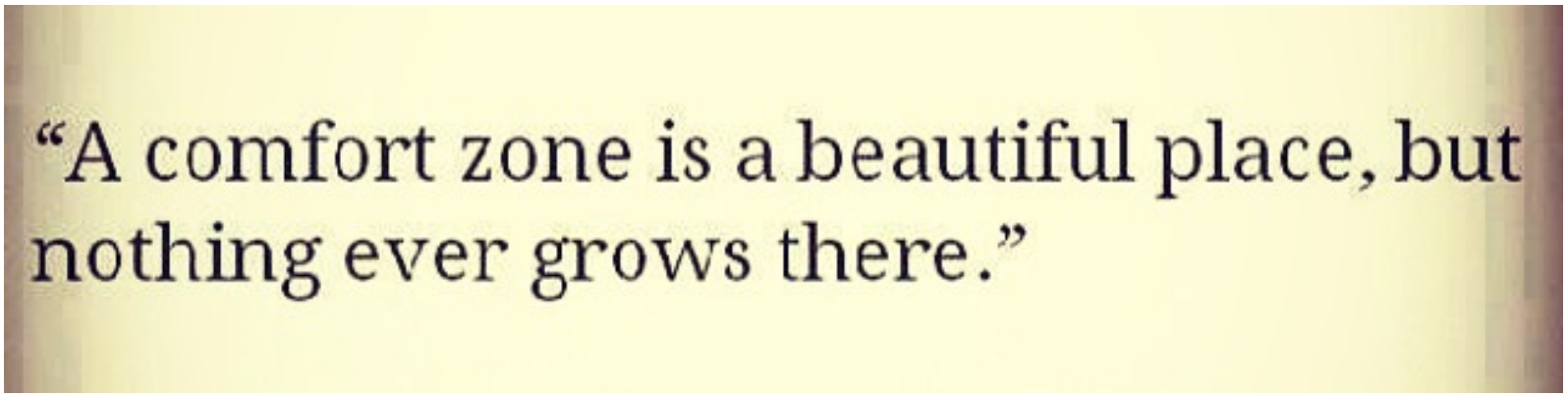
Lecture 13:

DNA replication in prokaryotes

The elements of DNA replication

Course 371

Lessons for life



AIMS

- Introduce the elements needed to replicate DNA.
- Introduce the chemistry of adding new DNA building block and form a new strand of DNA.
- Introduce the proteins and enzymes involved in the replication of DNA.
- Introduce the function of each enzyme and protein in the replication of DNA.

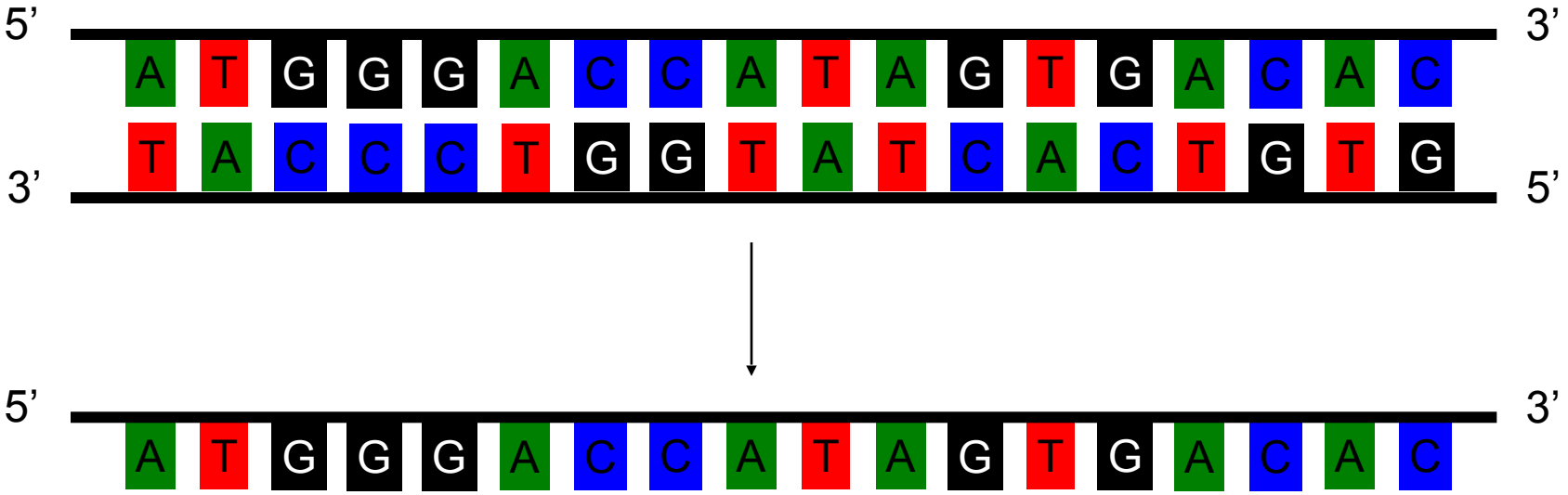
Replicating DNA

- What do we need to replicate DNA?
    1. DNA template.
    2. Building block of DNA.
    3. Builders (proteins and enzymes).
    4. 3'OH (primer).

We will go over each enzyme and its function but let's go over each one separately first.

DNA replication - What we need?

1. DNA template

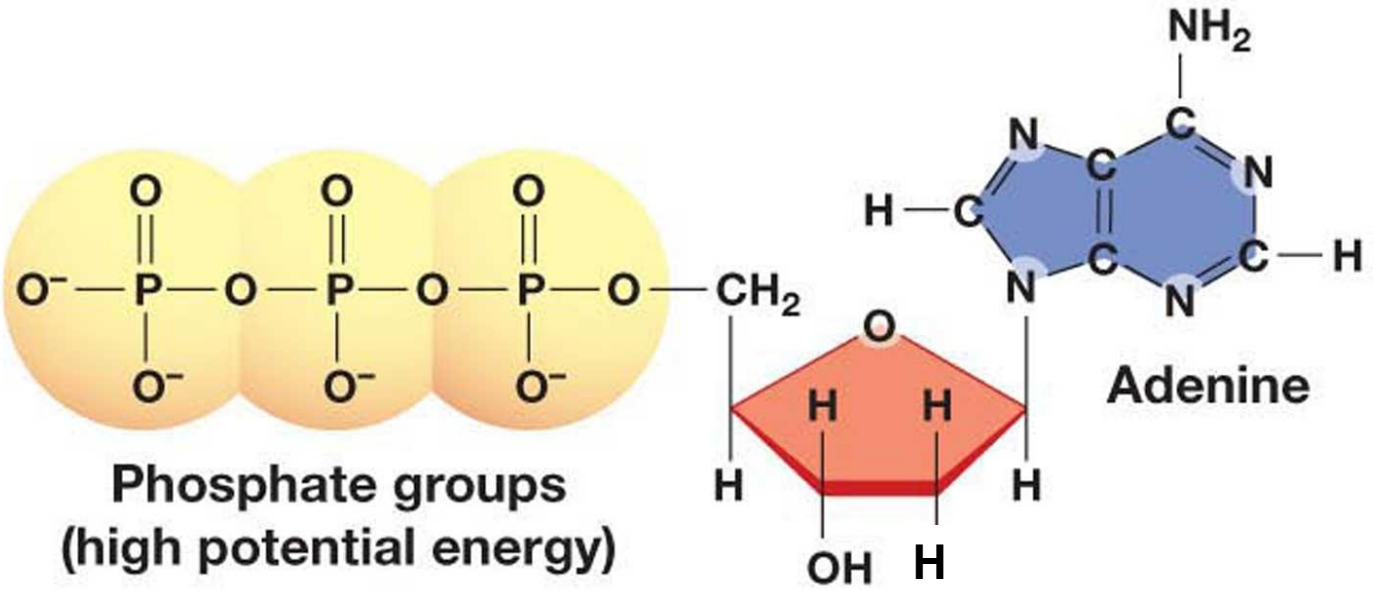


Each strand serves as a **template** for replication.

Remember complementary base-pairing!

DNA replication - What we need?

2. Deoxyribonucleoside triphosphate (dNTP)



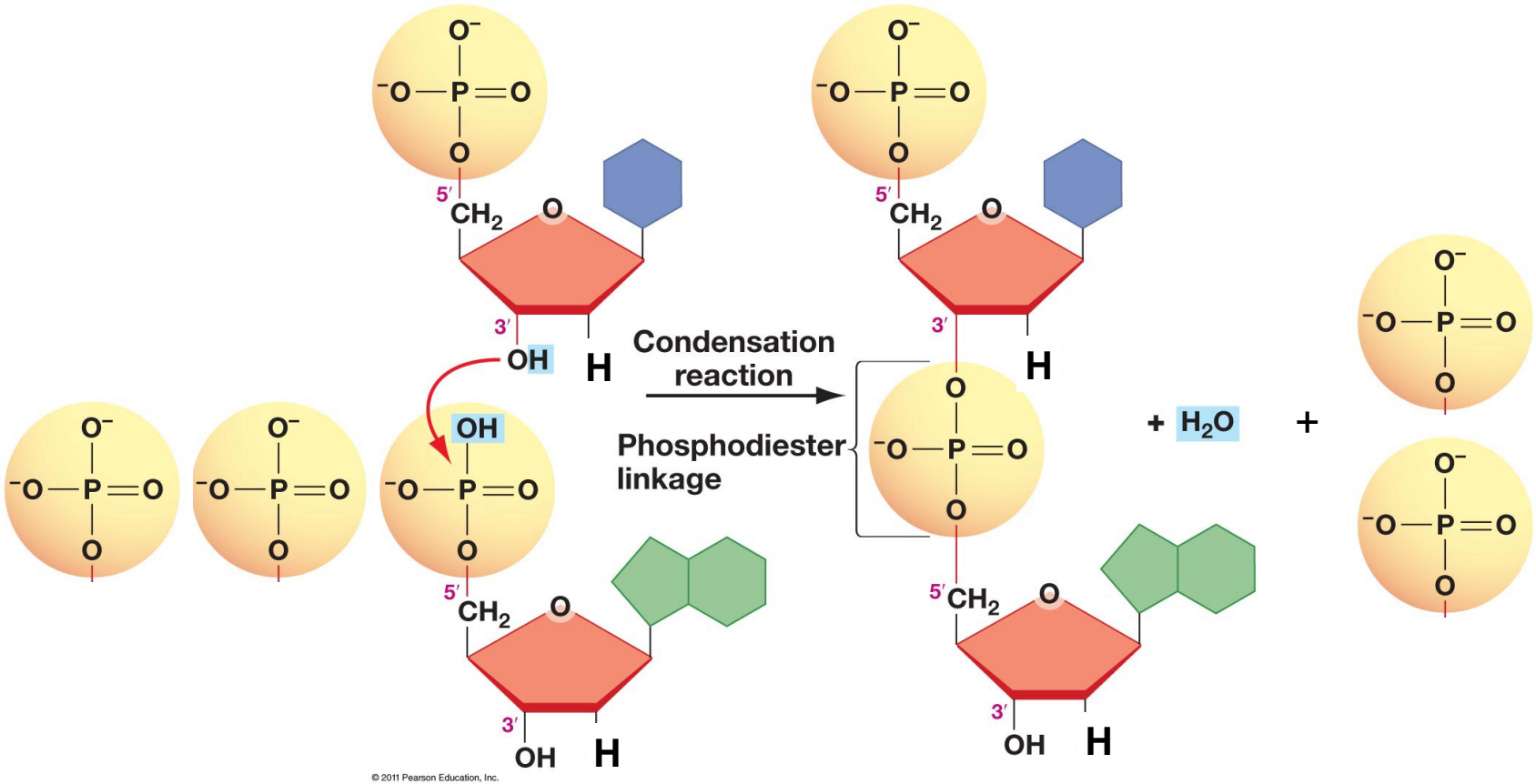
Four dNTPs serve as the building blocks of DNA (dATP, dTTP, dGTP, dCTP)

Remember **Nucleotides!**

DNA replication - What we need?

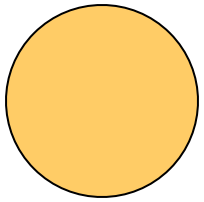
Why triphosphate?

For the energy required to for the phosphodiester bond

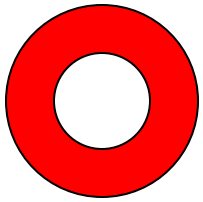


DNA replication - What we need?

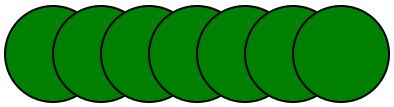
3. Builders (proteins and enzymes)



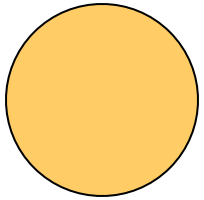
DNA polymerase I



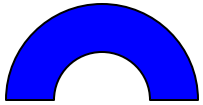
Helicase (DnaB)



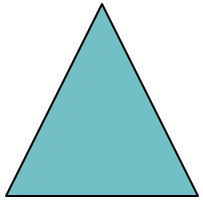
Single strand DNA binding proteins (SSB)



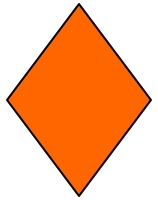
DNA polymerase III



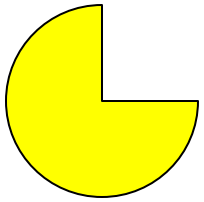
Gyrase



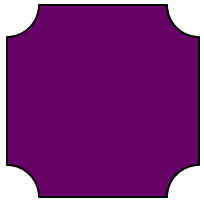
Initiation protein (DnaA)



Helicase loader (DnaC)



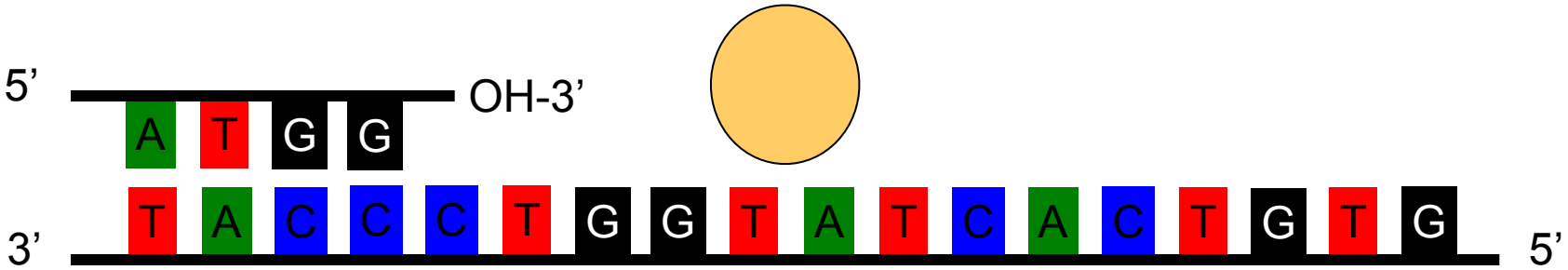
primase



Ligase

DNA replication - What we need?

4. Primers



In order for the DNA copying machine to work and add nucleotides,

a 3’-OH needs to be available to form a phosphodiester bond!

DNA replication - What we need?

What is a Primer?

Replicating DNA

- DNA is available in the cell.
- dNTPs are in the cell.
- Copying DNA done by enzymes with the help of proteins.
- 3’-OH is in the nucleotide structure.

We will go over the enzymes and their functions

Replication enzymes/proteins

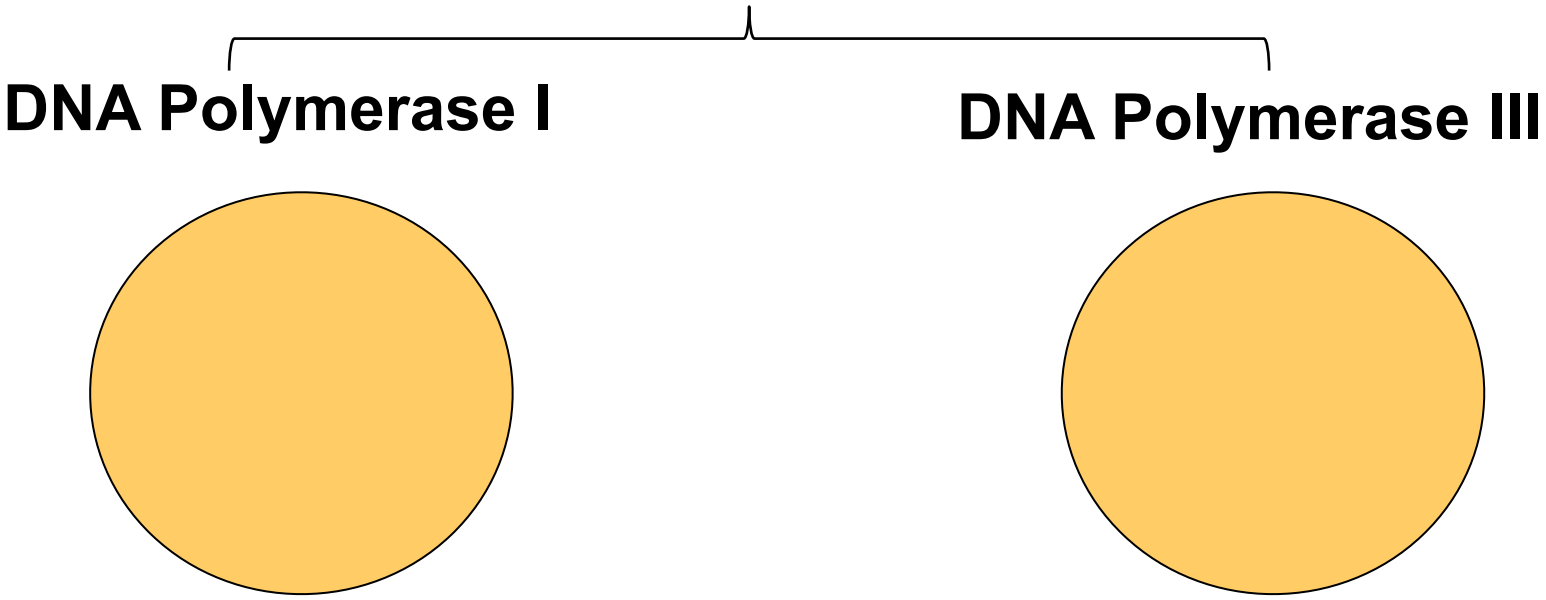
1. **DNA polymerase (DNA Pol)**
    - It is the DNA copier.
    - Uses the dNTPs (DNA building blocks) to make a complementary strand to the template.

Replication enzymes/proteins

1. **DNA polymerase (DNA Pol)**
    - Uses the available 3'-OH of a previous nucleotide and 5'phsphate from dNTP to form a phosphodiester bond.
    - Each time DNA Pol finds the correct complementary dNTP and catalyzes the reaction linking the new nucleotide.
    - **Remember DNA Pol needs 3'-OH**

Replication enzymes/proteins

DNA polymerase (DNA Pol)



Replication enzymes/proteins

1. DNA polymerase (DNA Pol)

DNA Polymerase I

DNA Polymerase III

1. Replicates DNA 5'➔3'.

2. Exonuclease activity 3'➔5' (when adding a wrong nucleotide can go back step(s) and remove them). This is called **Proofreading.**

3. Exonuclease activity 5'➔3' (if finds nucleotides in its way removes them.

A bit of review

DNA Polymerase Exonuclease activity

- **Nuclease:** an enzyme that cuts (digest) nucleotide(s).
- **Exo:** at the ends of a DNA molecule.
- **Endo:** in the middle of a DNA molecule.
- **Exonuclease:** ability to remove nucleotide(s) at the end of a molecule.
-

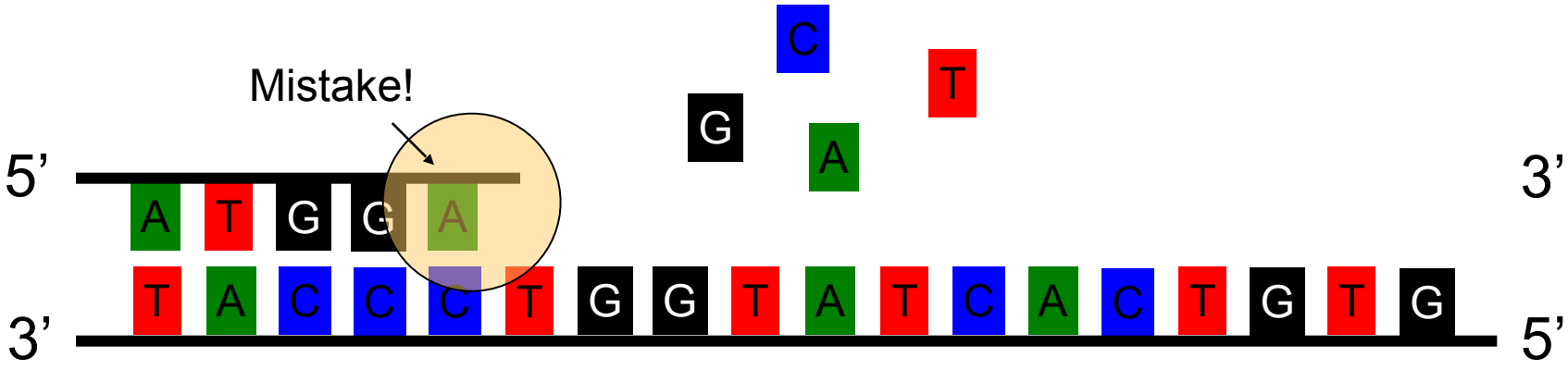
A bit of review

DNA Polymerase Exonuclease activity

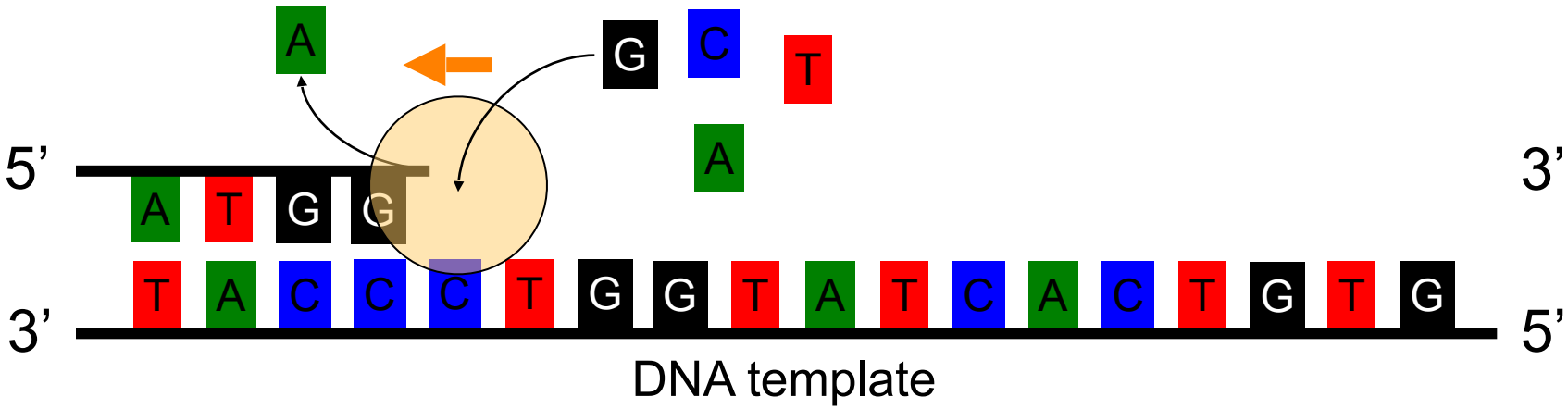
- **DNA Pol I:**
  - 3'→5' exonuclease activity
  - 5'→3' exonuclease activity
- **DNA Pol III:**
  - 3'→5' exonuclease activity

A bit of review

DNA Polymerase 3'→5' Exonuclease activity

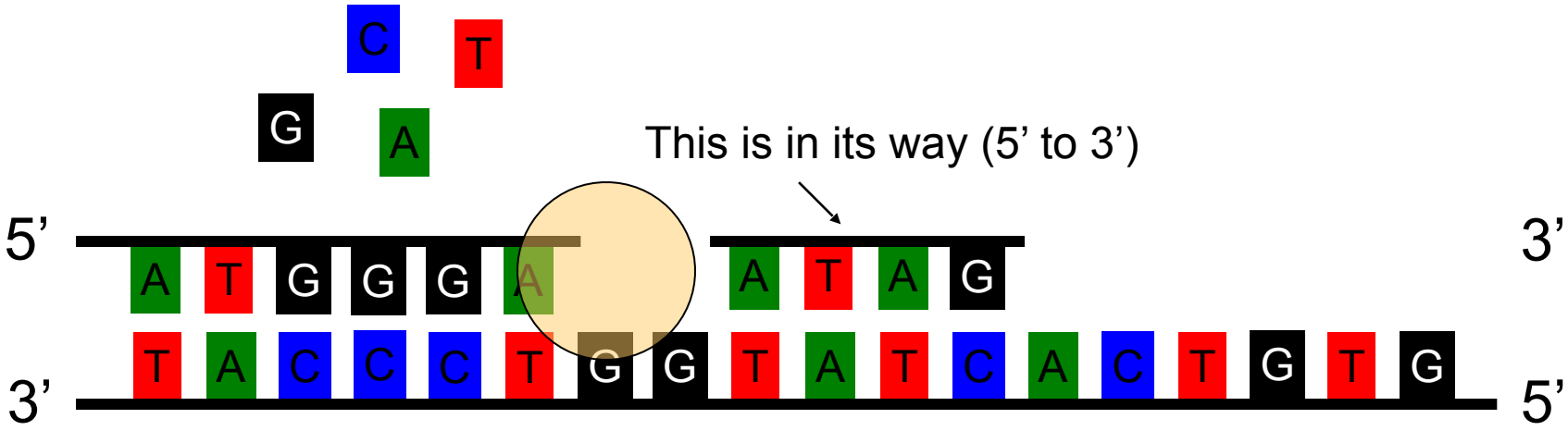


DNA polymerase goes back (**3' to 5'**) and removes wrong nucleotide then adds the correct one (**proofreading**)

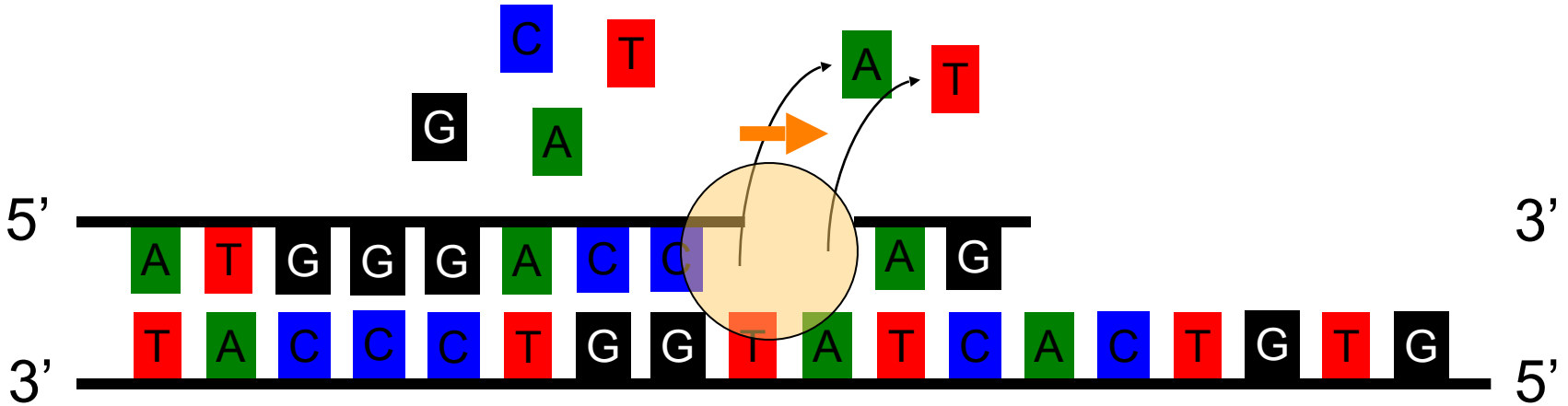


A bit of review

DNA Polymerase I 5'→3' Exonuclease activity

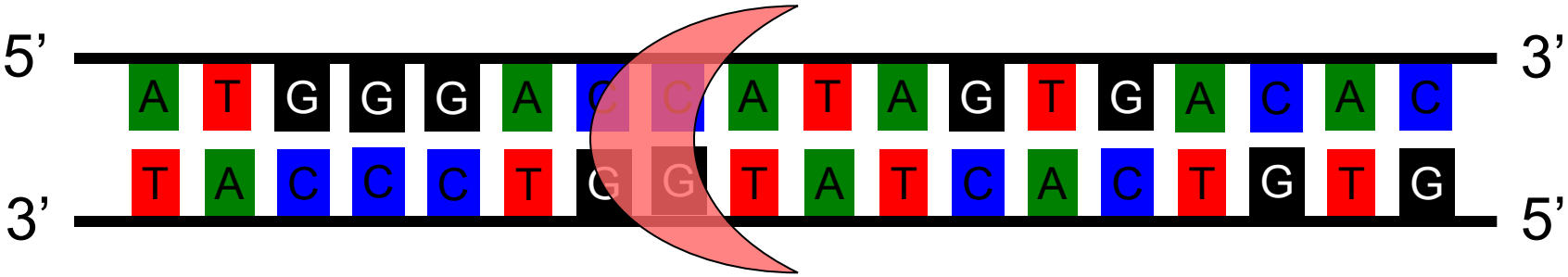


DNA polymerase I removes forward (**5' to 3'**) the nucleotides in its way and adds new ones

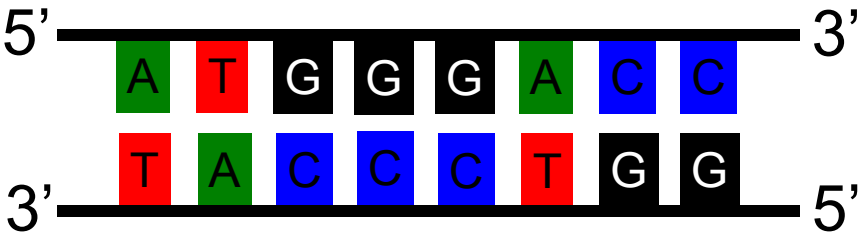


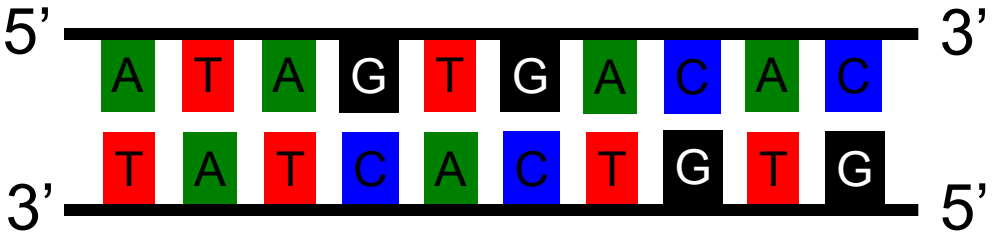
A bit of review

For later in the course (Endonuclease)



Endonuclease cuts the molecule in the middle





Replication enzymes/proteins

1. DNA polymerase (DNA Pol)

DNA Polymerase I

DNA Polymerase III

1. Replicates DNA 5'→3'.

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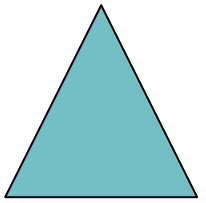
Replication enzymes/proteins

2. **Initiation protein (DnaA)**



Initiation protein (DnaA)

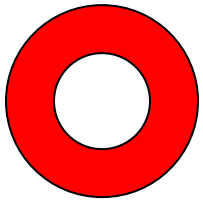
- Binds to AT repeat sequence in the double stranded DNA.
- The initiation protein **denatures** the double strands of DNA. Separating the two strands.
- This takes place in a specific location rich in AT sequence.

Remember AT hydrogen bonds!

Replication enzymes/proteins

3. **DNA Helicase (DnaB)**

- Helicase is placed on the denatured DNA.
- Helicase **untwist** DNA in two direction of the replication.
- Break hydrogen bonds between the bases and further exposing single stranded DNA.
- Calls and recruits an enzymes called **primase.**
- Pushes DNA replication forward.

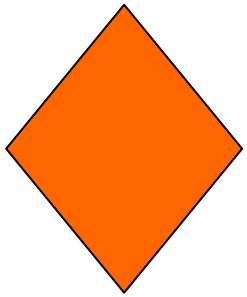


Helicase (DnaB)

Replication enzymes/proteins

4. **DNA Helicase loader (DnaC)**

- As the names suggests, this protein **loads** and places the DNA helicase on the denatured DNA.

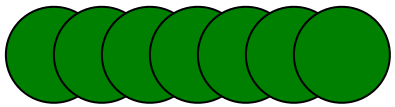


Helicase loader
(DnaC)

Replication enzymes/proteins

5. Single strand DNA binding protein (SSB)

- Binds to single stranded DNA template.
- SSB prevent the two denatured DNA strands from **re-annealing** (coming back together).

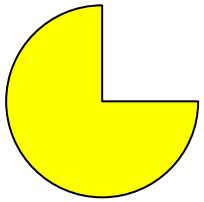


Single strand DNA binding proteins
(SSB)

Replication enzymes/proteins

6. **Primase**

- Primase adds a block of nucleotides (**primer**) to provide the polymerase with a 3'-OH needed for the synthesis of DNA.
- The block added is complementary to the template.
- Primase adds a single primer on one template.
- Primase adds multiple primers on the second template.

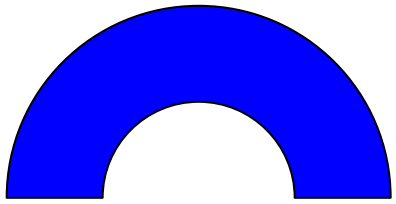


primase

Replication enzymes/proteins

7. **Gyrase**

- Gyrase is a type of topoisomerase.
- Relaxes the **tension** generated by the separation of the double strands and the untwisting of the double helix.

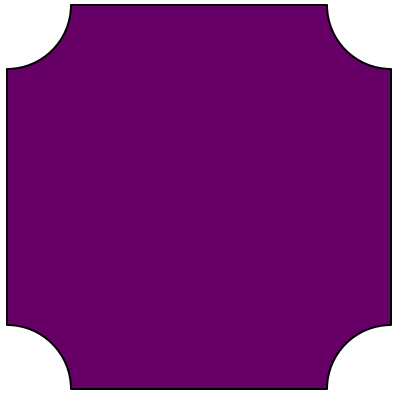


Gyrase

Replication enzymes/proteins

8. **Ligase**

- Joins to molecules that are disconnected.
- Seals the nicks in the replication process.



Ligase

Quiz

Which of the following enzymes is responsible for untwisting the DNA during replication

a) DNA Pol I

b) Lygase

c) Primase

d) Helicase

e) Gyrase

To study

dCTP

dATP

DNA polymerase I

Primase

Ligase

3’➔5’ exonuclease

DnaB

Primer

DnaC

DNA polymerase III

DNA Pol I

dGTP

Helicase

Gyrase

DNA Pol III

dTTP

Template

DnaA

dNTP

SSB

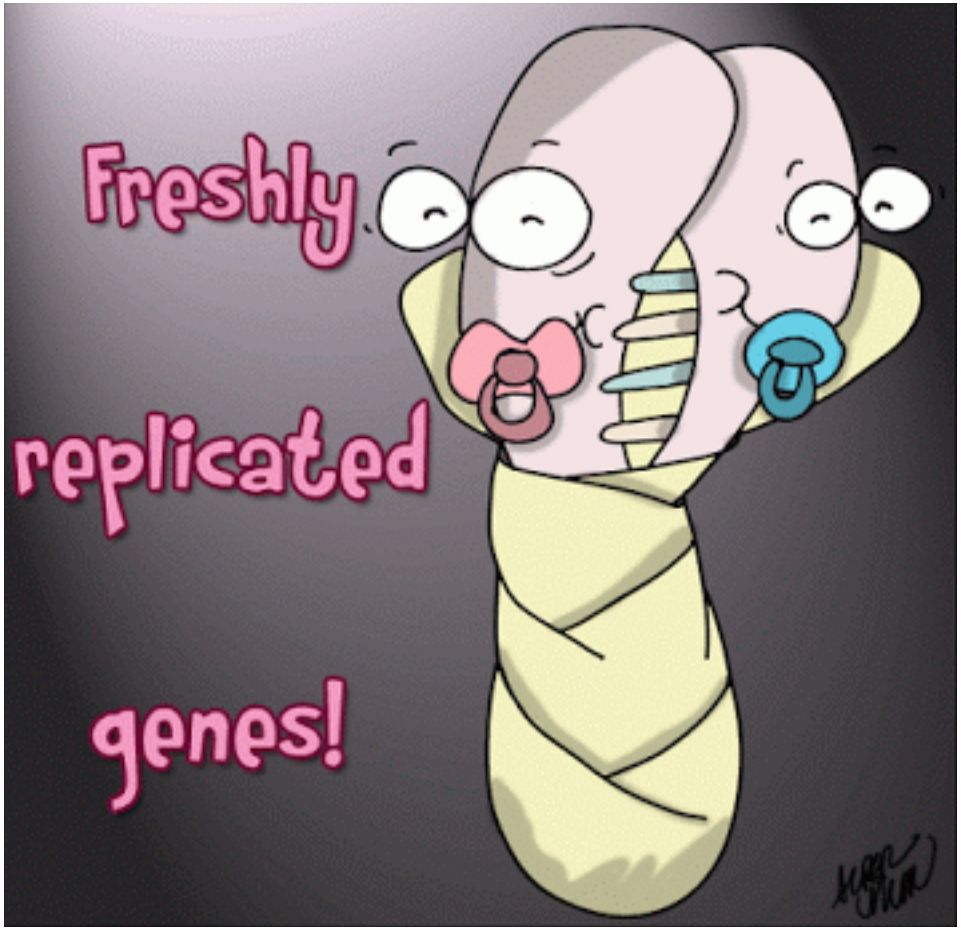
5’➔3’ exonuclease

Expectations

- You know what is needed for DNA replication and synthesis to take place.
- You know the building blocks of DNA.
- You know the enzymes/proteins in the process of replication.
- You know the function of each enzyme/protein.

Next lecture we will go over the process of DNA replication and connect it with what we learned today

For a smile



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