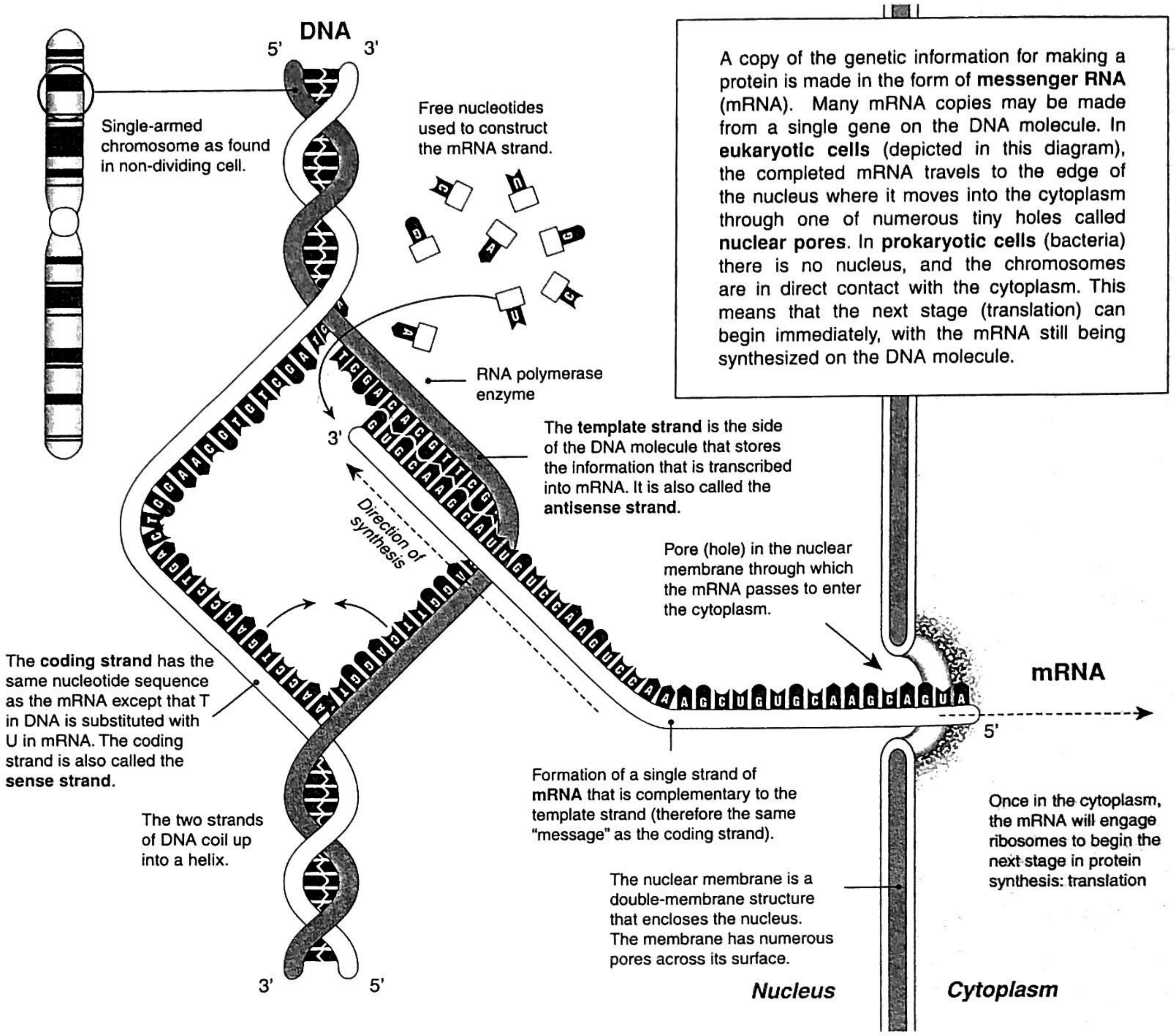
Transcription in Eukaryotes

Transcription is the process by which the code contained in the DNA molecule is transcribed (rewritten) into a **mRNA** molecule. Transcription is under the control of the cell's metabolic processes which must activate a gene before this process can begin. The enzyme that directly controls the process is RNA polymerase, which makes a strand of mRNA using the single strand of DNA (the **template strand**) as a template (hence the term). The enzyme transcribes only a gene length of DNA at a time and therefore recognizes start and stop signals (codes) at the beginning and end of the gene. Only RNA polymerase is involved in mRNA synthesis; it causes the unwinding of the DNA as well. It is common to find several RNA polymerase enzyme molecules on the same gene at any one time, allowing a high rate of mRNA synthesis to occur.



1. Explain the role of messenger RNA (mRNA) in protein synthesis: ______________________________

____

____

2. Explain the difference between the template and coding strands: ______________________________

____

3. For the following triplets on the DNA, determine the **codon** sequence for the mRNA that would be synthesized:

(a) Triplets on the DNA: T A C T A G C C G C G A T T T

Codons on the mRNA: ______________________________

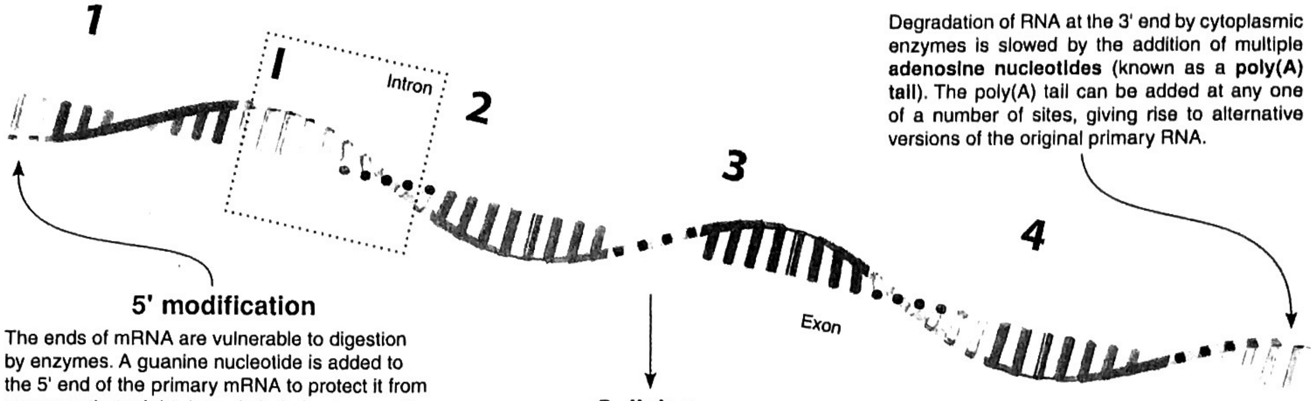
(b) Triplets on the DNA: T A C A A G C C T A T A A A A

Codons on the mRNA: ______________________________

Post Transcriptional Modification

Human DNA contains only 25,000 genes, but produces 90,000 different proteins. Each gene must therefore produce more than one protein. This is achieved by both **post transcriptional** and **post translational modification**. Primary mRNA molecules contain exons and introns. Usually **introns** are removed after transcription and the **exons** are spliced together, this is post transcriptional modification. However, the number of exons joined together and the way they are spliced together is not always the same. This creates variations of the polypeptide chain that results. These mechanisms allow for the production of the diverse range of proteins.

Post Transcriptional Modification



3' modification

Degradation of RNA at the 3' end by cytoplasmic enzymes is slowed by the addition of multiple **adenosine nucleotides** (known as a **poly(A) tail**). The poly(A) tail can be added at any one of a number of sites, giving rise to alternative versions of the original primary RNA.

5' modification

The ends of mRNA are vulnerable to digestion by enzymes. A guanine nucleotide is added to the 5' end of the primary mRNA to protect it from enzymes that might degrade it during transport from the nucleus to the cytoplasm. This process is called capping.

Splicing

1 2 4

2 3 4
1 3 4

1 I 2 3 4

Long binding site used → 1 2 3 4
Short binding site used → 1 2 3 4

Exon Skipping
During splicing, an exon may be skipped. This is a relatively common way to produce protein variants in mammals.

Mutually Exclusive Exons
In some cases, only one of two exons (but never both) will be incorporated into the mature mRNA.

Intron Retention
Introns are not always removed during the splicing process. In some rare cases the intron is retained in the mature mRNA.

Alternative Binding Sites
Exons may contain more than one site for binding to other exons. If the shorter version is used, the remaining code is discarded, and results in a shorter mRNA sequence.

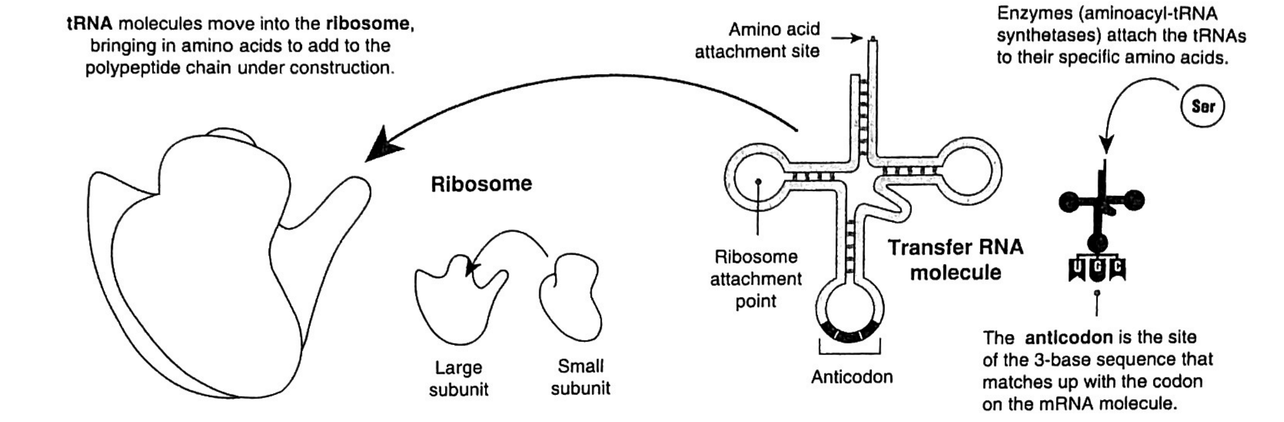
1. Explain why it is difficult to identify the true number of genes that are in the human genome: ____________________

2. Describe the ways in which mRNA can be modified to code for different proteins: ____________________

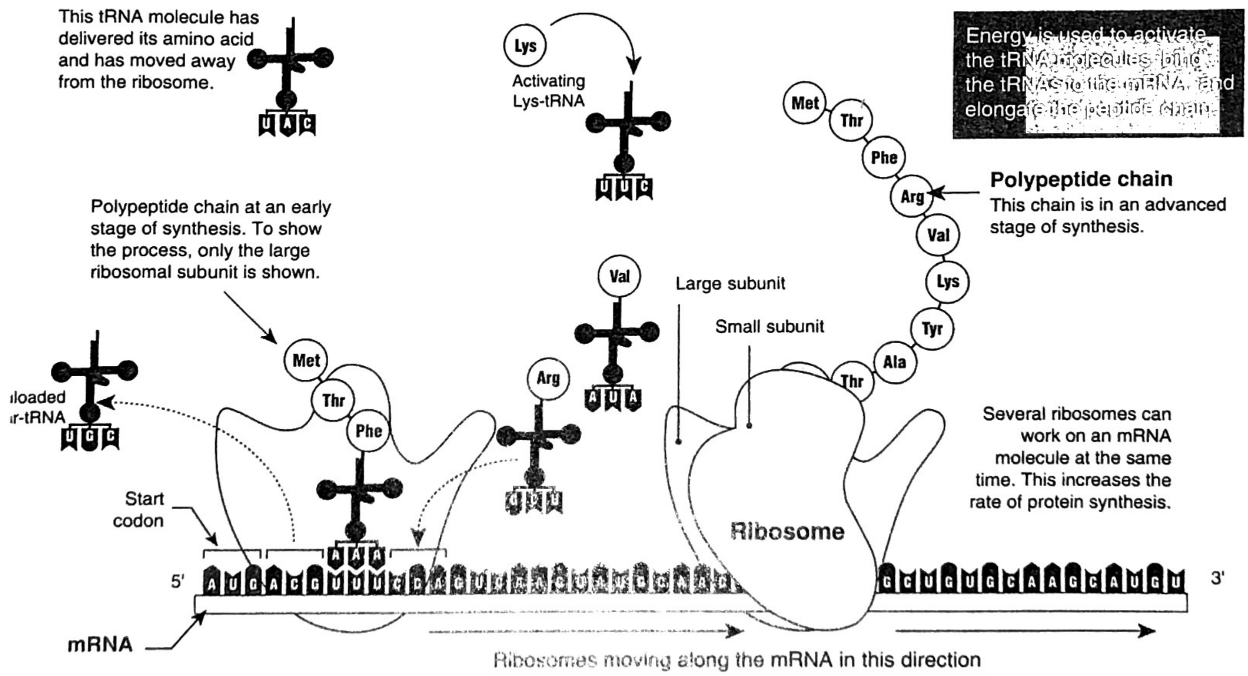
3. Explain the advantage of being able to modify the mRNA to produce different proteins: ____________________

Translation

The diagram at the bottom of the page shows the translation phase of protein synthesis. The scene shows how a single mRNA molecule can be 'serviced' by many ribosomes at the same time. The ribosome on the right is in a more advanced stage of constructing a polypeptide chain because it has 'translated' more of the mRNA than the ribosome on the left. The anticodon at the base of each tRNA must make a perfect complementary match with the codon on the mRNA before the amino acid is released. Once released, the amino acid is added to the growing polypeptide chain by enzymes.



Ribosomes are made up of a complex of ribosomal RNA (rRNA) and proteins. They exist as two separate sub-units (above) until they are attracted to a binding site on the mRNA molecule, when they join together. Ribosomes have binding sites that attract transfer RNA (**tRNA**) molecules loaded with amino acids. The tRNA molecules are about 80 nucleotides in length and are made under the direction of genes in the chromosomes. There is a different tRNA molecule for each of the different possible anticodons (see the diagram below) and, because of the degeneracy of the genetic code, there may be up to six different tRNAs carrying the same amino acid.



1. For the following codons on the mRNA, determine the **anticodons** for each tRNA that would deliver the amino acids:

   Codons on the mRNA: U A C   U A G   C C G   C G A   U U U

   Anticodons on the tRNAs: ______________________________

2. There are many different types of tRNA molecules, each with a different anticodon (HINT: see the mRNA table).

   (a) How many different tRNA types are there, each with a unique anticodon? ______________________

   (b) Explain your answer: ______________________________

   ____

   ____

The diagram opposite shows an overview of the process of protein synthesis. Each of the structures involved is labeled with a letter (**A-J**), while the major steps in the process are identified with numbers (**1-8**).

1. Using the word list provided below, identify each of the structures marked with a letter. Write the name of that structure in the spaces provided on the diagram.

   DNA, nuclear pore, free nucleotides, tRNA, RNA polymerase enzyme, amino acids, mRNA, ribosome, nuclear membrane, polypeptide chain

2. Match each of the processes (identified on the diagram with numbers 1-8) to the correct summary of the process provided below. Write the process number next to the appropriate sentence.

   ☐ tRNA molecule is recharged with another amino acid of the same type, ready to take part in protein synthesis

   ☐ tRNA molecule brings in the correct amino acid to the ribosome

   ☐ Unwinding the DNA molecule

   ☐ DNA rewinds into double helix structure

   ☐ Anti-codon on the tRNA matches with the correct codon on the mRNA and drops off the amino acid

   ☐ tRNA leaves the ribosome

   ☐ mRNA moves through nuclear pore in nuclear membrane to the cytoplasm

   ☐ mRNA synthesis: nucleotides added to the growing strand of messenger RNA molecule

3. Explain the purpose of protein synthesis: ______________________

4. Name the three different types of RNA involved in protein synthesis: ______________________

5. Outline three structural or functional differences between RNA and DNA:

   (a) ______________________

   (b) ______________________

   (c) ______________________

6. How are nucleic acids attached to tRNA? ______________________

7. (a) Name the general process taking place in the **nucleus**: ______________________

   (b) Name the general process taking place in the **cytoplasm**: ______________________

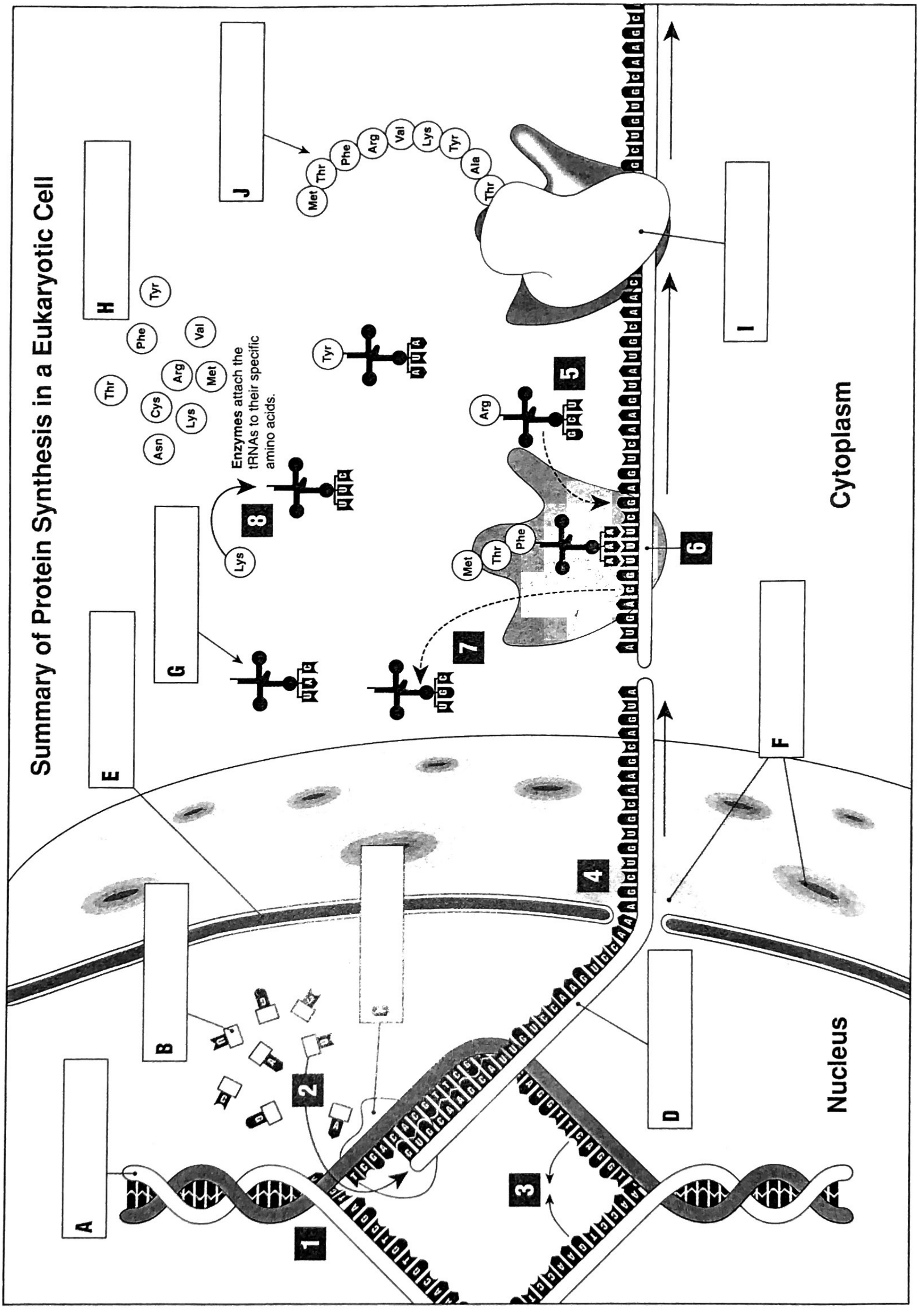
8. Consult the *mRNA-amino acid table* earlier in this workbook. Explain the result of a point mutation involving a change to the third base in a nucleotide as follows:

   (a) UUU changes to UUC: ______________________

   (b) UUU changes to UUA: ______________________

   (c) Which of these mutations is likely to result in a change to the protein produced? ______________________

Protein Synthesis: Translating the Code

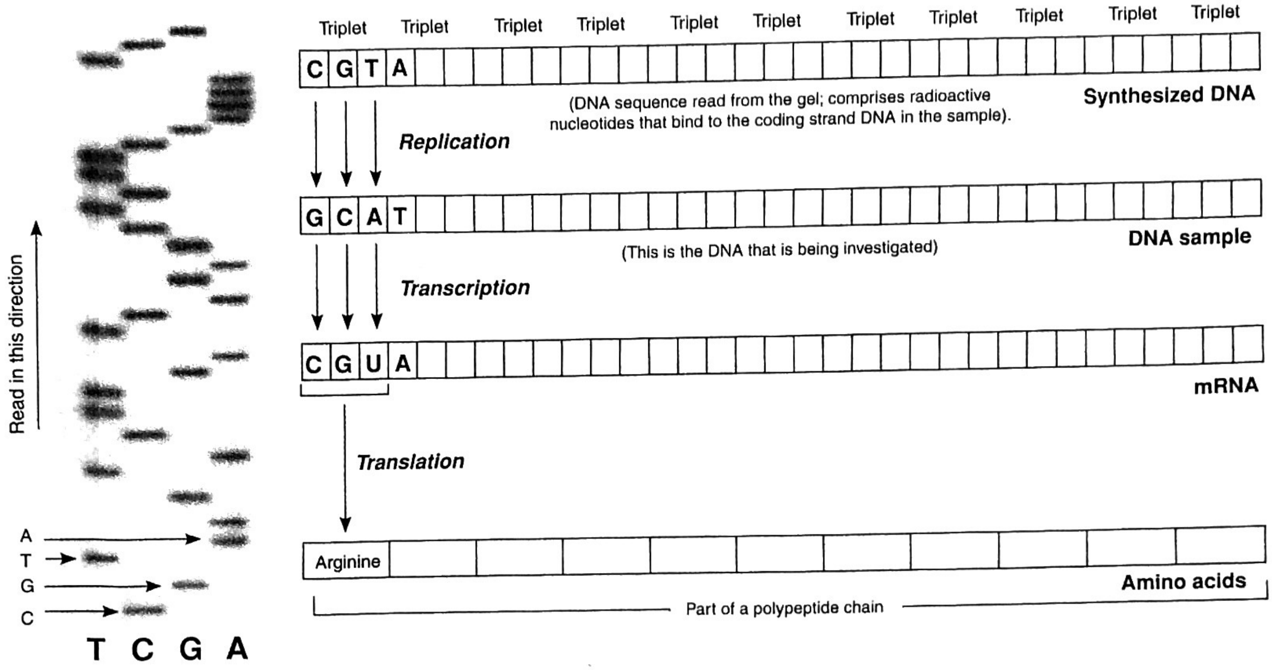


Analyzing a DNA Sample

The nucleotide (base sequence) of a section of DNA can be determined using DNA sequencing techniques The base sequence determines the amino acid sequence of the resultant protein therefore the DNA tells us what type of protein that gene encodes. This exercise reviews the areas of DNA replication, transcription, and translation using an analysis of a gel electrophoresis column. **Attempt it after you have completed the rest of this topic**. Remember that the gel pattern represents the sequence in the synthesized strand.

1. Determine the amino acid sequence of a protein from the nucleotide sequence of its DNA, with the following steps:
   (a) Determine the sequence of **synthesized DNA** in the gel
   (b) Convert it to the complementary sequence of the **sample DNA**
   (c) Complete the **mRNA** sequence
   (d) Determine the **amino acid** sequence by using the 'mRNA amino acid table' in this workbook.

   NOTE: The nucleotides in the gel are read from bottom to top and the sequence is written in the spaces provided from left to right (the first 4 have been done for you).



2. For each single strand DNA sequence below, write the base sequence for the **complementary DNA** strand:

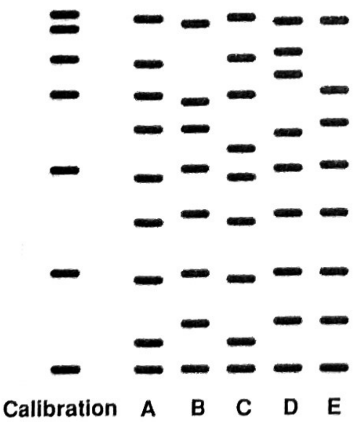
   (a) DNA: T A C T A G C C G C G A T T T A C A A T T

   DNA: ______________________________

   (b) DNA: T A C G C C T T A A A G G G C C G A A T C

   DNA: ______________________________

   (c) Identify the cell process that this exercise represents: ______________________________



3. Determine the relatedness of each individual (A-E) using each banding pattern on the set of DNA profiles (left). When you have done this, complete the dendrogram by adding the letter of each individual.