DNA Replication

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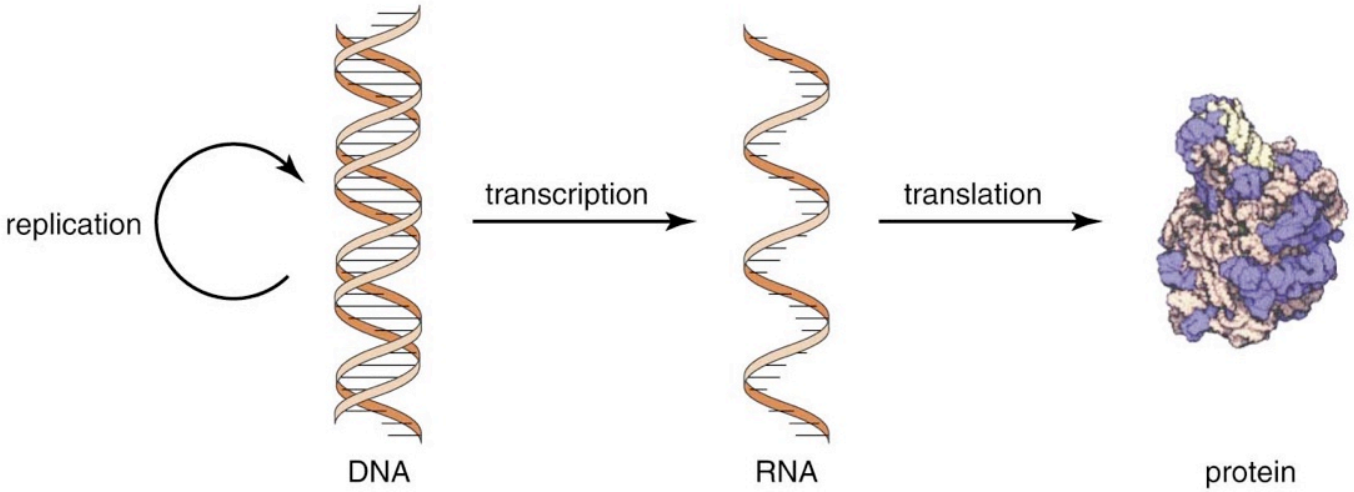
The Flow of Genetic Information



DNA Replication refers to the synthesis of new DNA using the existing DNA as a template.

Transcription refers to the synthesis of RNA using a gene sequence (DNA) as a template.

Translation refers to the synthesis of a protein using an mRNA transcript as the template.

Note that:

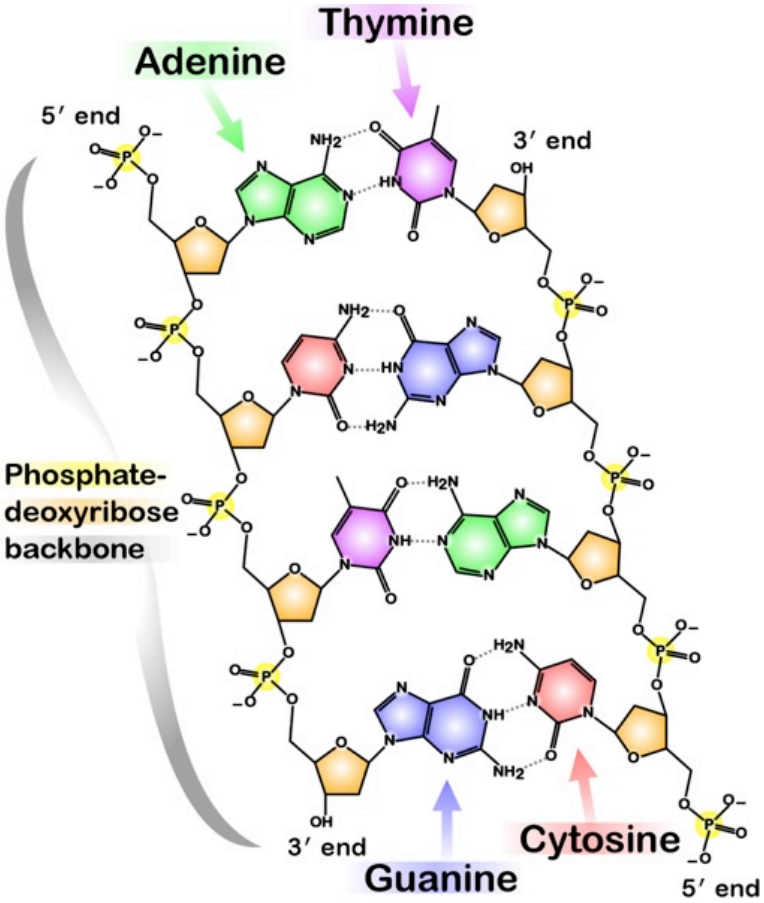
- most human DNA is never transcribed.
- not all RNA molecules are translated (e.g., rRNA, tRNA, others).
- RNA is sometimes ***reverse transcribed*** back to DNA.

The Structure of DNA is Fundamental to its Function

The function of DNA is the *storage of genetic information*.

DNA sequences known as **genes** specify the kinds of proteins that are made by cells.

Complementary base-pairing ensures redundancy and allows for genetic information to be copied.



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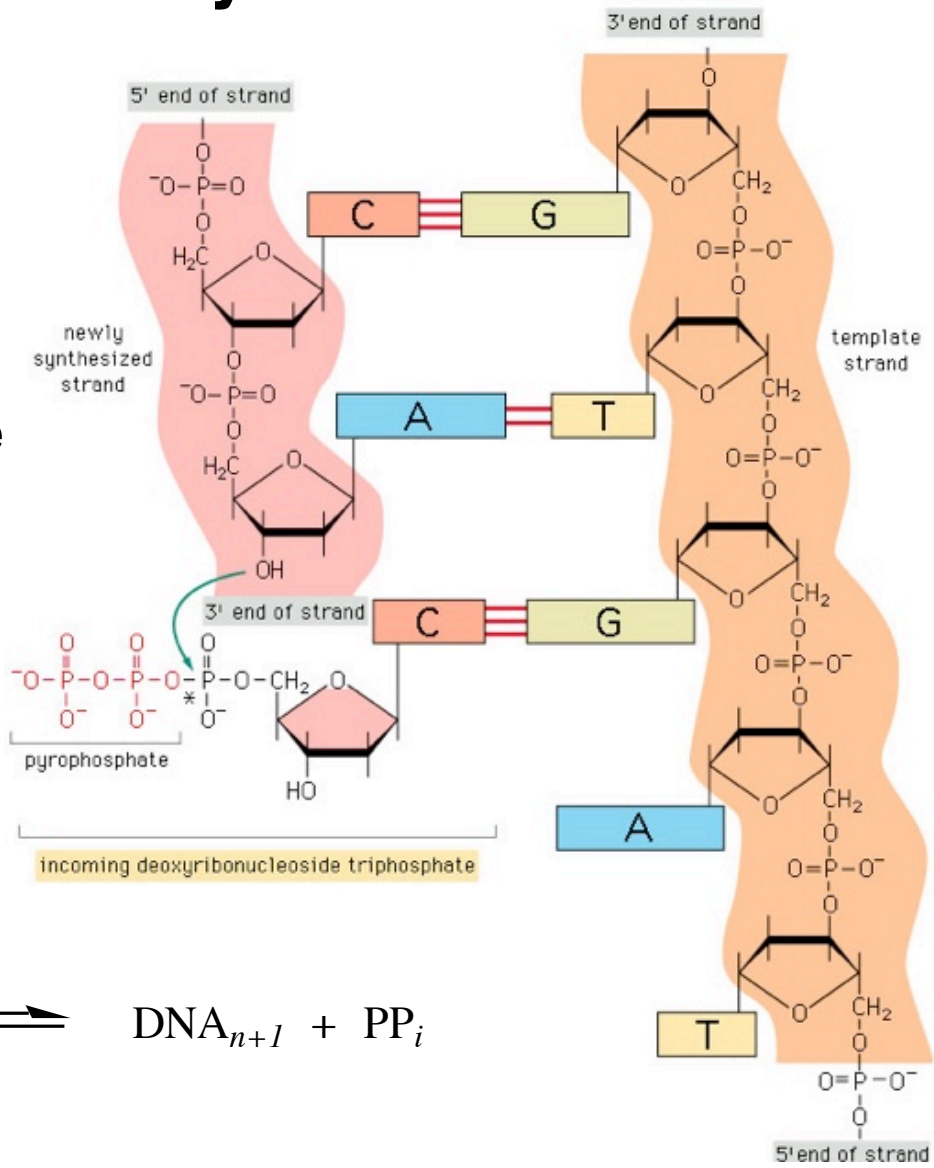
DNA Replication and DNA Polymerases

The replication of DNA is effected by enzymes known as **DNA polymerases**.

These enzymes catalyze the step-by-step addition of deoxynucleoside monophosphates to a DNA chain (the *primer strand*).



$$DNA_n + dNTP \xrightleftharpoons{Mg^{2+}} DNA_{n+1} + PP_i$$

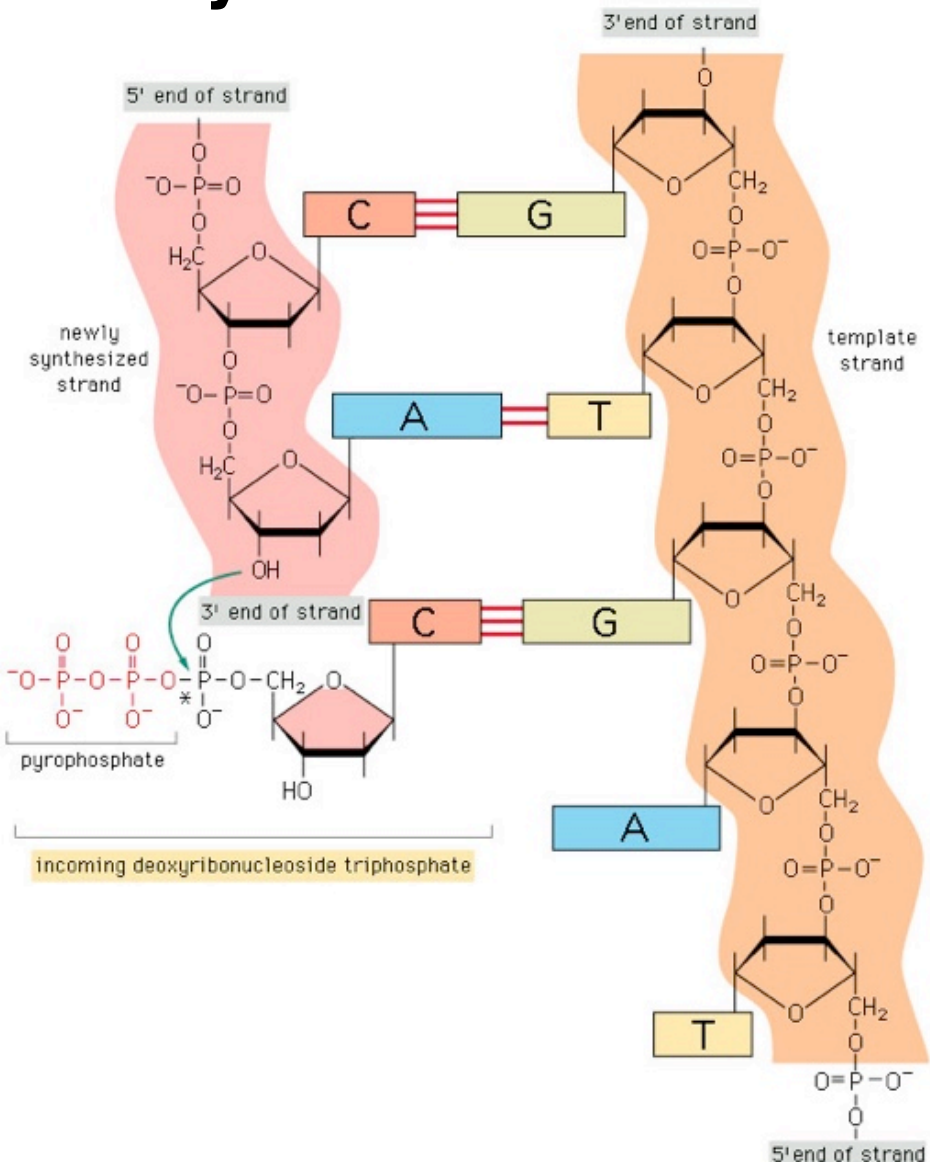
DNA Replication and DNA Polymerases

This reaction is a nucleophilic attack by the 3'-hydroxy group of the primer strand on the α-phosphate of the incoming deoxynucleoside triphosphate.

This results in the formation of a covalent phosphodiester bond in a ***transesterification*** reaction.

Note that polymerization proceeds in the 5'→3' direction.

A driving force is the subsequent hydrolysis of PP*i* by **inorganic pyrophosphatase.**



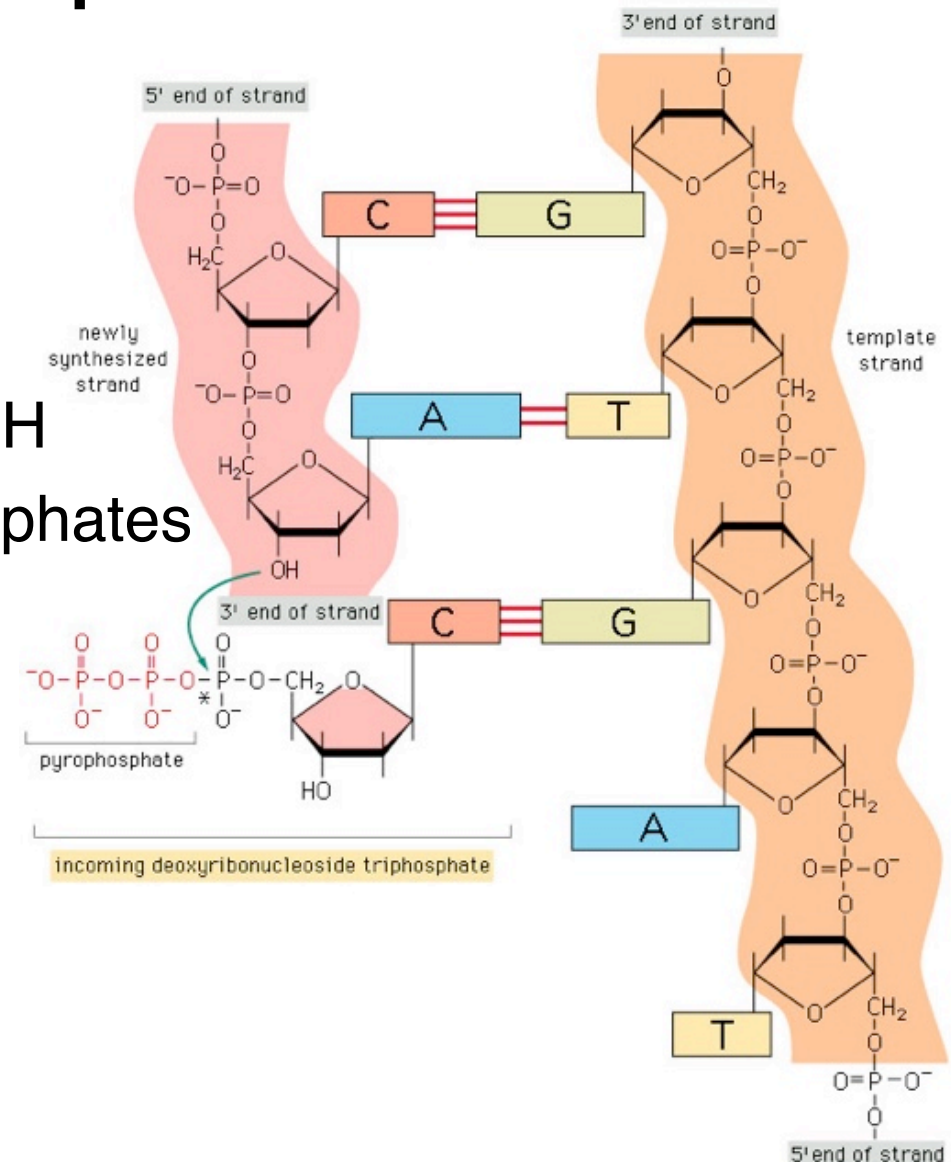
All DNA Polymerases Require:

Template strand

Primer strand with a free 3’ OH

2’-deoxynucleoside 5’-triphosphates

Mg^{2+}

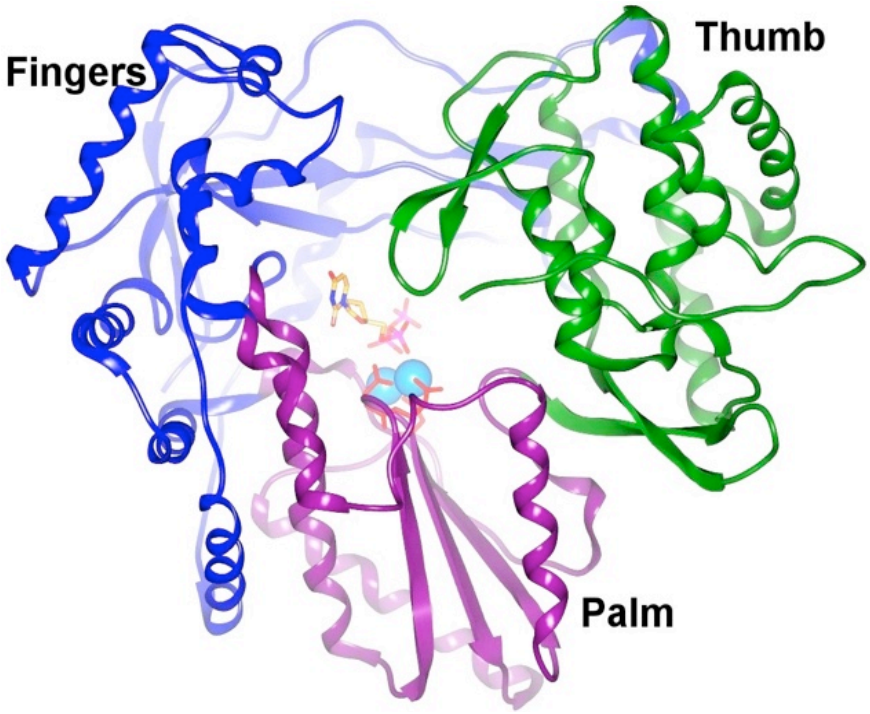


Prokaryotic DNA Polymerase Enzymes

The first DNA polymerase to be characterized was *DNA polymerase I* (**Pol I**) from *E. coli*.

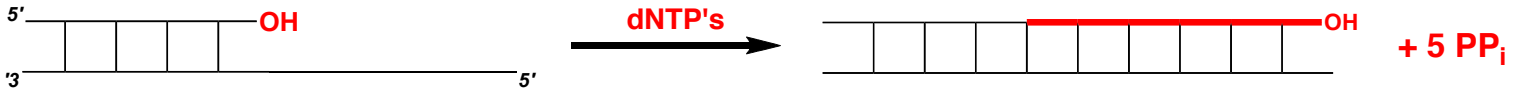
Pol I is a single 103 kDa polypeptide.

Not essential for replication, but important in DNA repair



Pol I has 3 distinct catalytic activities

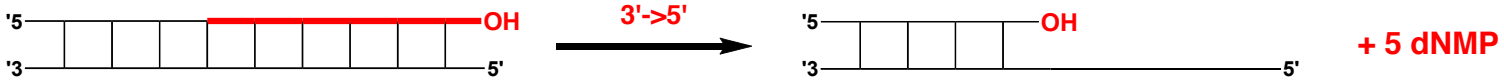
DNA Polymerase Activity



Replication function:

- Moderate processivity (adds ~20 bp per encounter with DNA)
- Moderate rate (adds ~25 bp/sec)
- Moderate fidelity (~1 error in 10^5 bp = ~10^{-5} errors/bp)

Exonuclease Activity 1



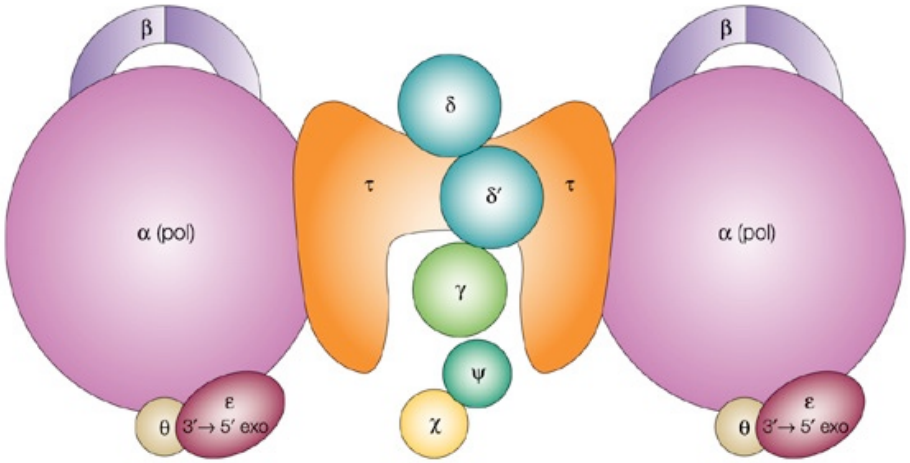
Editing function:

- Boosts fidelity to ~10^{-8} errors/bp)

Exonuclease Activity 2

Repair function

DNA Polymerase III replicates DNA for cell replication



A large, multiprotein complex composed of 8 different subunits

- Very fast: ~1000 bp/sec
- Very processive: 1000 bp/encounter
- Possesses 3' → 5' exonuclease activity, but *not* 5' → 3'
- Polymerase fidelity: ~10^{-5} errors/bp, boosted to 10^{-7}-10^{-8} by exonuclease proofreading

The DNA Replication Process in *E. coli*

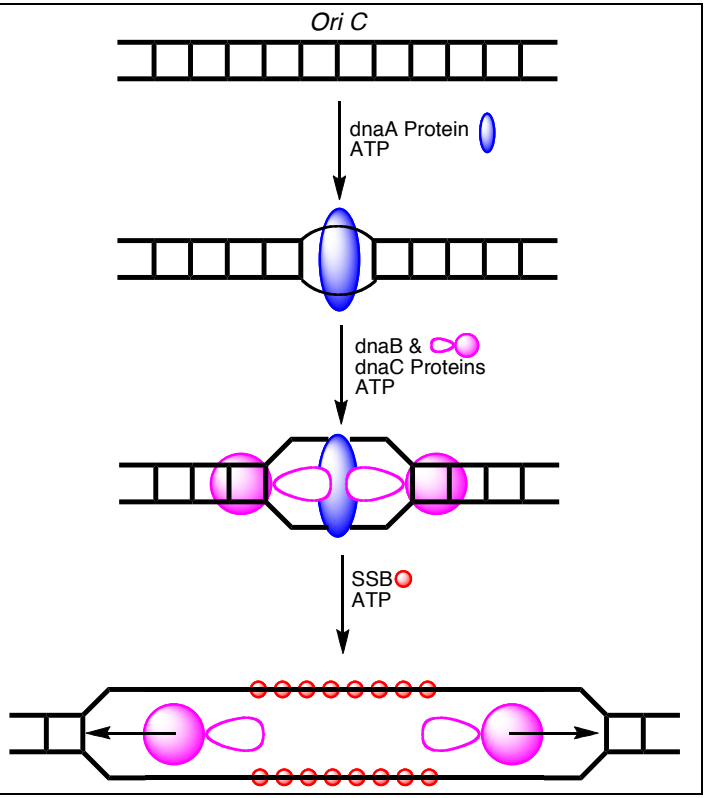
Initiation Phase. Replication of chromosomal DNA begins at a discrete site known as the origin of replication (in *E. coli*, this is known as ***oriC***).

Binding of **dnaA protein** to *oriC* results in unwinding of the DNA duplex.

The duplex is further unwound with the binding of **dnaB** and **dnaC** proteins.

The unwound DNA is stabilized and protected with the binding of single-stranded binding protein (**SSB**).

A bubble forms at *oriC* with the unwinding of DNA occurring in both directions.

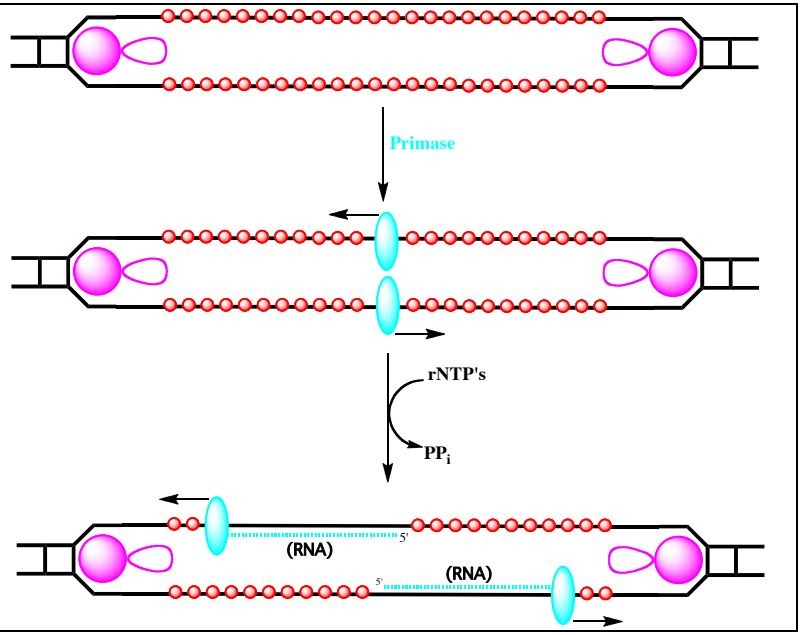


The DNA Replication Process in *E. coli*

Primase is an RNA polymerase that binds to single-stranded DNA in a multiprotein complex known as a **primosome** (seven proteins).

Primase synthesizes short (~ 5 – 10 base) **RNA primers**.

Primase dissociates and Pol III utilizes these RNA primers for the synthesis of DNA.

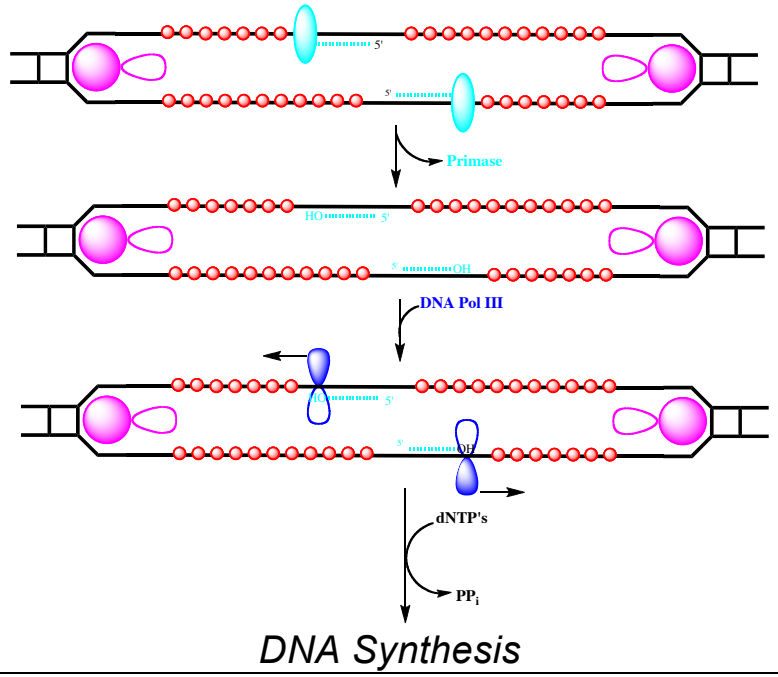


The DNA Replication Process in *E. coli*

Elongation Phase. Assembly of pol III complex onto duplex DNA results in the formation of a "***replication fork***".

Polymerase error rate ~ 10^{-5}

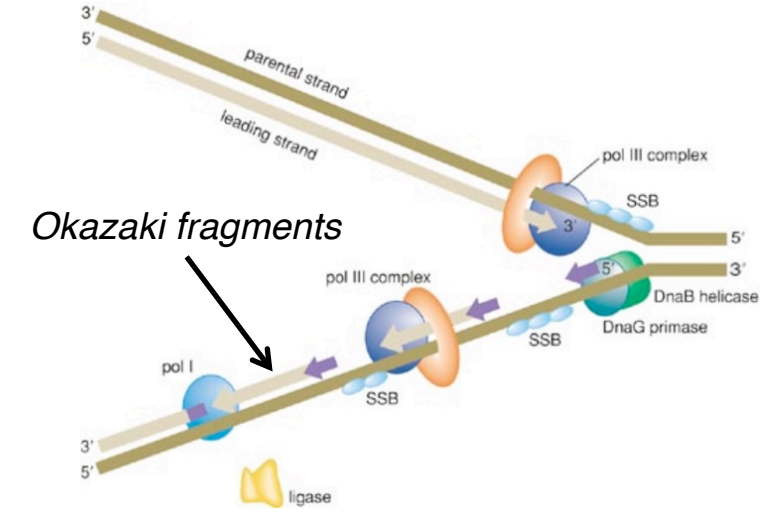
3'->5' exonuclease corrects > 99%
=> 10^{-7} to 10^{-8} overall error rate

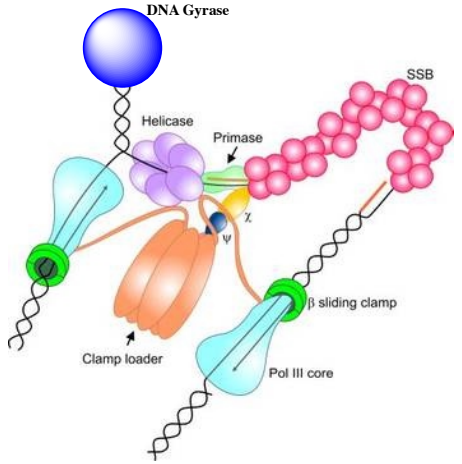


DNA Synthesis

The DNA Replication Process in *E. coli*

Remember "Leading Strand" vs. "Lagging Strand" synthesis





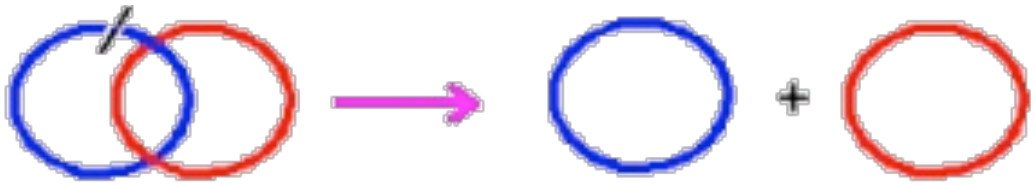
Positive supercoiling induced by unwinding of duplex DNA is prevented by ***DNA gyrase***; this enzyme introduces negative supercoils as it hydrolyzes ATP.

The DNA Replication Process in *E. coli*

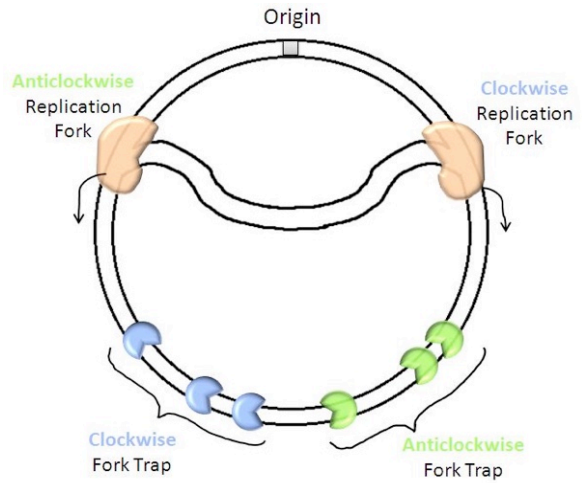
Termination of DNA Replication

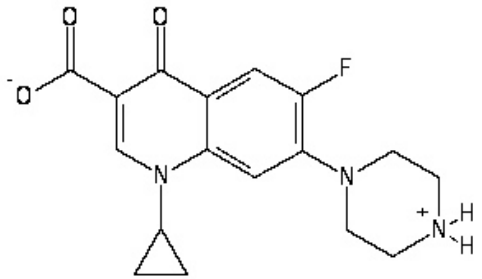
Replication of the *E. coli* genome ends when the replication forks meet at the *termination region* (***ter***).

The "concatanes" are resolved by **topoisomerase IV**.

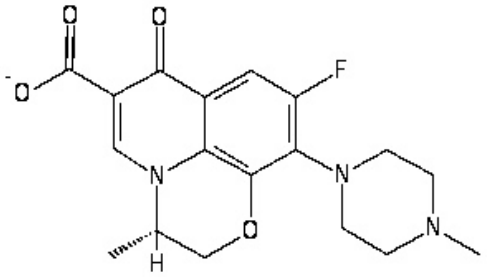


Fluoroquinolone antibiotics inhibit both **DNA gyrase** (gram negative) and **topo IV** (gram positive) enzymes.





Ciprofloxacin

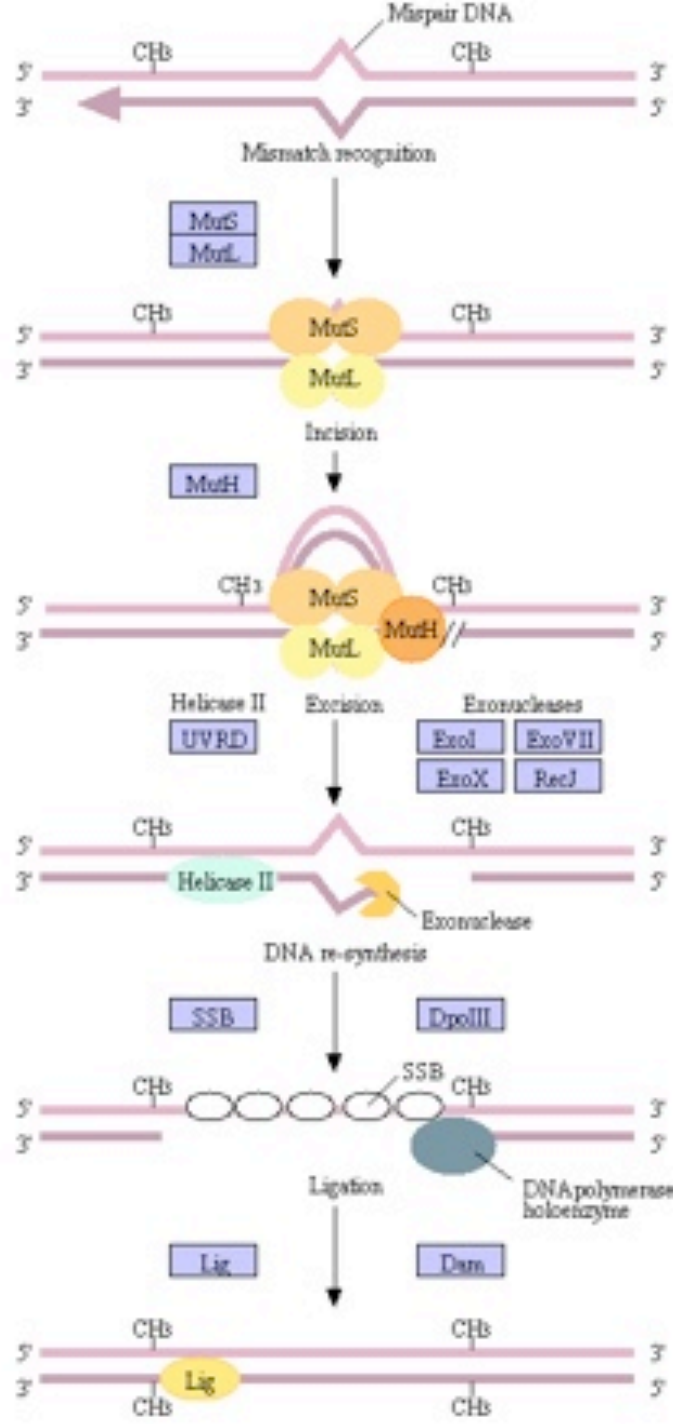


Levofloxacin

DNA Mismatch Repair

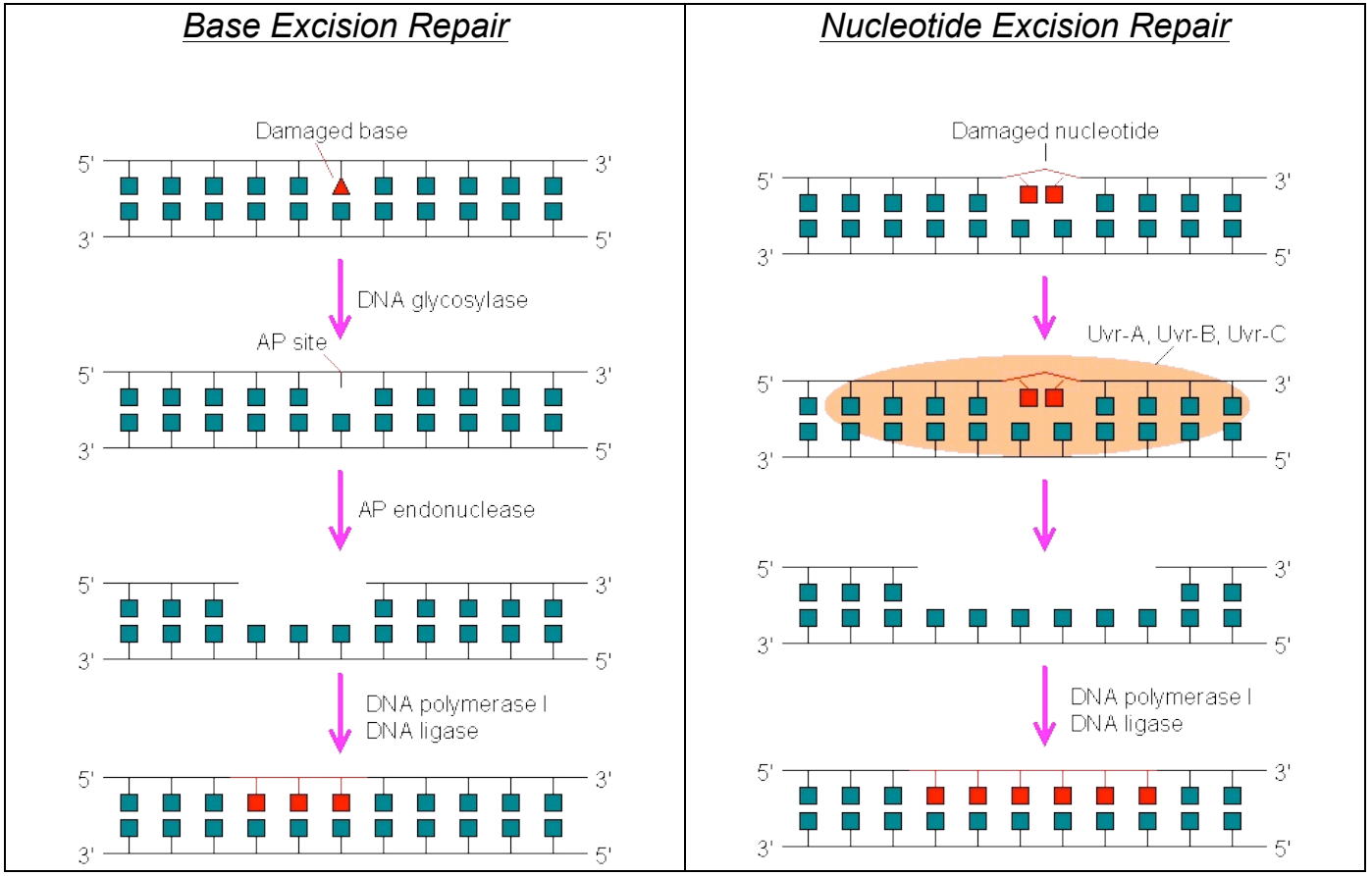
This system fixes >99% of the rare errors made by DNA polymerases, boosting the overall fidelity to 10^{-9} to 10^{-10} errors/bp

(*E. coli* genome: ~5×10^6 bp
Human genome: ~3×10^9 bp)



DNA Damage Repair

UV light, X-rays, reactive oxygen species (ROS), plant toxins, chemical carcinogens, and therapeutic alkylating agents can damage DNA.

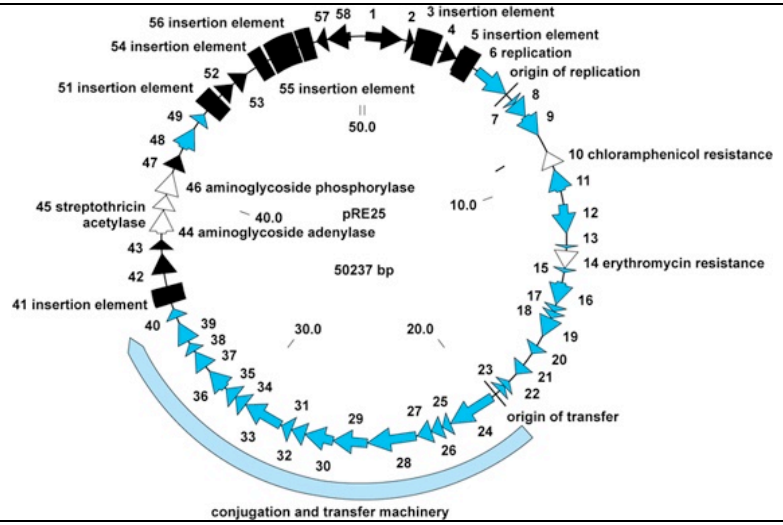


Bacterial Plasmids

Circular, extra-chromosomal, double-stranded DNA molecules that replicate autonomously

- The number of plasmid molecules per cell is determined by the "strength" of the replication origin

Plasmids may be transferred between bacteria and often carry genes responsible for antibiotic resistance and toxins



DNA Replication in Eukaryotes

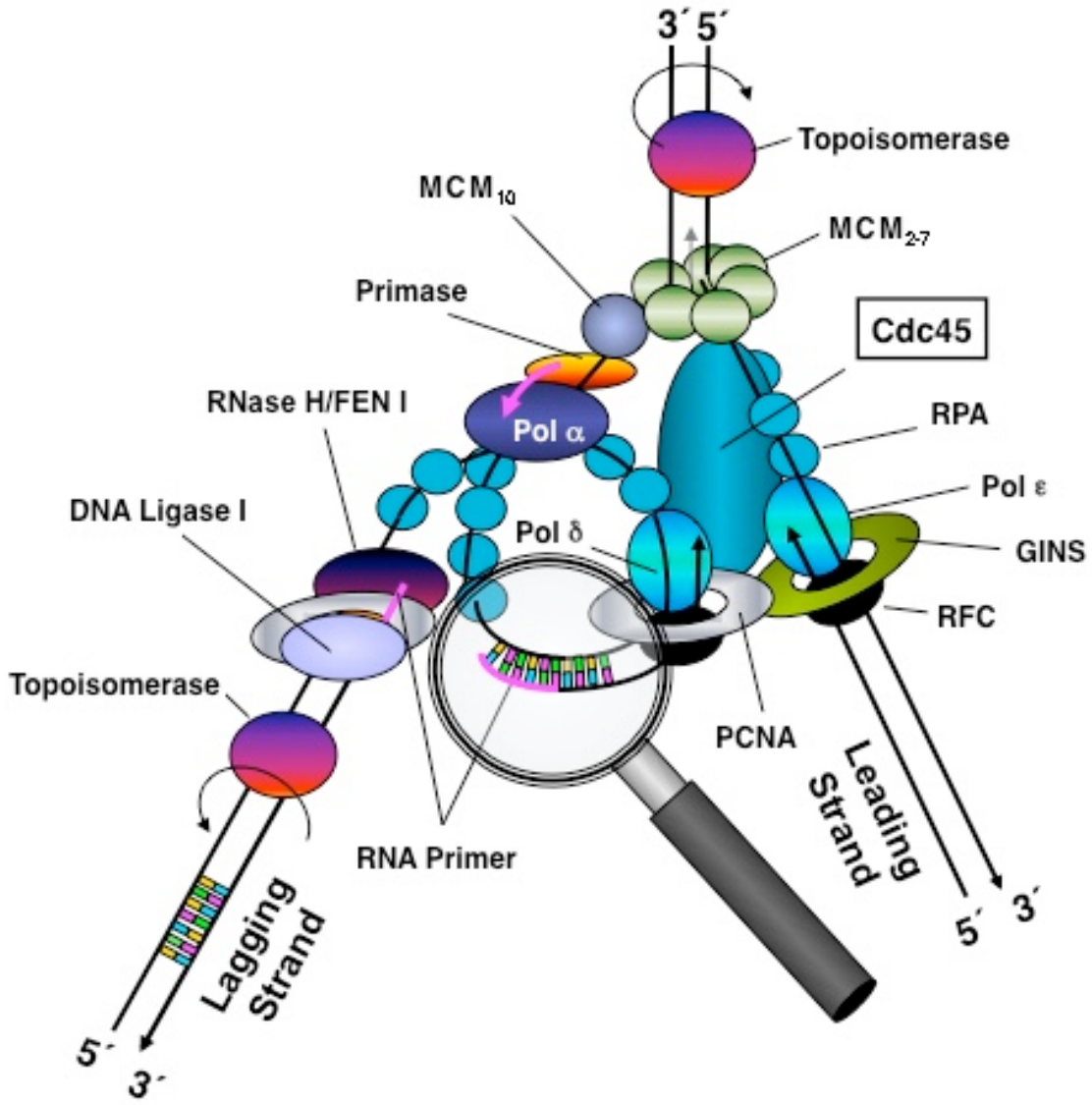
The process is similar, but more complex; there are at least 13 different eukaryotic DNA polymerases (named with Greek letters).

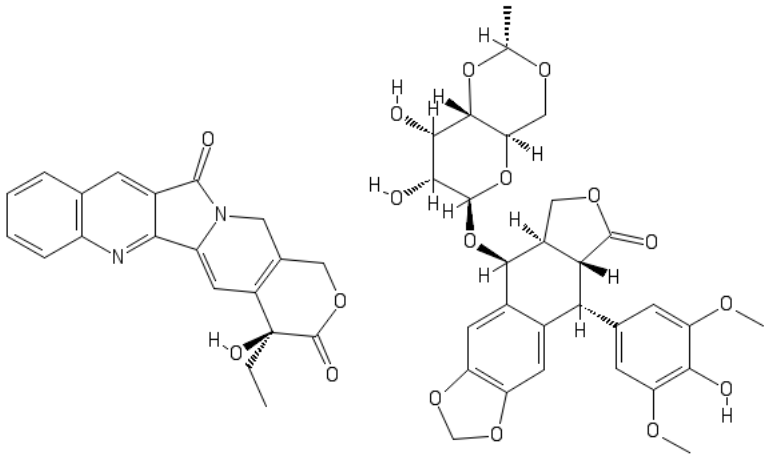
DNA polymerase α:primase complex synthesizes an RNA primer and then adds ~ 15 bases of DNA. This primer is removed by **FEN-1 nuclease**.

DNA pols δ and **ε** are the main polymerases involved in chromosomal replication.

Many of the other polymerases appear to function primarily in DNA repair pathways (e.g., **pol β**), plus those found in mitochondria (**pol γ**) and chloroplasts.

DNA Replication in Eukaryotes





Camptothecin and ***Etoposide*** are topoisomerase poisons (Topo I and Topo II, respectively). They trap the topo:DNA complexes and ultimately cause single and double-strand DNA breaks in the duplex. They are used in cancer chemotherapy.

Bottom Line

Know the requirements for DNA synthesis and *how* nucleotides are added to the primer strand (i.e.- what is the chemical reaction that takes place) and what makes the reaction "irreversible".

Know the multiple catalytic *activities* of pols I and III and their *function* in DNA replication and repair.

Understand the meaning of processivity and fidelity in DNA synthesis.

Know and be prepared to discuss the fidelity and processivity of pol I vs. pol III and how these features are well suited to their biological function.

Be prepared to describe the general features of initiation, replication and termination of genomic replication in *E. coli*. What roles do gyrase and topoisomerase IV play? What is the arrangement of DNA in a replication fork?

Be prepared to describe the essential features of DNA mismatch repair and DNA damage repair.

You should know what a plasmid is and why they are important to bacteria.

Know the common features between prokaryotic and eukaryotic DNA replication.

Know the correct spelling for any and all drugs and diseases discussed during the semester and the biochemistry behind them