Viruses

Bacteriophages

- dsDNA
- icosahedral capsid head (protein subunit capsomeres)
- no envelope
- Defence mechanism
    - Bacteria DNA mutates to code for receptor sites which are no longer complementary to phage attachment sites
    - Bacteria synthesises restriction enzymes which recognise foreign phage DNA and cleave it
    - Bacteria develops a lysogenic relationship with phage

T4 phage (lytic cycle)

- Attachment
    - Attachment sites on tail fibres adsorb to complementary receptor sites on bacterial cell surface
- Penetration
    - Lysozymes digest bacterial cell wall, releasing molecules that trigger change in shape of base plate proteins. This leads to the contraction of the tail sheath, driving the hollow core tube through the bacterial cell wall. When the tip reaches plasma membrane, phage DNA is injected into the host cell, capsid head remains outside
- Replication
    - Host cell macromolecular synthesising machinery is used to synthesise phage proteins. Host cell DNA is degraded, nucleotides are used to synthesise phage DNA. Phage enzymes and structural components are synthesised
- Maturation
    - Phage DNA and capsid assemble to form a DNA-filled head. Head, tail and tail fibres assemble independently and join in a specific sequence
- Release
    - Phage lysozyme synthesised within the host cell breaks down bacterial cell wall
    - Bacterial cell membrane lyses and releases the newly formed virions

Lambda phage (lysogenic cycle)

- Attachment
    - Attachment sites on tail fibres adsorb to complementary receptor sites on bacterial surface
- Penetration
    - Lysozymes digest bacterial cell wall, releasing molecules that trigger a change in the shape of the base plate proteins. This leads to the contraction of the tail sheath, driving the hollow core tube through the cell wall. When the tip reaches the plasma membrane, phage DNA is injected, leaving the capsid head outside
- Replication

    - Linear phage DNA circularises and is inserted into the host cell's genetic material catalysed by the enzyme integrase, forming a prophage. Repressor proteins repress the expression of viral DNA. Phage DNA replicates along with bacterial chr
    - Upon spontaneous induction (frequency enhanced by irradiation with UV light or exposure to agents that damage DNA → activates cellular proteases), cellular proteases destroy repressor proteins → prophage is excised from bacterial genome→ transcription occurs and it enters the lytic cycle
- Maturation
    - Phage DNA and capsid assemble to form a DNA-filled head. Head, tail and tail sheath assemble independently and join in a specific sequence
- Release
    - Phage lysozyme synthesised within the host cell breaks down bacterial cell wall. Bacterial cell membrane lyses and releases new virions

Animal virus (influenza): 8 segments of ssRNA (negative strand → template for mRNA synthesis) packaged with protein into a helical nucleoprotein. 3 polymerases form an enzyme complex: RNA-dependent RNA polymerase. Glycoproteins haemagglutinin and neuraminidase are embedded in the viral envelope→ gives rise to diff strands.

- Attachment
    - Haemagglutinin protruding from viral envelope binds to complementary sialic acid receptor sites on host cell membrane
- Penetration and uncoating
    - Virus enters via endocytosis. Host cell membrane invaginates and pinches off, placing virus in an endocytic vesicle. Virus then fuses with a lysosome, causing pH to drop. Within the vesicle, low pH stimulates viral envelope to fuse with lipid bilayer of vesicle membrane and nucleocapsid is released into the cytoplasm. Capsid is then degraded by cellular enzymes, leaving behind the helical nucleoprotein, which then enters the nucleus
- Replication
    - Viral genome is used as a template to synthesise the viral mRNA strands (+ve), catalysed by the viral RNA-dependent RNA polymerase. The mRNA produced in turn acts as a template for the synthesis of new viral RNA genome
    - mRNA strands are exit the nucleus into the cytosol and RER where they are translated into viral structural components e.g. glycoproteins to be incorporated into viral envelope (RER), and capsid proteins (cytosol)
- Maturation
    - Viral glycoproteins are then transported by vesicles from the ER and incorporated into the plasma memb
    - Capsid proteins associate with these glycoproteins at the plasma membrane
    - Viral genome associate with proteins to form helical nucleoprotein which then interacts with capsid proteins at plasma memb of host cell → interaction of capsid with nucleoprotein initiates budding process
- Release
    - Each new virus buds from cell wall, acquires host membrane with viral glycoproteins embedded
    - Release is facilitated by neuraminidase, which cleaves sialic acid from cell surface and progeny virions, facilitating release from infected cells

HIV: two copies of ssRNA (+ve stranded), RNA tightly bound to capsid proteins → contain two molecules of reverse transcriptase + integrase + protease → together forms virus core. Glycoproteins gp120 and gp41 protrude through the envelope (derived from host cell plasma memb) → specific conformation allows virus to bind to certain T4 helper cells

- Attachment
    - Viral particle comes into contact with cell carrying CD4 protein on its surface→ glycoprotein gp120 on viral particle surface interacts with CD4 on target cell, with help of a coreceptor
- Penetration and uncoating
    - With help of gp41, viral env fuses with host cell memb, capsid is released into the cell, leaving env behind → capsid and nucleocapsid protein are then degraded, releasing viral enzymes and RNA into cytoplasm
- Replication
    - Viral reverse transcriptase enzyme catalyse the conversion of viral RNA into DNA
    - First: catalyses synthesis of DNA strand complementary to viral RNA starnd, forming RNA-DNA hybrid
    - RNA strand is then degraded and a second DNA srand complementary to the first is synthesised to form a dsDNA molecule
    - Viral DNA enters host cell nucleus and is integrated into the genetic material of host catalysed by the enzyme integrase, becoming a provirus → once integrated, it can persist in a latent state for many years
    - Activation of host cell will result in transcription of viral DNA into mRNA
    - mRNA exits nucleus into cytoplasm where it is translated into viral polyproteins → cleaved into smaller functional proteins by viral protease enzyme\
    - envelope glycoproteins gp120 and gp41 are made in the ER and vesicles transport them to cell membrane
- Maturation
    - Capsid assembles around the viral genome and viral enzymes at cell membrane to form a new virus
- Release
    - Following assembly at cell surface, the virus buds off from the cell

Immune responses to viruses

- Immune system recognises viruses when antigens on surfaces o virus particles bind to immune receptors specific for these antiens → post-infection, body produces many virus-specific immune receptors which prevent re-infection by the strain of virus and produce acquired immunity
- Vaccine works by enabling immune system to recognise the antigens exhibited by virus
- However viral genomes are constantly mutating, producing new forms of antigens → new antigen no longer binds to receptors→ virus can evade immunity to original strain

Antigenic drift

- Continuous process of genetic and antigenic change among flu strains → influenza virus genes, made of RNA, are more prone to mutations than genes made of DNA

- Accumulation of mutations in genes encoding surface antigens of virus → result in virus having surface antigens that have diff conformation to previous virus strain → cannot be recognised by antibodies against previous strain → easier to infect host
- Occurs in influenza A and B viruses → antigens are surface proteins Haemagglutinin and neuraminidase
- Antigenic drift allows for evasion of these host immune systems by small mutations in the H and N genes that make the protein unrecognisable to pre-existing host immunity

Antigenic shift

- Process whereby there is a sudden and major change in the surface antigens of a virus → occurs when two or more diff strains of a virus/strains of two or more diff viruses combine to form a new subtype having a mixture of the surface antigens of the two or more original strands → specific form of genetic reassortment that confers phenotypic change
- Mainly occurs in influenza A
    - When 2 diff strains of influenza infect the same cell simultaneously, their protein capsids and lipid envelopes are rmoved, exposing their RNA, which is then transcribed to mRNA
    - Host cell forms new viruses that combine their antigens e.g. H3N2
    - Because, human immune system has difficultyrecognising the new influenza strain, it may be highly dangerous → can cause formation of highly virulent virus

Bacteria

Lac operon

- In absence of lactose, a basal level of b-galactosidase and permease is present in the cell because repression of the lac operon by the repressor is 'leaky' (weak interactions btwn repressor and operator)
- Permease → transports lactose rom surrounding medium into cell
- B-galactosidase → converts some lactose to its isomer allolactose
- Allolactose acts as an inducer molecule, binds to repressor protein at its allosteric site→ alters conformation of DNA-binding site of repressor → repressor inactivated and no longer complementary in shape and charge to operator, cannot bind
- RNA pol free to bind to promoter→ can move downstream to transcribe structural genes to form polycistronic mRNA
- Absence of glucose→ high level of cAMP→ formation of cAMP-CAP complex which attaches to CAP binding site in promoter, strengthening affinity of RNA pol to the promoter→ enhances transcription frequency
- Operon now turned on with increased synthesis of b-galactosidase, permease and transacetylase → lactose broken down into G+G

<u>Presence of glucose</u>

- Low cAMP levels→ CAP not activated, no CAP binding to CAP binding site in promoter, no upregulation of transcription of lac operon → B-galac not expressed to break down lactose/permease not expressed for lactose uptake

Conjugation

- F plasmid transferred through conjugation
- Sex pilus of F+ bacterial cell makes contact with F- cell and retracts to bring F- cell closer so a mating bridge can be formed between the 2 cells
- One of the 2 strands of plasmid DNA in F+ cell is nicked and transferred from the F+ cell to the F- cell through the mating bridge via rolling circle mechanism as the other DNA strand is used as a template for elongation
- Single strand F plasmid DNA circularises in F- cell and is used as a template for the synthesis of a complementary strand for a ds F plasmid DNA resulting in F+ cell

Operons

- Constitutive expression: codes for an enzyme that plays an ipt housekeeping role→ required for maintenance of basic cellular processes
- Inducible operon: catabolic processes, because substrate is not main energy source for bacteria, enzyme is only produced whenthe metabolite is present nd needed
- Repressible operon: anabolic process, with x being an essential molecule,its continuous synthesis is expected→ enzyme normally expressed until x conc becomes in excess

Mutation/inactivation

- Constitutive synthesis→ non-functional→ cannot recognise and bind to → RNA olymerase can/cannot bind to promoter to initiate transcription
- Binds irreversibly/permanently

Trp operon

- Tryptophan (corepressor) binds to trp repressor at its allosteric site to change the repressor to its active form→ binds to operator→ prevents binding of RNA polymerase to promoter→ operon switched off
- Trp repressor is a diffusible product which can diffuse to the location of the operator to exert its effect

Generalised transduction

- A phage infects a bacterium and injects its viral genome into host cell
- Bacterial DNA is degraded into small fragments, one of which may be randomly packaged into capsid head during the spontaneous assembly of new viruses
- Upon lysis, defective phage will infect another bacterium and inject bacterial DNA from the previous host cell into the new bacterium
- Foreign bacterial DNA can replace homologous region in recipient cell's chromosome if homologous recombi takes place, allowing expression of a diff allele from previous host

Specialised transduction

- A temperate phage infects a bacterium, injecting its viral genome into host cell
- Viral DNA is integrated into bacterial chr, forming prophage, which may be improperly excised to include adjacent segments of bacterial DNA during an induction event
- Bacterial DNA may be packaged into a capsid head during spontaneous assembly of new viruses
- Upon cell lysis, defective phage infects another bacterium, injecting bacterial DNA from perevious host into new bacterium

- Foreign bacterial DNA can replace homologous region in recipient cell's chr if homologous recombi takes place, allowing expression of diff allele from previous host

Transformation

- Fragments of naked foreign DNA from surrounding medium are taken up by bacterial cell via surface proteins
- Foreign DNA incorporated into bacterial chr via hmologus rcombination
- If foreign DNA contains diff allele that is now expressed in bacterial cel, bacterial cell has transformed

COPEG

Telomeres

- Non-coding tandem repeat sequences found at both ends of linear chromosomes
- Each round of DNA rep will result in the shortening of daughter molecules at the telomeres because dNA polymerase is unable to replace the RNA primers with DNA→ since telomeres are non-coding, this ensures that vital genetic info are not lost with each round of replication
- By forming a loop with 3'overhang, they protect and stablise terminal ends ofchromosme, hence prevent fusion of the ends with those of other chr→ prevent DNA machinery from recognising the ends of chr as DNA breaks, hence preventing apoptosis
- 3'overhang of telomeres allow their own extension, by providing an attachment point for the correct positioning o the enzyme telomerase in certain cels eg. Germ cells

Bacteria no telomeres→ circularDNA, chrm will not shorten with each rep cycle as DNA pol is able to replace the RNA primer with DNA

Telomerase

- Only germ cells undergo continuous clel division wherease somatic cells divide a limited number of times
    - Somatic cells undergo apoptosis whereas germ cells need to fithfully pass on intact enomes to its daughter cells over many generations
    - Telomerase catalsysses regeneration of telmoreres which allows many replication cycles toocur without loss of vital genetic info through erosion at chrmsmal ends
- Telomerase has an active site complementary in shape and charge to specific telomeric DNA sequence→ using telomerase RNA as a template, telmoerase reverse transcriptase forms a complementary DNA sequence through complementary base pairing, whereby adenine paris with U, T with A, C with G, G with C→ catalyses formation of phosphodiester bond between deoxyribonucleotides→ then translocates 6 base pairs in the 5'→ 3' direction of DNAoverhang to repeat above processes. After many roudns a series of tandem repeats of GGTTAG elongates the overhang, thus elongating the telomere
- Telomerase RNA forms complementary base pairs with ss overhang at 3'end of telomere→ aligns telomerase reverse transcriptase wrt the DNA→ serves as template for formation of complementary DNA seq→ a w u, t w a…
- How RNA is syntehsised: DNA carries genetc code for telomerase RNA→ RNA polymerase unzips egion of DNA containing gene for RNA to be synthesised→ free ribonucleotides then

assemble via complementary base pairing to DNA templat→ RNA pol catalsyses formation of phosphodiester bonds between adjacen ribonucleotides

In cancer cells

- Telomerase activity allows regeneration of telomeres lost at each division, whc ensures coding DNA is not lost, and allows for cancer cells to continue dividing idefinitley → telmoerase inhibitor blocks activity of telomerase, thus preventing extension of telomere. Normal shortening of telomere cocurs, and cancer cells will undergo apoptosis

Gene mutation

- Alteration in DNA nucleotide sequences → deletion/insertion mutation where one or several nucleotides are removed/added into the DNA nucleotide seq
- Substitution mutation, one nucleotide replaced by another
- Inversion mutation→ segment of nucleotides sequences separtes from allele and rejoins at original position, but is inverted
- Why some gene mutations have no effect
    - Genetic cod s degenerate wher more than one type of codon codes for the same amino acid
    - Mutation results in a codon coding for an amino acid with an R group having similar chemical properties to original amino acid
    - Mutation in the introns, which is spliced out and not translated, hence no change in aa seq
    - Mutation affets regiosna way from DNA binding site
    - No change in conformation and charge of DNA-binding site of p53→ stil able to bidn to same specific regions on DNA
- Exposure to UV/radioactivity, carcinogens, viruses→ increases chances of DNA damage and mutations, leading to loss of fxn mutation f TSG/gain-in-fxn mutation of proto oncogenes to form oncogenes→ disruption of regulatory genes leads to inability to repair damaged DNA and excessive cell growth and proliferation, resulting in formation of a malignant tumour capable of metastasising to other parts of body to form secondary tumours
- Gene amplification resulting in more copies of ..gene, therefore more protein transcribed and translated to ensure greater pumping of drugs out of cells so that drugs cannot accumulate and reach toxic levels for cells → greater resistance
    - To overcome: anti-sense RNA that will bind to mRNA transcripts of ..gene to prevent translation ofprotein so that number of proteins will decrease/molecular inhibitors that bind directly to potein to prevent theiractivity
- How dysregulation of cchekpoints may occur
    - Point mutation of gene to cause change in aa seq of prtein, thus protein is resistant to degradation
    - Prlonged activation of gene to cause more rounds of cell division resulting in excessive cel proliferation
    - Gene amplification of CDK gene to increase number of copies of gene resulting in increased amt of protein
    - Increase number of binding sites for proien to form increased amt of active CDK

Mutation in p53

- DNA binding domain of p53 protein has a conformation and charge complementary to specific parts of DNA molcuel which has a specific sequence of bases and hence a specific conformation
- Substitution mutation:
    - One nuc replaced with a diff one→ change in codon in mRNA, an amino acid with a R group of diff chemical property is coded or→ change in interactions involved in maintaining tertiary structure type and location of bonds formed eg ionic, disulphide, hydrogen, hydrophobic interactions, lead to alteration of 3D conformation of protein
- Frameshift mutation (insertion/deletion)
    - Due to deletion/insertion of nuc not in multiples of 3→ alteration of reading frame, all codons downstream of point of mutation will be read incorrectly, produce a diff sequence nd number of amino acids→ non-functional protein
- If mutation resulted in a stop codon, then a premature termination of translation would occur and the resultant protein would be truncated

TSG

- Loss in fxn mutation gives rise to non-functional protein→ effect can be masked by presence of normal dominant allele (fxnal copy of gene) that will result in sufficient quantities of the normal protein being synthesised to exert effect. Cancer only manifests when both TSGs are mutated (recessive mutation)

Proto oncogene

- Found in normal cells, gene that codes for a protein that is involvein normal cell growth and proliferation
- Oncogene: mutated form of p.o.g such that there is excessive production of protein or oncogene codes for a protein with increased activity, more resistant to degradation→ leading to uncontrolled cell proliferation resulting in cancer
- Translocation is a chromosomal mutation when myc gene being transferred from .. to… to be under the influence of an enhancer of …gene→ resulting in high level expression of Myc gene to poduce excessive amount of Myc protein→ gain in fxn mutation → excessive amt of protein stimulates cells to undergo greater cell division, leading to tumour formation
    - Long DNA potion of chr from chr8 is transferred to chr14→ regulation of gene expression is affected, but producing same gene product

Structure of DNA

- Make molecule of DNA more compact to fit in nucleus
- Prevent entanglement→ prevent DNA breakage/damage
- Regulation of gene expression/transcription → DNA wound around histones prevents transcription factors and RNA pol access to genes that are not meant to be expressed
- Histone methylation
    - Causes chromatin to be further compacted, preventing binding of transcription factors and RNA pol to promoter to for TIC, hence preventing transcription
- Most of chromatin comprises of many genes codign for proteins not required in differentiated cells→ histone methylation occurs on codign regions containing genes coding for proteings not requreid in ell to tightly package DNA into heterochromatin, preventing expression of genes → large percentage of genome is non-coding regions, wher histone

methylation occurs. Genome is of a large size, comprising both coding andnon-coding regions

Prokaryotes: gnees coding for proteins involved in same biochem pathway usually clustered togerher on one operon→repressor binds to operator, prevents rna pol from binding to promoter, reventing gene transcriptno

Transcription factors

- Have a DNA binding site complementary in shape and charge to TATA sequence→ comprises a specific sequence of bases and hence has a specific shape
- Unwinding of double helix of DA exposes template for complementary base pairing with free ribonucleotides→ RNA pol can catalyse formation of phosphodiester bonds between riboncu

Processing of pre mrna

- Splicing of pre-mRNA involves cutting out introns and joining exons to form a mature mRNA→ introns are non-coding regions to be removed. Mature mRNA comprises exons hic code for the sequene of amino acis in a protein

Centromeres

- Non-coding DNA consisting of tandem repeat sequences found at one location along length of chr→ allow sis chromatids to adhere to each other, allow kinetochore proteins to attach, subsequentlyspindle fibres to attach to kinetochores so that sis chromatids/homolog chrs can be separated to opposite poles

1. The causative agent increase chances of DNA damage and mutations in the genes which control regulatory checkpoints of the cell cycle in a single cell;

2. Loss-of-function mutation of tumor suppressor genes will result in inability to inhibit cell cycle, repair damaged DNA and promote apoptosis;

3. Gain-in-function mutation of proto-oncogenes to form oncogenes will result in overexpression of proteins/growth factors OR production of hyperactive/degradation resistant proteins/growth factors;

4. leading to excessive cell proliferation/division to form tumour;

5. Loss of contact inhibition enables cells to grow into a tumour;

6. Activation of genes coding for telomerase so that cells can divide indefinitely;

7. Angiogenesis must occur within the tumour so that the blood vessels formed can transport oxygen and nutrients for its growth;

8. Resulting in the formation of a maglinant tumour capable of metastasizing to other parts of body to form secondary tumours;

2. upon binding of activators binds to the enhancer, causing the bending DNA; 3. promoting assembly of/stabilized the transcription initiation complex, hence increasing the rate of

transcription; (Accepts if candidates express transcriptional initiation complex in terms of RNA polymerase + general transcription factors)

Each tRNA has a specific anticodon that is matched to a specific amino acid; 2. tRNA carries amino acid to the ribosome;

3. Specific base-pairing of anticodon on tRNA to codon on mRNA ensures that the sequence of bases on mRNA is translated into a specific sequence of amino acids in the forming polypeptide chain;

4. e.g. anticodon AGC in Fig. 3.1 base pairs with codon UCG in mRNA; 5. tRNA enters the A site or aminoacyl tRNA site;

6. Peptidyl transferase catalyses the formation of peptide bond between the amino acids; 7. It then moves to the P site or peptidyl tRNA site; 8. Finally it moves to the E site or Exit site where; (For 5-8 award only 1 mark) 9. tRNA that is released after peptide bond formation and is recycled;

Hydrophilic* charged glutamic acid* is replaced by hydrophobic*, non-polar valine*; 2 At low O2 concentrations, loss of O2 from HbS* results in an unusual conformational change that causes a hydrophobic patch to stick out; 3 This hydrophobic patch attaches to a hydrophobic patch on another HbS causing them to polymerise* into insoluble fibers*; (c) Describe the effects of this change in the haemoglobin. [3] 1 Long insoluble HbS fibers* within red blood cell* causes its shape to be distorted from a normal biconcave shape to a sickle* shape; 2 Sickle red blood cells are more fragile resulting in them having a shorter life span ◊ results in shortage of red blood cells and poor oxygen transport resulting in anaemia; 3 Sickle-shaped red blood cells, being pointed and elongated, may also get lodged in small blood vessels (capillaries) and therefore interfere with blood circulation. This may result in organ damage;

Sickle cell anaemia 2. result of a single base substitution in the gene which codes for the β-globin chain; CTC ◊ CAC (i.e. T is substituted by A) on the template strand, 3. 6th triplet codon is changed from GAG to GUG; 4. codes for the amino acid valine which is non-polar and hydrophobic instead of amino acid glutamate which is charged and hydrophilic; 5. at low Polymerisation of Hb S ◊ sickle cell RBC 6. Sickle red blood cells are more fragile ◊ break more easily ◊ shortage of red blood cells & poor oxygen transport;

DNA polymerase synthesizes DNA in 5' ◊ 3' direction* only as it will add nucleotides to the free 3'OH group; 2. Since the two parental strands DNA are antiparallel, the daughter strand is synthesized continuously using the 3' to 5' leading strand template and in short Okazaki fragments using the 5' to 3' lagging strand template.

1. Enzyme is aminoacyl-tRNA synthetase*; 2. has a specific active site* that has complementary shape/conformation and charge* to 3. a tRNA* with a specific anticodon* and 4. a specific amino acid* to be attached to corresponding tRNA

rRNA associates with a set of ribosomal proteins* to form large and small subunits of ribosomes*; 2. small ribosomal subunit can bind to mRNA* via complementary base pairing* whereby adenine base pairs with uracil and guanine base pairs with cytosine. 3. large ribosomal subunit enables binding of aminoacyl-tRNAs* to P site (peptidyl-tRNA binding site) and A site (amino-acyl tRNA binding site) 4. rRNA molecule forms peptidyl transferase* on large ribosomal subunit which catalyses formation of peptide bond* in the polypeptide

A specific sequence of nucleotides on a template DNA strand is transcribed by RNA polymerase to form mRNA by complementary base pairing*; 2. The mRNA contains triplet base codes known as codons* that each codes for a specific amino acid; 3. When the mRNA containing a specific sequence of codons which code for a specific amino acid sequence,is translated at ribosomes, polypeptides are formed.

Trna

- Cca stem at 3' end of tRNA allows for attachment of specific aa
- Each tRNA molcuel has a specifi anticodon complementary to codon o mRNA
- Allows for correct sequencing of aas on polypeptide cahin
- Different tRNAs have diff conformation and chage which is complementary to the active ste of corresponding aminoacyl-tRNA synthetase→ catalytic resuides cataysle formation of covalent bond between tRNA and aa