

DNA replication in eukaryotes

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Introduction

- ❑ DNA replication is the process of producing two identical copies from one original DNA molecules.
- ❑ It is the basis of inheritance and therefore it occurs in all living organisms.
- ❑ Fundamental mechanism of DNA replication is same, however there are some variation due to difference in molecular properties.
- ❑ DNA replication in eukaryotes (karyobionta) is more complex than the DNA replication in prokaryotes (akaryobionta).
- ❑ DNA in eukaryotes are highly condensed nucleosomal structure which needs to be unwound before replication.
- ❑ Majority of DNA replication (synthesis) occurs during S phase of the cell cycle (telomere is synthesized in late S phase).
- ❑ Due to complex nature of eukaryotic chromosomes, replication is not as well understood as that in prokaryotes.
- ❑ Replication is bidirectional as in the prokaryotes.

Introduction

Cell cycle

G1

Preparing for DNA replication
(cell growth)

S

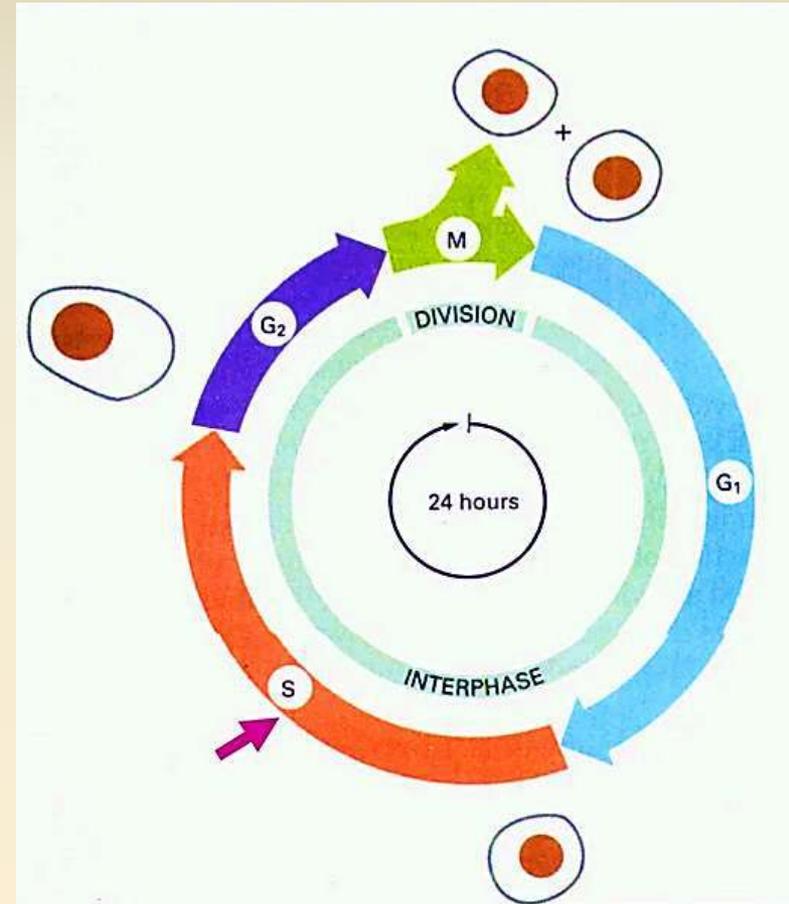
DNA replication

G2

A short gap before mitosis

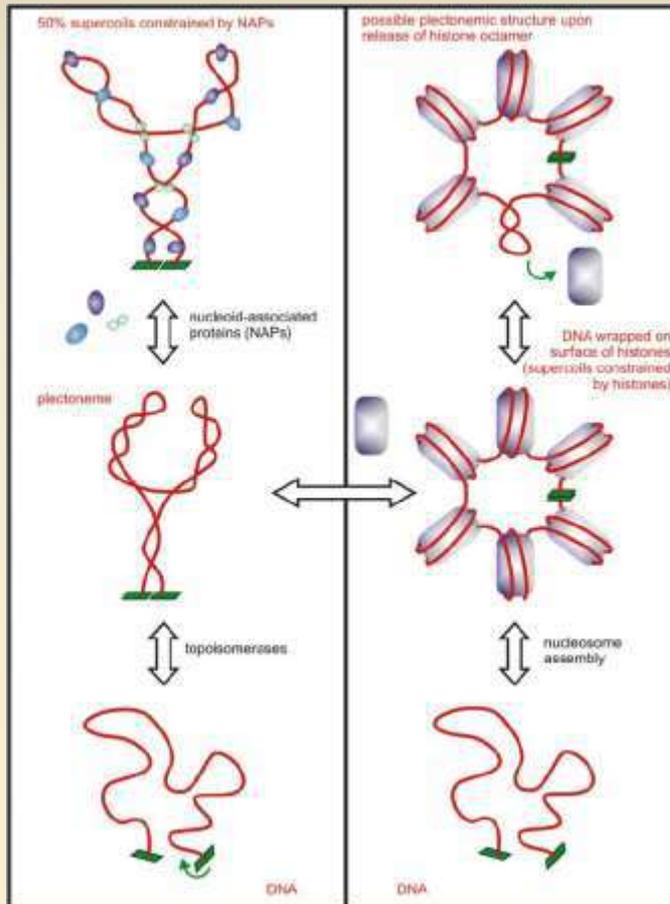
M

Mitosis and cell division



origin recognition complex (ORC)

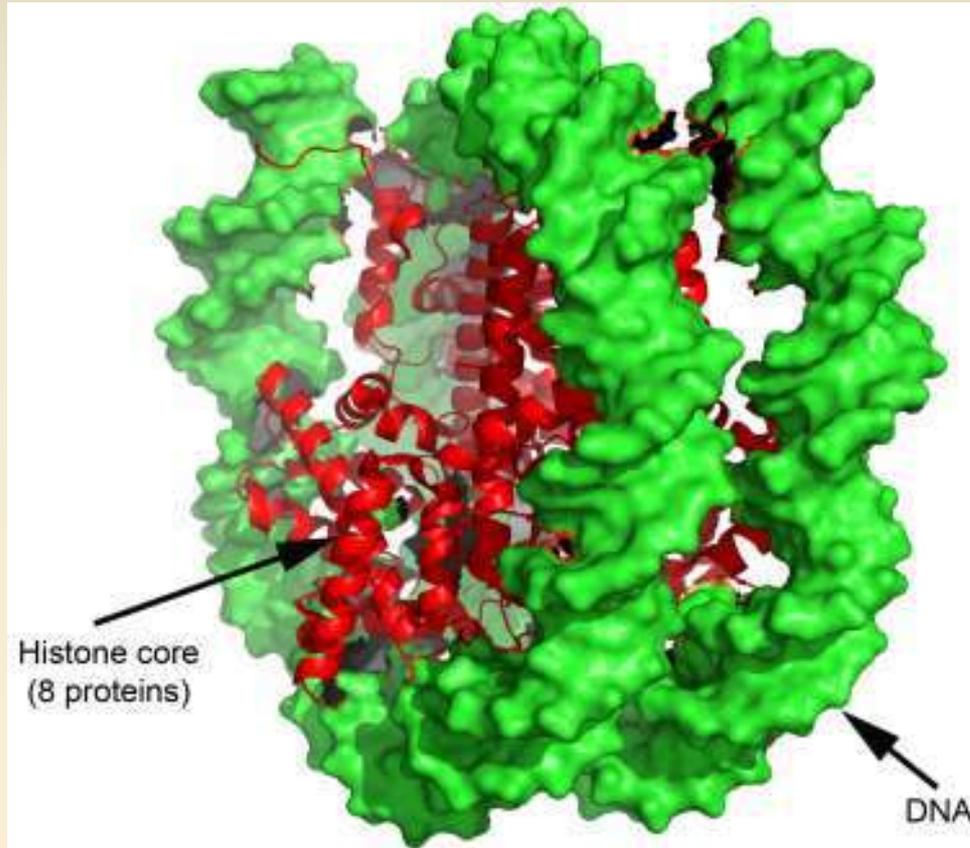
Prokaryotic and eukaryotic DNA supercoils



- ❑ Most prokaryotic genomes are less than 5 Mb in size (*Bacillus megaterium* has 30 Mb), while in eukaryotes, ranges from 10 Mb in some fungi to > 100,000 Mb in certain plants, salamanders and lungfishes).
- ❑ Prokaryotic chromosomes are circular, while eukaryotic one is linear.
- ❑ Chromatin is found in the eukaryotic chromosome.
- ❑ Opening DNA in prokaryotes is fast, while fork formation and movement is slow in eukaryotes.

DNA replication at heterochromatin region and telomeric region is slow, while at euchromatin and centromeric region, replication is faster.

Nucleosome formation

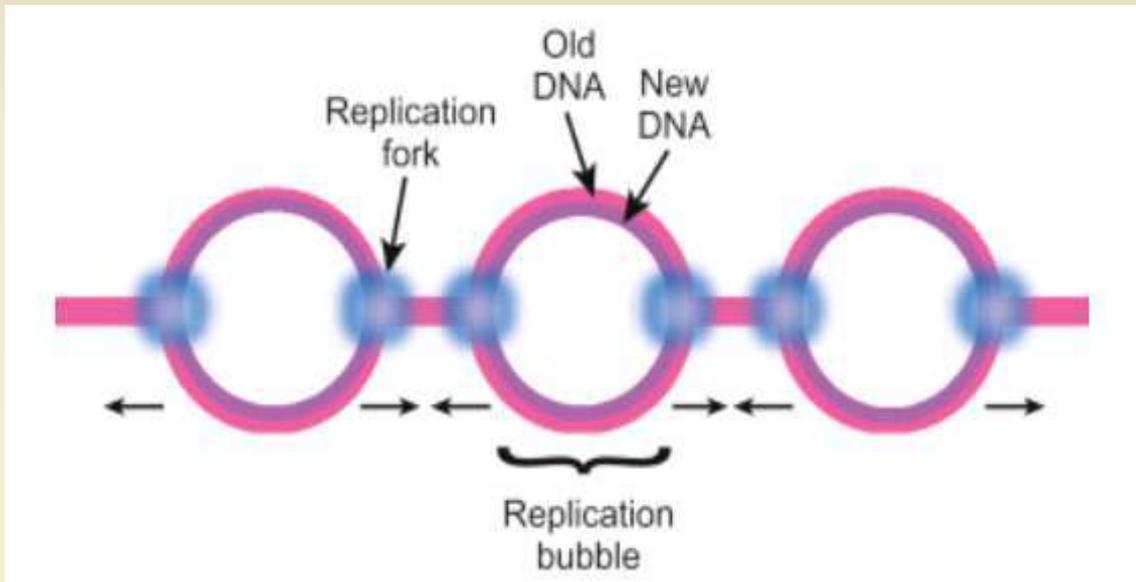


The positive charge of histones (lysine and arginine amino acids), is a major feature of the histone molecules enabling them to bind the negatively charged phosphate backbones of DNA. Pairs of four different histones (H2A, H2B, H3, and H4) combine to form an eight-protein bead around which DNA is wound (nucleosome)

Structure of a nucleosome

Bhagavan & Ha, in Essentials of Medical Biochemistry (Second Edition), 2015

Initiation of replication

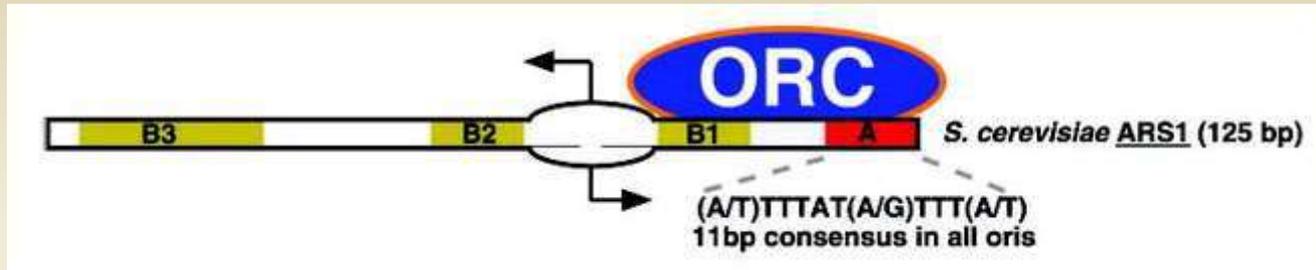


- G1-CDK is activated by cyclins.
- Activated G1-CDK activates S-phase specific CDK (S-CDK).
- **Activated** S-CDK then triggers the assembly of proteins at the origins of replication

Eukaryotic Chromosome Replication Bubbles

- **Nearly 10,000 and 100,000 replication origins may be found in a dividing human somatic cell.**
- **Each origin must initiate once only during each replication cycle in order to avoid duplication of DNA segments that have already been replicated.**

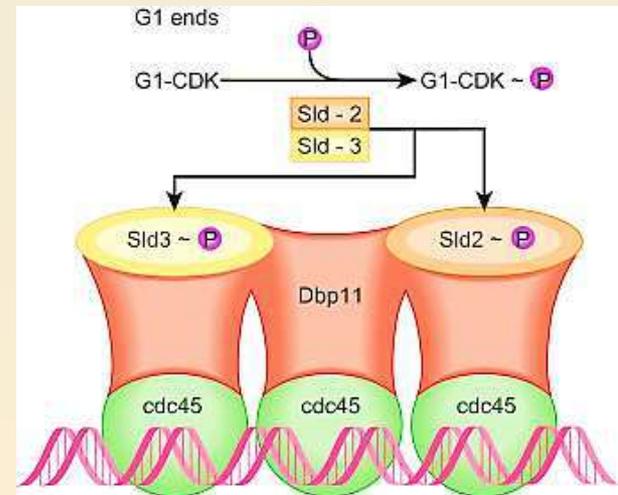
Initiation of replication



Replication origins in eukaryotes.

Gilbert D.M. Science. 2001 Oct 5; 294(5540): 96–100.

- ❑ After S-CDK activation, S-CDK transfers a phosphate to Sld2 and Sld3.
- ❑ Phosphorylated Sld2 and Sld3 bind to Dbp11, which acts as a scaffolding protein that holds the replication origin proteins in position.
- ❑ After that, cdc45 associates with the origin of replication to form the pre-loading complex (pre-LC).
- ❑ Then, a large number of different proteins, such as helicase, initiates unwinding of the DNA helix.



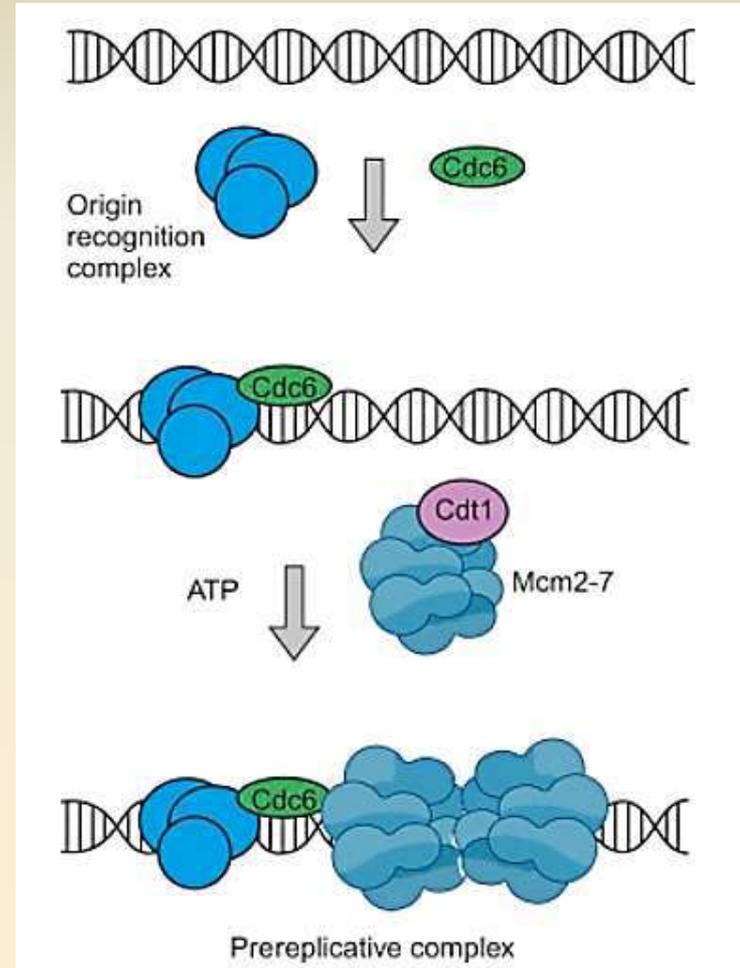
Clark & Pazdernik, in Molecular Biology (Second Edition), 2013

Initiation of replication

- ORC recruits Cdc6 and Cdt1 (also known as actor).
- Cdc6 and Cdt1 recruit the minichromosome maintenance (MCM) complex to form the pre-replicative complex (pre-RC).
- The MCM complex consists of 6 proteins (Mcm2 – Mcm7) that form a hexameric ring around the DNA.
- After activation (discussed later), MCM acts as a helicase to unwind the double helix and thus is equivalent to the bacterial helicase DnaB.

Licensing of origin

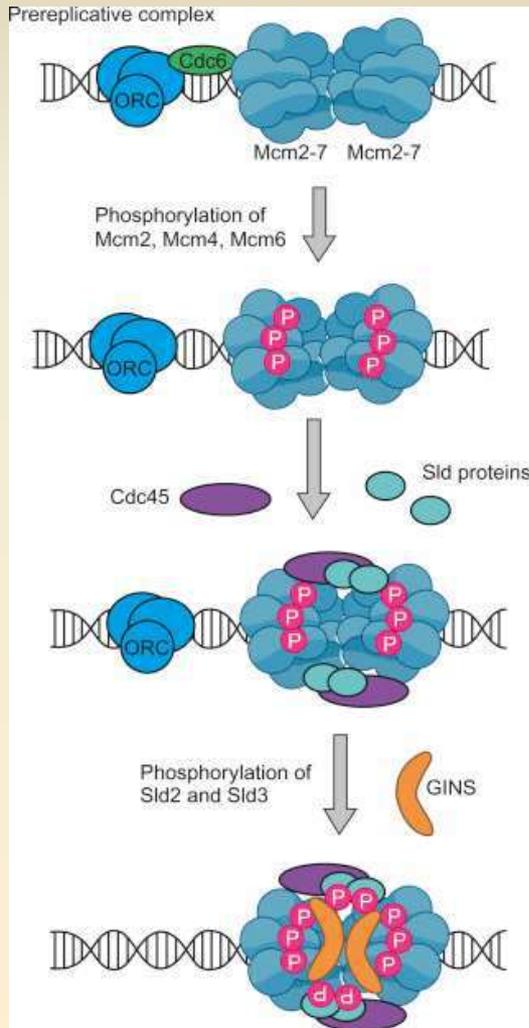
Clark et. al., in Molecular Biology (Third Edition), 2019



Formation of the Pre-replicative Complex

Initiation of replication

Formation of two active Replication Complex



- The origin recognition complex recognizes the origins of eukaryotic chromosomes.
- ORC is then joined by a series of other proteins, including MCM helicase, to form the pre-replicative complex.
- The MCM assemblies in the Pre-RC are phosphorylated by CDK.
- Phosphorylation promotes the binding of Cdc45 protein and the Sld proteins. CDK next activates Sld2 and Sld3.
- This enables the GINS complex (GINS complex – **Go**, **I**chi, **N**ii, and **S**an.) to bind to the Pre-RC. GINS complex is needed for the MCM helicase to operate.
- Finally, protein Mcm10 binds and the assemblage separates into two replication complexes or replisomes that proceed in opposite directions from the origin in a 3' to 5' direction.
- DNA polymerase also joins at this stage.

Clark et. al., in *Molecular Biology (Third Edition)*, 2019

Initiation of replication

Eukaryotes also contain multiple DNA polymerases

	DNA pol α	DNA pol β	DNA pol γ	DNA pol δ	DNA pol ϵ
3' exonuclease	No	No	Yes	Yes	Yes
Fidelity	10^{-4} - 10^{-5}	5×10^{-4}	10^{-5}	10^{-5} - 10^{-6}	10^{-6} - 10^{-7}
Processivity	Moderate	Low	High	High	High
Role	Lagging strand primer synthesis	DNA repair	Mitochondria I DNA replication	Lagging strand replication	Leading strand replication

Elongation

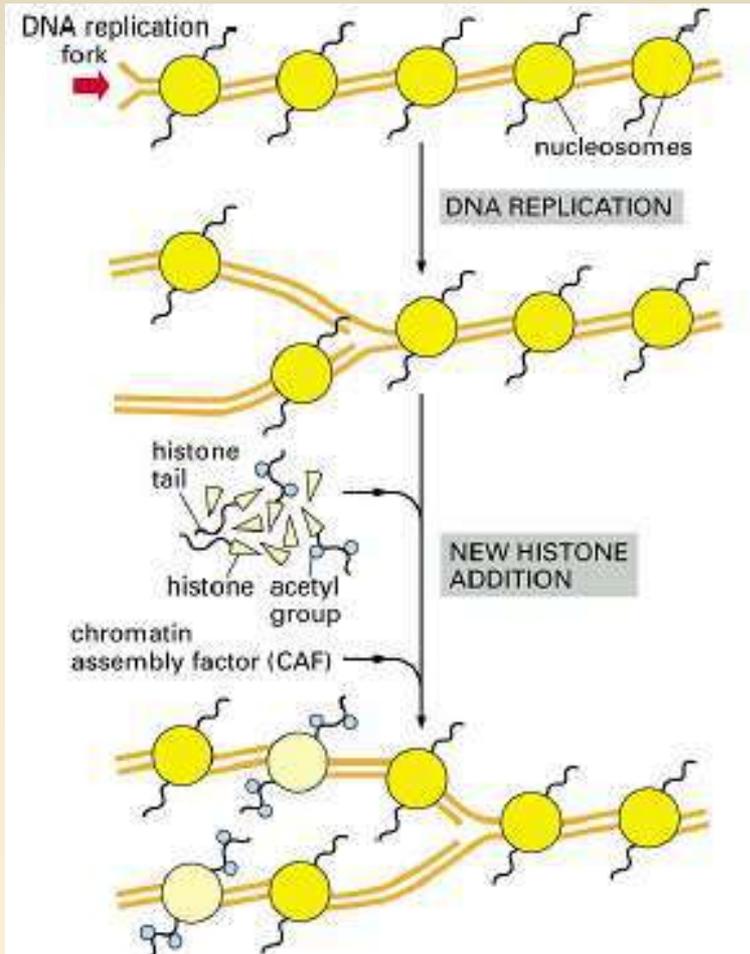


Figure 5-41 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

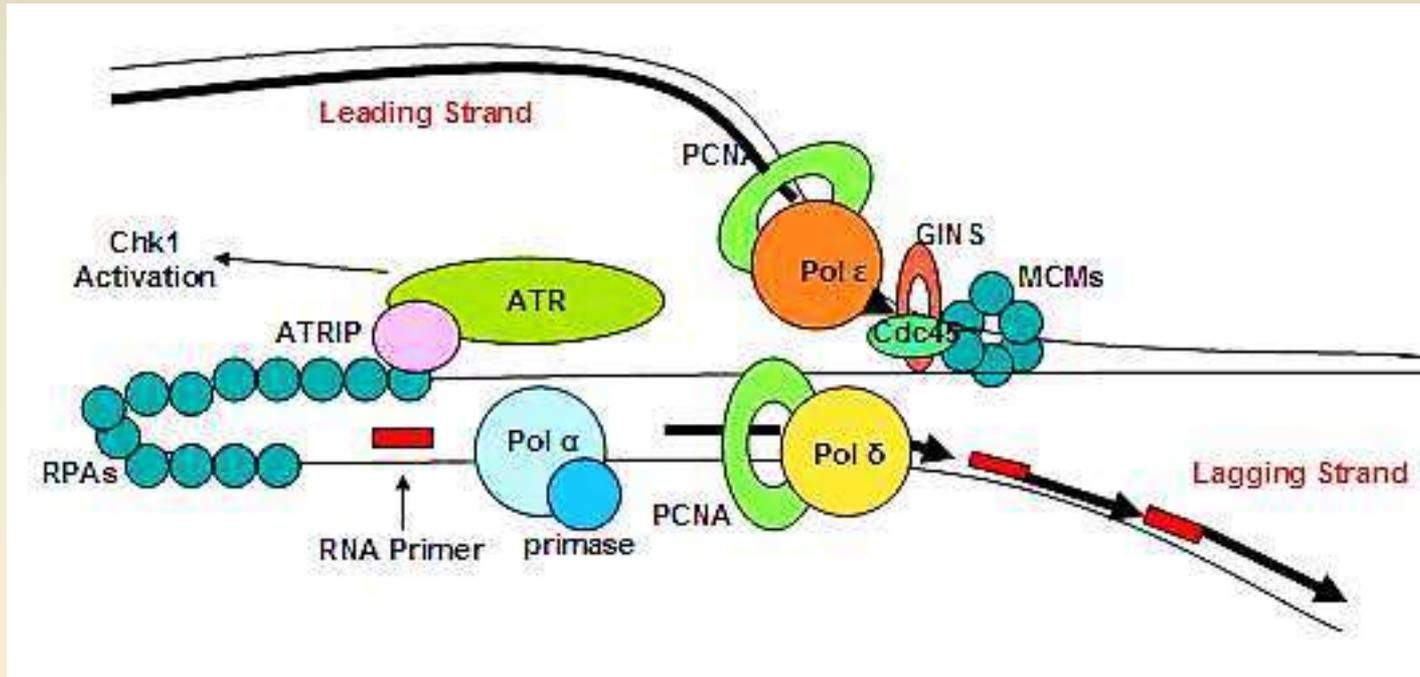
DNA polymerase starts synthesizing after its recruitment in Pre-Replicative complex (Pre-RC).

Histones are synthesized only during S phase and are added as replication proceeds.

Some histone parts are “inherited” and some are new.

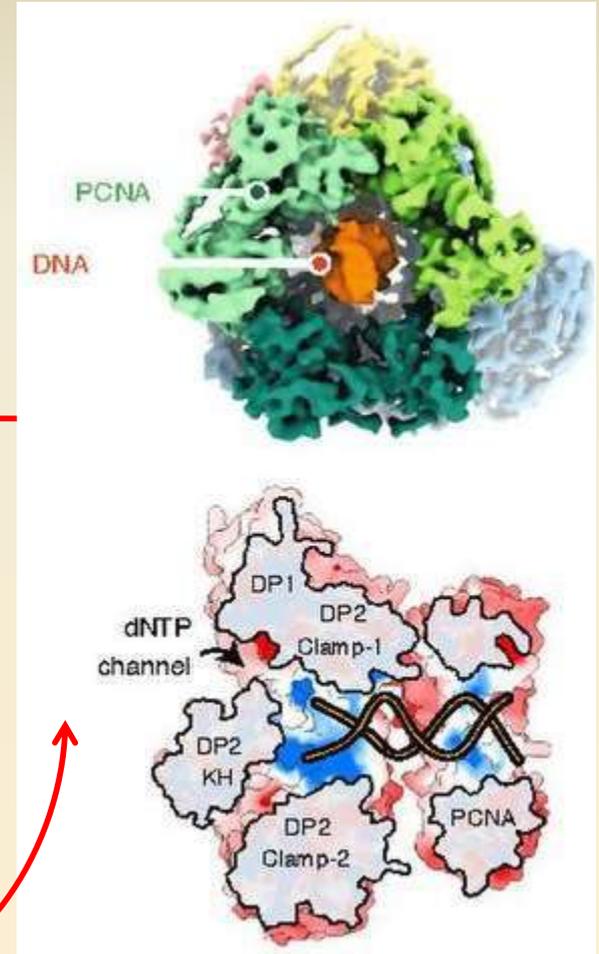
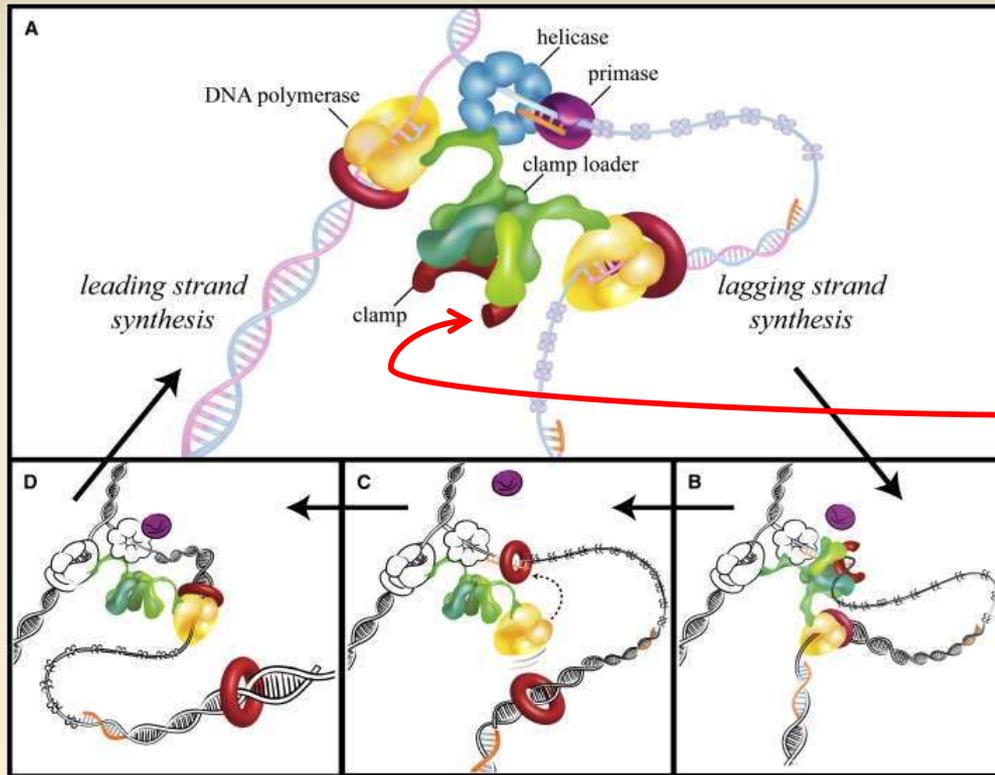
The spacing of histones every 200 nt might be the reason for the shorter Okazaki fragments in eukaryotes and the slower speed of replication.

Elongation



- DNA polymerase adds DNA nucleotides to the 3' end of the newly synthesized polynucleotide strand or primer synthesized by DNA primase (RNA polymerase).
- Proliferating cell nuclear antigen (PCNA) acts as DNA clamp and processivity factor for DNA polymerase δ .
- ATR is a serine/threonine kinase which maintains genome integrity by stabilizing replication forks.

Elongation



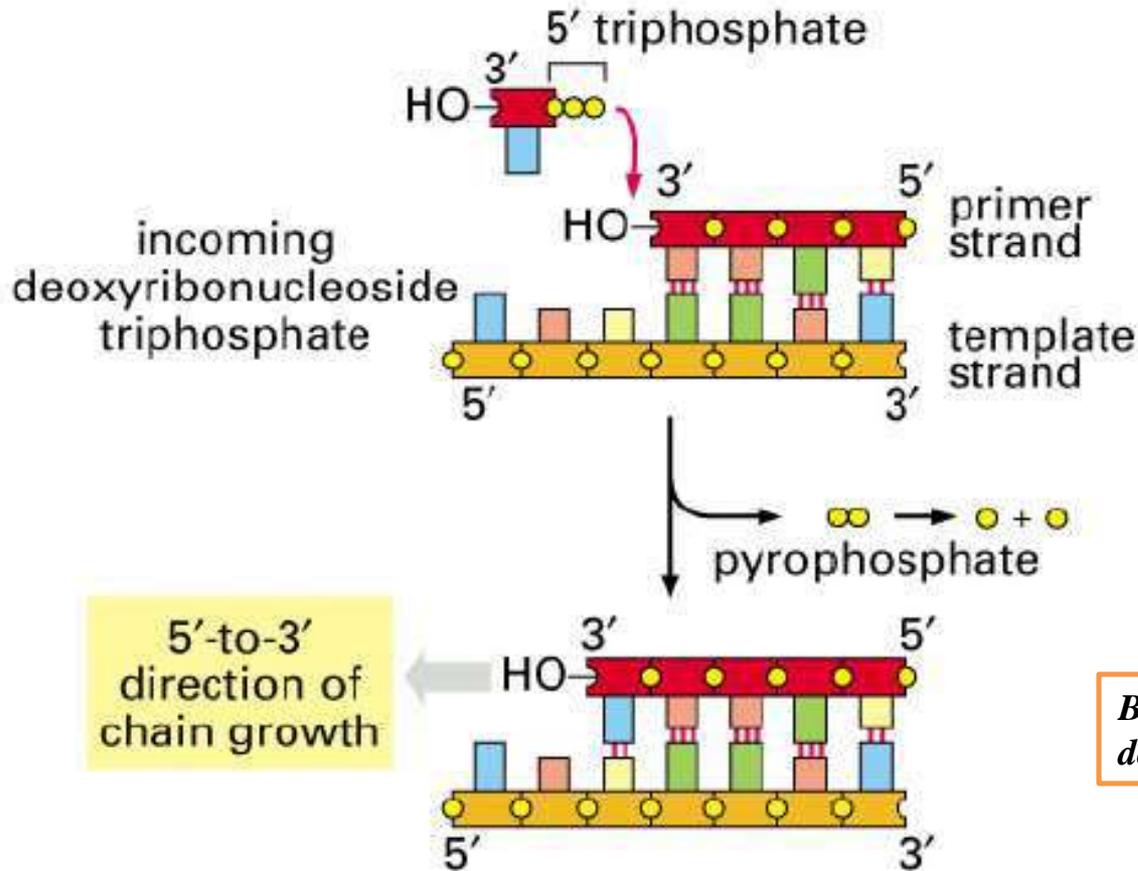
Replication complex and its moving on

Molecular Cell 2006; 23(2): 155-160.

DNA Clamp

Elongation

DNA is synthesized in 5' to 3' direction



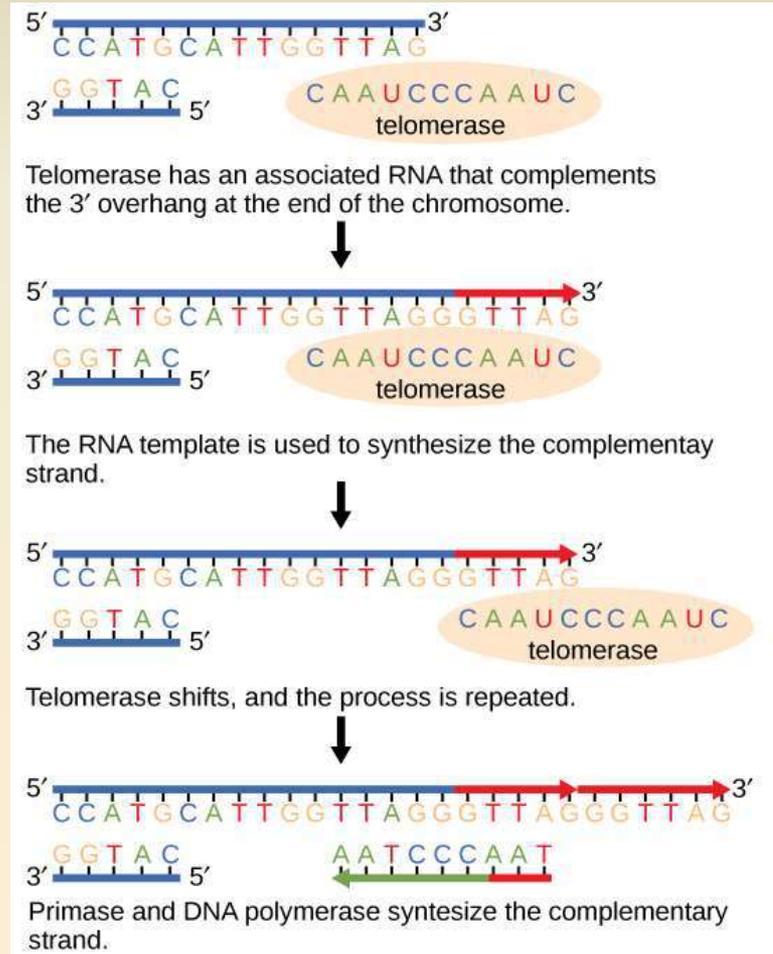
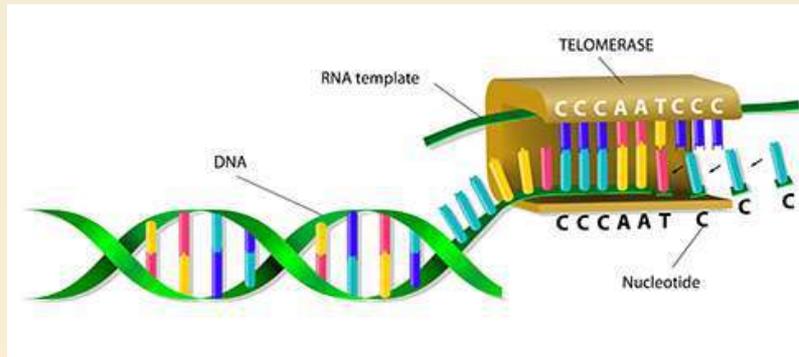
Bacterial chromosome doubles in 40 min

Molecular Biology of the Cell, 4th Edition.

Elongation

DNA synthesis at telomere

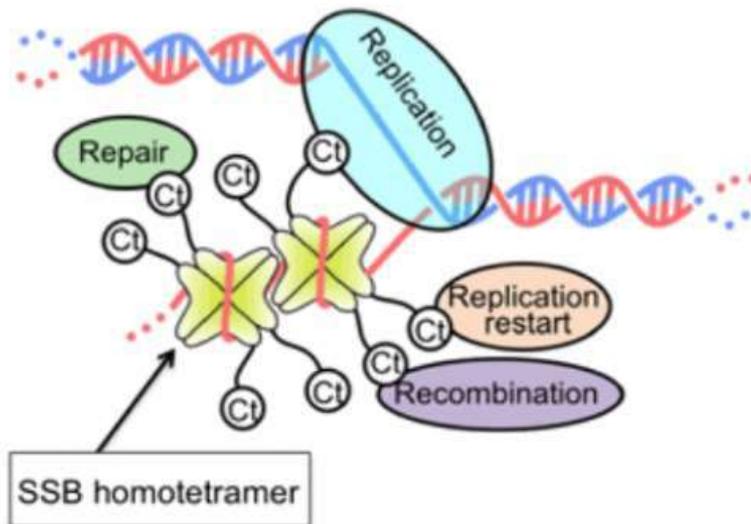
- Telomeres are repetitive sequences of TTAGGGs, these telomeres (Hexamers) cap the end of human chromosomes and are believed to prevent chromosome from undergoing degradation.
- Unlike DNA polymerase, telomeric regions are synthesized by telomerase enzyme activated at the late S phase of cell cycle.



Elongation

Single Strand Binding proteins are required to protect the unwound single strand DNA at replication fork or bubble.

SSB as an organizational hub for bacterial genome maintenance

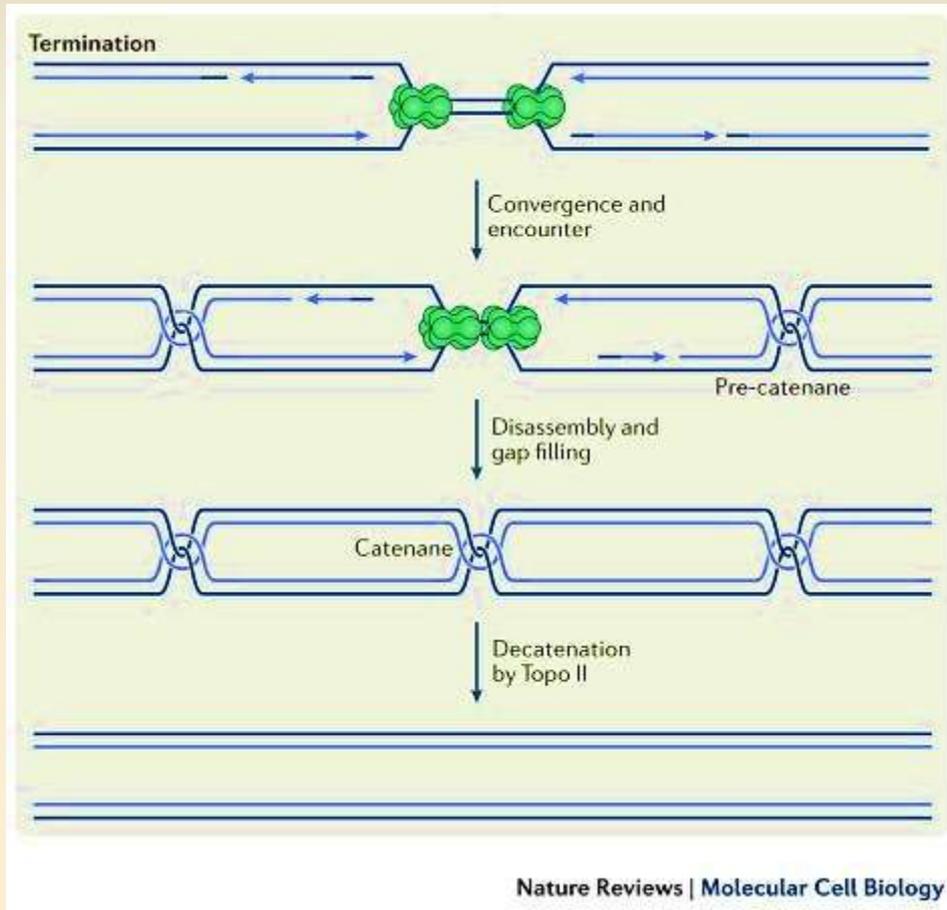


SSB-interacting protein	Require SSB-Ct?
DNA polymerase III, chi	Yes
Primase	Yes
Topoisomerase III	Yes
RecQ	Yes
RecO	Yes
RecJ	Yes
RecG	Yes
PriA	Yes
PriB	Unknown
Exonuclease I	Yes
Uracil DNA glycosylase	Yes
DNA polymerase II	Yes
DNA polymerase V	Yes
Exonuclease IX	Unknown
vRNA polymerase	Yes

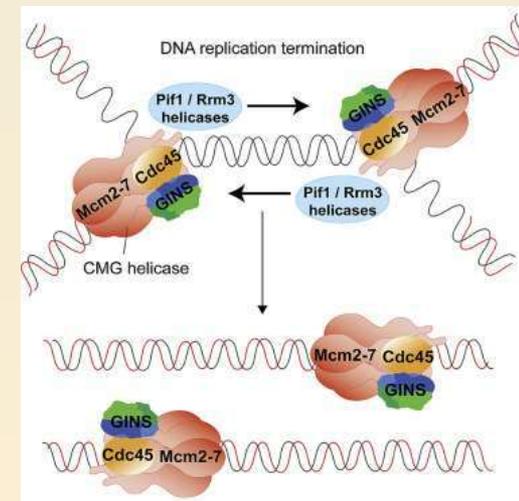
Shereda et al Crit Rev Biochem Mol Biol, 2008.

Termination

Termination of DNA replication is achieved convergence of advancing replication fork or bubble



- Catenation is removed by helicase, i.e., Pif1/Rrm3 helicase
- Gap is filled by DNA ligase



Molecular Cell 2019; 74(2): 231-244.e9

Further reading

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