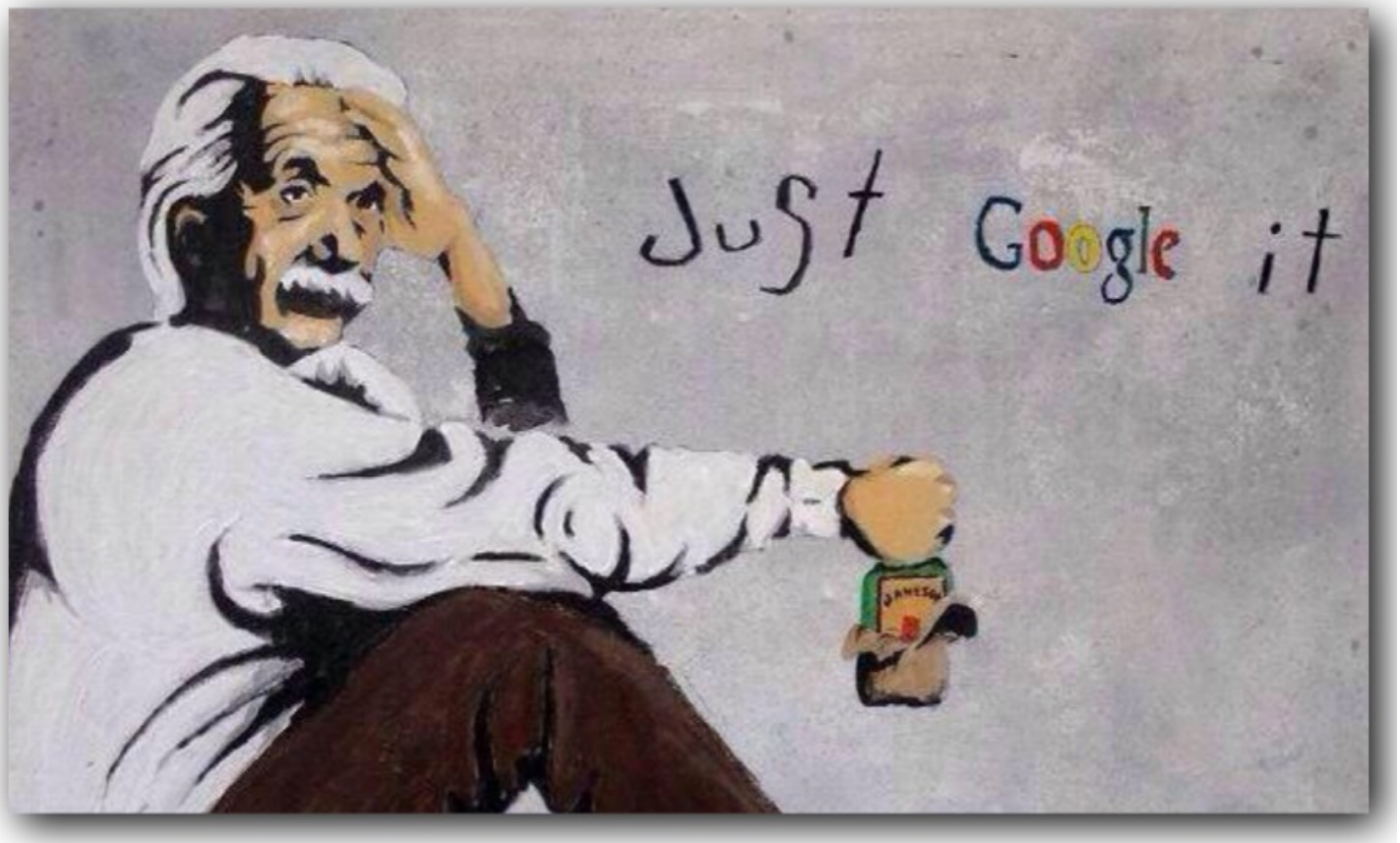
Lecture 15:

DNA replication in Eukaryotes and phage

Course 371

Lessons for life

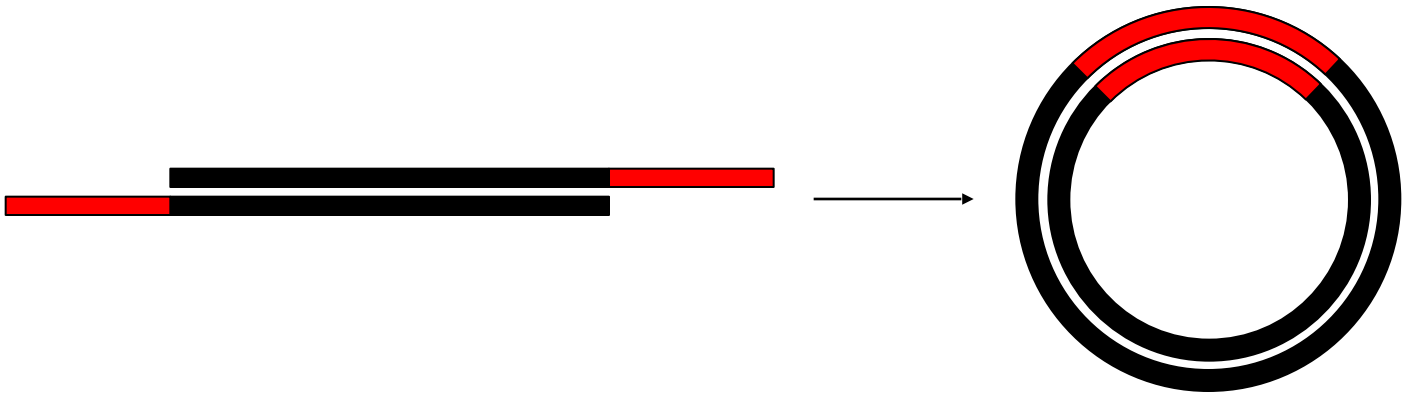


AIMS

- Understand the replication in bacteriophage and the benefits of such replication method for the virus.
- Understand the DNA replication in eukaryotes and how it differs from that of prokaryotes.
- Learn the names of the enzymes in the replication of eukaryotic genome and their equivalent in prokaryotes.
- Understand how the differences in the replication of eukaryotic DNA is fixed.

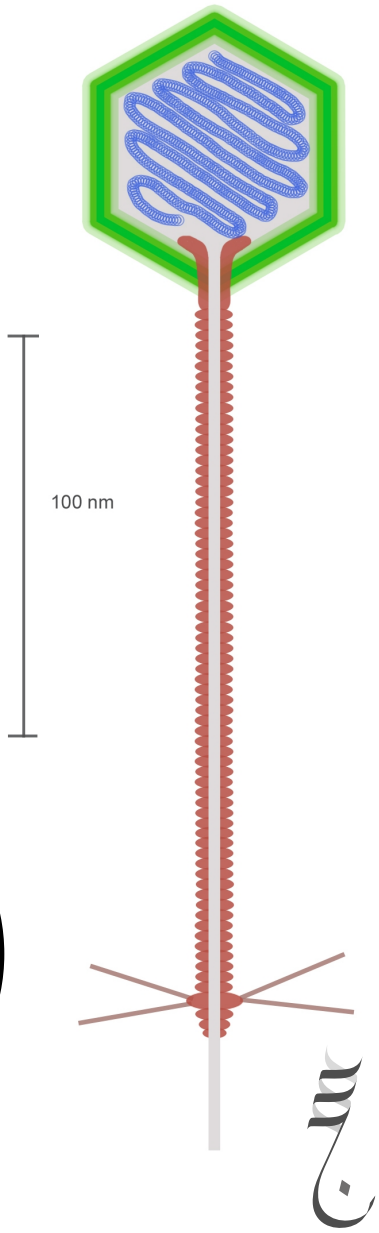
Replication in phages

- **Bacteriophage (lambda λ):** has a linear genome (ds DNA) enclosed a protein body.
- The linear genome contains sticky ends/overhang (**single strand DNA on each end**).



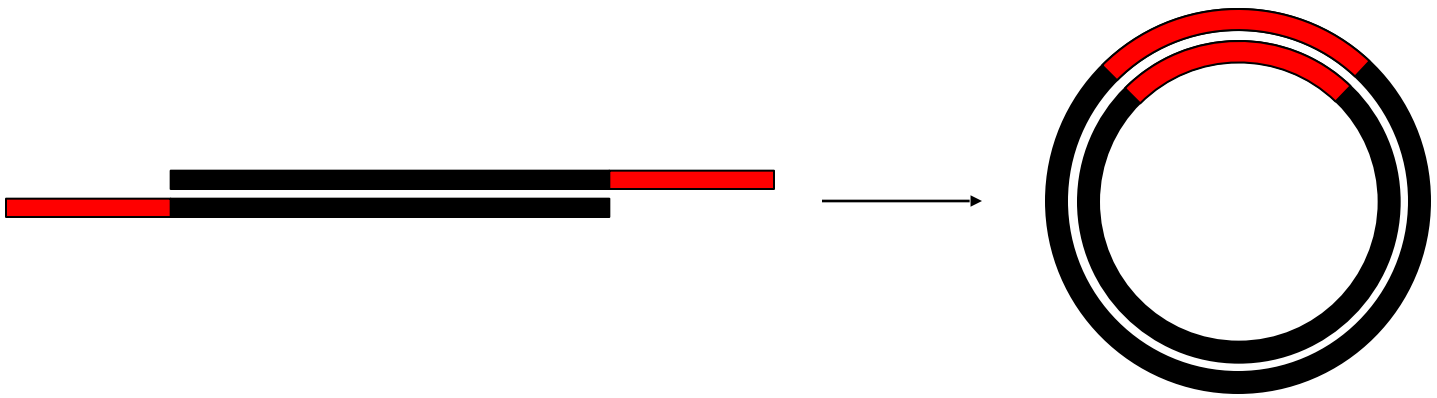
In phage body

In host cell

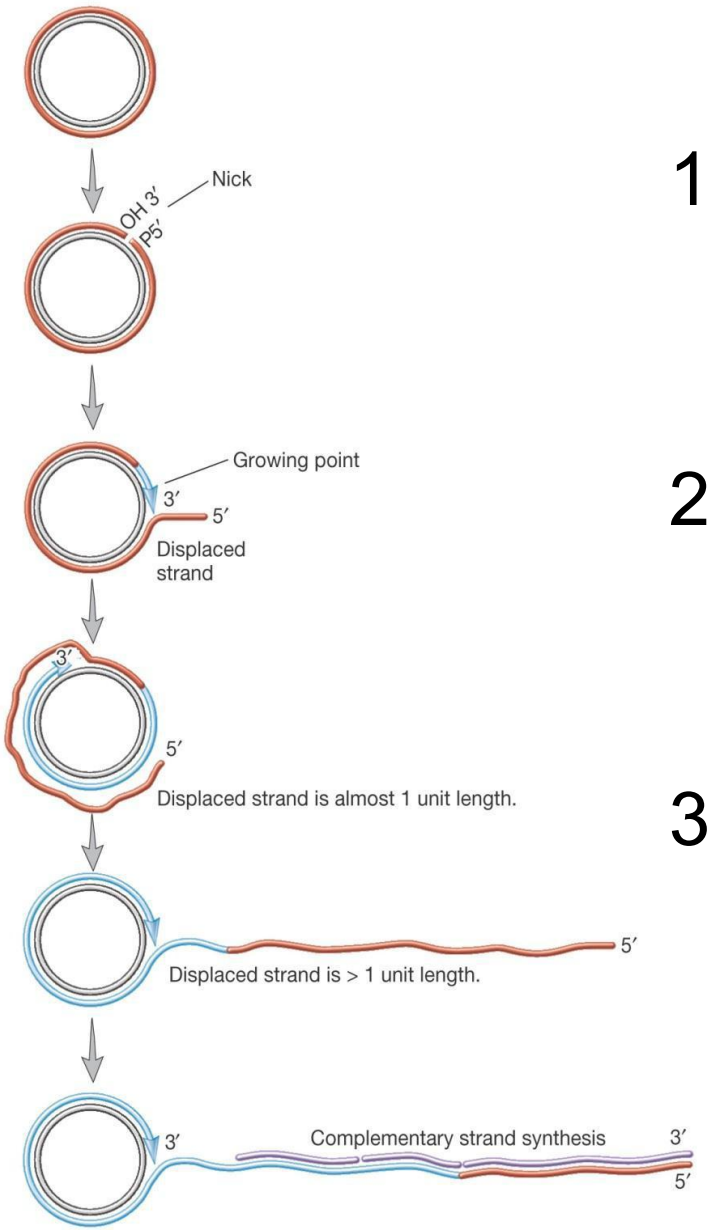


Replication in phages

- The sticky ends are complementary to each other.
- When the linear DNA of a phage is injected into a host cell, the sticky ends complement each other to make a circular DNA.

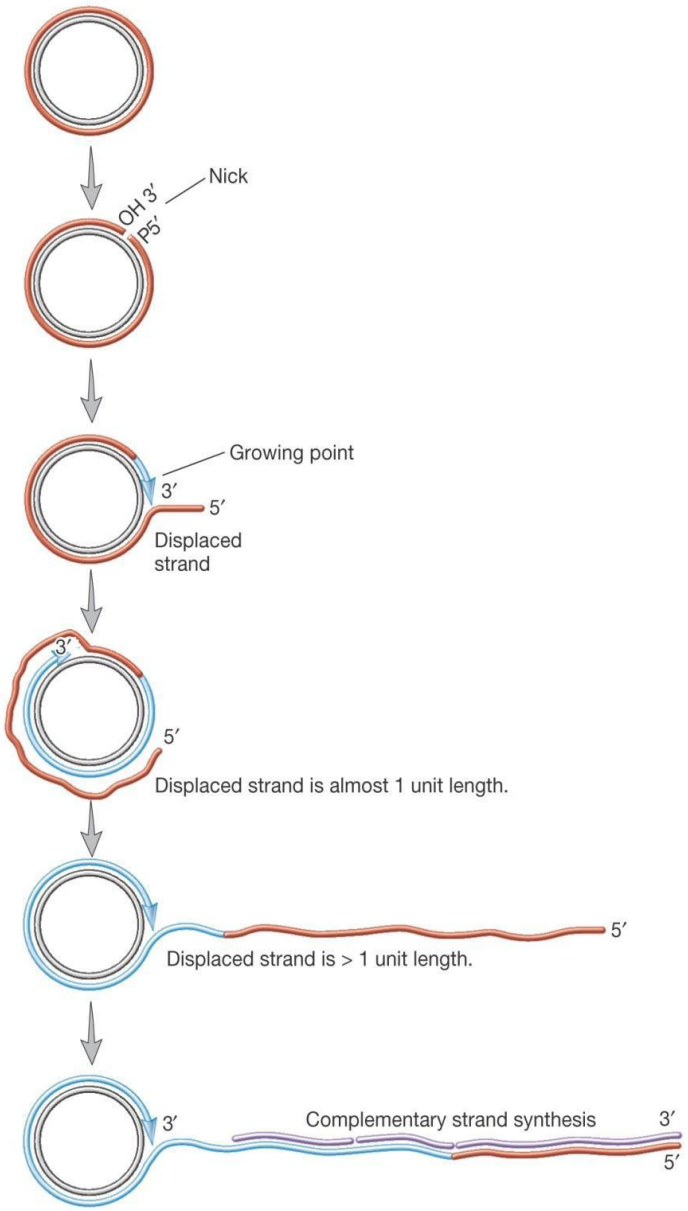


Rolling circle replication



1. Generate a nick (cut) in one of the strands at Ori.
2. The 5’ end is displaced with SSB.
3. The free 3’ end of the nick acts as a primer for DNA polymerase.

Rolling circle replication



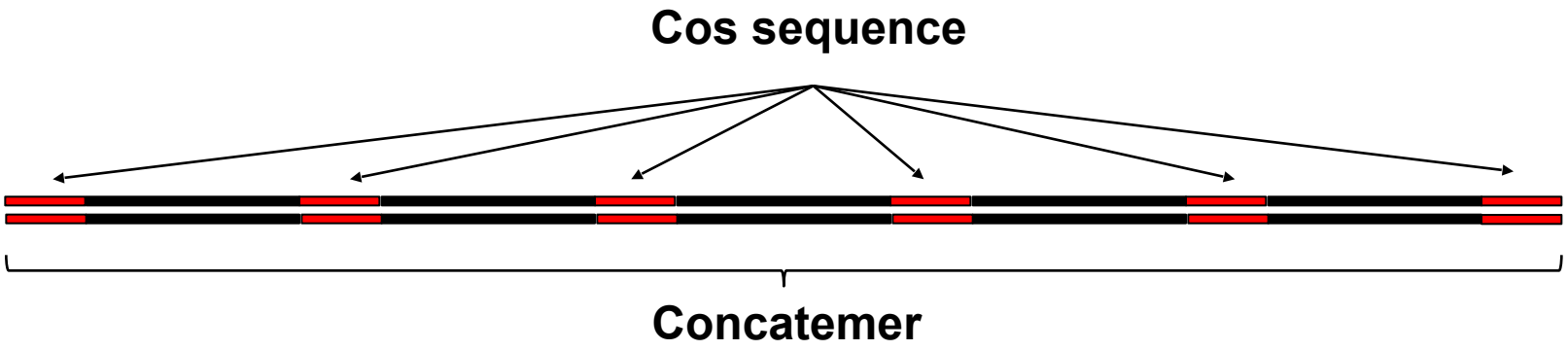
4. The single stand segment of the circular DNA acts as a template (**what kind?**).

5. Displaced single strand DNA rolls out as a free **tongue** as the replication go forward.

6. This rolls multiple times which generates many copies of the linear phage genome **all as a single molecule**.

Replication in phages

Result of the rolling circle replication is multiple copies of the virus DNA in one linear molecule called **concatemer.**

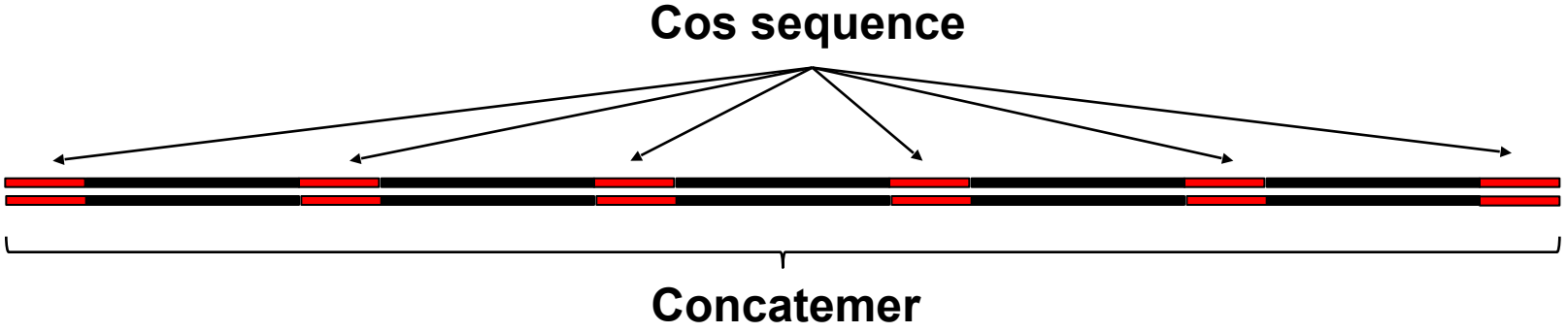


Replication in phages

• An **Endonuclease** comes to cut the **concatemer** into multiple genomes.

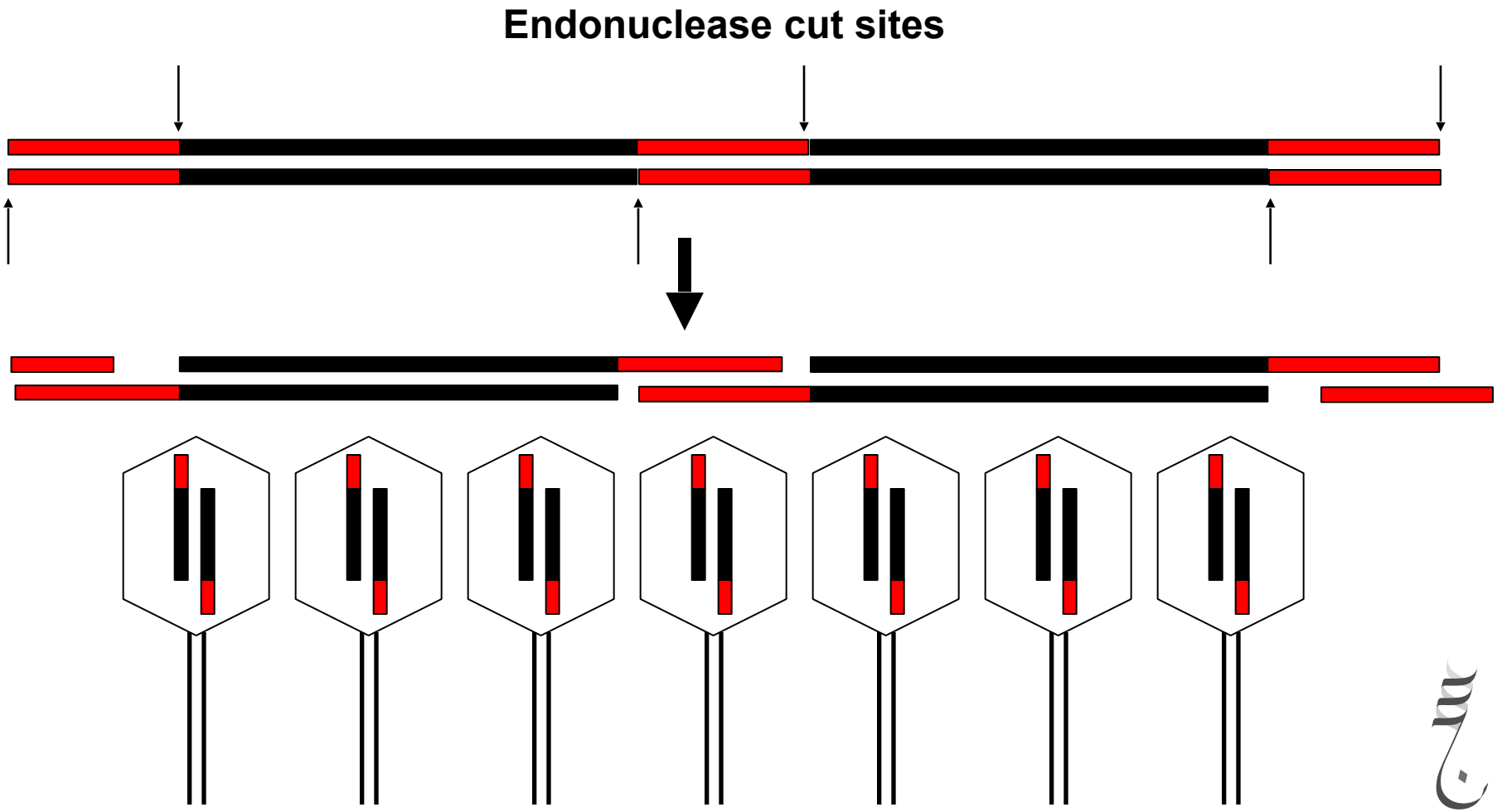
• The endonuclease binds to a specific sequence called **Cos sequence** and cuts DNA generating **sticky ends**.

• Each linear genome gets packaged into a protein body to make the progeny of phages.



Finishing phage genome

Endonuclease cuts the concatemer at the Cos sequence to generate multiple linear copies of the genome



Replication in Eukaryotes

Replication in eukaryotes is, generally similar to prokaryotes with some differences.

What are differences?

Replication in Eukaryotes

Prokaryotes	Eukaryotes
1. Single chromosome	1. Multiple chromosomes
2. Circular chromosome	2. Linear chromosomes
3. Chromosomes with **NO** ends	3. Chromosomes with ends
4. Small genome	4. Large genome
5. No nucleosomes	5. Nucleosome packaging

Replication in Eukaryotes

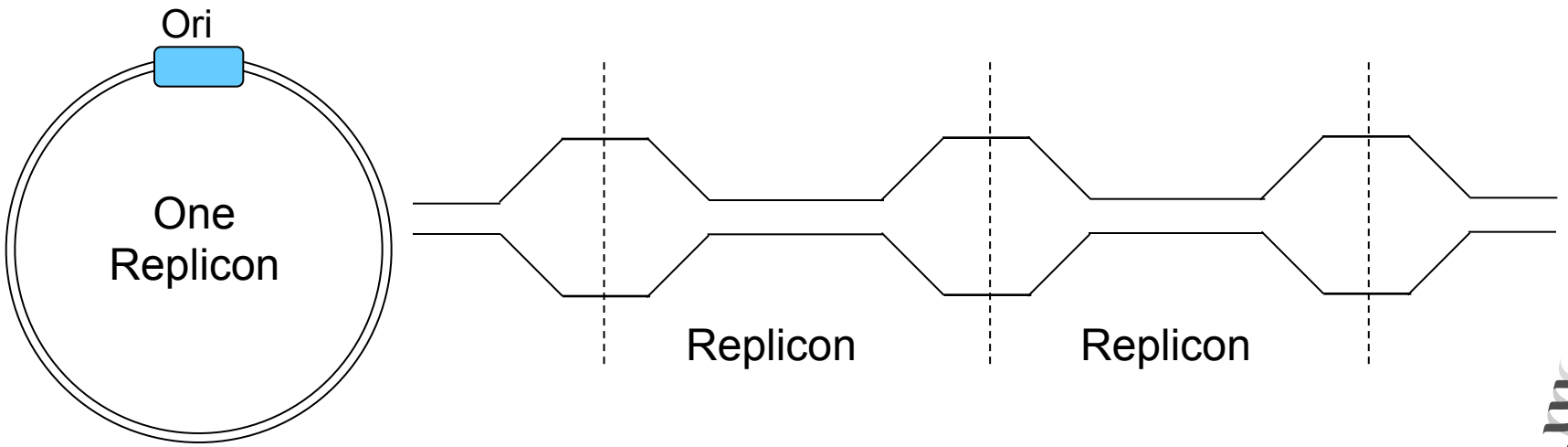
Difference #1: prokaryotes have a single origin of replication but eukaryotes have many.

- Many chromosomes → need many origins of replication.
- Large genome → need many origins of replication.

Replication in Eukaryotes

Why many Oris in Eukaryotes?

- *E.coli* genome is ~5Mb and the replication rate is very fast (1000bp/s).
- Human genome is ~3Gb and replication rate is slower (100 bp/s). To solve this (many replicons).



Initiation of replication

- DNA replication in eukaryotes start at a sequence called **autonomously replication sequence (ARS).**

What is the equivalent in prokaryotes?

- Initiation protein in eukaryotes is a multiunit complex called **origin recognition complex (ORC).**

What is the equivalent in prokaryotes?

Initiation of replication

Each origin of replication in eukaryotes is used once. Why?

Replication in Eukaryotes

Differences in the names of the enzymes

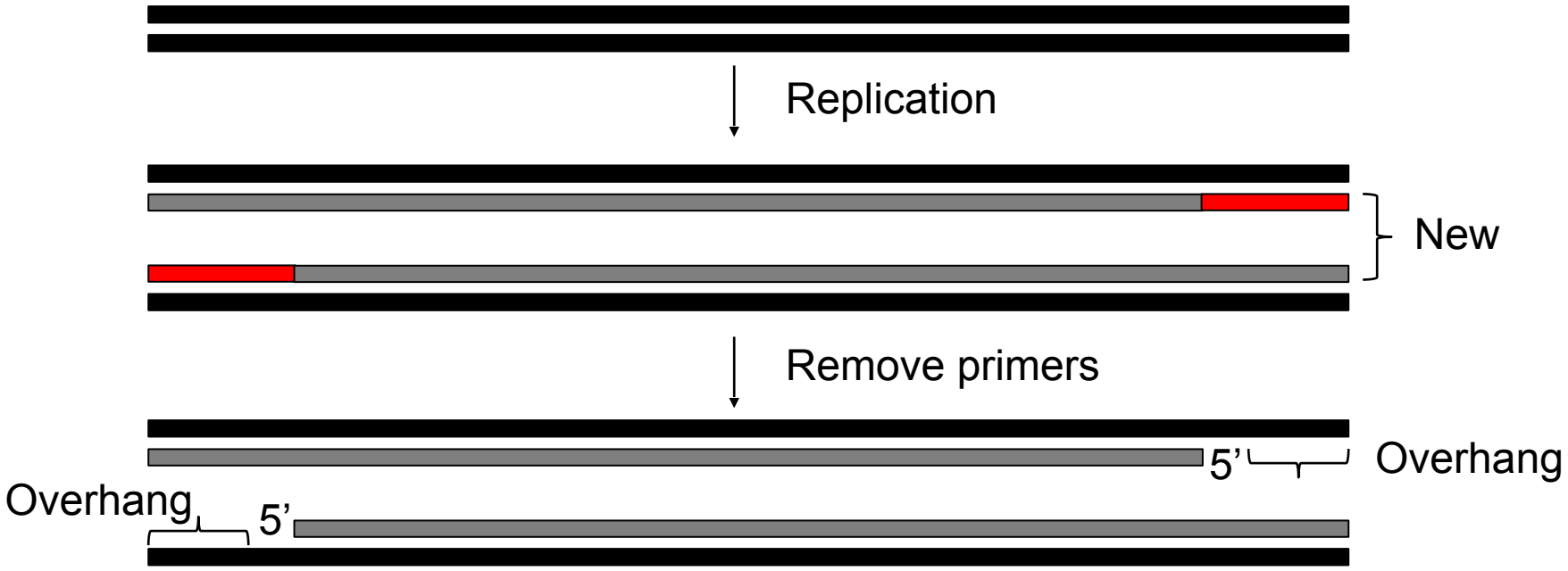
Prokaryotes	Eukaryotes
Primase	Pol (α) alpha
DNA Pol III	Pol (ε) epsilon
Pol I	Pol (δ) delta
Ligase	Eukaryotic Ligase

Replication in Eukaryotes

Difference #2: circular chromosome in prokaryote and linear chromosomes in eukaryotes.

Eukaryotic chromosomes are linear and have ends called **telomeres.**

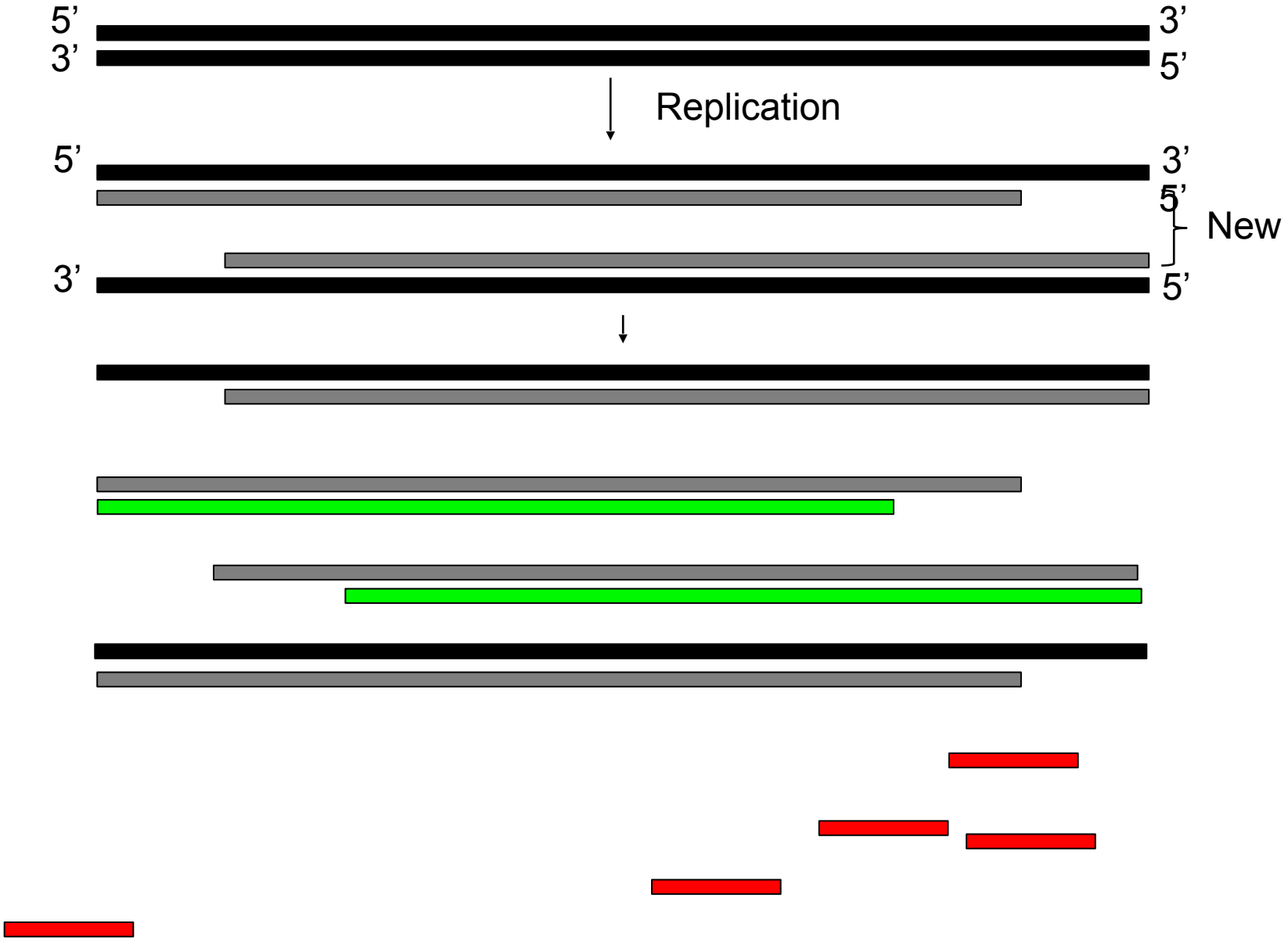
Replication in Eukaryotes



DNA Pol can not add nucleotides without 3’OH

If this does not get fixed chromosomes will get shorter every time DNA is replicated

Replication in Eukaryotes



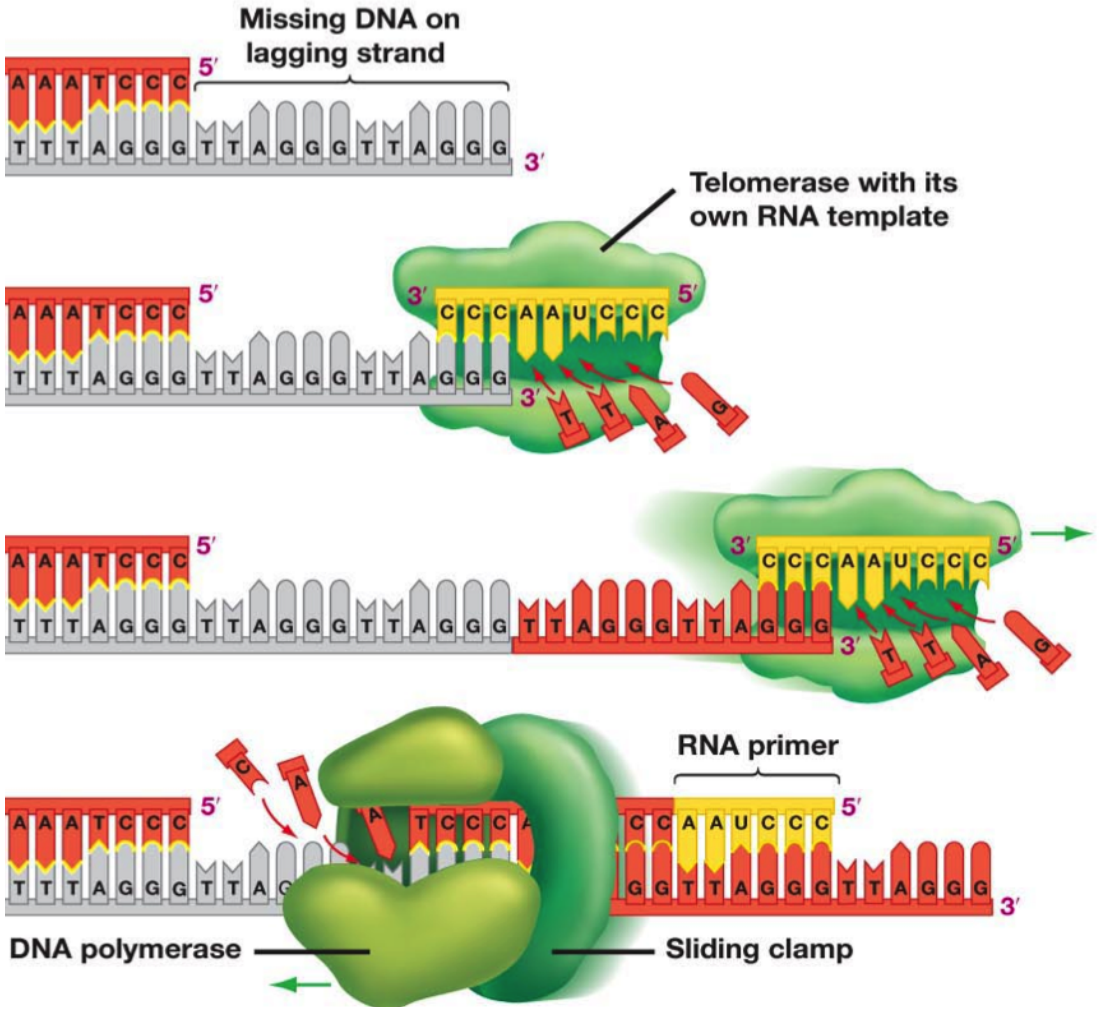
Solution: Telomere replication

- End of the chromosome (**telomere**) has a specific sequence of repeats.
- Telomere repeat sequence in human is 5’ TTAGGG 3’.
- A specific enzyme called **Telomerase** recognizes the repeat sequence (How?)
- **Telomerase is an enzyme composed of:**
  - Protein (polymerase)
  - RNA (template)

Solution: Telomere replication

- The RNA part of the **telomerase** is **complementary** to the repeat sequence of the telomere and acts as a template to regenerate the ends of the chromosomes.
- The addition of repeat sequences happens multiple times to extend the telomeres.

Solution: Telomere replication



1. End is unreplicated.

2. Telomerase extends unreplicated end.

3. Again, telomerase extends unreplicated end.

4. Lagging strand is completed.

Some Terms

- The process of making DNA from DNA template is called **Replication.**
- The process of making RNA from DNA template is called **Transcription.**
- The process of making DNA from RNA template is called **Reverse Transcription.**

So what is the process in which telomerase acts?

Nucleosome replication

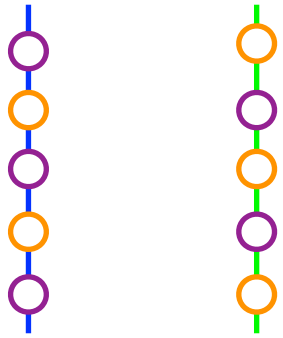
Difference #3: eukaryotic DNA is wrapped around nucleosomes.

- Nucleosomes are parts of DNA condensation in eukaryotes.

- 8 histone (octamer) proteins make a one nucleosome.
- H2A/H2B make one dimer while H3/H4 make the second dimer.

How many dimers in a single nucleosome?

Nucleosome replication



Nucleosome replication

- When replicating DNA the histones are removed to allow for the replication of DNA.
- When replication is done double the amount of nucleosomes needs to added to the old and newly replicated DNA.

So we need more histones, what do we do?

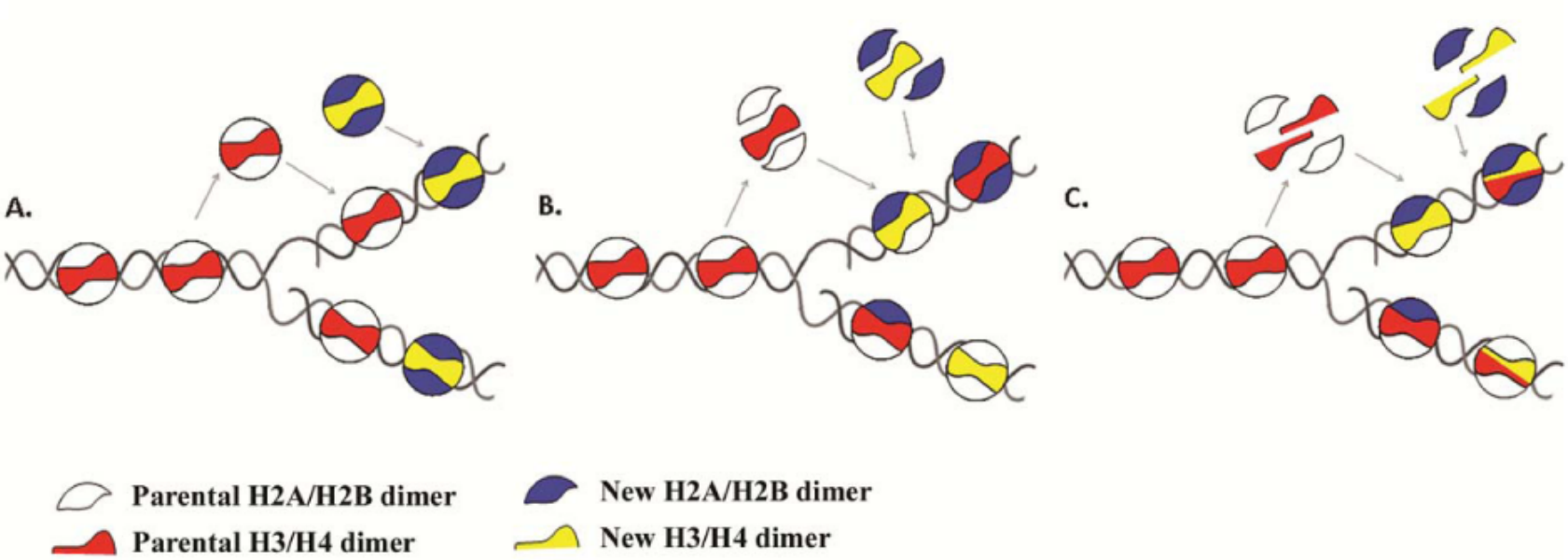
Nucleosome replication

- More histone proteins are made to accommodate the organization of two genomes.
- Nucleosomes are made of new and old dimers.
- On each genome different combination of dimers are generated:

New/new New/old Old/old

Nucleosome replication

The enzymatic machine that puts the nucleosomes to DNA is called **Histone Chaperone**



To study

Endonuclease

ORC

Replication

Cos sequence

Histone chaperone

Reverse Transcription

Pol (δ)

Telomerase

Pol (ε)

Concatemer

ARS

H2A/H2B dimer

Telomere

Replicon

Rolling circle replication

Pol (α)

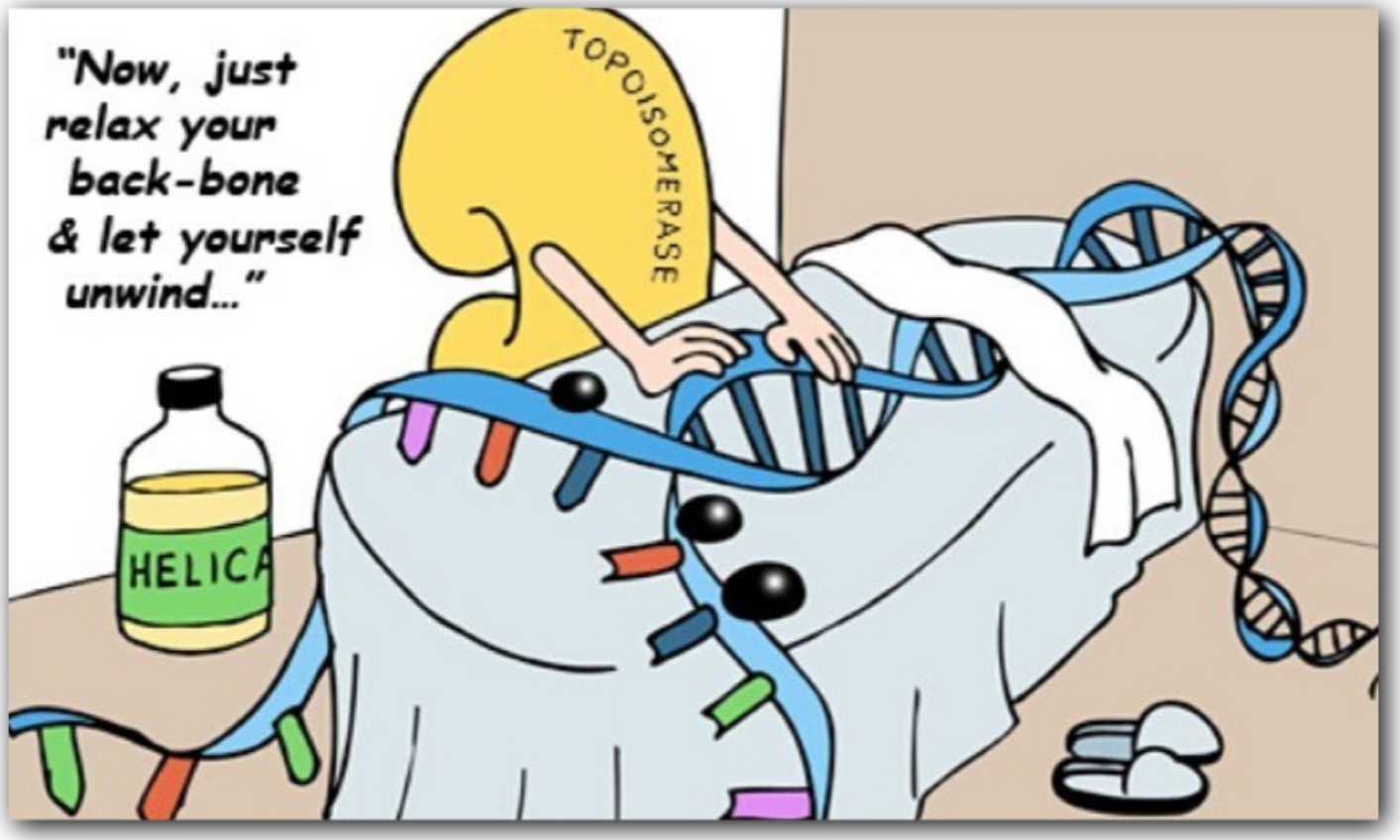
H3/H4 dimer

Transcription

Expectations

- You know the replication process in bacteriophage.
- You know that replication process in prokaryotes is similar to that of eukaryotes with some differences.
- You know the differences between eukaryotic and prokaryotic replication process and link this to the differenced in genome characteristics.
- You know the differences in enzyme names.
- You know how the ends of chromosomes are replicated.
- You know how nucleosomes are replicated.

For a smile


"Now, just relax your back-bone & let yourself unwind..."
TOPOISOMERASE
HELICA