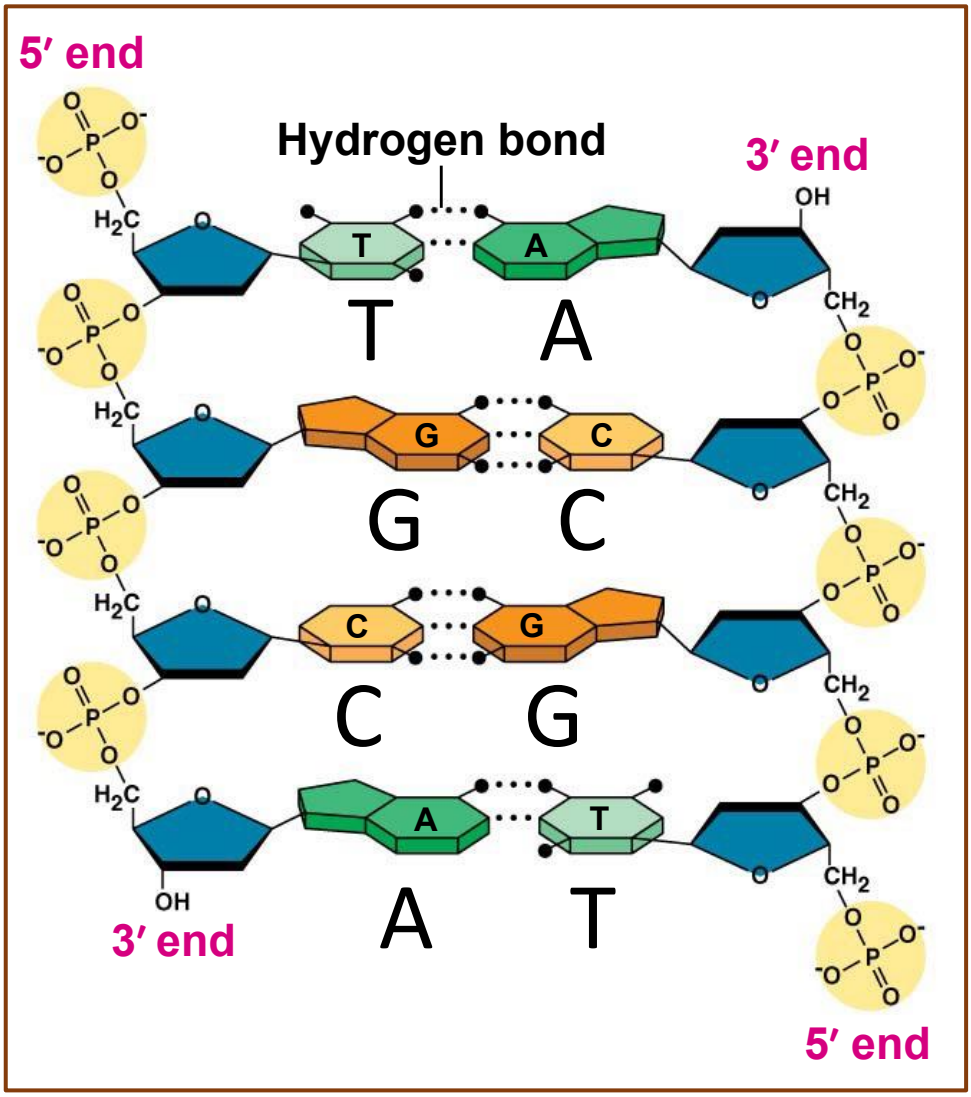
Tutorial 3

Covers Lecture 4

On 28th March 2023 for D4 and 30th March 2023 for D3

1. *Features of the double helix structure of DNA*

James Watson and Francis Crick proposed the double helix model for DNA in 1953. A schematic of the structure is shown below (figure 16.7b of Campbell Biology by Reece and others, 10th edition).

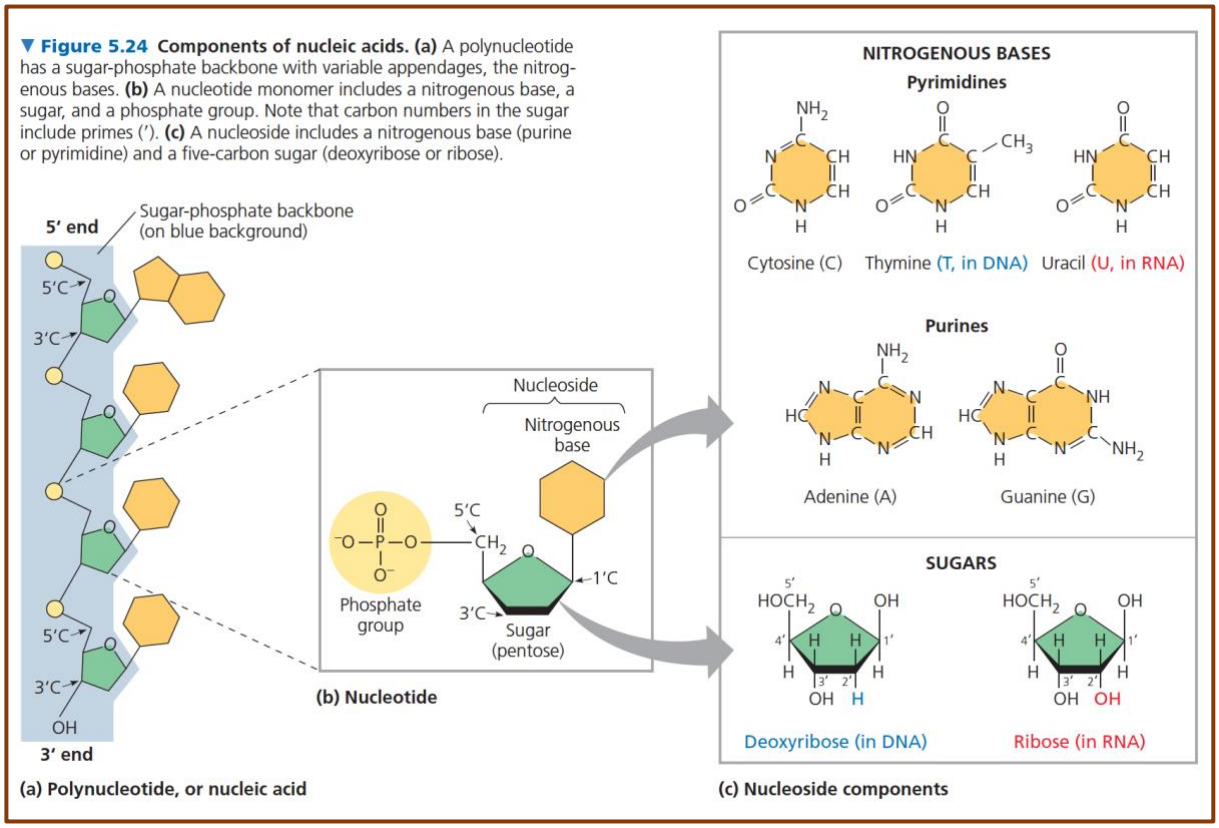


Key structural features of DNA are as follows:

(i) There are two strands.

(ii) Each strand is a polymer. Monomeric units are called nucleotides.

(iii) Each nucleotide consists of a nitrogenous base, deoxyribose (a 5-carbon monosaccharide), and phosphate.



▼ **Figure 5.24** **Components of nucleic acids. (a)** A polynucleotide has a sugar-phosphate backbone with variable appendages, the nitrogenous bases. **(b)** A nucleotide monomer includes a nitrogenous base, a sugar, and a phosphate group. Note that carbon numbers in the sugar include primes (′). **(c)** A nucleoside includes a nitrogenous base (purine or pyrimidine) and a five-carbon sugar (deoxyribose or ribose).

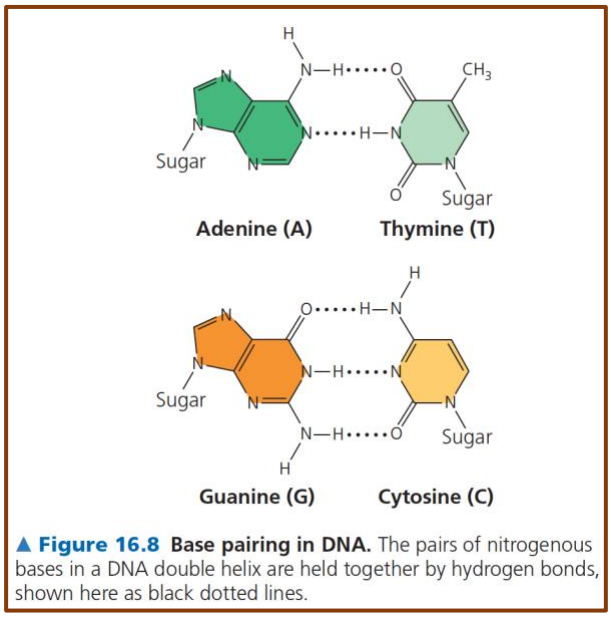
Note: Do NOT memorize detailed structures. Knowing key features 9as given in the text) suffices

(iv) Both strands have a 5’-end and a 3’-end.
Note: whenever you write a schematic of DNA, remember to label the 5’-end and 3’-ends.

(v) By convention, sequence of DNA is written from the 5’-end to the 3’-end.

(vi) Difference between DNA from two organisms lies in the sequence of the monomers; colloquially called as the DNA sequence, nucleotide sequence, or just sequence.

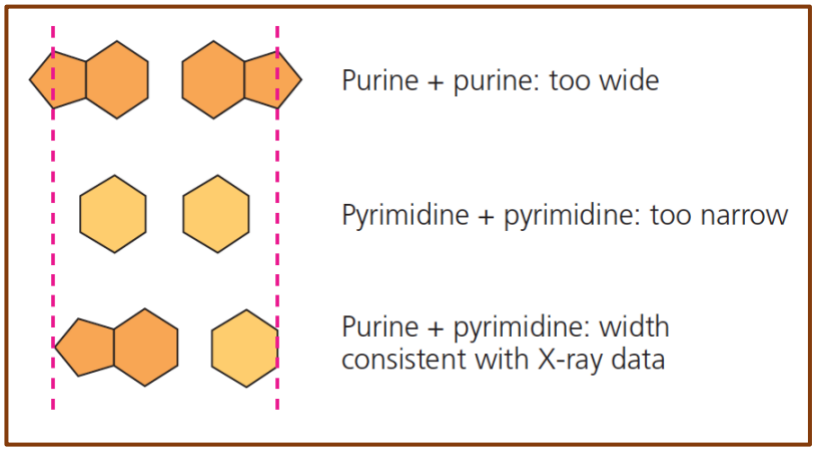
(vii) Nitrogenous base from one strand forms hydrogen bond (H-bond) with a base from the other stand. This H-bond pairing is very specific i.e., adenine (A) with thymine (T), and guanine (G) with cytosine (C).
This is called base complementarity.



▲ **Figure 16.8 Base pairing in DNA.** The pairs of nitrogenous bases in a DNA double helix are held together by hydrogen bonds, shown here as black dotted lines.

Note: you NEED NOT memorize the structures of the four bases.

(viii) Adenine (A) and guanine (G) have two rings (purines). Cytosine (C) and thymine (T) have one ring (pyrimidines). Because of the specific pairing, width of the double helix remains the same irrespective of the sequence.



(From page 318 of Campbell Biology, 10th edition; this schematic is not numbered in the book)

(ix) Knowledge of the sequence of one strand allows us to write the sequence on the complementary strand.

Suppose the sequence of one strand of DNA is as follows:
5'-ACGTCATCACGTGGCACTTC-3'
Write the sequence of its complementary strand.

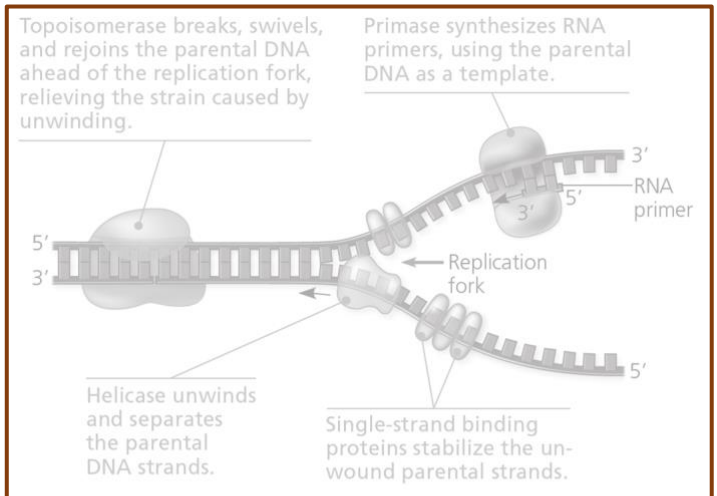
(x) The two strands of DNA double helix are anti-parallel.

2. *Biosynthesis of DNA*

Replication of DNA is the biosynthesis of a new and identical copy (= replica) of a double stranded DNA. Several molecular details of this process have been worked out extensively.

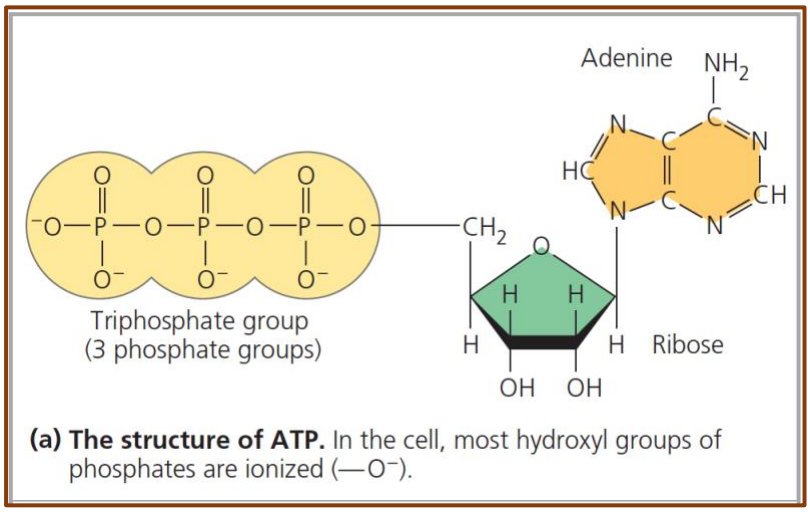
In this tutorial, we are concerned with two aspects of the biosynthesis of DNA.

(i) Separation of the two strands of double helix. Figure 16.13 is shown below. It has been greyed out to indicate to you that you are NOT expected to know the details given in this figure. Just that the two strands get separated as one of the first steps of replication.

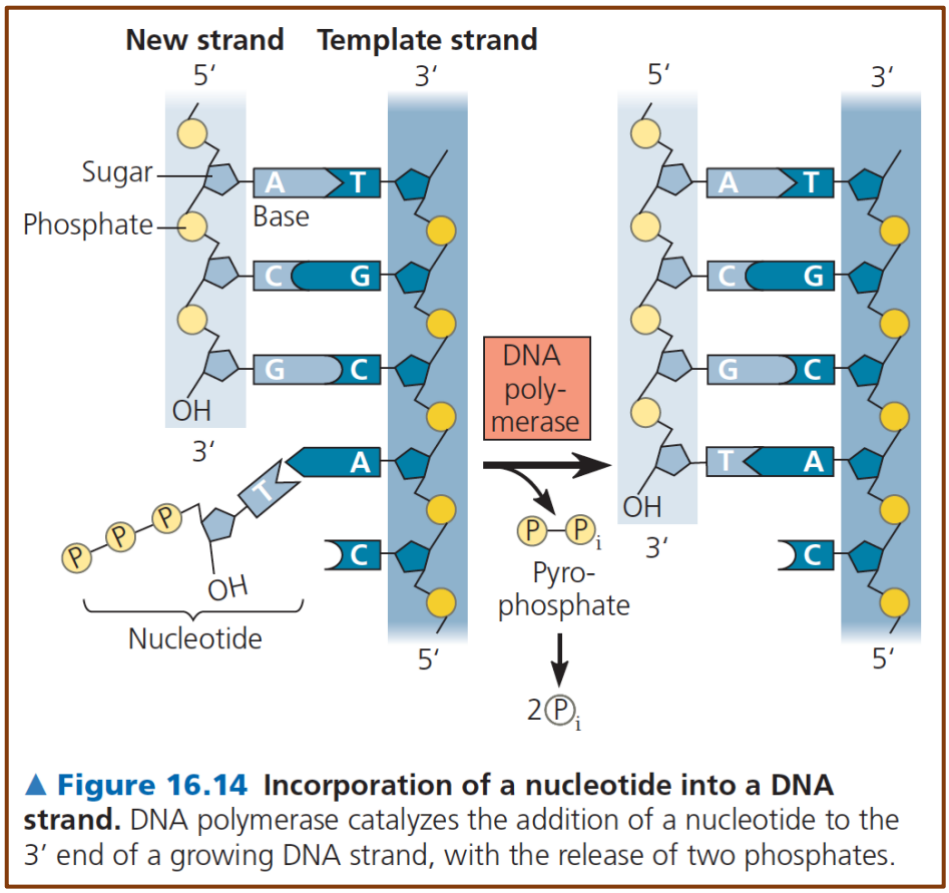


▲ **Figure 16.13 Some of the proteins involved in the initiation of DNA replication.** The same proteins function at both replication forks in a replication bubble. For simplicity, only the left-hand fork is shown, and the DNA bases are drawn much larger in relation to the proteins than they are in reality.

(ii) Synthesis of new strand using one of the strands as the template. Note that a nucleotide triphosphate (NTP) is added to the growing end of a strand; pyrophosphate is hydrolyzed. The three phosphate groups are identified as α-phosphate (linked to ribose), β-phosphate (middle phosphate), and γ-phosphate (terminal phosphate).



(a) The structure of ATP. In the cell, most hydroxyl groups of phosphates are ionized (—O^-).



▲ Figure 16.14 Incorporation of a nucleotide into a DNA strand. DNA polymerase catalyzes the addition of a nucleotide to the 3′ end of a growing DNA strand, with the release of two phosphates.

3. *The two strands of DNA in a double helix are anti-parallel*

One of the features of Watson-Crick's double helix model is that the two strands of DNA are anti-parallel. This was experimentally demonstrated by Arthur Kornberg in 1961.

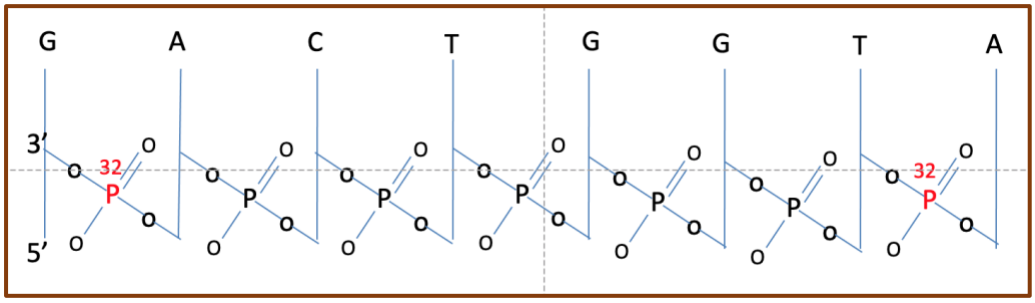
Note: Kornberg was awarded the Nobel Prize in Physiology or Medicine in 1959 for his discovery of DNA polymerase.

Reaction mixture consisted of DNA template, dNTPs, DNA polymerase, and other relevant ingredients (e.g., buffer of appropriate pH).

There were four tubes in which DNA synthesis was carried out. The contents of the four tubes differed in the dNTP which was α-^{32}P labelled:

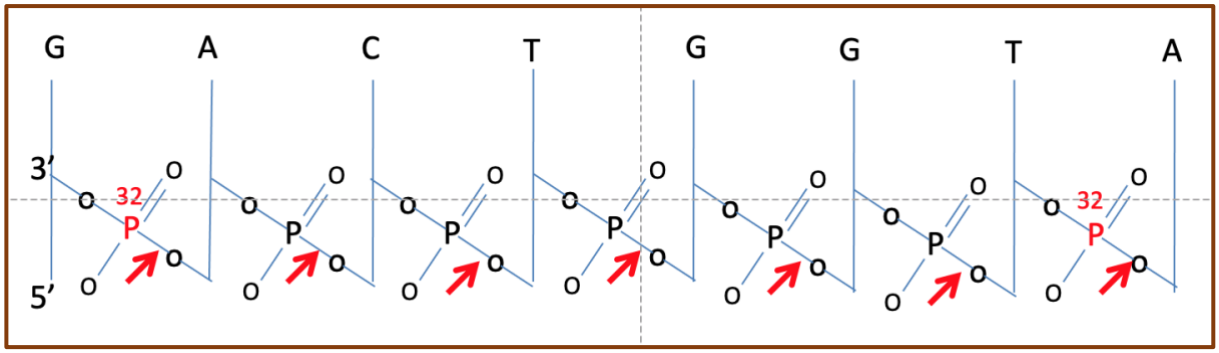
Reaction tube	Contents
No. 1	Reaction mixture + dATP (α-^{32}P)
No. 2	Reaction mixture + dTTP (α-^{32}P)
No. 3	Reaction mixture + dGTP (α-^{32}P)
No. 4	Reaction mixture + dCTP (α-^{32}P)

Schematic of the newly synthesized DNA from reaction tube no. 1 (i.e., dATP is α-^{32}P labelled):



Which phosphate groups are labelled in the newly synthesized DNA depends upon the labelled dNTP present in that reaction tube.

Treat newly synthesized DNA with DNase, an enzyme which hydrolyzes DNA giving rise to individual monomers as products. Note the bond that is cleaved by the DNase (marked by the red arrow):



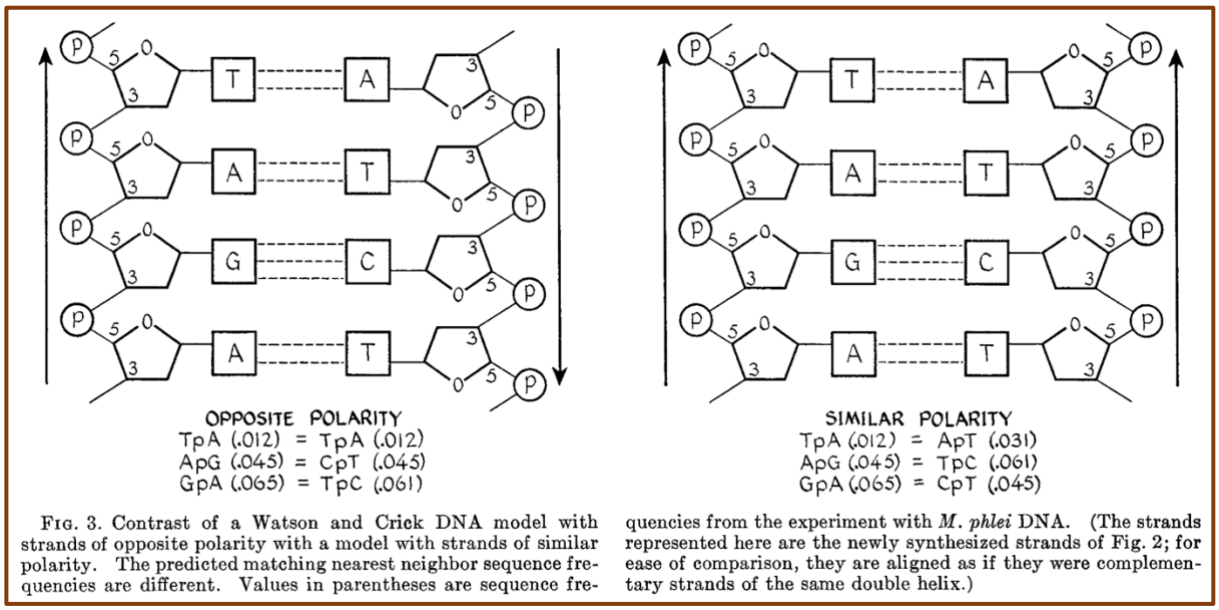


FIG. 3. Contrast of a Watson and Crick DNA model with strands of opposite polarity with a model with strands of similar polarity. The predicted matching nearest neighbor sequence frequencies are different. Values in parentheses are sequence frequencies from the experiment with *M. phlei* DNA. (The strands represented here are the newly synthesized strands of Fig. 2; for ease of comparison, they are aligned as if they were complementary strands of the same double helix.)

The two possible strand orientations viz., parallel and anti-parallel are shown below. Work out the differences in nucleotide frequencies that demonstrated that the two strands are anti-parallel.

ʚʛɔ E N D ʚʛɔ