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DNA, RNA AND PROTEIN SYNTHESIS

The genetic material is stored in the form of DNA in most organisms. In humans, the nucleus of each cell contains 3×10^9 base pairs of DNA distributed over 23 pairs of chromosomes, and each cell has two copies of the genetic material. This is known collectively as the human genome. The human genome contains around 30 000 genes, each of which codes for one protein.

Large stretches of DNA in the human genome are transcribed but do not code for proteins. These regions are called *introns* and make up around 95% of the genome. The nucleotide sequence of the human genome is now known to a reasonable degree of accuracy but we do not yet understand why so much of it is non-coding. Some of this non-coding DNA controls gene expression but the purpose of much of it is not yet understood. This is a fascinating subject that is certain to advance rapidly over the next few years.

The *Central Dogma of Molecular Biology* states that **DNA makes RNA makes proteins** (Figure 1).

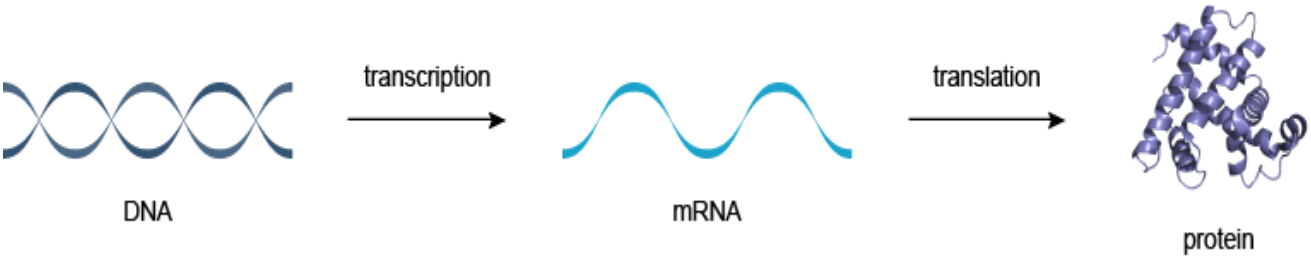


Figure 1 | **The Central Dogma of Molecular Biology: DNA makes RNA makes proteins**

The process by which DNA is copied to RNA is called transcription, and that by which RNA is used to produce proteins is called translation.

DNA REPLICATION

Each time a cell divides, each of its double strands of DNA splits into two single strands. Each of these single strands acts as a template for a new strand of complementary DNA. As a result, each new cell has its own complete genome. This process is known as *DNA replication*. Replication is controlled by the Watson-Crick pairing of the bases in the template strand with incoming deoxynucleotide triphosphates, and is directed by DNA polymerase enzymes. It is a complex process, particularly in eukaryotes, involving an array of enzymes. A simplified version of bacterial DNA replication is described in Figure 2.

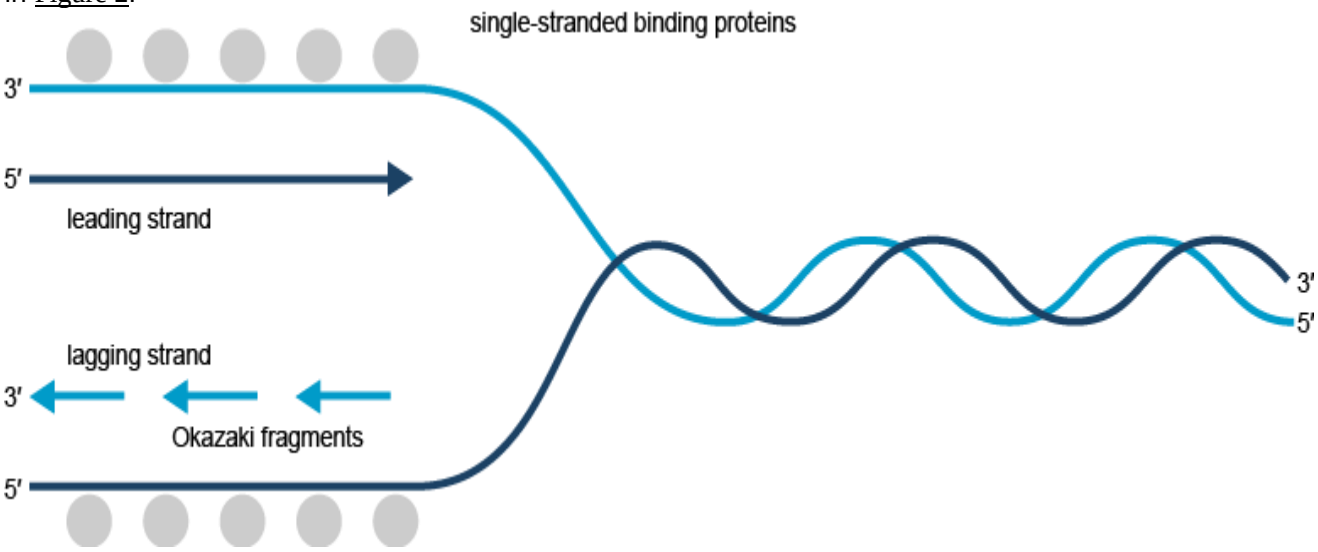


Figure 2 | DNA replication in bacteria Simplified representation of DNA replication in bacteria.

DNA biosynthesis proceeds in the 5′- to 3′-direction. This makes it impossible for DNA polymerases to synthesize both strands simultaneously. A portion of the double helix must first unwind, and this is mediated by *helicase* enzymes.

The leading strand is synthesized continuously but the opposite strand is copied in short bursts of about 1000 bases, as the lagging strand template becomes available. The resulting short strands are called *Okazaki fragments* (after their discoverers, Reiji and Tsuneko Okazaki). Bacteria have at least three distinct DNA polymerases: Pol I, Pol II and Pol III; it is Pol III that is largely involved in chain elongation. Strangely, DNA polymerases cannot initiate DNA synthesis *de novo*, but require a short primer with a free 3′-hydroxyl group. This is produced in the lagging strand by an RNA polymerase (called DNA primase) that is able to use the DNA template and synthesize a short piece of RNA around 20 bases in length. Pol III can then take over, but it eventually encounters one of the previously synthesized short RNA fragments in its path. At this point Pol I takes over, using its 5′- to 3′-exonuclease activity to digest the RNA and fill the gap with DNA until it reaches a continuous stretch of DNA. This leaves a gap between the 3′-end of the newly synthesized DNA and the 5′-end of the DNA previously synthesized by Pol III. The gap is filled by DNA ligase, an enzyme that makes a covalent bond between a 5′-phosphate and a 3′-hydroxyl group (Figure 3). The initiation of DNA replication at the leading strand is more complex and is discussed in detail in more specialized texts.

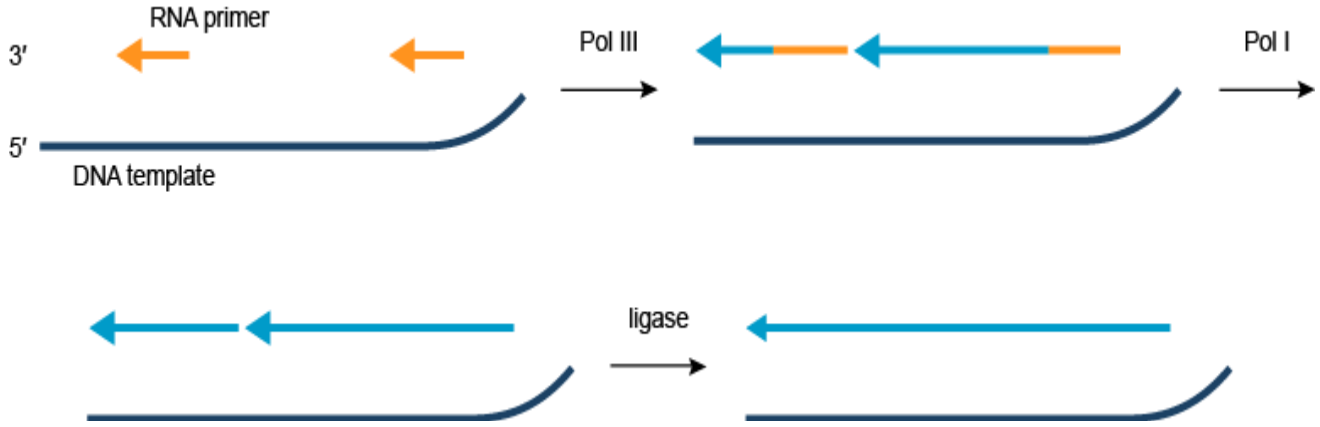


Figure 3 | **DNA polymerases in DNA replication**Simplified representation of the action of DNA polymerases in DNA replication in bacteria.

Mistakes in DNA replication

DNA replication is not perfect. Errors occur in DNA replication, when the incorrect base is incorporated into the growing DNA strand. This leads to *mismatched* base pairs, or*mispairs*. DNA polymerases have proofreading activity, and a DNA repair enzymes have evolved to correct these mistakes. Occasionally, mispairs survive and are incorporated into the genome in the next round of replication. These mutations may have no consequence, they may result in the death of the organism, they may result in a genetic disease or cancer; or they may give the organism a competitive advantage over its neighbours, which leads to evolution by natural selection.

TRANSCRIPTION

Transcription is the process by which DNA is copied (*transcribed*) to mRNA, which carries the information needed for protein synthesis. Transcription takes place in two broad steps. First, pre-messenger RNA is formed, with the involvement of RNA polymerase enzymes. The process relies on Watson-Crick base pairing, and the resultant single strand of RNA is the reverse-complement of the original DNA sequence. The pre-messenger RNA is then "edited" to produce the desired mRNA molecule in a process called*RNA splicing*.

Formation of pre-messenger RNA

The mechanism of transcription has parallels in that of DNA replication. As with DNA replication, partial unwinding of the double helix must occur before transcription can take place, and it is the RNA polymerase enzymes that catalyze this process.
Unlike DNA replication, in which both strands are copied, only one strand is transcribed. The strand that contains the gene is called the *sense* strand, while the complementary strand is the *antisense* strand. The mRNA produced in transcription is a copy of the sense strand, but it is the antisense strand that is transcribed.

Ribonucleotide triphosphates (NTPs) align along the antisense DNA strand, with Watson-Crick base pairing (A pairs with U). RNA polymerase joins the ribonucleotides together to form a pre-messenger

RNA molecule that is complementary to a region of the antisense DNA strand. Transcription ends when the RNA polymerase enzyme reaches a triplet of bases that is read as a "stop" signal. The DNA molecule re-winds to re-form the double helix.

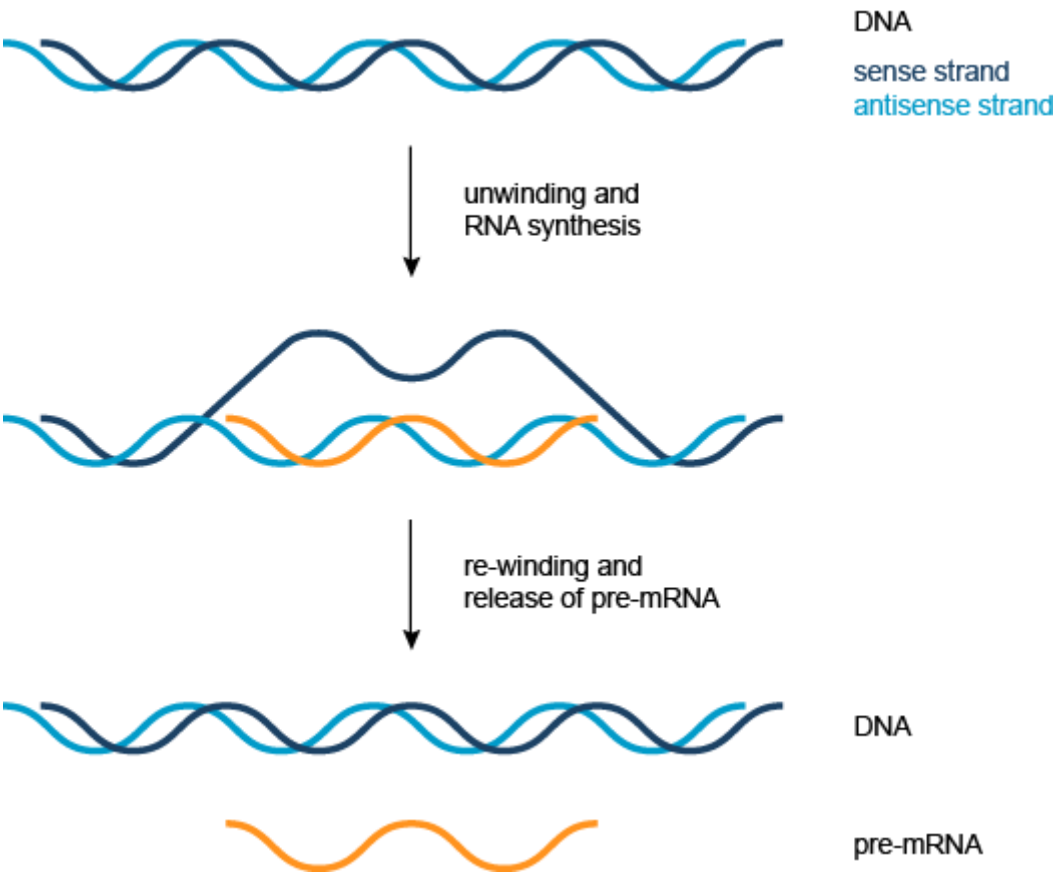


Figure 4 | **Transcription**Simplified representation of the formation of pre-messenger RNA (orange) from double-stranded DNA (blue) in transcription.

RNA splicing

The pre-messenger RNA thus formed contains introns which are not required for protein synthesis. The pre-messenger RNA is chopped up to remove the introns and create messenger RNA (mRNA) in a process called RNA splicing (Figure 5).

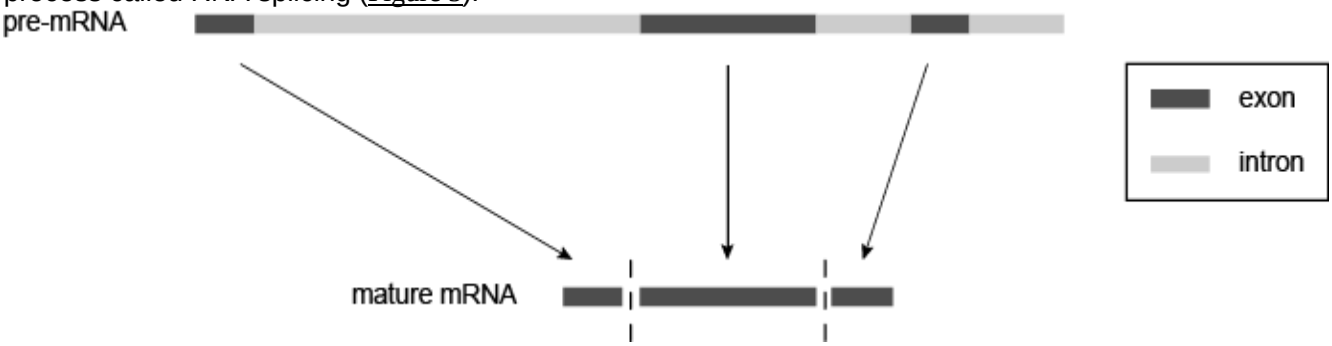


Figure 5 | **RNA splicing**Introns are spliced from the pre-messenger RNA to give messenger RNA (mRNA).

Alternative splicing

In alternative splicing, individual exons are either spliced or included, giving rise to several different possible mRNA products. Each mRNA product codes for a different protein isoform; these protein isoforms differ in their peptide sequence and therefore their biological activity. It is estimated that up to 60% of human gene products undergo alternative splicing. Several different mechanisms of alternative splicing are known, two of which are illustrated in Figure 6.

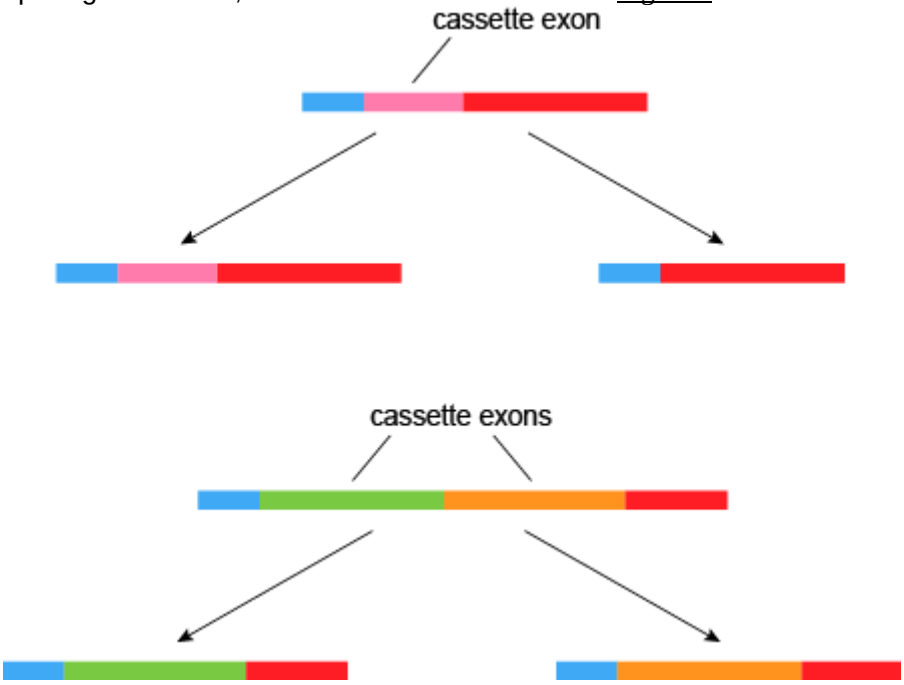


Figure 6 | **Alternative splicing**Several different mechanisms of alternative splicing exist − a cassette exon can be either included in or excluded from the final RNA (top), or two cassette exons may be mutually exclusive (bottom).

Alternative splicing contributes to protein diversity − a single gene transcript (RNA) can have thousands of different splicing patterns, and will therefore code for thousands of different proteins: a diverse proteome is generated from a relatively limited genome. Splicing is important in genetic regulation (alteration of the splicing pattern in response to cellular conditions changes protein expression). Perhaps not surprisingly, abnormal splicing patterns can lead to disease states including cancer.

Reverse transcription

In reverse transcription, RNA is "reverse transcribed" into DNA. This process, catalyzed by reverse transcriptase enzymes, allows retroviruses, including the human immunodeficiency virus (HIV), to use RNA as their genetic material. Reverse transcriptase enzymes have also found applications in biotechnology, allowing scientists to convert RNA to DNA for techniques such as PCR.

TRANSLATION

The mRNA formed in transcription is transported out of the nucleus, into the cytoplasm, to the ribosome (the cell's protein synthesis factory). Here, it directs protein synthesis. Messenger RNA is not directly involved in protein synthesis − transfer RNA (tRNA) is required for this. The process by which mRNA directs protein synthesis with the assistance of tRNA is called *translation*.
The ribosome is a very large complex of RNA and protein molecules. Each three-base stretch of mRNA (triplet) is known as a *codon*, and one codon contains the information for a specific amino acid. As the

mRNA passes through the ribosome, each codon interacts with the *anticodon* of a specific transfer RNA (tRNA) molecule by Watson-Crick base pairing. This tRNA molecule carries an amino acid at its 3′-terminus, which is incorporated into the growing protein chain. The tRNA is then expelled from the ribosome.Figure 7 shows the steps involved in protein synthesis.

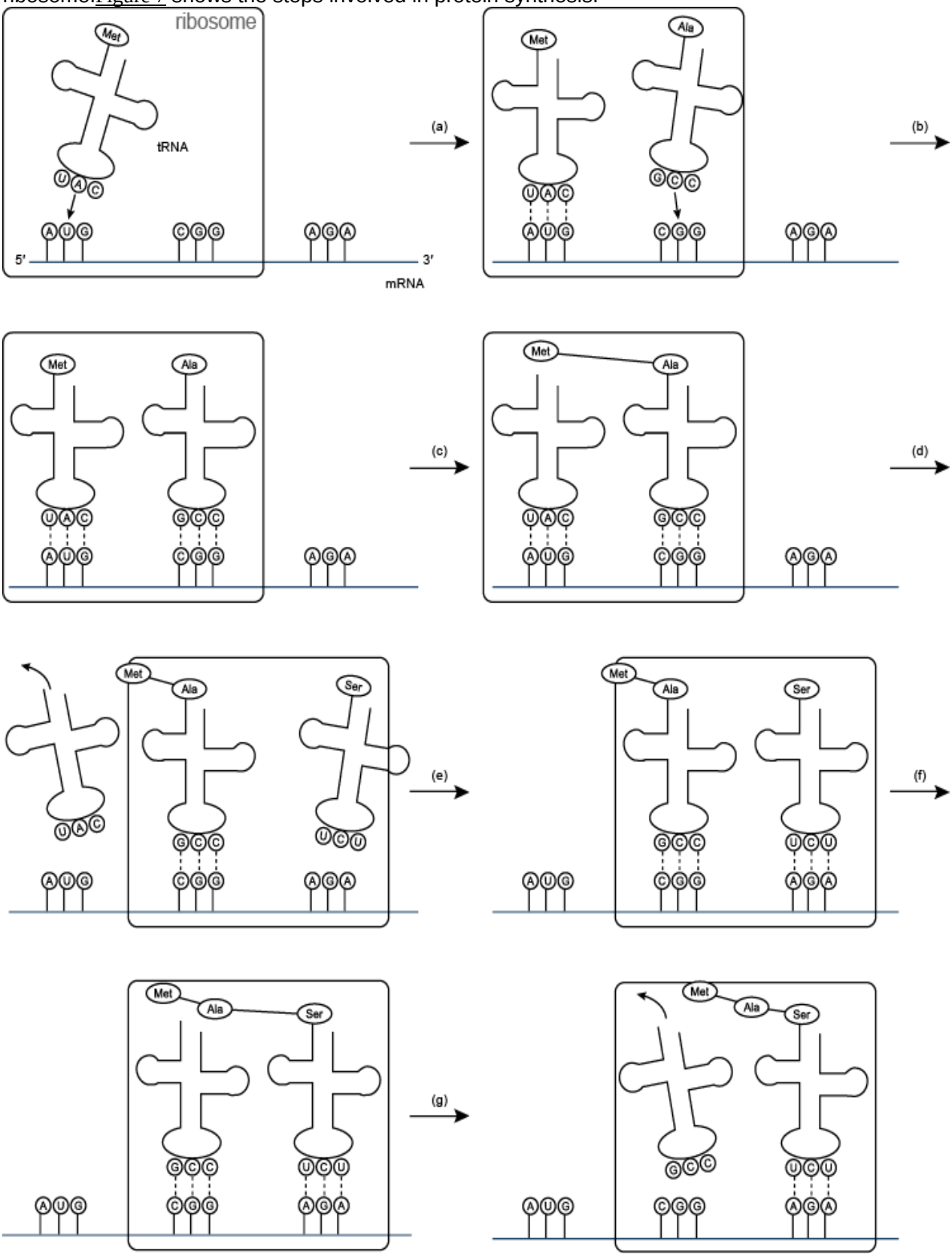
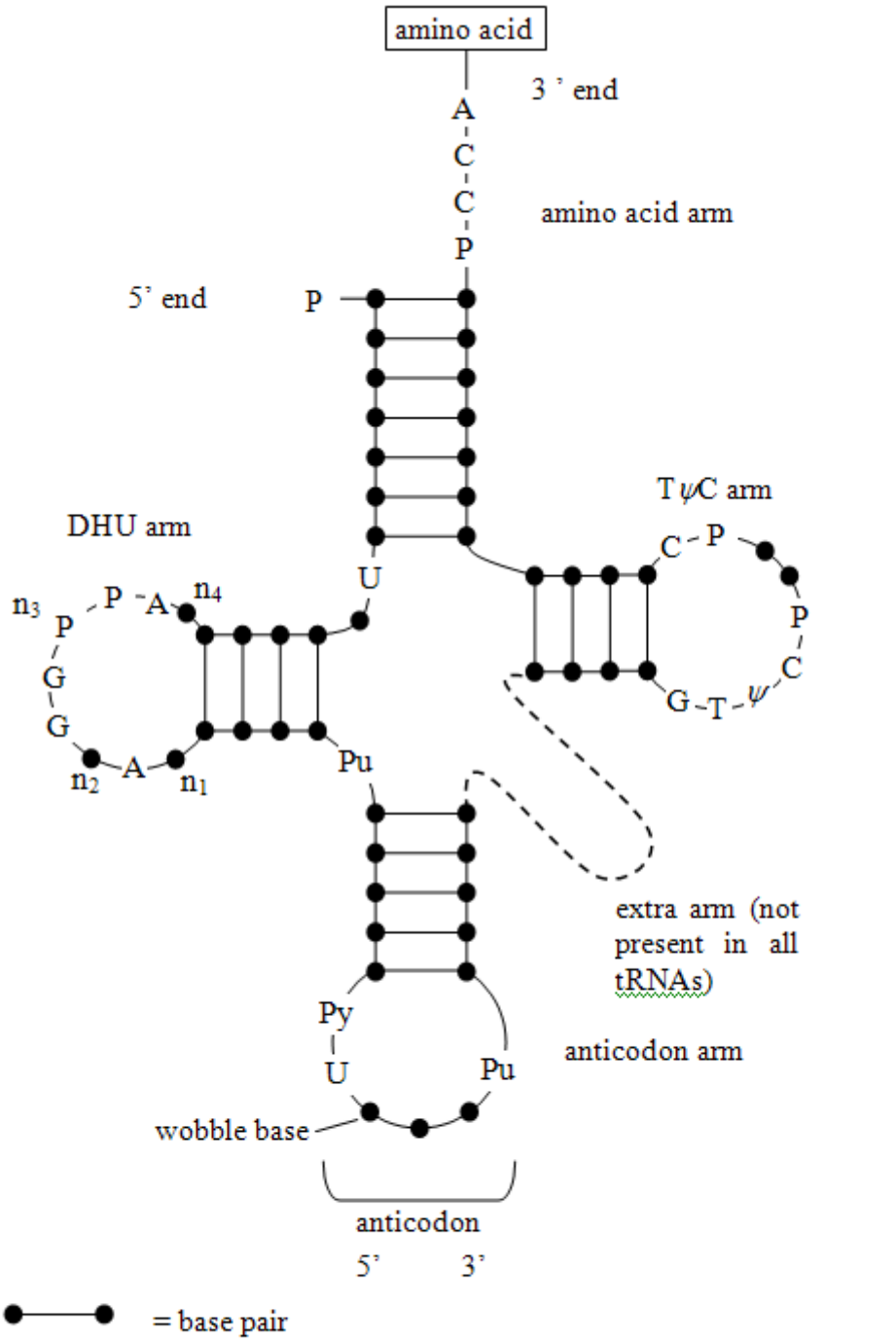


Figure 7 | **Translation**(a) and (b) tRNA molecules bind to the two binding sites of the ribosome, and by hydrogen bonding to the mRNA; (c) a peptide bond forms between the two amino acids to make a dipeptide, while the tRNA molecule is left uncharged; (d) the uncharged tRNA molecule leaves the ribosome, while the ribosome moves one

codon to the right (the dipeptide is translocated from one binding site to the other); (e) another tRNA molecule binds; (f) a peptide bond forms between the two amino acids to make a tripeptide; (g) the uncharged tRNA molecule leaves the ribosome.

TRANSFER RNA



Pu = purine, Py = pyrimidine, ψ = pseudouridine, G* = guanosine or 2'-O methyl guanosine, $n_1 = 0$ to 1, $n_2 = 1$ to 2, $n_3 = 2$ to 3, $n_4 = 3$ to 4 nucleoside residues in DHU arm depending on the tRNA. In some tRNAs the DHU arm has only 3 base pairs.

Figure 8 | **Two-dimensional structures of tRNA (transfer RNA)**In some tRNAs the DHU arm has only three base pairs.

Each amino acid has its own special tRNA (or set of tRNAs). For example, the tRNA for phenylalanine (tRNAPhe) is different from that for histidine (tRNAHis). Each amino acid is attached to its tRNA

through the 3′-OH group to form an ester which reacts with the α-amino group of the terminal amino-acid of the growing protein chain to form a new amide bond (peptide bond) during protein synthesis (Figure 9). The reaction of esters with amines is generally favourable but the rate of reaction is increased greatly in the ribosome.

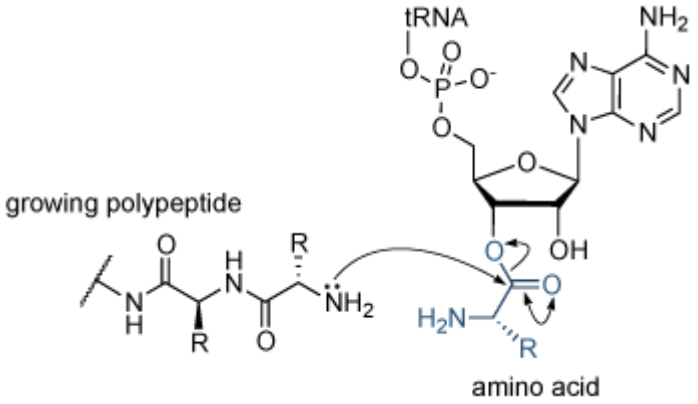


Figure 9 | **Protein synthesis**Reaction of the growing polypeptide chain with the 3′-end of the charged tRNA. The amino acid is transferred from the tRNA molecule to the protein.

Each transfer RNA molecule has a well defined tertiary structure that is recognized by the enzyme aminoacyl tRNA synthetase, which adds the correct amino acid to the 3′-end of the uncharged tRNA. The presence of modified nucleosides is important in stabilizing the tRNA structure. Some of these modifications are shown in Figure 10.

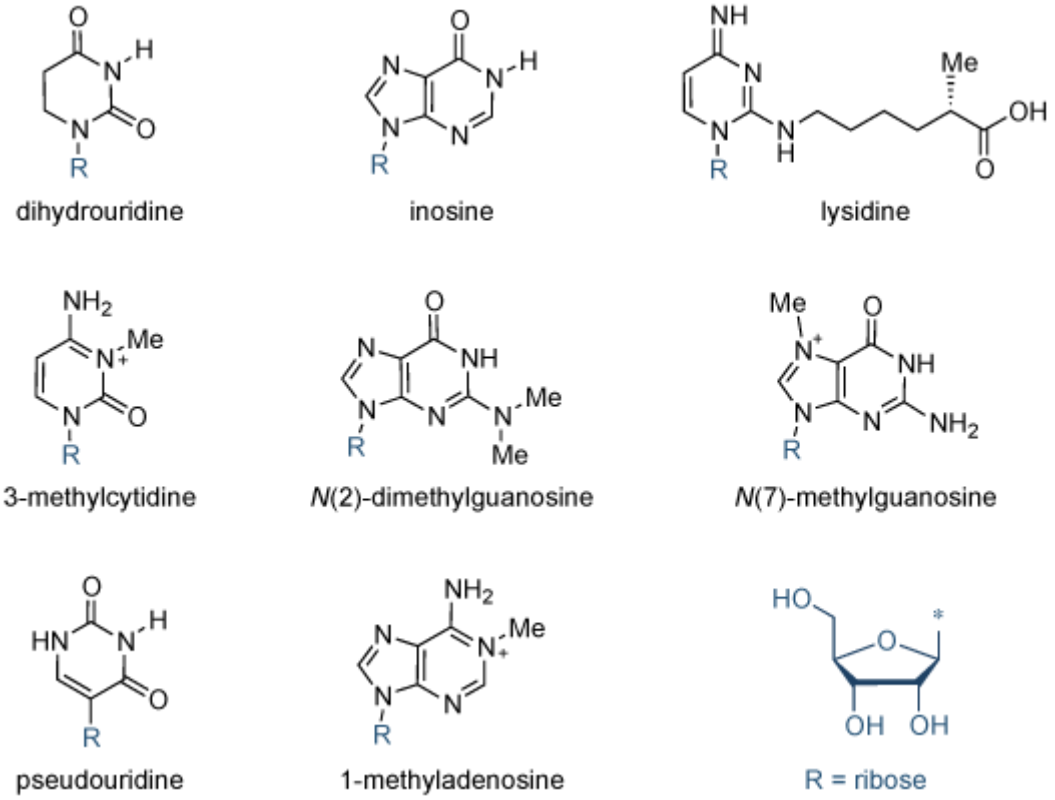


Figure 10 | **Modified bases in tRNA**Structures of some of the modified bases found in tRNA.

THE GENETIC CODE

The genetic code is almost universal. It is the basis of the transmission of hereditary information by nucleic acids in all organisms. There are four bases in RNA (A,G,C and U), so there are 64 possible triplet codes ($4^3 = 64$). In theory only 22 codes are required: one for each of the 20 naturally occurring

amino acids, with the addition of a start codon and a stop codon (to indicate the beginning and end of a protein sequence). Many amino acids have several codes (*degeneracy*), so that all 64 possible triplet codes are used. For example Arg and Ser each have 6 codons whereas Trp and Met have only one. No two amino acids have the same code but amino acids whose side-chains have similar physical or chemical properties tend to have similar codon sequences, e.g. the side-chains of Phe, Leu, Ile, Val are all hydrophobic, and Asp and Glu are both carboxylic acids (see Figure 11). This means that if the incorrect tRNA is selected during translation (owing to mispairing of a single base at the codon-anticodon interface) the misincorporated amino acid will probably have similar properties to the intended tRNA molecule. Although the resultant protein will have one incorrect amino acid it stands a high probability of being functional. Organisms show "codon bias" and use certain codons for a particular amino acid more than others. For example, the codon usage in humans is different from that in bacteria; it can sometimes be difficult to express a human protein in bacteria because the relevant tRNA might be present at too low a concentration.

First base (5'-end)	Middle base	Third base (3'-end)			
		U	C	A	G
U	U	Phe	Phe	Leu	Leu
	C	Ser	Ser	Ser	Ser
	A	Tyr	Tyr	Stop	Stop
	G	Cys	Cys	Stop	Trp
C	U	Leu	Leu	Leu	Leu
	C	Pro	Pro	Pro	Pro
	A	His	His	Gln	Gln
	G	Arg	Arg	Arg	Arg
A	U	Ile	Ile	Ile	Met
	C	Thr	Thr	Thr	Thr
	A	Asn	Asn	Lys	Lys
	G	Ser	Ser	Arg	Arg
G	U	Val	Val	Val	Val
	C	Ala	Ala	Ala	Ala
	A	Asp	Asp	Glu	Glu
	G	Gly	Gly	Gly	Gly

Figure 11 | The Genetic code − triplet codon assignments for the 20 amino acids. As well as coding for methionine, AUG is used as a start codon, initiating protein biosynthesis

An exercise in the use of the genetic code

One strand of genomic DNA (strand A, coding strand) contains the following sequence reading from 5′- to 3′-:

TCGTCGACGATGATCATCGGCTACTCGA

This strand will form the following duplex:

5′-TCGTCGACGATGATCATCGGCTACTCGA-3'
3′-AGCAGCTGCTACTAGTAGCCGATGAGCT-5'

The sequence of bases in the other strand of DNA (strand B) written 5′- to 3′- is therefore

TCGAGTAGCCGATGATCATCGTCGACGA

The sequence of bases in the mRNA transcribed from strand A of DNA written 5′- to 3′- is

UCGAGUAGCCGAUGAUCAUCGUCGACGA

The amino acid sequence coded by the above mRNA is

Ser-Ser-Ser-Arg-STOP

However, if DNA strand B is the coding strand the mRNA sequence will be:

UCGUCGACGAUGAUCAUCGGCUACUCGA

and the amino-acid sequence will be:

Ser-Ser-Thr-Arg-Ser-Ser-Gly-Cys-Ser-

THE WOBBLE HYPOTHESIS

Close inspection of all of the available codons for a particular amino acid reveals that the variation is greatest in the third position (for example, the codons for alanine are GCU, GCC, GCA and GCG). Crick and Brenner proposed that a single tRNA molecule can recognize codons with different bases at the 3′-end owing to non-Watson-Crick base pair formation with the third base in the codon-anticodon interaction. These non-standard base pairs are different in shape from A·U and G·C and the term *wobble hypothesis* indicates that a certain degree of flexibility or "wobbling" is allowed at this position in the ribosome. Not all combinations are possible; examples of "allowed" pairings are shown in Figure 12.

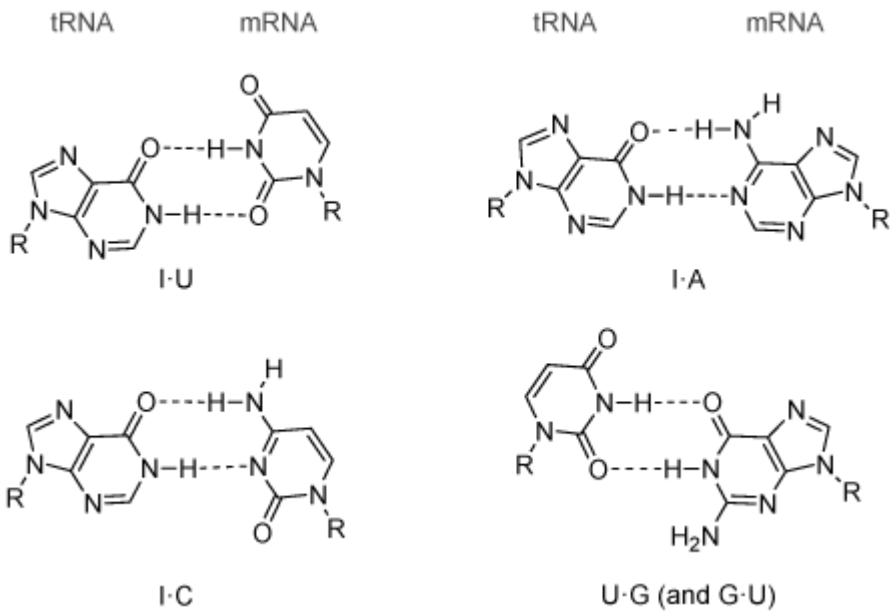


Figure 12 | **Structures of wobble base pairs found in RNA**

The ability of DNA bases to form wobble base pairs as well as Watson-Crick base pairs can result in base-pair mismatches occurring during DNA replication. If not repaired byDNA repair enzymes, these mismatches can lead to genetic diseases and cancer.