Protein synthesis amd central dogma

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Questions regarding DNA structure

Importance of the ratios of A to T and G to C to the structure of DNA

- The ratio of A:T and C:G is approximately 1:1 as specific complementary pairing occurs between A and T, C and G
- Hydrogen bonds hold two strands together which contributes towards the stability of the double helix
- A a purine (2 rings) is paired with a pyramidine (1 ring) which provides the same width of 2nm throughout double helix, contributing towards stability of DNA

Structure of DNA that makes it stable as a hereditary material / contribute to its stability

- Numerous hydrogen bonds that hold the two DNA strands together via complementary base pairings to stabilise the structure of the DNA double helix prevents them from DNA separation
- DNA has a double helix structure to protect nitrogenous bases from degradation by preventing enzyme access to the bases and more resistant to mutations
- DNA is double strand and each strand can serve as a template to repair any DNA damage to maintain structure integrity amd can be replicated accurately or one strand can serve as a template for repair if the other has a mutation on it hence there is a backup code
- Numerous hydrogen bonds hold the two strands together and adjacent nucleotides in each strand in each strand are joined by strong covalent phosphodiester bonds in sugar phosphate backbones to prevent breaking up of DNA nucleotide sequences on the DNA strand, generations of coded information can be passed on to next generation without any loss
- Base stacking stabalises double helix through hydrophobic interactions between the planar structure of bases
- Lack of hydroxyl group at carbon 2 of deoxyribose reduces reactivity of DNA

Hereditary material:

- Access to DNA is tightly regulated by histone proteins which packs and folds DNA to make it more compact into chromosomes to prevent DNA damage
- Coded information can be readily utilised as DNA strands are held by weak hydrogen bonds allowing the template strand to separate from non-template strand allowing transcription take place
- Complementary base pairings between complementary nucleotide sequences on each strands allow faithful transfer of information from DNA to RNA transcription, which will be translated to protein subsequently

Describe structure of DNA

Molecular level:

- DNA is a polymer of nucleotides
- Basic unit of DNA molecule is deoxyribonucleotides
- Nucleotides consists of a pentose sugar which is deoxyribose , phosphate group and a nitrogenous base
- Types of nitrogenous bases are adenine, guanine, thymine and cytosine
- Purines are nitrogenous bases with 2 rings while pyrimidines are nitrogenous bases with 1 ring. (Purines are purer and have 2 virginity rings)
- Purines include Adenine and guanine while pyrimidines include thymine and cytosine
- Nitrogenous base is joined to carbon 1 of deoxyribose sugar by covalent bond
- Phosphate group joined to carbon 5 of deoxyribose sugar by phosphodiester bond and they are negatively charged molecule
- Nucleotides are joined together by phosphodiester bonds to form poly nucleotides, whereby the 3 OH end of a nucleotide joins with the 5 phosphate end of adjacent nucleotide
- The sugar-phosphate backbone of a strand of polynucleotide is formed by deoxyribose and phosphate and lies on the outside of the molecule with the nitrogen-containing bases on interior

- Each strand of polynucleotide has a 5′ end and a 3′ end which refers to the phosphate group attached to the 5th carbon of the deoxyribose and OH group attached to the 3rd carbon of deoxyribose respectively
- DNA in prokaryotes and eukaryotes consists of two strands of polynucleotides and in virus can either be single stranded or double stranded
- In double stranded DNA, two strands are anti parallel to each other
- Polynucleotides in dsDNA held together by hydrogen bonds via complementary base pairings
- Adenine forms two hydrogen bonds with thymine
- Cytosine forms 3 hydrogen bonds with guanine
- Double stranded DNA forms a double helix structure with a minor and minor groove
- Helix is right handed

Role and property of DNA

- Function:store genetic information and pass it on from one generation to the next
- Property:
    - It can be replicated accurately so that information can be passed down to next generation, ensuring daughter cells have identical copies of DNA as parent cell
    - It is chemically stable to encode information without being easily degraded and passed to next generation without loss of coded information
    - There is backup of code as DNA is double stranded
    - Coded information readily utilised due to weak hydrogen bonds
    - Capable of variation to allow evolutionary changes in a population

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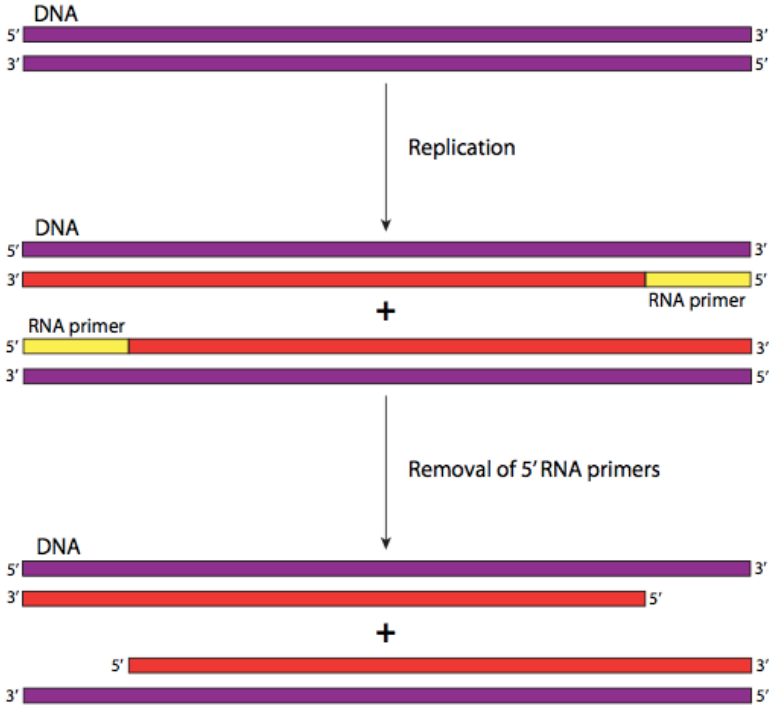
Questions regarding DNA replication

Why are two DNA polymerase needed for replication?

- The double stranded DNA is anti parallel in nature and since DNA polymerase is only synthesised in the 5′ to 3′ direction, necessary for two polymerase for more efficient replication
- Prevent degradation of single stranded DNA as efficiency is increased

How End replication problem arises and its consequences



- Occurs during replication or linear DNA
- Unable to fill gap after last RNA primer at the 5′ end is removed at the end of lagging strand as there is no pre-existing 3OH group that DNA polymerase can bind to and elongation occurs in the 5′ to 3′ direction forming a 3′ overhang
- This results in shortening of daughter strand compared to parental strand,3′ end not completely replicated
- However, since telomeres are non-coding, this ensures vital genetic information not lost with each round of replication
- If telomeres shorten to critical length, cell will be signalled to stop dividing

Describe DNA replication

- Occurs in the nucleus
- Enzyme helicase binds to the origin of replication and unwind the DNA helix by breaking the hydrogen bonds between complementary bases causing the parental strands to unzip and separate
- Single stranded binding proteins keep the strands apart so that they can act as the template for DNA synthesis
- Topoisomerase will relieve overwinding strain ahead of replication bubble by breaking and rejoining DNA strands
- An enzyme primase synthesis RNA primer which will then be added to each parental strand to provide a free 3 OH group for DNA polymerase iii to recognise and bind to as the 3D conformation of the active site of DNA polymerase is complementary to the 3OH group and hence initial of replication occurs from the 5′ to 3′ direction
    - This is because DNA polymerase **can only add nucleotides** to the new strand to a free 3 OH group **from a pre-existing strand** (in this case the RNA primer)
- DNA polymerase iii binds to the RNA primer, recruits and attaches free nucleotides to the new strand in a sequence complementary to the parental strand, catalysing phosphodiester bonds between adjacent daughter DNA nucleotides of the newly synthesised strand and it also prof reads as it moved along the template, removing and replacing any incorrect deoxyribonucleotides
- Base pairing occurs between adenine and thymine, cytosine and guanine
- DNA polymerase I will hydrolyse the RNA primers and replace it with deoxyribonucleotides
    - It will add nucleotides to the pre-existing 3′ OH end of the newly synthesised DNA strand
- DNA ligase seals the gap by catalysing formation of phosphodiester bond between the 3-OH of one nucleotide and 5′ phosphate of adjacent nucleotide

- DNA replication is semi conservative as each new dna molecules contains one original parental strand and one newly synthesised strand
- Only one strand called the leading strand is synthesised continuously
- the other strand is called lagging strand as it undergoes discontinuous replication as it is replicated away from the replication bubble forming Okazaki fragments and each fragment initiated by RNA primer before addition of DNA nucleotides. DNA polymerase I hydrolysed the primers and fills the gap with complementary deoxyribonucleotides with dna ligase catalysing the formation of a phosphodiester bond between 2 Okazaki fragments (How Dna replication of a lagging strand occurs)

Why are Okazaki fragments produced

- DNA strands are anti parallel
- DNA polymerase only add nucleotides to the free 3OH end
- Thus growing DMA strand can only elongate in the 5′ to 3′ direction
- As the replication bubble open, template for synthesis of lagging strand is exposed to the 5′ end of the newly synthesised daughter strand
- The lagging strand needs to be synthesised discontinuously in the form of Okazaki fragments in the direction opposite of the opening of the replication fork

Why is DNA replication carried out in 5′ to 3′ direction

- active site of DNA polymerase has a specific 3D conformation that is complementary to the free 3 OH of the growing polynucleotide
- Thus feee incoming nucleotides can only be added to the free 3OH end of growing polynucleotide strand

What is meant by semi-conservative replication?

- 2 strands of parental DNA molecules separate with the breakage of hydrogen bonds and each acts a template for synthesis of daughter DNA strands via complementary base pairing

- Daughter DNA molecules is made up of parental DNA strand and a newly made DNA strand

Importance of DNA ligase

- Catalyses the formation of phosphodiester bonds between adjacent DNA nucleotide of an Okazaki fragment and growing DNA chain inorder to produce continuous DNA strand

why leading and lagging strand is synthesised differently in DNA replication

- DNA polymerase lll only add deoxyribonucleotide to 3'OH of existing strand;
- DNA strands are antiparallel and DNA synthesis occurs bidirectional ;
- DNA polymerase moves towards replication fork in Box A while DNA polymerase moves away from replication fork in Box B;OR Daughter strand in Box A is synthesized continuously but daughter strand in Box B is synthesized discontinuously;
- As DNA unwinds, new primer is needed in box B, forming Okazaki fragments;

How tRNA acts an adaptor molecule for translation

(c) With reference to Fig. 2.1, explain how tRNA acts as an adaptor molecule for translation. [4]

1 translate base sequence into amino acid sequence
2 3′ CCA stem as attachment site for amino acid
3 anticodon determine specific amino acid attached
4 anticodon forms complementary base pairs with codon

▼ Notes

i. **Transfer RNA (tRNA)**

The translation of mRNA into protein depends on adaptor molecules that can recognise and bind both to the codon and to the amino acid. These adaptor molecules are tRNAs.

- tRNA has a cloverleaf secondary structure that contains three stem-loops and one stem.
- The secondary cloverleaf structure further folds to form a compact L-shaped tertiary structure.
- The site on the tRNA that recognises the mRNA codon is known as **anticodon**.
- The site that allows a covalent attachment of amino acid is the **acceptor stem** which has the sequence CCA at the 3′ end of tRNA.

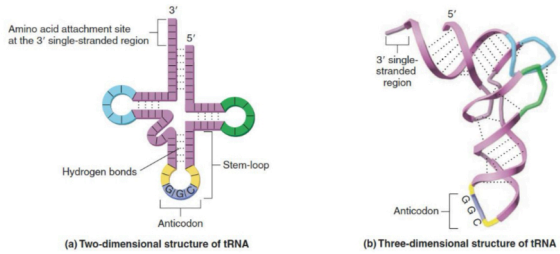


Figure 36: Structure of tRNA (Brooker, Widmaier, Graham, & Stiling, 2017)

Questions regarding to transcription

Describe transcription

- In eukaryotes , general transcription factors assemble along the promoter region to recruit RNA polymerase and position it correctly on the promoter region, forming a transcription initiation complex
- In prokaryotes, RNA polymerase recognises and binds to the Pribnow Box to initiate transcription
- TATA-binding proteins bind to the promoter at the TATA box, recruiting other general transcription factors and eventually RNA polymerase to bind to the promoter
- RNA polymerase causes the DNA double helix to unwind as hydrogen bonds between complementary bases are disrupted, exposing nitrogenous bases for complementary base pairing with free ribonucleotides
- One DNa strand acts as a template strand for synthesis of a RNA molecule
- As RNA polymerase reads the template strand from 3′ to 5′ direction, It adds Free ribonucleotides that carry corresponding base that aligns with DNA template sequence by Complementary bases pairing with the DNA template strand

- Adenine in DNA vase pairs with uracil in RNA, thymine DNA pairs with Adenine RNA. Cytosine DNA pairs with Guanine RNA and vice versa
- Incoming ribonucleitides are added to the 3 OH end and RNA polymerase catalyses the formation of phosphodiester bonds between ribonucletides
- RNA synthesised in the 5′ to 3′ direction
- As the transcription complex continues to move down the DNA double helix, unzipping the 2 strands, and synthesizing mRNA, the region of DNA that has just been transcribed, reanneals.
- Transcription stops when TNA polymerase reaches the terminator sequence AAUAAA and proteins bind downstream of the terminator sequence to cut pre-MRNA from the terminator sequence

Why are one of the strands of DNA not transcribed during transcription

- Non-template strand has a different nucleotide sequence which if expressed, would code for a different sequence of amino acids
- Results in different 3D conformation of protein which would result in a non-functional protein
- mRNA is single stranded so only one template strand is needed
- The promoter is at the 5′ end of the non-template strand hence impossible to read the non-template strand from 5′ to 3′

Possible roles for non-transcribed DNA

- contain promoter which binds RNA polymerase and general transcription factors to form the transcription initiation complex for transcription to take place
- May contain enhancer which binds to activators to promote the assembly of general transcription factors and RNA polymerase at the promoter of the gene to form the transcription initiation complex
- May contain silencer which binds to repressors to prevent assembly of general transcription factors and RNA polymerase at the promoter of the gene to form

transcription initiation complex

Compare transcription in eukaryotes and prokaryotes

Feature	Prokaryotes	Eukaryotes
Binding of RNA polymerase	Binds directly to promoter via sigma factor (sigma sigma boy sigma boy sigma boy)	Recruited by GTF to bind to promoter forming the transcription initiation complex
Genes transcribed	Several genes in an operon controlled by one promoter	One gene controlled by one promoter
mRNA formed	Polycistronic mRNA is formed	Monocistronic mRNA is formed
Upregulation of transcription	In lac operon, cAMP binds to catabolic activator protein which activates it and active CAP binds to CAP binding site in promoter region and increase affinity of RNA polymerase binding to the promoter, thus increasing frequency of transcription	Activator binds to enhancer, causing bending of spacer DNA and promoting the assembly of the transcription initiation complex which will increase frequency of transcription
Down regulation	Binding of repressor to operator prevents RNA polymerase binding to promoter and hence prevent transcription	Binding of repressor to silencer will prevent assembly of transcription initiation complex which will decrease frequency of transcription

Questions related to translation

Describe translation

- The translation of mRNA into proteins involves 4 stages: amino acid activation, initiation, elongation and termination
- During amino acid activation, amino-acyl tRNA synthase catalyses the attachment of a specific amino acids to the 3′ end of a specific tRNA
- During initiation, initiation factors binds to the small ribosomal subunit which would then recognise and bind to the 5′ end of the mRNA

- The anticodon carrying menthione attaches to the start codon AUG on the mRNA via complementary base pairing causing the large ribosomal subunit to then bind to the small ribosomal unit forming a translation initiation Complex
- The ribosome can cover only two codons on the mRNA at one time
- During elongation, the mRNA codon in the aminoacyl site of the ribosomes forms hydrogen bonds with anticodon of a second amino acyl tRNA complex
- Peptidyl transferase catalyses the formation of a peptide bind between the 2 amino acids
- The tRNA that was in the P site is moved to the excite site and leaves ribosome and released into cytoplasm
- The tRNA in the A site carrying the growing polypeptide chain is translocated to the P site to ensure that the mRNA is read in a 5′ to 3′ direction codon by codon
- The elongation process is repeated to assemble amino acids in the correct order as dictated by the template mRNA
- Termination occurs when a stop codon UGA, UAG,UAA on the mRNA is read
- The release factor will enter the A sites using hydrolysis of bond between polypeptide chain and the tRNA in the P site
- After translation, the polypeptide spontaneously coils and folds into its 3 dimensional shape and may undergo post translational modification in the endoplasmic reticulum

Explaining significance of Translation to produce a enzyme that can hydrolyse specific bond

- During translation, sequence of bases in mRNA converted into a sequence of amino acids in a polypeptide chain
- The primary structure of the enzyme contains information for folding of the chain into specific shape
- Polypeptide chain further bent, coiled, and folded extensively to a specific 3D conformation consisting of an active site to form a functional enzyme/protein

- The specific conform of active site confers to specificity of enzyme, making the substrate being complementary and being able to fit into active site

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Other CAQ

Explain how the information to synthesise polypeptides is coded for by DNA

- Each gene is made up of specific nucleotide sequence involving bases, adenine, thymine, cytosine and guanine
- The genetic code is passed down via complementary bases pairing to mRNA during transcription. Adenine pairs with uracil, thymine pairs with adenine and cytosine pair with guanine and vice versa
- The bases are then read as triplet codons by ribosomes during translation, specifying the amino acid to be incorporated into growing polypeptide chain

Compare ribosomes and RNA polymerase

- ribosome made up of 2 subunits a small subunit and a large subunit while RNA polymerase is made up of multiple subunits
- Ribosomes consists of protein and ribosomal RNA and RNA polymerase consists of only protein

Main features of genetic code

- Genetic code is made up of triple bases known as codon
- Each codon specifies one amino acids
- It is degenerate as there is usually more than one codon’s that encodes a specific amino acid
- The genetic code is is almost universal as the same codons encode the same amino acids in almost all organisms
- The Genetic code is non-overlapping meaning that successive triplets/codons are read in order without skipping any nucleotide

- The genetic code contains 3 stop codons that do not encode amino acids, UAA UGA UAG signal the termination of polypeptide synthesis

Structure and role of tRNA

Structure:

- Single stranded RNA
- It fold back upon itself and held in shape by hydrogen bonding between complementary base pairs at certain regions to form a 3D L shaped structure
- 3 bases form an anticodon on tRNA
- 3′ end with CCA stem is attachment site for a specific amino acid that corresponds to anticodon

Function

- Aminoacyl-tRNA synthesise catalyse the formation of bond between specific amino acid and tRNa at 3′ end with CCA stem with specific anticodon
- Different tRNA will have different specific conformation which are complementary to active site of corresponding aminoacyl-tRNA synthesase
- tRNA carries amino acid to the ribosome
- Each tRNA has a specific anticodon that binds to a specific to a specific mRNA codon via complementary base pairing where adenine pairs with uracil and cytosine base pairs with guanine by forming hydrogen binds
- The sequence of mRNA will then be translated into a specific sequence of amino acids in forming polypeptide chain

Explain what is non-coding DNA and its features

- They are DNA sequences that do not code for functional proteins or RNA
- They are sometimes tightly coiled DNA, preventing access by RNA polymerase and general transcription factors for transcription like centromeres

Explain why increased proportion of bases pairing cytosine and guanine increase melting temperature

- Base pairing forms 3 hydrogen bonds compared to 2 hydrogen binds between adenine and thymine
- Increase in hydrogen bonding allow dna to be more heat stable and higher temperatures is needed to increase kinetic energy of molecule to break more H bind

Why can transcription and translation occur simultaneously in bacterial cell

- Does not contain membrane bound nucleus and hence as soon as mRNA is transcribed the ribosomes can attach to mRNA and translation can be carried out
- Also, does not need to undergo post transcriptional modification and hence it can undergo transcription and translation simultaneously

How is ribosomes and RNA polymerase different in structure

- Ribosome is made of rRNA and protein while RNA polymerase made up of only protein
- Ribosome made up of 2 subunits while RNA polymerase is a single molecule
- Ribosome has an active site that is made of rRNA while RNA polymerase active site made of protein

Compare transcription and translation

	Transcription	Translation
Location	Occurs In nucleus	Occurs on ribosomes in cytoplasm
Process	DNA used as template	MRNA used as template
Enzymes that joins monomers	RNA polymerase links ribonucleotides	Peptidyl transferase on ribosomes joins amino acids
Linkages	Ribonucleotides linked by phosphodiester bonds	Amino acids limked by peptide bonds
Product	Single stranded mRNA	polypeptide

	Transcription	Translation
Direction read	DNA template read from 3′ to 5′ direction	mRNA read from 5′ to 3′ direction