



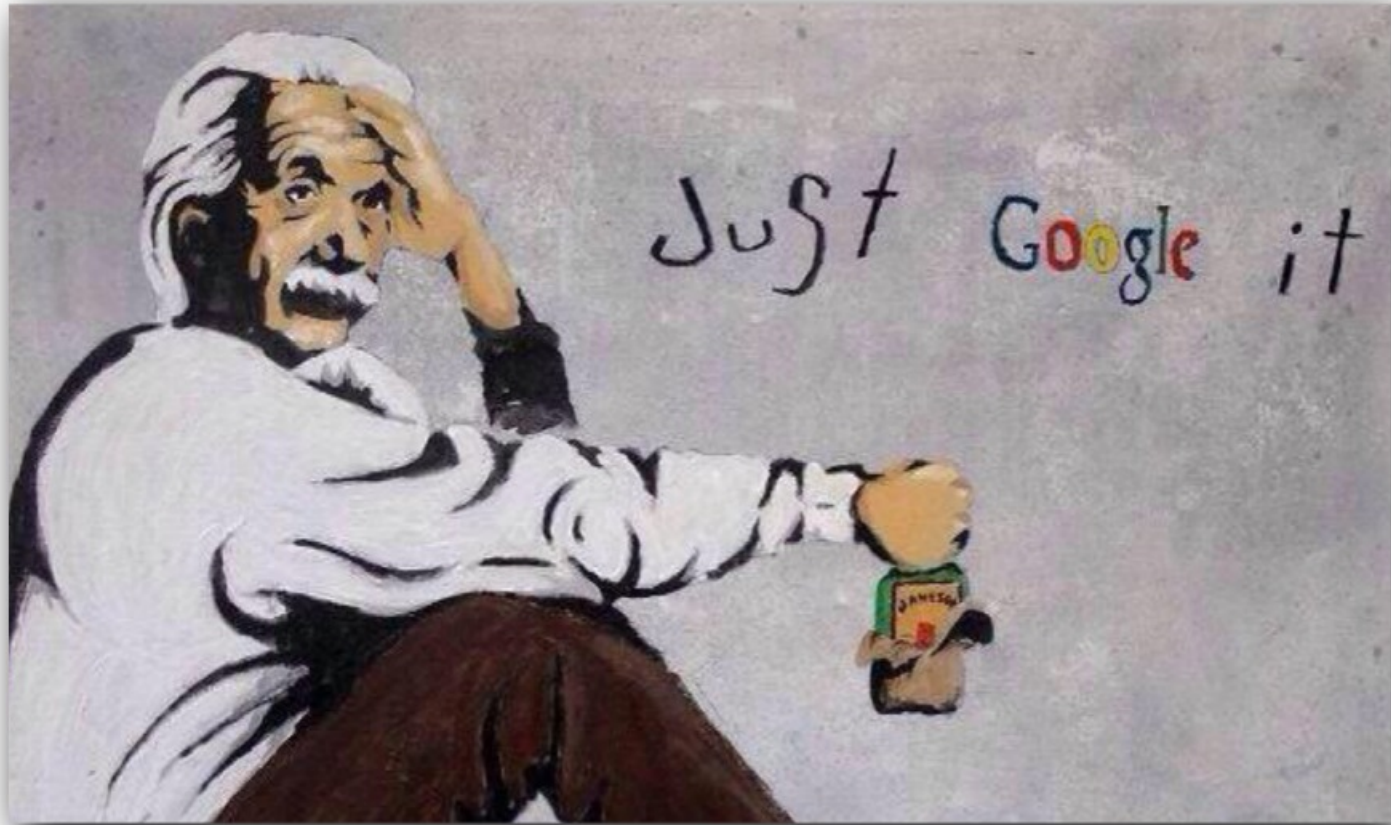
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# Lecture 10:

## DNA replication in Eukaryotes and phage

Course 281

# Lessons for life



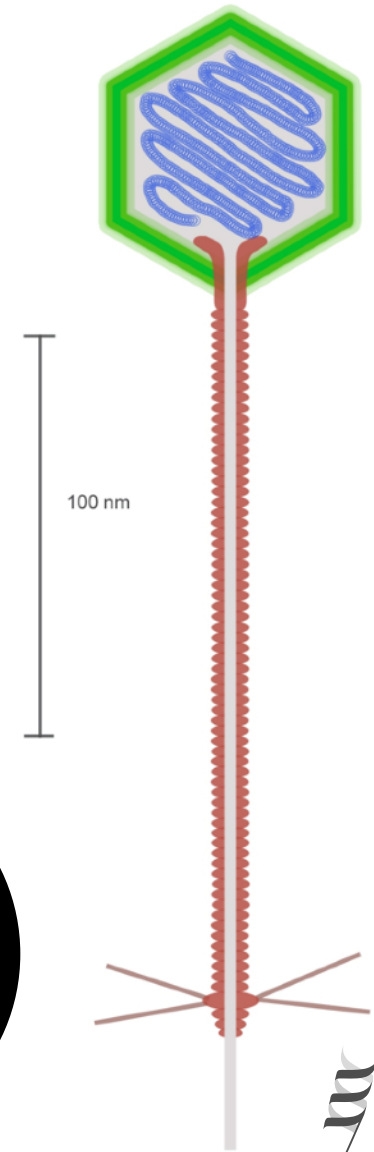
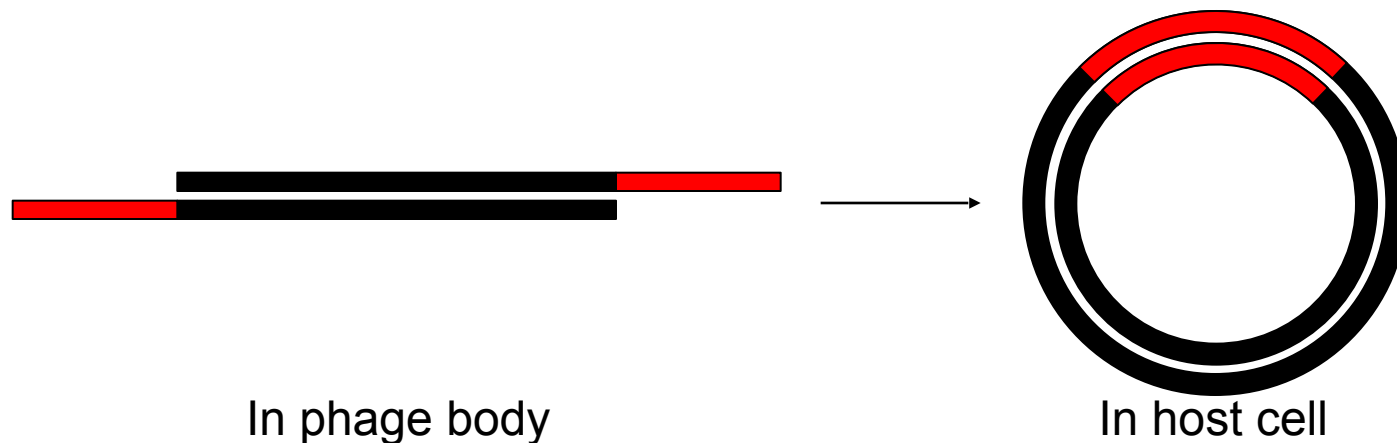
# AIMS

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- 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.

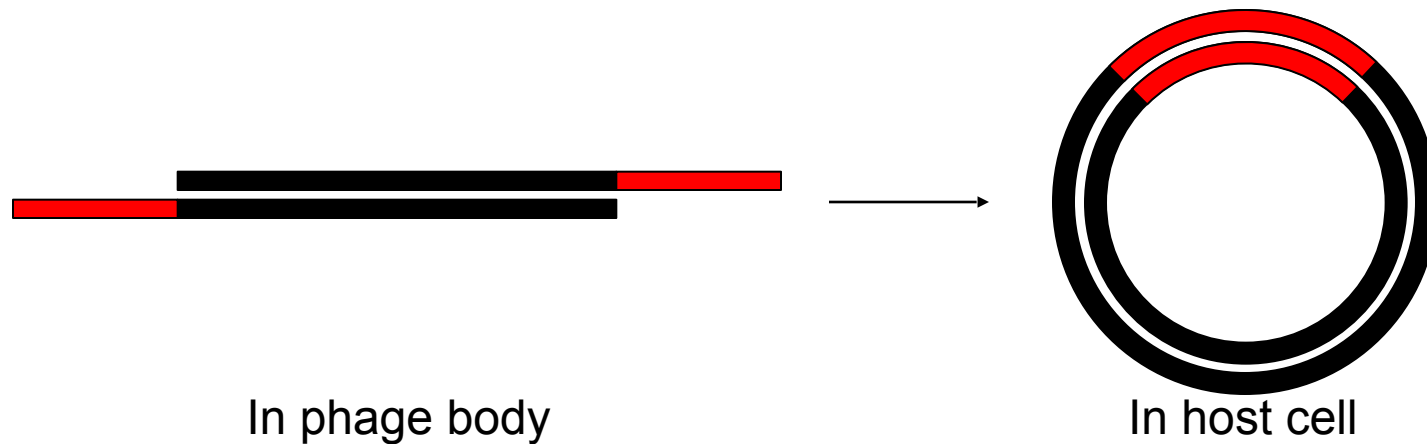
# Replication in phages

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

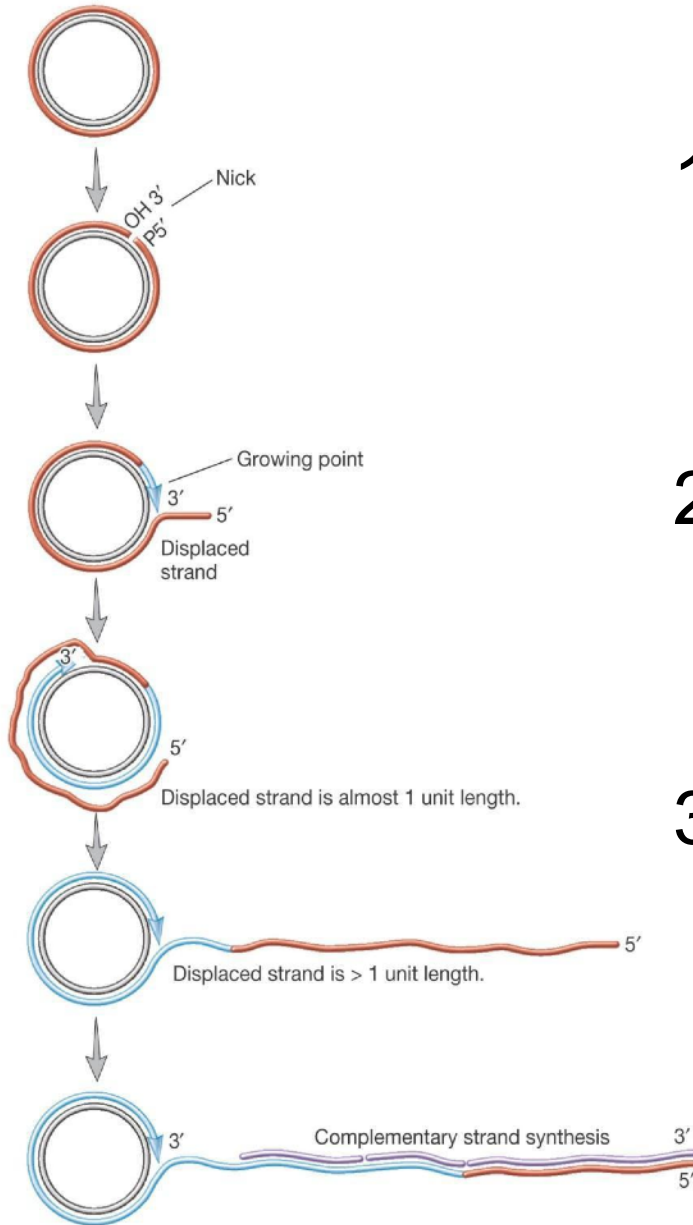


# Replication in phages

- 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.

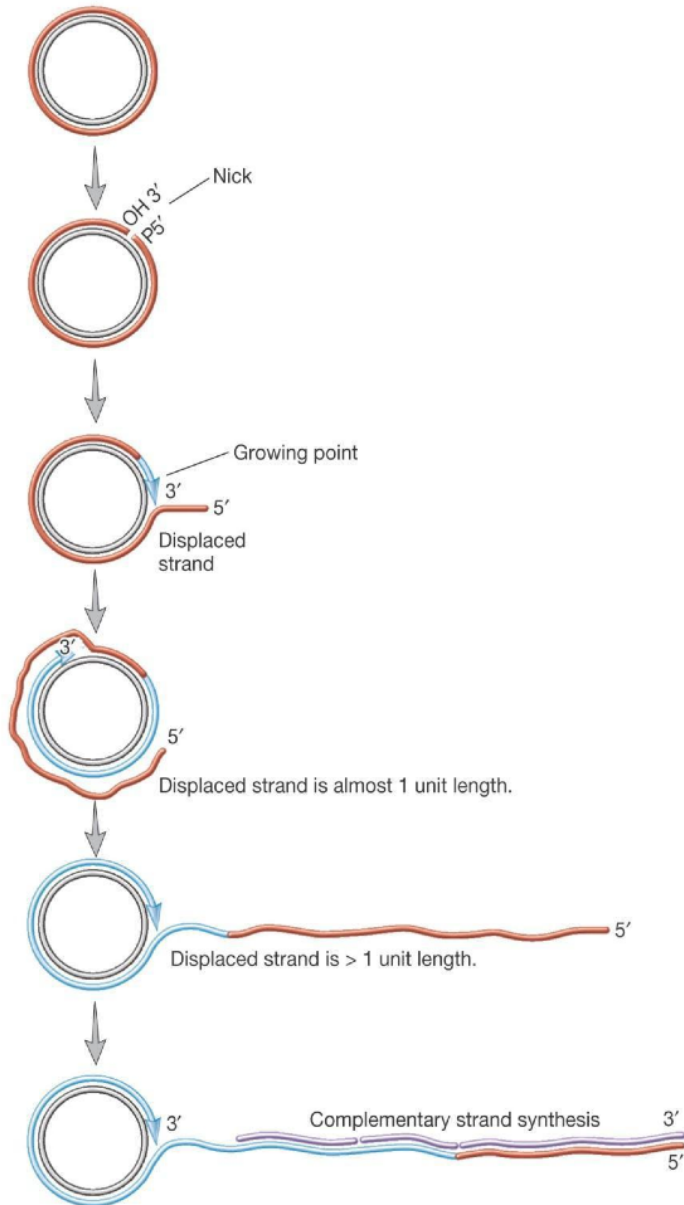


# Rolling circle replication



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



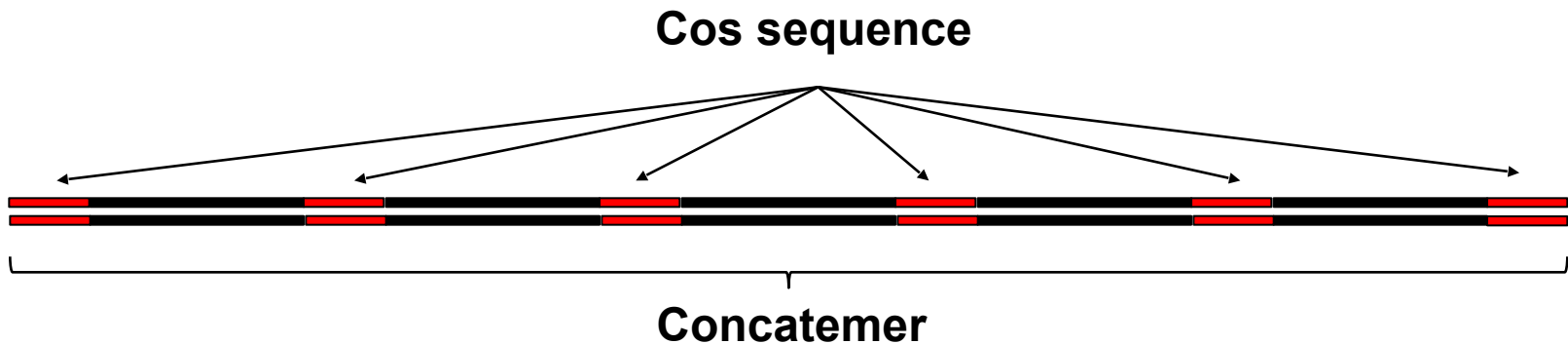
4. The single strand segment of the circular DNA acts as a template (**what kind?**).

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

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

# Replication in phages

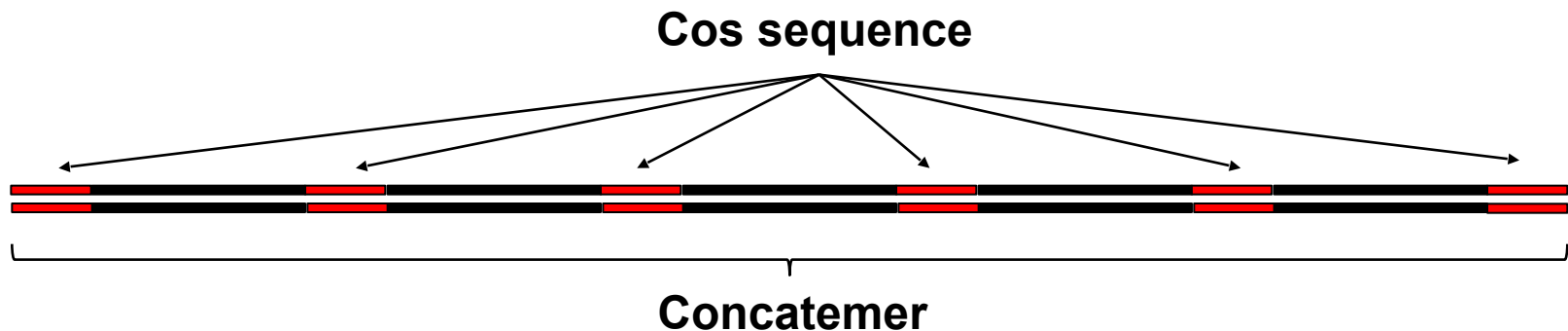
Result of the rolling circle replication is multiple copies of the virus DNA in one linear molecule called **concatemer**.





# Replication in phages

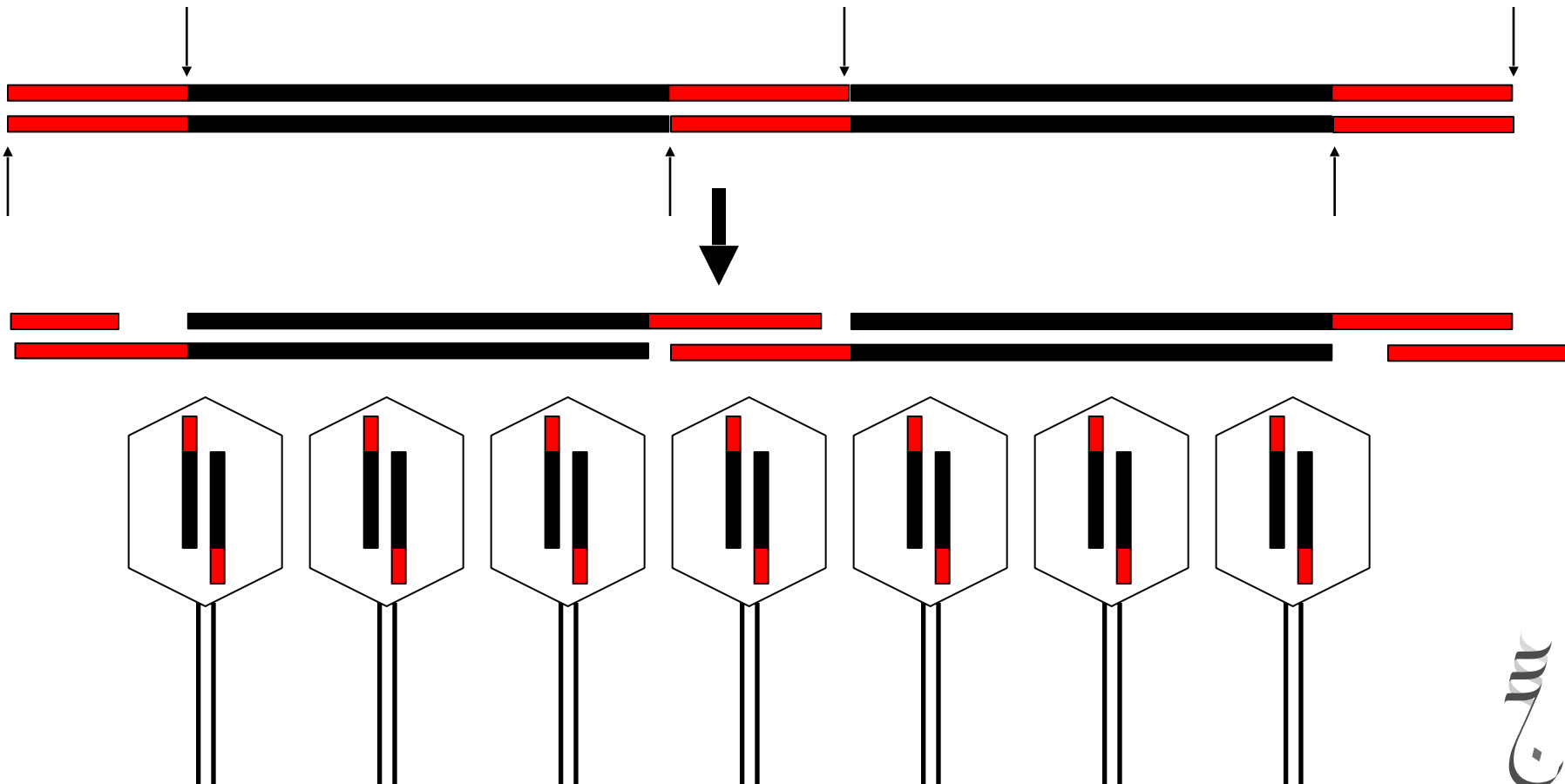
- An **Endonuclease** comes to cut the **concatemer** into multiple genomes.
- The endonuclease binds to a specific sequence called **Cos sequence** and cuts DNA generating **sticky ends**.
- Each linear genome gets packaged into a protein body to make the progeny of phages.



# Finishing phage genome

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

Endonuclease cut sites



# Replication in Eukaryotes

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

**What are differences?**

# Replication in Eukaryotes

## Prokaryotes

1. Single chromosome
2. Circular chromosome
3. Chromosomes with **NO** ends
4. Small genome
5. No nucleosomes

## Eukaryotes

1. Multiple chromosomes
2. Linear chromosomes
3. Chromosomes with ends
4. Large genome
5. Nucleosome packaging

# Replication in Eukaryotes



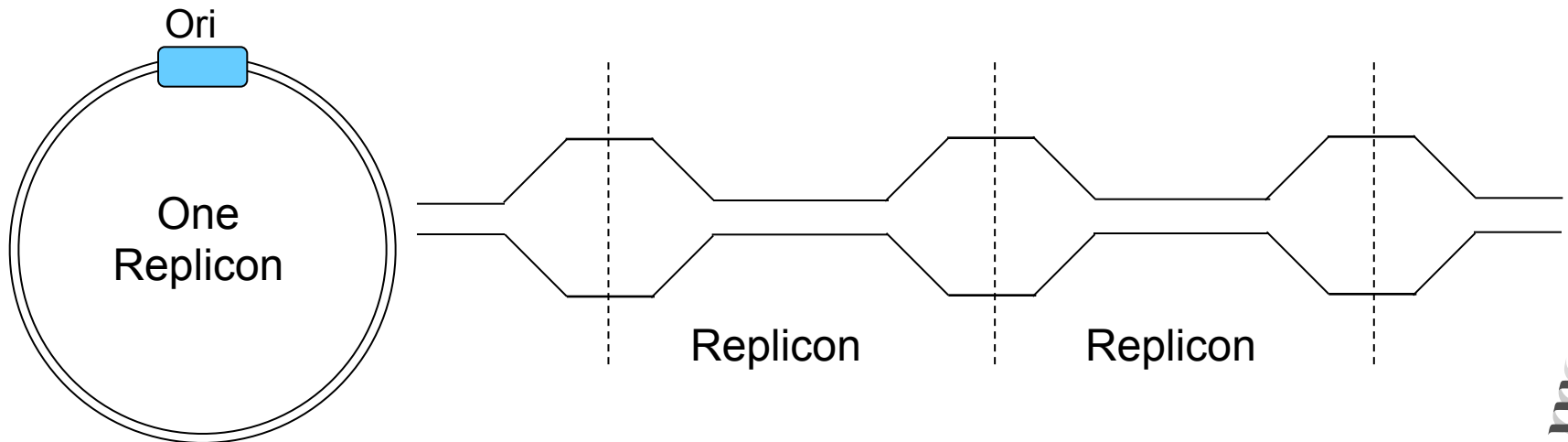
**Difference #1:** prokaryotes have a single origin of replication but eukaryotes have many.

- 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

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



# Replication in Eukaryotes

## Differences in the names of the enzymes

### Prokaryotes

Primase  
DNA Pol III  
Pol I  
Ligase

### Eukaryotes

Pol ( $\alpha$ ) alpha  
Pol ( $\epsilon$ ) epsilon  
Pol ( $\delta$ ) delta  
Eukaryotic Ligase

# Replication in Eukaryotes

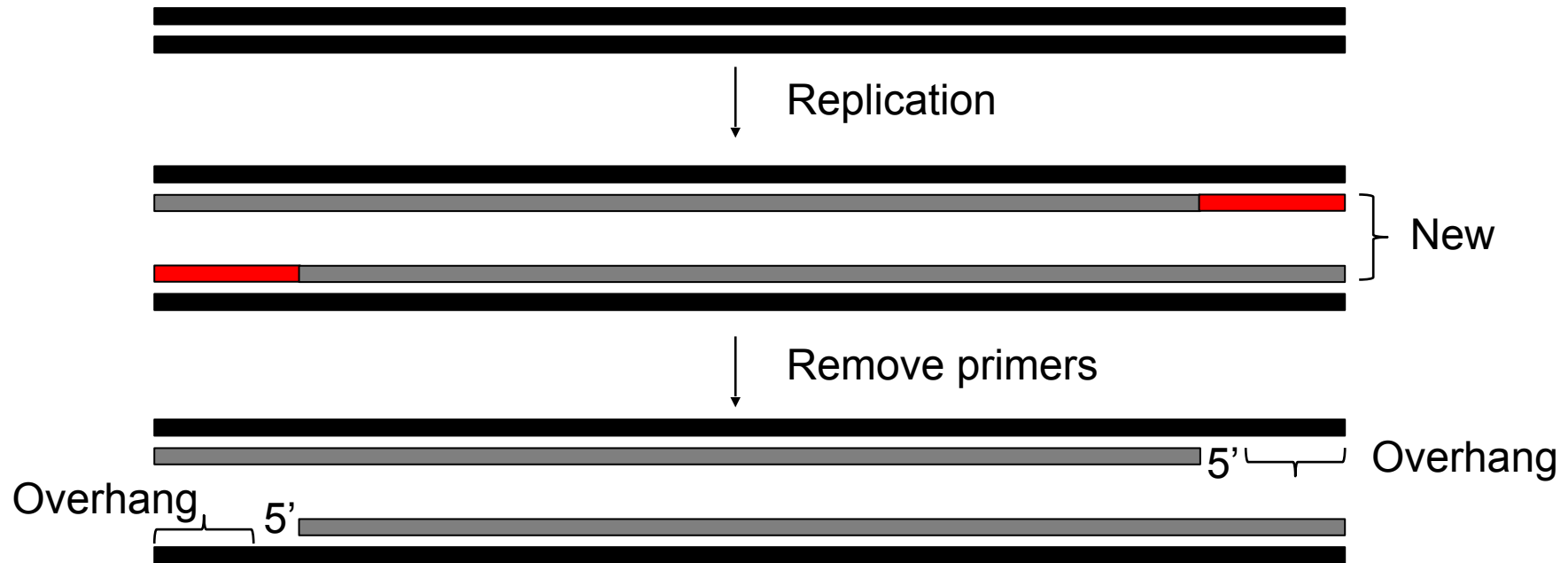


**Difference #2:** circular chromosome in prokaryote and linear chromosomes in eukaryotes.

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



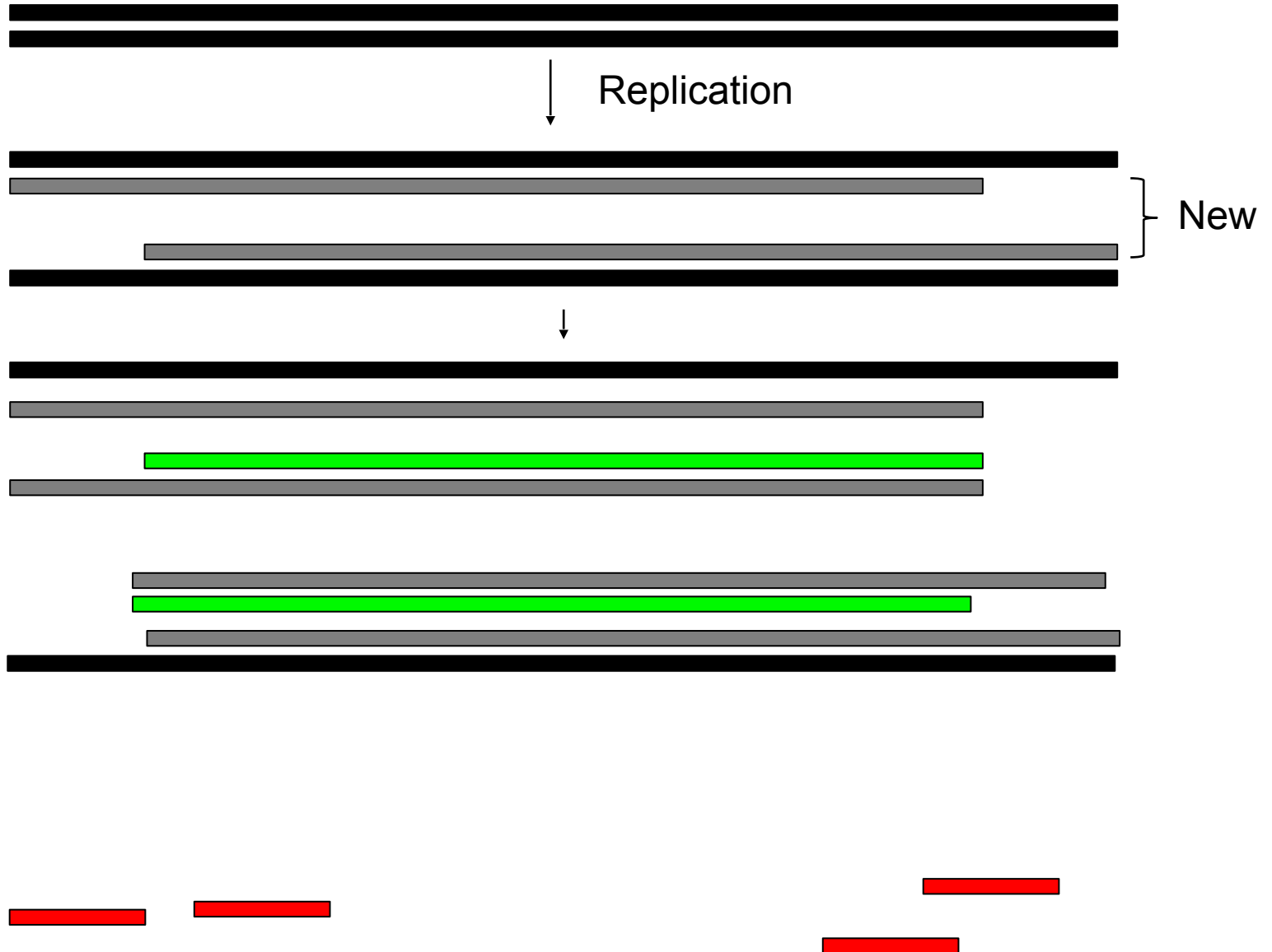
# Replication in Eukaryotes



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

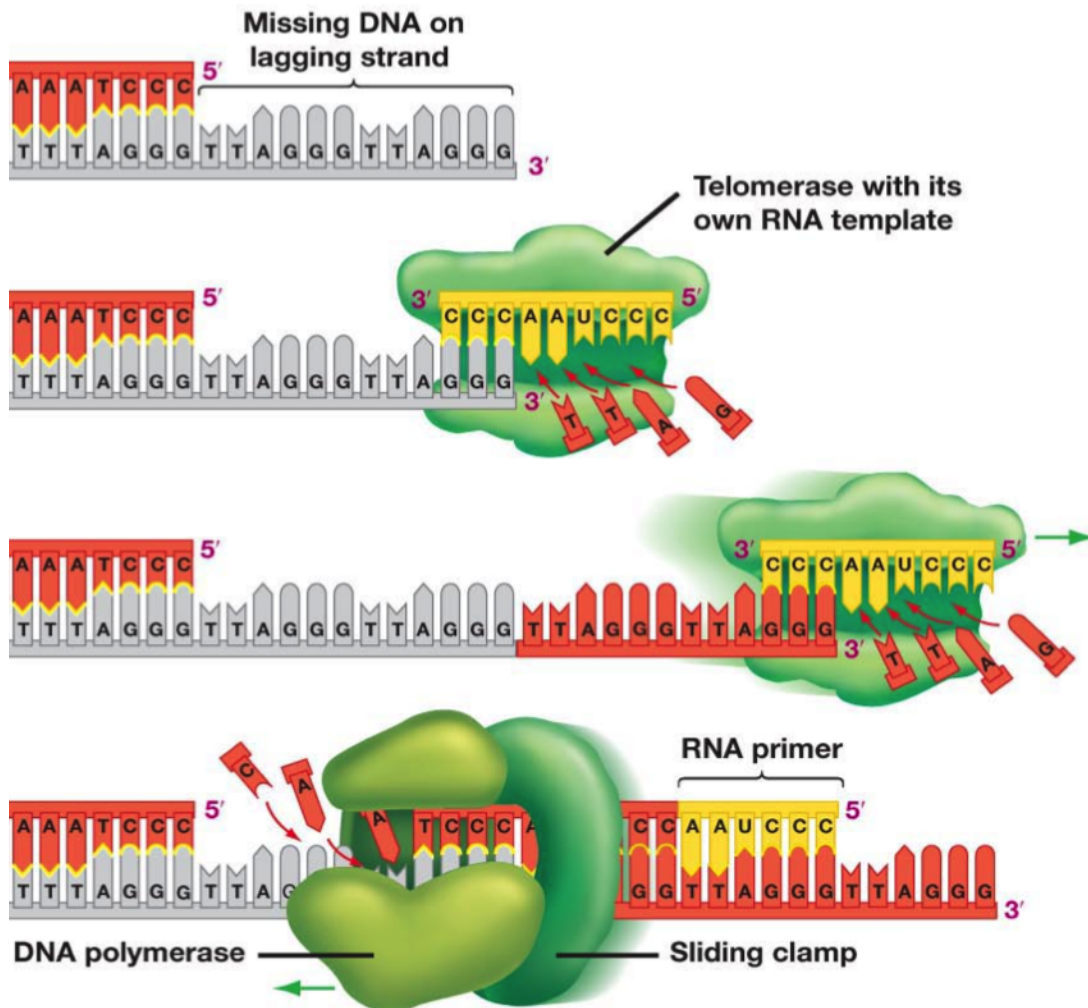
- End of the chromosome (**telomere**) has a specific sequence of repeats.
- Telomere repeat sequence in human is 5' TTAGGG 3'.
- A specific enzyme called **Telomerase** recognizes the repeat sequence (How?)
- **Telomerase is an enzyme composed of:**
  - Protein (polymerase)
  - RNA (template)

# 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

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- 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



- 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 be 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:

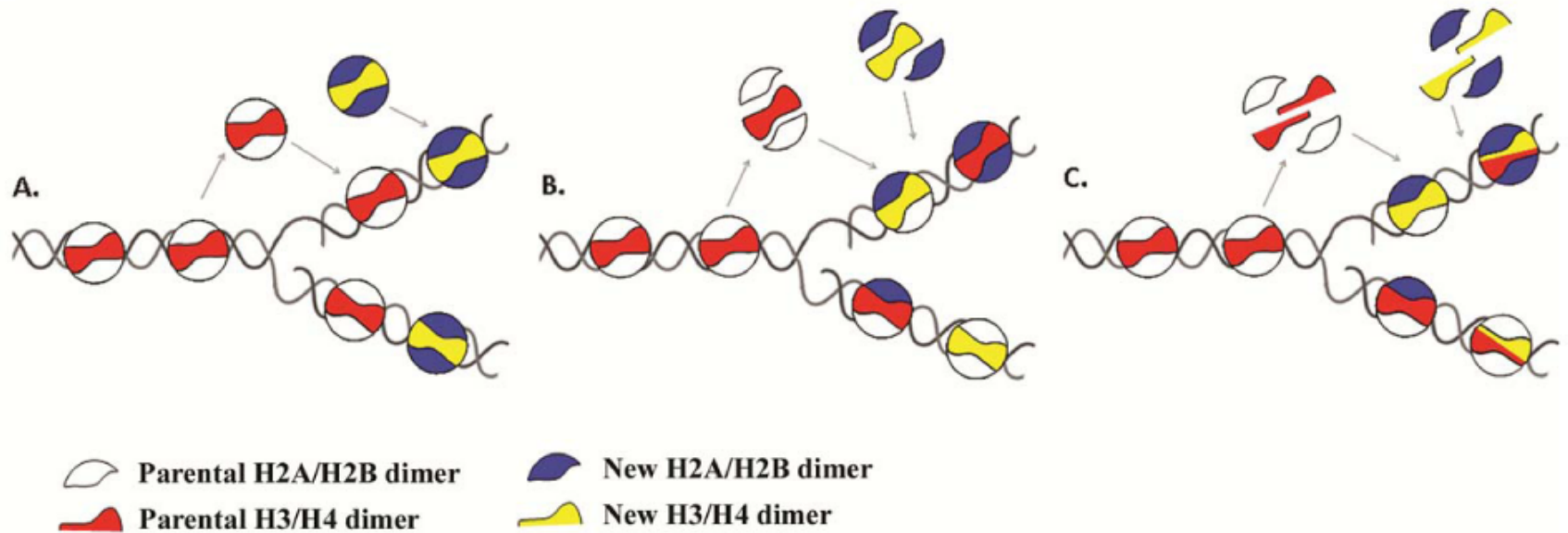
New/new

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 ( $\delta$ )

Concatemer

Telomerase

Pol ( $\epsilon$ )

ARS

H2A/H2B dimer

Telomere

Rolling circle replication

Replicon

Pol ( $\alpha$ )

H3/H4 dimer

Transcription

# Expectations

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- 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 differences 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

