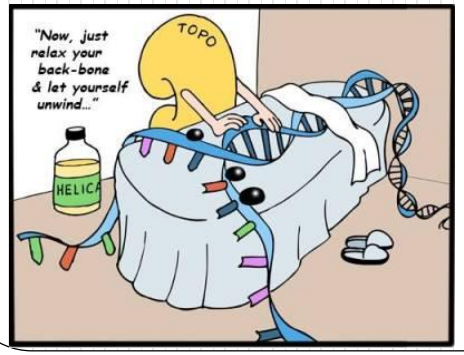
DNA replication

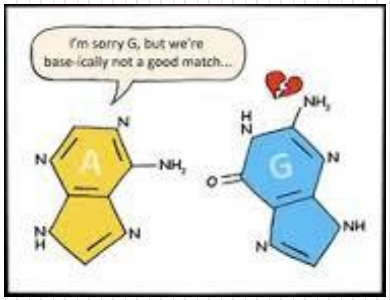
Lecture 10

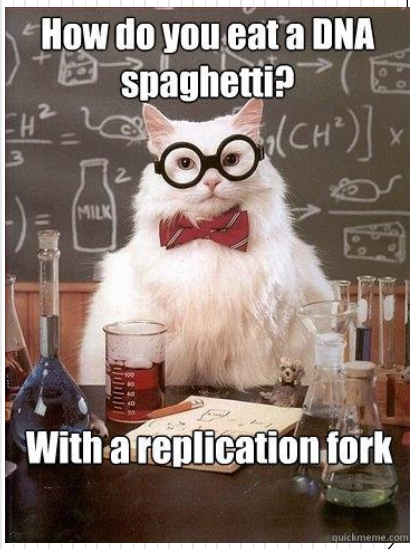
SLE254 Genetics and Genomics

Concepts of Genetics

Chapter 11 pp 276-300 (12th ed)



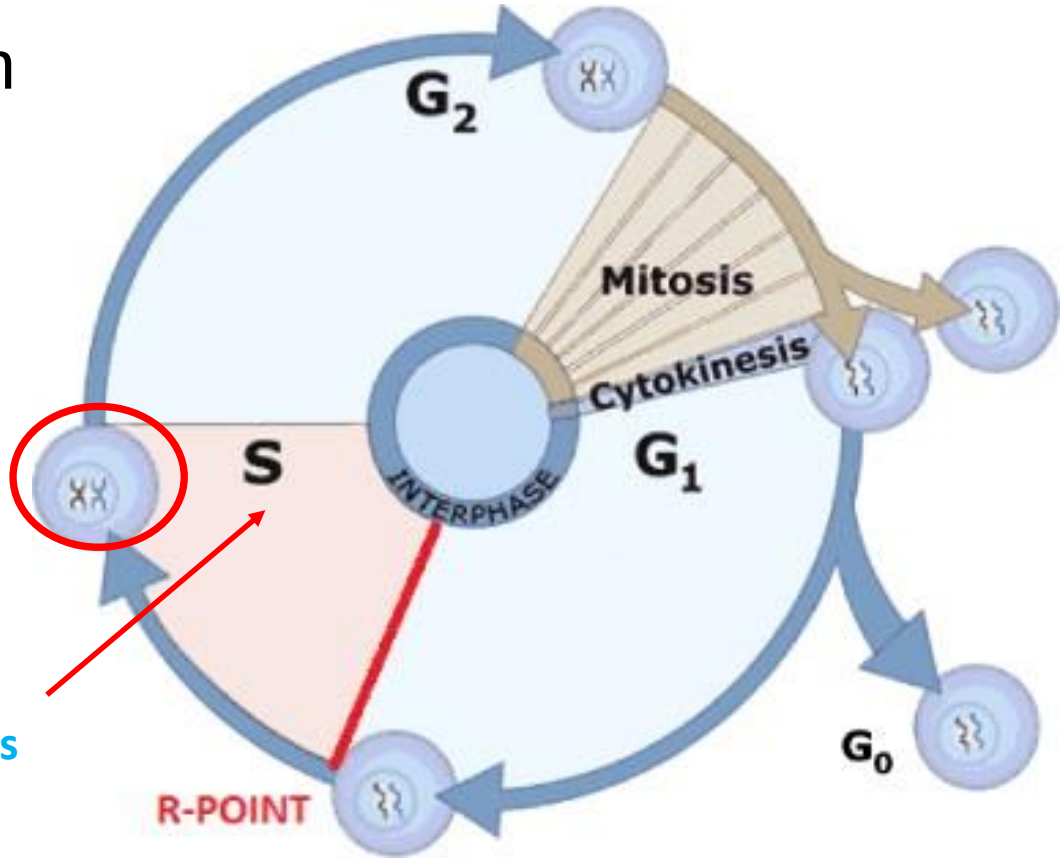




DNA replication

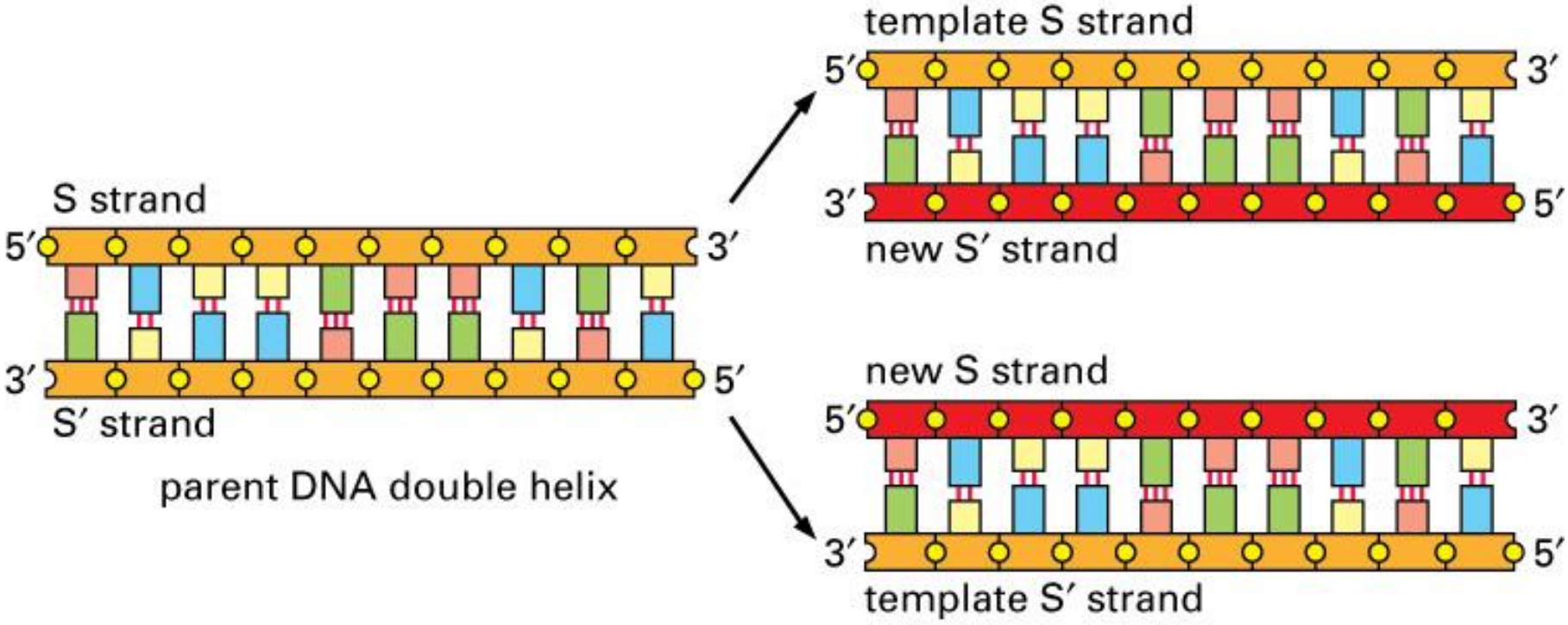
- https://www.youtube.com/watch?v=5qSrmeiWsuc

7.58 min



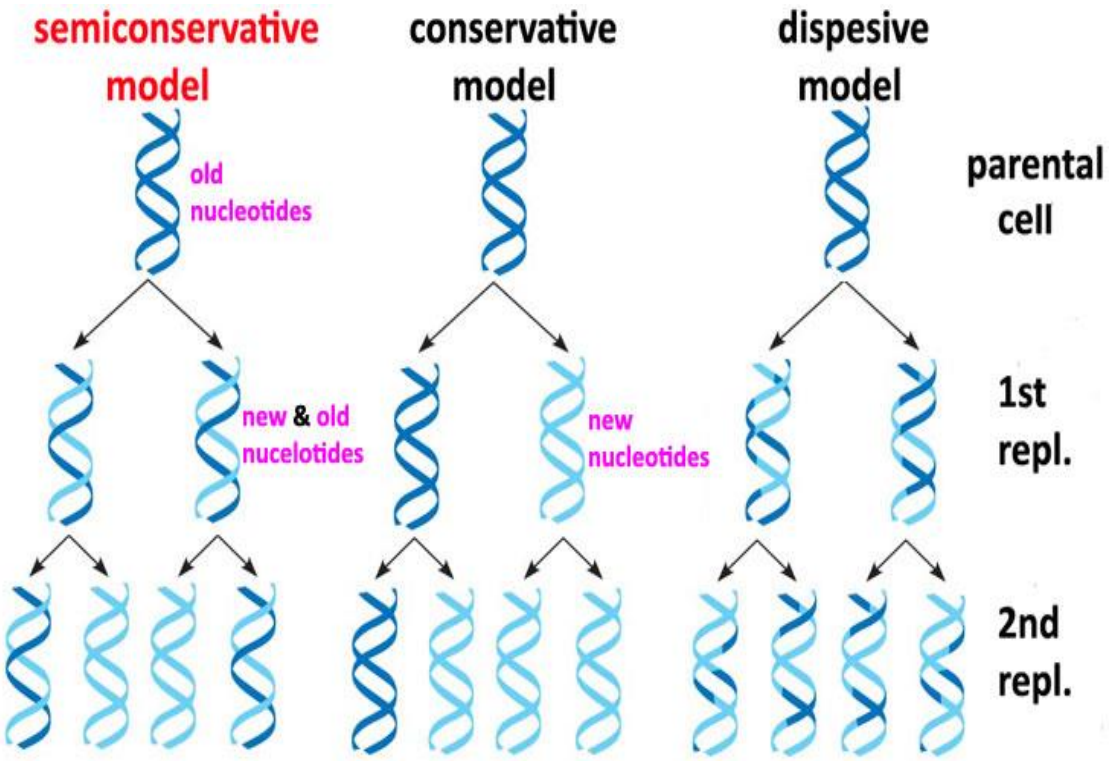
DNA replication

- To carry the genomic information to daughter cells the DNA molecule must replicate using itself as a template



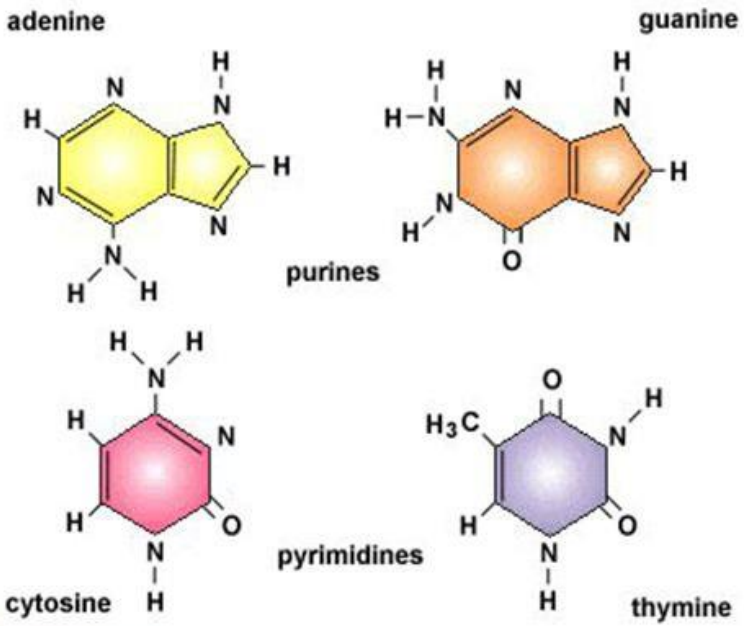
Three models of DNA replication

- Plausibly occurs three different ways:
  - Conservative – Daughter DNA doesn't contain any parent DNA
  - Dispersive – daughter DNA consist of strands each containing segments of both parental DNA strands
  - Semiconservative – Each daughter DNA contains 1 strand of parent DNA



Structure of DNA

- There are four Nitrogen-containing bases of DNA: adenine, guanine (purines- double ring) and thymine, cytosine (pyrimidines- single ring)



Meselson-Stahl experiment

- ^{15}N is a heavy isotope of Nitrogen
- Why nitrogen?
- DNA containing ^{15}N can be distinguished from ^{14}N using **sedimentation equilibrium centrifugation**
- Samples are forced by centrifugation through a gradient of heavy metal salt: cesium chloride
- DNA reaches equilibrium when their density = the density of the gradient medium
- ^{15}N will reach this point closer to the bottom of the tube than ^{14}N

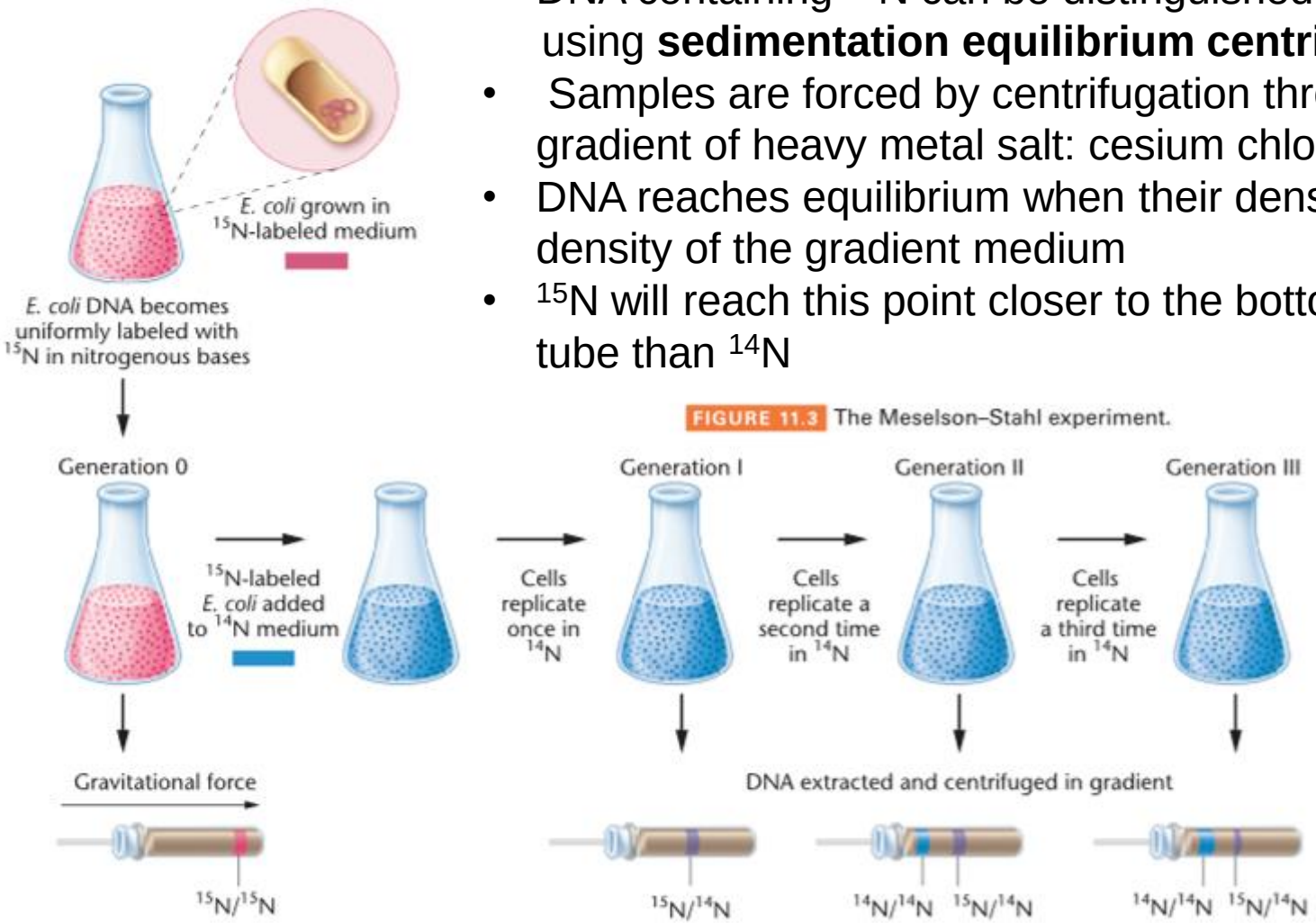


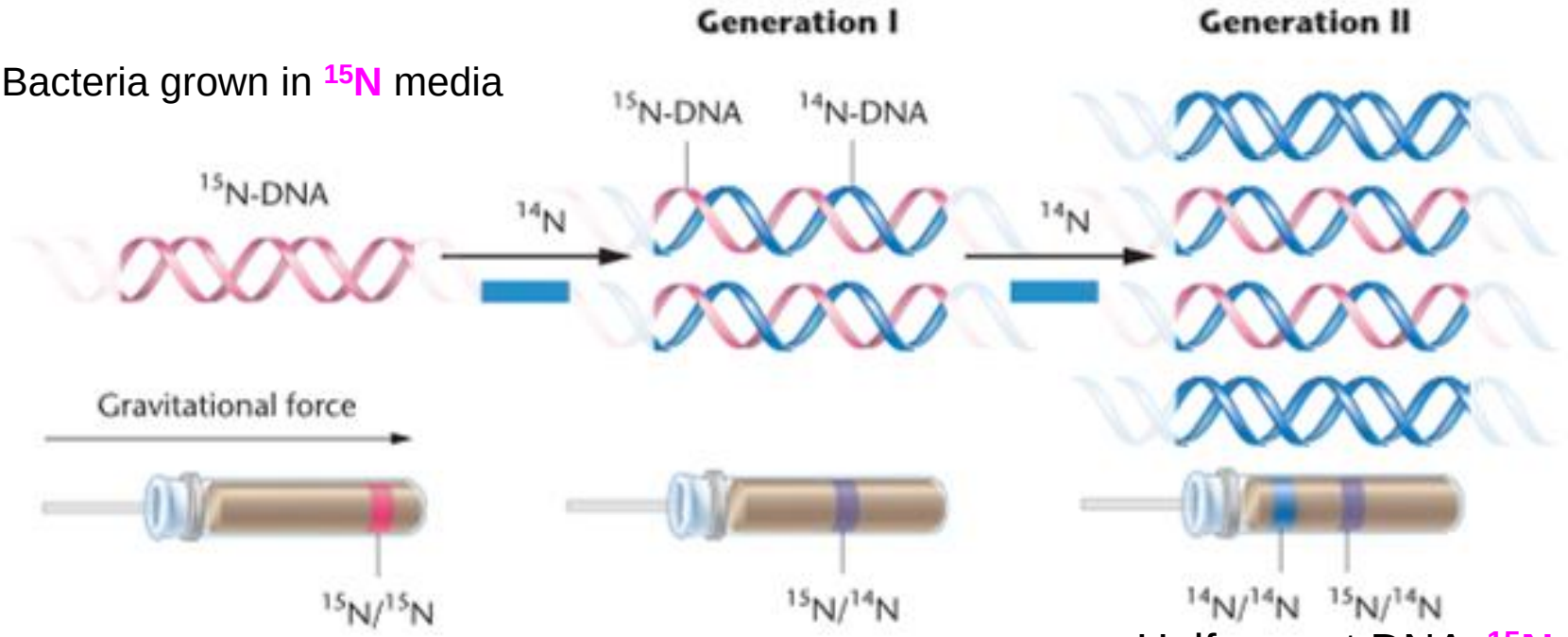
FIGURE 11.3 The Meselson–Stahl experiment.

Meselson-Stahl experiment

Bacteria grown in ^{15}N media

Bacteria grown in ^{14}N media

Bacteria grown in ^{14}N media



Parent DNA: ^{15}N heavy, equilibrium towards bottom of tube

Half parent DNA: ^{15}N half light daughter DNA ^{14}N mid tube equilibrium

Half parent DNA: ^{15}N half light daughter DNA ^{14}N mid tube equilibrium. Full daughter ^{14}N top tube equilibrium

Fig 11.4

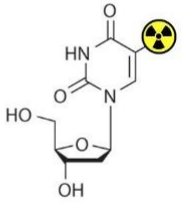
The tritiated thymidine experiment

- Evidence for semiconservative replication of eukaryotic chromosomes
  - Labelling of DNA in beans shoots with tritiated thymidine
  - The labelled DNA strands in the chromosomes is detected by autoradiography

^{3}H-thymidine

Labelled H

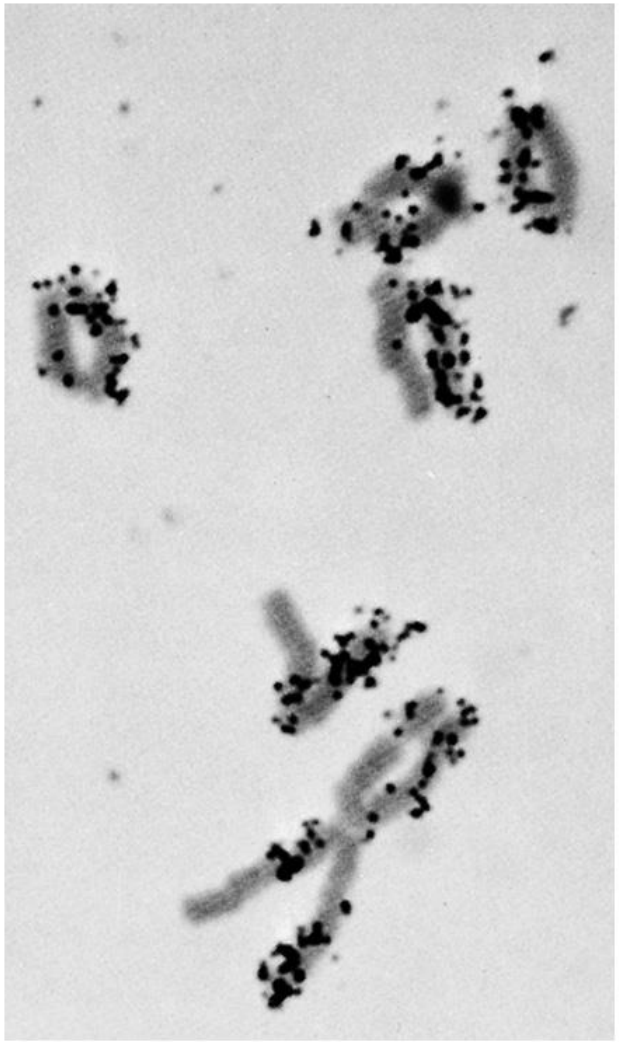
Deoxy nucleoside



Tritiated (3H) thymidine

After synthesis, first metaphase- nice and condensed

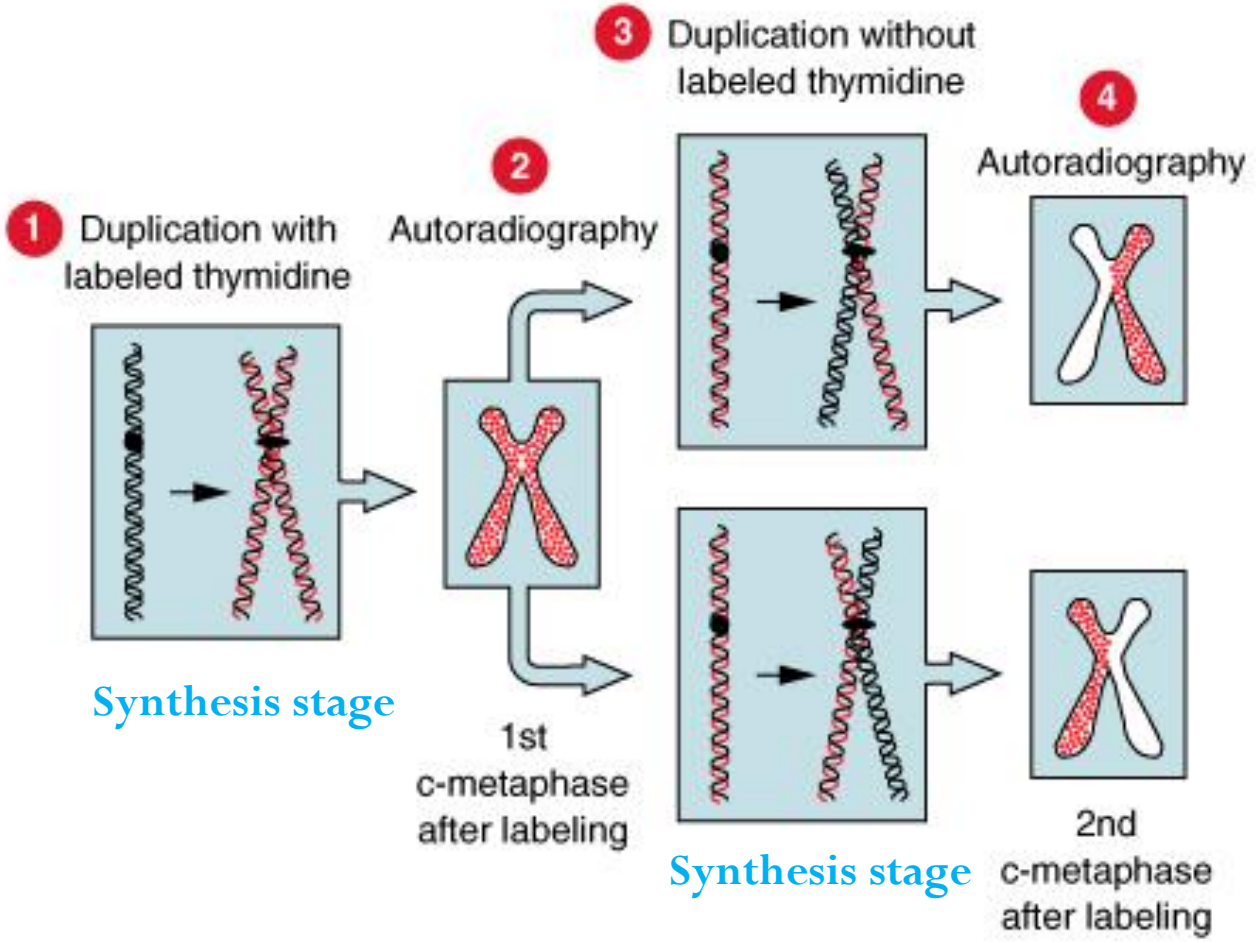
The tritiated thymidine experiment



- The second round of DNA replication was carried out **without the radioactive isotope so the new strands are not labelled.**
- Note that only one of each chromatid is labelled, as predicted from the **semiconservative replication model**.

After synthesis: the second metaphase – nice and condensed

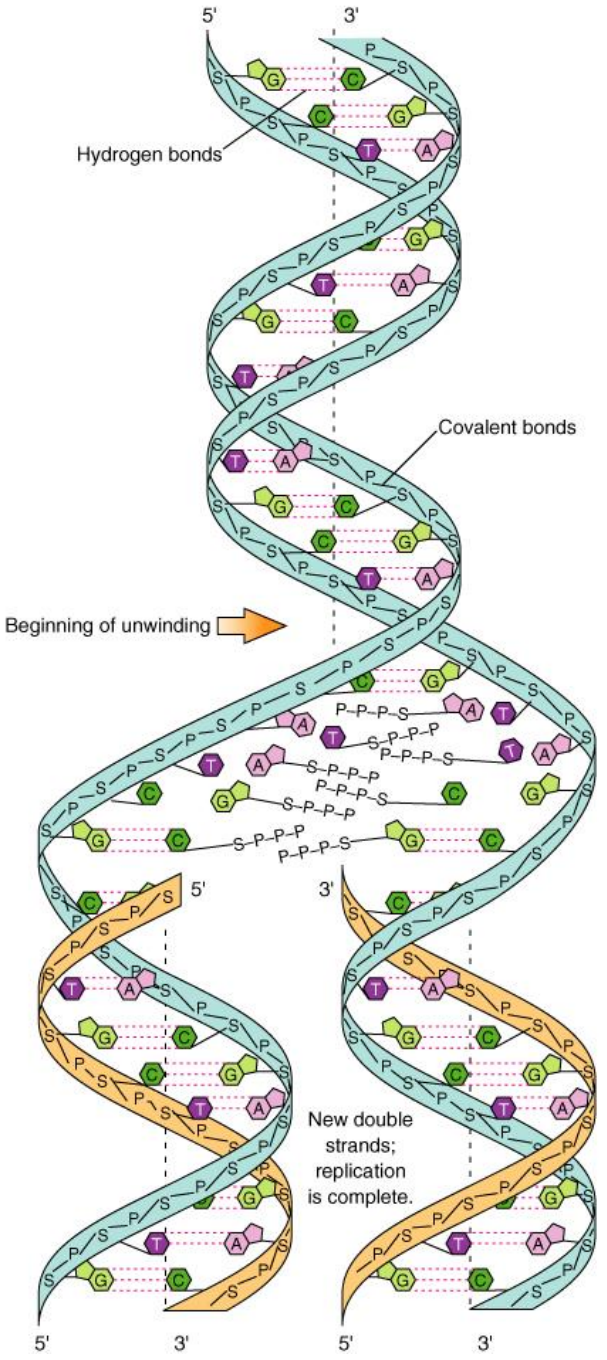
The tritiated thymidine experiment

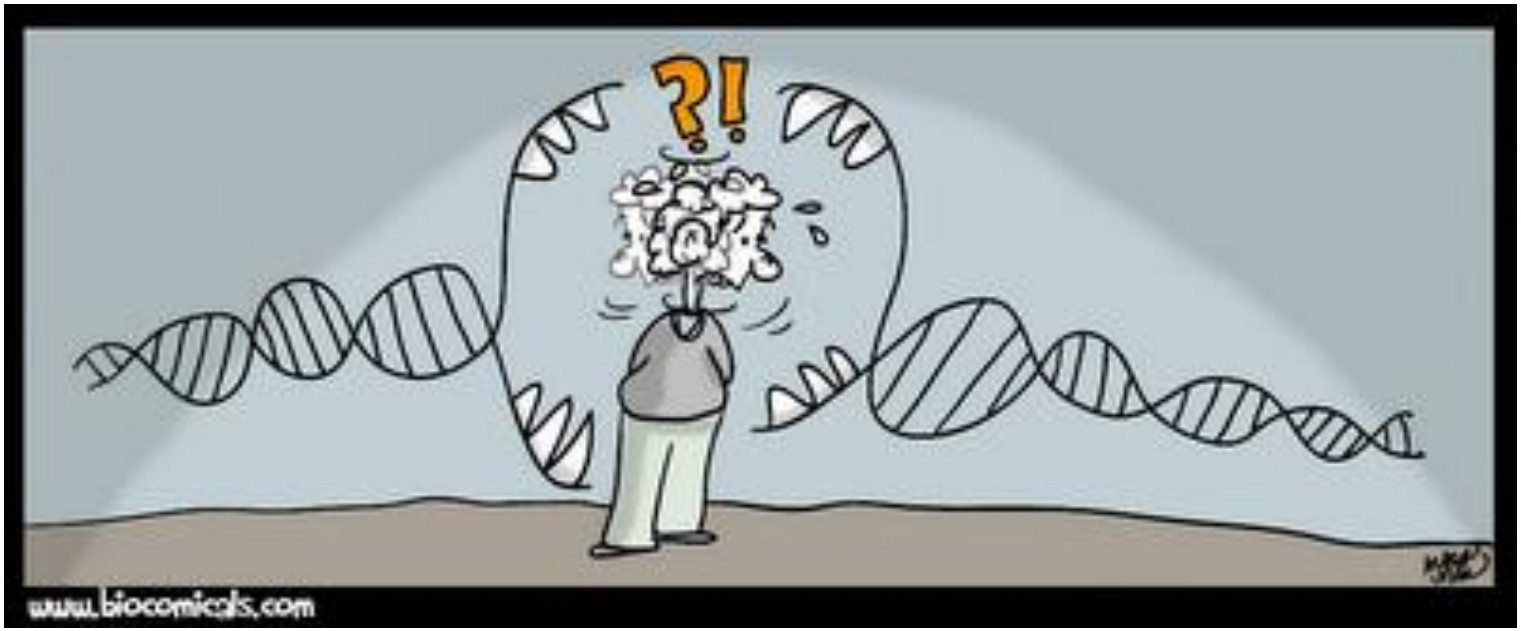


DNA replication is semiconservative

- Semiconservative means that one of each parental strand is conserved in the daughter double helicies

- A new daughter strand is synthesised from the parental template

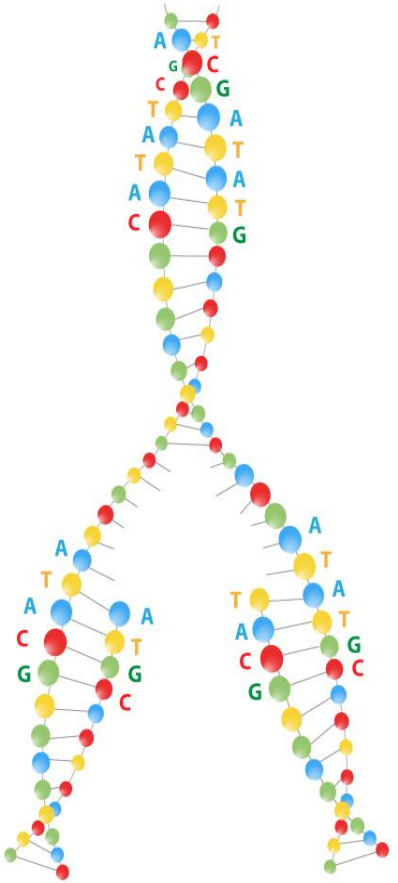




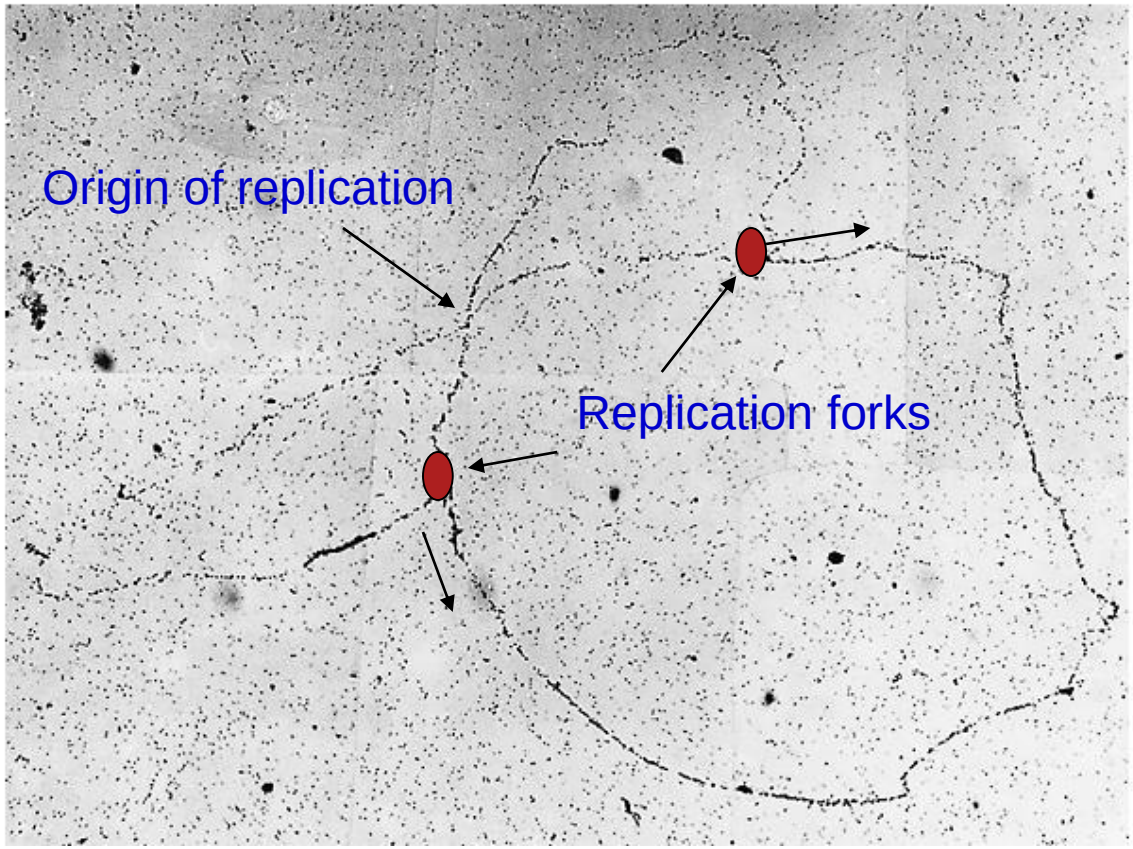
Replication fork

Replication fork

- The **point at which the DNA is being replicated**
- The replication fork can be visualised by labelling replicating DNA with **tritiated thymidine** and autoradiography
- This was first carried out with *E. coli* by John Cairns
  - *E. coli* was a good model: has a small circular chromosome, easy to purify



Cairns experiment showing the replication of *E. coli* DNA



Replicated bidirectionally - DNA Polymerase

The origin of replication

- DNA polymerase starts copying DNA at **unique points** called **origins of replication**
- The *E. coli* chromosome has **only one origin called *OriC***
- Origin of replications are often AT rich, because the double helix is more easily melted*
- Human chromosomes have **many** origins of replication- **we have more chromosomes must be replicated quickly..**

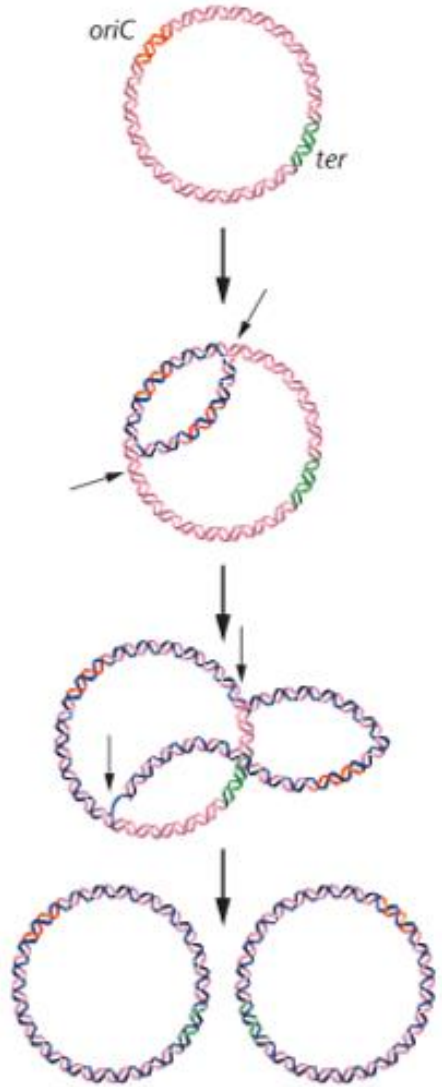
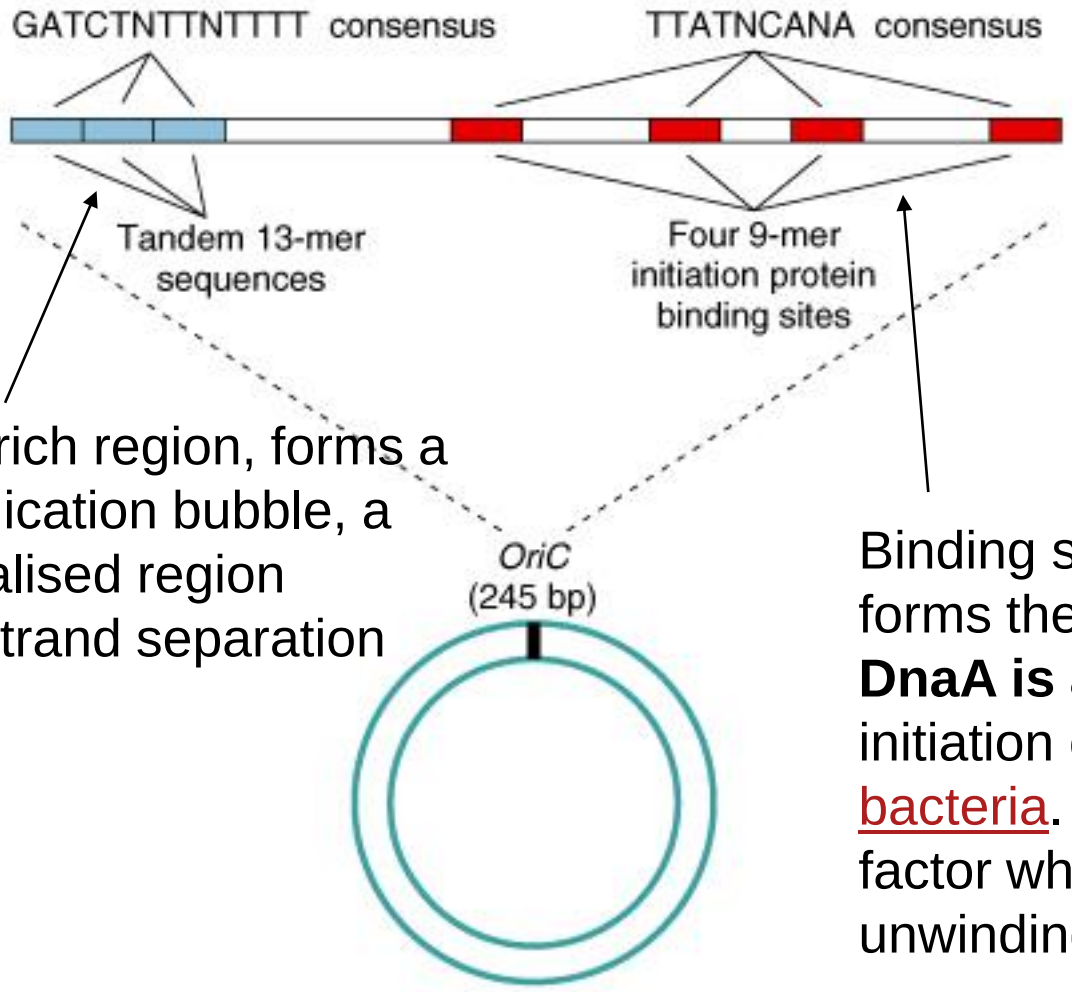


FIGURE 11.6 Bidirectional replication of the *E. coli* chromosome. The thin black arrows identify the advancing replication forks.

The origin of replication in *E. coli* (*OriC*)

OriC is 245 nucleotides long and has **two different repeat elements, the three 13-mer repeats are AT-rich.**

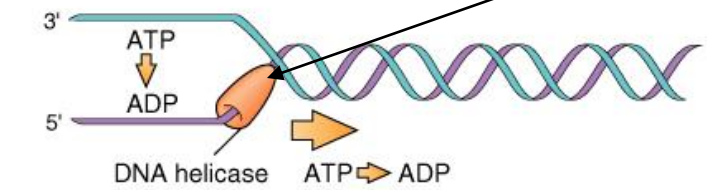
9-mer initiation site

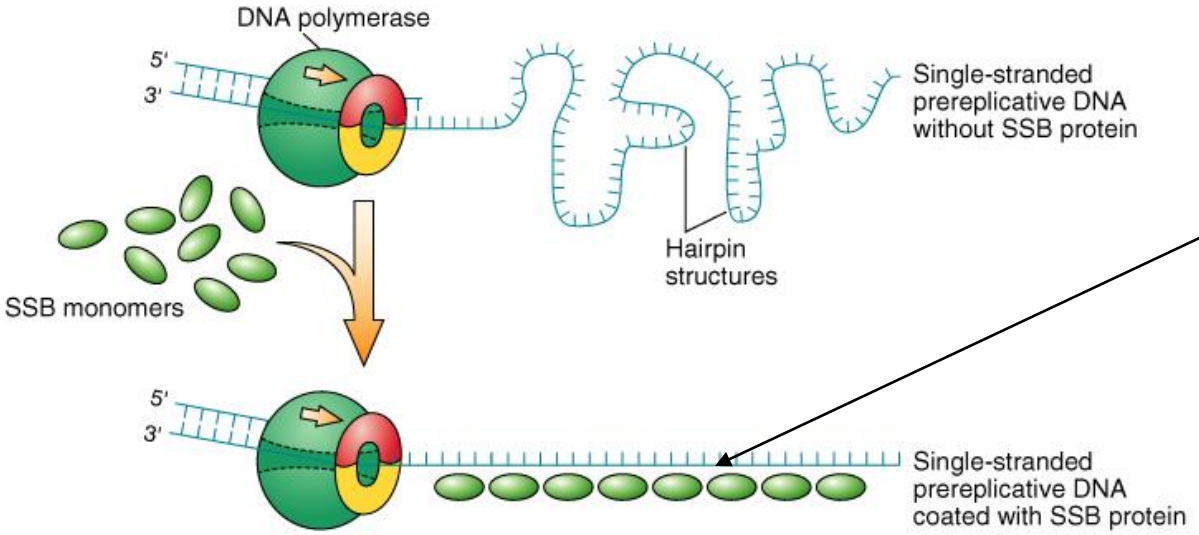


AT rich region, forms a replication bubble, a localised region of strand separation

Binding site for a protein that forms the replication bubble. **DnaA is a** protein that activates initiation of DNA replication in bacteria. It is a replication initiation factor which promotes the unwinding of DNA at oriC.

DNA must be **unwound** to allow synthesis: DNA helicase (DnaB protein)





Single stranded binding (SSB) protein attaches to the single stranded DNA to prevent reannealing

Topoisomerase enzyme

- DNA must be cut to allow it to be unwound and rotate during synthesis
  - **DNA topoisomerases (also called gyrase)**
- Topoisomerase I puts a single stranded break (phosphodiester bonds) in DNA ahead of the replication fork
- This allows the DNA to swivel as it is unwound

https://www.youtube.com/watch?v=k4fbPUGKurI

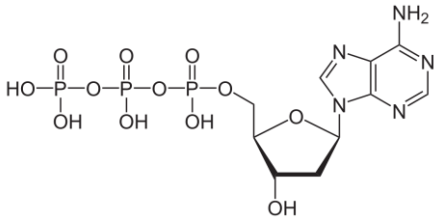
2 min

DNA Polymerases

- DNA polymerases are enzymes which catalyse the **synthesis of DNA from a DNA template**
- All DNA polymerases catalyse the addition of deoxyribonucleotide 5’-triphosphates (abbreviated dNTPs) **to the 3'-OH of the growing chain**
- As the name suggests dNTPs contain 3 phosphates
- DNA synthesis proceeds in the **5' to 3' direction**

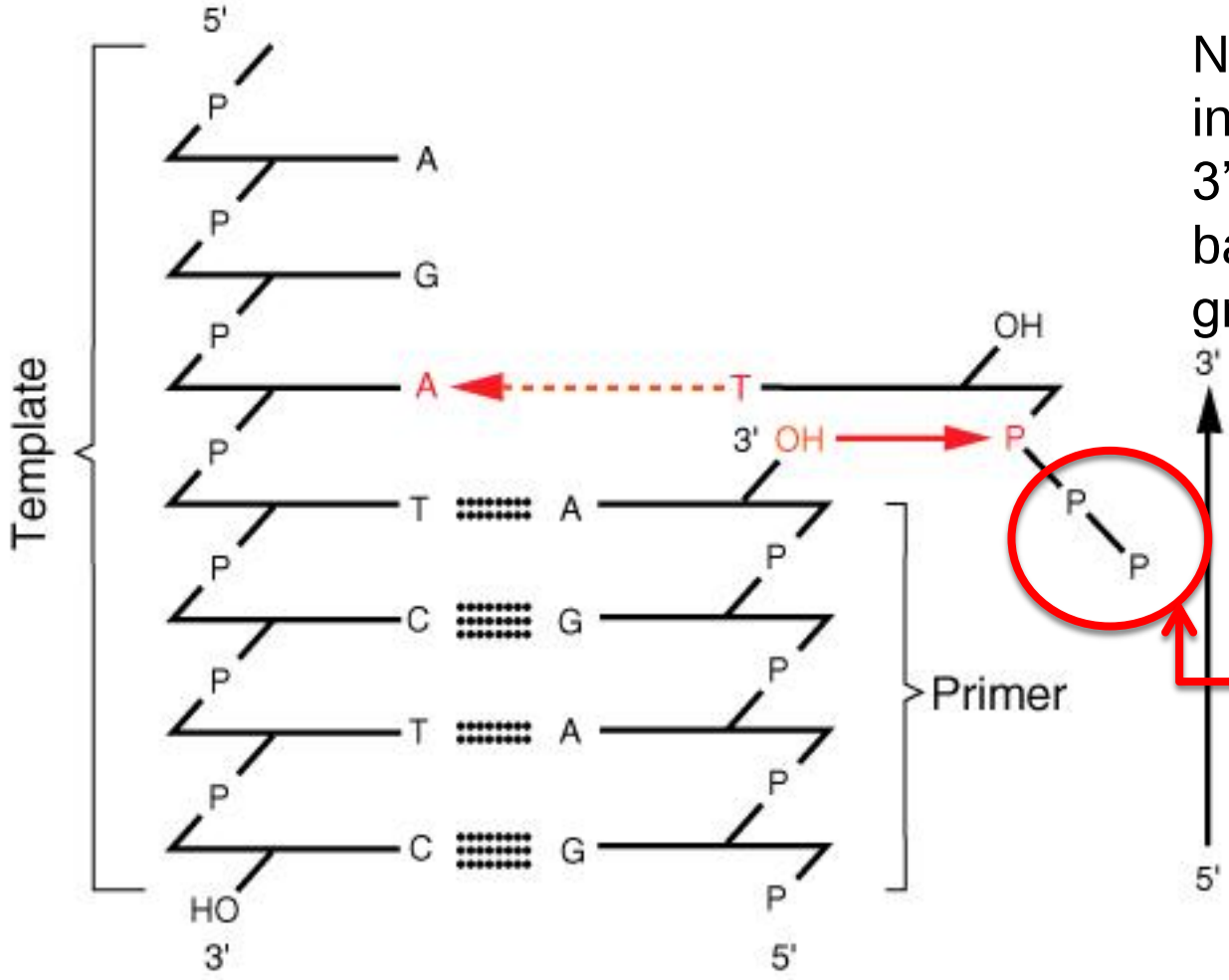
Basic rules of polymerases

- 1. Polymerisation occurs **only 5' to 3'**
- 2. Polymerisation requires a template to copy: the complementary strand.
- 3. Polymerisation requires 4 dNTPs: dATP, dGTP, dCTP, dTTP
- 4. Polymerisation requires a **pre-existing primer from which to extend**
  - The primer is **RNA** in most organisms



deoxyadenosine 5’-triphosphate

Basic mechanism of DNA polymerases

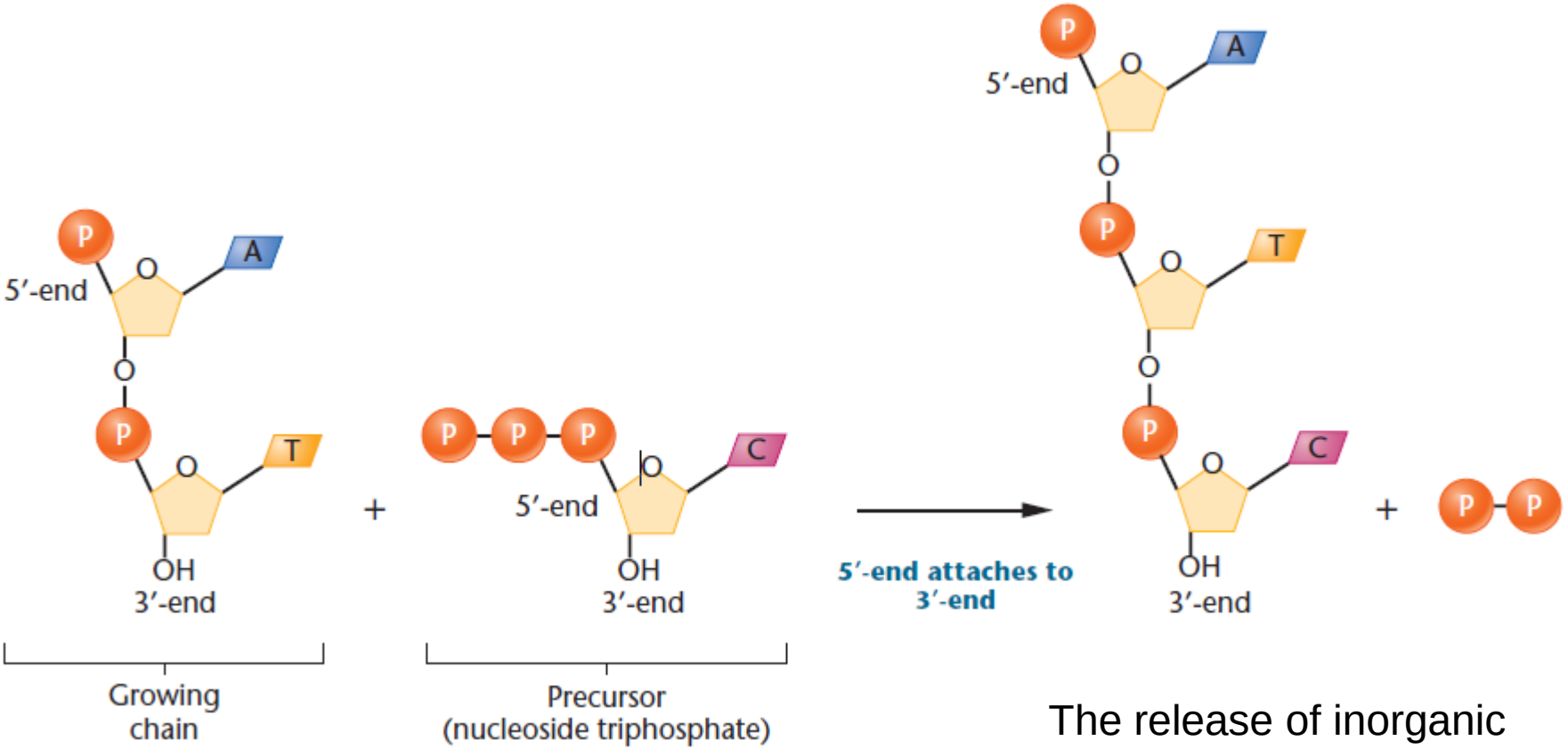


Note the addition of incoming dNTP to the 3'-OH, of the previous base, thus the chain grows from 5' to 3'

B and γ phosphates are removed to incorporate dTNP.

Basic mechanism of DNA polymerases

NEED TO KNOW



The release of inorganic pyrophosphate drives the reaction energetically

Fig. 11.8

DNA synthesis must start with a primer

- Chain synthesis by DNA polymerases must start from a **short oligonucleotide RNA primer** (10 to 12 nucleotides long in E.coli)
- DNA polymerase adds a dNTP to the 3'-OH of the primer, directed by complementary base pairing with the strand to be copied
  - the template strand is the strand that is copied

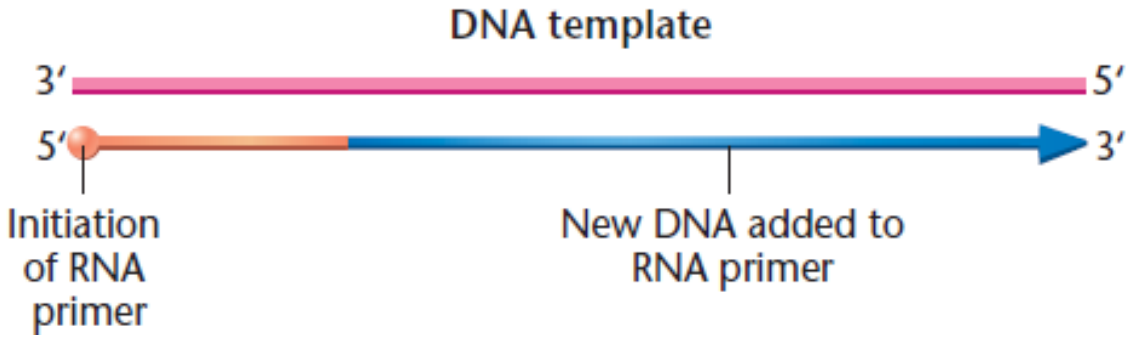
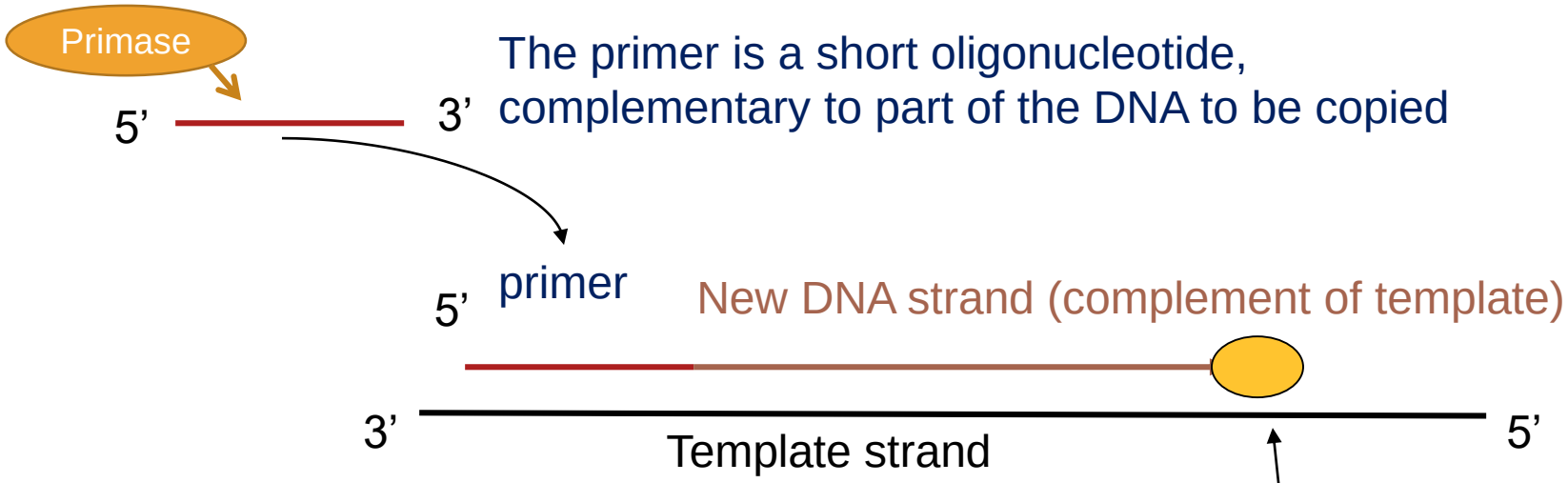


Fig. 11.10

DNA synthesis must **start** with a primer



Think of it this way: a new sugar phosphate backbone with attached complementary bases needs to be built in the daughter strand. The RNA primers job is to kick this off and provide the attachment sites for the growing chain

Types of DNA polymerases in *E. coli*

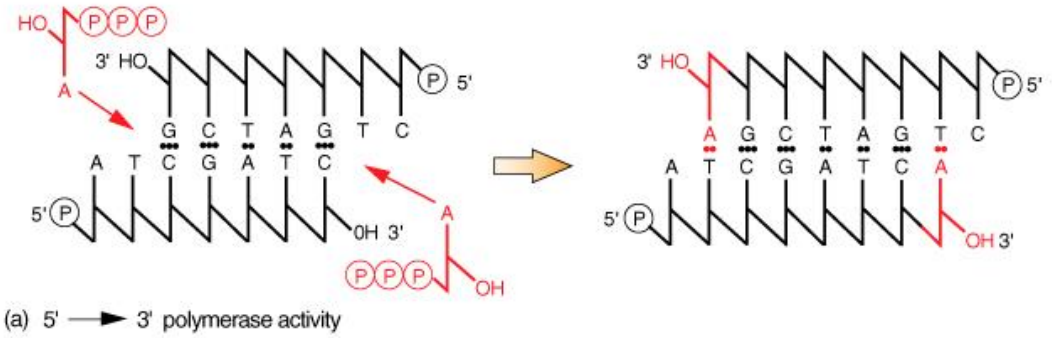
- There are **five DNA polymerases in *E. coli***
  - DNA polymerase I
  - DNA polymerase II
  - DNA polymerase III
  - DNA polymerases IV and V

  - Named by their discovery- all important
- Each polymerase has a **specific role in DNA replication**

DNA Polymerase I

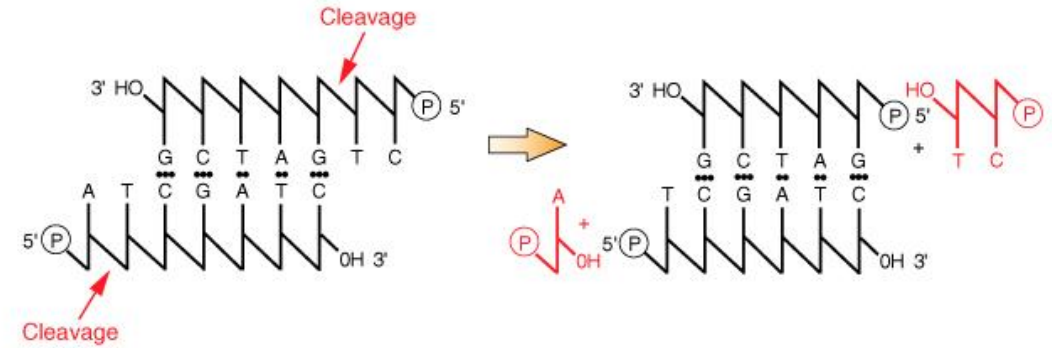
- First DNA polymerase isolated
- This enzyme has three activities:
  - **5' → 3' polymerase activity**
  - **5' → 3' exonuclease activity**
  - **3' → 5' exonuclease activity**
- Note that an **exonuclease is an enzyme which digests nucleic acids** (in this case DNA) from one end
- Why should a polymerase possess exonuclease activities?

The three activities of DNA Polymerase I



(a) 5' → 3' polymerase activity

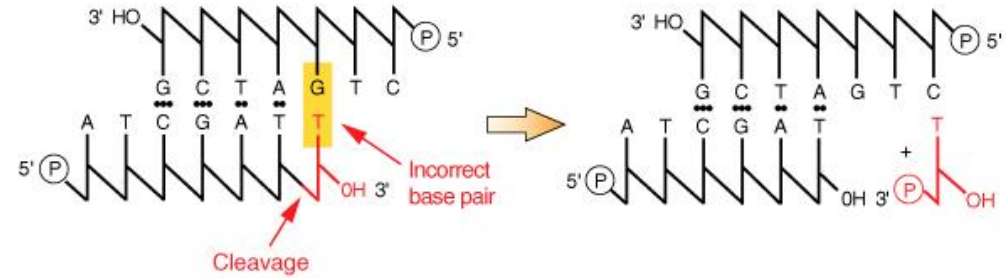
5' → 3' polymerase



(b) 5' → 3' exonuclease activity

5' → 3'exonuclease

Fix mistakes



(c) 3' → 5' exonuclease activity

3' → 5' exonuclease

Remove RNA primer

REMEMBER: DNA Polymerase I

- **DNA polymerase I is not the enzyme that replicates DNA**
  - Evidence for this: *E. coli* mutants lacking DNA polymerase I can still replicate DNA

- DNA polymerase I is required for **DNA repair, proof reading and removal of RNA primers**
  - Mutants with defective polymerase I are very sensitive to UV light
  - No proofreading - accumulating mutations

The other *E.coli* DNA polymerases

- DNA polymerase II is a minor enzyme involved in DNA repair
  - It has a 5' to 3' polymerase activity and a 3' to 5' exonuclease function
- DNA polymerases IV and V are minor repair enzymes

- **THE BIG PLAYER:**
- **DNA polymerase III is the enzyme that catalyses the semiconservative replication of DNA**

DNA polymerase III of *E. coli*

- This is a complex enzyme with many different protein subunits
  - The different subunits have specific roles in DNA replication
  - It has 5' to 3' polymerase and 3' to 5' exonuclease activities

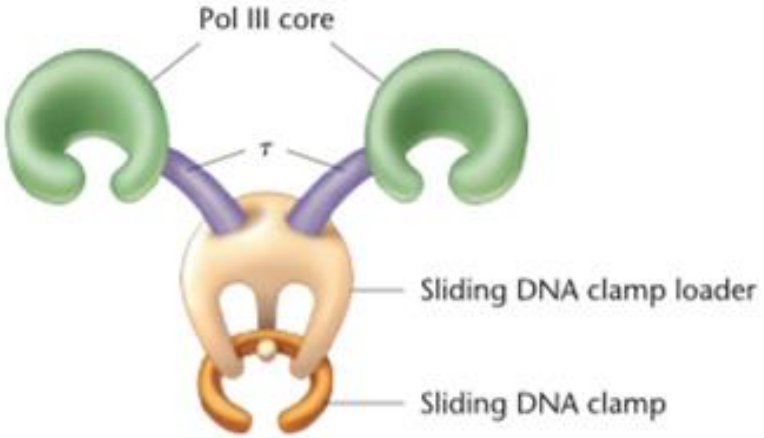


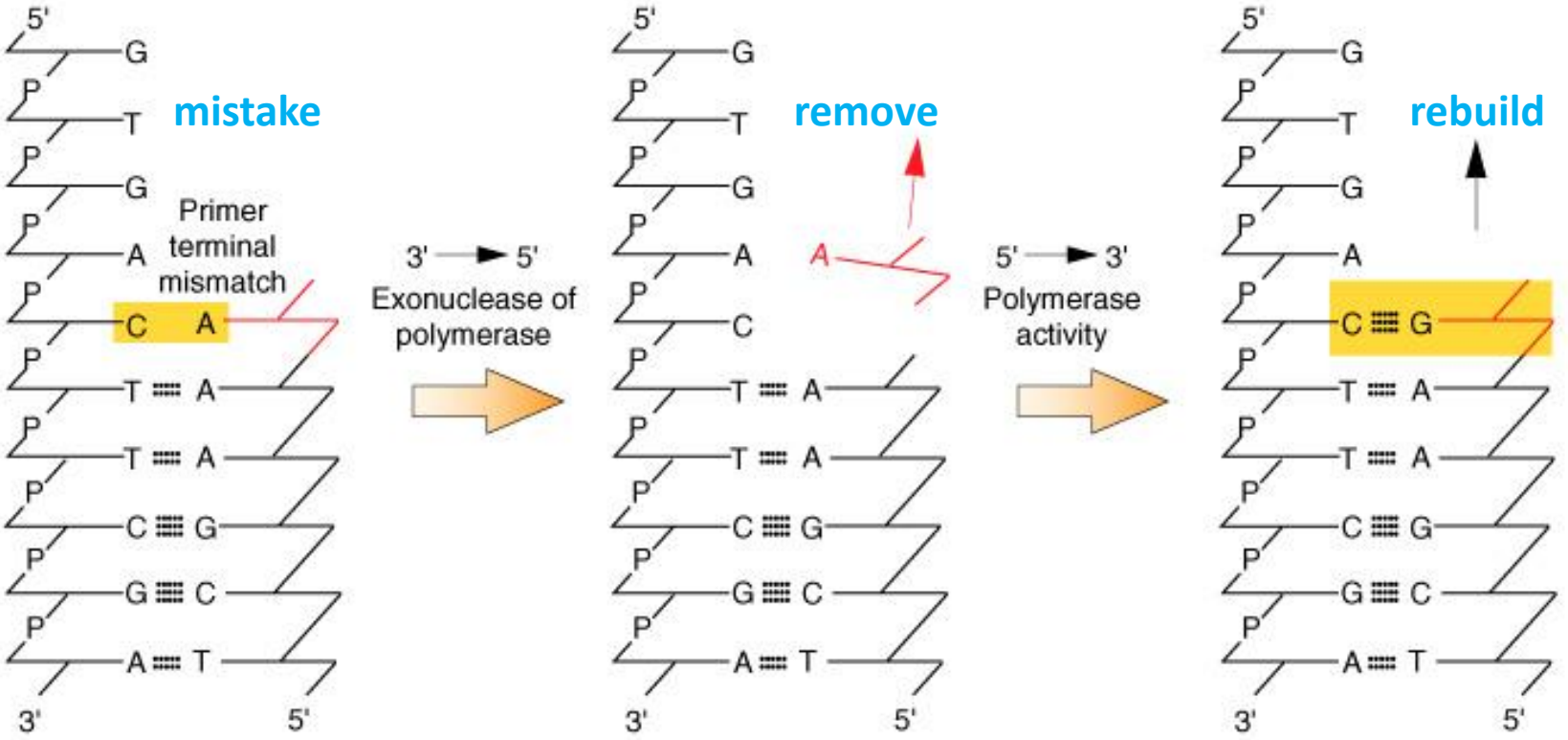
FIGURE 11.9 The components making up the DNA Pol III holoenzyme, as described in the text. While there may be three core enzyme complexes present in the holoenzyme, for simplicity, we illustrate only two.

Proof reading

- Maintains the fidelity of DNA replication
- The DNA polymerases have a proof reading ability that remove mismatched bases
  - Proof reading is one function of the exonuclease activity of the polymerases
  - This activity is built into the monomeric DNA polymerase I, but is carried out by the ε-subunit of DNA polymerase III
  - 6 billion bases that are replicated in human cells during DNA replication. Error rate of 1 in 10 billion.

Proof reading

- Uses the 3’ – 5’ exonuclease activity of DNA polymerase

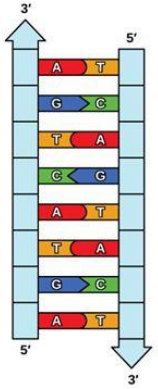


Summary of key points

- DNA synthesis is carried out by enzymes called DNA polymerases
- All DNA polymerases have an absolute requirement for a primer
- The primer is extended by the polymerase copying the template strand
- DNA synthesis occurs from a 3'OH of the primer and the chain is extended in the direction 5' – 3'
- The 3' – 5' exonuclease activities of DNA polymerases proofread new strands

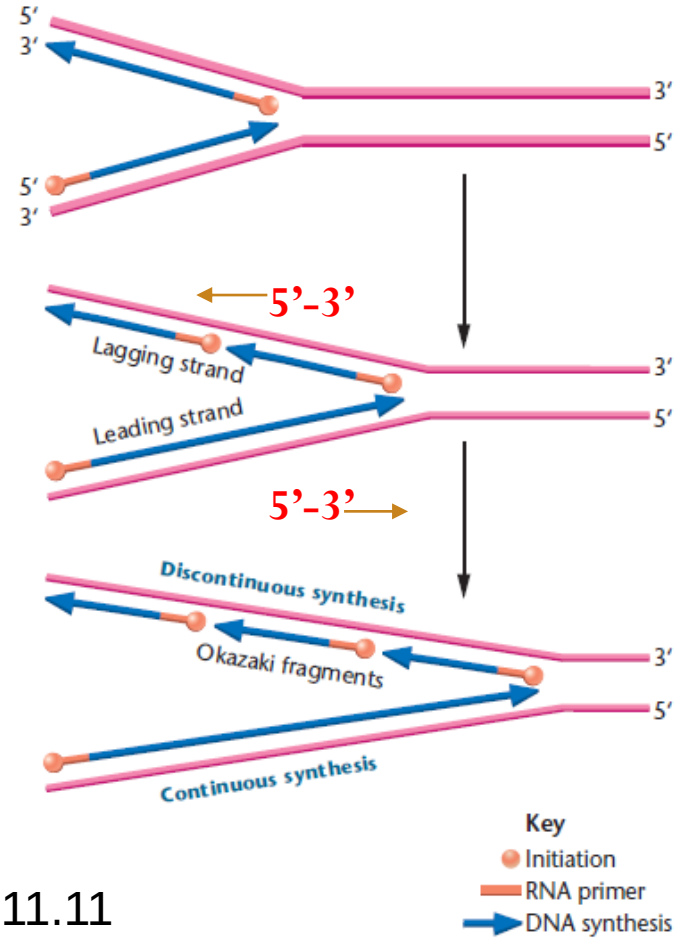
How can DNA polymerase extend both strands simultaneously?

- **All DNA polymerases synthesise DNA** only in the 5’ to 3’ direction
- Yet during replication of DNA, **both strands are being synthesised simultaneously**
- As the strands are **ANTIPARRALEL**, one strand is growing in the 3’ to 5’ direction
  - Extensive analysis has failed to find a DNA polymerase that synthesizes DNA in this direction
  - So how is this achieved??



The solution...

- Continuous synthesis on one strand, discontinuous on the other

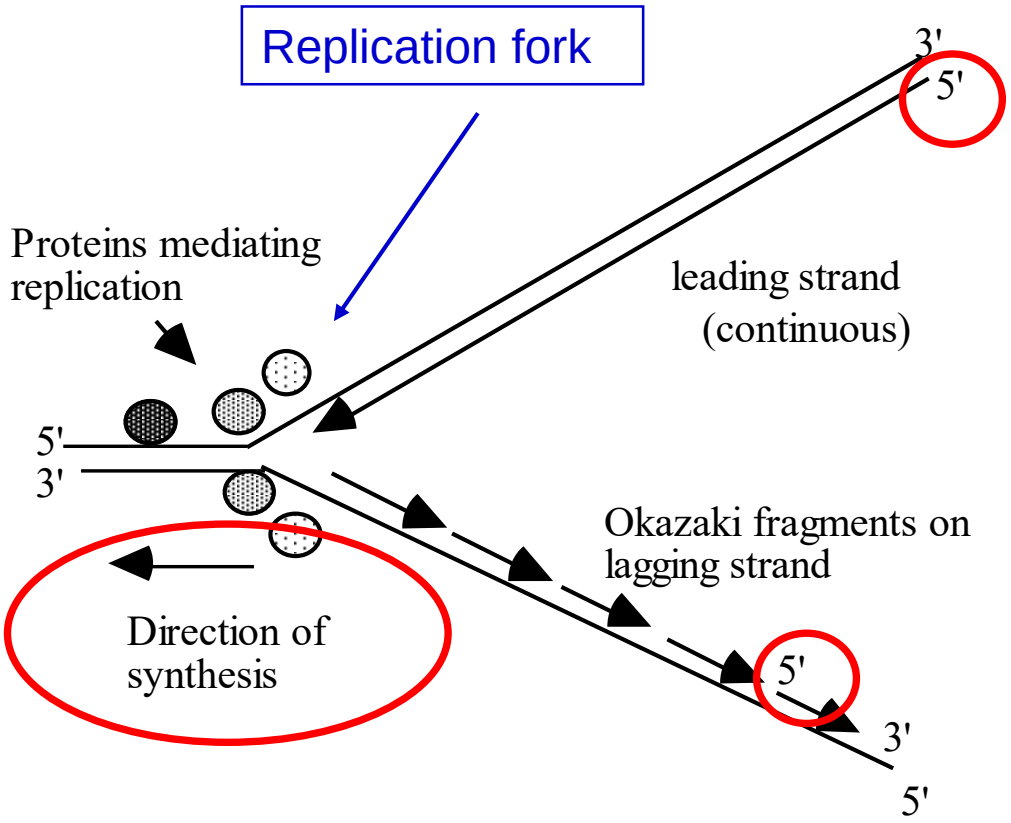


DNA synthesis on the lagging strand is actually proceeding in the opposite direction to the movement of the polymerase complex**, but is occurring in the correct 5‘ – 3' direction**

This reverse synthesis generates small DNA fragments **(Okazaki fragments)** which are then joined together (by an enzyme called DNA ligase)

Fig 11.11

The leading and lagging strands



The leading strand is being synthesised continuously.

The lagging strand synthesis is discontinuous

Lagging strand synthesis

- All DNA polymerases require a primer to start synthesis
  - The primer used is RNA and each Okazaki fragment starts from an RNA primer
- The RNA is removed by the exonuclease activity of DNA polymerase I
- The fragments are then joined by another enzyme, **DNA ligase**
  - The RNA primers are synthesised by an RNA polymerase called **DNA primase**

Lagging strand synthesis

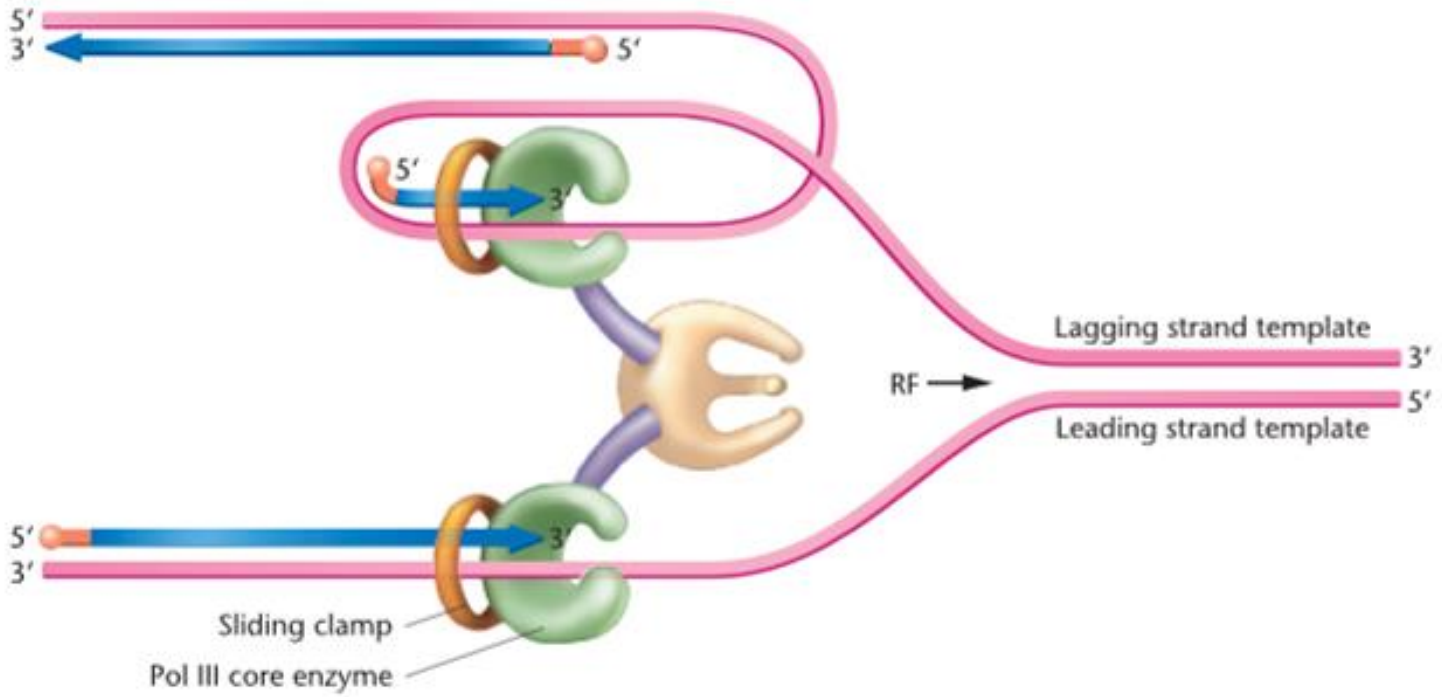


FIGURE 11.12 Illustration of how concurrent DNA synthesis may be achieved on both the leading and lagging strands at a single replication fork (RF). The lagging template strand is "looped" in order to invert the physical direction of synthesis, but not the biochemical direction. The enzyme functions as a dimer, with each core enzyme achieving synthesis on one or the other strand.

The replication fork in *E. coli*

NEED TO KNOW

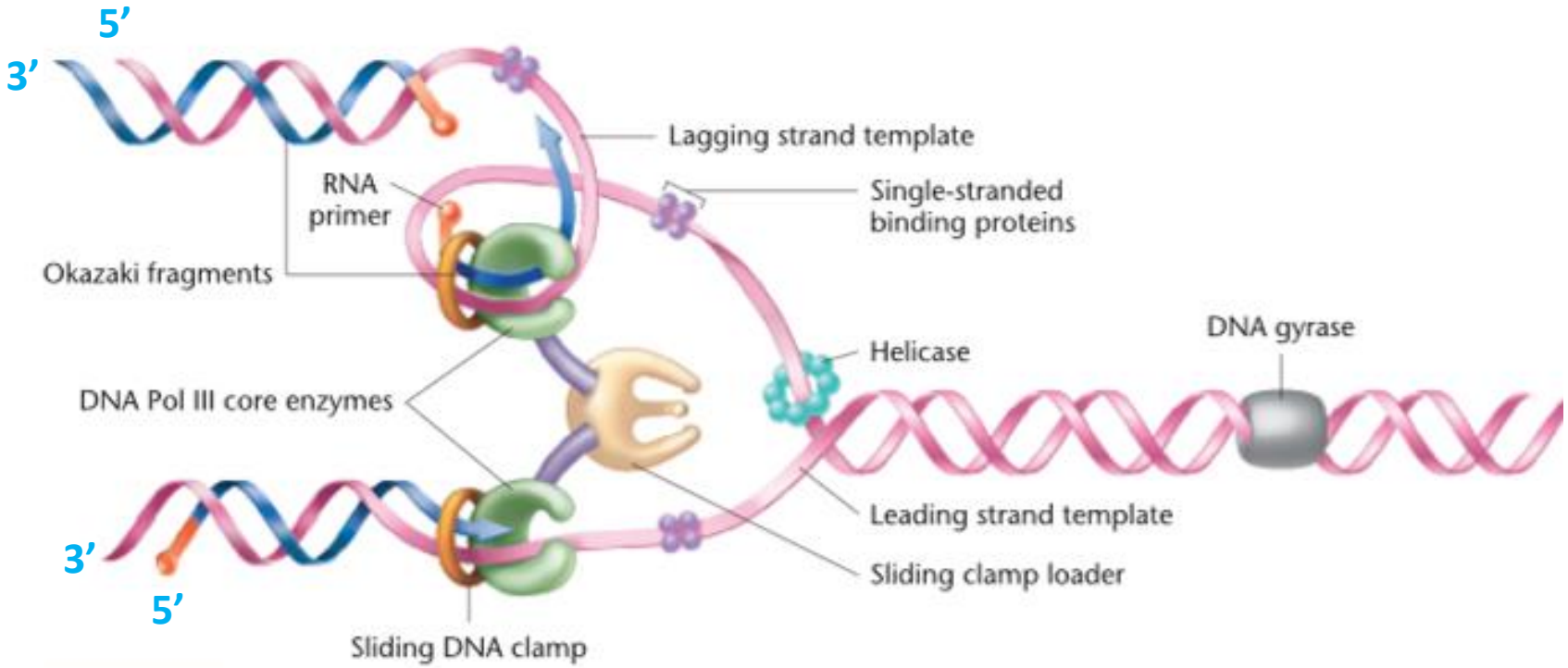


FIGURE 11.13 Summary of DNA synthesis at a single replication fork. Various enzymes and proteins essential to the process are shown.

Comparison of DNA replication in prokaryotes and eukaryotes

Prokaryotes

- RNA primers shorter (10 nucleotides)
- Okazaki fragments are longer
- Continuous DNA synthesis

Eukaryotes

- RNA primers longer (10-60 nucleotides)
- Okazaki fragments are shorter
- DNA synthesis only in S-phase

Comparison of DNA replication in prokaryotes and eukaryotes

Prokaryotes

- One origin of replication 2 replication forks
- An *E. coli* cell contains about 15 molecules of Pol III
- No nucleosomes
- Circular chromosome

Eukaryotes

- Multiple origins of replication and forks
- Multiple polymerases and different pol replicate the leading/lagging strands:
  - DNA polymerase α (alpha)*
  - DNA polymerase δ (delta)
  - Telomerase completes the synthesis of linear chromosomes

DNA Replication Rap – Music time !

https://www.youtube.com/watch?v=1L8Xb6j7A4w

3.57 min