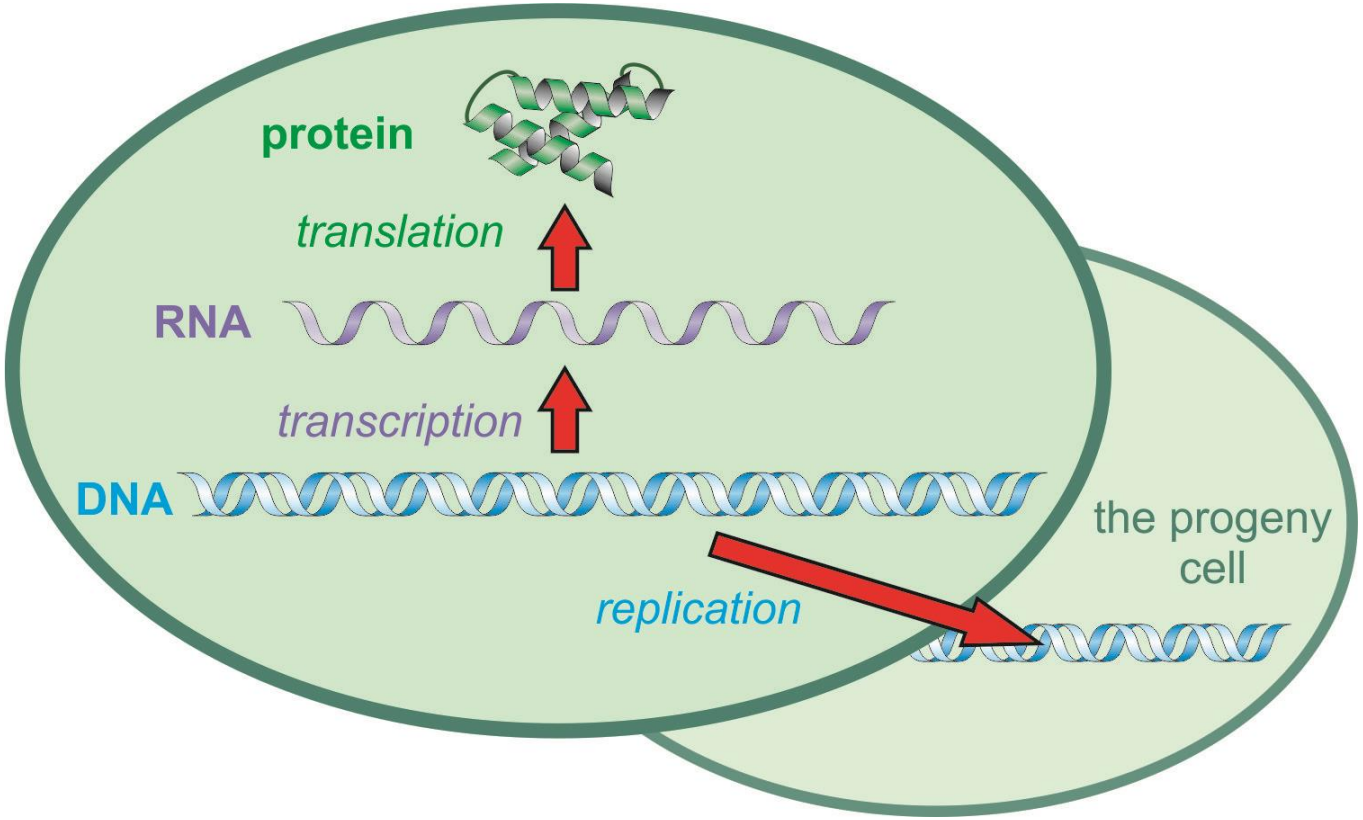
DNA and replication

dr hab. Krzysztof Leśniewicz

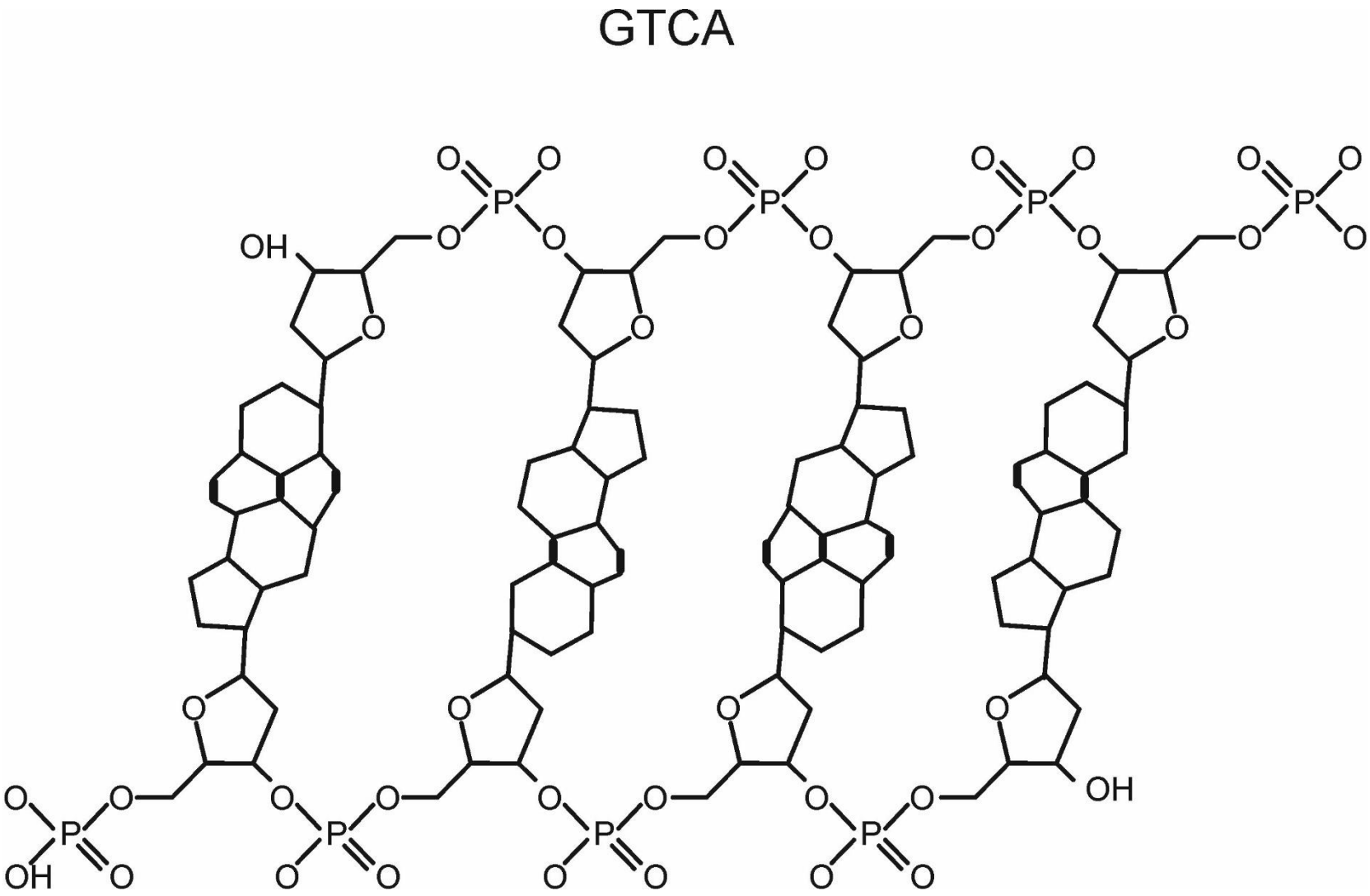
Course title: From molecules to cells

Central dogma of molecular biology

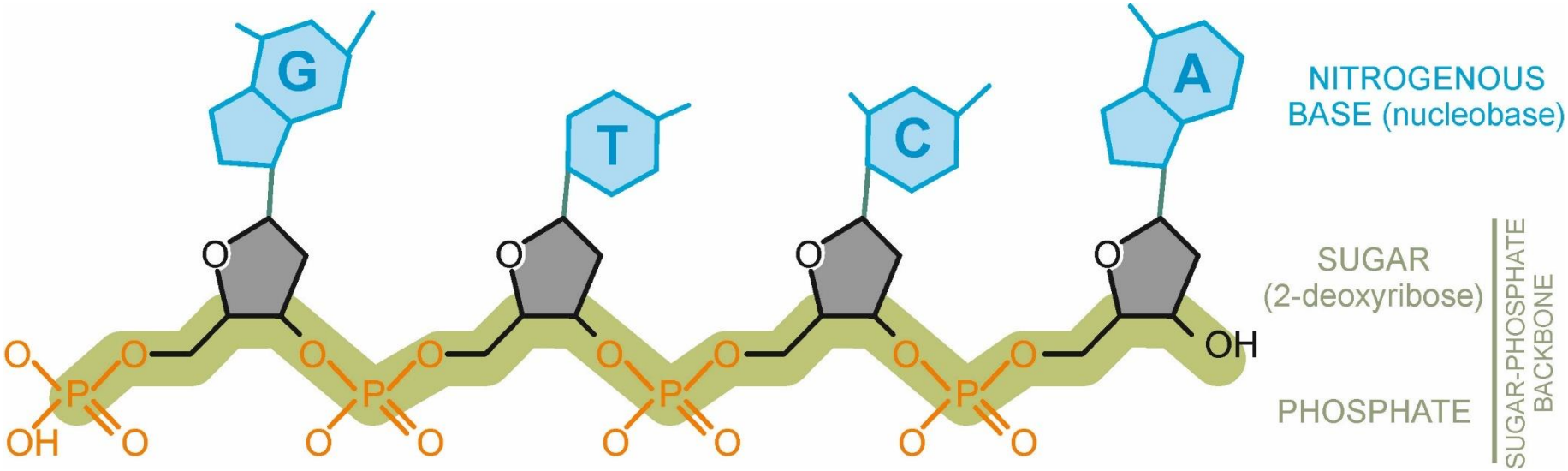
The transfer of genetic information within a biological system



What we need to know to understand how DNA works



DNA is a polymer composed of nucleotides



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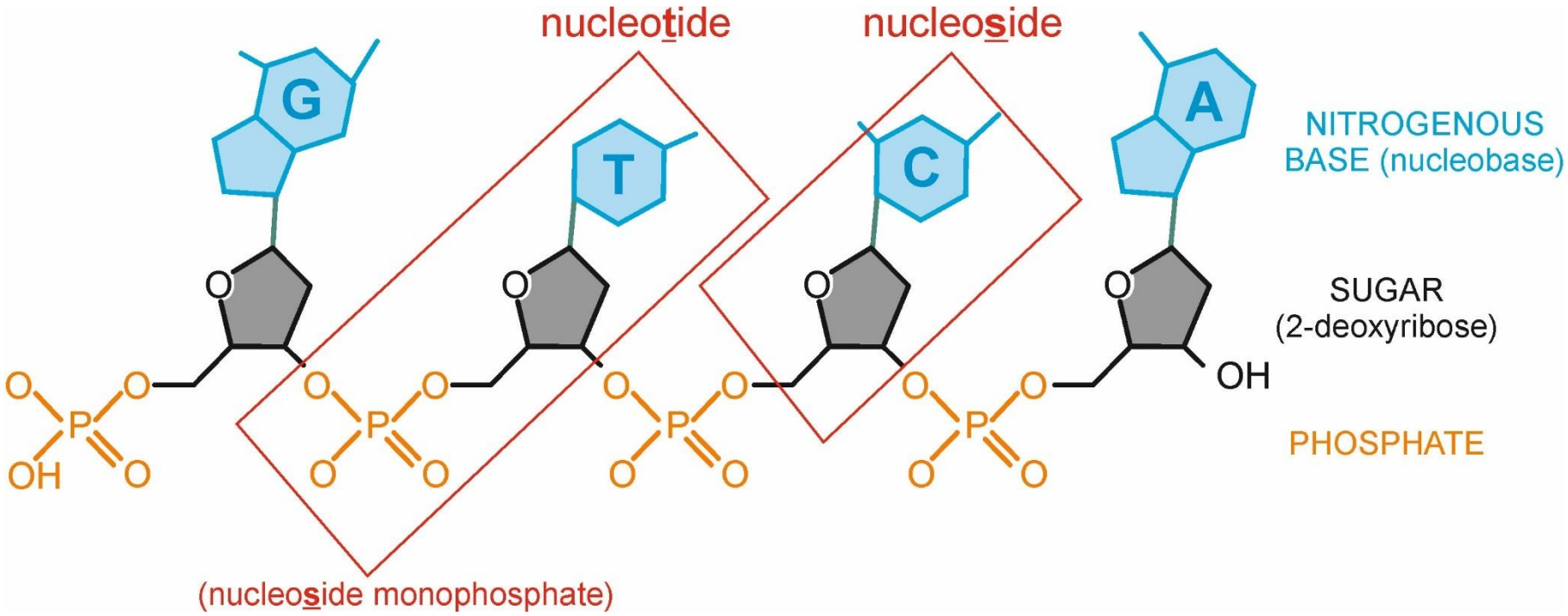
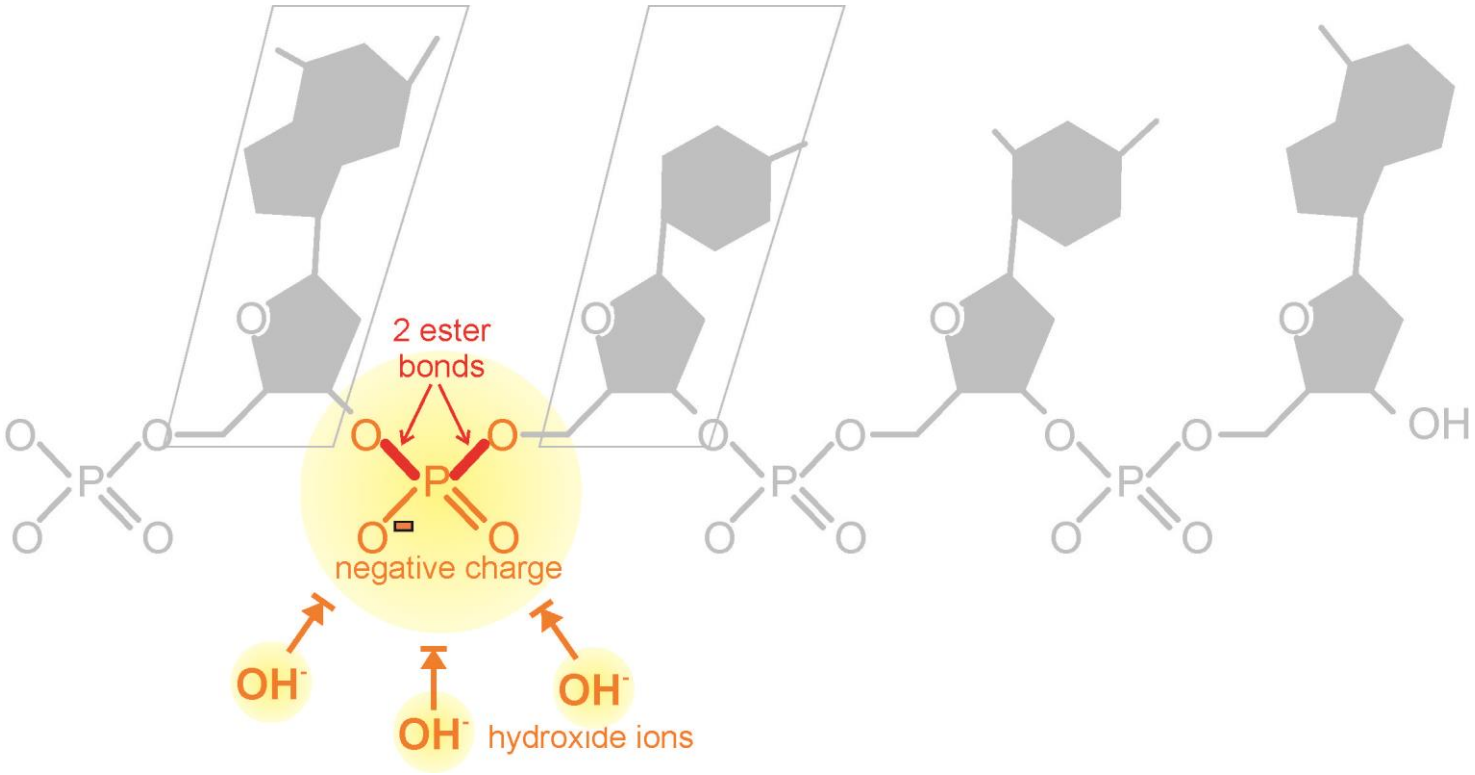
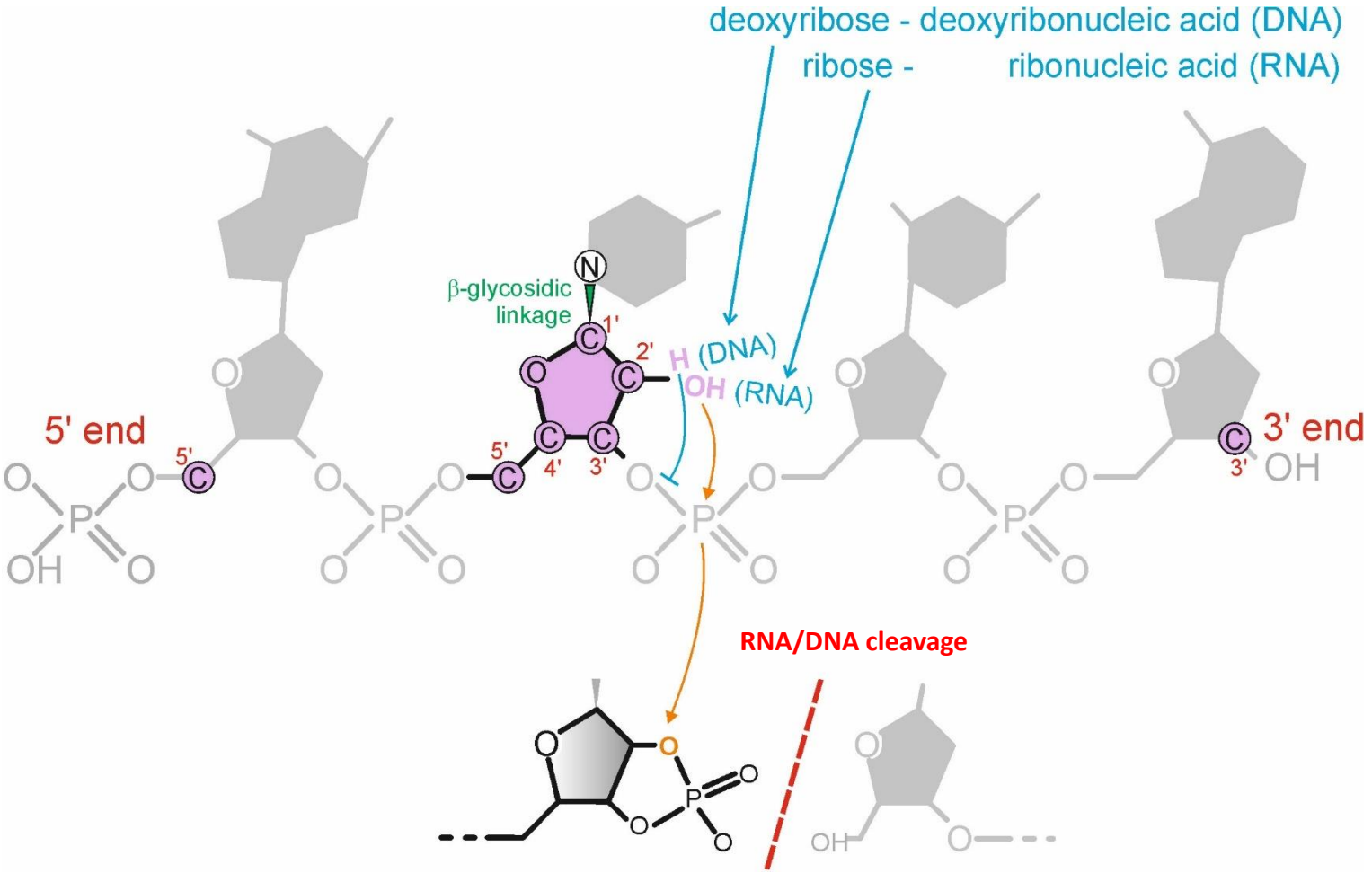


Phosphodiester bridge is negatively charged and give DNA molecules a negative charge

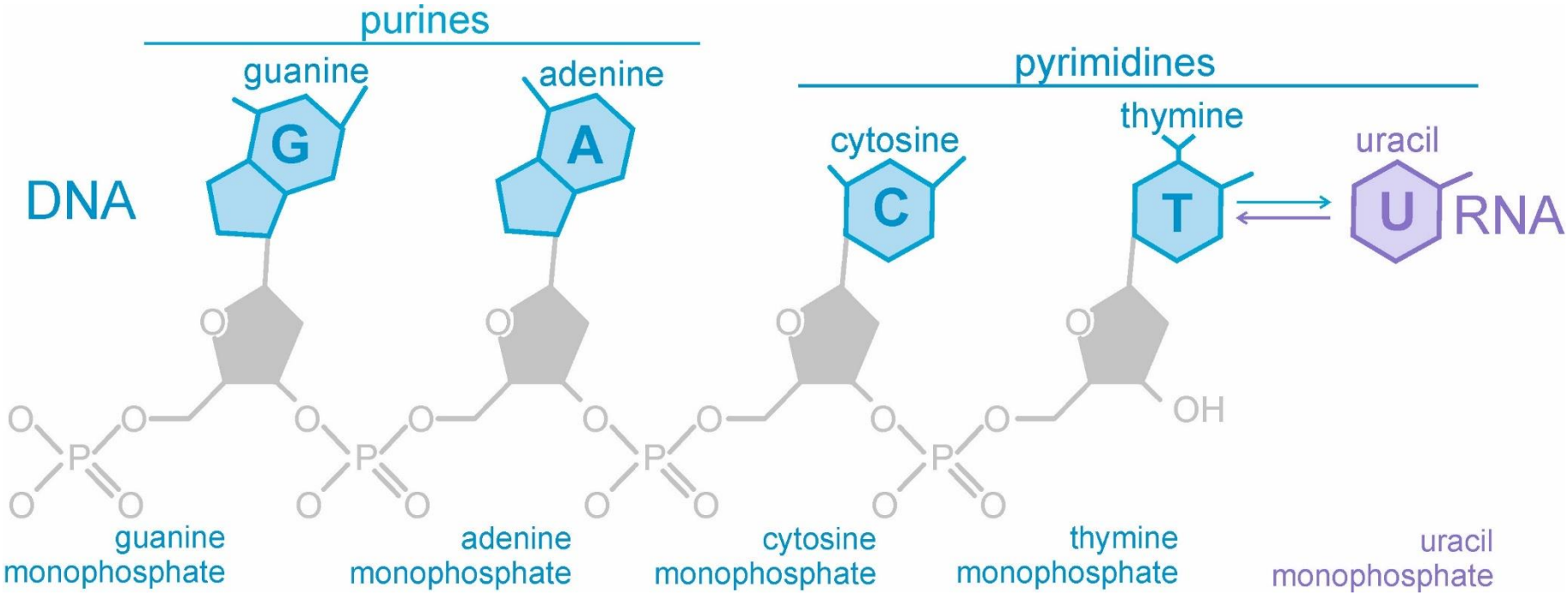


charge repulsion

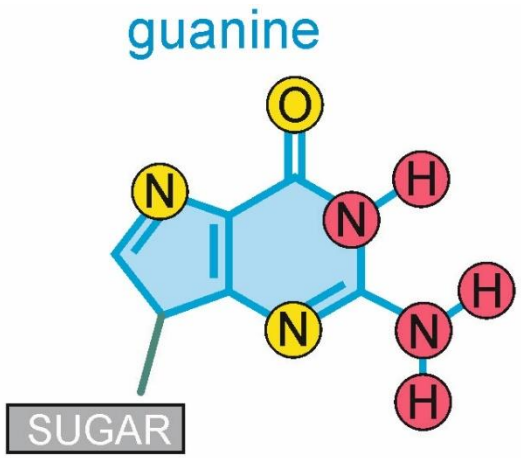
Ribose and deoxyribose are components of RNA and DNA, respectively.

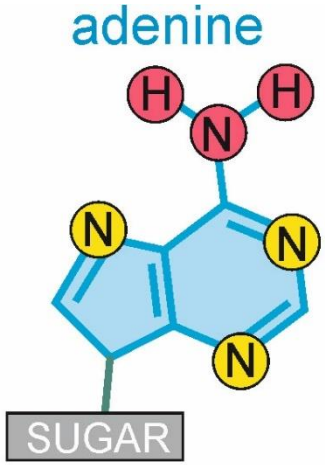


Genetic information is coded in the sequence of nitogenous bases



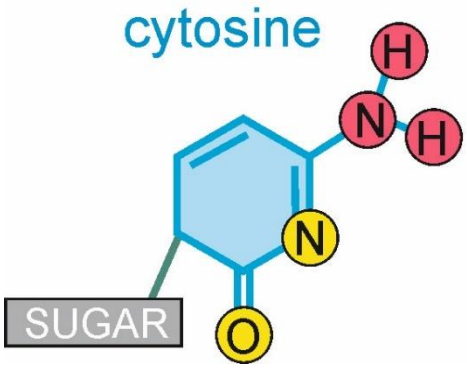
Nitrogenous bases can form hydrogen bonds

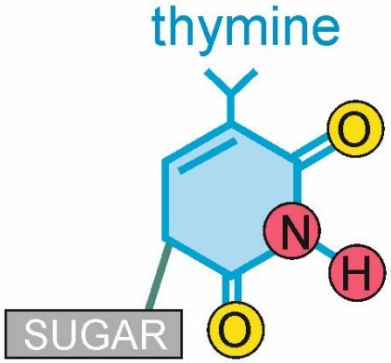


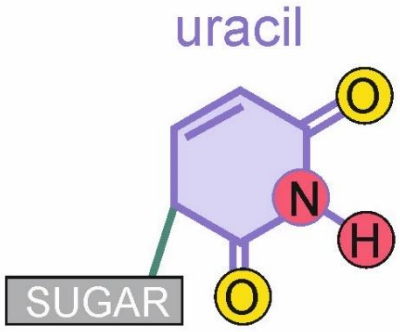


hydrogen bond donors
primary, secondary amine group

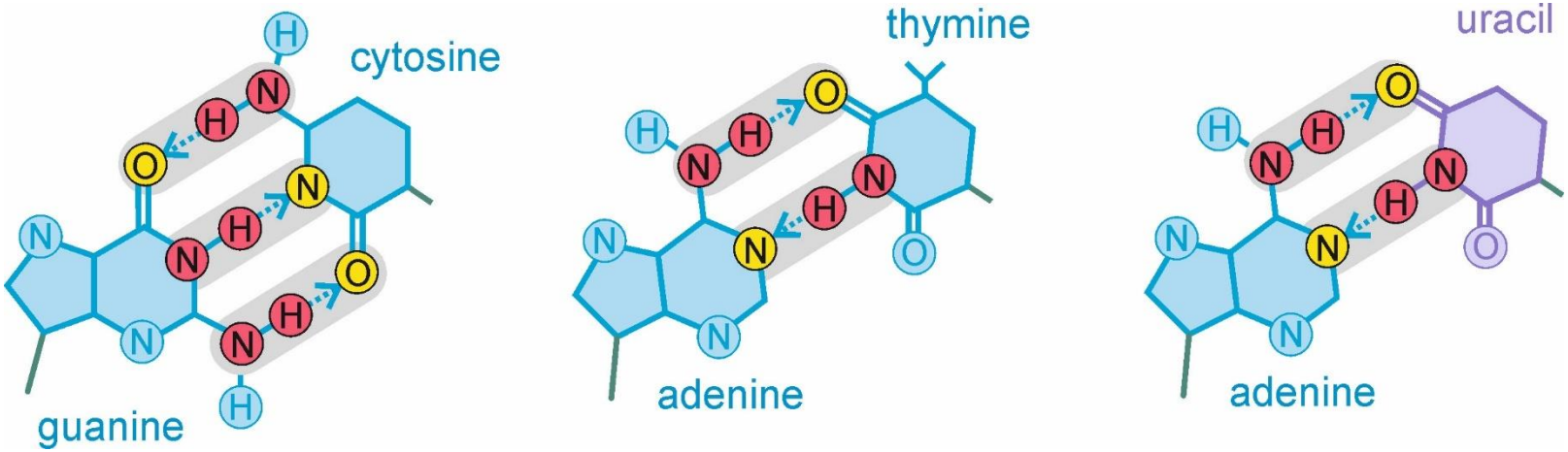
hydrogen bond acceptors
tertiary amine, carbonyl group

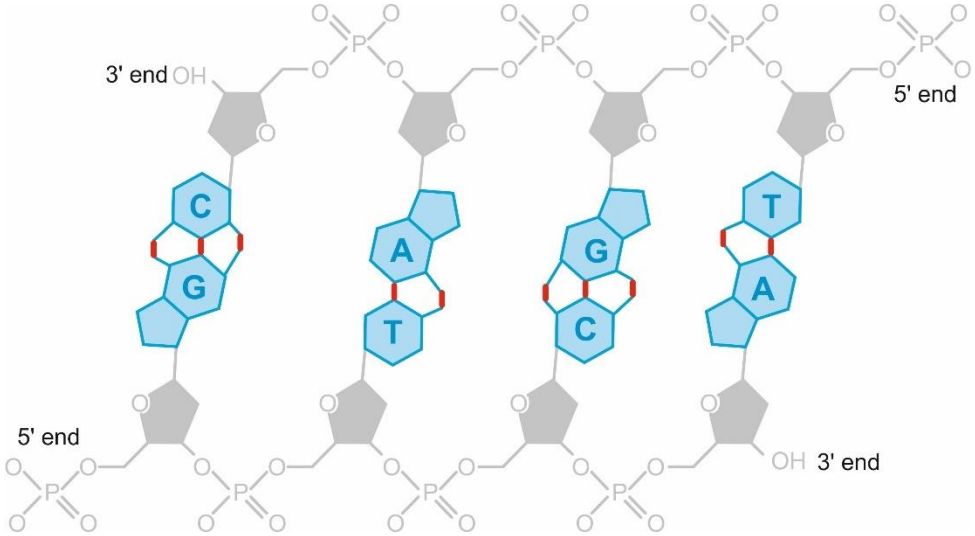






Nitrogenous bases pair with each other using hydrogen bonds





DNA $\xrightarrow{\text{replication}}$ DNA (dsDNA)

DNA $\xrightarrow{\text{transcription}}$ mRNA

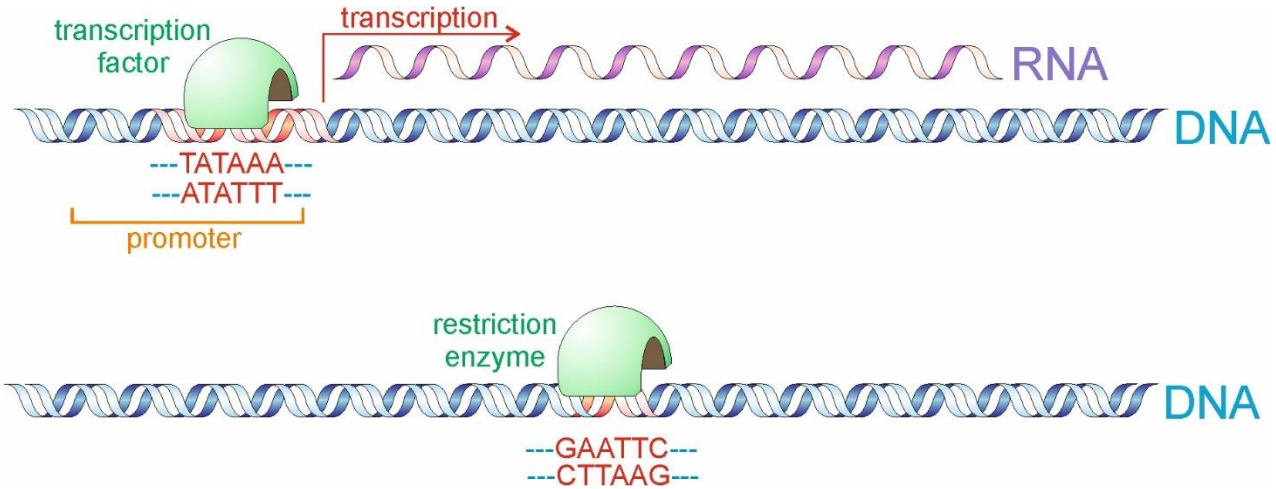
mRNA $\xrightarrow{\text{translation}}$ tRNA→protein

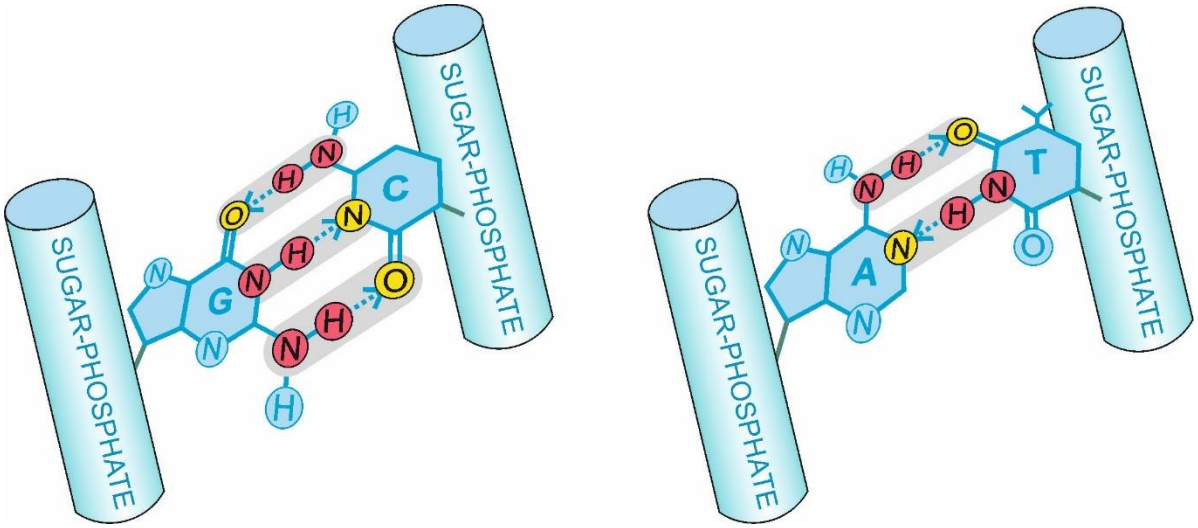
siRNA $\xrightarrow[\text{regulation}]{\text{RNAi}}$ mRNA

RNA $\xrightarrow[\text{transcrip}]{\text{reverse}}$ DNA

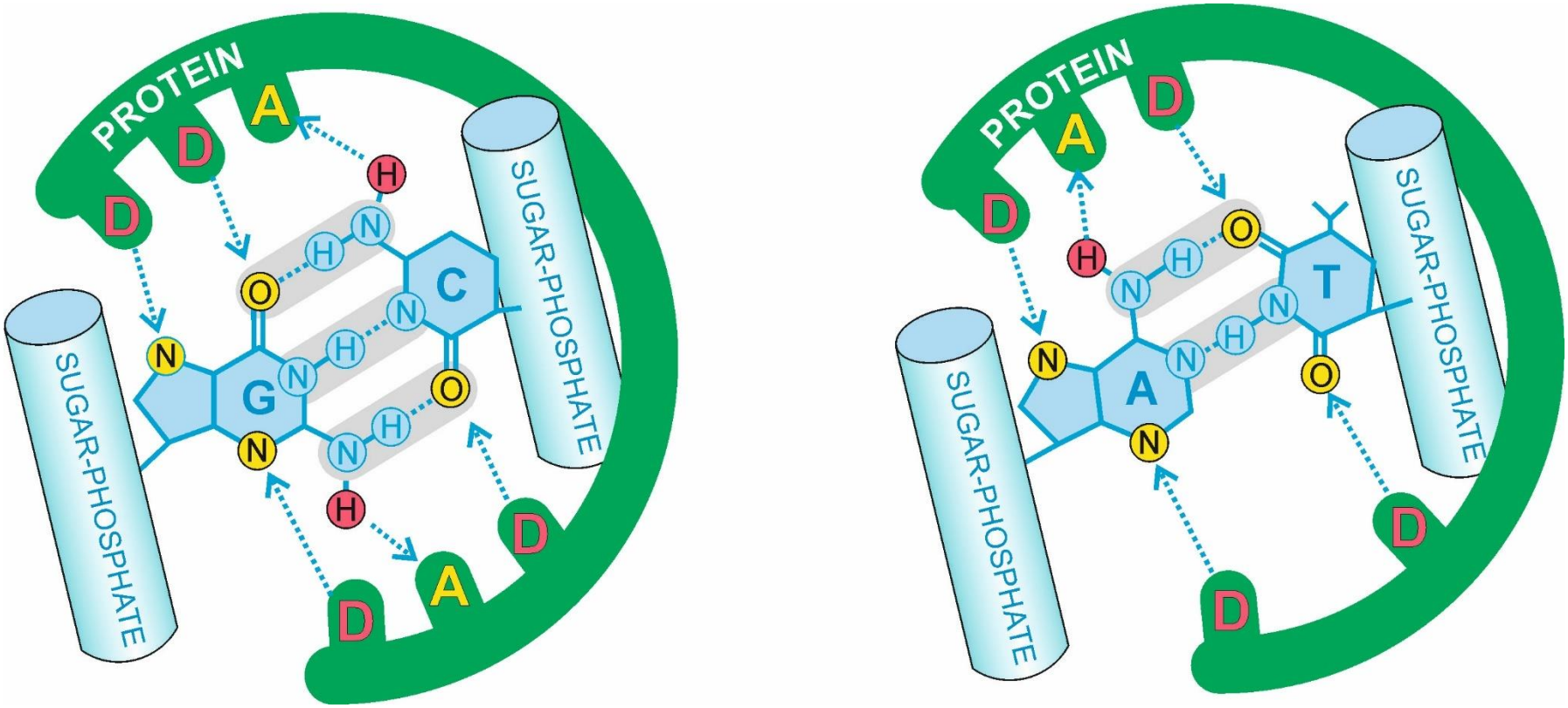
ssRNA $\xrightarrow[\text{replication}]{\text{RNA virus}}$ dsRNA

How proteins recognize and bind specific double-stranded nucleic acid sequences ?

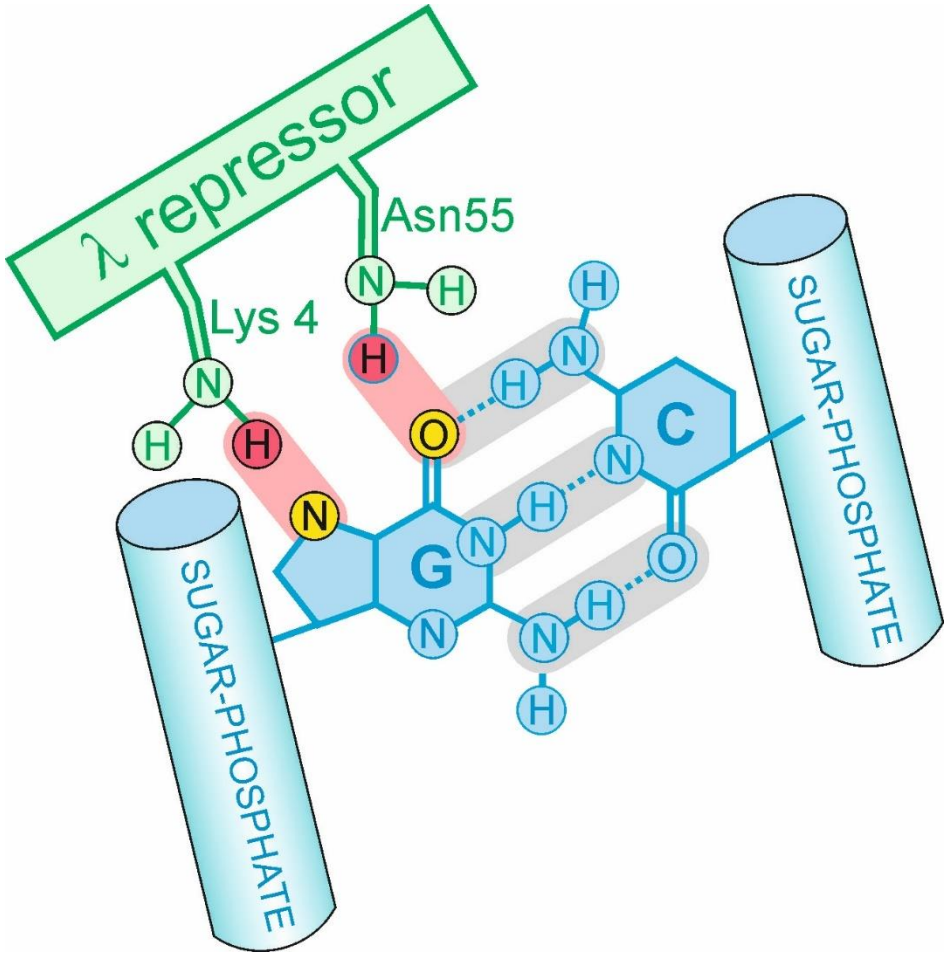




Proteins recognize specific DNA sequences by selective hydrogen bonds to the DNA bases



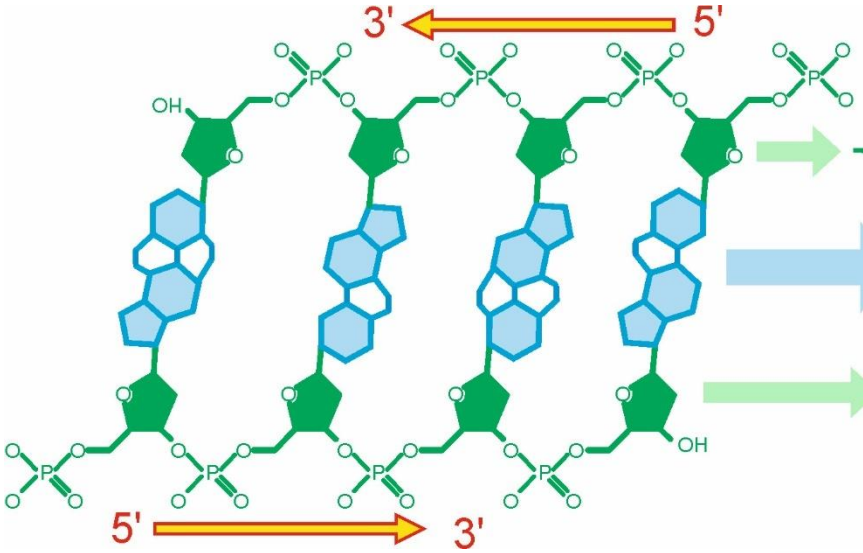
Lambda-repressor-like proteins recognize DNA by hydrogen bonds

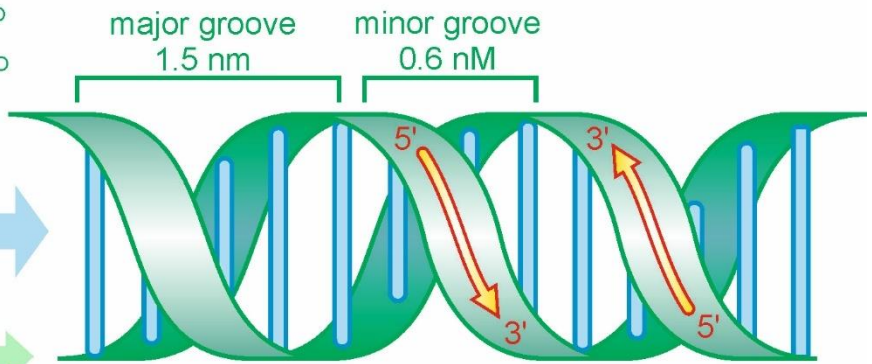


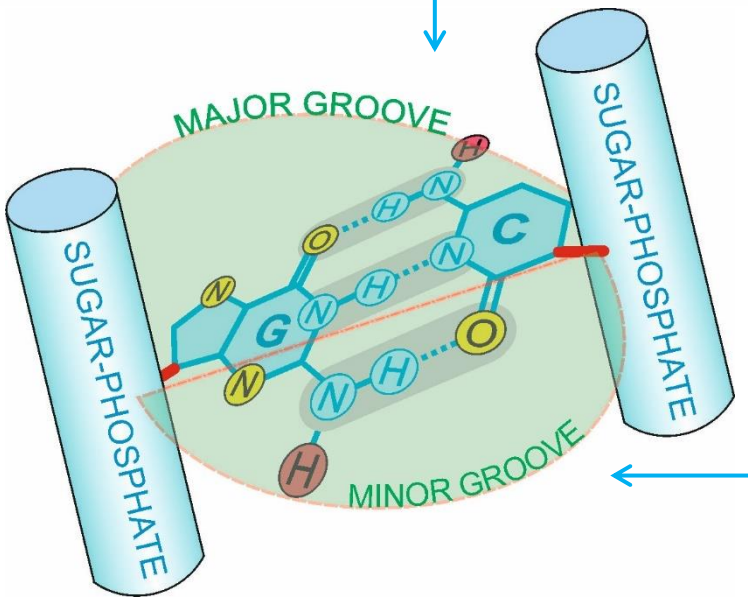
DNA has a double-helix structure

primary structure

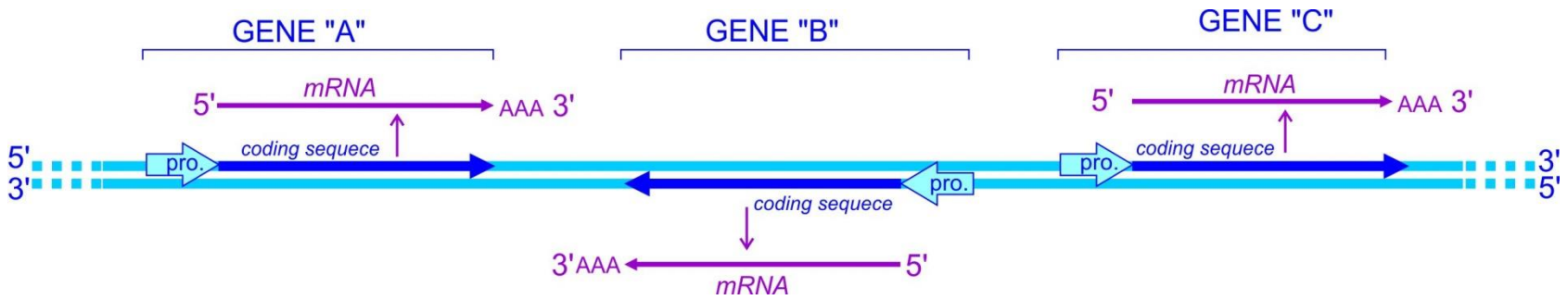
secondary structure



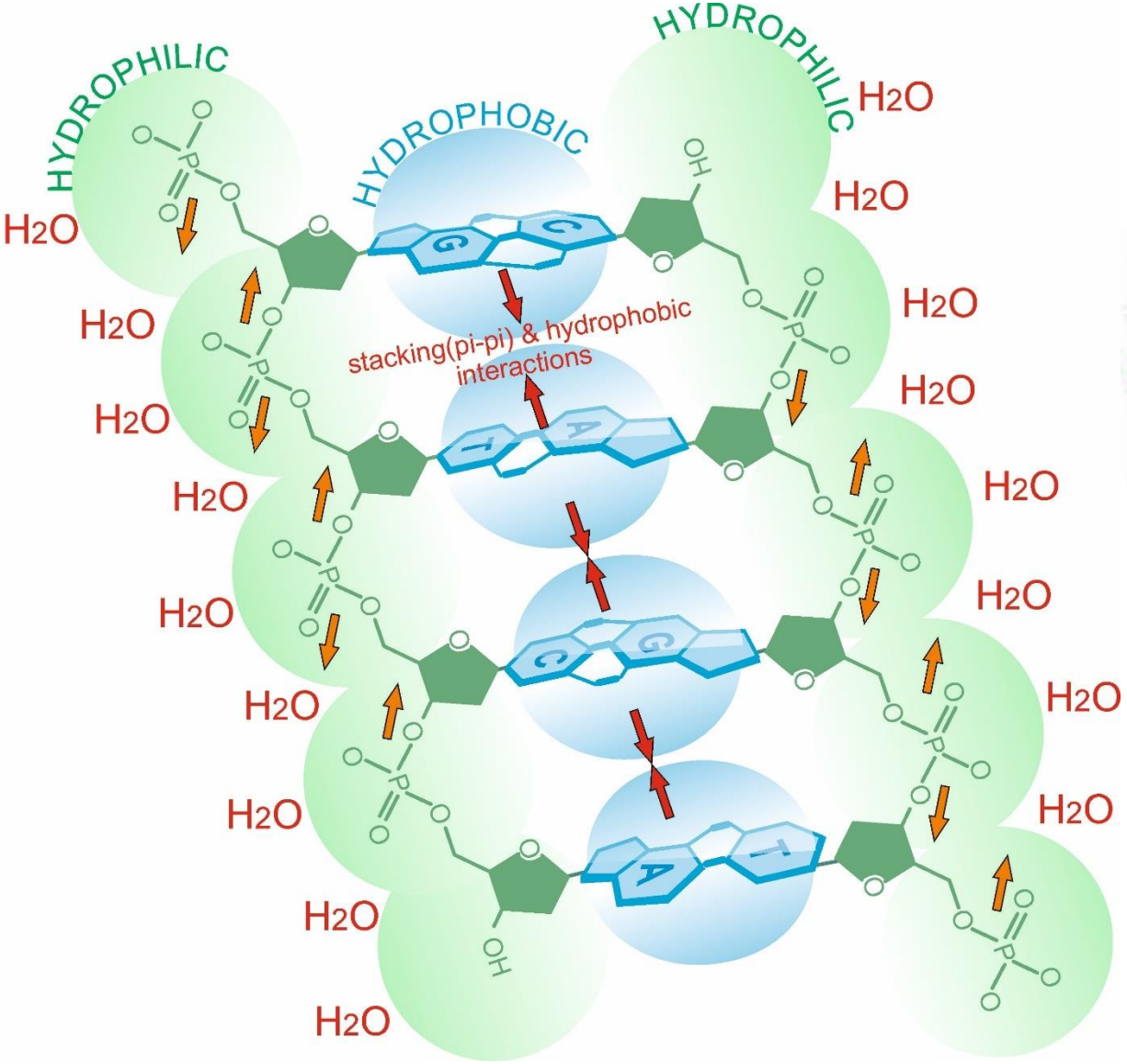


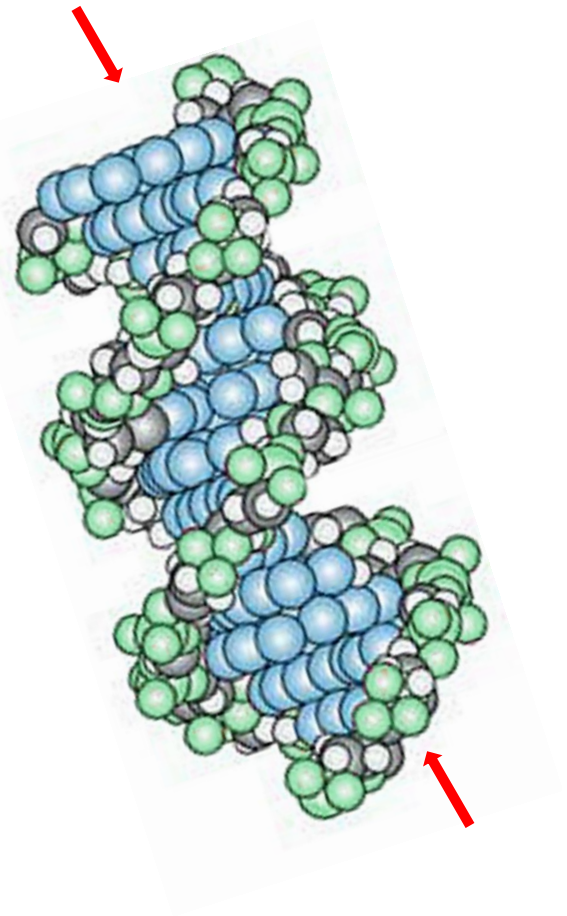


Coding sequences of different genes can be located on both strands of chromatin.

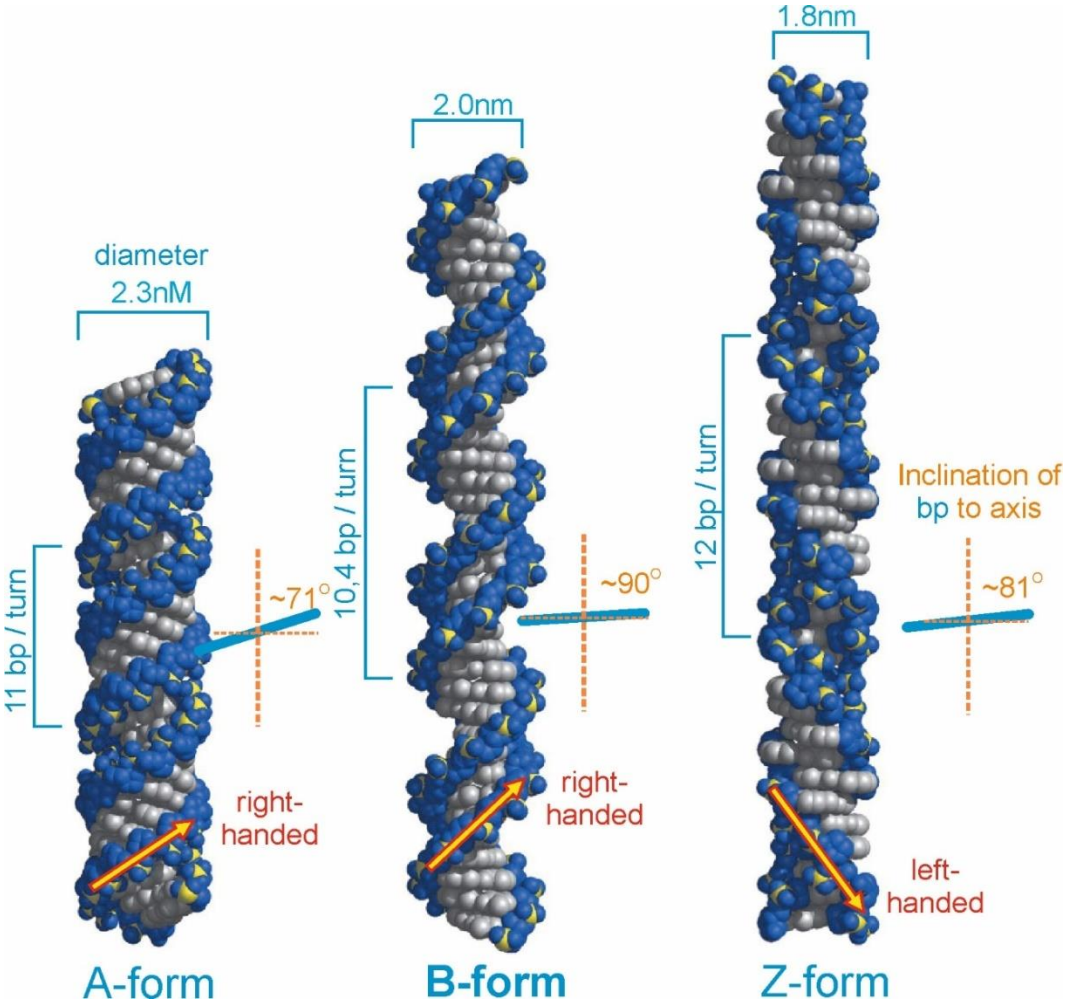


Water environment determines DNA double helix conformation





DNA can adopt three different forms



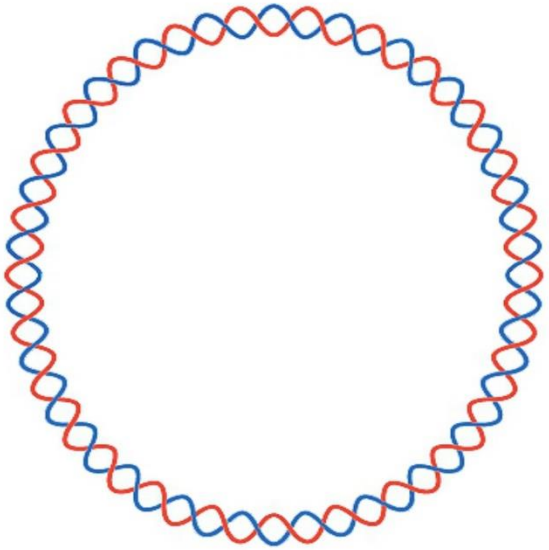
A-form
DNA/RNA, RNA/RNA duplexes
dsDNA under dehydrating cond.

B-form
dsDNA in physiological cond.

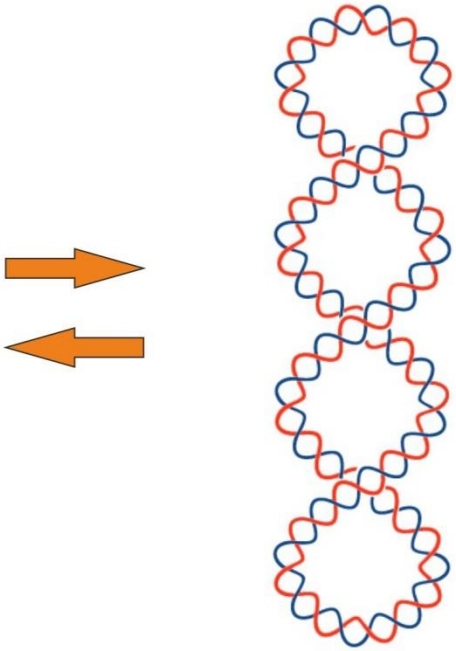
Z–form
pur/pir sequences (GCGCGC)
negatively supercoiled DNA

https://www.youtube.com/watch?v=o_-6JXLYS-k 4.30s

DNA can adopt tertiary structure colled supercoiling



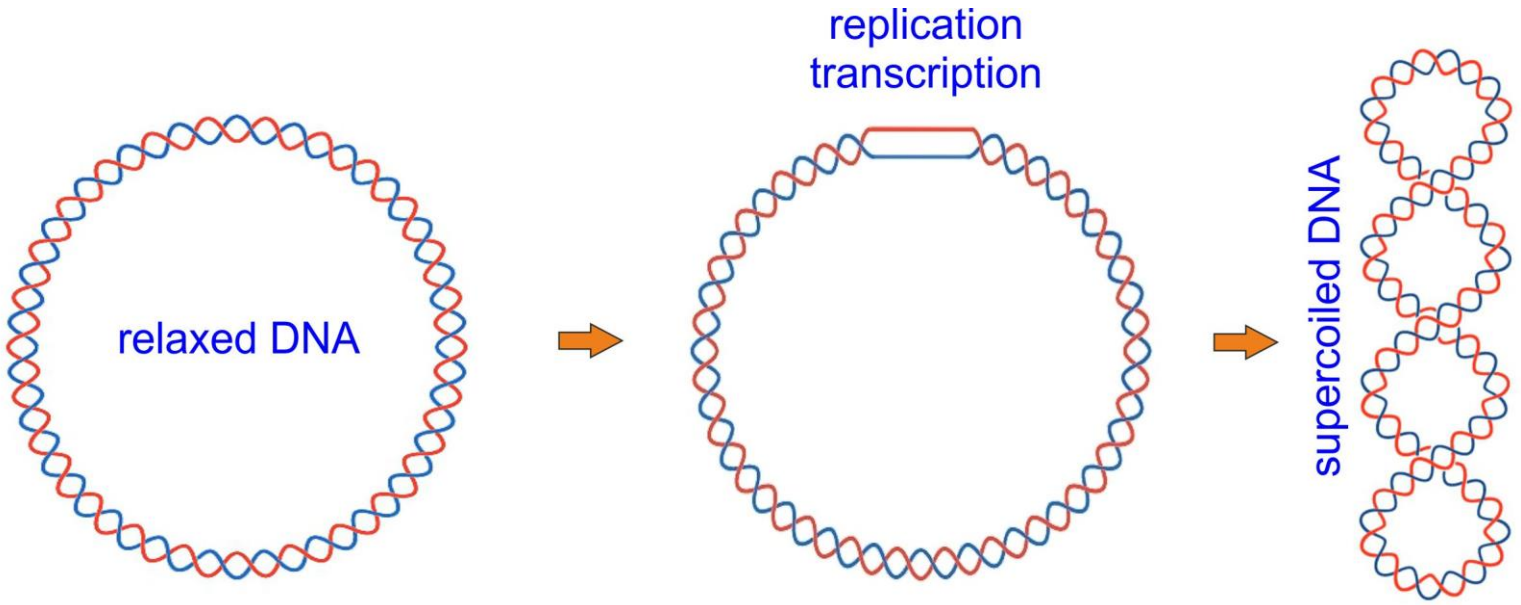
relaxed DNA



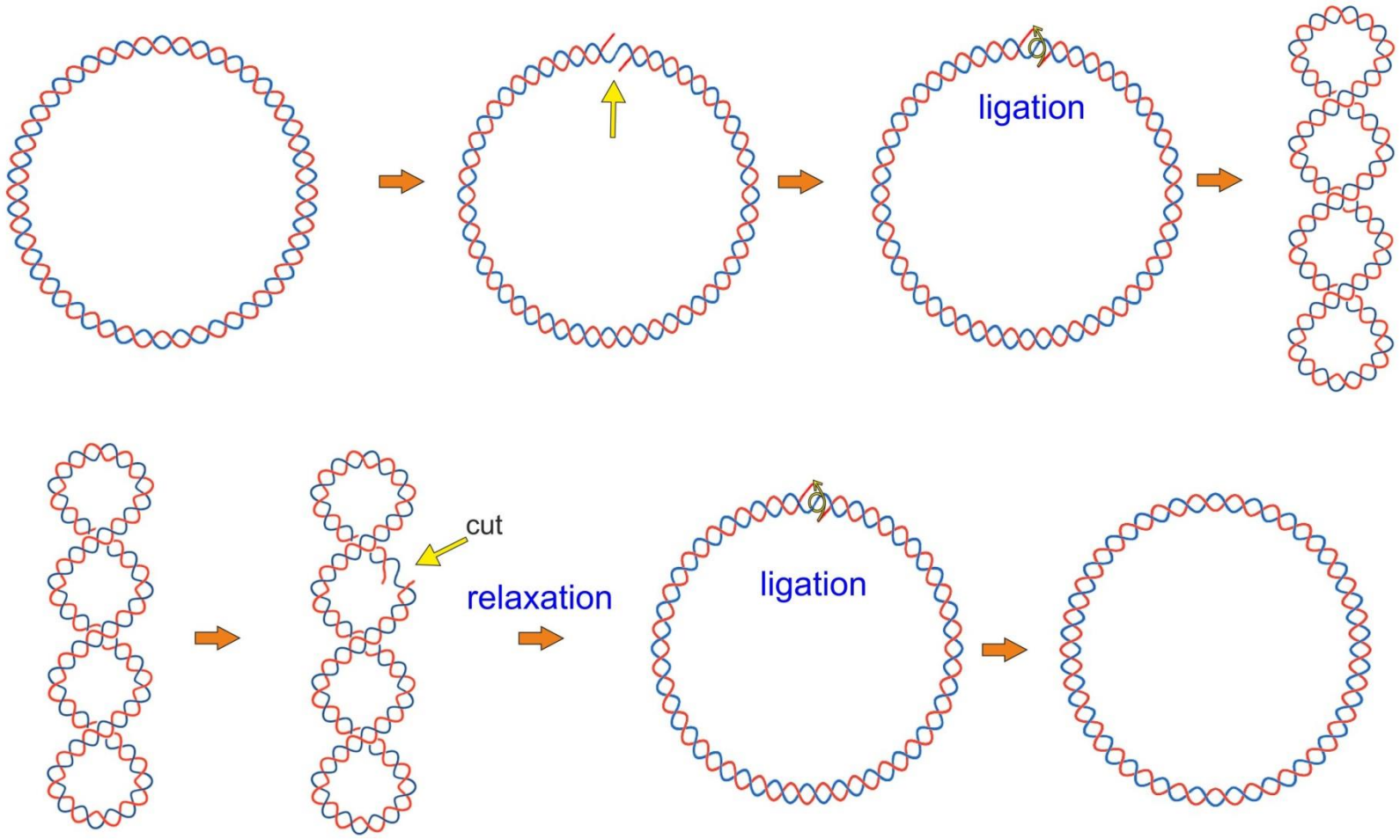
supercoiled DNA

telephone cord

Unwind of DNA during replication or transcription leads to DNA supercoiling

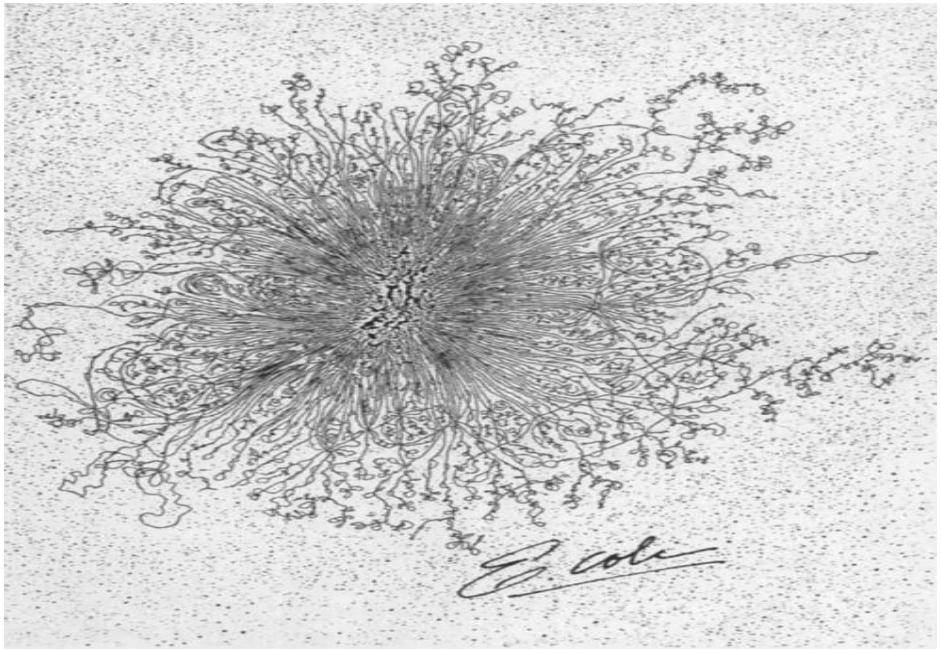


Topoisomerases are enzymes that regulate the tertiary structure of DNA



DNA supercoiling is a way to compact the bacterial chromosome in the cell

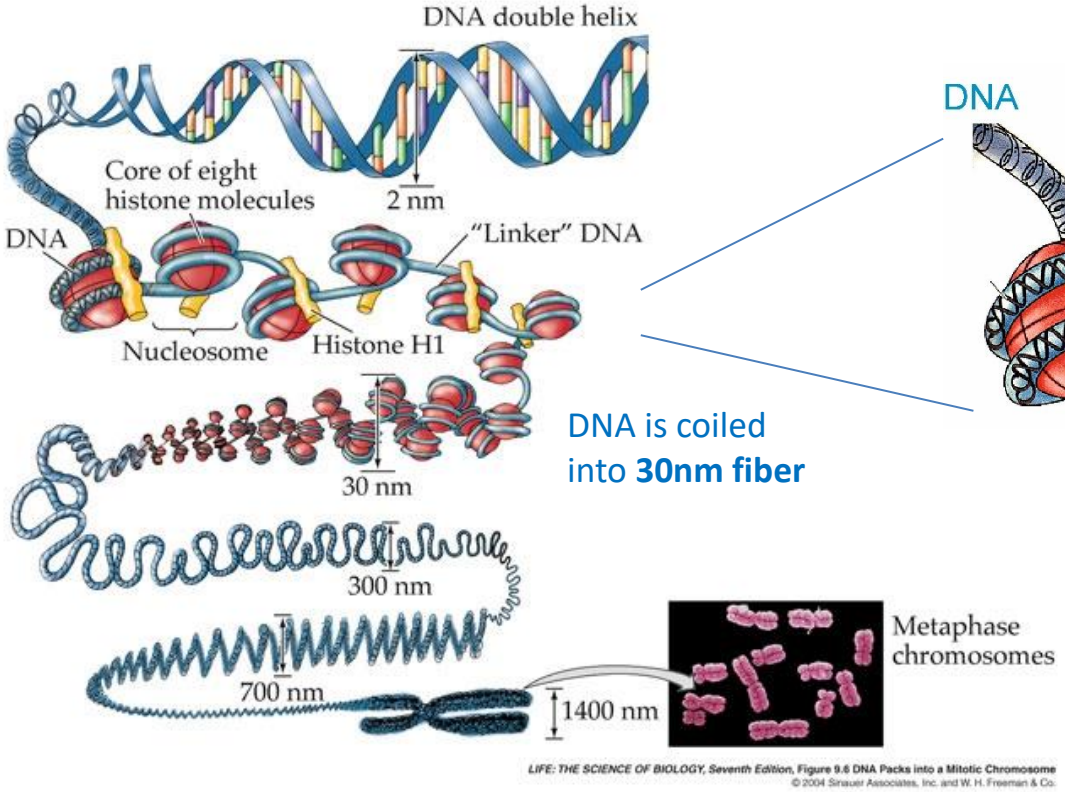
Bacterial genophore

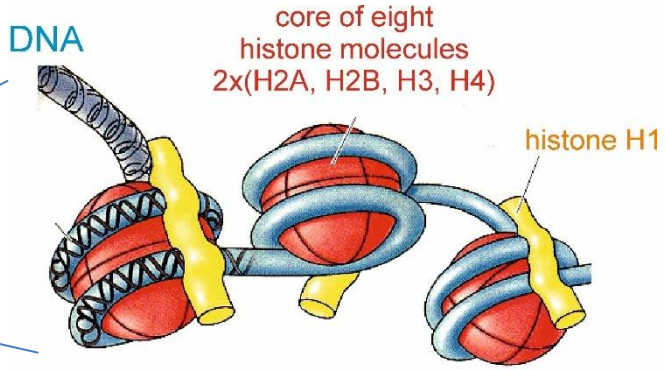


Bacterial DNA 1000x > bacterium diameter

https://www.youtube.com/watch?v=5hwaDamU-jo

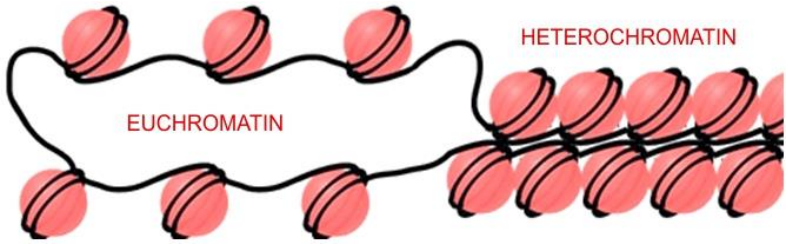
Eucaryotic DNA is wrapped around histones to form nucleosomes





DNA is coiled into **30nm fiber**

Cell diameter - 5 μm
One cell DNA lenght - 3.6 m



interphase chromatin

https://www.youtube.com/watch?v=OjPcT1uUZiE

Replication

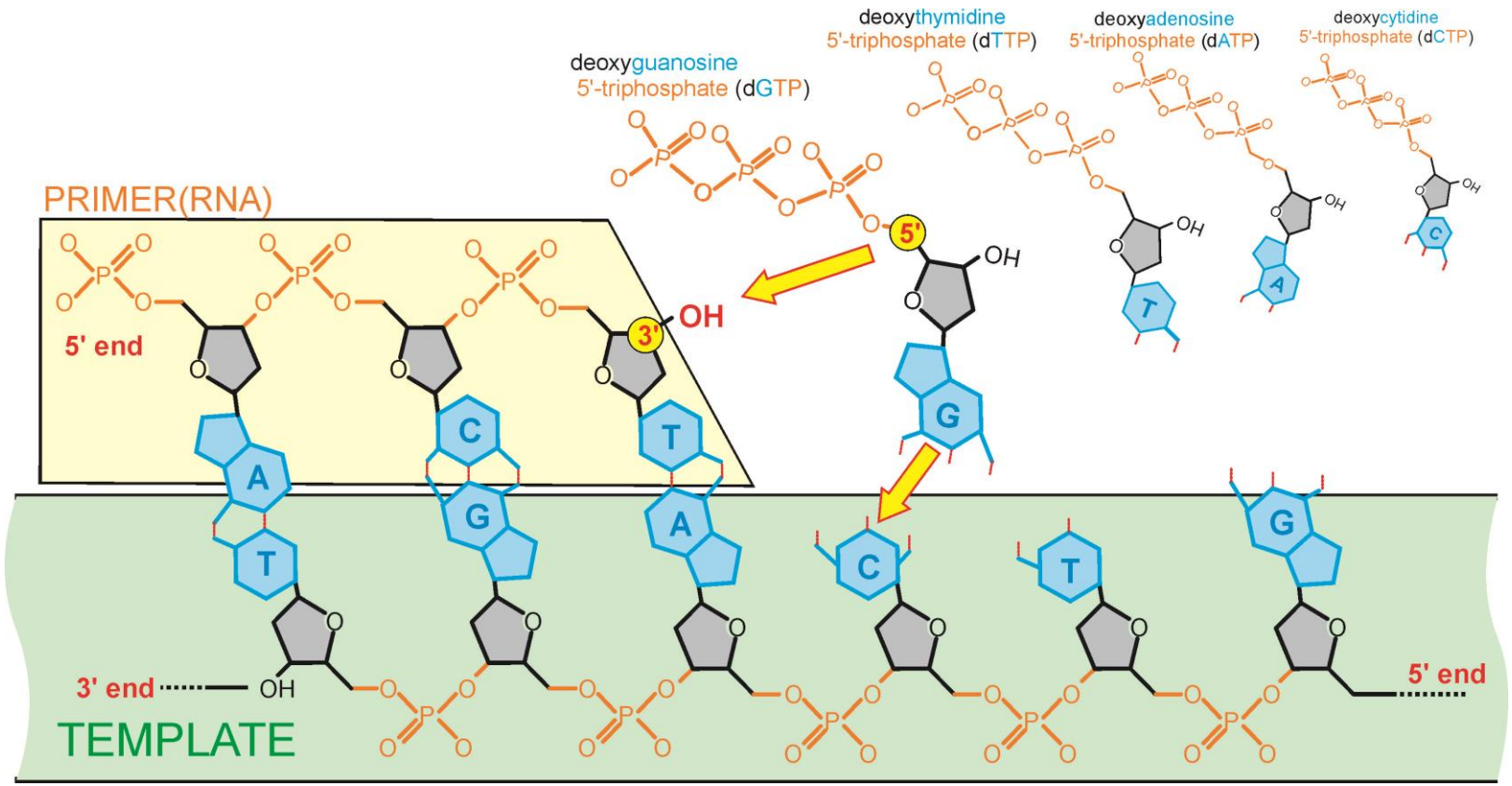
Life is distinguished by the capacity to:

- grow
- metabolize
- respond to stimuli
- adapt
- and **reproduce**

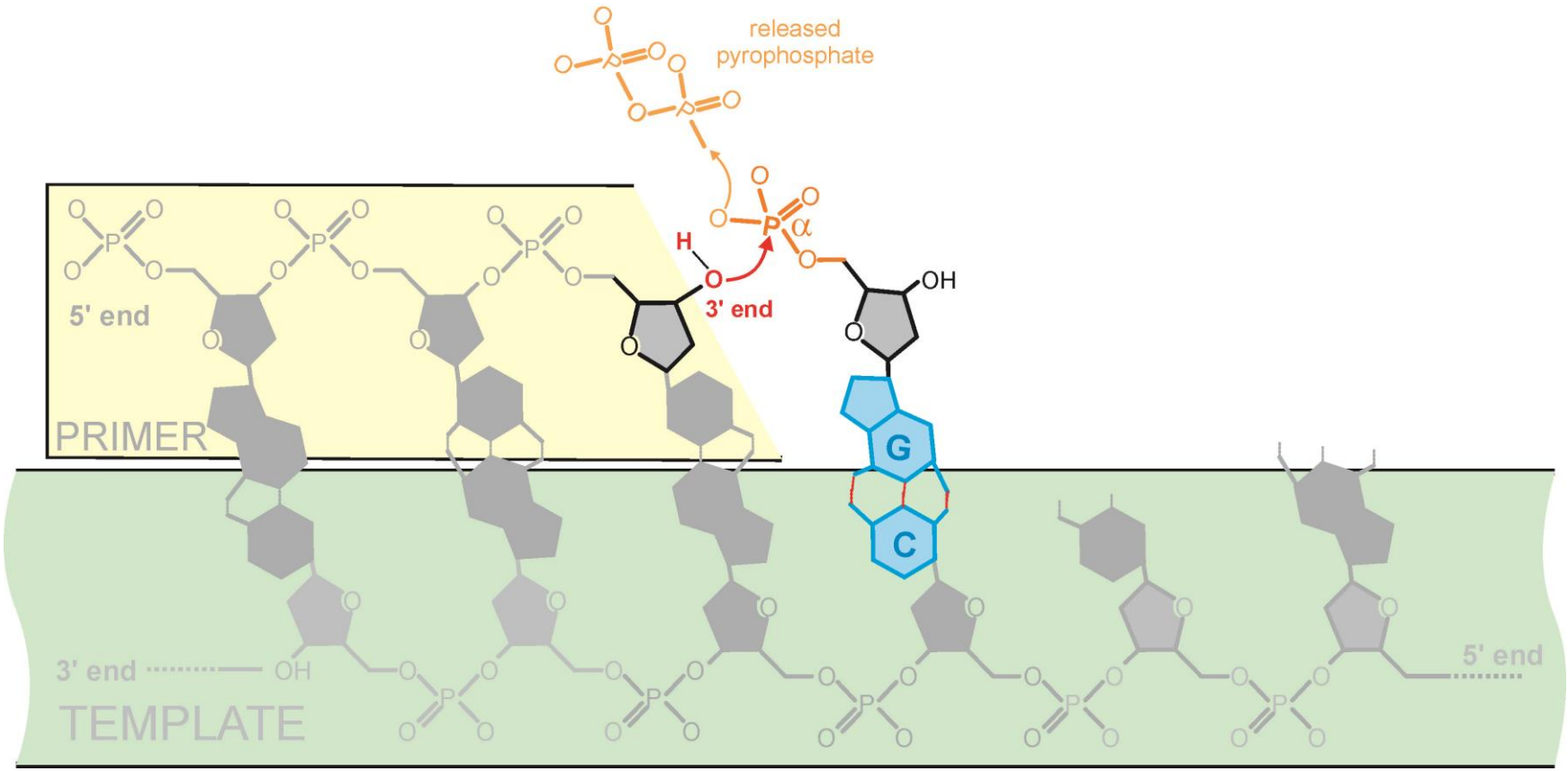
Bacteria have several DNA polymerases

Name	Function	Composition
DNA pol I	**REPLICATION and DNA repair**	**single polypeptide**
DNA pol II	DNA repair	
DNA pol III	**REPLICATION**	**composed of 10 subunits (Polymerase holoenzyme) α subunit – DNA synthesis**
DNA pol IV	DNA repair	
DNA pol V	DNA repair	

DNA polymerase requirement



DNA polymerase catalysis



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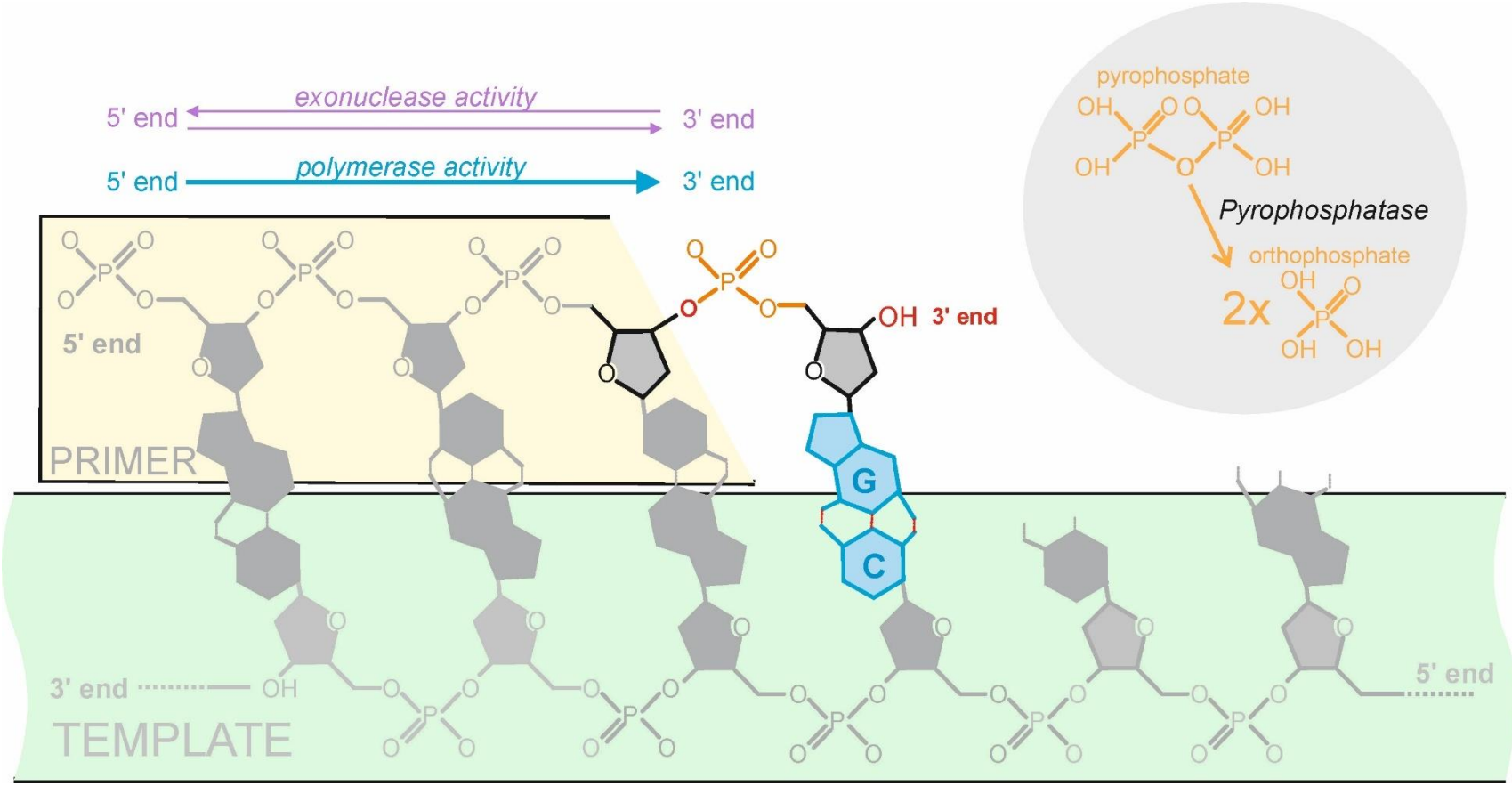
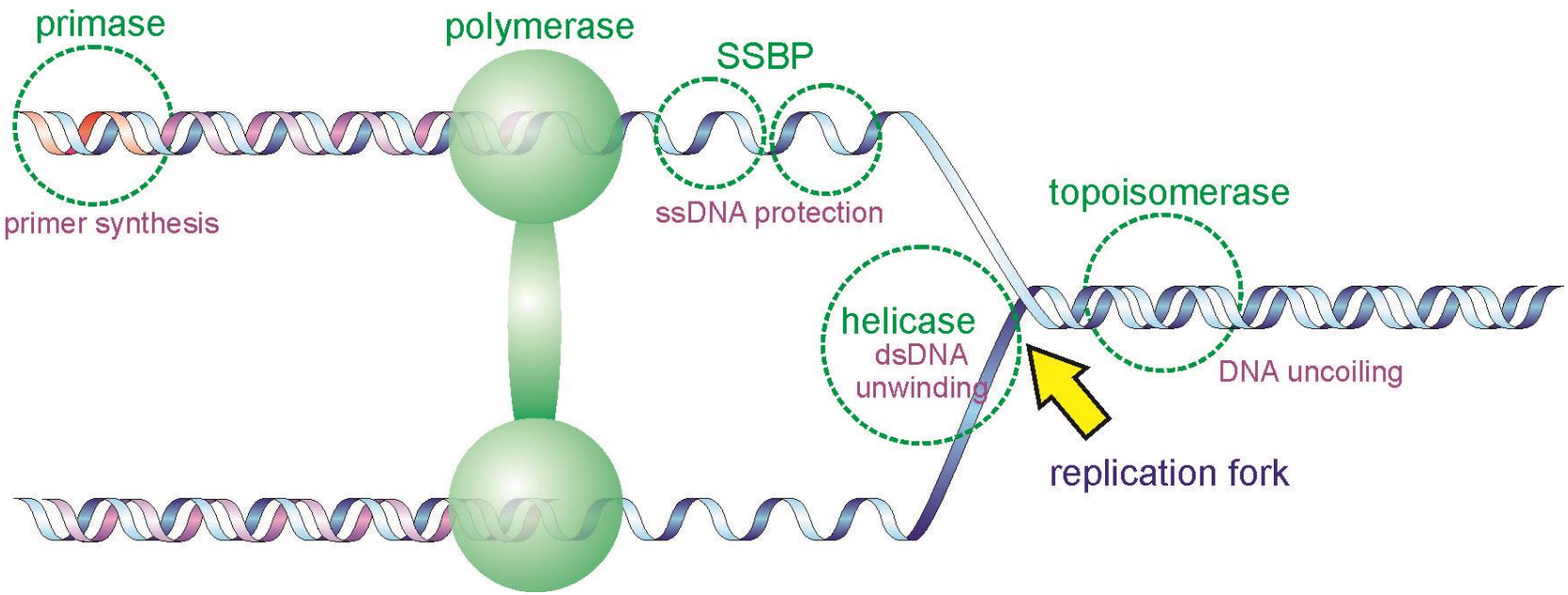
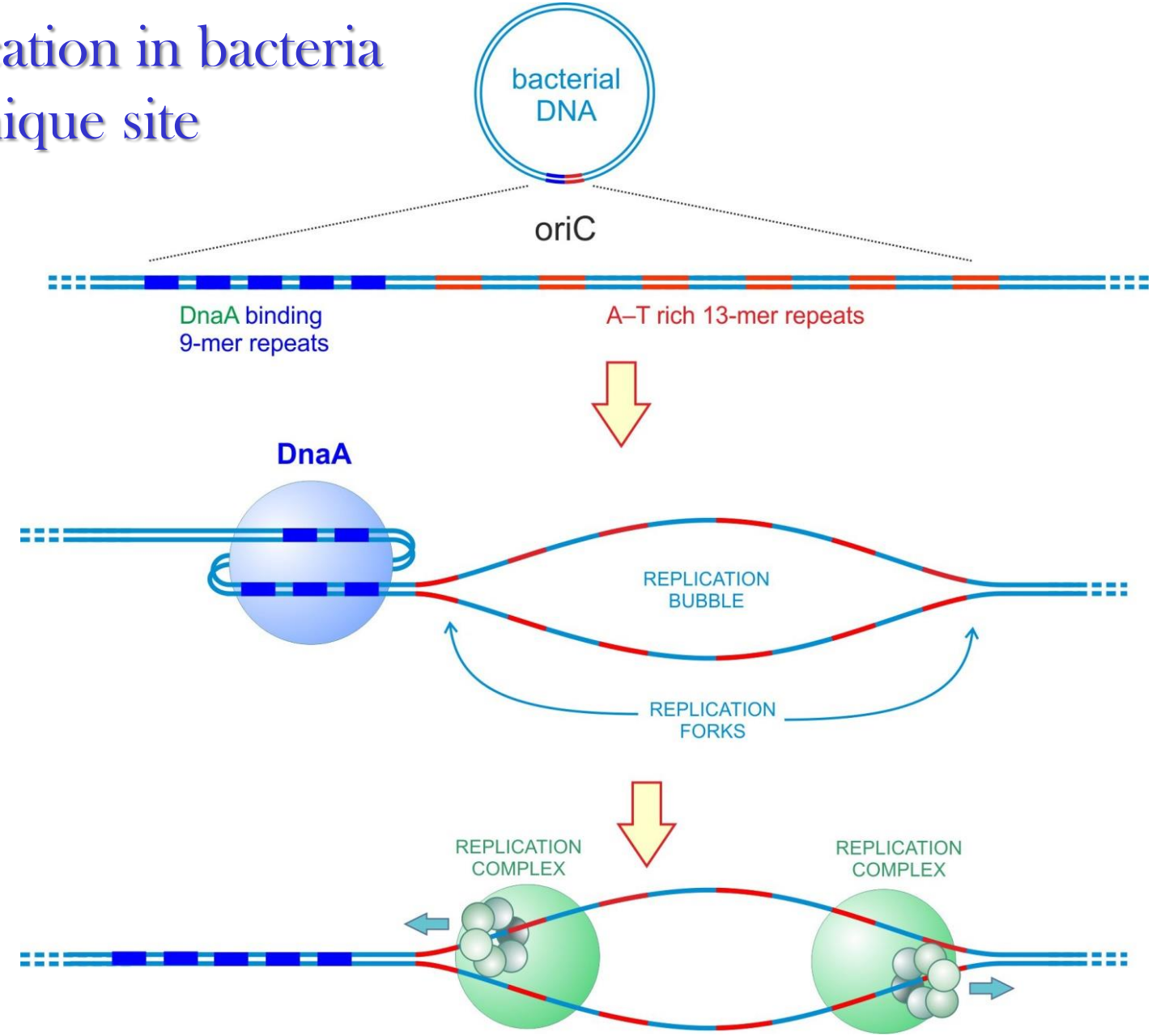


Other enzymes cooperating with DNA polymerase

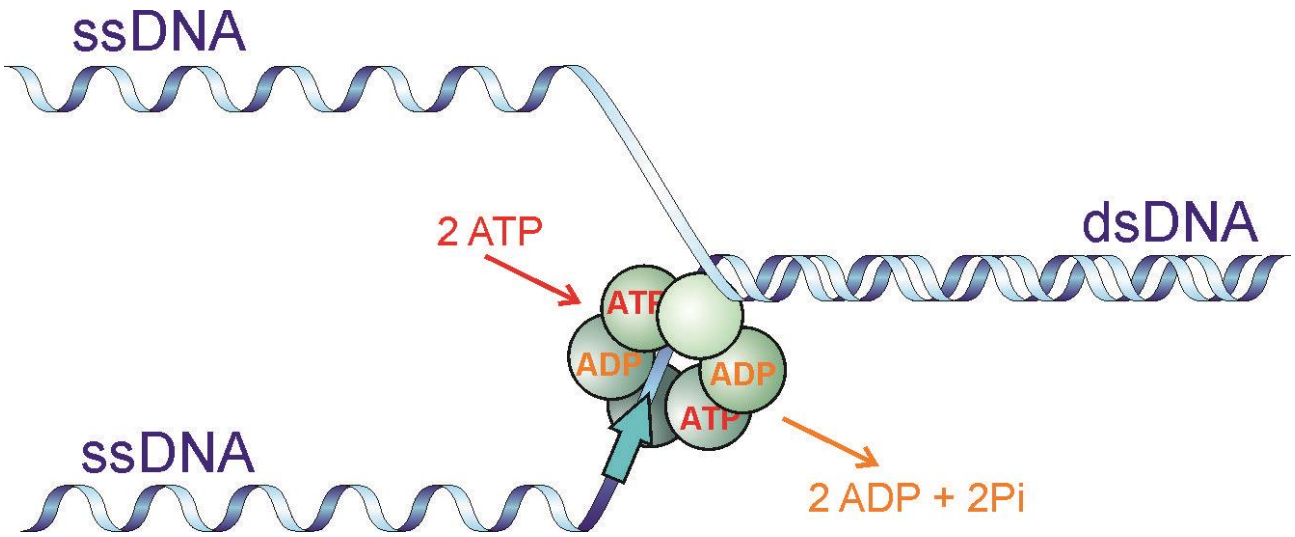


DNA replication in bacteria begins at unique site



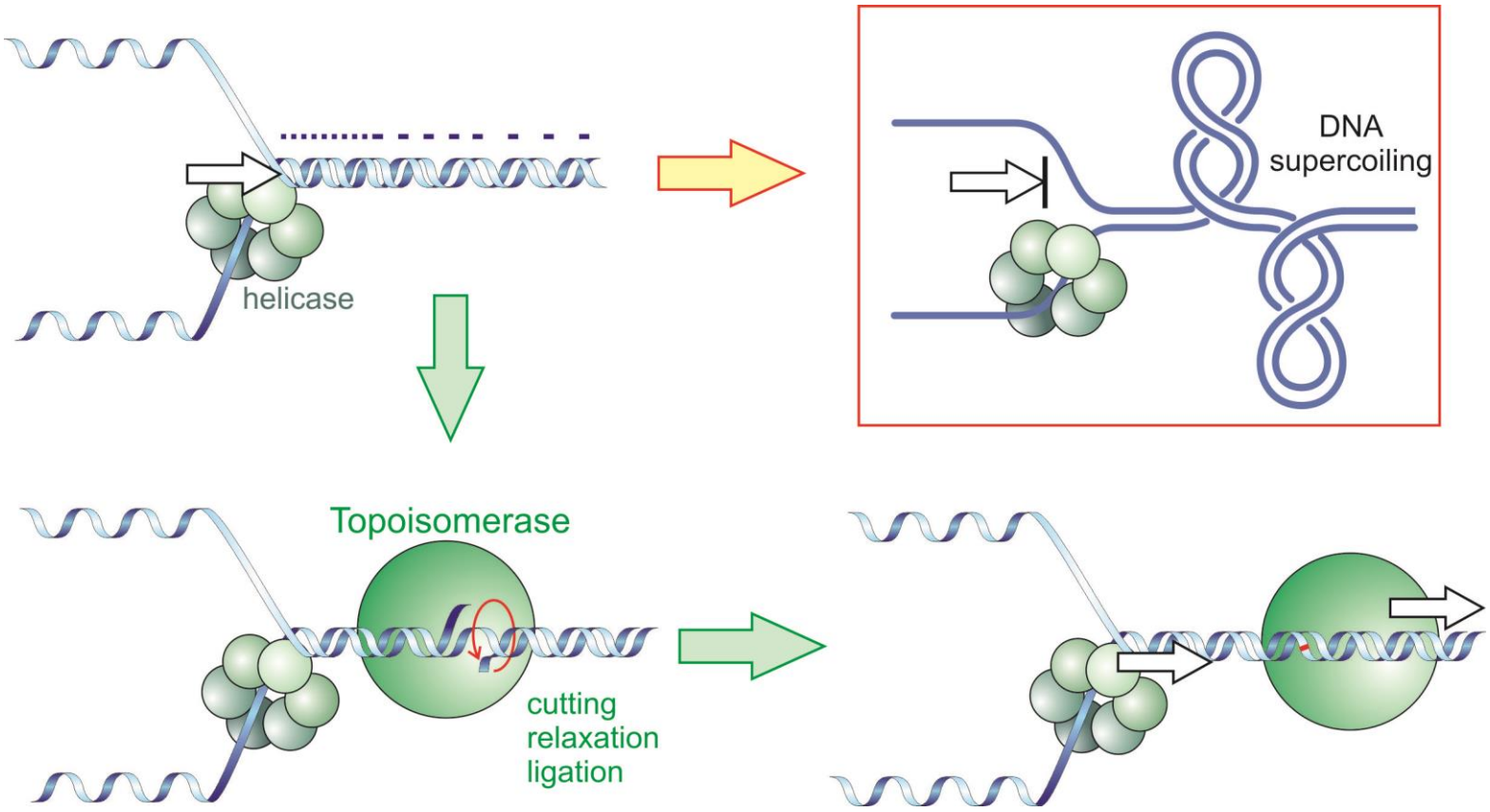
https://www.youtube.com/watch?v=0Ha9nppnwOc
https://www.youtube.com/watch?v=TNKWgcFPHqw

Helicase catalyzes the unwinding and separation of double-stranded DNA



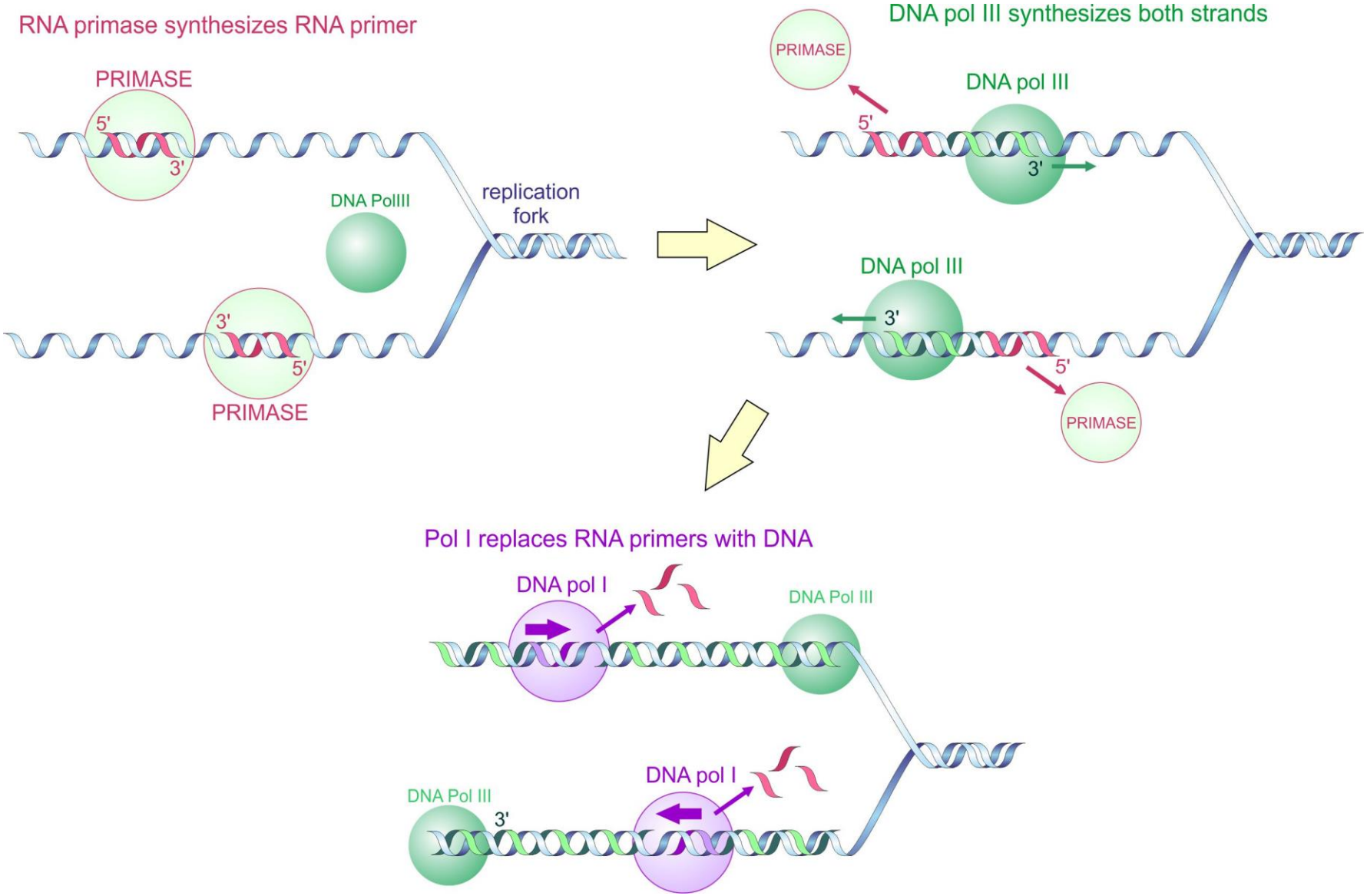
https://www.youtube.com/watch?v=Z9ER-04WEBk

DNA topoisomerase I catalyzes the relaxation of supercoiled DNA ahead of a replication fork

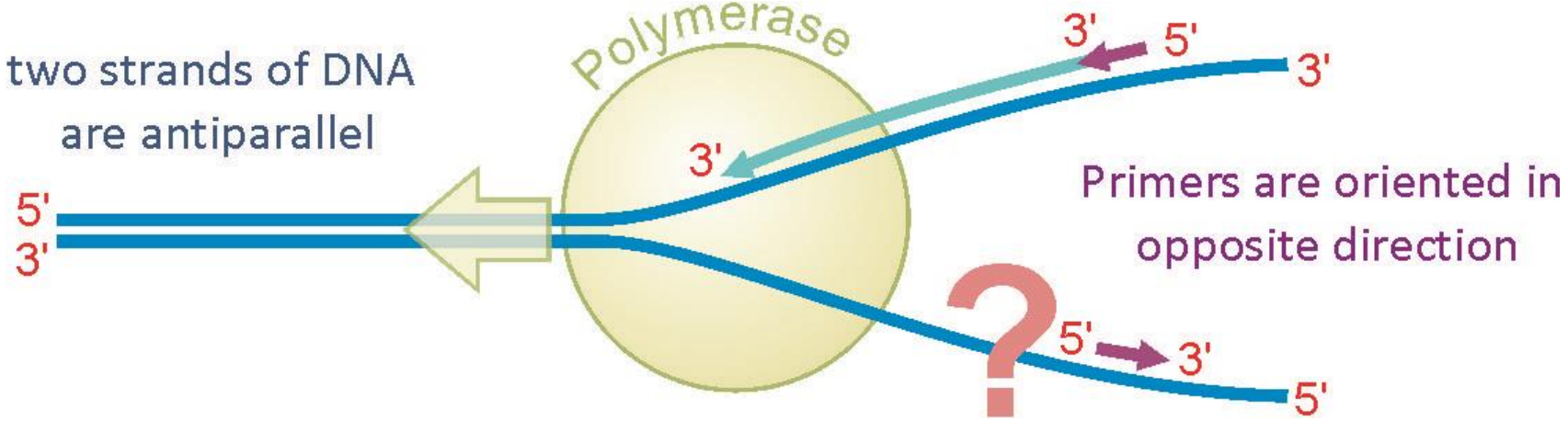


https://www.youtube.com/watch?v=MTZVYL9eBxQ

RNA primase is an enzyme that synthesizes primer for replication to start.



One strand is made continuously and other strand is synthesized in fragments



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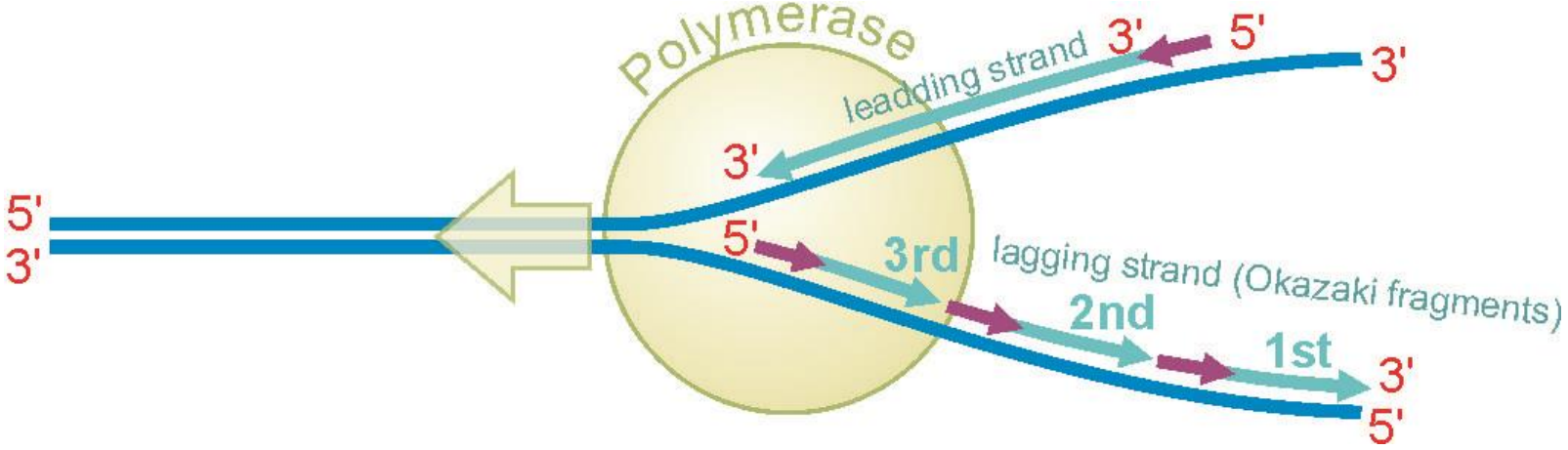
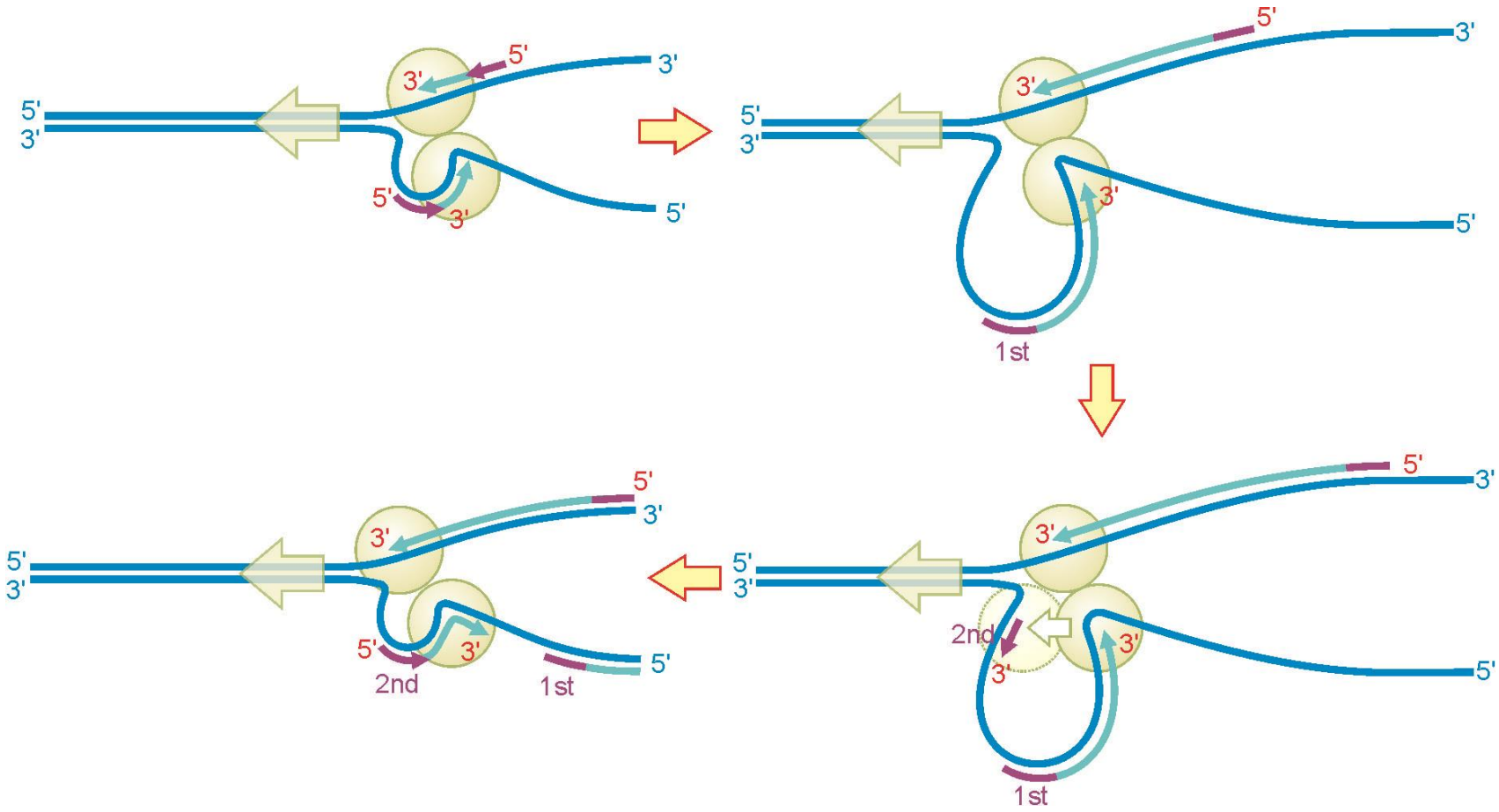
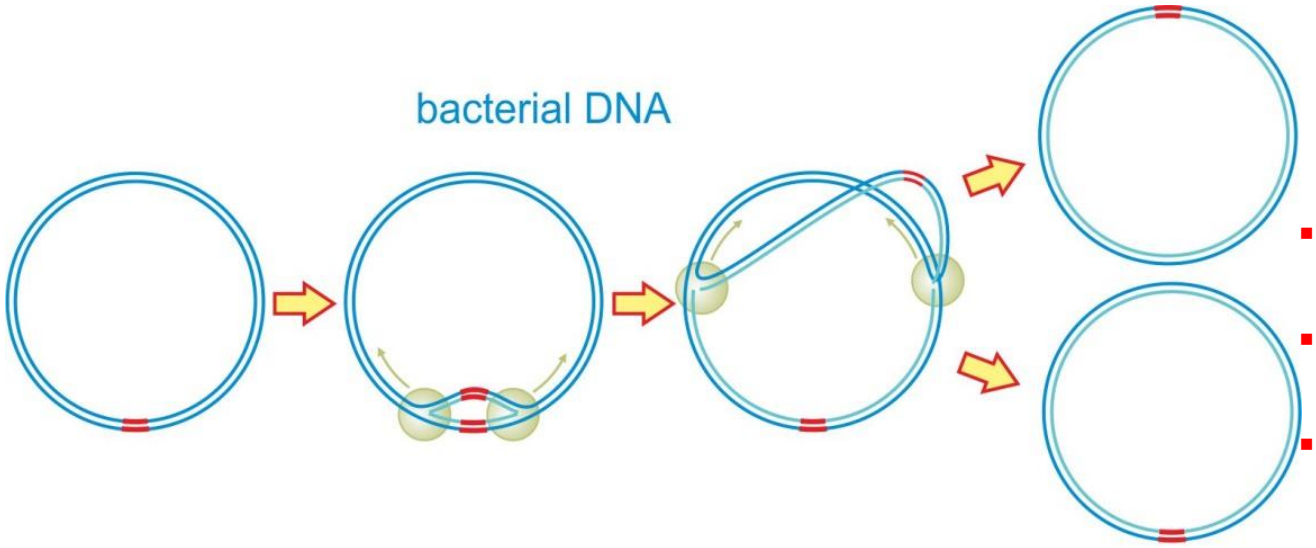


The synthesis of lagging-strand

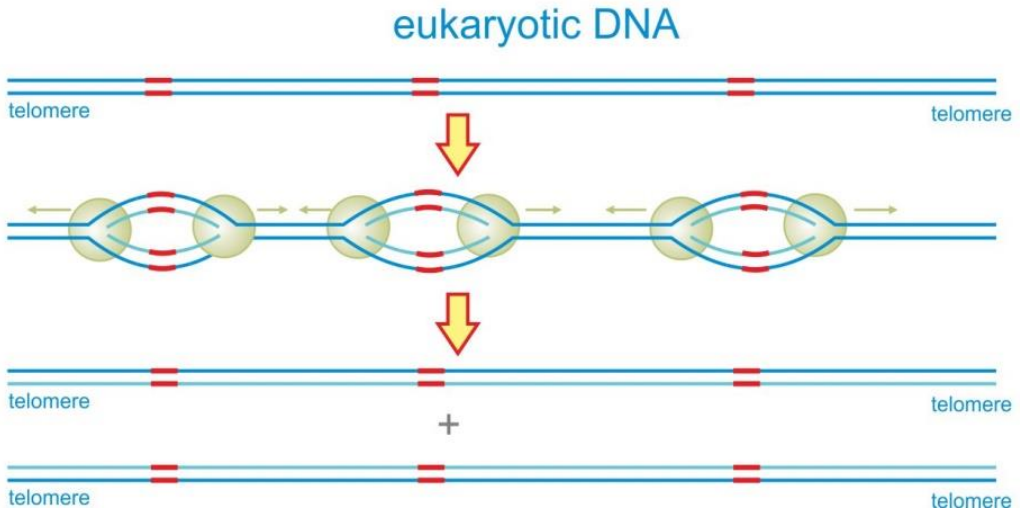


https://www.youtube.com/watch?v=QMX7IpME7X8
https://www.youtube.com/watch?v=5VefaI0LrgE

Differences between prokaryotic and eukaryotic replication



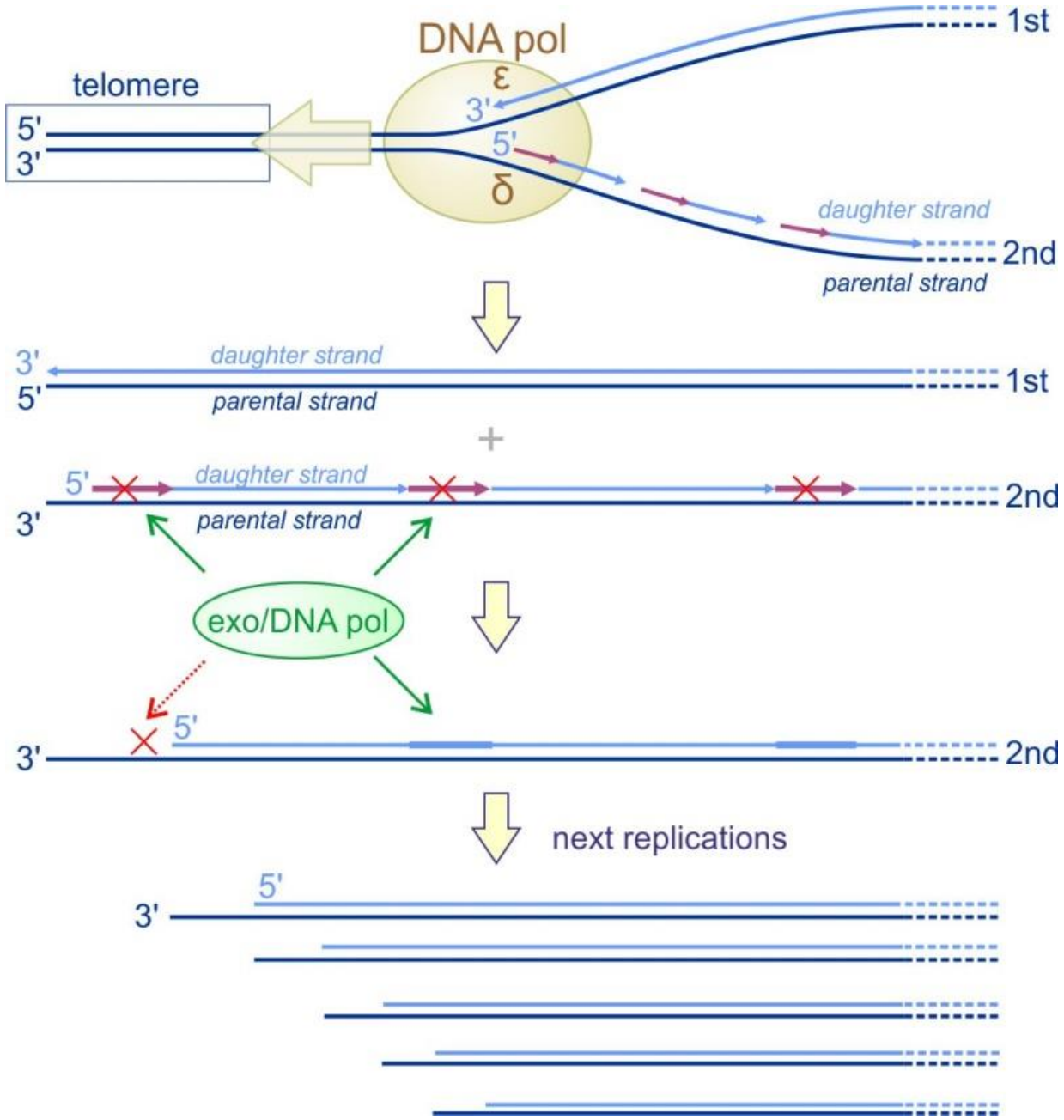
- Circular chromosome (**genophore**)
- Size of genome - about 4.6 million bp
- Single origin of replication (replication bubble)



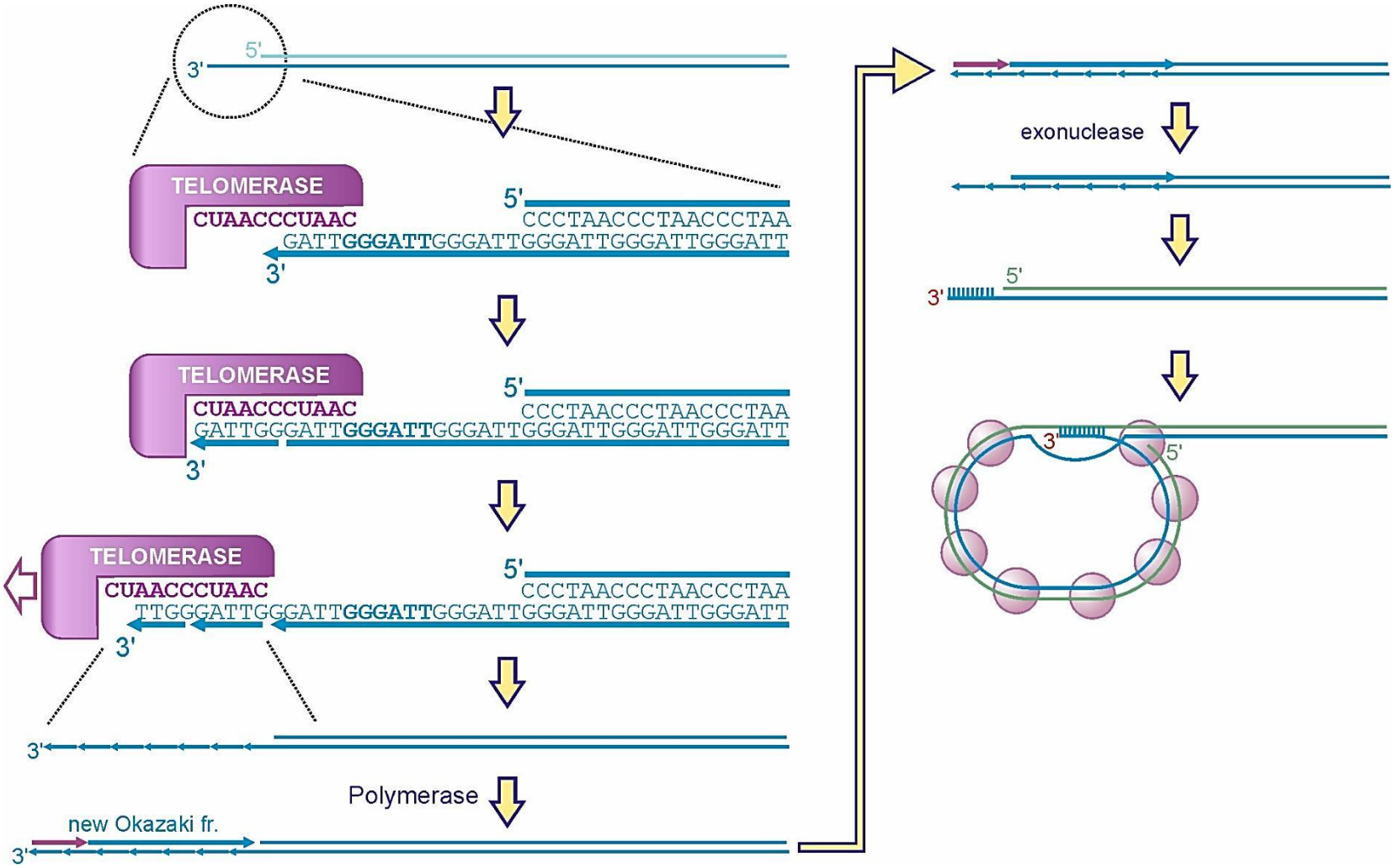
- Size of genome - about 6 billion bp
- multiple origin of replication (several hundred/chromosome)
- Linear chromosome with ends

Semiconservative replication produce two copies that each contained one of the original strands and one new strand

During replication telomeres are shortened



Telomerase reconstructs the telomeres



https://www.youtube.com/watch?v=i6nE6gUp2cw