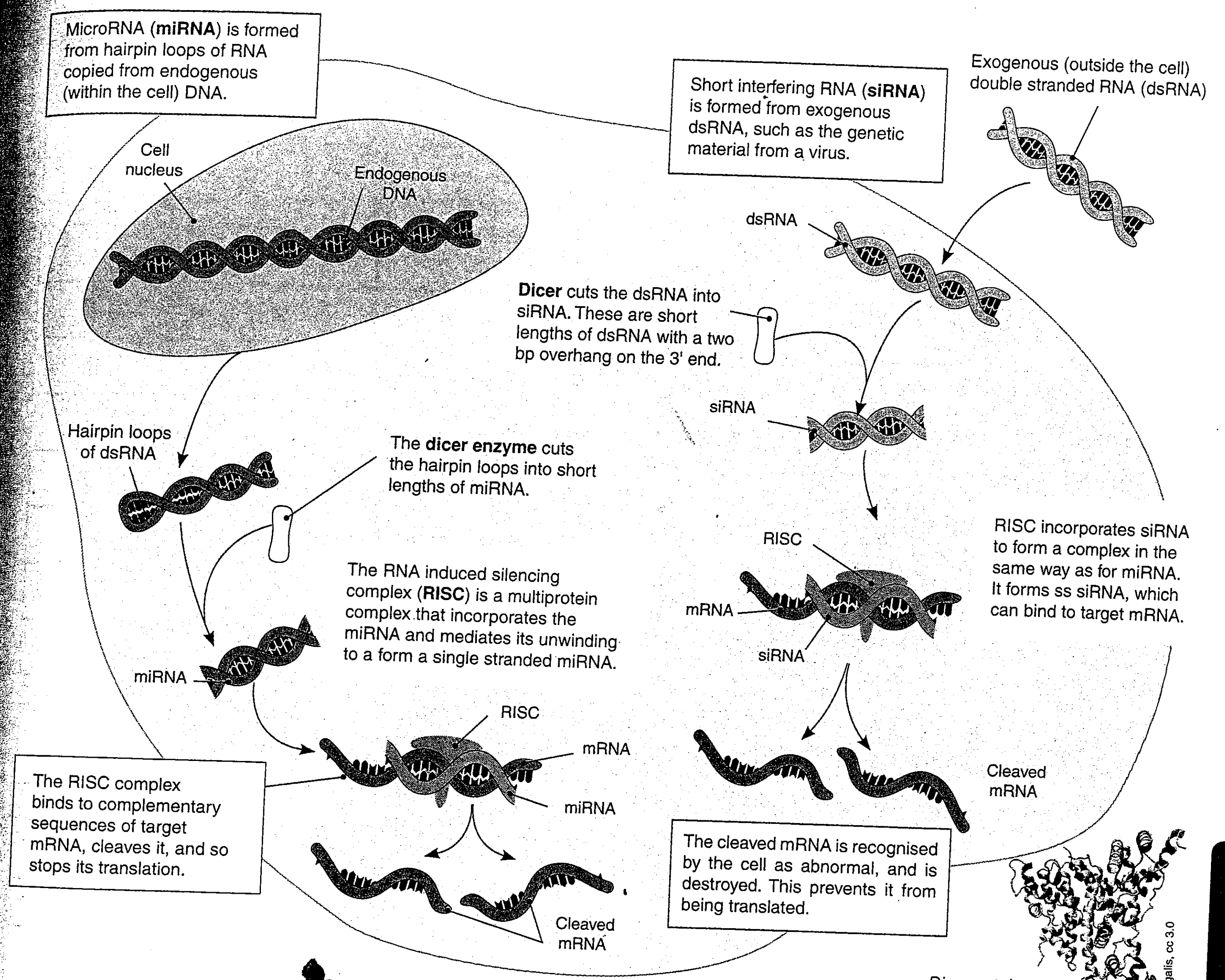
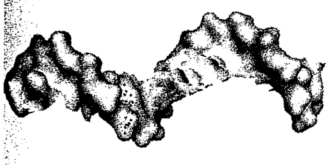
RNA Molecules

RNA plays vital roles in transcribing and translating DNA, forming messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA). RNA is also involved in processes such as modifying mRNA after transcription and regulating translation.

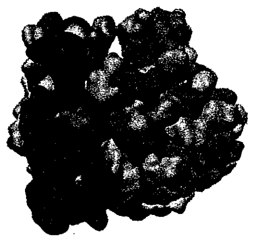
Regulation of translation is achieved by destroying specific mRNA targets using short RNA lengths, which may be exogenous (short interfering RNAs) or endogenous (microRNAs). Mechanisms of **RNA interference** (RNAi) are illustrated below.



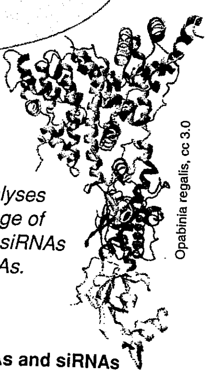


Messenger RNA (above) is transcribed from DNA. It carries a copy of the genetic instructions from the DNA to ribosomes in the cytoplasm, where it is translated into a polypeptide chain.

Transfer RNA (above) carries amino acids to the growing peptide chain. One end of the tRNA carries the genetic code in a three-nucleotide sequence called the anticodon. The amino acid links to the 3' end of the tRNA.



Ribosomal RNA (above) forms ribosomes from two separate ribosomal components (the large and small subunits) and assembles amino acids into a peptide chain.



Dicer catalyses the cleavage of dsRNA to siRNAs and miRNAs.

miRNAs and siRNAs

RNA interference (**RNAi**) regulates gene expression through miRNAs and siRNAs, which bind to specific mRNA sequences, causing them to be cleaved. RNAi is important in gene regulation during development and in defence against viruses, which often use double-stranded RNA as an infectious vector.

DNA and the Genetic Code

1. Briefly describe the roles of RNA: ______________________________

____

2. How does RNAi regulate gene expression? ______________________________

____

____

____

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Related activities: *Transcription in Eukaryotes, Translation*

The Genetic Code

The genetic information that codes for the assembly of amino acids is stored as three-letter codes, called **codons**. Each codon represents one of 20 amino acids used in the construction of polypeptide chains. The **mRNA-amino acid table** (bottom of page) can be used to identify the amino acid encoded by each of the mRNA codons. Note that the code is **degenerate** in that for each amino acid, there may be more than one codon. Most of this degeneracy involves the third nucleotide of a codon. The genetic code is **universal**; all living organisms on Earth, from viruses and bacteria, to plants and humans, share the same genetic code (with a few minor exceptions representing mutations that have occurred over the long history of evolution).

Amino acid	Codons that code for this amino acid	No.	Amino acid	Codons that code for this amino acid	No.
Ala Alanine	GCU, GCC, GCA, GCG	4	**Leu** Leucine		
Arg Arginine			**Lys** Lysine		
Asn Asparagine			**Met** Methionine		
Asp Aspartic acid			**Phe** Phenylalanine		
Cys Cysteine			**Pro** Proline		
Gln Glutamine			**Ser** Serine		
Glu Glutamic acid			**Thr** Threonine		
Gly Glycine			**Try** Tryptophan		
His Histidine			**Tyr** Tyrosine		
Iso Isoleucine			**Val** Valine		

1. Use the **mRNA-amino acid table** (below) to list in the table above all the **codons** that code for each of the amino acids and the number of different codons that can code for each amino acid (the first amino acid has been done for you).

2. (a) How many amino acids could be coded for if a codon consisted of just two bases? ________________

   (b) Why is this number of bases inadequate to code for the 20 amino acids required to make proteins?

3. Describe the consequence of the degeneracy of the genetic code to the likely effect of a change to one base in a triplet:

mRNA-Amino Acid Table

How to read the table: The table on the right is used to 'decode' the genetic code as a sequence of amino acids in a polypeptide chain, from a given mRNA sequence. To work out which amino acid is coded for by a codon (triplet of bases) look for the first letter of the codon in the row label on the left hand side. Then look for the column that intersects the same row from above that matches the second base. Finally, locate the third base in the codon by looking along the row from the right hand end that matches your codon.

Example: Determine **CAG**

C on the left row,
A on the top column,
G on the right row
CAG is Gln (**glutamine**)

Read second letter here — Read first letter here — Read third letter here

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

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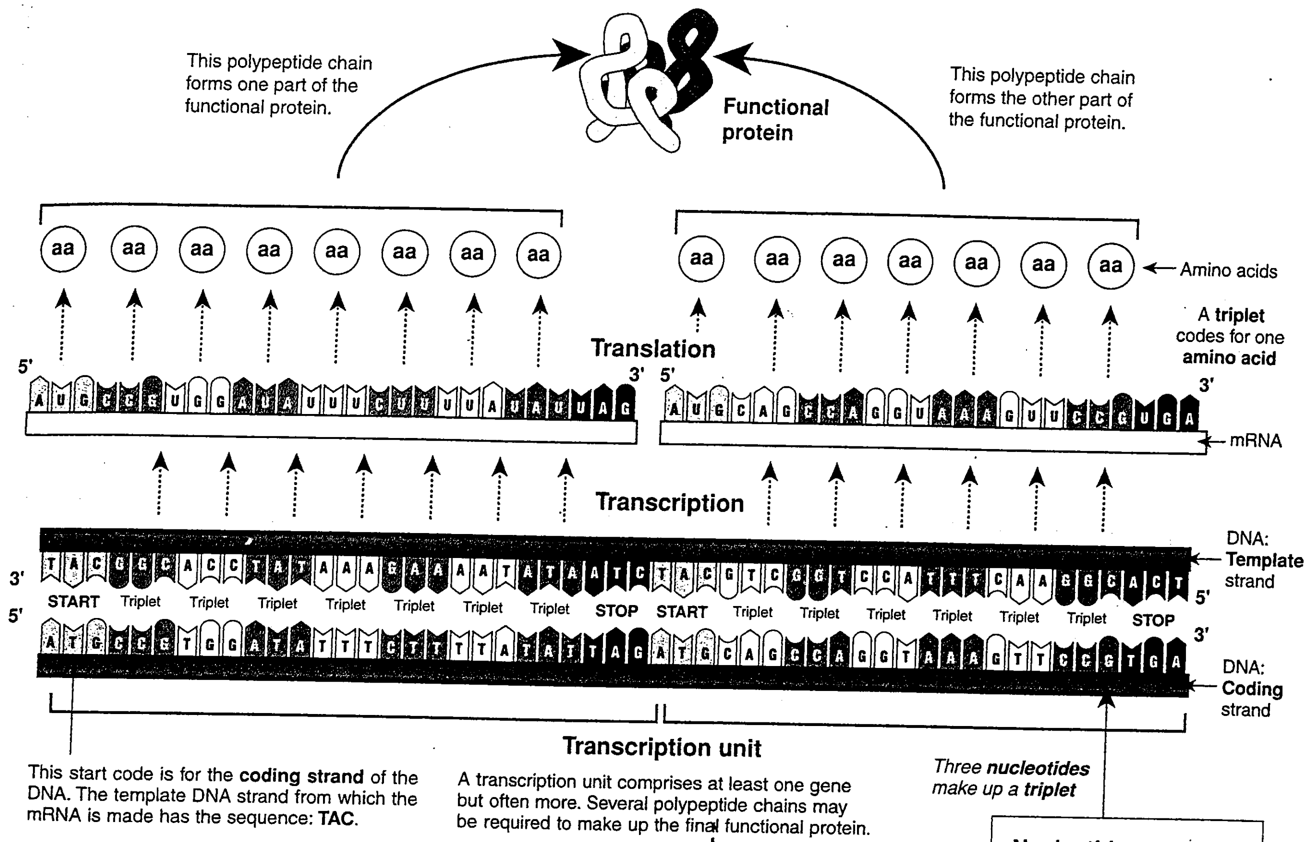
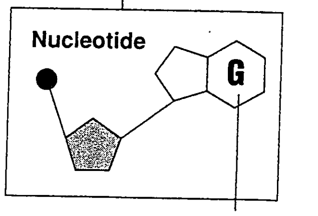
The Simplest Case: Genes to Proteins

The traditionally held view of genes was as specific sections of DNA encoding proteins (including **enzymes**). This simple definition was revised as the one gene-one polypeptide hypothesis in recognition of the fact that several polypeptide chains may contribute to the functional protein. Even more recently, geneticists have discovered that genes also encode functional RNAs that have regulatory roles in the cell. Much of the nonprotein-coding DNA there fore has a purpose and is not "junk" DNA as was previously assumed. Although our concept of what constitutes a gene is changing rapidly and now encompasses all those segments of DNA that are transcribed (to RNA), the concept of one gene-one polypeptide is still useful. This activity considers this simplest scenario: that in which the gene codes for a functional protein. The **nucleotides** are read as **triplets**, which are equivalent to **codons** on the mRNA. Punctuation codons mark the start and end points for the gene. The genes required to form a functional end-product (in this case, a functional protein) are collectively called a **transcription unit**.





In models of nucleic acids, nucleotides are denoted by their base letter. (In this case: **G** is for guanine)

1. Describe the purpose of the start and stop codons on a strand of DNA:

2. (a) The mRNA strand is transcribed from which DNA strand? ______

   (b) Explain how the bases differ between the DNA strand and the mRNA strand that results from transcription:

3. Describe the steps involved in forming a functional enzyme comprising two polypeptide chains: ______

4. Recall the anti-parallel nature of DNA, with the strands orientated in opposite directions. Explain its significance:

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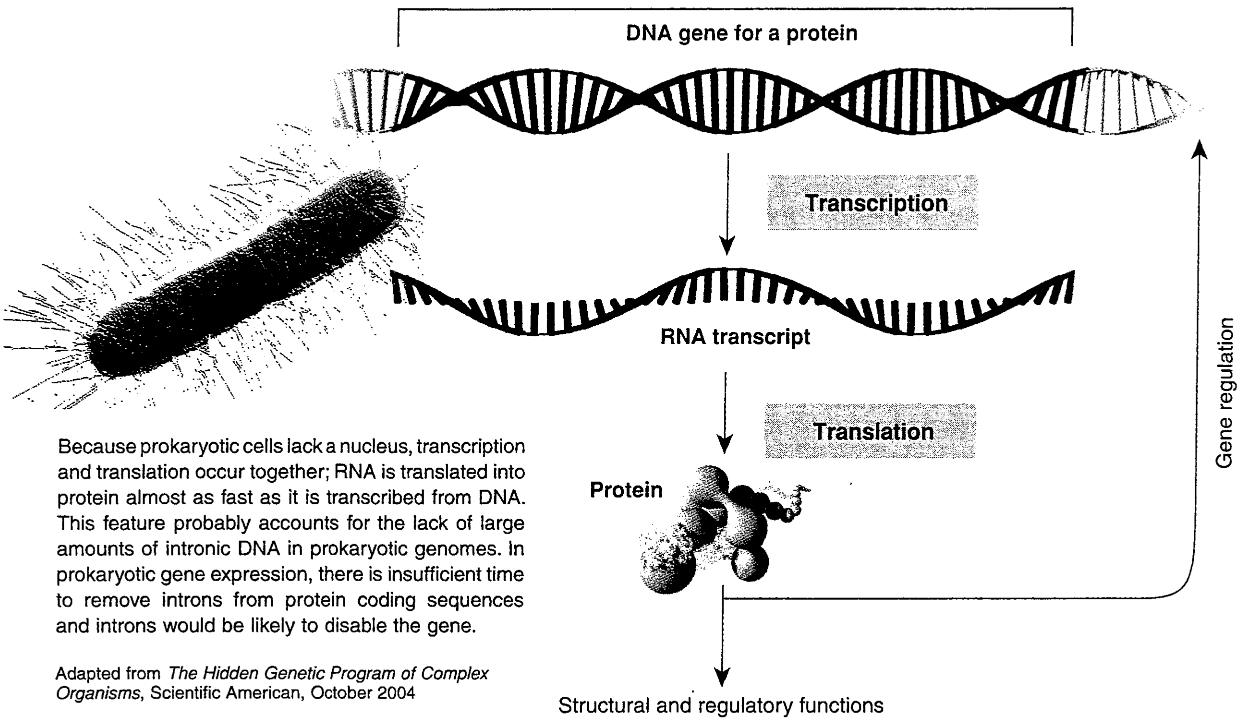
Gene Expression in Prokaryotes

The process of transferring the information encoded in a gene to its functional gene product is called **gene expression**. The central dogma of molecular biology for the past 50 years or so has stated that genetic information, encoded in DNA, is transcribed as molecules of RNA, which are then translated into the amino acid sequences that make up proteins. The established opinion was often stated as "one gene-one protein" and proteins were assumed to be the main regulatory agents for the cell (including its gene expression). The one gene-one protein model is supported by studies of prokaryotic genomes, where the DNA consists almost entirely of protein-coding genes and their regulatory sequences.

Genes and Gene Expression in Prokaryotes



Because prokaryotic cells lack a nucleus, transcription and translation occur together; RNA is translated into protein almost as fast as it is transcribed from DNA. This feature probably accounts for the lack of large amounts of intronic DNA in prokaryotic genomes. In prokaryotic gene expression, there is insufficient time to remove introns from protein coding sequences and introns would be likely to disable the gene.

Adapted from *The Hidden Genetic Program of Complex Organisms*, Scientific American, October 2004

1. Describe the important features of gene expression in prokaryotes: ____________________

____

____

____

2. The traditional (old) view of gene expression in eukaryotes (table right) was based on a modification of the one gene-one protein model. This model does not adequately explain gene expression in eukaryotes, but it is probably still appropriate for prokaryotes. Suggest why:

____

____

____

____

____

____

Gene Expression in Eukaryotes

The Old View	The New View
Introns are spliced out of a primary RNA transcript	Introns are spliced out of a primary RNA transcript
All the exon RNA is translated into proteins.	Not all exon RNA is translated into proteins. Non-protein-coding exonic DNA may have its own function or may contribute to microRNAs
Introns are junk DNA with no function; they are degraded and recycled.	Introns are processed into microRNAs which are involved in regulating development.

3. How is gene expression in prokaryotes fundamentally different from gene expression in eukaryotes?

____

____

____

Related activities: *The Simplest Case: Genes to Proteins*

Periodicals: *Gene structure and*

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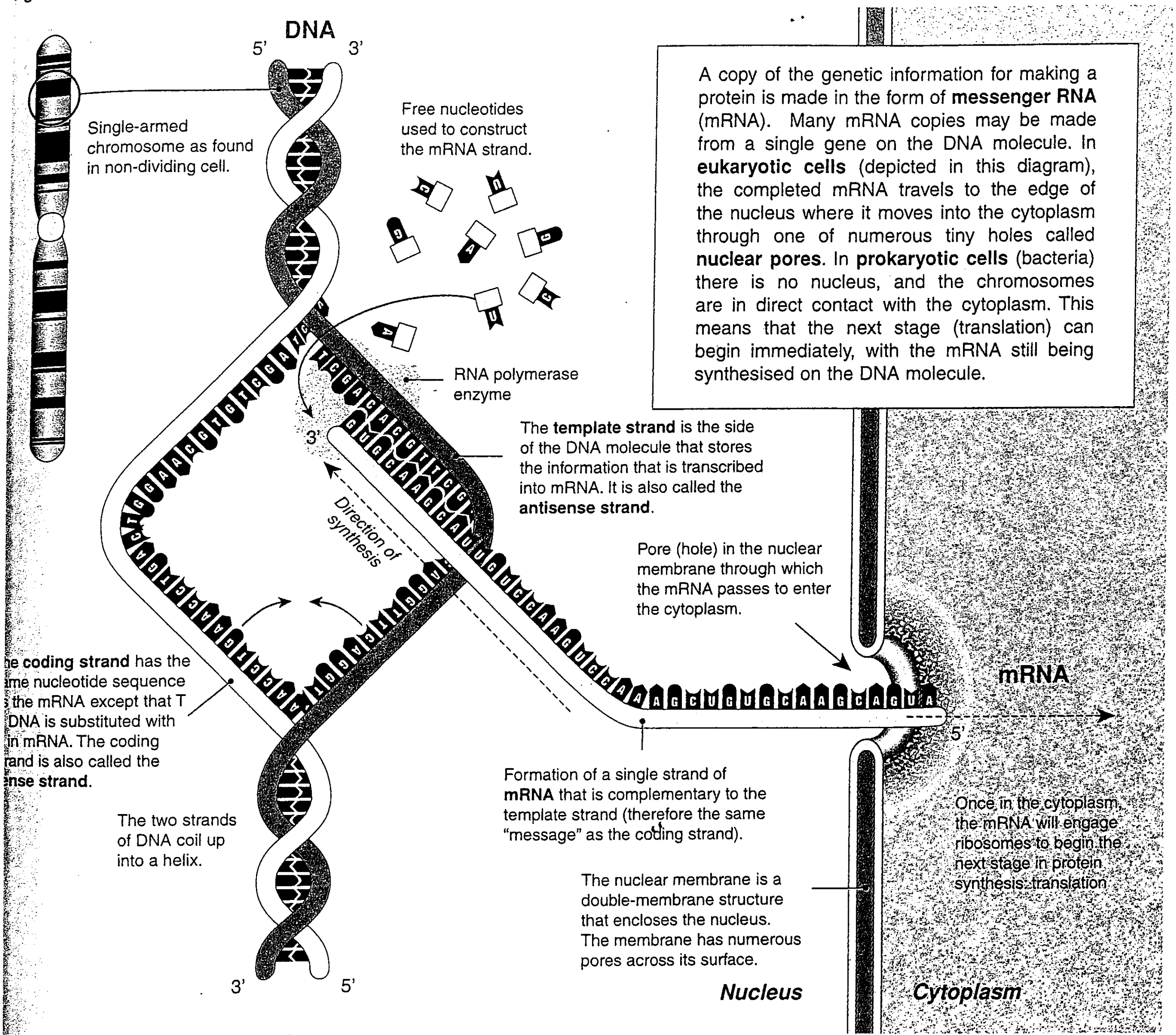
ranscription in Eukaryotes

'anscription is the process by which the code contained in ie DNA molecule is transcribed (rewritten) into a **mRNA** iolecule. Transcription is under the control of the cell's ietabolic processes which must activate a gene before this rocess can begin. The enzyme that directly controls the process RNA polymerase, which makes a strand of mRNA using the ngie 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 recognises start and stop signals (codes) at the beginning and end of the gene. Only RNA polymerase is involved in mRNA synthesis as it unwinds 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.



Explain the role of messenger RNA (mRNA) in protein synthesis: ______________________

____

____

The genetic code contains punctuation codons to mark the starting and finishing points of the code for synthesis of polypeptide chains and proteins. Consult the mRNA–amino acid table earlier in this workbook and state the codes for:

(a) Start codon: ______________ (b) Stop (termination) codons: ______________

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

(a) Triplets on the DNA: TAC TAG CCG CGA TTT

Codons on the mRNA: ______________________

(b) Triplets on the DNA: TAC AAG CCT ATA AAA

Codons on the mRNA: ______________________

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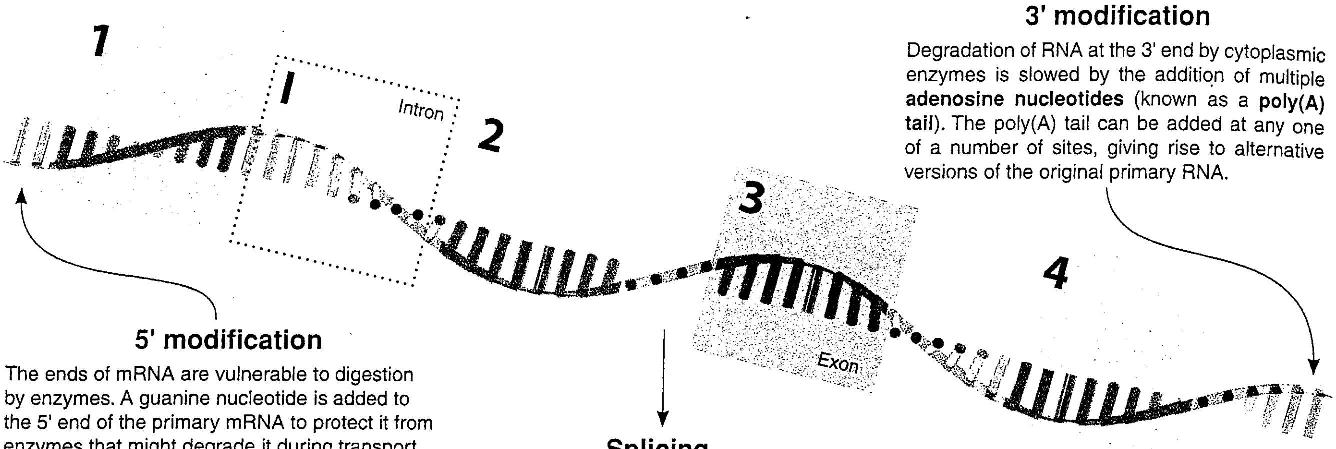
Post Transcriptional Modification

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. 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

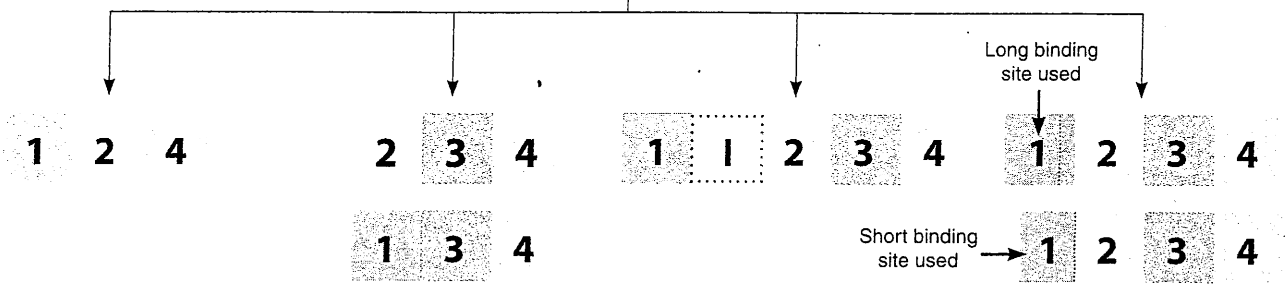
Degradation of RNA at the 3' end by cytoplasmic enzymes is slowed by the addition of multiple **adenosine nucleotides** (known as a **poly(A) tail**). The poly(A) tail can be added at any one of a number of sites, giving rise to alternative versions of the original primary RNA.



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



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. Why is it difficult to determine the true number of genes in the human genome? ______________________

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

3. What is the advantage of being able to modify the mRNA to produce different proteins? ______________________

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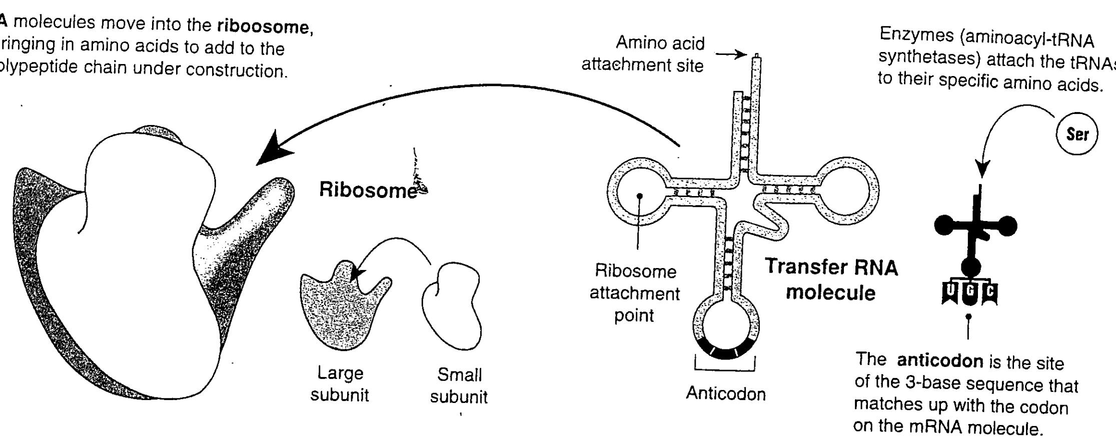
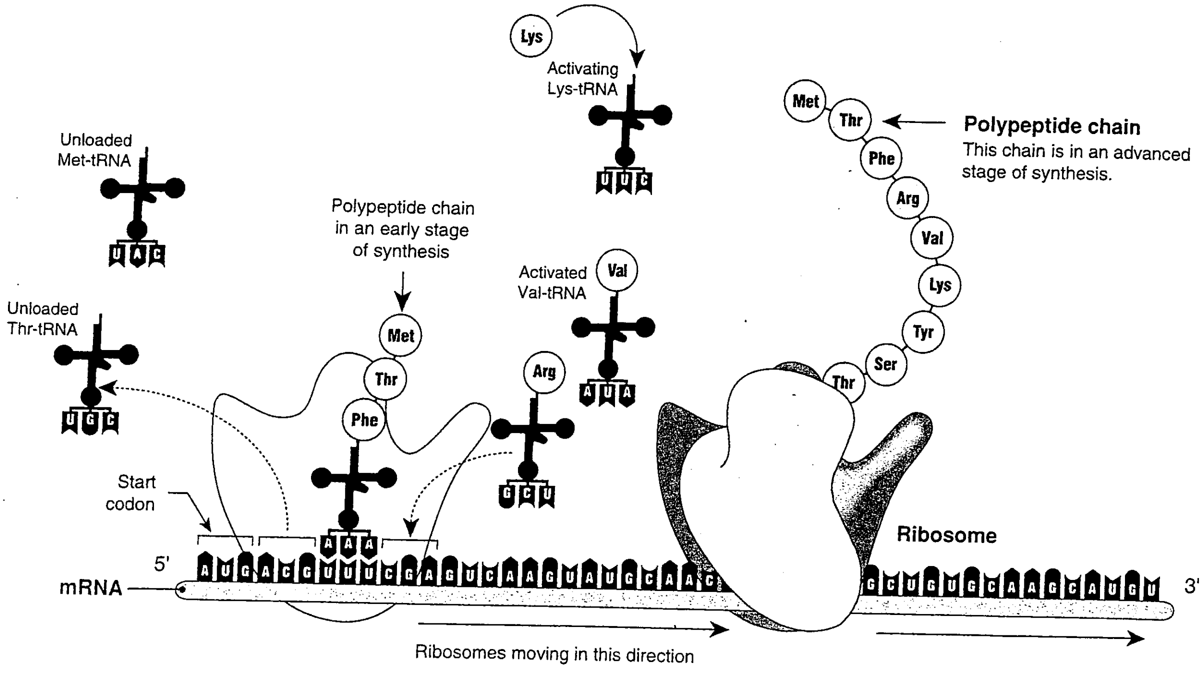
Translation

The diagram below 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: ______________________

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