PROTEIN STRUCTURE AND FUNCTION

The folding of a polypeptide (PP) to form a protein with a unique 3D shape is determined by its sequence of amino acids (AA).

STRUCTURE

Primary Structure: A linear sequence of AA.

Secondary Structure: Hydrogen bonds of the peptide backbone causes the AA to fold into a repeating pattern. Most common type is alpha-helix and beta-sheet.

Tertiary Structure: 3D folding pattern of a protein due to chemical interactions and repulsions between AA.

Quaternary Structure: More than one PP chain.

EXAMPLE OF PROTEINS

Enzymes, hormone receptors, some hormone receptors and antibodies.

IMPORTANCE OF STRUCTURE

Retains the important genetic information, base pairings in PP strands can separate for replication and transcription.

H bonds between comp. bases ensure DNA molecule does not form an irregular shape that will affect its function in the cell.

ENZYMES

Enzymes are specific for their substrate and increase rate of reaction by lowering activation energy.

Induced-Fit Model: As an enzyme's active site binds with its specific and complementary substrate, their 3D shape changes to allow a perfect complementary fit between the active site and substrate.

FACTORS

Temperature

Low:Slow movement-Less collisions-Decreased reaction rate

Optimal: Fast movement-More collisions-Increased reaction rate/until plateau

High: Decreased reaction rate-Tertiary structure destabilised-Denaturated (can be reversed if minimal damage and moved to optimal)

pH

Non-optimal: Changes charge of AA-Alters shape 3D so cannot bind to comp. substrate-Decreased reaction rate

Optimal: Increased reaction rate

Inhibitors

Non-competitive: Attaches to enzyme-Chemically alters enzyme's active site-Substrate cannot bind

ENZYMES (cont)

Competitive: Competes with substrate-Imitates 3D shape of substrate-Stops substrate from binding

Co-factors

Required to complete enzyme's active site to help substrate bind.

Concentration of enzyme

Increased concentration-More active sites-More collisions-Increased reaction rate/until plateau

Concentration of substrate

Increased concentration-More collisions-Increased reaction rate/until plateau

DNA STRUCTURE

Stores and transmits genetic info. and functions the same way in all living organisms.

DNA is a helical double-stranded molecule.

Eukaryotes	Prokaryotes
Bound to histones in linear chromosomes- Found in nucleus, chloroplasts and mitochondria.	Unbound, circular, single chromosome- Found in the nucleoid.
Simple Genome	Complex Genome
1 copy of gene	2 copies of gene
No homologous pairs	Chromosomes in homologous pairs-observed as karyotypes
Made of only DNA	Made of chromatin, nucleoprotein
Copies its chromosomes and divides immediately after	Copies chromosomes, then cells grow, goes through mitosis, organise chromosomes into 2 equal groups

ISSUES OF COLLECTING GENETIC INFO.

Discrimination	Insurance companies and employers discriminate based on genetic health.
Ownership	Genetic info. collected should be legal property of the individual obtained from.

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ISSUES OF COLLECTING GENETIC INFO. (cont)

Privacy/Confidentiality	Shared with other people without permission.
Emotional Impact	Results can be distressing, leading to counselling for incurable genetic illnesses.
Family Members	Could expose infidelities within the family, causing emotional damage.
Young children	Cannot consent to being tested.
Social Implications	Results can make an individual socially awkward due to stigmas against genetic illnesses.
Reproductive Choices	Genetic illnesses affecting reproductive organs affect people having kids. Influenced by society, culture and religion.
Limitations	Does not predict severity of genetic illnesses or age. Environment affects development of genetic diseases.
Inaccuracies	Possibility of misinterpreted or inaccurate results. False info. about individual's genetic health.
Reliability	Can be unreliable indicator for genetic diseases, other studies required to assess reliability of genetic testing in the accurate diagnosis of genetic disease.

GENES

A unique sequence of nucleotides that code for a functional protein or RNA molecule.

Exons:	Code for protein
Introns:	Do not code for protein

RNA SPLICING

Immature mRNA contains exons and introns-Introns are removed-Exons spliced together-Forms **mature MRNA**

PROTEIN SYNTHESIS

Transcription of mRNA-Translation of mRNA into an AA sequence at ribosomes.

TRANSCRIPTION (In the Nucleus)

1. RNA unwinds DNA strands-Template and Coding strand-Bases are exposed

2. Free **RNA nucleotides** BP to comp. bases on **T strand**

3. RNA Polymerase join positioned bases to form **mRNA** (messenger RNA)

4. mRNA peels away- DNA rewinds

TRANSLATION

1. mRNA leaves nucleus via **nuclear pores**-attaches to ribosome in cytoplasm-2 codons inside at a time

2. tRNA carries specific AA and anticodon comp. to codon-form H bond between anticodon and codon

3.mRNA moves to next codon-2nd tRNA transfers AA to ribosome--peptide bonds join AA together-forming PP chain

4.tRNA keeps bringing AA to ribosome until STOP codon on mRNA-PP released-forms protein

DNA REPLICATION

Allows for genetic info. to be inherited.

Base pairings (BPs) and method of DNA replication are universal

Structure: A phosphate group, deoxyribose sugar, nucleotide base (A,T,C,G).

H Bonds: Very weak bond between strands of DNA allow for replication.

Importance of BP:

A only binds to T. C only binds to G. Ensures the genetic info. is completely and correctly transferred to next generation.

Semi-conservative Replication:

1 OG DNA=2 daughter DNA-Helicase separates OG strands to become template for newly synthesised strands of DNA-Each daughter molecule has one OG strand.

DNA PROFILING

DNA EXTRACTIONS FROM TARGET CELL

Cell lysis: Detergents added-Breaks down cell and nuclear membranes-Releases DNA

Protein Removal: Protease and RNAase added-Removes proteins and RNA-Centrifuge-Forms a pellet of cell debris

DNA Precipitation: Add ice-cold ethanol-DNA precipitates from solution

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DNA PROFILING (cont)

DNA Purification: DNA is washed-Removes impurities

POLYMER CHAIN REACTION (PCR): Amplifies target gene

Requires: DNA Polymerase, Free nucleotides, Primers, Target gene

Process

Denaturation: Heated-H bonds break-DNA unwinds-Bases are exposed

Annealing: Cooled-Primers bind to separated strands of target DNA through H bonds between comp. bases-DNA nucleotides bind to exposed bases

Extension: Heated- DNA Polymerase joins nucleotides to produce new DNA

Repeated 25-30 times until sufficient amount of DNA fragments

ELECTROPHORESIS

Used to separate DNA molecules/fragments of different sizes.

Process

DNA fragments are negatively charged-Attracted to positive-Length travelled determines size of fragment- Marker DNA determines (Smaller=Faster & Larger=Slower)-Fluorescent dye used for DNA Profile

Is a DNA-based pattern composed of a series of bands correspond to DNA fragments of different sizes.

DNA profile is unique to each individual. Approx. half from mother and half from father.

Introns contain highly repetitive sequences of bases called **Short Tandem Repeats (STRs)** .

STRs can determine: number of repeats in each allele at a locus, total length of STR

STRs containing variable numbers of repeating nucleotides are called **Variable Number Tandem Repeats (VNTRs)** (6-100bp).

VNTRs has 2 types: Minisatellites (10-70 nucleotides) and Microsatellites (Less than 10 nucleotides))

Single Nucleotide Polymorphism (SNPs): Detects types of changes due to mutations from one generation to next.

Found throughout genome-Looks at allele and determines sequence change between 2 genes.

If less than 1% of population does not carry the same nucleotide at same position in DNA sequence-Classified as SNPs.

DNA SEQUENCING

Nucleotide base sequence of target DNA molecule can be determined.

Chain termination method

ddNTPs (deoxy-nucleo-tides)	Modified DNA nucleotide- cannot form sugar phosphate bond with other dNTPs or ddNTP-last nucleotide on fragment-terminating the strand
dNTPs	Normal DNA nucleotide

Process

1. 4 test tubes- many copies of target DNA molecule

2. All 4 dNTPs added to each tube in excess

3.Different ddNTP added to each tube

4.Primers & DNA polymerase added

5.Thermocycler

6. DNA replication stops when ddNTPs joins to strand-produces many incomplete template DNA

7.Contents poured into 4 separate wells in gel-separated through electrophoresis-relative DNA sequence of target DNA revealed

ELECTROPHEROGRAM

Can determine base sequence of unknown segment of DNA-Observe change in base sequence of different genomes for genetic diseases-For forensics, disease detection, paternity

Follows same principle of electrophoresis-gel, DNA fragments etc.

Capillary Tubes inside a DNA sequencer-Different colour associated with ddNTPs-Fragments pass through light-ddNTPs absorb light then emits light which enters a detector

Detector graphs light into an electric current-producing Electropherogram-height represents amount of light absorbed and emitted-represents sequence of target DNA molecule

TERMS

Allele	An individual inherits two alleles, one from each parent, for any given genomic location where such variation exists. If the two alleles are the same, the individual is homozygous for that allele. If the alleles are different, the individual is heterozygous.
Genome	Entire set of DNA found in a cell.

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TERMS (cont)

Locus	Particular position or place where something occurs or is situated.
rRNA	Molecule in ribosome and is exported to the cytoplasm to help translate the information in mRNA into protein.
Coding vs. Template	Coding strand determines the correct nucleotide sequence of mRNA. Template strand acts as a base for mRNA transcription.
DNA vs. RNA Codon	The DNA codons are identical to the RNA codons, except for the one base thymine (T), which replaces uracil (U) in the RNA codons.

EPIGENETICS

GENE EXPRESSION

Genes are used to direct protein synthesis. **Housekeeping Genes** are expressed continuously, involved with general cellular maintenance and energy provision. Others are switched on or off in certain cells at particular times according to the function of the cell. Others are permanently switched off.

CELL DIFFERENTIATION

Results from the regulation of gene expression. All organisms that reproduce sexually start life as a fertilised egg or zygote. As this single cell begins to divide, stem cells differentiate into specialised cells e.g. nerve cells, epithelial cells, muscle cells, sex cells etc.

DNA METHYLATION

A methyl group (CH3) is added to cytosine bases in the DNA strand-**switches off** a gene by **blocking RNA polymerase and preventing transcription**. **Demethylation** activates a gene and **allows transcription** to occur.

HISTONE MODIFICATION

DNA is packaged with histone proteins to form chromatin. When chromatin is tightly wound and packaged RNA polymerase is unable to bind to DNA and as such, transcription cannot occur and the gene is ‘switched off’. When acetyl groups are added to histones, chromatin is unwound so that RNA polymerase can bind, transcription can occur and the genes are ‘switched on’. Acetyl groups can be added and removed.

TRANSCRIPTION FACTORS

EPIGENETICS (cont)

Transcription factors are proteins that control the rate of transcription. These proteins either promote or prevent the binding of RNA polymerase to the gene to be transcribed which changes the gene expression. Some transcription factors, called activators bind to DNA and activate or increase the rate of transcription, whereas others, called repressors bind to DNA and slow or stop transcription.

TRANSLATION FACTORS

Class of proteins that control the rate of translation through the activation or inhibition of ribosomes. Non-coding RNAs (ncRNA) act on mRNA already transcribed and modify or destroy mRNA molecules so that they are not translated. For example: Micro RNAs prevent translation by bonding with complementary bases on target mRNA molecules-Small interfering RNA causes mRNA to be degraded after transcription-Long non-coding RNA regulates the activity of proteins involved in the transcription of genes.

PROCESSING IMMATURE MRNA MOLECULES

Regulating the production of RNA molecules with different splicing of RNA sections: Some transcripts can undergo alternative splicing, making different mRNAs and proteins from the same RNA transcript.

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