DNA and Genomics

1. 04/2/7 Outline the differences in structure between amino acids and nucleotides. [6]

(N2004 P2 07)

(a)

Features	Amino acids	Nucleotides
Components	• Each amino acid has an amino group, a carboxyl group, a H atom and a R group attached to a central alpha carbon atom.	• Each nucleotide is made up of a pentose sugar, a nitrogenous base and a phosphate group. • There may be up to three phosphate groups in each 'free' nucleotide.
Ionization	• In water, the carboxyl and amine groups ionize to form a negatively charged and a positively charged group, respectively. • The negatively charged carboxyl group can accept a proton while the positively charged amino group can donate a proton. • This amphoteric property of amino acids allows them to act as pH buffer.	• In water, the phosphate group is negatively charged which allows the nucleotide to be soluble in water. • This also gives rise to the negative charges of RNA and DNA polynucleotides. • The negative charges along the sugar phosphate backbone of the DNA allow them to migrate toward the positive electrode during gel electrophoresis.
Types	• The 20 naturally occurring amino acids are distinguished by the identity of the R groups. • This helps to categorize the amino acids into polar uncharged, non-polar uncharged and charged (basic or acidic) amino acids.	• The 4 different types of nucleotides in DNA are distinguished by the base - cytosine, guanine, thymine and adenine. • In RNA, the bases are the same except for thymine which is replaced by uracil.
Formation of bonds	• The different R groups allow for the formation of ionic, H, disulfide bonds or hydrophobic interactions between amino acids. • These interactions give rise to the secondary, tertiary and quarternary structures of a protein.	• The H bonds between complementary bases (C and G, A and T), allows for the formation of the double helix structure of DNA.

2. [11/2/9c] Outline the main features of DNA replication. [8] (refer to ans in next qns)

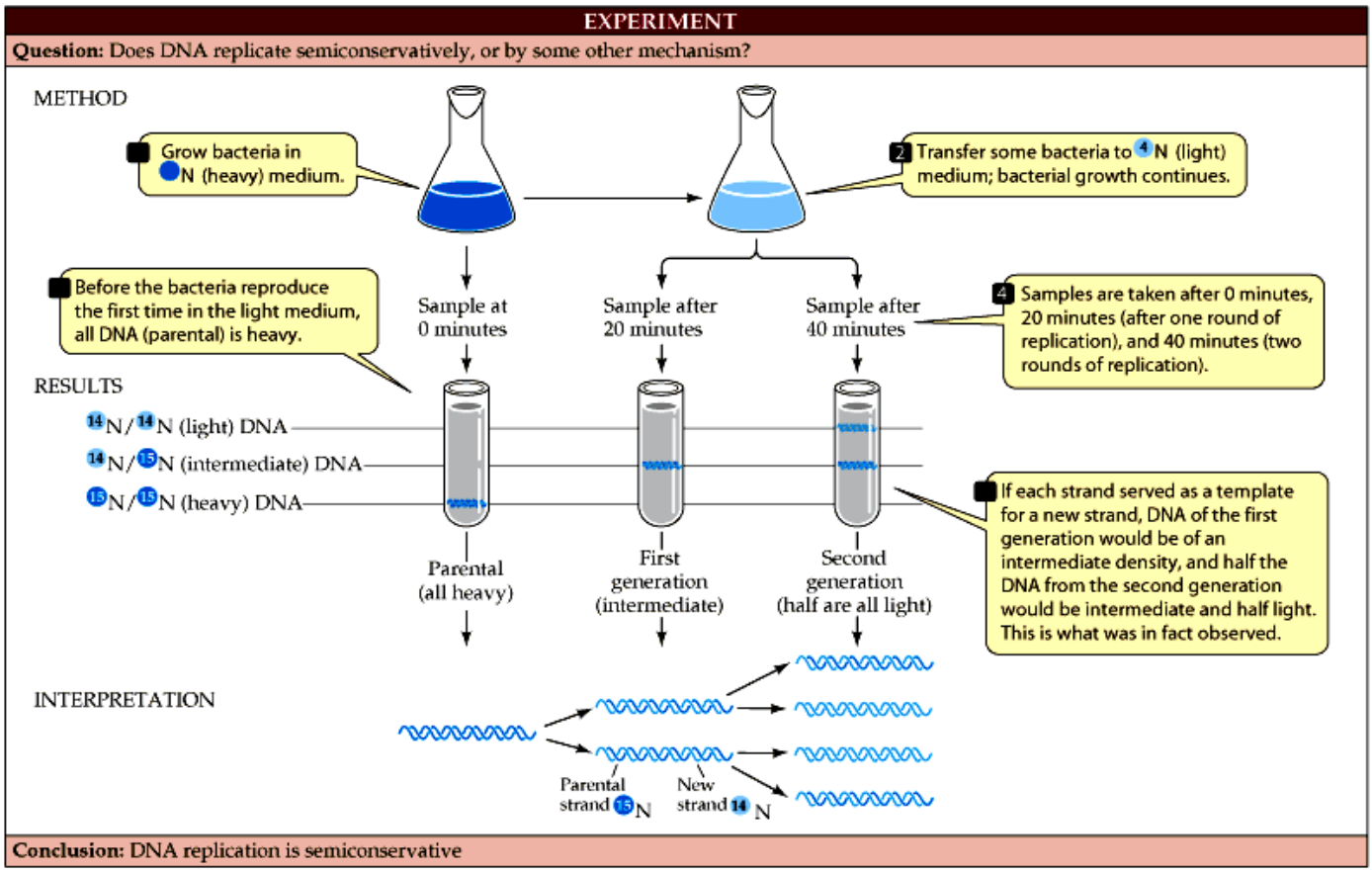
Describe the process of DNA replication and the experimental evidence for semi-conservative replication. [15]

(b)

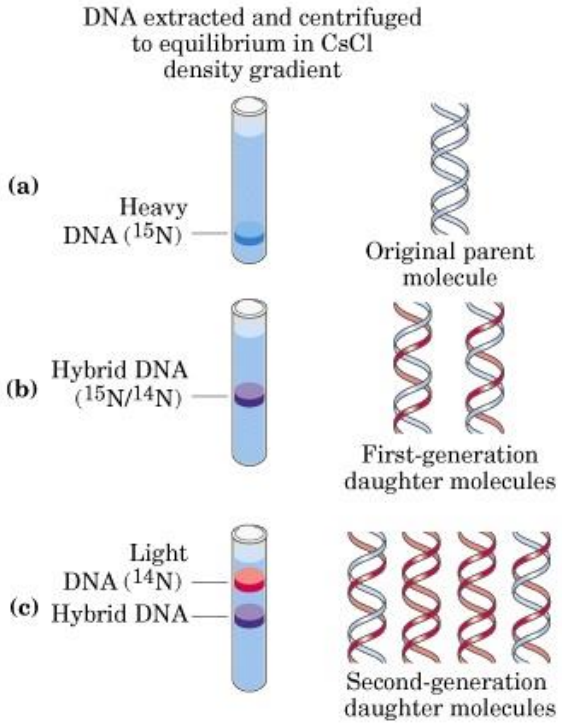
1. A portion of double helix is unwound and unzipped at the origin of replication by DNA helicase and hydrogen bonds between parental strands are broken.
2. Each strand is bound and stabilised by single-stranded binding proteins, preventing them from rewinding behind the replication fork.
3. DNA topoisomerase introduces a break in a single strand, thus allowing the strand to rotate around the break, and reseals the strand, thus eliminating positive supercoil in front of the replication fork.
4. Each parental strand acts as a template for the synthesis of new daughter strand.
5. Primase synthesises RNA primers in the 5' to 3' direction by adding ribonucleotides via complementary base pairing using parental DNA strand as template.
6. DNA polymerase adds deoxyribonucleotides to 3'OH end of RNA primer, via complementary base-pairing with the parental strand and catalysing the formation of phosphodiester bonds between nucleotides.
7. Adenine (A) always pairs with thymine (T) with two hydrogen bonds and guanine (G) always pairs cytosine (C) with three hydrogen bonds.

8. The leading strand is synthesised continuously in the 5’ to 3’ direction and the lagging strand is synthesised discontinuously, via a series of Okazaki fragments in the 5’ to 3’ direction.
9. The RNA primers are excised and replaced with deoxyribonucleotides by another DNA polymerase.
10. DNA ligase catalyses the formation of phosphodiester bond between the two Okazaki fragments.
11. The product of semi-conservative replication is two DNA daughter molecules formed from one original parental DNA molecule and each daughter molecule contains one strand conserved from the parental molecule and one newly synthesised strand.

Experimental evidence for semi-conservative replication



1. Grow *Escherichia coli* in a medium containing ammonium chloride (NH_4Cl) with a heavy nitrogen isotope / ^{15}N isotope, for many generations;
2. Every time a cell divides, its DNA replicates and ^{15}N is incorporated into nucleotides which are used to synthesize new DNA. All the bases in the DNA molecules contain ^{15}N and their DNA will be 'heavy'.
3. Bacteria were transferred into a medium containing only the lighter nitrogen isotope / ^{14}N isotope and allowed to grow. At various times of one, two or more generations after the transfer, samples of bacteria were collected;
4. DNA samples are extracted from bacteria of each generation and put into a caesium chloride solution and spun at 40000 g in a centrifuge;
5. The caesium chloride molecules sink to the bottom of the test tubes creating a density gradient. The DNA molecules will position at their corresponding level of density where its density equals to caesium chloride solution;
6. DNA molecules containing ^{15}N are heavier than those containing ^{14}N, so they ended up nearer the base of the tubes.
7. The tubes are observed under UV rays. DNA appear as fine layers in the test tubes at different heights according to their density;
8. By semi-conservative replication, DNA of first generation would be of intermediate density as all DNA molecules comprise one ^{15}N strand and one ^{14}N strand.
9. Half of DNA molecules from **second generation** would be of **intermediate density** and half of DNA molecules would be of **light density** as 50% of DNA molecules comprise one ^{15}N strand and one ^{14}N strand and 50% of DNA molecules comprise two ^{14}N strands.
10. Annotated diagram.

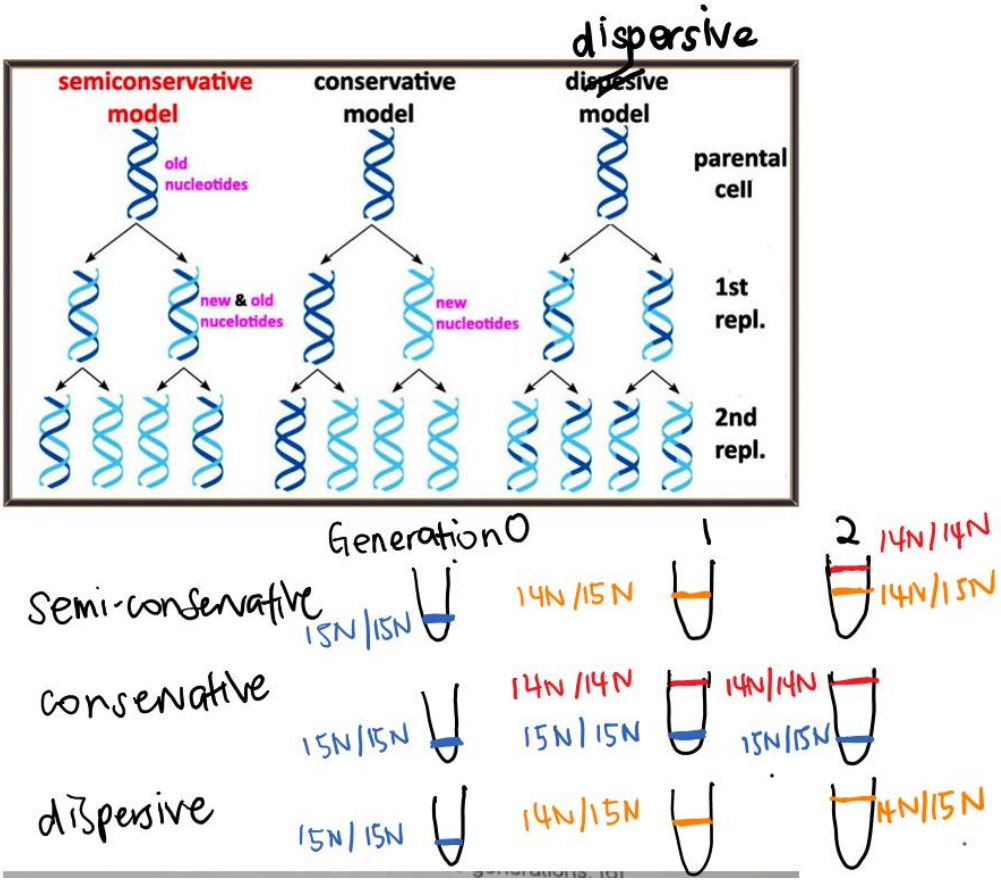


Results proving DNA replication is semi-conservative

Marker's comments:
Many students misinterpreted the question and went out to describe the process of semi-conservative replication.

For those students who managed to elaborate on the experimental evidence, many are confused about the sequence of the usage of ^{14}N and ^{15}N in the Meselson and Stahl experiment. Many had the misconception that DNA was extracted from the cell and grown in the ^{14}N / ^{15}N medium. Most students were not able to describe the centrifugation progress and identified the generations incorrectly.

3. Predict and explain the results of the 3 models of DNA replication up to 2 generations.[10]



In semi-conservative replication model, original parental molecule consisting of both N15 strands separate, both then serve as template for G1;

In the first generation / G1, all DNA molecules consist of one original parental N15 strand and a newly synthesized N14 strands, which will be seen as one N14N15 band of intermediate density;

In the second generation / G2, both strands in G1 molecule separate, each strand of N14 and N15 then serves as template for synthesis of new complementary strand

such that 50% G2 molecules consist of N14N14 (one N14N14 band of light density) and 50% consist of N14N15 (one N14N15 band of intermediate density);

In conservative model, in G1 there will be a N14N14 band and a N15N15 band of equal thickness;

This is because the parental molecule remains intact and the resulting daughter molecule is formed from two newly synthesized DNA strands from N14;

In G2, there will still be a N15N15 band as the two parental strands reassociate to restore the parental molecule, but a thicker N14N14 band as both strands of the DNA molecule act as templates for the synthesis of an entirely new DNA molecule using N14 so there will still be more DNA molecules made up of only N14;

In dispersive model, in G1 there will be only one N14N15 band;

This is because the parental DNA molecule breaks up into short segments, which act as templates for the synthesis of DNA. The segments are then joined together, resulting in both old and new DNA interspersed along each strand in both daughter DNA molecules. Hence each strand of both daughter molecules contains a mixture of old and newly synthesised DNA;

In G2, there will still be only one band but due to more incorporation of lighter N14, the single band will still be lighter than a N14N15 band but heavier than a N14N14 band;

4. Describe the structure and roles of DNA and RNA (tRNA, rRNA and mRNA).

1. DNA is made up of (deoxyribo)nucleotides, which comprise of a phosphate group, nitrogenous bases and deoxyribose as its pentose sugar;
2. DNA molecule consists of 2 chains / strands / polynucleotides that are anti-parallel and spiral around an imaginary axis to form a double helix; NOT wound around each other!
3. It has a uniform width of 2 nm with nitrogenous bases stacked 0.34 nm apart and there are 10 base pairs in each turn of the helix;
4. Hydrophobic nitrogenous bases face the interior while the hydrophilic sugar-phosphate backbones face the exterior / nucleotides linked by phosphodiester bonds to form sugar-phosphate backbone;
5. 2 chains / strands held together by hydrogen bonds which are formed via complementary base-paring, Adenine = Thymine and Guanine ≡ Cytosine;
6. Each DNA molecule carries genes and each gene is a unit of inheritance / stores coded instructions for the synthesis of RNA or protein / carries genetic information that is inherited;
7. RNA is made up of (ribo)nucleotides, which comprise of a phosphate group, nitrogenous bases and ribose as its pentose sugar; RNA does NOT necessarily have complementary base-pairing of A=U, G≡C! This base-pairing only occurs in secondary RNA structures. RNA is NOT used to synthesize mRNA, tRNA and rRNA! It EXISTS as either mRNA, tRNA or rRNA.
8. Messenger RNA is a single-stranded RNA which is transcribed in the nucleus and transported into the cytoplasm for translation by ribosomes;
9. Ribosomal RNA is a single-stranded RNA synthesized in the nucleolus and assembled with ribosomal proteins to form ribosomal subunits for translation; rRNA does NOT code for ribosomal proteins!
10. Transfer RNA is a single-stranded RNA, which is folded into a clover leaf structure then into its three-dimensional structure.
11. tRNA possess an anticodon which base pairs complementarily with codon on mRNA.
12. tRNA has an acceptor stem / 3' end for amino acid attachment, thus allowing tRNA to transfer the amino acid to the ribosome during translation;

Contrast between the structure of DNA with that of RNA. [5]

Features	DNA	RNA
Number of polynucleotide chains ;	Double, always present in the form of double helix	Single strandedRNA
Type of pentose sugar in nucleotide ;	Deoxyribose – Carbon-2 is attached to 2 H atoms	Ribose – Carbon-2 is attached to 1 H atom & 1 -OH group
Type of pyrimidine bases in nucleotide ;	thymine (T) & cytosine (C)	uracil (U) & cytosine (C)
Stability	Very stable – serves as a unit of inheritance	Less stable - degraded after it serves its function
Molecular mass ;	Relatively large	Relatively small

Describe the roles of mRNA, tRNA and rRNA in protein synthesis. [8] ***Messenger RNA (mRNA)***

- One codon codes for one amino acid;
- mRNA (5'UTR) has ribosome attachment site;
- Codons are non-overlapping
- Has spliceosome recognition sites (5' & 3' splice sites) to allow for excising of introns;
- Mature mRNA consists of exons/ no introns;
- Has START/ INITIATOR (AUG) codon and STOP (UAA, UGA, UAG) codons;
- 5' 7-methyl guanosine cap and 3' poly-A tail to ensure mRNA stability;
- Single-stranded, Complementary base pairing between codon and anticodons; (award only once in either mRNA or tRNA discussion)

Transfer RNA (tRNA)

- Has (3' CCA) end to attach to amino acid/ has amino acid attachment site;
- At least 20 different tRNAs - one for each amino acid
- Has anticodons to complementary bp with mrna codons;
- Has shape complementary to amino acyl tRNA synthetase for activation of amino acid;
- Internal hydrogen bonds to form clover leaf shape/ have specific 3D configuration/ stabilize the molecule;

Ribosomal RNA (rRNA)

- rRNA and ribosomal proteins are assembled into the large and the small subunits of ribosomes;
- Ribosomes are the sites of protein synthesis;
- rRNA of the large subunit has the peptidyl transferase activity that catalyses peptide bond formation;
- rRNA is involved in binding to mRNA and tRNA;

- Due to hydrogen bonding/ complementary base pairing within;
- With complementary base-pairing at certain regions of RNA, resulting in formation of hairpin loop and stem-loop structures.
- Gives rise to a variety of ribosomal types (e.g. 70S, 80S, 50S, 30S, etc);

[03/2/8a] Describe the role of messenger RNA in protein synthesis. [8]

[14/2/10a] Describe the structure and role of tRNA. [7]

1. Transfer RNA is a single-stranded RNA, which is folded into a clover leaf structure then into its three-dimensional structure.
2. It is folded through the formation of hydrogen bonds between complementary base pairs within its ribonucleotide sequence -> stabilize the molecule;
3. tRNA possess an anticodon which base pairs complementarily with codon on mRNA during translation.
4. The complementary base pairing between the mRNA codons and tRNA anticodons allow for the specific amino acid sequences specified by the coding sequence of the mRNA. Which is in turn determined by gene sequence.
5. tRNA has an acceptor stem / 3' end (3' CCA end) for amino acid attachment, thus allowing tRNA to transfer the amino acid to the ribosome during translation;
6. At least 20 different tRNAs - one for each amino acid.
   Has shape complementary to aminoacyl tRNA synthetase for activation of amino acid. The enzyme catalyses the covalent attachment of a tRNA to a specific amino acid before the tRNA can take part in the translation process;
7. Apart from anticodons, other parts of tRNA also associate with ribosome's tRNA binding sites to increase stability. In this way, tRNAs bring specific amino acids to the ribosome to be joined together into a polypeptide, according to the codons in the mRNA nucleotide sequence. When the amino acid carried by the tRNA has been attached to the growing polypeptide chain by the action of peptidyl transferase, the tRNA is released and free to be attached to another amino acid molecule.

Describe how the structures of RNA are adapted for translation. [8]

5. Describe how the information on DNA is used to synthesise polypeptides in prokaryotes and eukaryotes [20]

(c)

Transcription ***(in prokaryotes)***

1. During transcription initiation, sigma factor of RNA polymerase recognises and binds to double-stranded DNA at Pribnow box of promoter;
2. DNA is unwound and separated to make the template strand available, for complementary base pairing with free nucleotides;
3. During transcription elongation, RNA polymerase moves along DNA and catalyzes the phosphodiester bonds between ribonucleotides;
4. During transcription termination, a terminator sequence halts the transcription process and the short RNA-DNA hybrid is separated either by rho-dependent or rho-independent mechanism;

Transcription ***(in eukaryotes)***

1. During transcription initiation, TATA-binding protein (TBP) recognises the TATA box of promoter and recruits general transcription factors and RNA polymerase to initiate transcription;
2. DNA is unwound and separated to make the template strand available, for complementary base pairing with free nucleotides;
3. During transcription elongation, RNA polymerase moves along DNA and catalyzes the phosphodiester bonds between ribonucleotides;
4. During transcription termination, a polyadenylation **signal** sequence halts the

transcription process and the short RNA-DNA hybrid is separated;

Post-transcriptional modification / RNA processing (only in eukaryotes)

WHY eukaryotic pre-mRNA is processed

5. Euk pre-mRNA must undergo post-transcriptional modifications in the nucleus after transcription to yield functional, mature mRNA;
6. Only mature mRNA can exit the nucleus for translation to take place in the cytoplasm at the ribosomes;
7. via addition of 5'methylguanosine cap, RNA splicing, addition of 3' Poly-A tail

HOW eukaryotic pre-mRNA is processed

8. Addition of 5' methylguanosine cap confers stability as it protects the mRNA from 5' exonucleases;
   - Addition of methyl guanosine nucleoside triphosphate to the first nucleotide by a 5'-5' triphosphate linkage;
   - Catalysed by guanylyl transferase;

   Function

   - Facilitate the export of the mature mRNA from the nucleus into the cytoplasm;
   - **Protects** the mRNA from degradation by 5' exonucleases, hence confers stability to the mRNA;
   - Facilitates in binding of ribosomes to mRNA, specifically the small subunit of ribosome to bind and initiate translation;
9. Splicing involves the removal of introns and ligation of exons to produce mature mRNA.;
   - Introns are non-coding sequences on pre-mRNA, interspersed between exons which are coding sequences;
   - Spliceosome recognises specific splice sites flanking an intron, cleaves both ends of the introns therefore introns are removed, and the exons that previously flanked the intron are spliced/ joined together, forming the mature mRNA;
   - Forming the continuous coding mature mRNA seq for translation to take place

   Function

   - Introns are non-coding -> needs to be removed in order for transcription of mature RNA to form normal, functional protein
   - Alternative splicing is carried out whereby different exons are joined in different combinations. Different mature mRNA is produced from the same pre-mRNA, therefore different polypeptides are produced from a single gene without increasing the genome size;
10. Addition of 3' poly-A tail, which is about 200 adenine residues are added to the 3' end of the pre-mRNA;
    - Catalysed by poly-A polymerase;

    Function

    - Facilitate the export of the mature mRNA from the nucleus into the cytoplasm.
    - **Slow down** degradation by 3' exonucleases. The longer the poly-A tail, the longer is

      the half-life of mRNA.
      - Determines the stability / half-life of mRNA, thereby determining the amount of polypeptide translated;

Translation

11. During amino acid activation, each amino acid is joined to the correct tRNA by aminoacyl-tRNA synthetase forming aminoacyl tRNA;
12. During translation initiation, small ribosomal subunit binds to both mRNA and a specific initiator tRNA, which carries methionine (eukaryotes) / N-formyl-methionine (prokaryotes);
13. The small subunit scans along the mRNA until it reaches the start codon / AUG and the large ribosomal subunit attaches / binds to mRNA, forming translation initiation complex, together with initiator tRNA;
14. Initiator tRNA sits in the peptidyl (P) site of ribosome and vacant aminoacyl (A) site is ready for next aminoacyl tRNA;
15. During translation elongation, anticodon of incoming aminoacyl tRNA complementary base pairs with mRNA codon in the aminoacyl (A) site;
16. Peptidyl transferase catalyzes the formation of peptide bonds between the (amino end) new amino acid in the A site and the carboxyl end of the growing polypeptide in the P site;
17. Ribosome translocates by advancing three nucleotides / a codon along mRNA, and the empty tRNA in exit (E) site is released;
18. Translation termination occurs when a stop codon / UAG / UAA / UGA reaches the A site of ribosome and a release factor binds to stop codon;
19. Release factor causes the addition of water which hydrolyzes the completed polypeptide from the tRNA in the P site;

[ACJC 2015/P2/9b] Describe the process of translation. [8]

Initiation

1. **The specific amino acid is attached to the sequence 3' CCA at end of tRNA;**
2. **by amino acyl-tRNA synthetase to form aminoacyl-tRNA;**
3. **Small ribosomal subunit binds to mRNA at the 5'/ modified guanosine cap and moves downstream to locate the start codon/ AUG;**
4. **Aminoacyl-tRNA carrying methionine/ Aminoacyl-tRNA with anticodon UAC (binds to the start codon AUG on mRNA);**
5. **Large ribosomal subunit then binds and methionine aminoacyl-tRNA is positioned at the P site of the large ribosomal subunit;**

Elongation

6. **The second aminoacyl-tRNA is held at the A site of the large ribosomal subunit.;**
7. **The two amino acids are joined by a peptide bond catalysed by peptidyl transferase;**
8. **And first amino acid (methionine) is now transferred to the aminoacyl-tRNA at the A site, forming peptidyl-tRNA;**
9. **Ribosome translocates in the 5' to 3' direction, (tRNA originally at the P site is relocated to the E site where it is ejected from the ribosome into the cytoplasm);**

Termination

10. **When stop codon/ UGA/ UAG/ UAA is reached, release factor protein binds and polypeptide is released from ribosome;**
11. **Ribosome disassembles into its large and small subunits.;**

@1m, max 8

6. RI 2016/Prelim/Q9c With reference to the structure of ribosome, describe the role of the ribosome in translation. [8]

1. rRNA in small subunit of ribosome;
2. has a mRNA binding site / which allows it to bind ***mRNA****;
3. Large ribosomal subunit contains A, P and E sites; (note: mark can be given if either A / P / E site mentioned)
4. A site binds to incoming aminoacyl-tRNA during translation;
5. A site binds ***release factors**** when stop codon is reached / during translation termination;
6. This results in hydrolysis of the bond between the tRNA and polypeptide chain in the P site;
7. P site binds to initiator tRNA during initiation of translation;
8. P site binds to the tRNA attached to a growing polypeptide chain during translation;
9. E site binds tRNA that are subsequently released into cytosol;
10. rRNA in large ribosomal subunit makes up ***peptidyl transferase****;
11. which catalyse the formation of ***peptide bond**** in the polypeptide chain;

7. Compare and contrast replication and transcription. [12]

1 Both involved the formation of phosphodiester bonds between nucleotides
2 Both process synthesises polynucleotide strands in the 5' to 3' direction;
3 Both occurs in the nucleus in eukaryotes;

	Features	Replication	Transcription

4	Product	Uses DNA as template to synthesise double stranded DNA @ DNA double helix	Uses DNA as template to synthesise single stranded RNA molecules (e.g. mRNA, tRNA and rRNA)
5	Polymerase involved in addition of nucleotides	DNA polymerase	RNA polymerase
6	Monomers	Deoxyribonucleotides are added to synthesise DNA	Ribonucleotides are added to synthesise RNA
7	Template	Both strands of DNA serve as template for replication	Only 1 strand of DNA serves as a template for transcription
8	Extent of Template	Entire DNA strand serve as a template so that entire DNA molecule is replicated	Only a selected stretch of 1 DNA strand serves as a template for transcription
9	Occurrence	During synthesis phase of interphase	Occurs during protein synthesis
10	Types of monomers	4 different deoxyribonucleotides containing either adenine, thymine, guanine or cytosine as the nitrogenous bases	4 different ribonucleotides containing either adenine, uracil, guanine or cytosine as the nitrogenous bases
11	Enzyme for unwinding and unzipping DNA	Helicase	RNA polymerase
12	Site of initiation	Origin of replication	TATA box of promoter in eukaryotes and -35 and -10 sequences (Pribnow box)of promoter in prokaryotes
13	End of process / Termination	Entire DNA molecule is replicated	Polyadenylation signal sequence in eukaryotes, rho-dependent or rho-independent termination in prokaryotes

8. Contrast between transcription and translation. [8]

Transcription uses DNA as a template to synthesize mRNA while translation uses mRNA as a template to synthesize polypeptide chains / proteins;

Transcription results in the formation of phosphodiester bonds by RNA polymerase within the mRNA while translation results in the formation of peptide bonds by peptidyl transferase within the polypeptide chains / proteins;

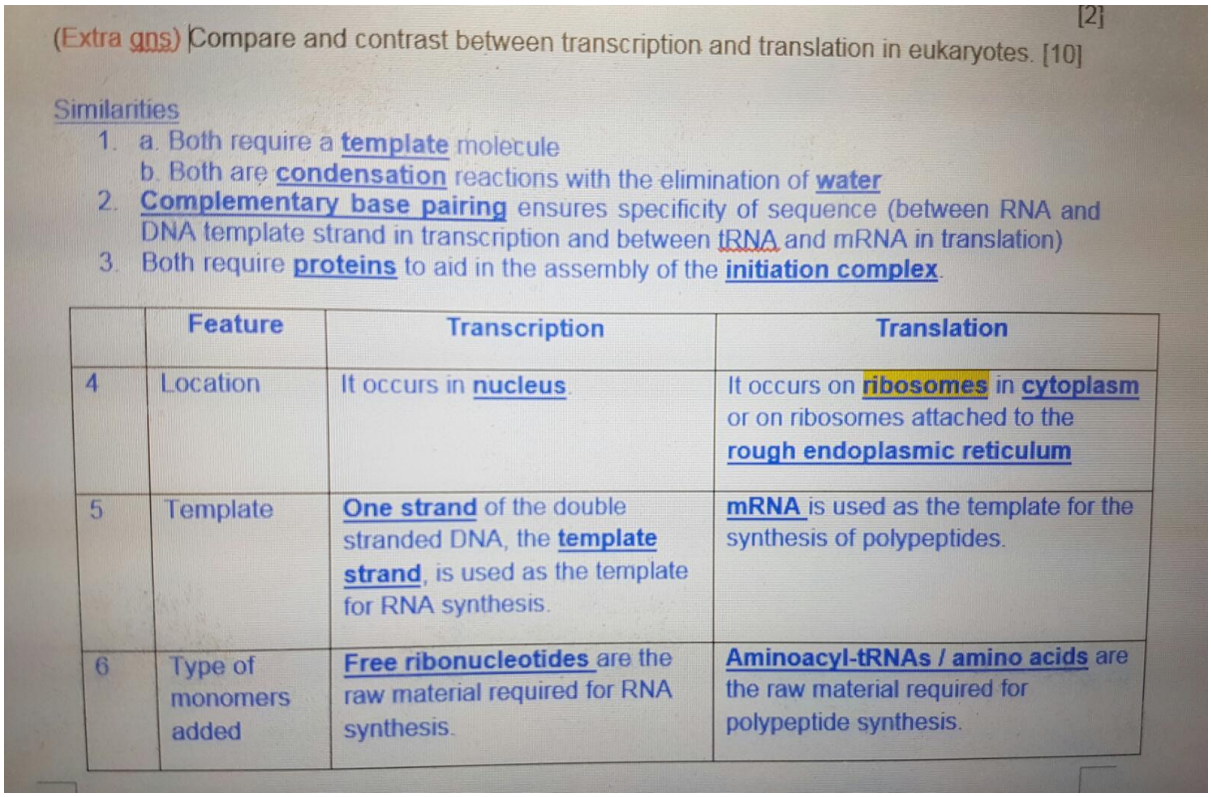
Transcription uses ribonucleotides as monomers to build up into polymers while translation uses amino acids as monomers to build up into polymers;

Transcription occurs in nucleus while translation occurs in ribosomes in cytoplasm;

Features of comparison

- Template
- Monomer required
- Product / polymer formed
- Enzyme(s) needed for polymerisation
- Type of bonds formed between monomers
- Location
- (initiation) Components of initiation complex
- Where does it start?
- (termination) How does it terminate?

**Elaboration is required, meaning EXPLAIN. Don't just state.*



[2]

(Extra qns) Compare and contrast between transcription and translation in eukaryotes. [10]

Similarities

1. a. Both require a **template** molecule
   b. Both are **condensation** reactions with the elimination of **water**
2. **Complementary base pairing** ensures specificity of sequence (between RNA and DNA template strand in transcription and between tRNA and mRNA in translation)
3. Both require **proteins** to aid in the assembly of the **initiation complex**.

	Feature	Transcription	Translation
4	Location	It occurs in **nucleus**.	It occurs on **ribosomes** in **cytoplasm** or on ribosomes attached to the **rough endoplasmic reticulum**
5	Template	**One strand** of the double stranded DNA, the **template strand**, is used as the template for RNA synthesis.	**mRNA** is used as the template for the synthesis of polypeptides.
6	Type of monomers added	**Free ribonucleotides** are the raw material required for RNA synthesis.	**Aminoacyl-tRNAs / amino acids** are the raw material required for polypeptide synthesis.

7	Type of bonds formed between monomers	Phosphodiester bonds are formed between 3' OH group of ribose of one ribonucleotide and the 5' phophate group of the next / incoming ribonucleotide.	Peptide bonds are formed between the carboxyl group of one amino acid and the amino group of the next amino acid.
8	Enzyme	RNA polymerase catalyses the formation of phosphodiester bonds.	Peptidyl transferase in the large ribosomal subunit catalyses the formation of peptide bonds.
9	Type of products	mRNA, rRNA and tRNA are the products.	Polypeptide is the product.
10	Direction of movement along template	RNA polymerase moves along the DNA template strand from its 3' to 5' end.	Ribosomes moves along the mRNA from its 5' to 3' end.
11	Need for ribosomes	No ribosomes are involved.	Ribosomes are involved.
12	Need for tRNA	No tRNA is involved.	tRNA are involved in carrying amino acid to the ribosome.
13	Need for other protein factors	transcription factors and RNA polymerase assemble to form the transcription initiation complex	Initiation factors, ribosome and initiator tRNA assemble to form the translation initiation complex
14	Termination	Termination of transcription occurs when RNA polymerase reaches the termination site on the DNA template	Termination of translation occurs when STOP codon (UGA, UAA, UAG) enters the A-site and a release factor binds to the A-site.

9. [9283/07/2/7] Outline how enzyme synthesis is controlled by DNA. [7]

10. [ACJC 2015/P2/10b] Explain how a deletion mutation in the gene for DNA polymerase III will prevent the occurrence of DNA replication. [6]

1. **Deletion of nucleotides not in multiples of three will lead to a frameshift mutation;**
2. **Which result in change in codon sequence/ result in premature stop codon;**
3. **And hence different sequence of amino acids in the polypeptide/ resulting in truncated polypeptide;**
4. **Deletion of nucleotides in multiples of three will result in the deletion of essential amino acids (binding residue/ catalytic residue);**
5. **Ref. to change in bonds between R groups in polypeptide;**
6. **Resulting in change in 3D configuration of DNA polymerase III such that active site is no longer complementary in shape to the deoxyribonucleotides;**
7. **Hence DNA polymerase III will no longer be able to catalyse the condensation reaction/ phosphodiester bond between deoxyribonucleotides;**
8. **No formation of daughter DNA strand/ DNA molecule;**

OR

9. Deletion of nucleotides in promoter;
10. Resulting in change in 3D conformation of promoter region
11. Not complementary to RNA polymerase/ cannot bind
12. No transcription and translation of DNA polymerase III;
13. Hence DNA polymerase III will no longer be able to catalyse the condensation reaction/ phosphodiester bond between deoxyribonucleotides;
14. No formation of daughter DNA strand/ DNA molecule;

@1m, max 6

[14/2/10b] Explain how gene mutations may affect the protein coded for by a gene. [7]

1 Gene mutations are changes to the DNA nucleotide sequence which could be a single nucleotide substitution or the addition or deletion of nucleotides;

2 When changes to the DNA sequence occur within the coding regions of a gene, the mRNA sequence that is transcribed using this DNA as a template will be different.

3 When this mRNA sequence is translated, the changes in the codons in mRNA may lead to changes in the amino acid sequence of the polypeptide product.

4 Since different amino acids have different R groups, the changes in the amino acid sequences may lead to changes in the interactions and bonds that hold the protein in its tertiary structure and therefore cause changes to its function, usually making it less functional or non-functional. Sometimes, mutations may cause the protein product to become more active in its function, though this is not as common.

5 These mutations may also result in a premature stop codon (UAA, UGA or UAG) in the mRNA sequence. As a result, the polypeptide formed will be truncated and probably non-functional.

6 In cases where the mutation involves the addition or deletion of a number of nucleotides not in multiples of three, it will result in a frameshift where all th downstream of the mutation will be changed.

7 This results in an extensive change in amino acid sequence and will almost definitely produce a non-functional polypeptide;

11. [03/2/8b] Explain how substitution of one base in DNA may change the phenotype of an organism. [6]

8 Substitution is the replacement of one nucleotide pair for another nucleotide pair;

9 The substitution in the DNA will result in a change in the codon of mRNA;

10 Triplet of nucleotide bases code for one amino acid and substitution may change the amino acid coded for;

11 This could affect the way in which the protein folds due to different R / side groups and bonds formed,

12 The 3-dimensional conformation / structure / tertiary structure of the protein will be changed / altered;

13 This will affect the physical and chemical properties of the proteins and the altered proteins may affect metabolism or development;

OR

1 Substitution is the replacement of one nucleotide pair for another nucleotide pair;

2 Sickle-cell anemia is due to substitution of a single base pair in the gene that codes for one of the polypeptides of haemoglobin;

3 The substitution of a single nucleotide, from CTT to CAT in the DNA's template strand of chromosome 11 leads to a change in the codon of mRNA;

4 The original amino acid coded for is hydrophilic glutamate, which is changed to hydrophobic valine at the sixth position;

5 The mutated haemoglobin tends to polymerise into long rigid chains when not bound to oxygen;

6 The long fibres distort the cell surface membrane of the red blood cell giving it its distinct sickle shape;

[14/2/10c] For a named genetic disease, describe the causal mutation and outline its effect on the phenotype of an organism. [6]

For sickle-cell disease / anaemia

1. Substitution is the replacement of one nucleotide pair for another nucleotide pair + Sickle-cell anemia is due to substitution of a single base pair in the gene that codes for one of the polypeptides of haemoglobin;
2. Substitution of a single nucleotide, from CTT to CAT in the DNA's template strand of chromosome 11 leads to a change in the codon of mRNA;
3. Changed the original amino acid coded for, which is glutamate, a hydrophilic amino acid, to valine, a hydrophobic amino acid, at sixth position;
4. Mutated haemoglobin tends to polymerise into long rigid chains when not bound to oxygen;
5. The long fibres distort the membrane of red blood cell giving it its distinct sickle shape;
6. The sickle shaped RBCs result in decrease in surface area of cell, thus decreases efficiency of carrying oxygen / lack of oxygen being transported to respiring cells + Resulting in lack of rigidity, obstructing blood vessels and restricting blood flow to cells;
7. Sickled shaped RBCs has shorter lifespan than normal RBCs / Less oxygen transported may result in organ damage;

OR

For cystic fibrosis

1. Mutation occurs in the gene that codes for Cystic Fibrosis Transmembrane Conductance Regulator (CFTR);
2. Deletion of 3 consecutive nucleotide (reject deletion of a codon) in the DNA of chromosome 7 results in the loss of phenylalanine in CFTR protein;
3. This will affect the way in which the protein folds due to different side groups and bonds formed, in turn the 3-dimensional conformation / configuration / structure of the protein will be distorted + This will affect the physical and chemical properties of the proteins and altered proteins may affect metabolism or development;
4. CFTR which functions as a Cl^- channel is now defective and no longer able to transport chloride ions out of the cells;
5. Accumulation of chloride ions in the cell causing thick mucus accumulating on surface of epithelial cells of lungs / pancreas / reproductive system;
6. In the lungs, the thick mucus decreases gaseous exchange. In the respiratory tract, this sticky mucus secretion accumulates which increases bacterial adherence to airway epithelial cells. The patient is therefore prone to chronic respiratory infections. Impaired CFTR may also affect the pancreas, with the thick mucus causing

obstruction to its ducts and preventing digestive enzymes from reaching the intestines. Obstruction to the reproductive tract may also cause infertility, especially in males.

Explain how a change in the sequence of the DNA nucleotide (gene mutation) may affect the amino acid sequence in a protein, and hence the phenotype of the organism, e.g. sickle cell anaemia and cystic fibrosis. (Knowledge of substitution, addition, deletion and frameshift mutation may be required.)

For sickle-cell disease / anaemia

8. Mutation of a single base pair occurs in the gene that codes for one of the polypeptides of haemoglobin;
9. Triplets of bases code for one amino acid and change of one base for another base may change the amino acid coded for;
10. Substitution of a single nucleotide, from CTT to CAT in the DNA's template strand of chromosome 11 leads to a change in the codon of mRNA;
11. Changed the original amino acid coded for, which is glutamate, a hydrophilic amino acid, to valine, a hydrophobic amino acid, at sixth position;
12. This will affect the way in which the protein folds due to different side groups and bonds formed, in turn the 3-dimensional conformation / configuration / structure of the protein will be distorted;
13. This will affect the physical and chemical properties of the proteins and altered proteins may affect metabolism or development;
14. Mutated haemoglobin tends to polymerise into long rigid chains when not bound to oxygen;
15. The long fibres distort the membrane of red blood cell giving it its distinct sickle shape;
16. The sickle shape RBC result in less oxygen being carried to tissues and easily stuck in small blood vessels;

For cystic fibrosis

7. Mutation occurs in the gene that codes for Cystic Fibrosis Transmembrane Conductance Regulator (CFTR);
8. Triplets of bases code for one amino acid and removal of three bases will result in loss of one amino acid coded for;
9. Deletion of 3 consecutive nucleotide in the DNA of chromosome 7
10. Result in the loss of phenylalanine in CFTR protein;
11. This will affect the way in which the protein folds due to different side groups and bonds formed, in turn the 3-dimensional conformation / configuration / structure of the protein will be distorted;
12. This will affect the physical and chemical properties of the proteins and altered proteins may affect metabolism or development;
13. CFTR which functions as a Cl^- channel is no longer able to transport chloride ions out of the cells;
14. Accumulation of chloride ions in the cell causing thick mucus accumulating on surface of epithelial cells of lungs / pancreas / reproductive system;

Note: When a question asks to state the type of gene mutation, the answer will be either 'substitution', 'addition' or 'deletion', and NOT 'missense' or 'nonsense' mutation. Both 'missense' and 'nonsense' will are consequences of the said three mutations. 'Frameshift mutation' results from 'addition' or 'deletion' and NOT 'substitution'.

12. Explain the significance of the change in amino acid to the properties of haemoglobin.

At the sixth position of a polypeptide in haemoglobin, amino acid is changed from hydrophilic glutamate to hydrophobic valine;

Alters / disrupts the three-dimensional structure / conformation of haemoglobin;

At low oxygen concentration, hydrophobic areas on different molecules would stick together

Decreases the solubility of haemoglobin in aqueous medium / decreases the affinity of haemoglobin for oxygen;

[3]

13. [NJC 2015/P2/10] Define the terms gene and allele and explain how they differ. [4]

- ***Gene is a heritable factor / unit of inheritance***
- ***Gene is composed of DNA***
- ***Gene controls a specific characteristic / codes for a polypeptide / protein***

[Max. 2 marks]

- ***Allele is a form of a gene***
- ***Alleles of a gene occupy the same gene locus / same position on chromosome***
- ***Occur in pairs in a diploid cell***
- ***Causes slightly different phenotypes for the same characteristic***
- ***Alleles differ (from each other) by one or a small number of bases / base pair(s)***

[Max. 2 marks]

[NJC 2015/P2/10] Describe the chemical structure of the gene. [8]

- ***Genes are composed of units of DNA***
- ***Name 3 out of the 4 nitrogenous bases (A, T, C, G)***
- ***N bases are paired (A-T, G-C)***
- ***Components of nucleotide / DNA***
- ***A / G bases are purines and T / C are pyrimidines***
- ***DNA is a double stranded helix***
- ***H-bonds between N bases in base-pairing***
- ***The two DNA strands are antiparallel***
- ***DNA codes for amino acid sequence in proteins***
- ***Each gene has a start and stop nucleotide sequence***