryo and placed in mother

– problems of successful conservation

1. numbers reach beyond available capacity of ecosystem
2. can be controlled by culling
3. can be controlled by birth control:
   – vasectomy
   – chemical contraceptives in form of vaccine; targets zona pellucida; stimulates immune response that produces antibodies for these

glycoproteins; antibodies then attach to female's own eggs, to block from sperm

botanic gardens

– tissue culturing or cloning

– role:

1. protect endangered plant species
2. research methods of reproduction and growth for optimal environment
3. research conservation methods for reintroduction
4. reintroduce species to natural habitats
5. educate public

– seed bank

1. carry out germination every five years; if fewer than 85% germinate, plants grown to produce fresh seeds
2. seeds that cannot be dried or frozen (recalcitrant seeds); collect seeds and grow successive generations of plants or keep them as tissue culture
3. coconut is large, embryo too large to freeze successfully; collectors remove embryos and culture them in sterile tubes and eventually plant them

CONTROLLING ALIEN SPECIES

– alien or invasive species are those that have moved from one ecosystem to another where they were previously unknown

– introduction of alien species:

1. trading of animals and plants
2. carrying them on ships
3. intentionally used as biological control agents
4. introduced for sport
5. escapees

– problems:

1. become successful predators
2. become pests
3. few controls
4. introduce diseases

5. invasive plants on water blocks sunlight and reduces oxygen
6. compete for food or resources
7. occupy similar niches and push original species to extinction

INTERNATIONAL CONSERVATION ORGANISATIONS

CITES

– Convention on International Trade in Endangered Species of Wild Flora and Fauna
– considers evidence presented to it about endangered species and assigns them to one of three Appendices
– if trade in species or its products become illegal, price can rise, likely make it worthwhile to break the law

WWF

– World Wide Fund for Nature
– best known campaign groups for wildlife
– funds conservation projects
– publicises environmental issues and campaigns to save ecosystems from degradation and species from extinction

RESTORING DEGRADED HABITATS

– important part of conservation
– mangrove forest being replanted in many parts of the world to provide protection from storm damage, flooding, rising sea levels
– mangrove forests also important nursery grounds for young fish

CHAPTER 19 GENETIC TECHNOLOGY

GENETIC ENGINEERING

– aim is to remove a gene from one organism and transfer it to another so that gene is expressed in its new host
– recombinant DNA (rDNA) is the DNA that has been altered and contains lengths of nucleotides from two different organism; made by joining pieces from two or more different sources
– organism which now expresses new gene is transgenic organism or genetically-modified organism (GMO)
– provides a way to overcome barriers to gene transfer between species
– genetic engineering often results in transfer of a single gene

overview of gene transfer

1. gene that is required is identified; may be cut from chromosome, made from mRNA by reverse transcription or synthesised from nucleotides
2. Multiple copies of gene are made using PCR
3. gene inserted into vector which delivers gene to cells of organism
4. vector takes gene into cell
5. cells that have new gene are identified and cloned

TOOLS FOR THE GENE TECHNOLOGIST

restriction enzymes

– restriction endonuclease
– class of enzymes from bacteria which recognise and break down DNA of invading viruses known as bacteriophages
– bacteria make enzymes that cut phage DNA into smaller pieces; these enzymes cut sugar phosphate backbone of DNA at specific places within molecule
– role in bacteria is to restrict viral infection

– restriction enzyme binds to specific target site on DNA and cuts at that site
– bacterial DNA protected from such an attack either by chemical markers or by not having target sites
– target sites are specific sequences of bases (sequence is palindromic, read the same way from either directions)
– either cut straight across sugar-phosphate backbone to make blunt ends, or cut in staggered fashion to give sticky ends
– sticky ends are short lengths of unpaired bases; known as sticky ends because they can easily form hydrogen bonds with complementary sequences of bass on either pieces of DNA cut with same restriction enzyme

vectors

1. inserting a gene into plasmid vectors

– vectors must be used to get new gene into recipient cell
– plasmids are small, circular pieces of double-stranded DNA; occur naturally in bacteria, often contain genes for antibiotic resistance
– gene can be exchanged between bacteria/bacteria species
– to get plasmids, bacteria containing them are treated with enzymes to break down cell walls; 'naked' bacteria then spun at high speed in centrifuge, so relatively large bacterial chromosomes are separated from smaller plasmids
– circular DNA of plasmid cut open using restriction enzyme; same enzyme used to cut out the gene should be used, so sticky ends are complementary
– if restriction enzyme gives blunt ends, sticky ends need to be attached to both gene and plasmid DNA
– opened plasmids and lengths of DNA mixed together; some plasmid sticky ends pair with those on new gene
– DNA ligase links together sugar-phosphate backbones of DNA and plasmid, producing closed circle of double-stranded DNA containing new gene; this is recombinant DNA
– pUC groups of plasmids have:
    1. low molecular mass, readily taken up by bacteria
    2. origin of replication so they can be copied
    3. several single target sites for different restriction enzymes in short length of

DNA called polylinker

4. one or more marker genes, allowing identification of cells that have taken up plasmid

2. getting plasmids into bacteria

– bacteria treated by putting them into a solution with high concentration of Ca+, then cooled and given heat shock to increase chances of plasmids passing through cell surface membrane
– small proportion of bacteria take up plasmids with gene, and are transformed
– the rest either take up plasmids that have closed without incorporating a gene or do not take up plasmids at all

3. identifying bacteria with recombinant DNA

– importance is so that they can be used to make gene product
– DNA polymerase in bacteria copies plasmids; bacteria then divide by binary fission so that each daughter cell has several copies of plasmid
– bacteria transcribe new gene and may translate it to give required gene product

4. insulin production

– problems in locating locating/isolating gene coding for human insulin from all the rest of the DNA in human cell
– researchers extracted mRNA for insulin from pancreatic ß cells, only cells that express insulin gene; contain large quantities of mRNA for insulin
– mRNA then incubated with reverse transcriptase which comes from group of retroviruses
– enzyme reverses transcription, using mRNA as template to make single-stranded DNA
– single-stranded DNA then converted to double-stranded DNA using DNA polymerase to assemble nucleotides to make complementary strand
– advantage of this form of insulin is that its now a reliable supply available to meet increasing demand; supply not dependent on meat trade

5. other genetic markers

– to identify successfully-transformed bacteria
– uses enzymes that produce fluorescent substances, taken from jellyfish, making protein called green fluorescent protein)
– fluoresces under ultraviolet light
– gene for the enzyme inserted into plasmid
– to identify bacteria that took up plasmid is to shine UV light on it
– glowing ones are GMO

promoters

– region of DNA to which RNA polymerase binds as it starts transcription
– controls expression of genes
– to express the gene inserted into bacterium, use appropriate promoter
– ensures that it recognises which of two DNA strands is template strand
– within sequence of nucleotides in promoter region is the transcription start point; the first nucleotide of gene to be transcribed
– promoter can be said to control and ensure high level of gene expression
– in eukaryotes, various proteins known as transcription factors are also required to bind to promoter region or RNA polymerase before transcription can begin

gel electrophoresis

– technique used to separate different molecules; used in analysis of proteins and DNA
– involves placing mixture of molecules into wells cut into agarose gel and applying electric field
– movement of charged molecules within gel in response to electric field depends on factors:
    1. net charge — negatively charged molecules move to anode (+), positive to cathode (–); highly charged molecules move faster
    2. size — smaller molecules move through gel faster
    3. composition of gel — polyacrylamide for proteins, agarose for DNA; size of ‘pores’ within gel determines speed of which proteins and fragments of DNA move

1. electrophoresis of proteins

– charge on proteins dependent on ionisation of R groups on amino acid residues;

whether R groups are charged depends on pH

– when proteins separated by electrophoresis, procedure carried out at constant pH using buffer solution
– usually proteins have net negative charge
– gel electrophoresis used to separate polypeptides produced by different alleles of genes
– two variants of ß-globin for sickle cell anaemia/normal haemoglobin have different net charges; sickle cell anaemia lower negative charge, do not move as far

2. electrophoresis of DNA

– DNA fragments carry small charge due to negatively charged phosphate groups
– DNA fragments move through gel towards anode
– one use is genetic profiling (fingerprinting)
    1. two different restriction enzymes cut DNA
    2. fragments are selected and multiplied
    3. DNA fragments put into gel and separated by electric field
    4. radioactive probe added to bind to invisible bands of DNA so they can blacken X-ray film
– region of DNA that is known to vary between different people is chosen; these regions often contain variable numbers of repeated DNA sequences and are known as variable number tandem repeats (VNTRs)
– DNA can be extracted from almost anything; quantity of DNA increased using PCR
– DNA then chopped into pieces using restriction enzymes known to cleave it close to VNTR regions
– when current turned off, gel contains DNA fragments that have ended up in different places
– to make fragments visible, they are transferred onto absorbent paper placed on top of gel
– paper then heated to make two strands in DNA molecule separate from one another
– short sequence of single-stranded DNA (probes) added; they have base sequence complementary to VNTR region, contain radioactive phosphorus isotope
– when paper placed on X-ray film, radiation emitted by probes make film go dark

– ends up with pattern of dark stripes

– probes may be labelled with fluorescent stain that glows under UV light

polymerase chain reaction

– method for rapid production of large number of copies of particular fragment of DNA; virtually unlimited quantities can be produced from smaller quantity of DNA

– steps:

1. denaturing — double-stranded DNA is denatured by heating at 95 ºC, to produce single-stranded DNA
2. annealing — attaching primers to ends of single-stranded DNA, requires temperature of 65 ºC
3. elongation — building up new complete DNA strands using DNA polymerase; polymerase used comes from microorganisms that live in hot environments

– once DNA has been copied, mixture is then heated again to separate DNA strands and repeat the process

– primer is used to begin process; primer is a short length of DNA, about 20 base pairs long, with base sequence complementary to start part of DNA to be copied; primer attaches to start of DNA strand, and DNA polymerase continues to add nucleotides all along rest of it

– *taq* polymerase from thermophilic bacterium used because

1. it is heat stable — not destroyed by denaturation step, does not have to be replaced during each cycle
2. its high optimum temperature means temperature for elongation step does not need to be dropped below that of annealing process, efficiency maximised

microarrays

– tool to identify genes present in organism's genome and to find out which genes are expressed within cells

– based on a small piece of glass or plastic

– short lengths of single-stranded DNA are attached to this support in regular 2D pattern, with many different positions; each individual position has multiple copies of same DNA probe

– used to compare genes present in two different species

1. DNA collected from each species and cut up into fragments, denatured to give

single-stranded DNA, and labelled with fluorescent tags (different species different colour)

2. labelled DNA samples mixed together and allowed to hybridise with probes on microarray; any DNA that does not bind can be washed off
3. microarray then observed under UV light, causing tags to fluoresce
4. original colours indicate genes belonging to 2 species; new colour derived from original ones indicate that two species have DNA with exact same sequence; no colour means no DNA hybridised, particular gene not present on either species
5. microarray then scanned so data can be read by computer

– can also detect which genes are expressed at any specific time in each cell

– used to compare which genes are active by identifying genes being transcribed into mRNA

1. mRNA from 2 types of cell collected and reverse transcriptase used to convert mRNA to cDNA; amount of cDNA may need to be increased by PCR
2. cDNA labelled with fluorescent tags, denatured to give single-stranded DNA, and hybridised with probes on microarray
3. spots on microarray that fluoresce indicate genes that were being transcribed
4. intensity of light emitted by each spot indicates level of activity of each gene
    – high intensity — many mRNA present in sample
    – low intensity — few mRNA present in sample

bioinformatics

– the collection, processing and analysis of biological information and data using computer software

– combines biological data with computer technology and statistics

– builds up databases and allows links to be made between them

– databases hold gene sequences, sequences for complete genomes, amino acid sequences of proteins and protein structures

– computer technology facilitates collection and analysis of mass information

– Ensembl (eukaryotic genomes), UniProt (primary sequences/functions of proteins), BLAST (algorithm to compare primary biological sequence information)

– when a genome has been sequenced, comparisons can be made with other known genomes

– comparisons can be made between amino acid sequences or structure of proteins

– microarrays used to find where and when genes are expressed, then information about those genes and proteins they code for are accessed

GENETIC TECHNOLOGY AND MEDICINE

– allows products specific to humans to be made (e.g. human growth hormone, thyroid stimulating hormone, factor VIII)
– use bacteria, yeast, cultures of mammalian cells
– advantages:
    1. simple nutritional requirement
    2. large volumes of product produced
    3. production facilities require little space; process can be carried out anywhere
    4. few practical/ethical problems
– disadvantages:
    1. bacteria do not modify their proteins in same way as eukaryotes

genetic screening

– analysis of person's DNA to check for presence of particular allele
– can be done in adults, fetes, embryo
– adult woman with family history of breast cancer (Brca-1 and Brca-2) may choose to be screened; positive result leads to decision of elective mastectomy
– designer baby made by pre-implantation genetic diagnosis (PGD) and IVF
    1. mix father's sperm and female egg in a dish
    2. at eight-cell stage, one cell removed to analyse DNA in it
    3. embryo with faulty allele discarded

ethics of genetic screening

– in UK law 2004, addition of allele to egg, sperm, zygote not allowed
– people believe that law too relaxed, others believe otherwise
– controversy of sex preselection and termination
– fetus can be screened for genetic disease by:
    1. amniocentesis — obtaining sample of amniotic fluid at 15-16 weeks of pregnancy; hypodermic needle inserted away from fetus, umbilical cord, placenta, aided by ultrasound scanning

2. chronic villus sampling — carried out between 10-13 weeks of pregnancy; small part of placenta (chorion) removed by needle, monitored by ultrasound scanning; small increase in miscarriage risk

– therapeutic abortion: termination of pregnancy due to medical reasons

– ethical dilemma of knowing that one carries a disease, or live obliviously

GENE THERAPY

– can cure disorders by inserting 'normal' alleles into cells

– problems lie in getting normal alleles to work properly in host cells

– most common vector used is virus (retrovirus/lentivirus)

1. defect in SCID involves inability to make adenosine deaminase (ADA vital for immune system to function)
2. child's T-lymphocytes removed and inserted with normal alleles for ADA using virus as vector
3. cells then replaced
4. not permanent, requires regular transfusions
5. gene therapy using stem cells caused leukaemia as result of using retrovirus as vector
6. retroviruses insert genes into host's genome randomly; may insert their genes with another gene, or into regulatory sequence of gene; may activate nearby gene to cause cancer
7. lentiviruses used instead, can be modified to inactivate replication
8. adeno-associated virus also used but it does not insert genes into host genome; cannot be passed on to daughter cells

– work on vectors led to successful gene therapies

1. bad eyesight caused by Leber congenital amaurosis (retinal cells die) improved
2. normal allele for ß-globin inserted to blood stem cells, corrects ß-thalassaemia
3. people with haemophilia B has reduced symptoms
4. successful SCID treatments

cystic fibrosis

– abnormally thick mucus produced in lungs and other parts of body

– person with CF prone to bacterial infections in lungs because mucus difficult to remove,

allows breeding of bacteria

– thick mucus affects other parts of body; pancreatic dust blocked; men are often sterile

– caused by recessive allele of gene coding for transporter protein in alveoli cell membrane, CFTR; this protein should allow Cl^- to pass out of cells, but doesn't in CF

1. cells lining airways pump out Cl^- through channel protein formed by CFTR
2. results in high conc. of Cl^- outside cells, reducing water potential
3. water moves out of cell via osmosis, down water potential gradient
4. water mixes with mucus to make it thin enough for easy removal by cilia

CFTR gene

– found on chromosome 7, consists of 250 000 bases

– mutations in this gene produces defective alleles; most common is deletion of 3 bases, missing one amino acid

– fault CFTR allele is recessive, heterozygous people are symptom-free carriers

– caused by a single gene, so good candidate for gene therapy

– trials include insertion of normal alleles into liposomes and sprayed as aerosol into noses; this only lasted a week, cells have short lifespan

– using different vector, harmless viruses, to carry allele into passages of gas exchange system; this caused side-effects due to viral infection

– allele needs to enter many cells throughout respiratory system; has not been achieved

– some cases of CF, mutation simply replaced on base with another, creation stop codon; translation on ribosomes stop when reaching this codon, so only short length of CFTR protein made; drug PTC124 allows translation to keep going even after stop codon

– some occasions, DNA inserted without vectors into tissue; this method removes problems associated with using vectors

somatic and germ cell gene therapy

– gene therapy involves introduction of correct allele into cells (often somatic/body cells)

– insertion of allele into germ cells is gene therapy on sex cells (gametes, embryo)

– germ cell gene therapy illegal in humans, but has been successful in animals

– problem is that all cells of child are produced from genetically-engineered zygote; child grows and produces sex cells which contain modification, offspring too ('germ line')

GENETIC TECHNOLOGY AND AGRICULTURE

genetically modified plants

– gene technology used to produce herbicide-resistant strains

– allows fields to be sprayed with herbicide after crop has germinated, eliminated weed that could compete for light, space, water, ions

– increases crop yield

– detrimental effects on environment of herbicide-resistant crops:

1. GM plants become agricultural weed
2. pollen will transfer gene to wild relatives, producing offsprings of invasive weeds
3. herbicide-resistant weeds will evolve as so much of same herbicide used

1. insect resistant crops

– GM plants protected against attack by insect pests

– maize from corn borer, cotton from boll weevil

– detrimental effects on environment:

1. evolution of resistance by insect pests
2. damaging effect on other insect species
3. transfer of added gene to other plant species

– less pesticides used to reduce risk of spray carrying to/affecting non-target insect species in other areas

– gene for Bt toxin (lethal to insets, harmless to other animals) taken from bacterium; different strains of bacterium, different toxins produced, used against different insect species

– crop plants contain Bt toxin gene, produces their own insecticides

– Bt resistance in corn borers recessive allele

– leaves from GM Bt maize end up in streams and may be eaten by other insects

– Bt plant pollen not viable after two hours from release from anthers

– GM crop seed is expensive; growers need to buy new seeds each season

– danger of losing biodiversity

2. Golden Rice

– vitamin A deficiency in places where people are poor and rice a diet staple

– vitamin A deficiency causes blindness and immune deficiency syndrome

– vitamin A made from carotene (orange carotenoid pigment) in out bodies
– GM rice contains lots of carotene in its endosperm
– gene taken from daffodils and soil bacterium
– this gene, with promoter, inserted into plasmids, plasmids inserted into bacteria, which naturally infect plants so could introduce GM plasmid into rice cells; mixed with rice embryos in Petri dish; infection occurs and embryos carry GM plasmid
– controversy:
    1. Golden Rice is seen as wrong way to solve poverty
    2. better to lift people out of poverty, but cannot be quickly achieved
– Golden Rice not commercially available, needs to be approved by national authorities

genetically modified animals

– GM animals for food production rarer than GM plants
– Atlantic salmon
    1. growth hormone regulating gene from Pacific Chinook salmon and promoter from another fish species taken and injected into fertilised Atlantic salmon egg
    2. salmon able to grow all year, reaches market size in 18 months instead of 3 years
    3. rears only sterile females, farmed in land-based tanks
    4. characteristics reduce ability to compete with wild salmon in natural environment

social implications of using genetically modified organisms in food production

– can genetically modified organisms be used safely?
– are there damaging effects on human societies?
– concerns:
    1. GM plants become agricultural weeds/invade natural habitats
    2. introduced genes transferred by pollen to wild relatives, offspring more invasive
    3. introduced gene transferred by pollen to unmodified plants growing on organic certified farms
    4. GM plants direct hazard to others, being toxic/produce allergies
    5. herbicide leaves toxic residues on crop
    6. GM seeds and herbicides expensive; farmers buy seeds each season
    7. traditional varieties lost