⇒ Nucleic acids have distinct ends

- 3' end: free hydroxyl group on a 3' carbon
- 5' end: free phosphate group on a 5' carbon
- These are antiparallel; and run in opposite directions

⇒ e.g. 5' → 3' downwards on left side, upwards on right side

∴ 5' end of one strand is opposite 3' end of the other

Distinguish between DNA and RNA

1. DNA has deoxyribose sugar, RNA has ribose sugar
2. Thymine is in DNA, uracil is in DNA
3. DNA is double-stranded; forms a helix

Lecture 16: DNA Synthesis

DNA replication occurs during the *S phase* of the cell cycle

⇒ Semi-conservative: each separate strand provides the template

∴ High degree of fidelity

⇒ Nucleotides added sequentially by deoxyribosenucleoside triphosphates (dNTPs)

⇒ Synthesis occurs in 5' → 3' direction relative to the strand being synthesised

Outline enzymes involved in replication

TOPOISOMERASE:	Relaxes supercoiling of DNA
HELICASE:	Unwinds double strand of DNA
PRIMASE:	Synthesis of primers
DNA POLYMERASE:	Adds and proofreads new bases
LIGASE:	Links the added bases

Describe prokaryotic DNA synthesis

BACTERIA

- Single circular chromosome
- Replication begins at a single *origin of replication*, where unwinding begins
- Replication forks form on either side of the origin
- Daughter DNA molecules separated once replication is complete

Describe eukaryotic DNA synthesis

Eukaryotic cells contain more, complex DNA

∴ Require multiple origins of replication

Two replication forks are formed at each origin

∴ The process is <u>bidirectional</u>

⇒ At each unit of replication (replicon), synthesis moves from 5' → 3'

⇒ Leading strand moves continuously in this direction

Lagging strand forms in the same direction, but *discontinuously* behind leading strand

⇒ Okazaki DNA fragments are formed

⇒ DNA polymerases construct each fragment
⇒ DNA ligase joins each Okazaki fragment

Describe how errors in replication occur

DNA synthesis proceeds at ~3000 nucleotides/min
⇒ ~1 mistake/billion bases
∴ Replication errors accumulate over time
⇒ Explains differences between identical twins over time

Outline Huntington's disease as an example of replication error

Huntington's Disease is caused by an autosomal dominant trait
⇒ But only affects 1 in 10,000

All humans have Hungtingtin gene – HTT = codes for Huntingtin protein
⇒ Part of this gene has a repeated section- trinucleotide repeat (CAG_
⇒ CAG = glutamine
⇒ Repetition: polyglutamine expansion
⇒ Length reaches a certain threshold – mutant protein produced (mHTT)
⇒ This protein *increases the rate of neuronal decay*

Extended polyglutamine misfolds and forms aggregates
⇒ Inhibits proteolytic degradation and apoptsis
⇒ Causes over production of reactive oxygen species

PROGNOSIS & SYMPTOMS:
- Begins at ~age 35
- Slight impairment of muscle co-ordination, initially
- Eventually, voluntary and involuntary movements become uncontrollable
- 'Chorea' = jerky, writhing movements
- Death often follows within 15 years (pneumonia, choking, heart failure)

Lecture 17: DNA to Protein

Proteins are the link between genotype and phenotype
⇒ DNA dictates protein synthesis
∴ DNA controls phenotype

This is known as gene expression
⇒ Process involves two stages:
- Transcription
- Translation

Outline transcription

Transcription: production of messenger mRNA from a specific sequence of DNA
⇒ Occurs in the nucleus

Requires:
- RNA Polymerase
- Nucleoside triphosphates
- DNA template strand

RNA polymerase moves complementary bases onto the template strand
⇒ This forms a complementary strand of mRNA
⇒ Begins at *promoter* (site of initiation)
⇒ Moves from 5' → 3'

When it is completely formed, mRNA exits nucleus and moves into ribosomes

Outline translation

mRNA threads through ribosome until start codon (AUG) is found
⇒ AUG codes for methionine
∴ Met is always the first amino acid in virtually all proteins

- The following amino acids added are dictated by the base sequence
- Amino acids are added until a stop codon is reached; protein synthesis is terminated
    - ⇒ Amino acids are transferred by tRNA
    - ⇒ Anticodon of tRNA molecule binds with complementary codon of mRNA
        - ⇒ 3' end of tRNA carries corresponding amino acid
    - ⇒ Two amino acids attach at a time – peptide bonds form between them

Lecture 18: Genetic Engineering

Explain how foreign genes are isolated

The desired gene is isolated from the genome by *restriction enzymes*
⇒ Endonucleases are bacterial enzymes that 'cut' DNA at specific sequences

Explain how the foreign gene is incorporated into DNA

The bacterial plasmid is cut with the *same* endonuclease
⇒ This will leave 'sticky' ends that are complementary to the ends of the gene
∴ Compatible, will bind effectively

DNA ligase is required to covalently link the sugar-phosphate backbone (ligation)

- Only some of the plasmids will contain the foreign gene
⇒ Some foreign genes may circularise themselves; leaving original plasmid as is

Describe how a genetically modified bacterium may be isolated

Only *some* bacteria take up the plasmid – this is transformation

⇒ These bacteria need to be isolated from others

⇒ Most plasmids used for engineering contain genes resistant to antibiotics

∴ Antibiotics distinguish between 'transformed' bacteria and wild-type

To distinguish between reformed plasmids and foreign gene plasmids:

⇒ LacZ activity is compared

⇒ Incorporated foreign gene *disrupts LacZ gene*

⇒ LacZ hydrolyses beta-galactosides [such as X-Gal]

⇒ This reaction produces a colour change [blue]

∴ Successful plasmid bacteria will NOT be blue

⇒ Bacterial colonies without LacZ activity = white

Lecture 19: Viruses

Outline how viruses were first discovered

Mayer (1883) ⇒ Disease could be transmitted through sap from plant to plant

Ivanowski (1892) ⇒ Identified that the infectious agent was smaller than bacteria

Beijerinck (1898) ⇒ Showed the agent could replicate

Stanley (1935) ⇒ Crystallised agent, named as TMV – tobacco mosaic virus

Outline the general characteristics of a virus

- Very small – typically less than 1 micrometre
- Obligate intracellular parasites ⇒ require a host to survive
- DNA (single/double stranded) or RNA genome, surrounded by protein coat/envelope
- Can infect all types of organisms

Outline the structural terminology associated with viruses

- CAPSID: protein coat that surrounds the genetic material
- CAPSOMERE: protein subunits that make up the capsid
- NUCLEOCAPSID: the name given to the capsid + enclosed genome
- ENVELOPE: (only some viruses) covers the capsid; made of lipids, carbs and proteins

A completely assembled and infectious virus: VIRION

Explain viral replication

Viruses use the host cells' DNA, RNA and protein synthesis machinery to replicate

⇒ ATTACHMENT: Viruses attach to cell membrane

⇒ PENETRATION: By endocytosis or fusion

⇒ UNCOATING: By viral or host enzymes

⇒ BIOSYNTHESIS: Production of nucleic acids and proteins (by host machinery; ribosomes etc.)
⇒ MATURATION: Nucleic acid and capsid proteins assemble into newly formed virions
⇒ RELEASE: by budding, or lysis

The host range = those which a virus can attach to ⇒ determined by the proteins on its surface

Outline the modes of transmission of viruses

- Person-to-person contact [saliva, blood, bodily fluids etc.]
- Aerosols (influenza)
- Food or water contamination (Rota virus)
- Insect bites (Ross River Fever)
- Animal carriers (Hendra virus)

Influenza: Case study

- Enveloped viruses, 80-120 nm with 8 segments
- Three types: A, B and C
    ⇒ Many subtypes
- Attach to host cells via haemagluttin (HA)
- Release via budding, by the action of neuraminidase (NA)

∴ Neuraminidase inhibitors can work as a treatment
⇒ NA contains 4 enzymatic pockets; as determined by x-ray crystallography
    ⇒ *Relenza* blocks NA by inhabiting these pockets; inhibiting enzymic activity

VACCINE
⇒ Attenuated influenza virus, grown in chicken eggs
⇒ Provokes immune response and production of memory cells

Genomes (especially HA and NA) are highly variable
∴ New vaccines are needed each year