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Principles of Genetics

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Section no. (3)

DNA: The Genetic Material

- Even though Morgan was able to confirm that the genes are on the chromosomes , the scientists still did not know what genes consisted of , they knew that the genetic material must be :
- 1-able to store information that is used to control both the development and metabolic activities of the cell or organism .
- 2-stable so that it can be replicated with high fidelity during cell division and be transmitted from generation to generation .
- 3-able to undergo rare changes called Mutations to generate genetic variability that is acted upon during evolution .

The Discovery of DNA

- DNA was first identified in 1869 by Friedrich Miescher, a Swiss biologist, removed the nuclei of pus cells (these cell have little cytoplasm) and found that they contained a chemical material , he called nuclein, which , he said , rich in phosphorous and had no sulfur, that properties distinguished it from protein , and separated the substance into a basic part (which we now know is DNA) and an acidic part (a class of acidic proteins that bind to basic DNA).
- Later the scientists realized that there are two types of nucleic acids : DNA (deoxyribonucleic acid) and RNA (ribonucleic acid)

Transformation of Bacteria :

- In 1928 , the bacteriologist Frederick Griffith performed an experiment with a bacterium (*streptococcus pneumoniae*) that causes pneumonia in mammals .
- He noticed that when these bacteria are grown on culture plate , some , called S strain bacteria , produce shiny , smooth colonies and others called R strain bacteria , produce colonies that have a rough appearance under the microscope , S strain bacteria have a mucous coat but R strain don't .

- When Griffith injected mice with the S strain bacteria they died , and when he injected mice with the R strain bacteria , they did not die .
- In an effort to determine if the smooth coat was responsible for the virulence (ability to kill) of the S strain , he injected mice with heat –killed S strain bacteria , the mice did not die .
- Finally , Griffith injected mice with a mixture of heat-killed S strain and live R strain bacteria , unexpectedly , the mice died and living S strain bacteria were recovered from their bodies .

- He concluded that some substance necessary to produce a mucous
- Coat which cause virulence must have passed from the dead S strain to the living R strain . So that the R strain bacteria were transformed
- Now the question a rise what the transforming substance which cause transformation ?

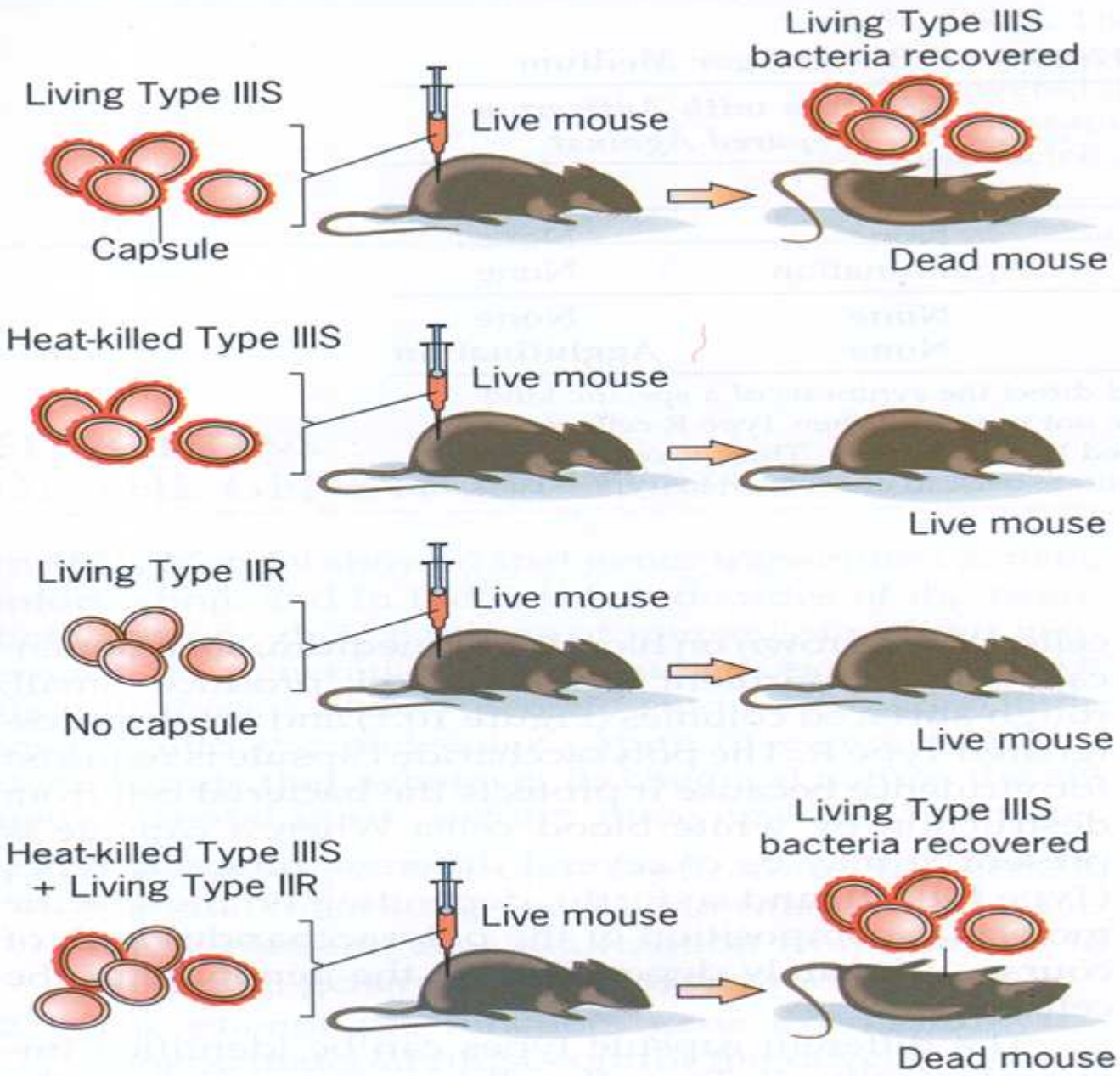


Figure 10.2 Griffith's discovery of transformation in *Streptococcus pneumoniae*.

Avery , Macleod & Maclyn Experiment :

- The next group of investigators , led by Oswald Avery worked *invitro* (in laboratory glassware) , this group published a paper that demonstrated that the transforming substance is DNA . the details as following :
- In 1943, Oswald Avery, Colin Macleod, and Maclyn McCarty, at the Rockefeller Institute, discovered that different strains of the bacterium *Strepotococcus pneumonae* could have different effects on a mice. One **virulent** strain could kill an injected mice, and another **avirulent** strain had no effect. When the virulent strain was heat-killed and injected into mice, there was no effect. But when a heat-killed virulent strain was coinjected with the avirulent strain, the mice died. What **transforming substance** was the dead virulent strain giving to the avirulent strain to make it lethal

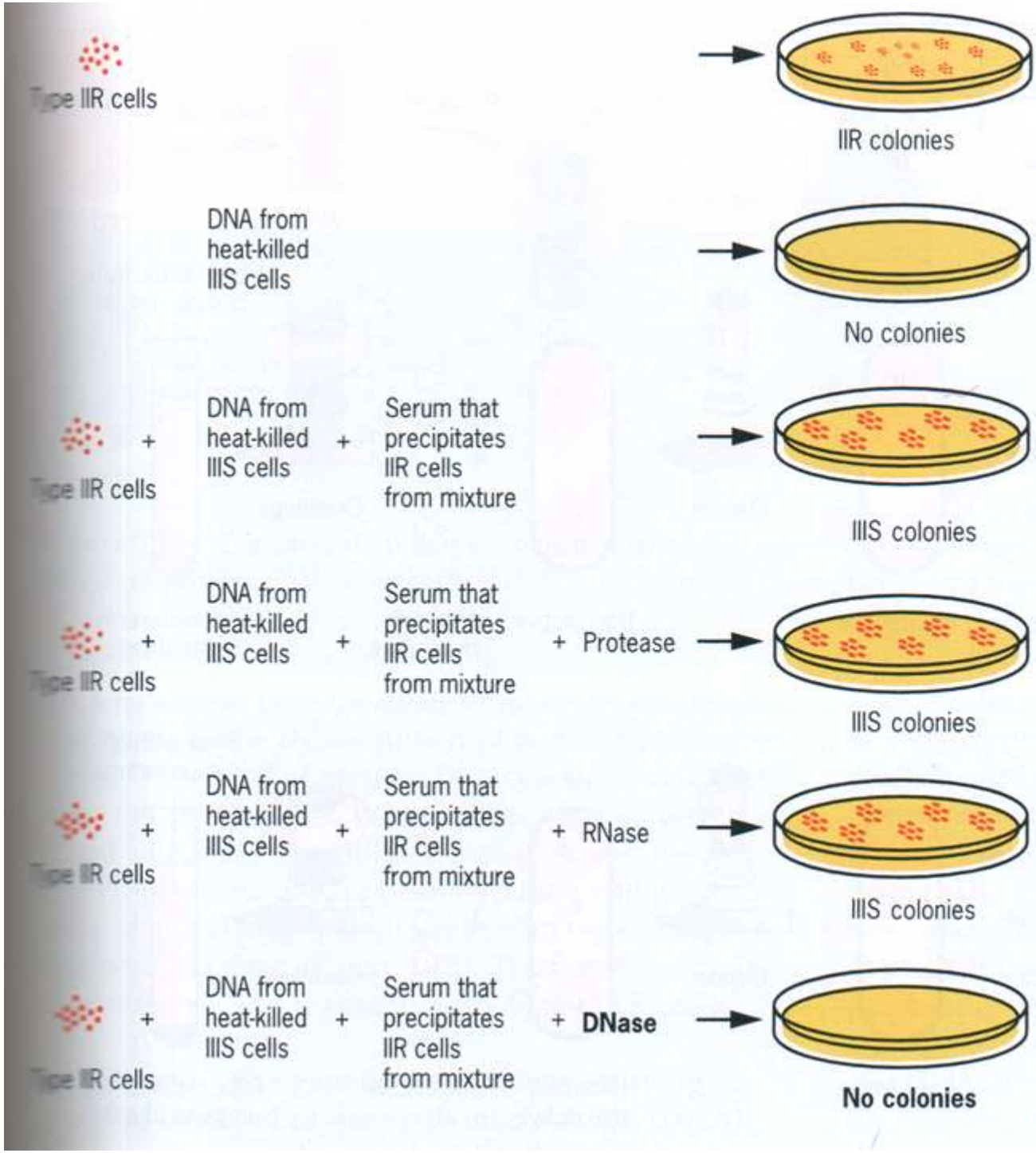
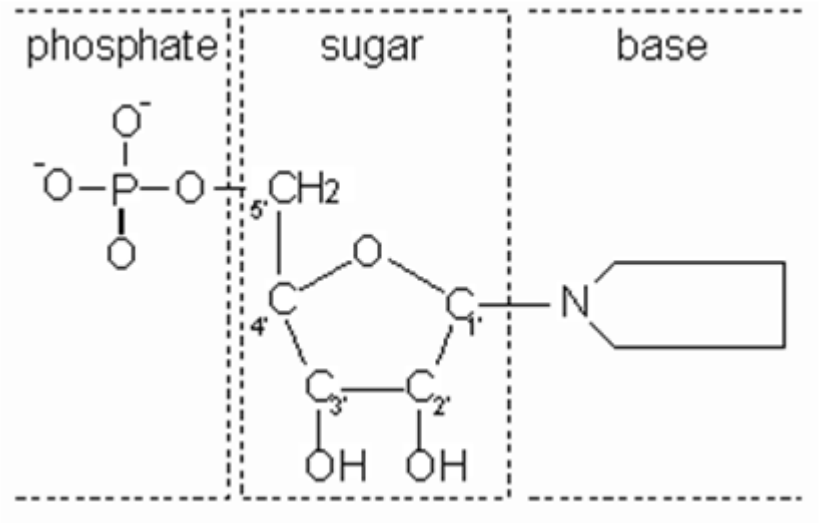


Figure 10.3 Avery, MacLeod, and McCarty's proof that the "transforming principle" is DNA.

Nucleic Acids

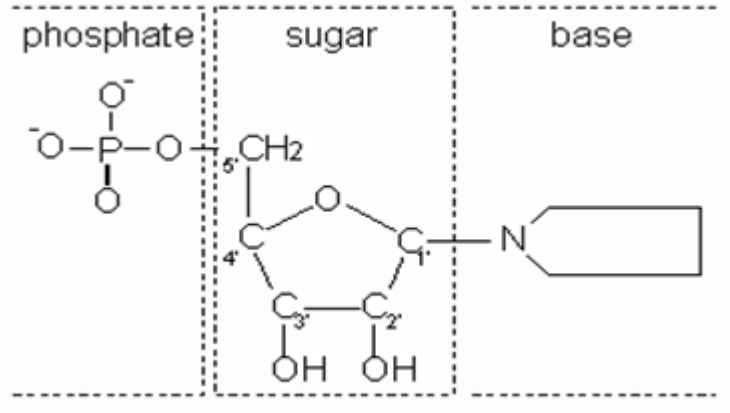
- DNA and its close relative RNA are perhaps the most important molecules in biology. They contains the instructions that make every single living organism on the planet, and yet it is only in the past 50 years that we have begun to understand them. DNA stands for deoxyribonucleic acid and RNA for ribonucleic acid, and they are called nucleic acids because they are weak acids, first found in the nuclei of cells. They are polymers, composed of monomers called **nucleotides**.

Nucleotides



- Nucleotides have three parts to them:
- 1-a phosphate group, which is negatively charged, and gives nucleic acids their acidic properties.
- 2-a pentose sugar, which has 5 carbon atoms in it. By convention the carbon atoms are numbered as shown to distinguish them from the carbon atoms in the base.

Nucleotide parts



- If carbon 2 has a hydroxyl group attached (as shown), then the sugar is ribose, found in RNA. If the carbon 2 just has a hydrogen atom attached instead, then the sugar is deoxyribose, found in DNA.

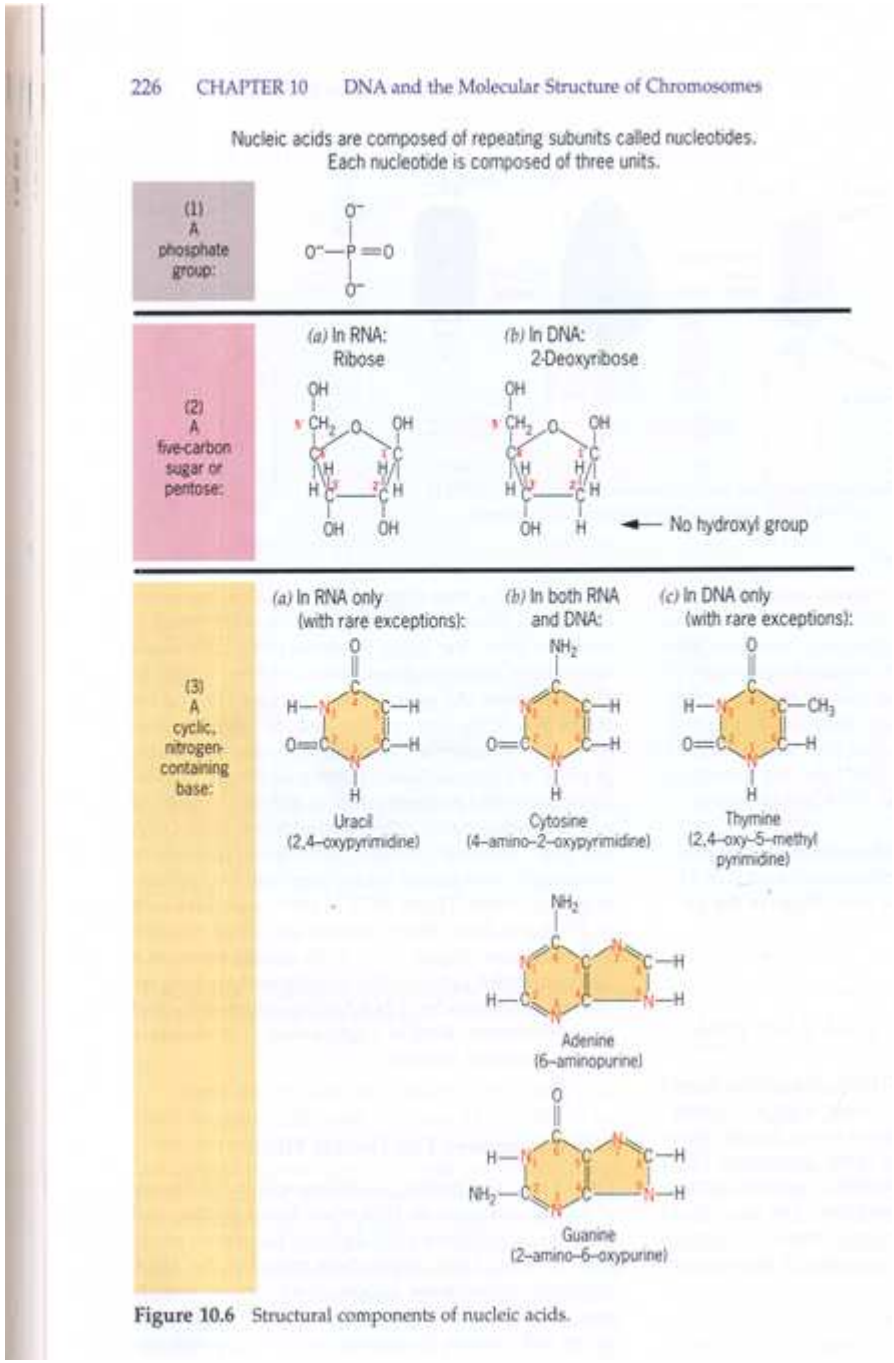


Figure 10.6 Structural components of nucleic acids.

- 3-a nitrogenous base. There are five different bases (and you don't need to know their structures), but they all contain the elements carbon, hydrogen, oxygen and nitrogen. The bases are usually known by there first letters only,

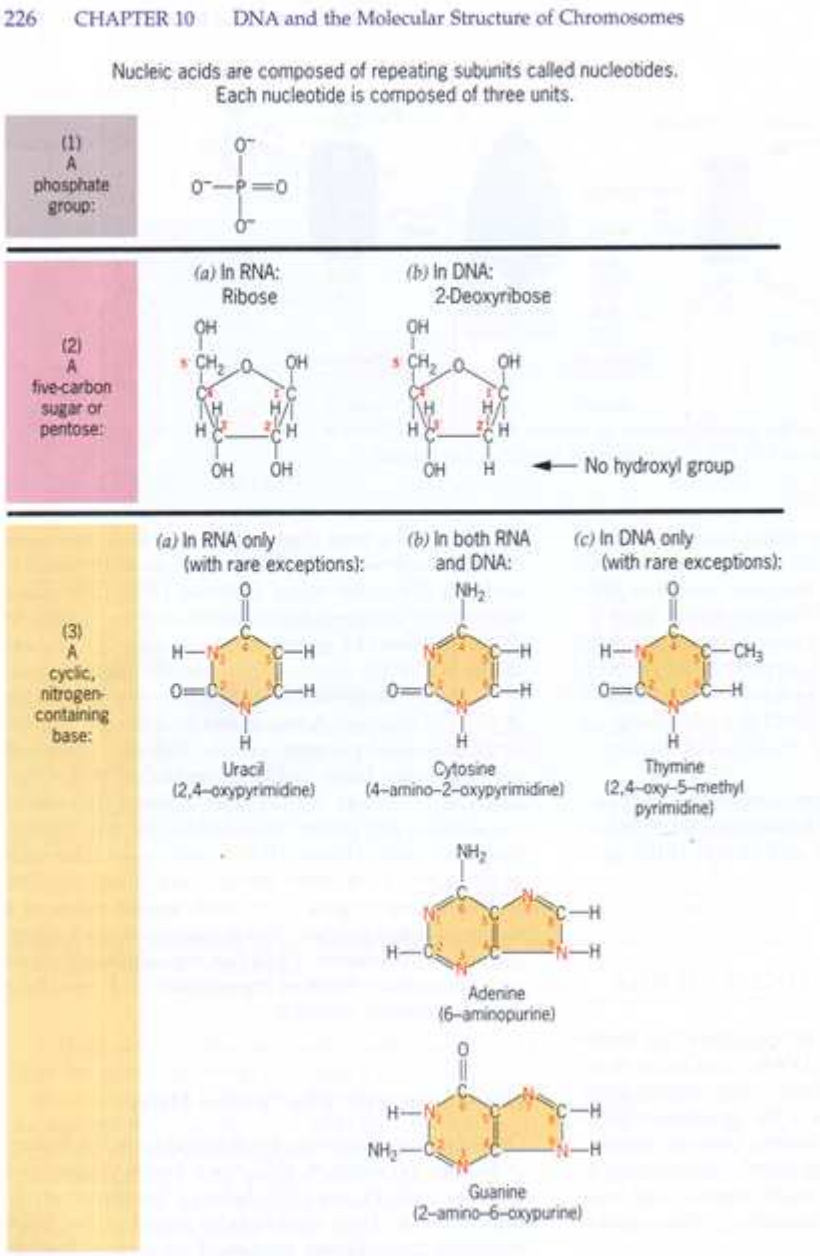
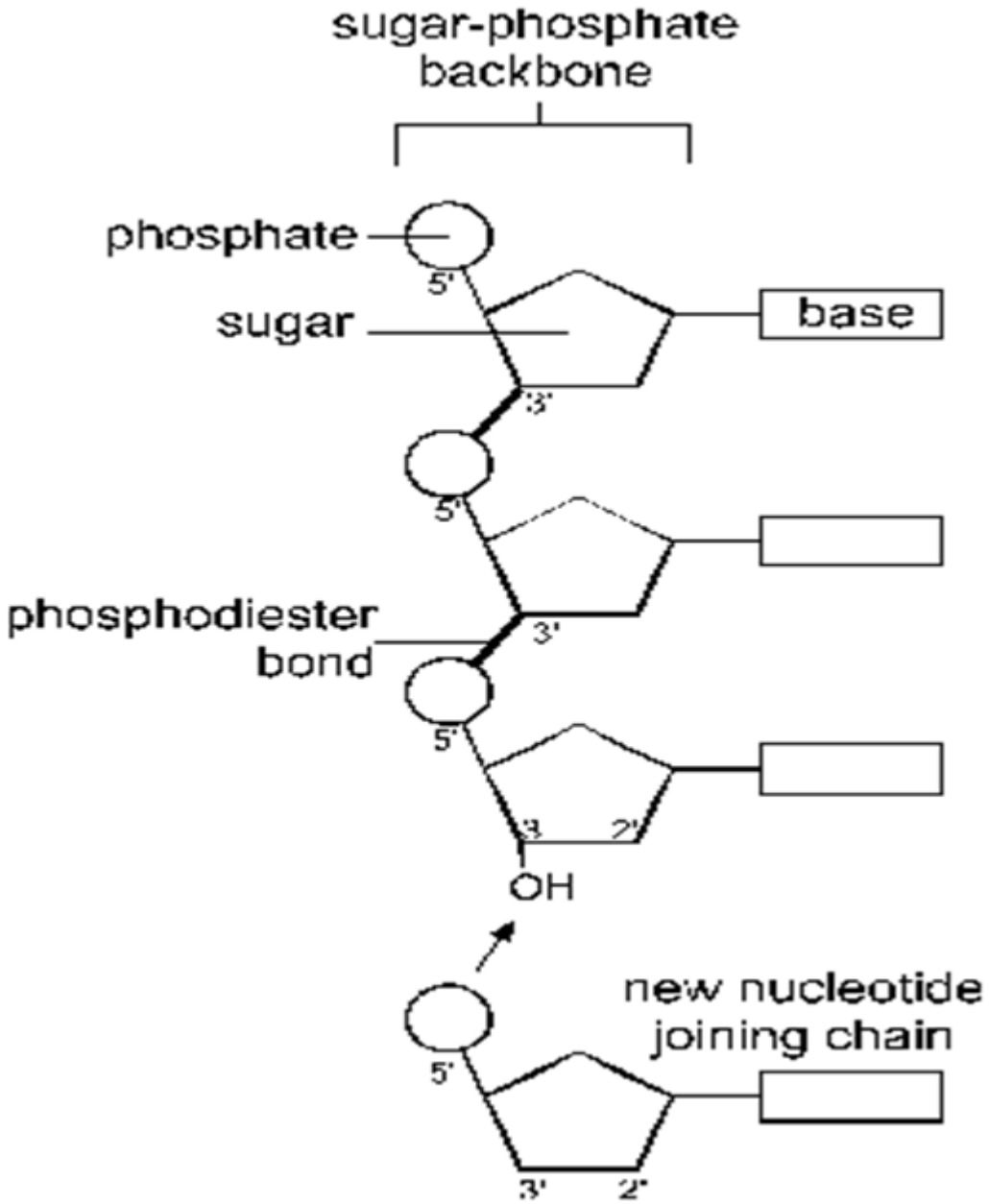


Figure 10.6 Structural components of nucleic acids.

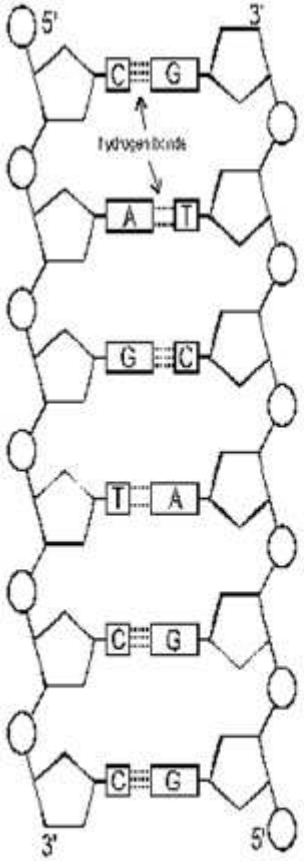
- so you don't need to learn the full names. The base thymine is found in DNA only and the base uracil is found in RNA only, so there are only four different bases present at a time in one nucleic acid molecule. Nitrogen Bases are:
- Adenine (A)
- Guanine (G)
- Cytosine (C)
- Uracil (U)
- Thymine (T)

Nucleotide Polymerisation

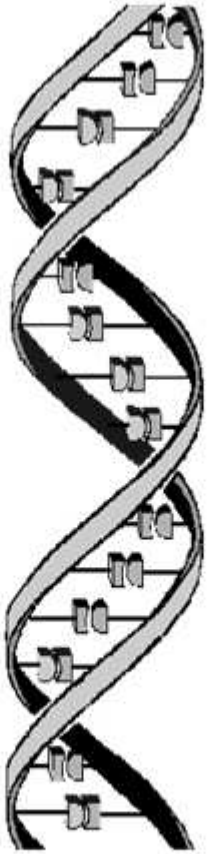
- Nucleotides polymerise by forming bonds between carbon 3 of the sugar and an oxygen atom of the phosphate. This is a condensation polymerisation reaction. The bases do not take part in the polymerisation, so there is a sugar-phosphate backbone with the bases extending off it. This means that the nucleotides can join together in any order along the chain. Many nucleotides form a polynucleotide.
- A polynucleotide has a free phosphate group at one end and a free OH group at the other end.

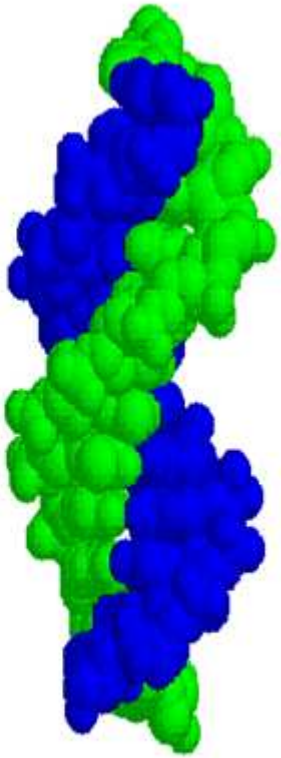

sugar-phosphate
backbone
phosphate
sugar
base
5'
3'
phosphodiester
bond
OH
new nucleotide
joining chain

Structure of DNA

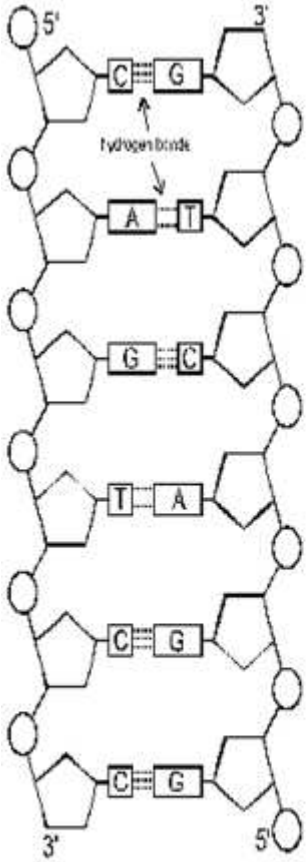


DNA showing the complementary base pairing between antiparallel strands

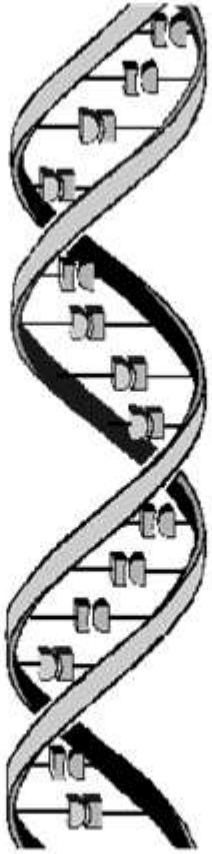

DNA showing the double helix


spacefilling model of the double helix

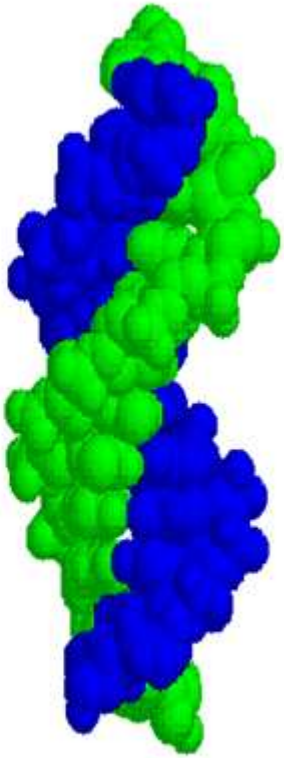
- The three-dimensional structure of DNA was discovered in the 1950's by Watson and Crick. The main features of the structure are:
- DNA is <u>double-stranded</u>, so there are two polynucleotide stands alongside each other.



DNA showing the complementary base pairing between antiparallel strands

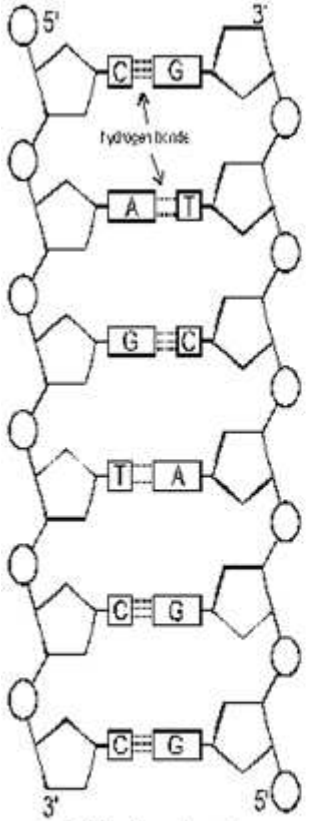


DNA showing the double helix

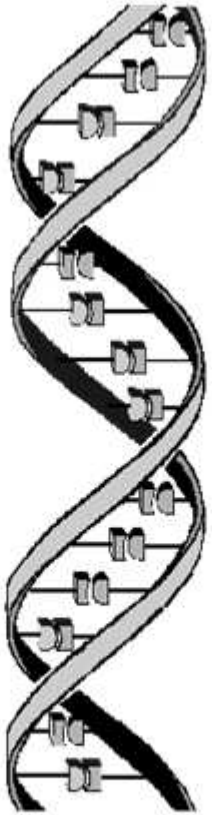


spacefilling model of the double helix

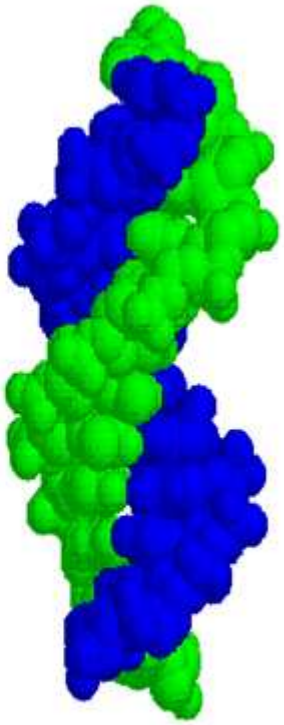
- The strands are <u>antiparallel</u>, i.e. they run in opposite directions.
- The two strands are wound round each other to form a <u>double helix</u>.
- The two strands are joined together by <u>hydrogen bonds</u> between the bases. The bases therefore form <u>base pairs</u>, which are like rungs of a ladder.



DNA showing the complementary base pairing between antiparallel strands



DNA showing the double helix

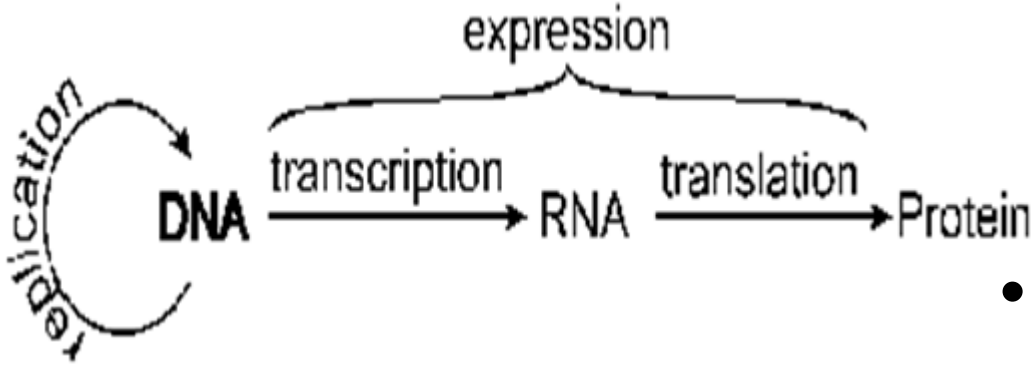


spacefilling model of the double helix

- The base pairs are specific. A only binds to T (and T with A), and C only binds to G (and G with C). These are called <u>complementary base pairs</u>. This means that whatever the sequence of bases along one strand, the sequence of bases on the other strand must be complementary to it. (Incidentally, complementary, which means matching, is different from complimentary, which means being nice.)

Function of DNA

- DNA is the genetic material, and genes are made of DNA. DNA therefore has two essential functions: replication and expression.
- Replication means that the DNA, with all its genes, must be copied every time a cell divides.
- Expression means that the genes on DNA must control characteristics. A gene was traditionally defined as a factor that controls a particular characteristic (such as flower color), but a much more precise definition is that a gene is a section of DNA that codes for a particular protein. Characteristics are controlled by genes through the proteins they code for.



- Expression can be split into two parts: transcription (making RNA) and translation (making proteins).
- These two functions are summarised in this diagram (called the central dogma of genetics).

- No one knows exactly how many genes we humans have to control all our characteristics,
- the latest estimates are 60-80,000. The sum total of all the genes in an organism is called the genome.

The table shows the estimated number of genes in different organisms:

Species	Common name	length of DNA (kbp)*	no of genes
phage l	virus	48	60
Eschericia coli	Bacterium	4 639	7 000
Drosophila melaogaster	fruit fly	165 000	~10 000
Homo sapiens	Human	3 150 000	~70 000

- Amazingly, genes only seem to comprise about 2% of the DNA in a cell. The majority of the DNA does not form genes and doesn’t seem to do anything.

RNA

RNA is a nucleic acid like DNA, but with 4 differences:

- 1-RNA has the sugar ribose instead of deoxyribose
- 2-RNA has the base uracil instead of thymine
- 3-RNA is usually single stranded
- 4-RNA is usually shorter than DNA

Messenger RNA (mRNA)

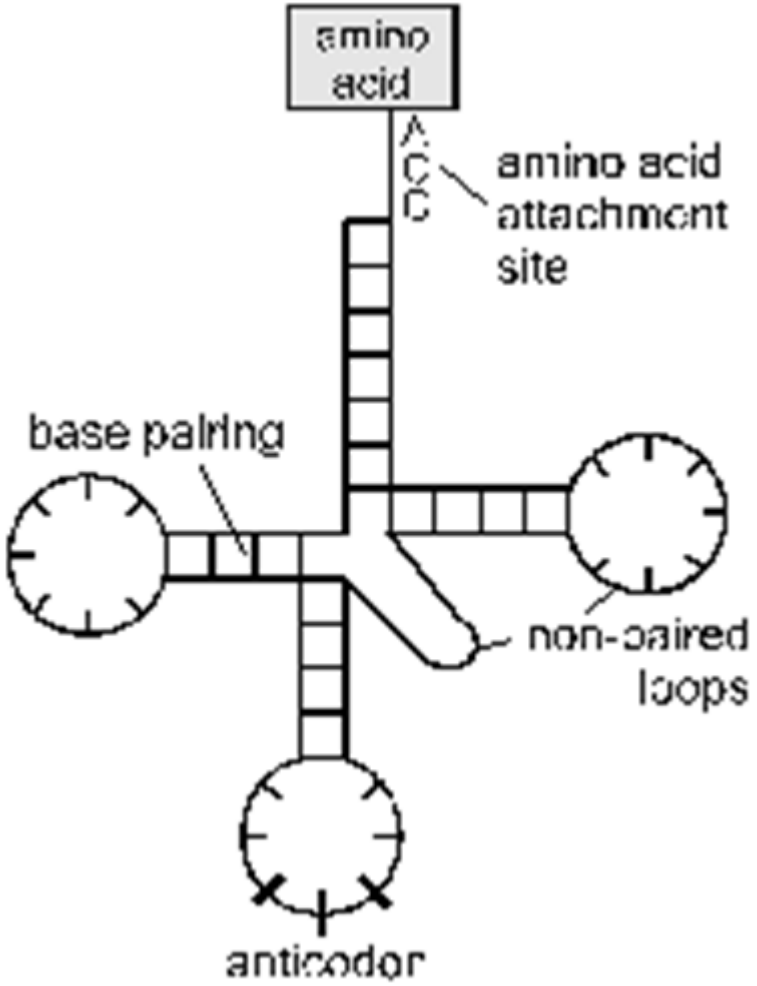
- mRNA carries the "message" that codes for a particular protein from the nucleus (where the DNA master copy is) to the cytoplasm (where proteins are synthesised). It is single stranded and just long enough to contain one gene only. It has a short lifetime and is degraded soon after it is used.

Ribosomal RNA (rRNA)

- rRNA, together with proteins, form ribosomes, which are the site of mRNA translation and protein synthesis. Ribosomes have two subunits, small and large, and are assembled in the nucleolus of the nucleus and exported into the cytoplasm.

Transfer RNA (tRNA)

- tRNA is an "adapter" that matches amino acids to their codon. tRNA is only about 80 nucleotides long, and it folds up by complementary base pairing to form a looped clover-leaf structure. At one end of the molecule there is always the base sequence ACC, where the amino acid binds. On the middle loop there is a triplet nucleotide sequence called the anticodon. There are 64 different tRNA molecules, each with a different anticodon sequence complementary to the 64 different codons. The amino acids are attached to their tRNA molecule by specific enzymes. These are highly specific, so that each amino acid is attached to a tRNA adapter with the appropriate anticodon.



Transfer RNA (tRNA)

The Genetic Code

- The sequence of bases on DNA codes for the sequence of amino acids in proteins. But there are 20 different amino acids and only 4 different bases, so the bases are read in groups of 3. This gives 4^3 or 64 combinations, more than enough to code for 20 amino acids. A group of three bases coding for an amino acid is called a codon, and the meaning of each of the 64 codons is called the genetic code.

The Genetic Code (mRNA codons)			
UUU, UUC: phe UUA, UUG: leu	CUU, CUC, CUA, CUG: leu	AUU, AUC, AUA: ile AUG: start/met	GUU, GUC, GUA, GUG: val
UCU, UCC, UCA, UCG: ser	CCU, CCC, CCA, CCG: pro	ACU, ACC, ACA, ACG: thr	GCU, GCC, GCA, GCG: ala
UAU, UAC: tyr UAA: stop UAG: stop	CAU, CAC: his CAA, CAG: gln	AAU, AAC: asn AAA, AAG: lys	GAU, GAC: asp GAA, GAG: glu
UGU, UGC: cys UGA: stop UGG: trp	CGU, CGC, CGA, CGG: arg	AGU, AGC: ser AGA, AGG: arg	GGU, GGC, GGA, GGG: gly

There are several interesting points from this code:

- 1-The code is degenerate, i.e. there is often more than one codon for an amino acid. The degeneracy is on the third base of the codon, which is therefore less important than the others.
- 2-One codon means "start" i.e. the start of the gene sequence. It is AUG.
- 3-Three codons mean "stop" i.e. the end of the gene sequence. They do not code for amino acids.
- 4-The code is only read in one direction along the mRNA molecule.

Replication - DNA Synthesis

- DNA is copied, or replicated, before every cell division, so that one identical copy can go to each daughter cell. The method of DNA replication is obvious from its structure: the double helix unzips and two new strands are built up by complementary base-pairing onto the two old strands.

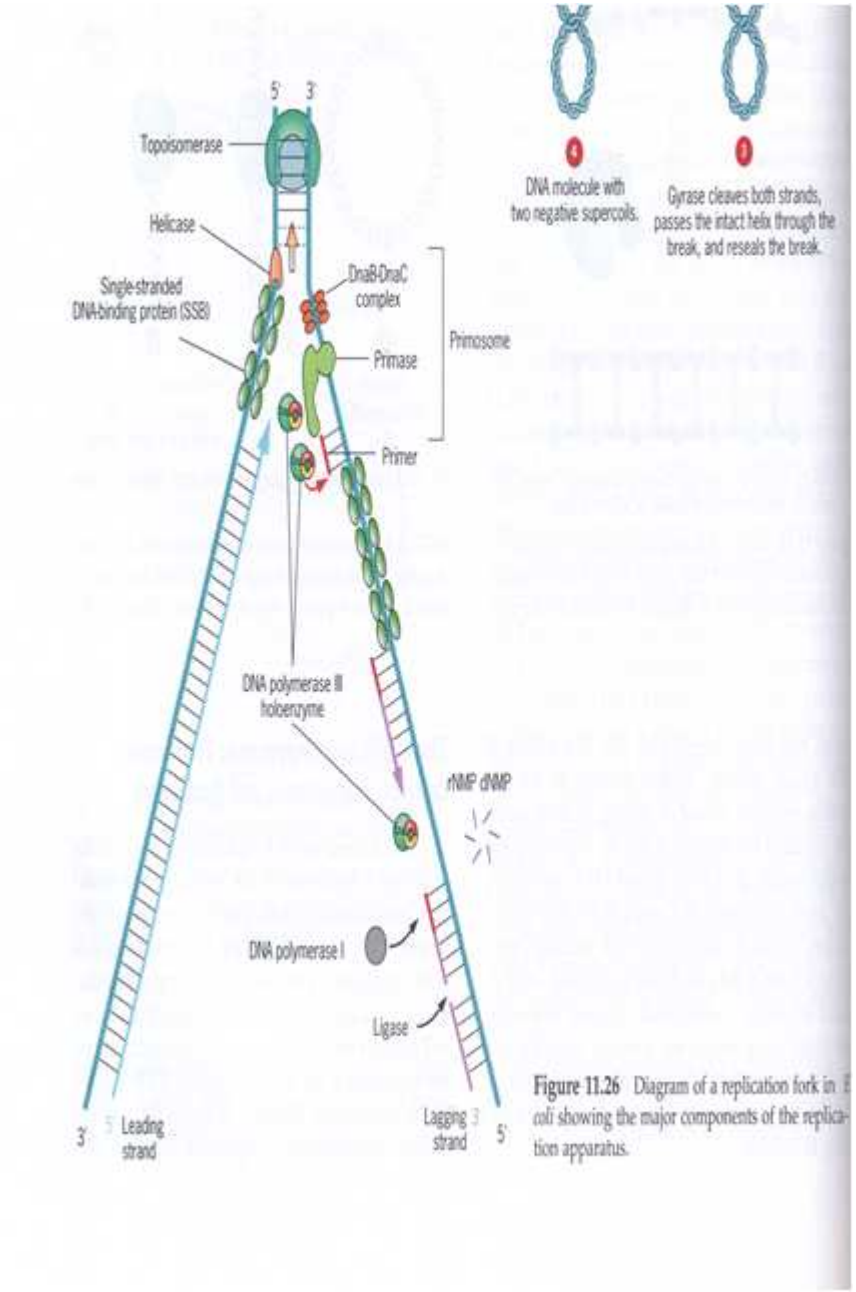


Figure 11.26 Diagram of a replication fork in E. coli showing the major components of the replication apparatus.

- Replication starts at a specific sequence on the DNA molecule called the <u>replication origin</u>.
- An enzyme unwinds and unzips DNA, breaking the hydrogen bonds that join the base pairs, and forming two separate strands.

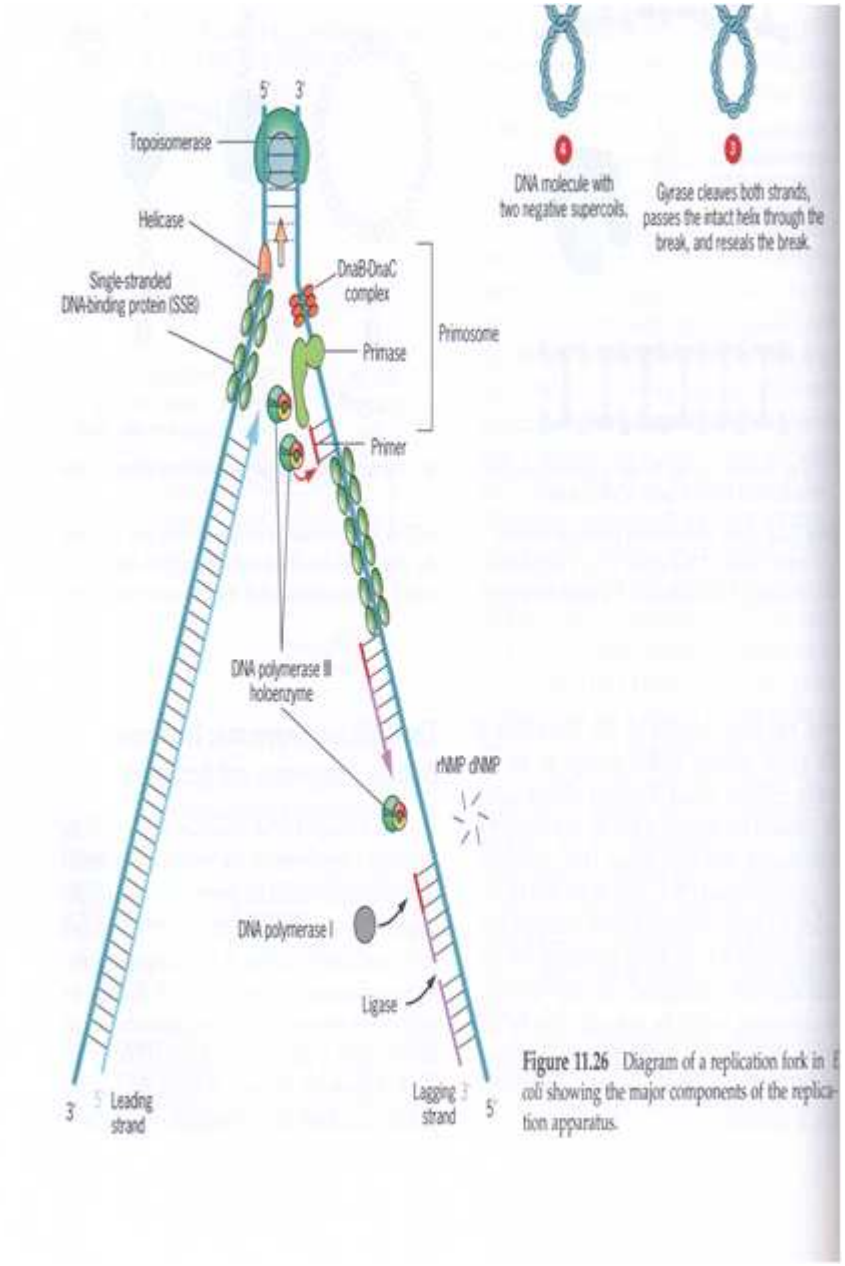


Figure 11.26 Diagram of a replication fork in E. coli showing the major components of the replication apparatus.

- The new DNA is built up from the four nucleotides (A, C, G and T) that are abundant in the nucleoplasm.
- These nucleotides attach themselves to the bases on the old strands by complementary base pairing. Where there is a T base, only an A nucleotide will bind, and so on.

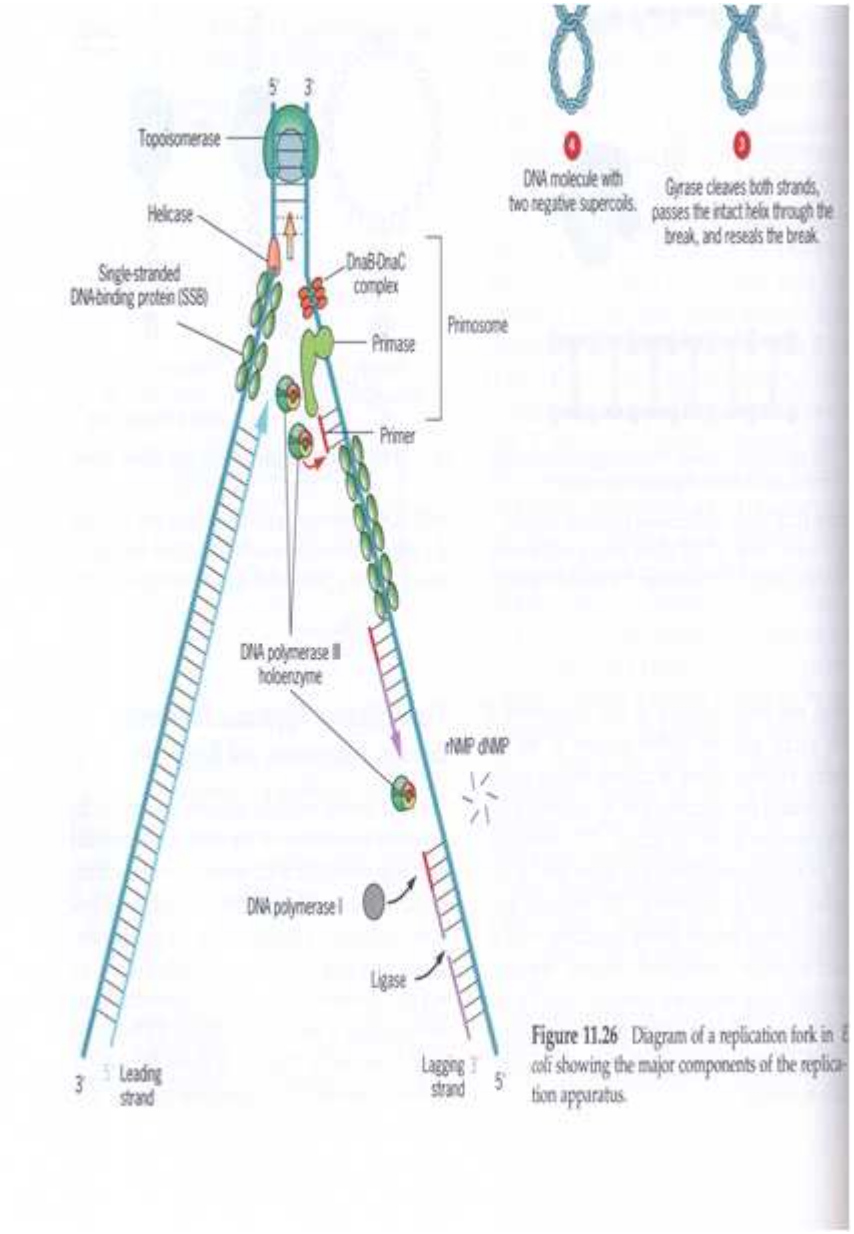


Figure 11.26 Diagram of a replication fork in *E. coli* showing the major components of the replication apparatus.

- A winding enzyme winds the new strands up to form double helices.
- The enzyme <u>DNA polymerase</u> joins the new nucleotides to each other by strong covalent bonds, forming the sugar-phosphate backbone.
- The two new molecules are identical to the old molecule

. •

- DNA replication can takes a few hours, and in fact this limits the speed of cell division. One reason bacteria can reproduce so fast is that they have a relatively small amount of DNA.

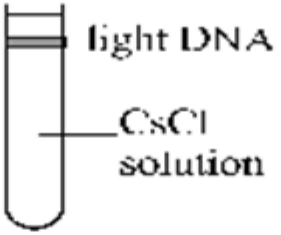
The Meselson-Stahl Experiment

- This replication mechanism is sometimes called semi-conservative replication, because each new DNA molecule contains one new strand and one old strand. This need not be the case, and alternative theories suggested that a "photocopy" of the original DNA could be made, leaving the original DNA conserved (conservative replication. The evidence for the semi-conservative method came from an elegant experiment performed in 1958 by Meselson and Stahl. They used the bacterium *E. coli* together with the technique of density gradient centrifugation, which separates molecules on the basis of their density.

The Meselson-Stahl Experiment

1. Grow bacteria on medium with normal $^{14}NH_4$

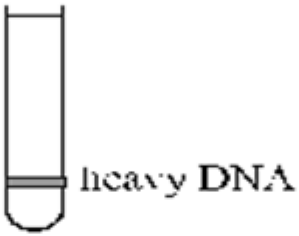
purify DNA and centrifuge →



These first two steps are a calibration. They show that the method can distinguish between DNA containing ^{14}N and that containing ^{15}N.

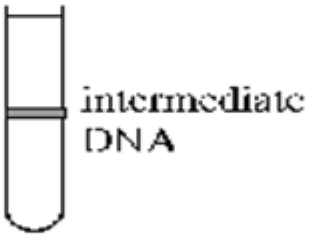
2. Grow bacteria for many generations on medium with ^{15}NH

purify DNA and centrifuge →



3. Return to $^{14}NH_4$ medium for 20 mins (one generation)

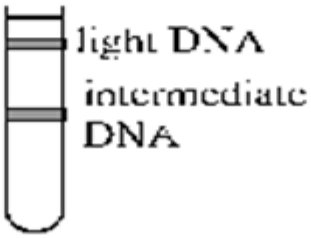
purify DNA and centrifuge →



This is the crucial step. The DNA has replicated just once in ^{14}N medium. The resulting DNA is not heavy or light, but exactly half way between the two. This rules out conservative replication.

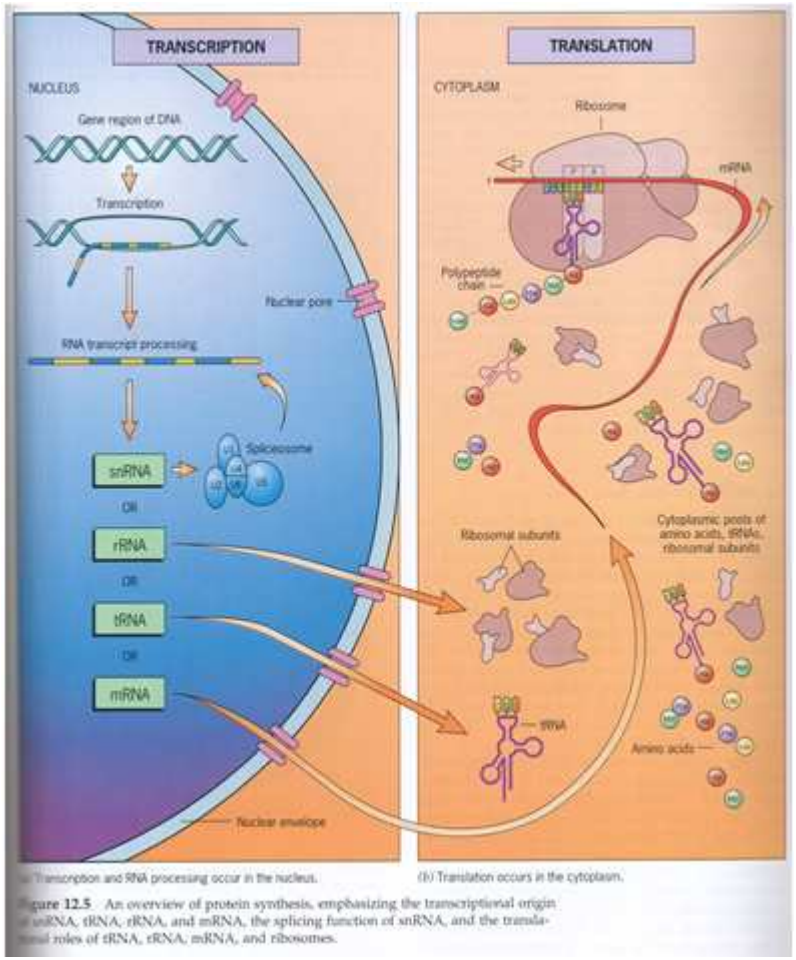
4. Grow on $^{14}NH_4$ medium for 40 mins (two generations)

purify DNA and centrifuge →



After two generations the DNA is either light or half-and-half. This rules out dispersive replication. The results are all explained by semi-conservative replication.

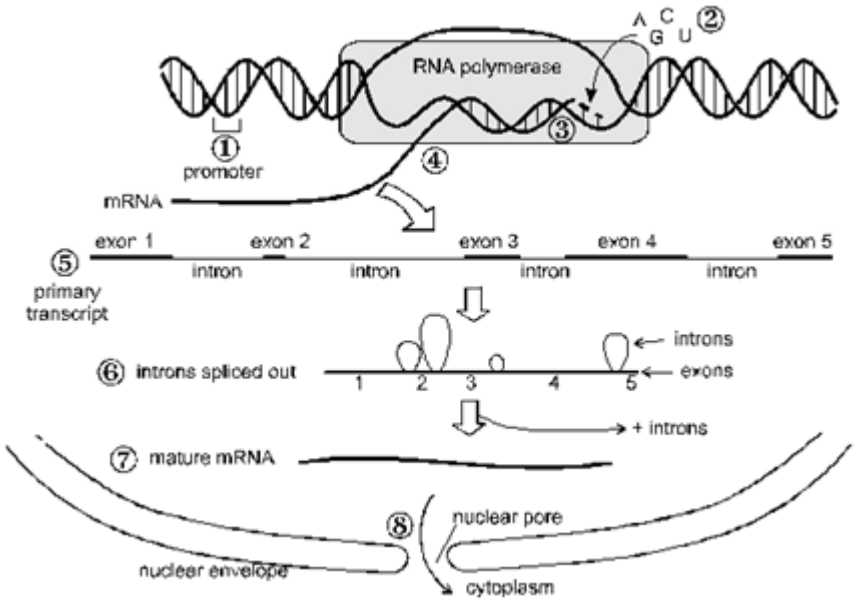
Transcription - RNA Synthesis



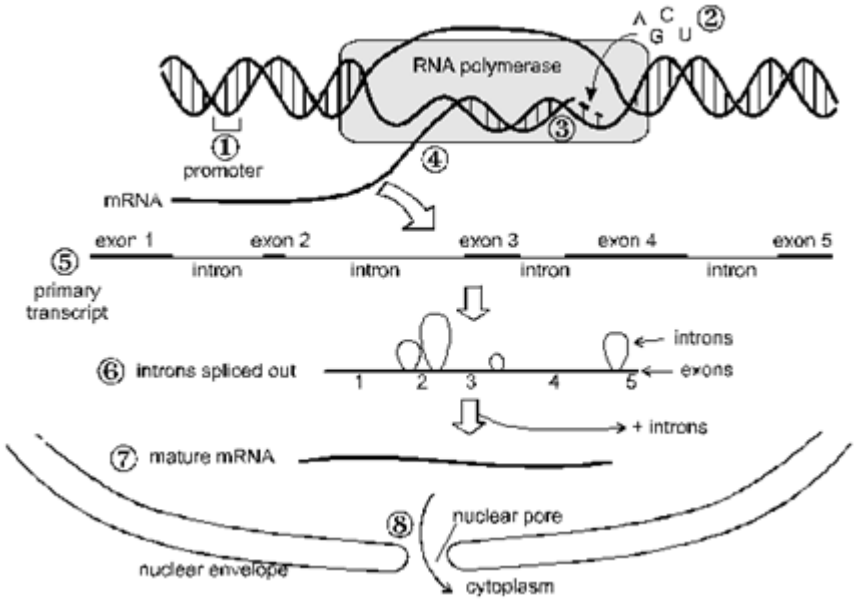
(a) Transcription and RNA processing occur in the nucleus. (b) Translation occurs in the cytoplasm.

Figure 12.5 An overview of protein synthesis, emphasizing the transcriptional origin of snRNA, tRNA, rRNA, and mRNA, the splicing function of snRNA, and the translational roles of tRNA, rRNA, mRNA, and ribosomes.

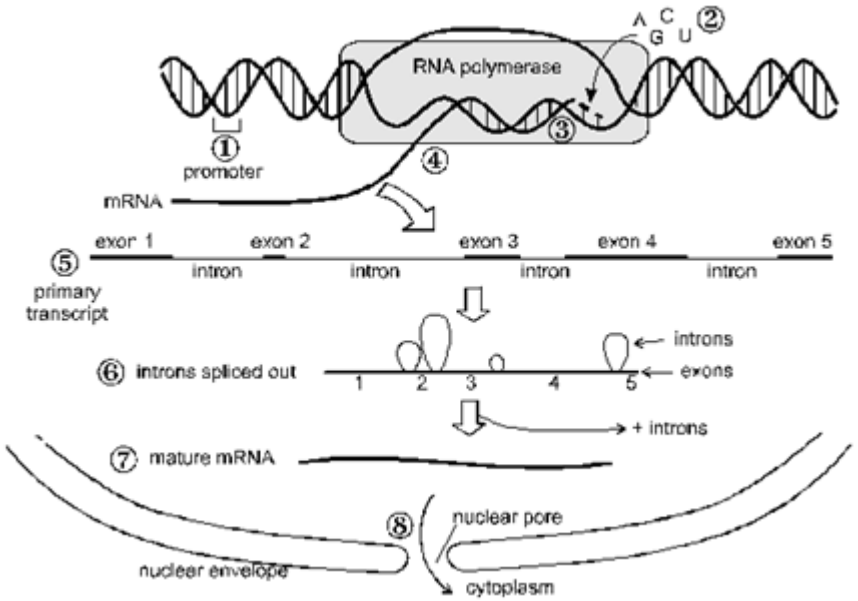
- DNA never leaves the nucleus, but proteins are synthesised in the cytoplasm, so a copy of each gene is made to carry the "message" from the nucleus to the cytoplasm. This copy is mRNA, and the process of copying is called transcription.



- The start of each gene on DNA is marked by a special sequence of bases.
- The RNA molecule is built up from the four ribose nucleotides (A, C, G and U) in the nucleoplasm. The nucleotides attach themselves to the bases on the DNA by complementary base pairing, just as in DNA replication.



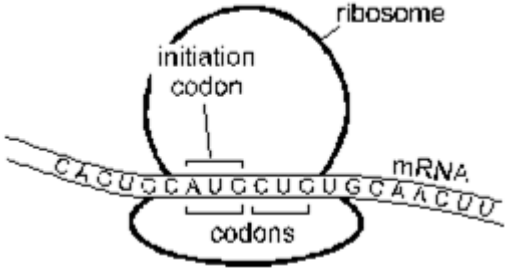
- However, only one strand of RNA is made. The DNA stand that is copied is called the <u>template</u> or <u>sense strand</u> because it contains the sequence of bases that codes for a protein. The other strand is just a complementary copy, and is called the <u>non-template</u> or <u>antisense strand</u>.
- The new nucleotides are joined to each other by strong covalent bonds by the enzyme <u>RNA polymerase</u>.



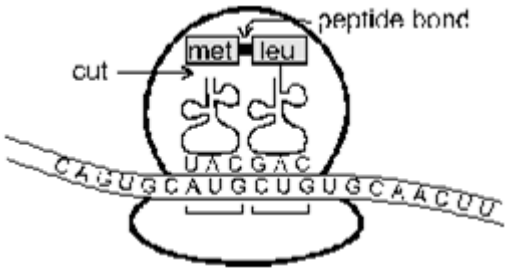
- Only about 8 base pairs remain attached at a time, since the mRNA molecule peels off from the DNA as it is made. A winding enzyme rewinds the DNA.
- The initial mRNA, or primary transcript, contains many regions that are not needed as part of the protein code. These are called introns (for interruption sequences), while the parts that are needed are called exons (for expressed sequences). All eukaryotic genes have introns, and they are usually longer than the exons.

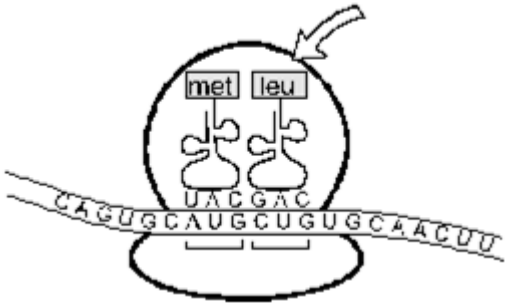
- The introns are cut out and the exons are spliced together by enzymes
- The result is a shorter mature RNA containing only exons. The introns are broken down.
- The mRNA diffuses out of the nucleus through a nuclear pore into the cytoplasm.

Translation - Protein Synthesis

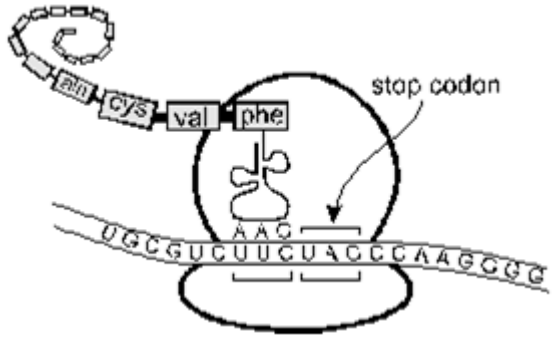


- 1. A ribosome attaches to the mRNA at an initiation codon (AUG). The ribosome encloses two codons.
- 2. met-tRNA diffuses to the ribosome and attaches to the mRNA initiation codon by complementary base pairing.

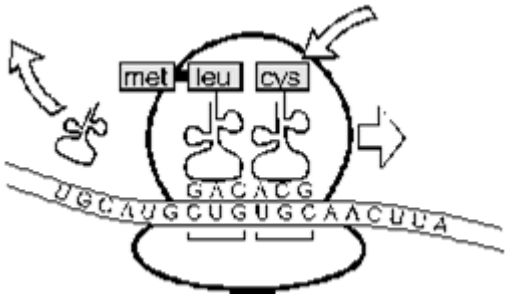




- 3. The next amino acid-tRNA attaches to the adjacent mRNA codon (leu in this case).
- 4. The bond between the amino acid and the tRNA is cut and a <u>peptide bond</u> is formed between the two amino acid
-



- 5. The ribosome moves along one codon so that a new amino acid-tRNA can attach. The free tRNA molecule leaves to collect another amino acid. The cycle repeats from step 3.



- 6. The polypeptide chain elongates one amino acid at a time, and peels away from the ribosome, folding up into a protein as it goes. This continues for hundreds of amino acids until a stop codon is reached, when the ribosome falls apart, releasing the finished protein.

- A single piece of mRNA can be translated by many ribosomes simultaneously, so many protein molecules can be made from one mRNA molecule. A group of ribosomes all attached to one piece of mRNA is called a polysome.

Post-Translational Modification

- In eukaryotes, proteins often need to be altered before they become fully functional. Modifications are carried out by other enzymes and include: chain cutting, adding methyl or phosphate groups to amino acids, or adding sugars (to make glycoproteins) or lipids (to make lipoporteins).