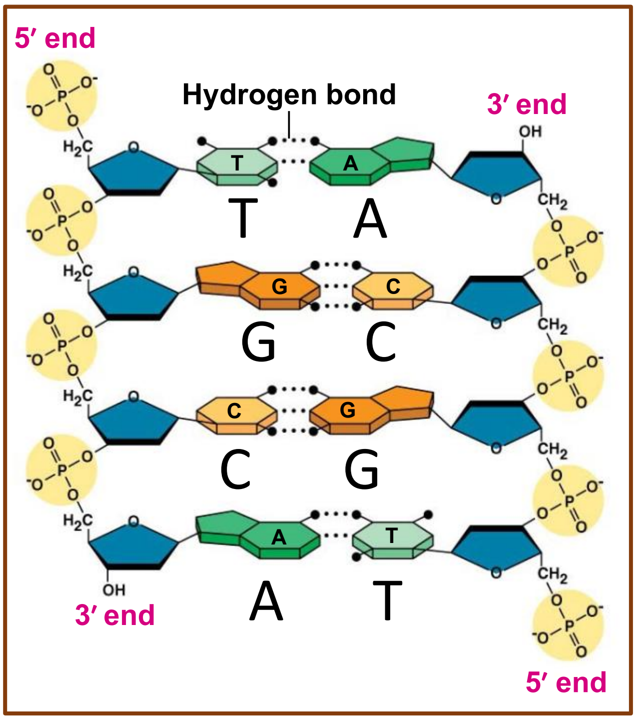
Answer key for Tutorial – 3

Covers Lecture 4

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

Demonstrating that the two strands of a double helix are anti-parallel

Convention used for writing the nucleotide sequence of one strand of a DNA double helix:



The sequence of the DNA fragment shown above may be written as follows:

p <u>5’-T-3’</u> p <u>5’-G-3’</u> p <u>5’-C-3’</u> p <u>5’-A-3’</u> OH

Each nucleotide is underlined. The phosphate that connects two nucleotides is shown in between.

The above can be further simplified as

5’-TpGpCpA-3’

It is a common practice to simplify further by omitting even “p”. In other words, the above sequence will be written as 5’-TGCA-3’.

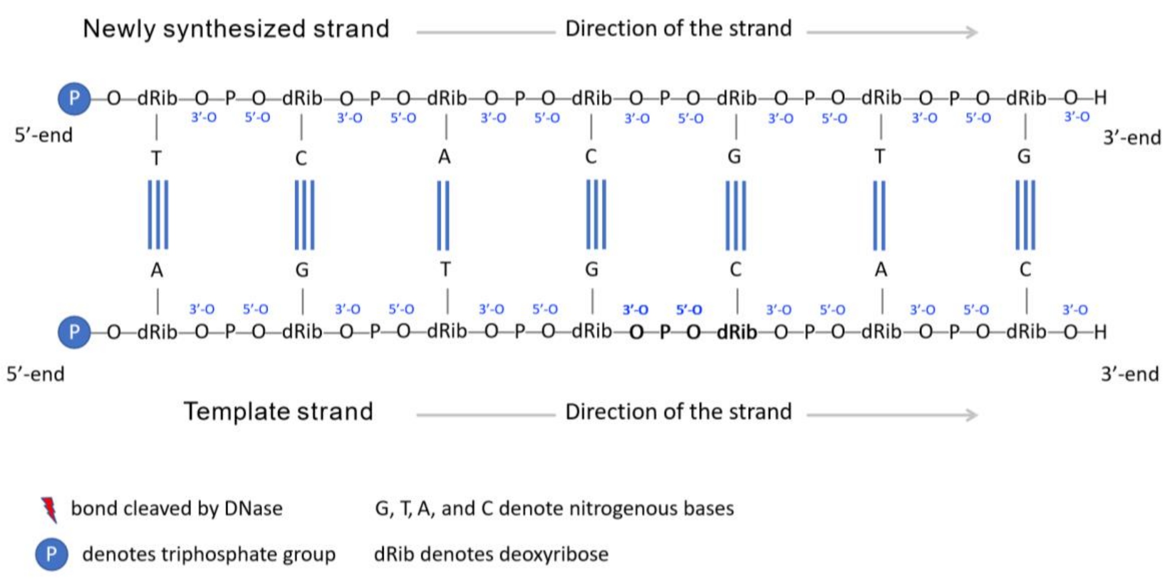
Orientation	Sequence of the two strands	Possible dinucleotides from both strands
Parallel	5’-AGCTAGCTGGATCA-3’ 5’-TCGATCGACCTAGT-3’	5’-ApC-3’ 5’-ApG-3’ 5’-ApT-3’ 5’-CpA-3’ 5’-CpC-3’ 5’-CpG-3’ 5’-CpT-3’ 5’-GpA-3’ 5’-GpC-3’ 5’-GpG-3’ 5’-GpT-3’ 5’-TpA-3’ 5’-TpC-3’ 5’-TpG-3’
	Dinucleotide frequencies which should be the same ApG = TpC GpC = CpG CpT = GpA TpA = ApT TpG = ApC GpG = CpC GpA = CpT CpA = GpT	
Anti-parallel	5’-AGCTAGCTGGATCA-3’ 3’-TCGATCGACCTAGT-5’	~~5’-ApC-3’~~ 5’-ApG-3’ 5’-ApT-3’ 5’-CpA-3’ 5’-CpC-3’ ~~5’-CpG-3’~~ 5’-CpT-3’ 5’-GpA-3’ 5’-GpC-3’ 5’-GpG-3’ ~~5’-GpT-3’~~ 5’-TpA-3’ 5’-TpC-3’ 5’-TpG-3’
	Dinucleotide frequencies which should be the same ApG = CpT CpT = ApG TpG = CpA GpG = CpC GpA = TpC CpA = TpG GpC, TpA, ApT: need not be same as any other	

Experimental data showed that the dinucleotide frequences match as expected for anti-parallel orientation.

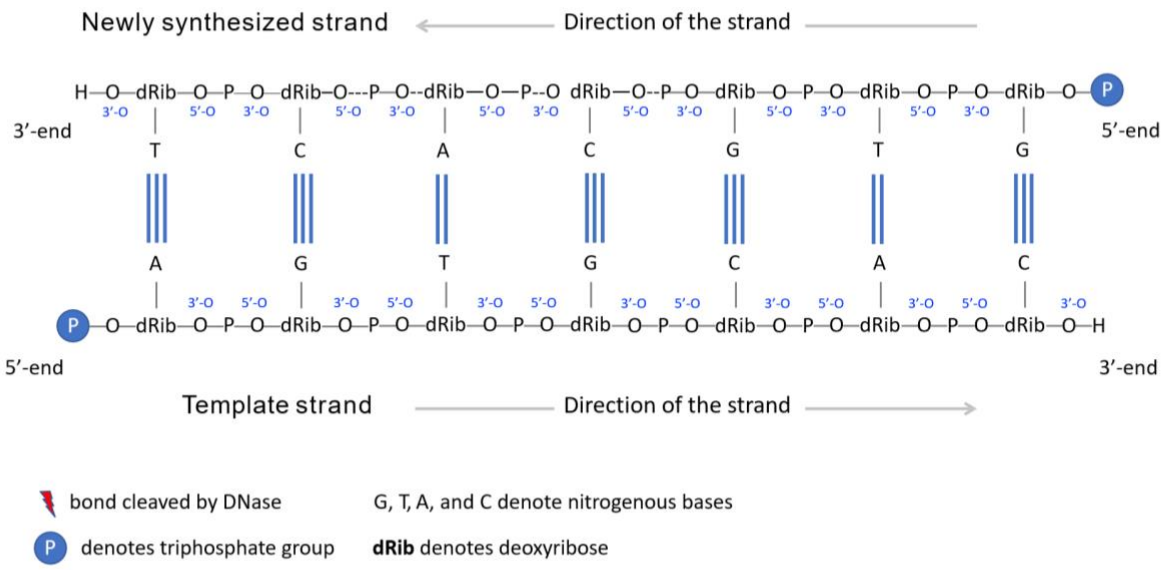
Ans – As we know that DNA contains two polymers (poly-nucleotide) strands having H-bonds in between the nucleotide bases of opposite strands, which are complementary by nature. Hence, Adenine (A) binds to Thymine (T) with two H-bond in between them and Guanine (G) binds to Cytosine (C) with three H-bond in between. DNA polymer strands have a 5' side and 3' side reflecting the presence of a free P-group and free OH-group respectively.

In order to prove the fact of DNA strand polarity, Kornberg took advantage of nucleotide base complementarity and specificity of DNase enzyme to design the experimental setup. Here, he also used radiolabeled α-^{32}P (present in the phosphate group of each nucleotide) to tag a specific nucleotide base during DNA replication and its detection (detection of α-^{32}P with the bound nucleotide). If we think about the polarity of the two strands of DNA (when it was totally unknown), there are two possibilities (or options to the asked question):

1. DNA strands can be parallel to each other (meaning having 5' end of both the strands in the same side), or



2. DNA strands can be anti-parallel to each other (meaning having 5' end of both the strands in opposite sides)



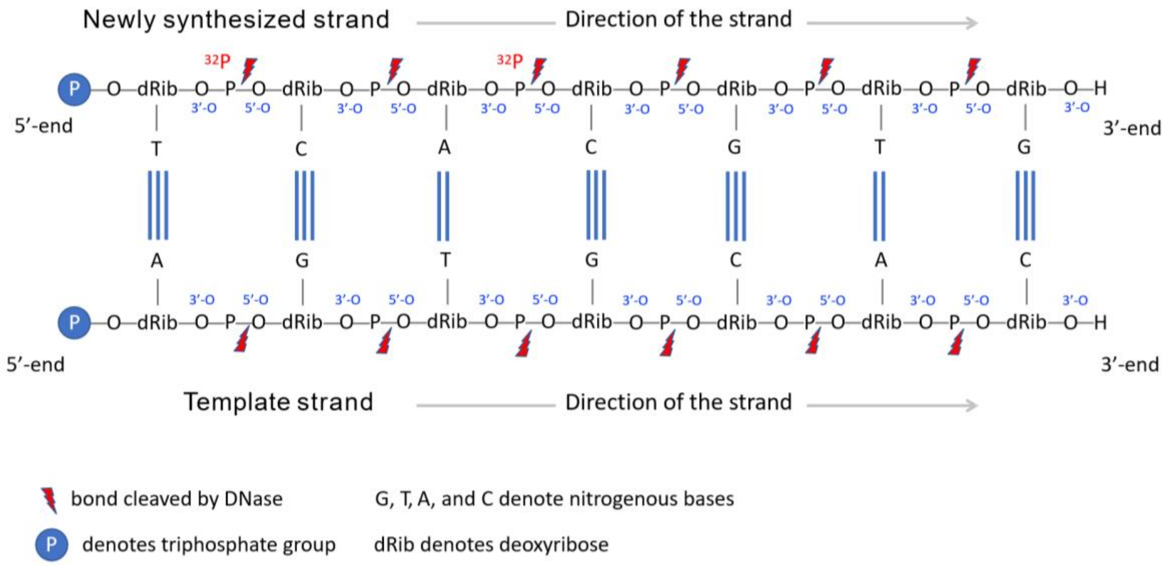
Experimental setup by Arthur Kornberg:

Reaction tube	**Contents**
Tube 1 –	**dATP (α-^{32}P)** + dTTP + dCTP + dGTP
Tube 2 –	dATP + **dTTP (α-^{32}P)** + dCTP + dGTP
Tube 3 –	dATP + dTTP + **dCTP (α-^{32}P)** + dGTP
Tube 4 –	dATP + dTTP + dCTP + **dGTP (α-^{32}P)**

Taken for DNA replication and then was cleaved by DNase enzyme. And the frequency of the outcomes was compared with each other. And depending on the experimental frequency he answered the question.

Let, for example, take tube 3 for the above-mentioned steps, he should get the results as stated below.

1. If DNA strands are parallel to each other (same polarity)



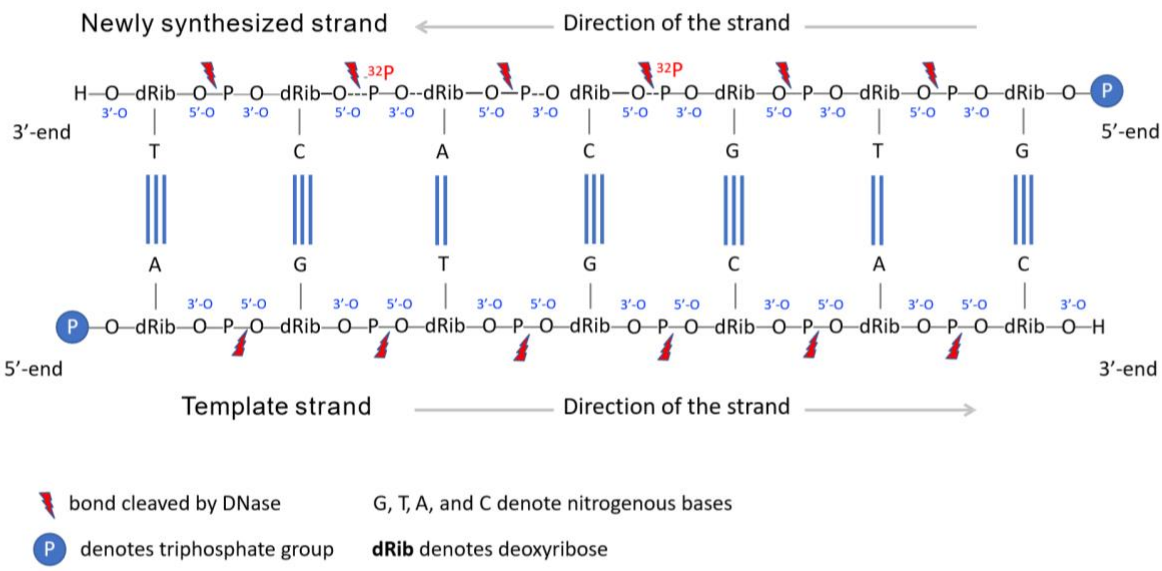
Then, considering a frame of two nucleotides having one α-^{32}P C;

TpC = ApG (TpC means, α-^{32}P from C transferred to 5’ neighbour T)
ApC = TpG (ApC means, α-^{32}P from C transferred to 5’ neighbour A)

TpC / ApC
ApG / TpG (showing complementarity – possible in the same direction)

Hence, the transfer of α-^{32}P of C to the neighbouring 5’ nucleotide is always towards the same direction in both cases.

2. If DNA strands are anti-parallel to each other (opposite polarity)



Then, considering a frame of two nucleotides having one α-^{32}P C;

ApC = GpT (ApC means, α-^{32}P from C transferred to 5′ neighbour A)
GpC = GpC (GpC means, α-^{32}P from C transferred to 5′ neighbour G)

ApC / GpC
GpT / GpC (Showing non-complementarity – possible in the opposite direction)

Hence, the transfer of α-^{32}P of C to the neighbouring 5′ nucleotide is always towards the opposite direction in both cases.

So, it was concluded that every time he performed this experiment, he got equal frequencies as described in point 2, every single time. And this is only possible when strands are anti-parallel to each other or are in opposite polarity.

Q. 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.

Ans - 5'-ACGTCATCACGTGGCACTTC-3'
3'-TGCAGTAGTGCACCGTGAAG-5'