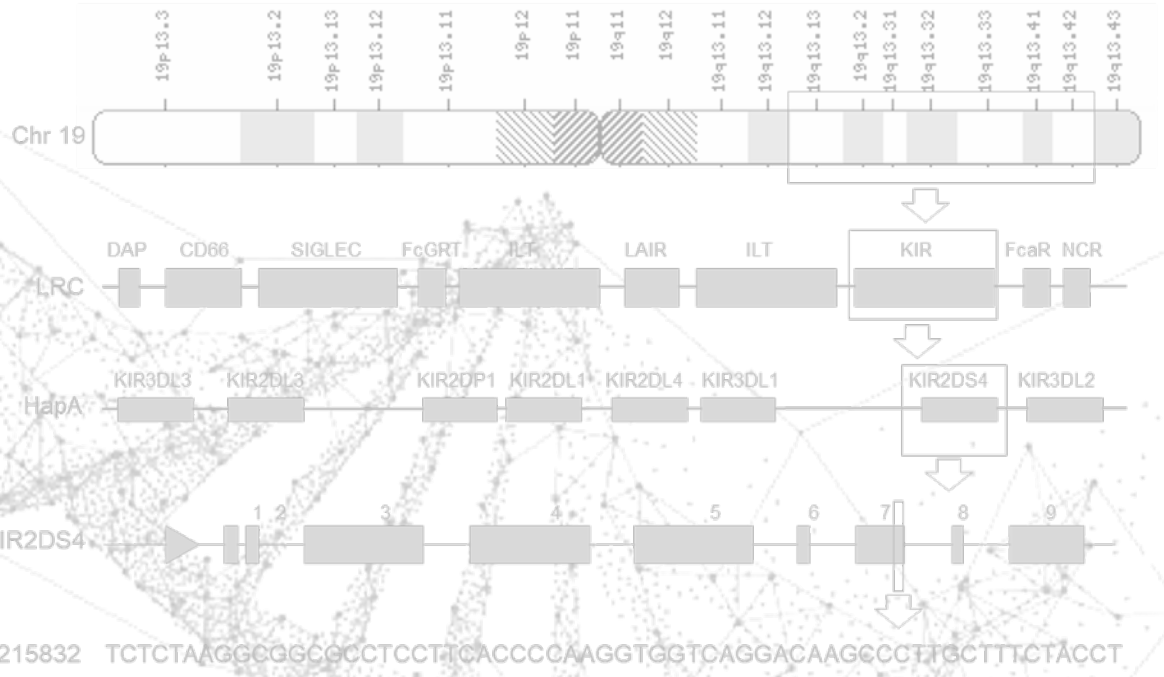

Replicons, Replication and DNA Polymerases

San Luis Potosi State University (UASLP) Mexico
Molecular Biology Course, Faculty of Medicine post-graduate program

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Viral & Human Genomics BSL-3 Laboratory

Last updated October 07, 2025 v2

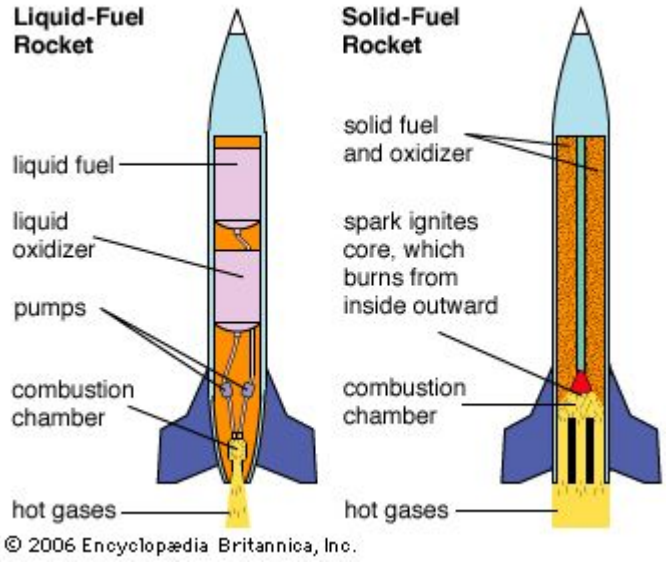
Introduction

Regardless of the number of chromosomes a cell has (1 in a bacterium or 1400 in Ophioglossum), the entire genome must be duplicated (replicated) only once and faithfully with each cell division.

The initiation of DNA replication forces the cell (prokaryotic or eukaryotic) to divide.

Replication is controlled ONLY during initiation, once started it cannot be regulated or turned off, it continues until the entire genome is copied.

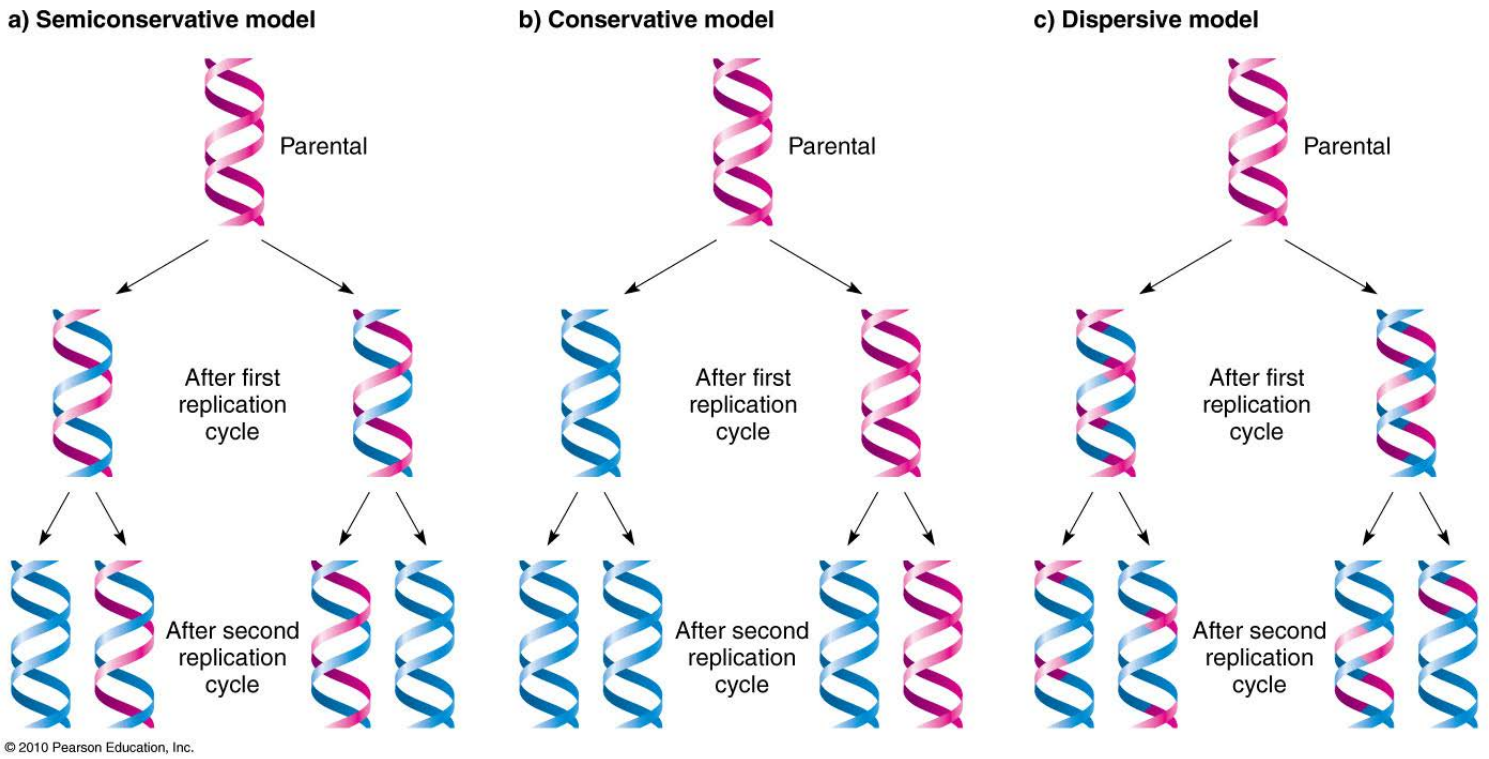
Any cell with defects in replicative machinery is condemmed to die.



Semiconservative replication

Proposed by James Watson and Francis Crick in 1953 based on their double-helix model.

Confirmatory discovery credited to Matthew Meselson and Franklin Stahl in 1958.

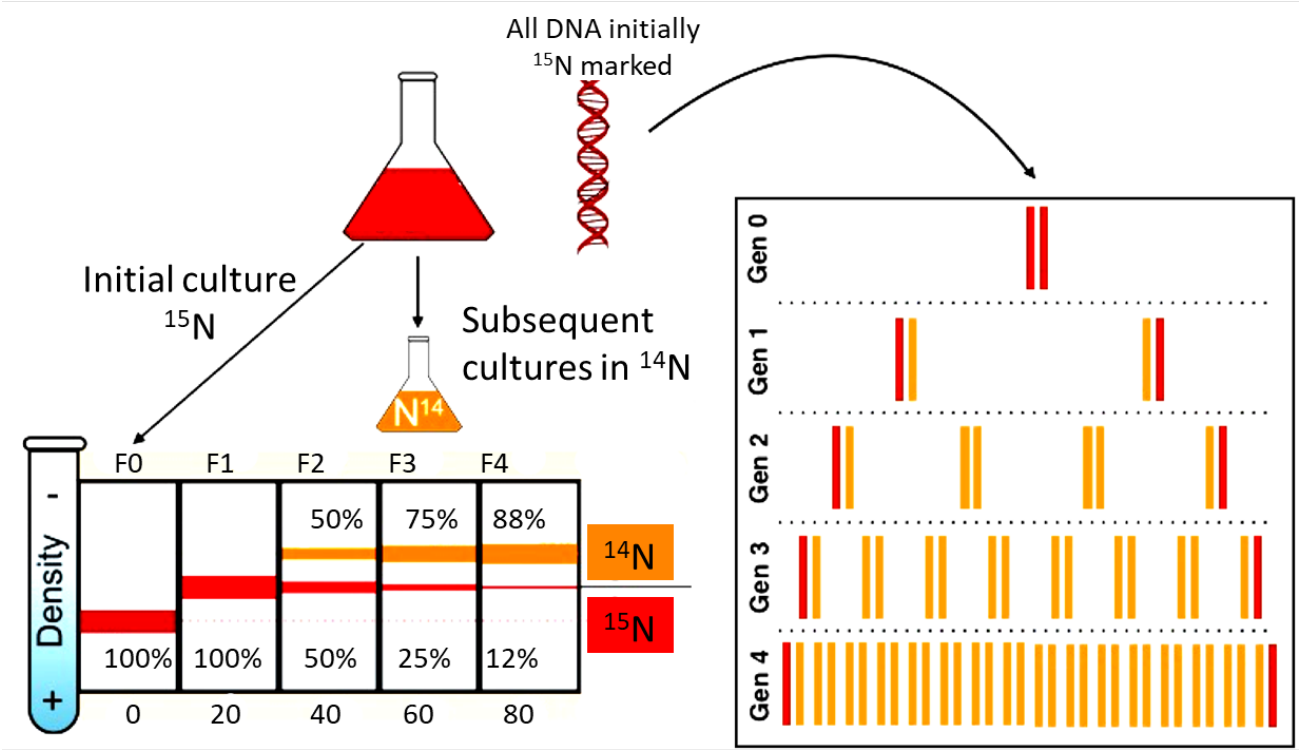


Meselson and Stahl experiment

Groundbreaking experiment, using isotopes of nitrogen (^{15}N and ^{14}N) to label E. coli DNA.

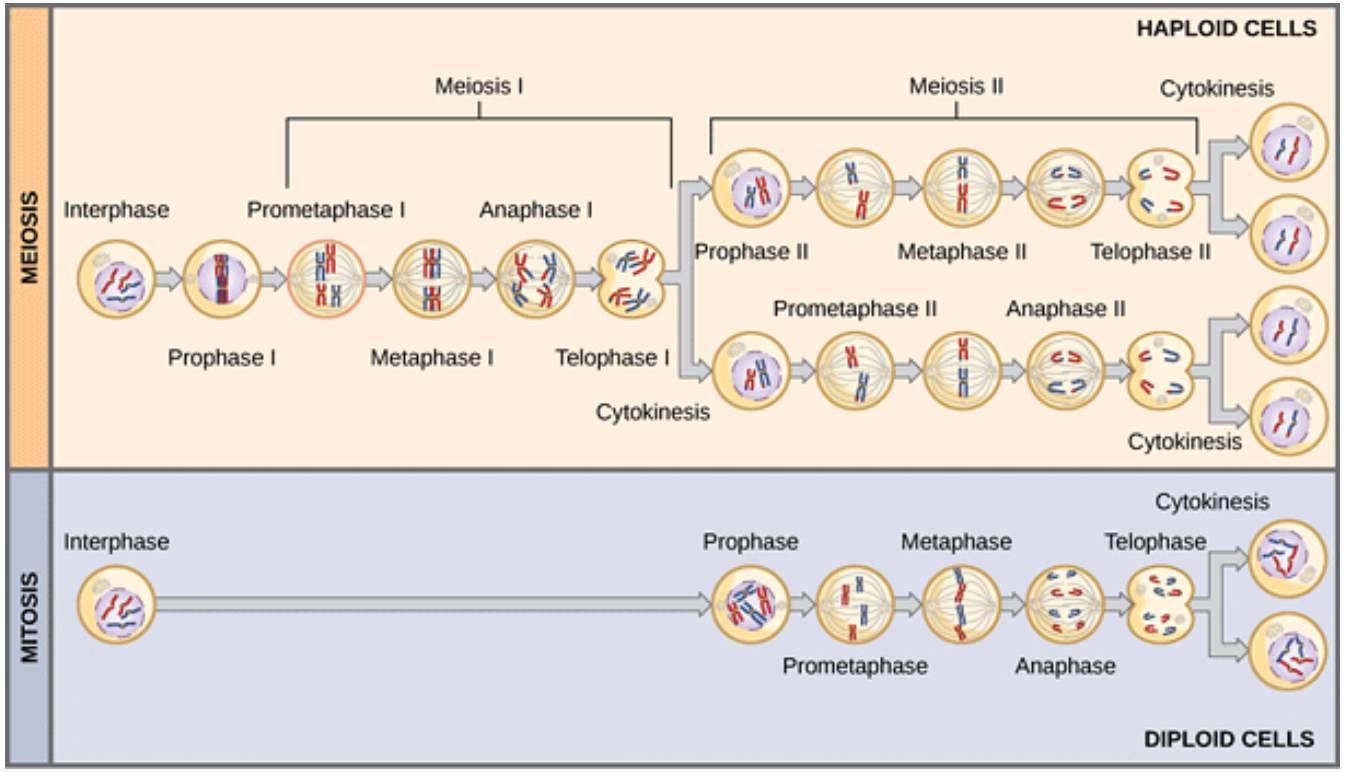
Centrifugation in cesium chloride gradient separates DNA based on its density.

Their results showed that, after one round of replication, DNA molecules consisted of one heavy strand (^{15}N-labeled) and one light strand (^{14}N-labeled).



Meiosis and mitosis

Feature	Mitosis	Meiosis
Purpose	Growth, repair, and asexual reproduction	Production of gametes for sexual reproduction
Divisions	1 division	2 divisions
Daughter cells	2	4
Genetic composition	Genetically identical to parent cell	Genetically diverse (crossing over)
Chromosomes	Maintains same chromosome number as parent (diploid)	Reduces chromosome number by half (haploid)
Occurs in	Somatic (body) cells	Germ cells (to form sperm and eggs)
Crossing over	Does not occur	Occurs during prophase I
Homologous chromosomes	Do not pair	Pair up during prophase I
Role in life cycle	Enables growth and tissue repair	Promotes genetic diversity & ensures euploidy



Mitosis

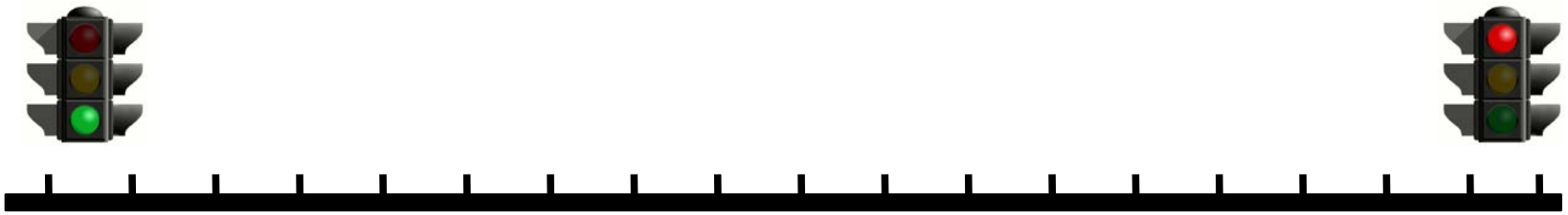
https://www.youtube.com/watch?v=5bq1To_RKEo

Meiosis

https://www.youtube.com/watch?v=kQu6Yfrr6j0&t=5s

Replicons

Working definition: Any DNA sequence flanked by an origin and a termination site.



The origin and terminus constitute DNA sequences with cis effects (on the same DNA strand) that allow the initiation and termination of replication.

1. The unit of DNA in which replication takes place.

1. A portion of DNA that is replicated by a single origin and that has the necessary elements to initiate replication (origin and end).

Prokaryote replicon

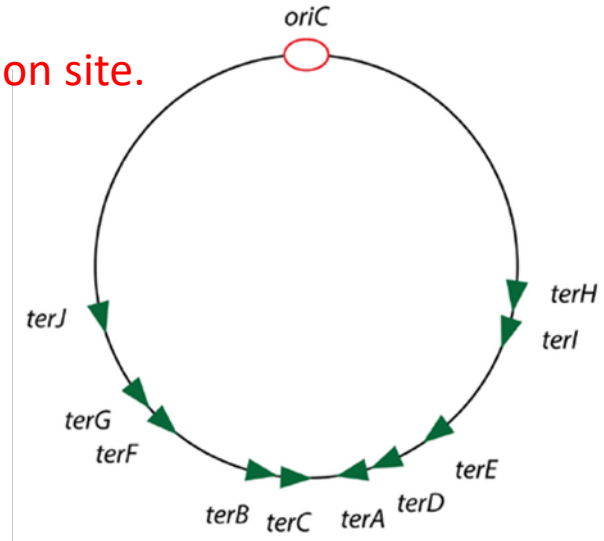
Replication involves only one chromosome and a single initiation site.

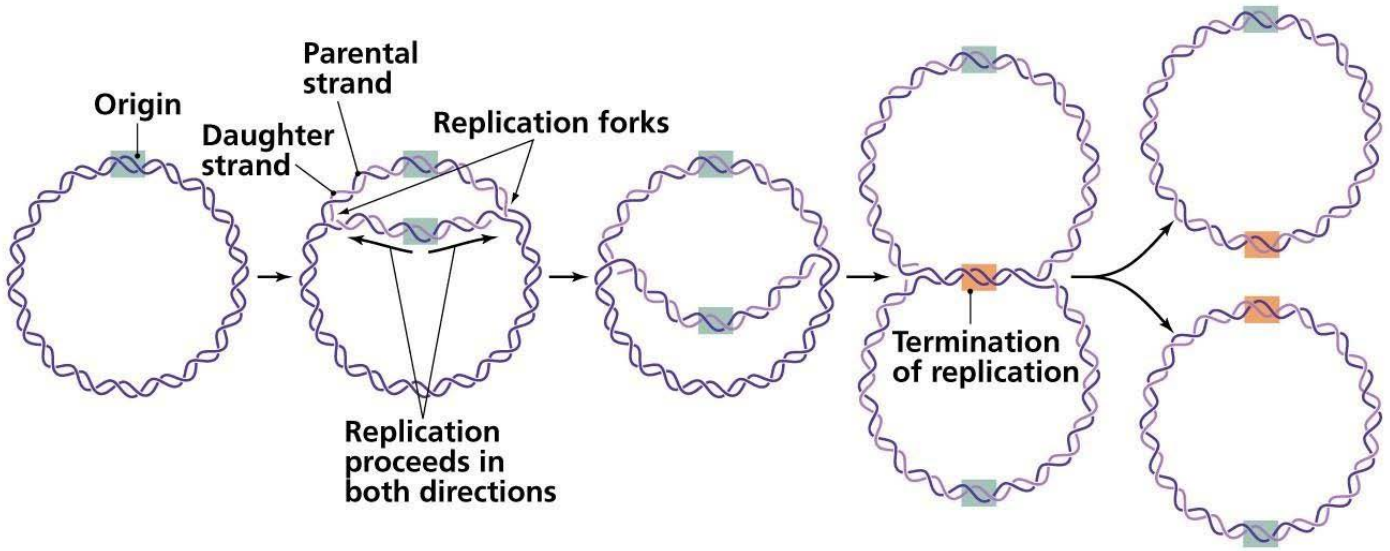
Have *OriC* origin of replication.

Have *ter* sequences for termination.

Form theta (θ) intermediate.

Fast replication 50,000 bps per minute.





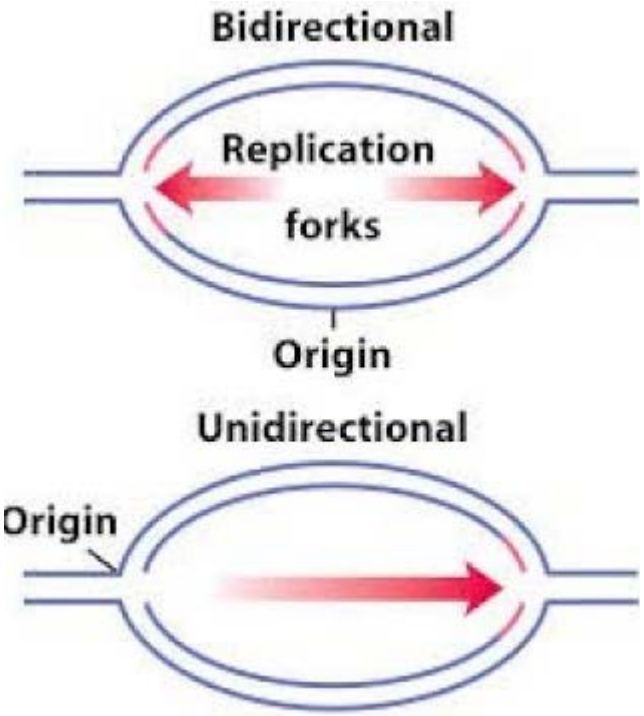
Unidirectional and bidirectional origins

The replicative eye can move in one or both directions **(UNIDIRECTIONAL and BIDIRECTIONAL).**

Unidirectional = a single replication fork makes use of the site of origin until it reaches a terminus (or falls off the DNA strand if it is too close to the chromosome end).

Bidirectional = Two replication forks make use of the same origin, extending the DNA in opposite directions, **THE MOST COMMON IN EUKARYOTES.**

In any case, the corners of the replicative eyes extend to encompass the entire replicon (or the entire chromosome).



Prokaryote segregation

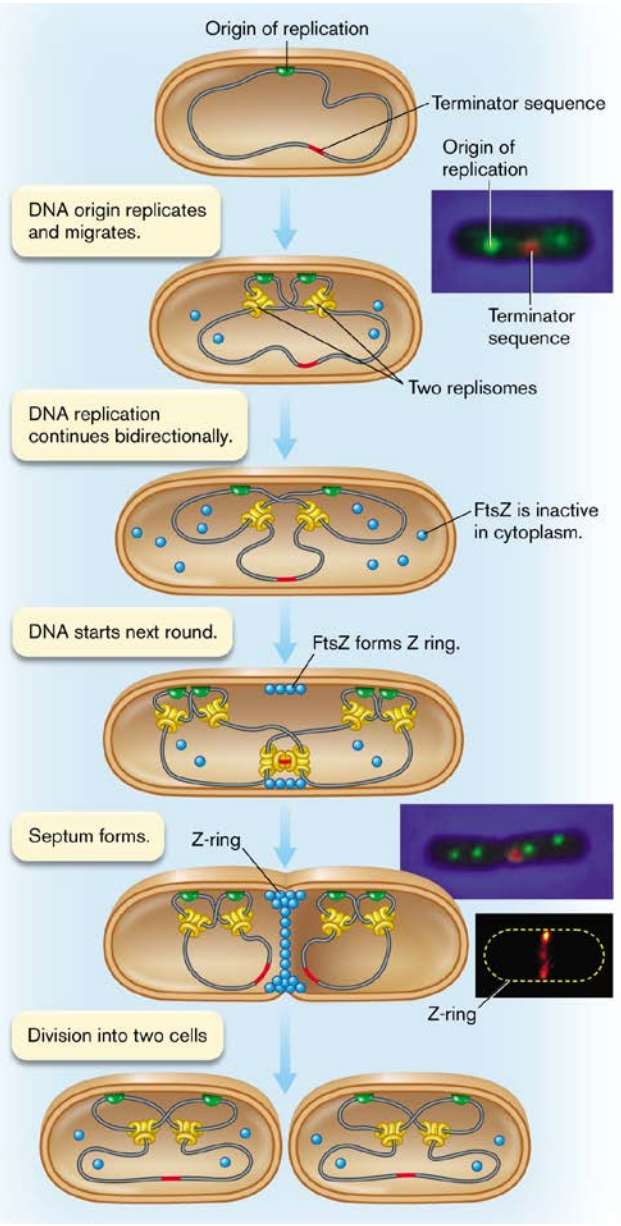
Replication Initiates Segregation:
As the DNA is replicated, the two newly synthesized chromosomes are actively segregated.

Attachment to the Cell Membrane:
Origins of replication (OriC) are attached to the cell membrane or partitioning machinery.

Chromosomal Movement:
Segregation occurs as replication progresses, likely involving **Par (partitioning) proteins**:

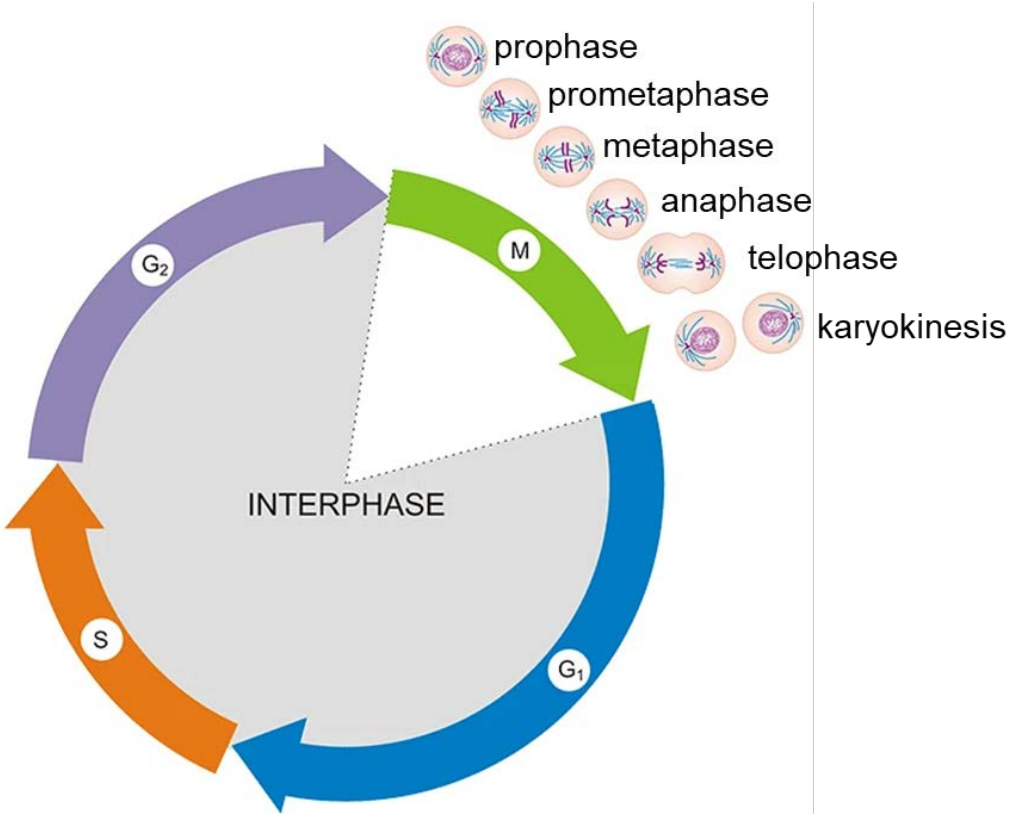
- **ParA/ParB** systems ensure precise positioning of chromosomes.

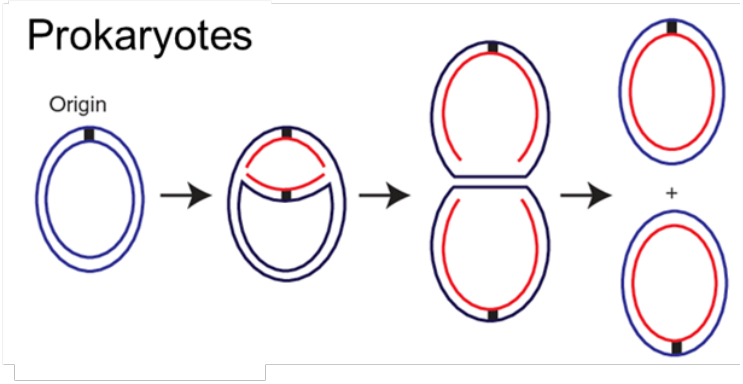
Cytokinesis and Division:
After segregation, the **FtsZ protein** forms a contractile ring at the cell midpoint (Z-ring) to initiate septum formation and divide the cell into two daughter cells.

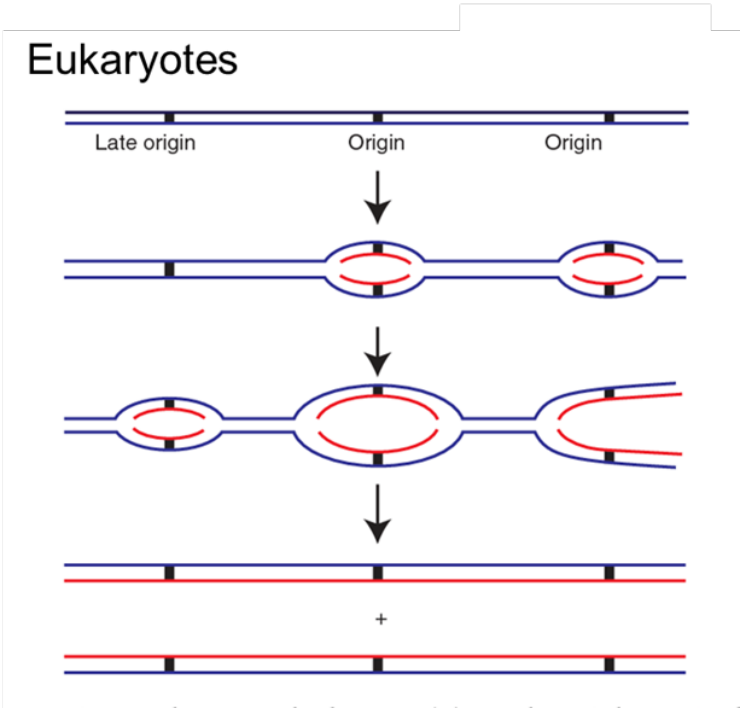


Eukaryote replicon

In eukaryotes, replication is carried out during the S phase of the cell cycle (synthesis) through multiple initiation sites, cell division requires mitotic reorganization of the cell (MTOCs, etc.).





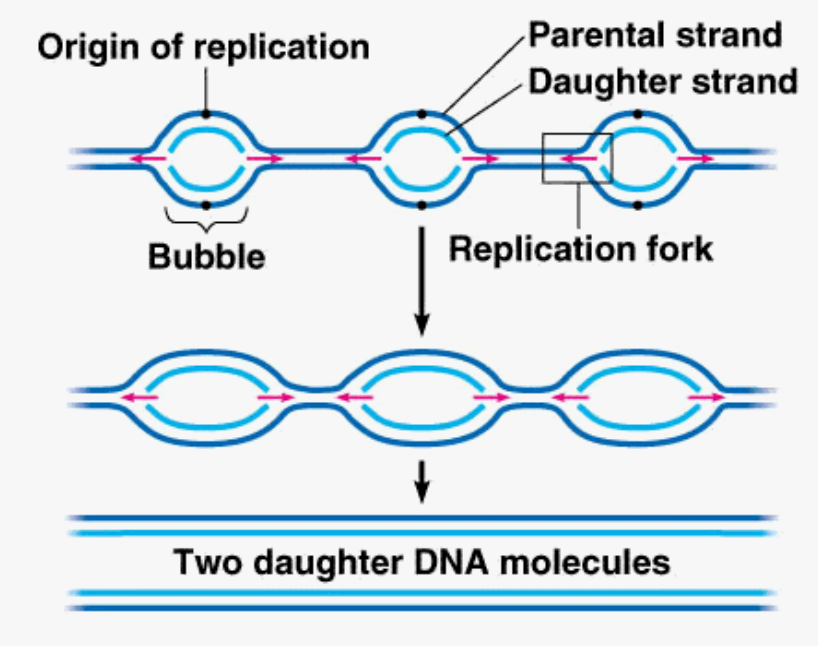


Eukaryote replicon

One of the differences that distinguish us from prokaryotes is that we HAVE MULTIPLE REPLICONS.

The segregation unit (chromosome) has multiple replicative units (replicons).

Multiple replicons for each chromosome, each of which is “fired” only once during the cell cycle although not simultaneously.



Some type of signal must distinguish “fired” from “non-fired” replicons.

Some type of signal must tell the cell that all the replicons have been fired and that it can continue cell division.

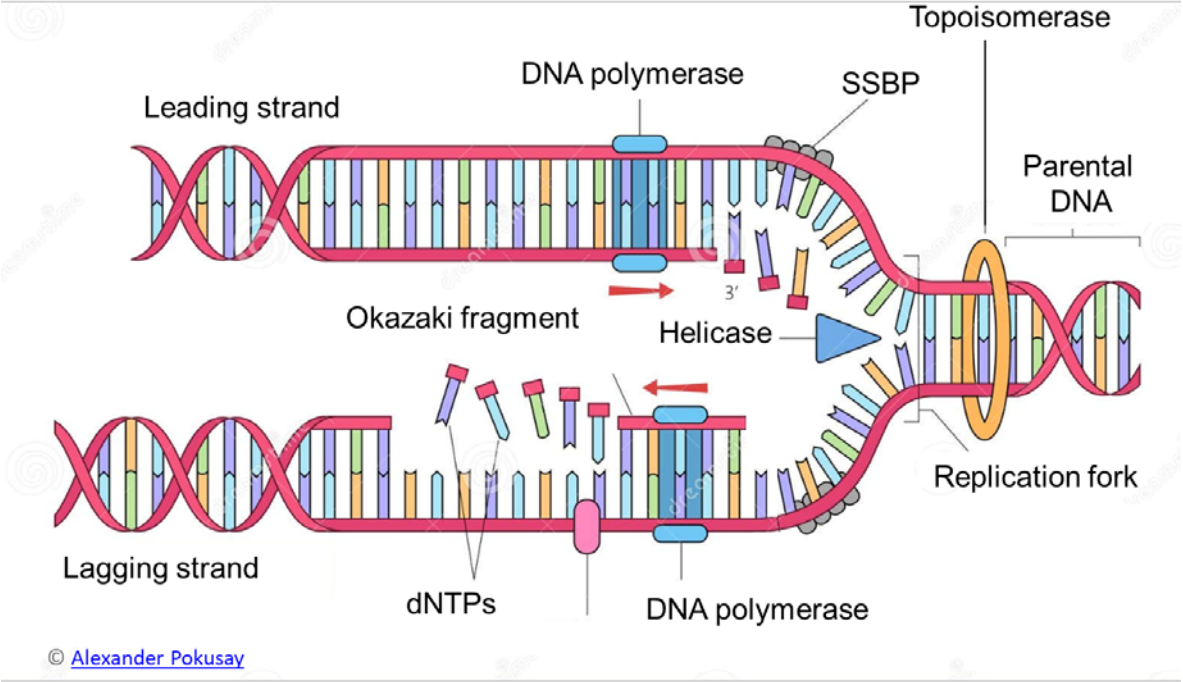
Replication forks

It is the **site where replication occurs.**

The classic image of **the replicon flanks** is that of the replication fork.

Loss **of higher compaction hierarchies.**

Denaturation of DNA duplexes to give access to the replicative complex



Eukaryote replicon

In eukaryotes, DNA replication is restricted to the S phase of the cell cycle.

Speed of prokaryotic replication forks: 800 b/sec (50,000 b/min).

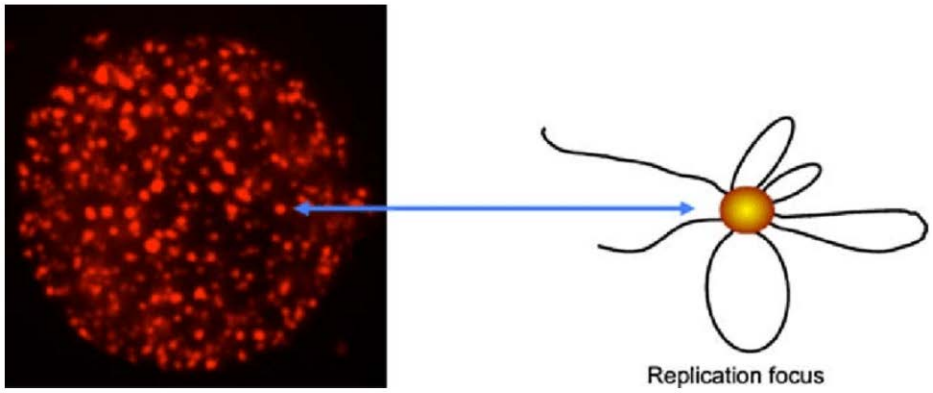
Speed of eukaryote forks is 30 b/sec (2000 b/min).

$3x10^9$ bases/2000 = 1,500,000 minutes = 25,000 hours = 1,041 days = 2.8 years

HoSa uses 30,000 - 90,000 replicons which should allow full replication to be achieved in less than 45 minutes!

In reality, only 15% of the replicons are on at the same time... so the S phase takes approx. 6 hours (to reduce entropy).

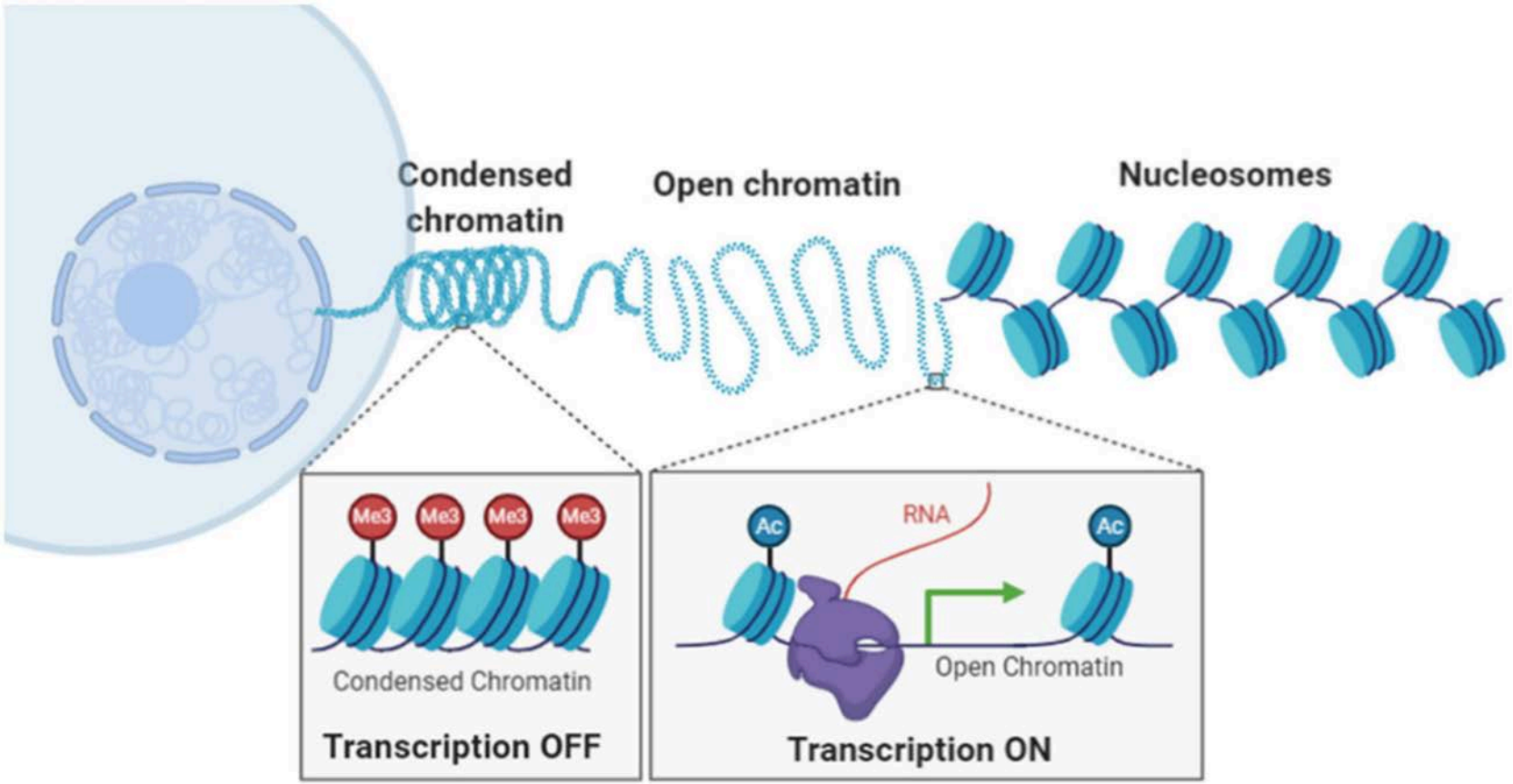
There are between 100 and 300 replicative foci in eukaryotes, each focus has >300 replication forks.



DNA replication foci and clusters of replication origins. Replication foci were identified by biotin-dUTP pulse during DNA synthesis in Xenopus egg extract. A single nucleus is shown. Replication foci are thought to occur by association of several replicons that are synchronously activated in each focus

Genome compaction during replication

Illustration of hierarchical chromatin compaction status and genomic availability.



Xu Jianquan, Liu Yang. Probing Chromatin Compaction and Its Epigenetic States in situ With Single-Molecule Localization-Based Super-Resolution Microscopy. Frontiers in Cell and Developmental Biology 2021, Vol 9

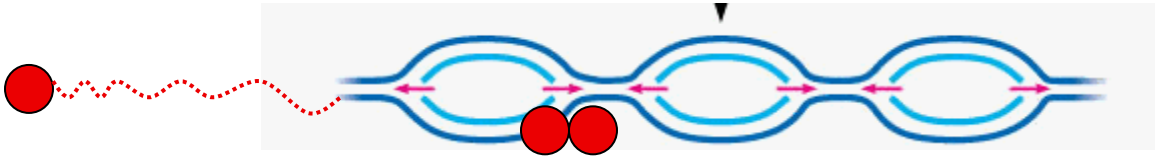
Eukaryote replicon

Prokaryote replicons are large (between 4 and 10 million bp).

Eukaryotes replicons are smaller and variable (from 4kbp to 100 kbp, mean 40kbp).

Unlike prokaryotic circular replicons, eukaryotic replicons do not have ter sequences, the DNA polymerase simply extends the chain until it encounters a DNA pol in the opposite direction, after which it detaches...

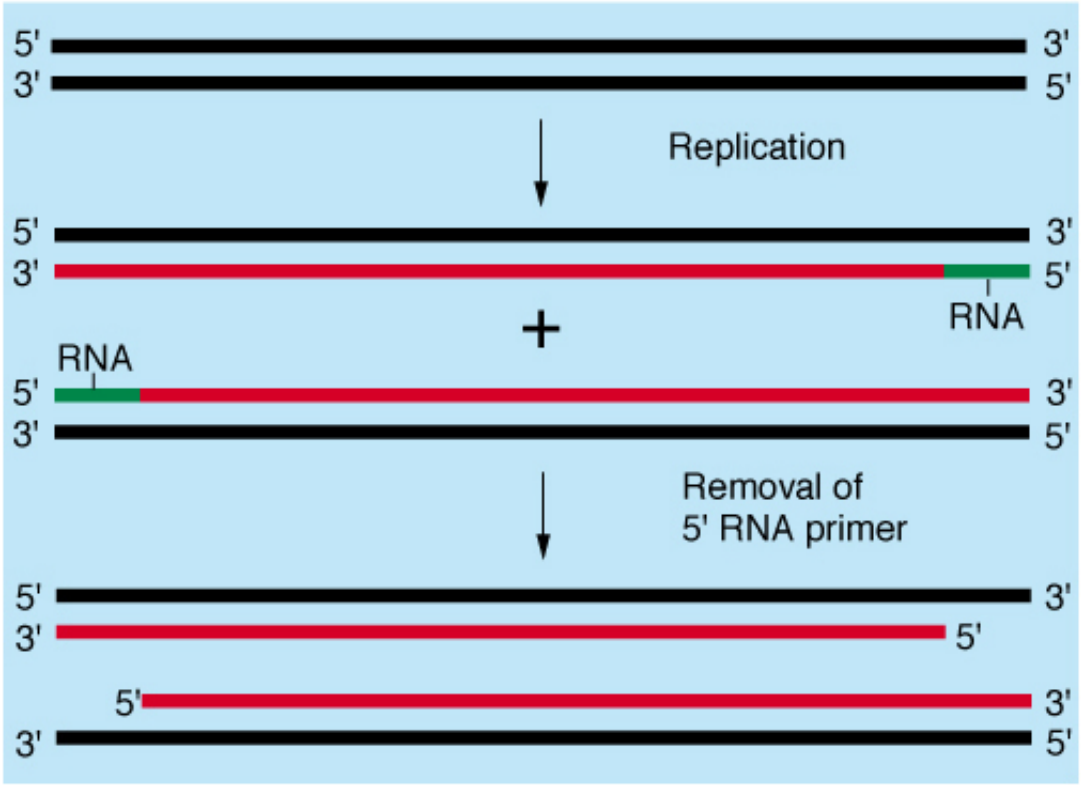
...or “falls off” the chromosome once it has reached its terminal end.



Linear replication paradigm

The RNA primers are removed after serving their function.

Hence the paradigm of the replication of a linear DNA fragment (necessarily leads to its gradual shortening).



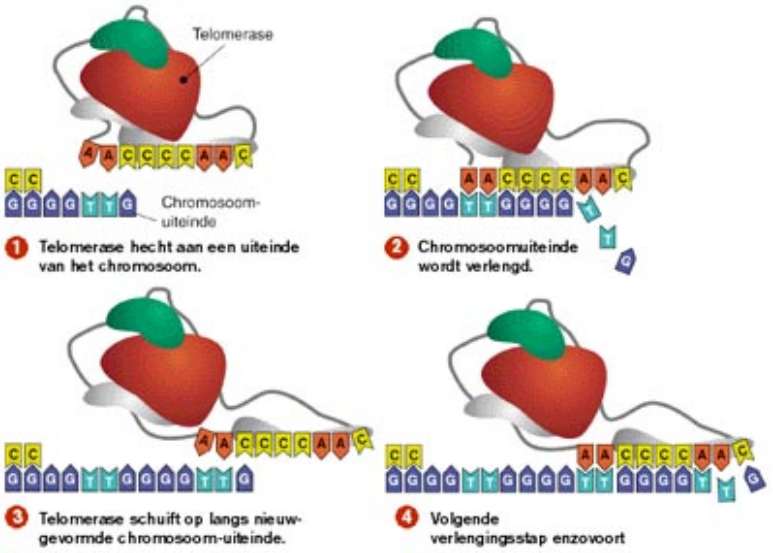
Linear replicon paradigm

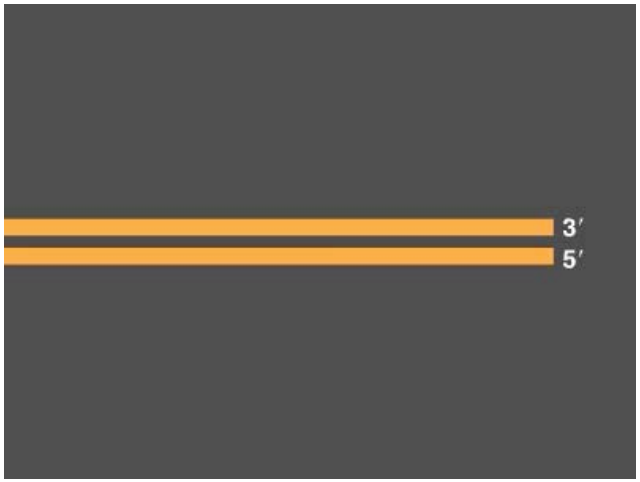
1.- **Circularize genome** (as bacteria and T4 or Φ phages do).

2.- Formation of **terminal loops** (variant of the first), used in the mitochondrial genome of Paramecium and our telomeres.

3.- Use of a terminal **protein that provides** a free non-nucleotide OH-3', just as Adenoviruses and polioviruses do.

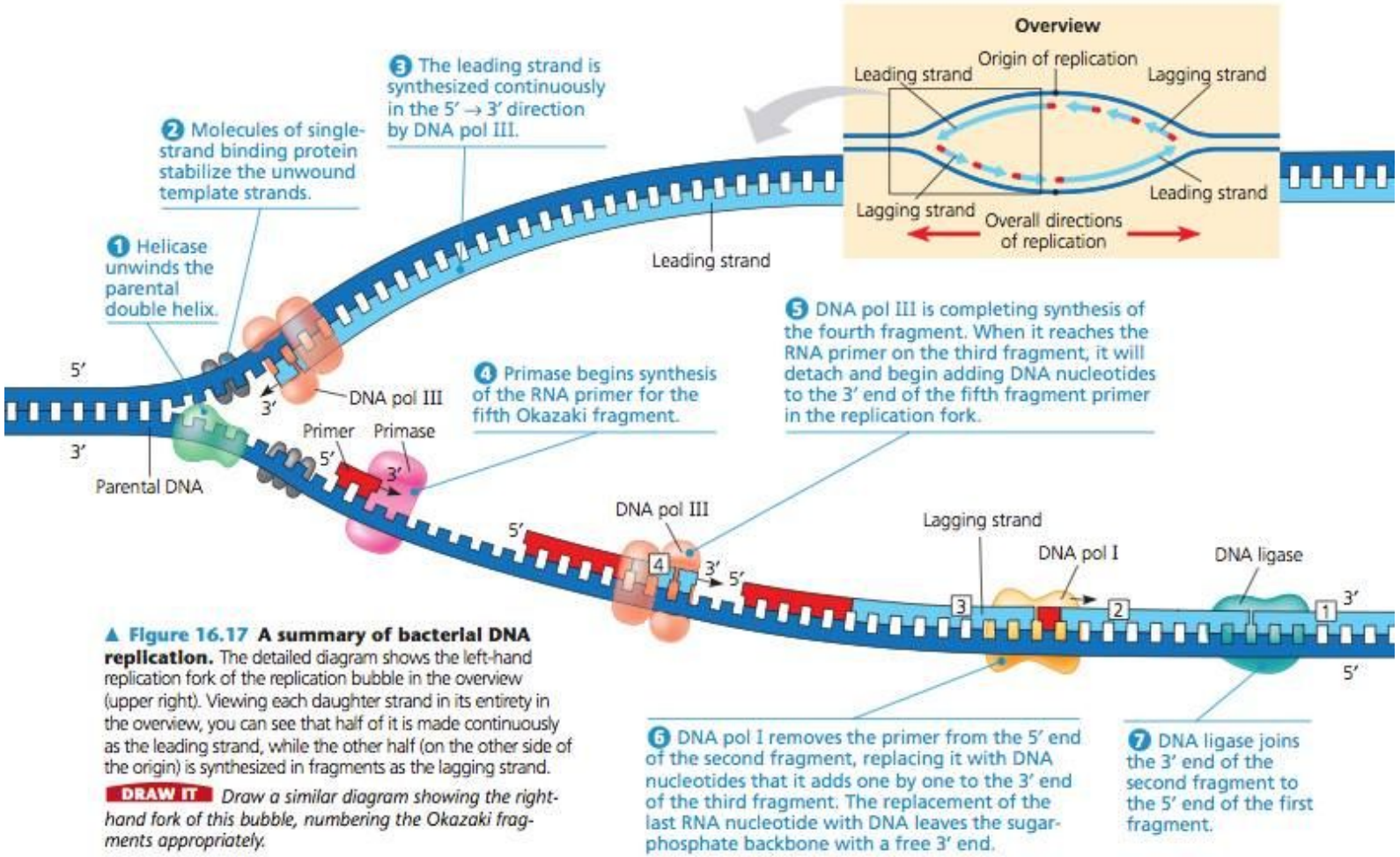
4.- Repetitive sequences and telomerases to extend the ends: **eukaryotic telomeres.**





Replications phases

It has three stages: Initiation, elongation and termination.



▲ **Figure 16.17 A summary of bacterial DNA replication.** The detailed diagram shows the left-hand replication fork of the replication bubble in the overview (upper right). Viewing each daughter strand in its entirety in the overview, you can see that half of it is made continuously as the leading strand, while the other half (on the other side of the origin) is synthesized in fragments as the lagging strand.

DRAW IT *Draw a similar diagram showing the right-hand fork of this bubble, numbering the Okazaki fragments appropriately.*

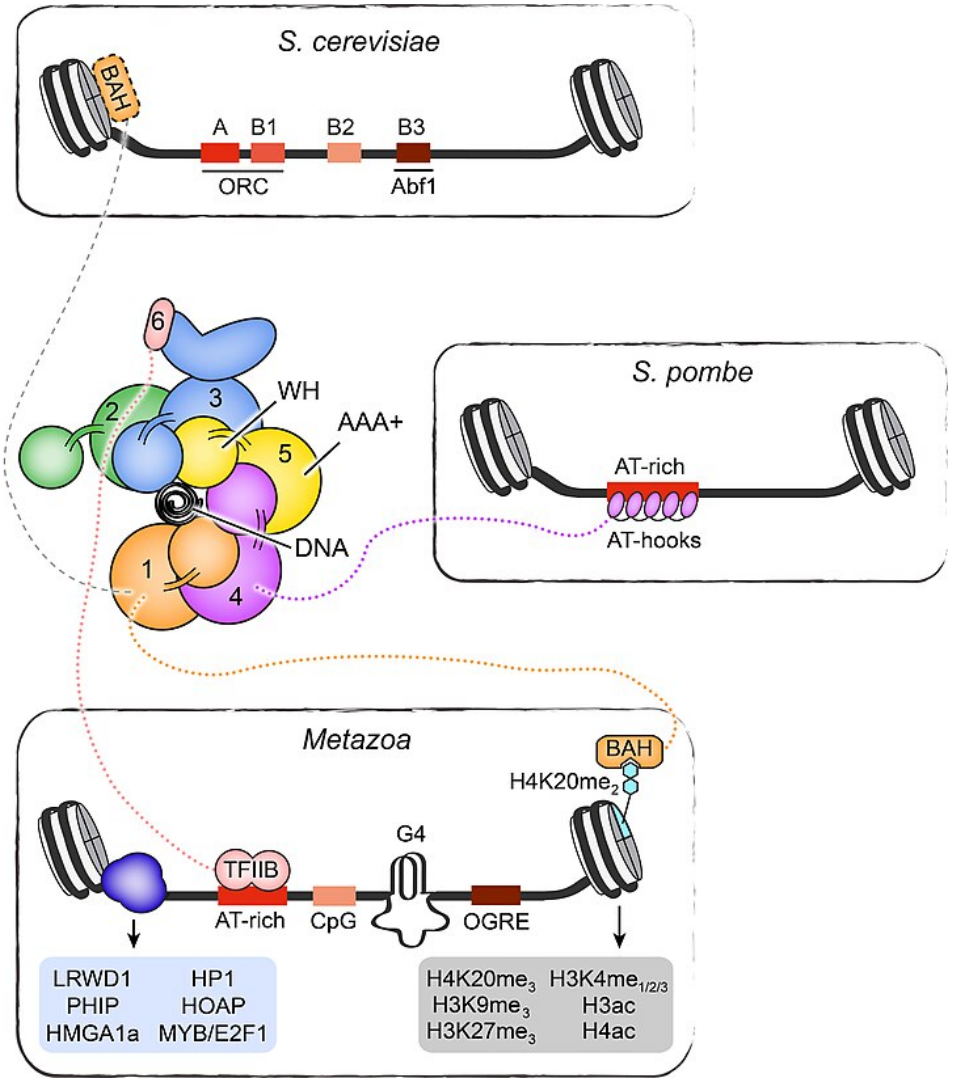
Initiation phase

The origin recognition complex (ORC), a heteromeric six-subunit protein, is a central component for eukaryotic DNA replication.

The ORC binds to DNA at replication origin sites in an ATP-dependent manner and serves as a scaffold for the assembly of other key initiation factors.

Specific DNA elements and epigenetic features involved in ORC recruitment.

BAH domain in S. cerevisiae Orc1 binds nucleosomes.



Initiation phase

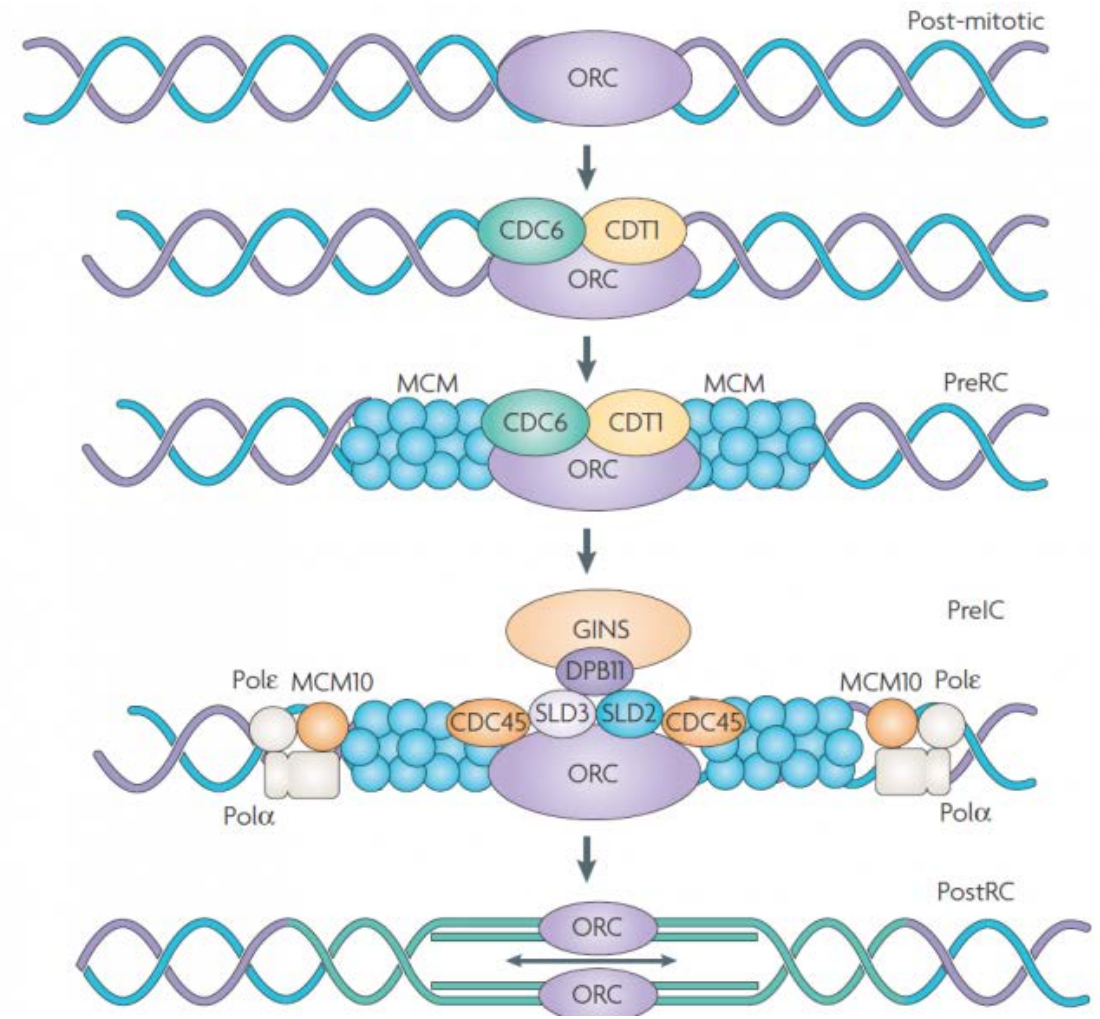
Cyclin CDC6 and the replicative licensing factor CDT1 recruit MCM complexes to the ORC during G1.

MCM: Minichromosome maintenance protein complex

Mcm's Complex denatures DNA to allow entry of DNA Polymerases.

Cyclin-dependent kinases CDC, SLD and DPB activate the pre-replisomal complex.

Active replisome.



Elongation phase

Polymerization of daughter chains.

In charge of another **protein complex called the replisome**.

The replisome **does not constitute an isolated protein complex** (like **ribosomes**) but results from the **aggregation of several proteins** at the level of the replication fork and includes not only the proteins but also the DNA that serves as a reference.

During this phase the parental DNA strands are unwound and daughter strands are synthesized.

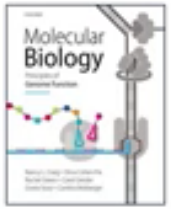
Termination phase

All those events that occur from the moment the new daughter molecule is synthesized until chromosome segregation.

Union of fragments belonging to adjacent replicons.

Dissolution of protein complexes involved in the initiation and elongation of replication.

Summary video



Molecular Biology: Principles of Genome Function

Second Edition

OXFORD UNIVERSITY PRESS

Animation 1:
DNA replication

Animation produced by Connor Hendrich
© Oxford University Press 2014

https://youtu.be/0Ha9nppnwOc?si=KyLZrhqihudMXTFP

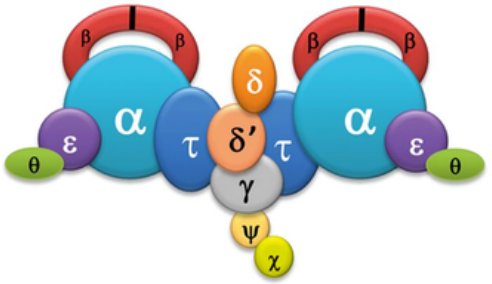
DNA Polymerases in prokaryotes

Enzymes that synthesize a new DNA strand from a reference strand.

Only a few DNA pol's participate in DNA replication (Replicases).

Most DNA pol's involved in DNA repair (Repairases).

	Pol I	Pol II	Pol III	Pol IV	Pol V
DNA polymerase family	A	B	C	Y	Y
Activity	5'-3' polymerase 3'-5' exonuclease 5'-3' exonuclease	5'-3' polymerase 3'-5' exonuclease	5'-3' polymerase 3'-5' exonuclease	5'-3' polymerase	5'-3' polymerase
Number of molecules/ cell					
– SOS	400	50 - 75	10 - 20	150 - 250	< 15
+ SOS	400	350 - 1000	10 - 20	1200 - 2500	200
Biological functions in the cell	DNA replication, Okazaki fragment maturation, DNA repair	DNA replication (backup DNA polymerase), DNA repair, TLS	DNA replication DNA repair	TLS	TLS



DNA synthesis does not depend on a single enzyme but on a multimeric enzyme complex.

Replicative activity is just one of the functions associated with the replisome.

DNA Polymerases in eukaryotes

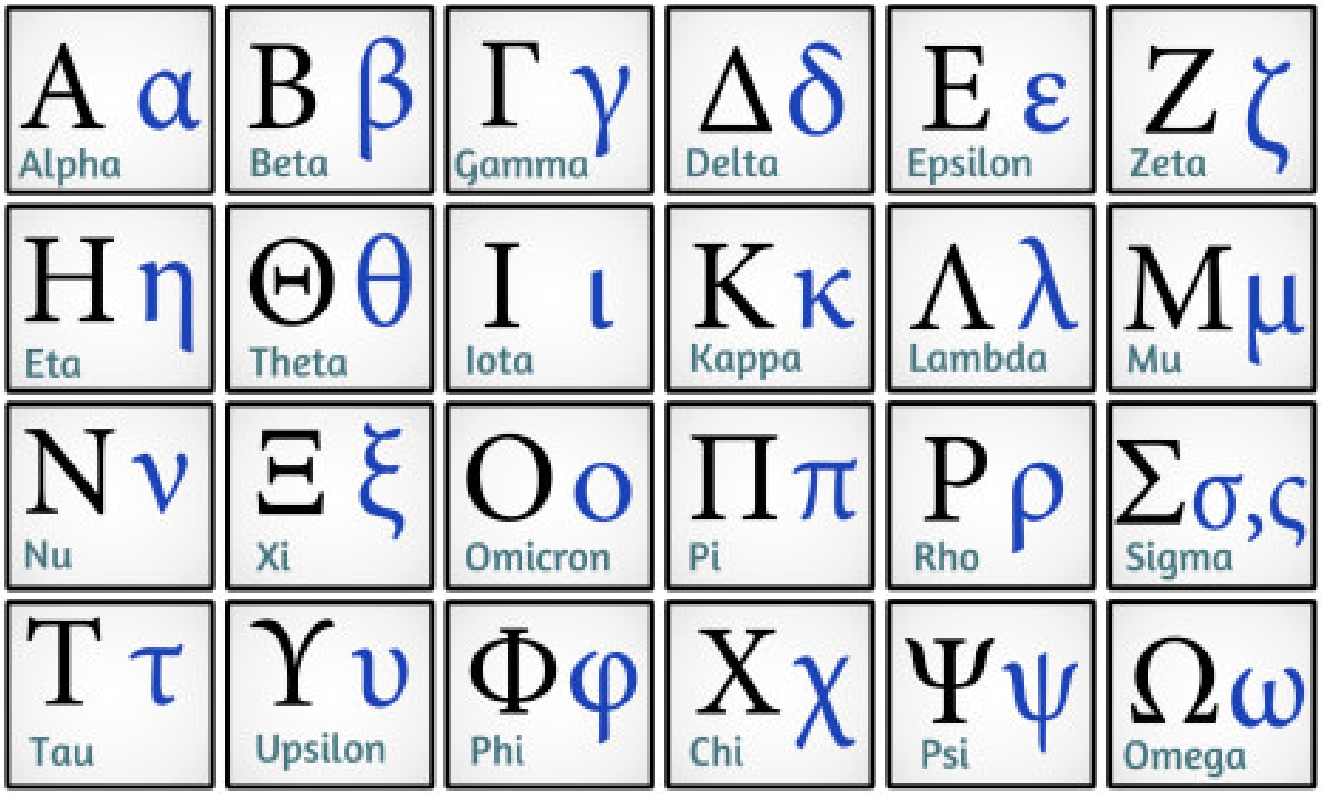
Polymerase[a]	Family	Catalytic subunit				Associated activities	Proposed functions
		Molecular mass (kDa)[b]	Human gene (alias)	Chromosomal location[c]	Yeast gene[d] (alias)		
α (alpha)	B	165	*POLA*	Xp22.1-p 21.3	*POL1* (*CDC17*)	Primase	chromosomal replication, S-phase checkpoint, DSB repair
β (beta)	X	39	*POLB*	8p11.2	-	dRP & AP lyase	BER, single strand break repair
γ (gamma)	A	140	*POLG*	15q25	*MIP1*	3'→5' exonuclease, dRP lyase	mitochondrial replication, mitochondrial BER
δ (delta)	B	125	*POLD1*	19q13.3	*POL3* (*CDC2*)	3'→5' exonuclease	chromosomal replication, NER, BER, MMR, DSB repair
ε (epsilon)	B	255	*POLE*	12q24.3	*POL2*	3'→5' exonuclease	chromosomal replication, NER, BER, MMR, DSB repair, S-phase checkpoint
ζ (zeta)	B	353	*POLZ* (*REV3*)	6q21	*REV3*		TLS, DSB repair, ICL repair?, SHM
η (eta)	Y	78	*POLH* (*RAD30*, *RAD30A*, *XPV*)	6p21.1	*RAD30*		TLS, SHM
θ (theta)	A	198	*POLQ*	3q13.33	-		ICL repair?
ι (iota)	Y	80	*POLI* (*RAD30B*)	18q21.1	-	dRP lyase	TLS?, BER?, SHM
κ (kappa)	Y	76	*POLK* (*DINB1*)	5q13	-		TLS
λ (lambda)	X	66	*POLL*	10q23	*POL4* (*POLX*)	dRP lyase	DSB repair, BER?
μ (mu)	X	55	*POLM*	7p13	-	TdT	DSB repair
σ (sigma)	X	60	*POLS* (*TRF4-1*)	5p15	*TRF4*		sister chromatid cohesion
REV1	Y	138	*REV1*	2q11.1-q11.2	*REV1*	TdT (for dC)	TLS

Shcherbakova PV, et al. Functions of Eukaryotic DNA Polymerases. Sci Aging Knowledge Environ. 2003.

Greek alphabet

Α α	Β β	Γ γ	Δ δ	Ε ε	Ζ ζ
Η η	Θ θ	Ι ι	Κ κ	Λ λ	Μ μ
Ν ν	Ξ ξ	Ο ο	Π π	Ρ ρ	Σ σ,ς
Τ τ	Υ υ	Φ φ	Χ χ	Ψ ψ	Ω ω

Greek alphabet



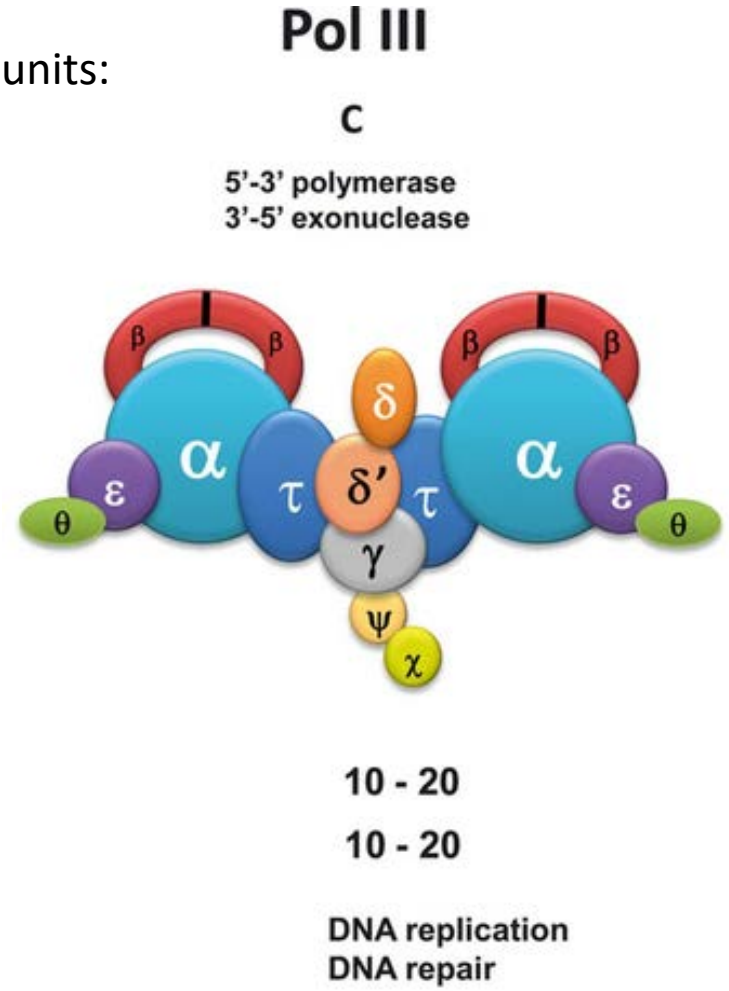
DNA polymerase III

The holoenzyme is a 900 kD complex with several subunits:

Catalytic cores
Subunit α (5' ⇨ 3' synthetic activity).
Subunit ε (3' ⇨ 5' exonuclease activity).
Subunit θ (complex stabilizer) and e function.

Processivity component (Clamp β)
Subunit γ,
Subunit δ,
subunit δ',
Subunit χ,
Subunit ψ,

Dimmerization component
Subunit τ.



DNA polymerase III

DNA polymerase at the replication fork

Three essential components in the figure replisome:
1.- **Helicase** (DnaB)
2.- **Primase** (DnaG)
3**.- DNA polymerase III** dimer

Helicase advances **in front of the replication fork grasping a strand of ssDNA** by opening the DNA duplex located in front of it.

Gyrase to alleviate super-coiling ahead of helicase (not shown in this figure).

The **Helicase "pulls"** the DNA polymerase III dimer in the 5' - 3' direction.

At the site of origin, **the primase places the oligonucleotide** primer on the leader reference, as the replication fork advances, the DNA polymerase of the leader strand extends it without problems.

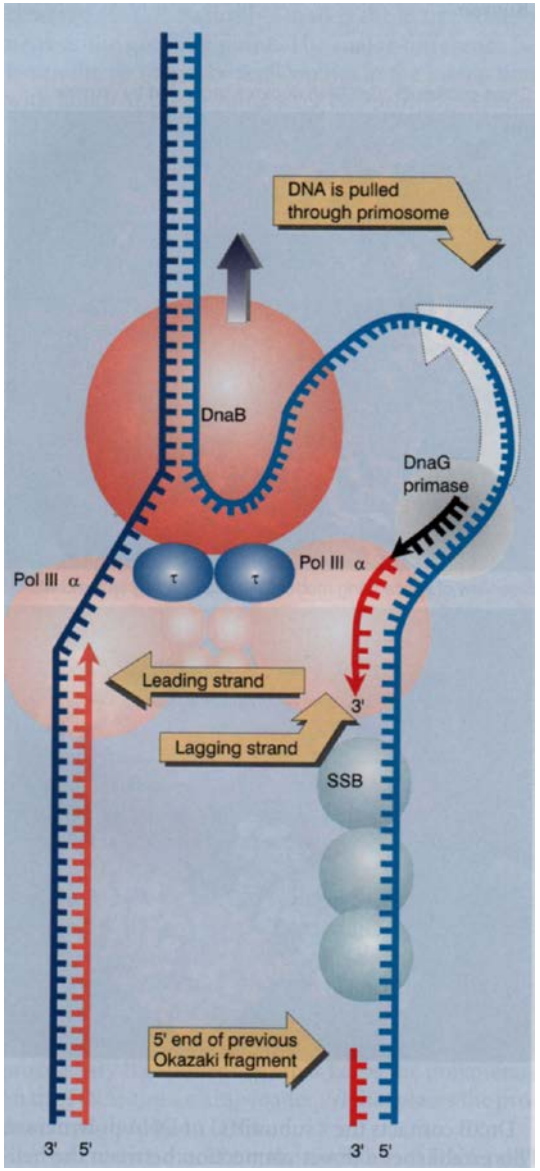
DNA polymerase III

The extension of the leading strand causes a **loop** in front of the DNA polymerase **corresponding to the lagging strand**.

Periodically, **DnaB (helicase) recruits DnaG (primase)** to prime the next Okazaki fragment.

Once the Okazaki fragment is primed, **the primase is released** and the corresponding DNA polymerase extends the Okazaki fragment until it meets the previously synthesized one...this process **increases even more the size of the ssDNA loop** in front of the ipsilateral complex.

Once the DNA polymerase has finished the Okazaki fragment, **Clamp β dissociates** to re-form at a site close to the replication fork (where DnaGprimase has been recruited again by DnaB (helicase).



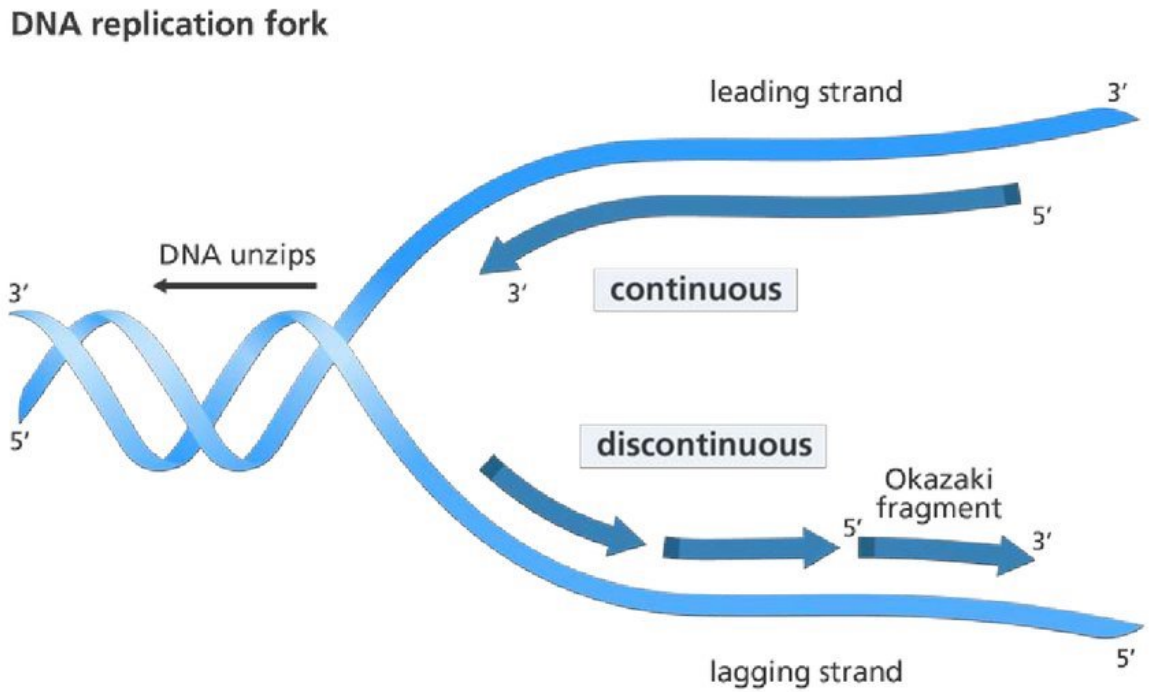
Semidiscontinuous replication in eukaryotes

Chains are antiparallel.

During replication both chains must be replicated at the same time.

No problem with the leader chain, moves in the same direction as the fork.

Problem with delayed chain, “paradoxical” retrograde movement to fork.



Semidiscontinuous replication in eukaryotes

The oligo removal process in prokaryotes is very similar to nick-translation, except that instead of replacing DNA with DNA, RNA is replaced with DNA.

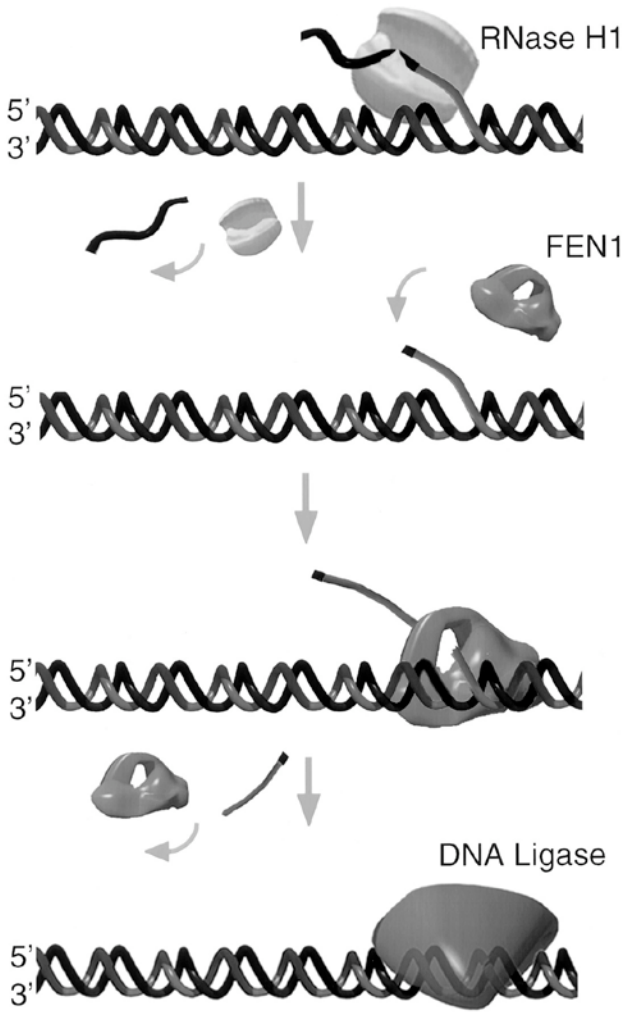
In mammals (whose DNA pol lacks exonuclease activity in the 5’ - 3' sense) this process is more complex:

1.- An enzyme called RNase H1 identifies DNA:RNA hybrids and nicks oligo end.

2.- FEN1 flap-endonuclease excises the the RNA oligo.

3.- DNA polymerase (repairase) fills in RNA oligo with DNA.

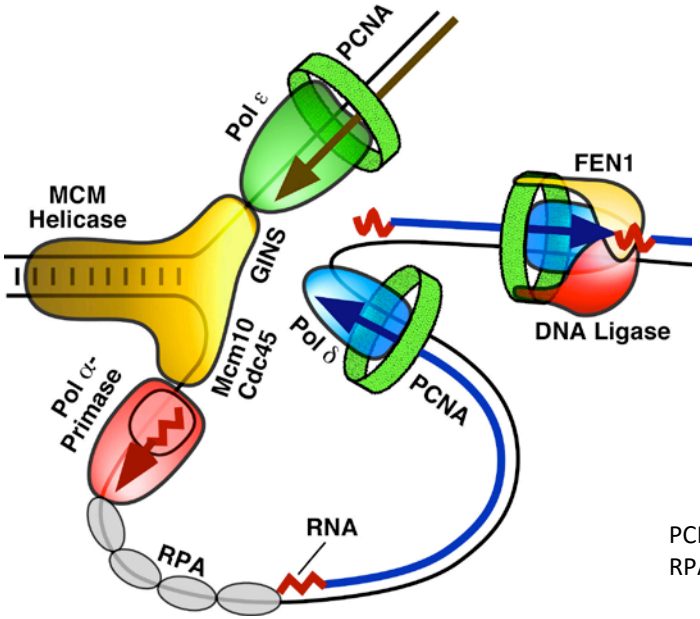
4.- DNA Ligase joins DNA strands.

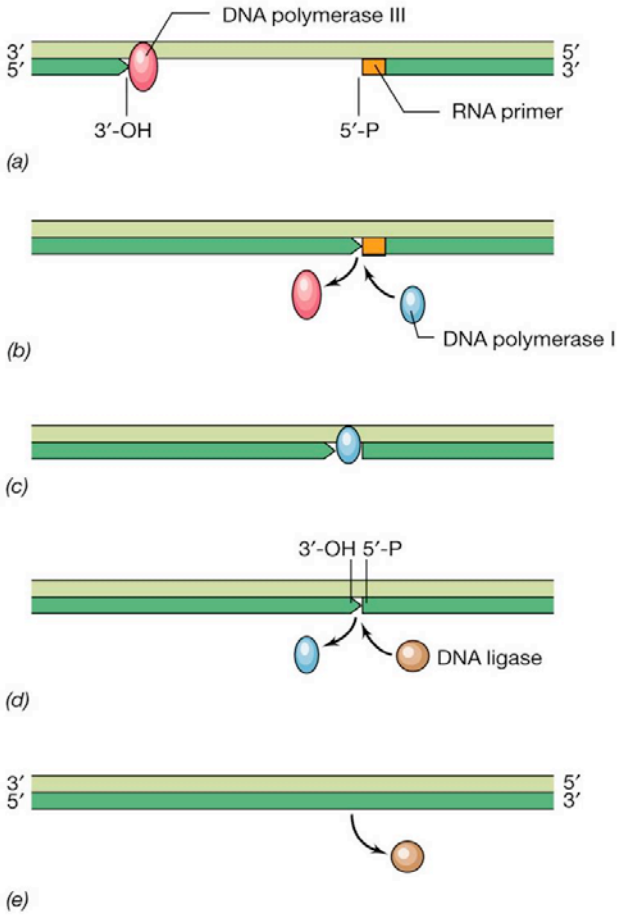


Okazaki fragmetn processing

Once this is achieved (in both pro- and eukaryotes), the Okazaki fragments must be joined together.

This responsibility falls to an enzyme called ligase, which joins the 3' terminal hydroxyl of an Okazaki fragment to the 5' phosphate of the adjacent fragment.





PCNA: Proliferating Cell Nuclear Antigen (DNA clamp)
RPA: Replication Protein A (SSB)

Summary video

https://www.youtube.com/watch?v=bee6PWUgPo8

Laboratorio de Genómica Viral y Humana

Instalaciones de Alta Contención Biológica Nivel de Bioseguridad 3 (BSL-3) CDC-certificadas

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