Protein Biochemistry

Protein Functions	Structure	Post-translational modification & Targeting
Different structures reflect unique function	Proteins are made up of amino acids with various side chains	Reversible (addition) or irreversible (removal)
Recognition of specific molecules: hormones, antibodies, DNA binding proteins	Amino acids have a hydrogen, central carbon, amino group, side chain, and a carboxyl group	Methylation is adding a CH3 group (eg histones to regulate gene expression)
Movement of molecules: porin, ferritin	Side chains: positive or negative charge, polar or nonpolar, different shapes and sizes	Glycosylation is adding sugar molecules (eg cell surface proteins)
Structural functions: components of the cytoskeleton such as microtubules	Primary structure: order of amino acids in a polypeptide chain, joined by peptide bonds (which are rigid), have a C and N-terminus	Ubiquitination is adding a 76 amino acid polypeptide which denotes protein is ready to be degraded
Enzymes: speed up chemical reactions by lowering the activation energy required	Secondary structure: alpha helix or beta pleated sheet, stabilised by hydrogen bonds	Phosphorylation is adding PO3 group, regulates enzyme function
	Tertiary structure: tightly packed 3D structure, noncovalent interactions between side chains	Targeting is when proteins are transported to where they need to go in a cell
	Quaternary structure: complex with 2 or more subunits which can be identical or different	Many proteins have a short signal or localisation sequence indicating where they need to go, this is then removed

Protein Biochemistry (cont)

Many proteins contain several different tightly packed domains, each carries out a specific function

DNA Structure

DNA Structure	Experimental Evidence	Chromosome Structure	DNA- Binding Proteins
DNA is made up of nucleotides	Chargaff used paper chromatography and looked at base proportions. % purine = % pyrimidine	Chromosomes are long DNA molecules containing genetic information, have regulatory sequences for proper expression and replication	Proteins bind to specific domains which can have a general affinity for DNA, or are sequence specific
Nucleotides have: deoxyribose ring, nitrogenous base, phosphate group	Wilkins and Franklin used X-Ray crystallography, found DNA is a helix with even structure	Eukaryotic chromosomes are linear, have a; centromere, and telomeres	Transcriptional regulators bind regulatory sequences near promoters to block or stimulate transcription (eg lac operon in E.coli)
Purines (adenine, guanine) have 2 rings, pyrimidines (cytosine, thymine, uracil) have 1 ring	Watson and Crick made a model: A-T and G-C hydrogen bonded base pairs, antiparallel strands, right handed double helix, one helical turn every 10.5 base pairs (3.4 nm), major and minor grooves	Bacteria have a smaller single circular chromosome	Restriction endonucleases are enzymes that cut DNA at specific sequences. Bacteria use them to restrict virus action, they can be used in the lab to manipulate DNA

DNA Structure (cont)

DNA is written from 5' to 3'	Plasmids in prokaryotes can be passed between cells via conjugation	Histones are proteins that DNA wraps around to form chromatin. Not sequence specific

2 H bonds between adenine and thymine, 3 H bonds between cytosine and guanine

DNA as Genetic Material

Chromosomal Inheritance	Transforming Principle	Hershey-Chase Experiment
Sutton & Boveri investigated where genetic material is carried using cytology, and microscopy	Griffith worked on S. pneumoniae; S strain are pathogenic (have capsule), R strain is not	Bacteriophage T2 inject genetic material inside E.coli, investigated what this material is
Sutton used grasshoppers, Boveri used Ascaris worms (roundworms). Their chromosomes are large and few in number, making them easy to observe	When cell extract of dead S strain is injected to mice- no illness. When combined with live R strain and injected- illness	Labelled bacteriophage with radioactive isotopes. 32P for DNA, 35S for protein to deduce which is genetic material
Discovered chromosomes are important in reproduction and development	Bacteria are being transformed when combined, hereditary material is being passed	Allowed bacteriophage to inject unlabelled bacteria. Separated phage from bacteria using blender
Discoveries matched those of Mendel's, and provided physical basis for his theories	Tested which molecule carries hereditary material, used enzymes which destroy specific molecules.	Centrifuged. Tested infected bacteria pellet with Geiger counter.

DNA as Genetic Material (cont)

Suggested different combinations of chromosomes could cause variation; discovered genes, and the linear structure of chromosomes	Discovered DNA is responsible for transformation. Gene coding for the capsule is passed to R strain from S strain, making them pathogenic	Bacteriophage labelled 32P had made the bacteria radioactive, indicating DNA is genetic material

DNA Replication

Semi- Conservative Replication	Process of Replication	Enzymes for Replication	Leading and Lagging Strands & Telomeres
DNA strands are complementary	DNA strands separate and are used as templates for new strands	Polymerase adds nucleotides in a 5' to 3' direction, needs primer to start	Leading strand is 5' to 3', while the lagging strand is 3' to 5' direction
3 theories for replication: conservative, semi-conservative, dispersive	Replication fork- region where DNA is being copied	Primase generates primer (usually RNA), a small stretch of nucleotides in a 5' to 3' direction. Removed afterwards and the gap is filled in (by polymerase)	Replication in lagging strand leads away from fork and is discontinuous. Strand is primed many times, so Okazaki fragments form.
Meselson-Stahl used nitrogen isotopes to test which theory is correct. Grew E.coli in 15N (to make heavy DNA) and transferred to 14N	Origin of replication- where the hydrogen bonds are broken and the strands are pulled apart so replication can start	Single stranded binding proteins separate the DNA strands and prevent reannealing	Primer removal at the end of Okazaki fragments causes erosion of genetic material, telomeres solve this

DNA Replication (cont)

Separated heavy and light DNA by ultrac-entrifugation, obtained a liquid gradient.	Humans have multiple origins of replic-ation, E.coli have one	Helicase breaks the hydrogen bonds between bases and unwinds the helix	Telomeres- short stretches of repetitive DNA sequences at the end of chromo-somes, some is lost after replic-ation
Observed using UV light, after 1 generation DNA was hybrid. After 2+ generations it became lighter, proving semi-conservative replic-ation	Replic-ation is bidire-ctional	Ligase joins the stretches of DNA together into a single strand	Telomeres are effective where DNA needs to be passed on perfectly
		Topoisomerase relieves pressure from overwinding around the replication bubble by making and resealing breaks in the DNA	