Name: ______________________

Bio Factsheet

April 1998 Number 22

Protein synthesis I - Nucleic Acids

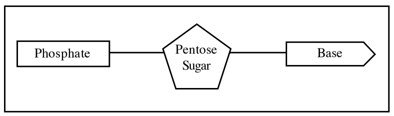
Proteins are large, organic molecules which play a fundamental role in metabolic activities including nutrition, respiration, transport, sensitivity, co-ordination and reproduction.

The characteristics of cells and organisms are determined by the particular proteins which are present. The synthesis of these proteins involves two types of nucleic acid; DNA and RNA. **DNA** is contained within the nucleus of a cell and carries the code to determine which particular proteins are made. Various forms of **RNA** then carry this information to the cytoplasm of the cell and assemble the protein. To understand protein synthesis, you must first have an understanding of DNA and RNA.

Nucleic acids

DNA and RNA are both nucleic acids. Nucleic acids are macromolecules (large molecules) made up of chains of individual units called **nucleotides**. Each nucleotide is made up of 3 parts (Fig 1):

Fig 1. Diagrammatic representation of a nucleotide



1. A **phosphate group** (H_3PO_4), which is the same in all nucleotides.
2. A **pentose (5 carbon atoms) sugar**. This sugar can either be **ribose** sugar ($C_5H_{10}O_5$) or **deoxyribose** sugar ($C_5H_{10}O_4$)
3. One of five **nitrogenous bases**. These bases are divided into two types, depending on their structure (Fig 2):
   (a) **Purines** - Bases made up of one six-sided ring and one five-sided ring.
   (b) **Pyrimidines** - Bases made up of a single six-sided ring. The details of these rings is given in Table 1.

Fig 2. The ring structure of pyrimidines and purines

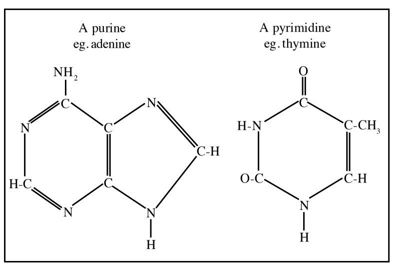
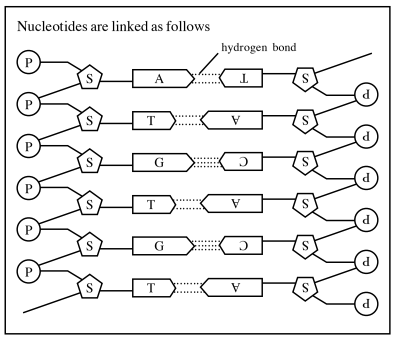


Table 1. Nitrogenous bases in nucleic acids

Ring structure	Base	Symbol	Nucleic acid
Purine (double)	Adenine Guanine	A G	DNA/RNA DNA/RNA
Pyrimidine (single)	Cytosine Thymine Uracil	C T U	DNA/RNA DNA RNA

The three components of nucleotides are joined together by **condensation** reactions (through the removal of water). Individual nucleotides are then joined together by similar condensation reactions between the phosphate group of one nucleotide and the pentose sugar of another (Fig 3). This linkage of nucleotides forms long chains, called **polynucleotides**, which make up nucleic acids.

Fig 3. Formation of a polynucleotide



From Fig 3, it can be seen that polynucleotides have a 'backbone' of phosphate and sugar, with the nitrogenous bases projecting inwards.

> ***Exam hint** - Not all Examination Boards require candidates to be able to recognise purines and pyrimidines but all expect candidates to know that purines are larger molecules than pyrimidines and that A and G are purines etc.*

Comparing DNA & RNA

DNA and RNA are both vital in protein synthesis. Table 2 summarises the similarities and differences between these two macromolecules:

Table 2. Comparison of DNA and RNA

DNA	RNA
Formed in nucleus	Formed in nucleus
Predominantly found in nucleus	Found throughout the cell
Double strand of nucleotides - coiled into a double helix. The two strands are linked by hydrogen bonding between the bases (Fig 3): Cytosine with Guanine, Adenine with Thymine	Single strand of nucleotides which can be folded into different shapes
Pentose sugar present - Deoxyribose	Pentose sugar present - Ribose
Bases present: Cytosine, Guanine, Adenine, Thymine	Bases present: Cytosine, Guanine, Adenine, Uracil
Larger molecule	Smaller molecule
One basic form	Three main forms: messenger RNA, transfer RNA, ribosomal RNA
Ratio of 1:1 for adenine:thymine, and cytosine:guanine	Ratio of adenine:thymine, and cytosine:guanine variable

> ***Exam hint** - Do **not** confuse thymine with thiamine.*

To summarise, DNA and RNA are both made up of nucleotides. In DNA, there are two nucleotide strands which are wound around each other at approximately every ten bases. Thus DNA forms a helix. The strands are **anti-parallel** - i.e. they run in opposite directions to each other. The two strands of nucleotides which make up the DNA double helix are held together by the **hydrogen bonding** between nitrogenous bases. This paring is always as follows:

- **Adenine with Thymine (A-T)**
- **Cytosine with Guanine (C-G)**

The different structures of the bases result in two hydrogen bonds being formed A to T (A=T), and three hydrogen bonds between C to G (C≡G).

The bonding of the nitrogenous bases ensures that purines always bond with pyrimidines, and more specifically, A to T and C to G. The precise nature of this bonding is biologically important for two reasons:

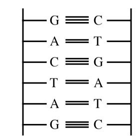
1. The structure of DNA remains exact and regular. This is vital since DNA carries the heredity material for an individual.
2. DNA can exist as a very long sequence of bases, with an enormous variety in order, to carry the large amount of genetic information for an individual.

DNA Replication

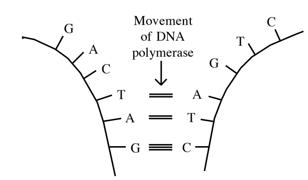
The replication of DNA takes place shortly before cell division, during a phase of the cell cycle called **interphase**. DNA replication is said to be **semi-conservative**. This means that when two new double helixes of DNA are produced, one of the strands of each helix is from the original (parental) DNA strand and the other is new. The sequence of diagrams in Fig 4 illustrate the replication of DNA.

Fig 4. Replication of DNA

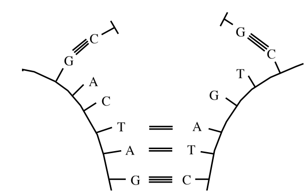
1. A portion of the DNA double helix about to be replicated



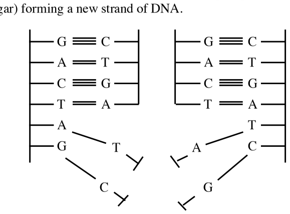
2. Replication has started. The enzyme **DNA polymerase** moves along the DNA double helix unwinding it and 'unzipping it' by breaking the hydrogen bonds between the nitrogenous bases.



3. **Free nucleotides** in the nucleoplasm of the nucleus are attracted to the exposed complementary bases and form new hydrogen bonds with them.



4. DNA polymerase continues to move along the DNA, exposing the bases for free nucleotides to come into and bond. Once these new nucleotides are in place they bond together (phosphate to deoxyribose sugar) forming a new strand of DNA.



5. Replication is now complete, forming two identical strands of DNA which are exact copies of the original strand. This method is said to be semi-conservative, since each strand retains **half** of the original DNA material.

Evidence for semi-conservative DNA replication

The evidence for semi-conservative DNA replication came from experiments by **Matthew Meselsohn** and **Franklin Stahl**, two scientists at the California Institute of Technology, using the bacterium *Escherichia coli*. Matthew and Franklin experiments can be explained in the following series of steps:

1. *E. coli* were cultured in a growth medium containing nitrogen in the form of the isotope ^{15}N (known as 'heavy nitrogen').
2. By leaving the E. coli in the culture for a long enough period of time, all DNA in the *E. coli* became made up of 'heavy nitrogen'. This meant that the molecular weight of the DNA in these *E. coli* was measurably greater.
3. The *E. coli* containing the 'heavy nitrogen' were then placed into a medium containing normal nitrogen (^{14}N), so that any new DNA manufactured would be from this normal nitrogen.
4. The *E. coli* was allowed to divide once and the first generation cells were then collected.
5. When the DNA was extracted from these cells and the relative weight determined using a centrifugation technique, the molecular weight of the DNA was found to be **intermediate** between heavy and light types. This confirmed that the DNA was made up of one original (heavy) strand of DNA and one new (light) strand of DNA - Semi-conservative replication.

Practice Questions

1. Define the following terms:
   (a) DNA double helix (3 marks)

   (b) complementary base pairing (3 marks)

   (c) semi-conservative replication of DNA (2 marks)

2. (a) Read through the following account of DNA replication, then find the most appropriate word or words to complete the account.

During DNA replication, the enzyme .... binds to the DNA double ....This causes the DNA to .... and breaks the .... bonds between the nucleotides. These nucleotides are bound together at .... bases. The base adenine binds with .... and .... binds with guanine. Free nucleotides found in the .... bind with the exposed bases producing two strands of DNA. The process is said to be .... because in both of the two DNA strands produced, one sequence of nucleotides is new and the other is from the ....DNA. (10 marks)

(b) When a sample of DNA is extracted from the nucleus of a cell, chemical analysis showed that 38% of the bases were adenine. What percentage of the bases are guanine (3 marks)

3. DNA and RNA are major molecules involved in the transfer of hereditary material and protein synthesis.
   (a) To which group of molecules do DNA and RNA belong? (1 mark)

   (b) DNA and RNA are both composed of nucleotide sub-units. Describe the structure of a nucleotide. (3 marks)

   (c) State four similarities and four differences between a DNA molecule and an RNA molecule (8 marks)

Answers

Marking points are shown by semicolons

1. (a) Two strands of nucleotide;
   held together by hydrogen bonding;
   coiled or twisted around each other (approximately every 10 bases).

   (b) hydrogen bonding between pairs of organic bases;
   (projecting from the sugar-phosphate backbone of nucleic acids);
   pairing occurs between adenine-thymine, guanine-cytosine in DNA;
   pairing between adenine-uracil, guanine-cytosine in RNA.
   (Any 3)

   (c) Half of the original parent molecule is retained/conserved;
   half is composed of new nucleotide molecules.

2. (a) DNA polymerase;
   helix;
   unwind;
   hydrogen;
   nitrogenous/exposed;
   thymine;
   cytosine;
   nucleoplasm/nucleus;
   semi-conservative;
   parental/original.

   (b) 38% adenine, ∴ 38% thymine;
   remaining 24% is cytosine and guanine (50% each);
   ∴ 12% guanine.

3. (a) nucleic acids.

   (b) phosphate;
   ribose/5C sugar;
   nitrogenous base;
   components joined by condensations reactions

   (c) (see Table 2)

Acknowledgements;
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ISSN 1351-5136

Bio Factsheet

Name: ______________________________

AS Biology- Genetic Control

Number 49

Protein Synthesis II - Mechanisms

Before studying this Factsheet the student should have fully mastered the information in Factsheet Number 22 (Protein synthesis I, April 1998).

This Factsheet summarises the key aspects of the mechanisms of protein synthesis.

1. **The nature of the genetic code.**
2. **The relationships of transfer RNA (tRNA) to amino acids and their role in polypeptide synthesis.**
3. **The roles of messenger RNA (mRNA), rough endoplasmic reticulum(RER) and ribosomes in polypeptide synthesis (transcription and translation).**
4. **The modification of polypeptides into proteins in the RER and Golgi body.**

Questions on this topic usually test knowledge and understanding, by using flow diagram, tick box, 'fill in the missing word' or continual prose questions.

The nature of the genetic code
The genetic code can be found on DNA and on mRNA.

> ***Remember*** *- DNA contains the base thymine but mRNA contains uracil so the letters T or U must be used accordingly.*

This genetic code is universal to all life forms. Fig 1 illustrates the genetic code in its mRNA form.

> ***Exam Hint*** *- A frequent exam error is to say that 'protein synthesis occurs at the ribosomes'. Remember, protein synthesis is a two step process,* ***polypeptide*** *synthesis occurs at the ribosomes, but the assembly of* ***proteins*** *occurs in the spaces of the rough endoplasmic reticulum and Golgi body.*

The triplets of bases shown in Fig 1 are **codons**. A codon is the unit of the genetic code and each codon will always relate to the same amino acid. There are 64 possible codons but only 20 amino acids found in proteins, thus some amino acids have several codons. Because of this, the code is said to be **degenerate** and **redundant**. The code is also **non- overlapping**, meaning that adjacent codons do not share bases.

Fig 1. The genetic code on mRNA

First base	Second base: U		C		A		G		Third base
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys	U
	UUC	Phe	UCC	Ser	UAC	Tyr	UGC	Cys	C
	UUA	Leu	UCA	Ser	UAA	Stop	UGA	Stop	A
	UUG	Leu	UCG	Ser	UAG	Stop	UGG	Trp	G
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U
	CUC	Leu	CCC	Pro	CAC	His	CGC	Arg	C
	CUA	Leu	CCA	Pro	CAA	Gln	CGA	Arg	A
	CUG	Leu	CCG	Pro	CAG	Gln	CGG	Arg	G
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser	U
	AUC	Ile	ACC	Thr	AAC	Asn	AGC	Ser	C
	AUA	Ile	ACA	Thr	AAA	Lys	AGA	Arg	A
	AUG	Met	ACG	Thr	AAG	Lys	AGG	Arg	G
G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly	U
	GUC	Val	GCC	Ala	GAC	Asp	GGC	Gly	C
	GUA	Val	GCA	Ala	GAA	Glu	GGA	Gly	A
	GUG	Val	GCG	Ala	CAG	Glu	GGG	Gly	G

U = uracil
C = cytosine
A = adenine
G = guanine

It is not necessary to learn this by heart, or to remember the amino acids

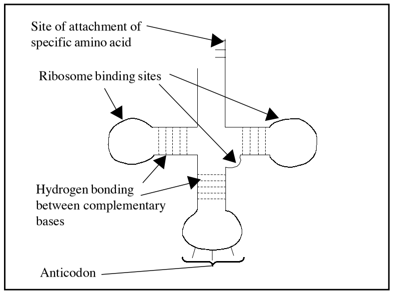
A gene is a length of DNA or mRNA which codes for the assembly of a specific **polypeptide**, and so the sequence of codons which make up the gene will determine the sequence in which amino acids are assembled into that polypeptide. This sequence of amino acids is the **primary structure** of the polypeptide. This will govern how the polypeptide folds and cross bonds into its **secondary structure** (alpha-helix or beta-pleated sheet) and **tertiary structure** (globular form) at the ribosomes, and how it will assemble into its **quaternary structure** (the arrangement and joining of polypeptides together) in the rough endoplasmic reticulum and Golgi body.

Three codons mark the end of genes and are responsible for the release of the polypeptides into the spaces of the rough endoplasmic reticulum. They are referred to as **chain termination codons** or **stop** codons. They may also mark the start of the next gene along the DNA or mRNA molecule.

> **Typical Exam Question**
> An interesting task is to imagine that life in another solar system has the same code but that it is overlapping. Compare the polypeptides made from identical base sequences with a non-overlapping code and an overlapping code. One exam board has asked a question on this theme.

tRNA and its roles in polypeptide synthesis
Transfer RNA is found in the cytoplasm. It is about 80 nucleotides long and is clover leaf in shape (Fig 2). There are 20 types of tRNA molecule, one for each amino acid. One end contains a triplet of exposed nucleotides called the **anticodon**, which is complementary to one of the codons found on the mRNA (Fig 1). The other end of the tRNA molecule has a site for the attachment of a specific amino acid. The amino acid which becomes attached must correspond to the anticodon at the other end, and thus also to the codon on the mRNA.

Fig 2. The structure of tRNA



> ***Remember - Transcription*** *is the copying of genentic code from DNA onto mRNA.* ***Translation*** *is the assembly of a polypeptide from the genetic code on the mRNA.*

Each molecule of tRNA thus picks up its own amino acid, and by matching its anticodon to the complementary codon on the mRNA the amino acids can be assembled into the correct sequence.

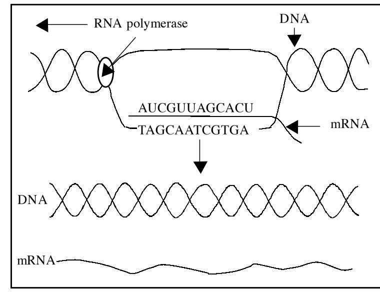
> ***Remember*** *- complementary bases will join by hydrogen bonding, A to U or A to T and C to G. This is essential knowledge to work out some exam answers.*

Before amino acids can join with tRNA they have to be activated using ATP as an energy source. The activation and combination with tRNA occurs in the cytoplasm. Thus protein synthesis is an **anabolic** or energy requiring process.

The roles of mRNA and ribosomes in polypeptide synthesis
The genetic code on the DNA is passed onto mRNA by a process of **transcription**. In this process the DNA helix unwinds for the part of its length which contains the genes to be copied, and one of its strands (called the coding strand) acts as a template for the synthesis of a complementary single strand or mRNA. The enzyme **RNA polymerase** catalyses the process.

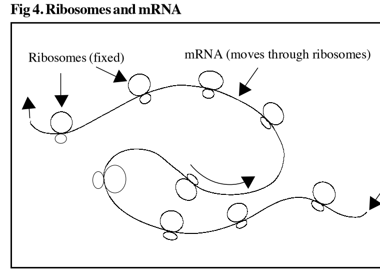
The process of transcription is shown in Fig 3. The mRNA is synthesised from free complementary nucleotides in the surrounding nuclear sap.

Fig 3. Transcription of mRNA from DNA



After transcription the DNA returns to its double stranded form and the new mRNA passes through the pores in the nuclear membrane into the cytoplasm to become associated with the ribosomes that are fixed on the rough endoplasmic reticulum. Fig 4 shows the association between mRNA and ribosomes.

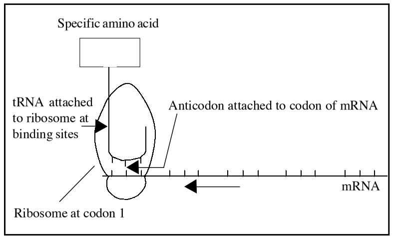
Fig 4. Ribosomes and mRNA



The process of **translation** can now take place. This is the synthesis of a specific polypeptide by the ribosomes using the genetic code on the mRNA to assemble the amino acids in the correct sequence.

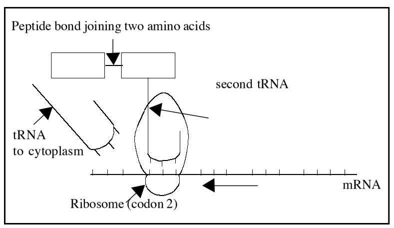
In the first step of translation codon 1 of the first gene is covered by the ribosome. This enables the complementary tRNA to attach to the codon with its anticodon, by hydrogen bonding and so the first specific amino acid is brought into place (Fig 5).

Fig 5. Translation Step 1



In the second step of translation the mRNA moves so that codon 2 of the gene is covered by the ribosome. This enables the second tRNA molecule to attach to the second codon by an anticodon-codon link and so the second specific amino acid is carried into place. The enzyme **peptide synthetase** in the ribosome catalyses the condensation reaction to form a **peptide bond** to join the first and second amino acids into a dipeptide. The first tRNA molecule is then released back to the cytoplasm for reuse (Fig 6).

Fig 6. Translation Step 2



Similar steps are repeated as each successive codon of the gene is covered by the ribosome, and so a polypeptide is assembled, the amino acid sequence of which is related to the codon sequence of the gene. At the end of the gene is a chain termination (stop) codon. When this is covered by the ribosome there is no complementary tRNA to join the codon and so the synthesised polypeptide is released into the spaces of the rough endoplasmic reticulum. The process of translation then proceeds along gene 2 of the mRNA.

***Remember -** It is now known that the ribosome covers two codons of the mRNA at a time.Thus two tRNA molecules with their amino acids can be held in place while a peptide bond forms.*

The process of polypeptide synthesis is **amplified** by having the length of mRNA attached to several or many ribosomes at a time so that they can all carry out translation at the same time. Such an assembly of mRNA and ribosomes attached to the rough endoplasmic reticulum is called a **polyribosome**. The same length of mRNA can pass through the same assembly of ribosomes time and time again. The polyribosomes in an activated plasma cell enable the production of around 2000 antibody molecules per cell per second for 4 to 5 days.

(The mRNA and associated ribosomes illustrated in Fig 4. is a polyribosome system).

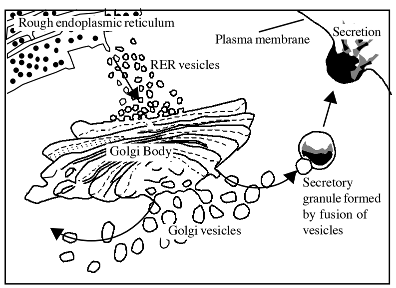
Modification of polypeptides into protein

The synthesised polypeptides are transferred to the Golgi body in vesicles which bud off from the rough endoplasmic reticulum, migrate through the cytoplasm and fuse with the cisternae (cavities) of the Golgi body. Here (and also in the rough endoplasmic reticulum and its vesicles) the polypeptides couple by hydrogen bonding and sulphur bonding, between amino acid side chain groups, to form proteins. Examples of proteins formed in this way are **lysozyme** and **catalase**.

The Golgi body also allows the assembly of other protein derivatives. For instance, carbohydrates may be joined to proteins to make **glycoproteins** such as mucus, lipids may be joined to proteins to make **lipoproteins,** iron containing haem groups may be joined to proteins to make molecules such as **haemoglobin, myoglobin** and **cytochromes.**

The products of the Golgi body are budded off as Golgi vesicles.They either remain in the cytoplasm as, for example, lysosomes (containing lysozyme) and peroxisomes (containing catalase), or fuse together into secretory granules. These can then fuse with the plasma membrane to secrete their contents out of the cell, for example, antibodies, plasma proteins, digestive system enzymes. This process is called **exocytosis**. The functions of the Golgi body are shown in Fig 7.

Fig 7. The functions of the Golgi body



Practice Questions

1. Read through the following account of protein synthesis and then fill in the spaces with the most **appropriate word or words.**

   Messenger RNA formed by ______________ from the nuclear DNA passes through pores in the _________________ and attaches to ________ fixed to the _____________________. __________________ amino acids are brought to the mRNA by the molecules of ____________ which attach to the ___________ of the mRNA by their ___________________. Adjacent amino acids then join by _____________________ to form a ___________________. These assemble into proteins either in the spaces or vesicles of the _______________________ or are transported to the _________________ for assembly there.

   (12 marks)

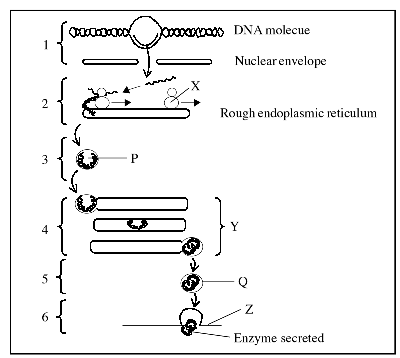
2. The table below refers to some features of mRNA and tRNA. If a feature is correct mark the relevant box with a tick and if it is incorrect mark the box with a cross.

Feature	mRNA	tRNA
Contains anticodons		
May contain several genes or alleles		
Has a clover leaf shape		
Can associate with any amino acid		
Contains uracil instead of thymine		
A short molecule 70 –90 nucleotides long		

.

(6 marks)

3. The diagram below shows some of the stages involved in the secretion of an enzyme by a stomach cell. The stages are labelled 1 to 6.



(a) Name the structures X, Y and Z.

(b) Name the processes occuring in stages 1, 2, 4 and 6.

(4 marks)

(c) Distinguish between vesicles P and Q and their contents.

(4 marks)

4. The following sequence of codons is from the gene on DNA which codes for part of the haemoglobin molecule.

   CAT GTA AAT TGA GGA CTT CTC
   DNA

   (a) Using the genetic code shown on page I work out the haemoglobin gene codons on the mRNA and the sequence of amino acids found in the haemoglobin molecule.

   (3 marks)

   (b) If the DNA base T, marked with an arrow was substituted with A, how would the haemoglobin chain differ?

   (1 mark)

Answers

Semicolons indicate marking points.

1. transcription; nuclear membrane; ribosomes; rough endoplasmic reticulum; specific; tRNA; codons; anticodons; peptide bonds/ condensation/peptide links; polypeptide; rough endoplasmic reticulum; Golgi body;

2.

Feature	mRNA	tRNA
Contains anticodons	✗	✓
May contain several genes or alleles	✓	✗
Has a clover leaf shape	✗	✓
Can associate with any amino acid	✗	✗
Contains uracil instead of thymine	✓	✓
A short molecule 70 –90 nucleotides long	✗	✓

3. (a) X = ribosome; Y = vesicle of RER; Z = Golgi vesicle;

   (b) 1 = transcription; 2 = translation; 4 = protein assembly/modification; 6 = exocytosis;

   (c) P is a vesicle from the rough endoplasmic reticulum; Q is a vesicle from the Golgi body;

   P contains polypeptides/proteins assembled in RER: Q contains proteins assembled in Golgi body/modified proteins/ glycoproteins/any correct example;

4. (a) GUA CAU UUA ACU CCU GAA GAG;; (deduct 1 mark per error)

   Val His Leu Thr Pro Glu Glu ;

   (b) last but one amino acid/penultimate amino acid would be valine/ Val instead of glutamic acid/Glu;

Acknowledgements;
This Factsheet was researched and written by Martin Griffin.
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ISSN 1351-5136