SOS in Biochemistry, Jiwaji University, Gwalior
M.Sc. II Semester (2019-20)
Paper BCH 205: Fundamentals of Molecular Biology (Unit 1)

ENZYMOLOGY
of
DNA REPLICATION - II

Eukaryotic DNA Polymerases

EUKARYOTIC DNA REPLICATION

Eukaryotic DNA replication is not as well understood as bacterial replication

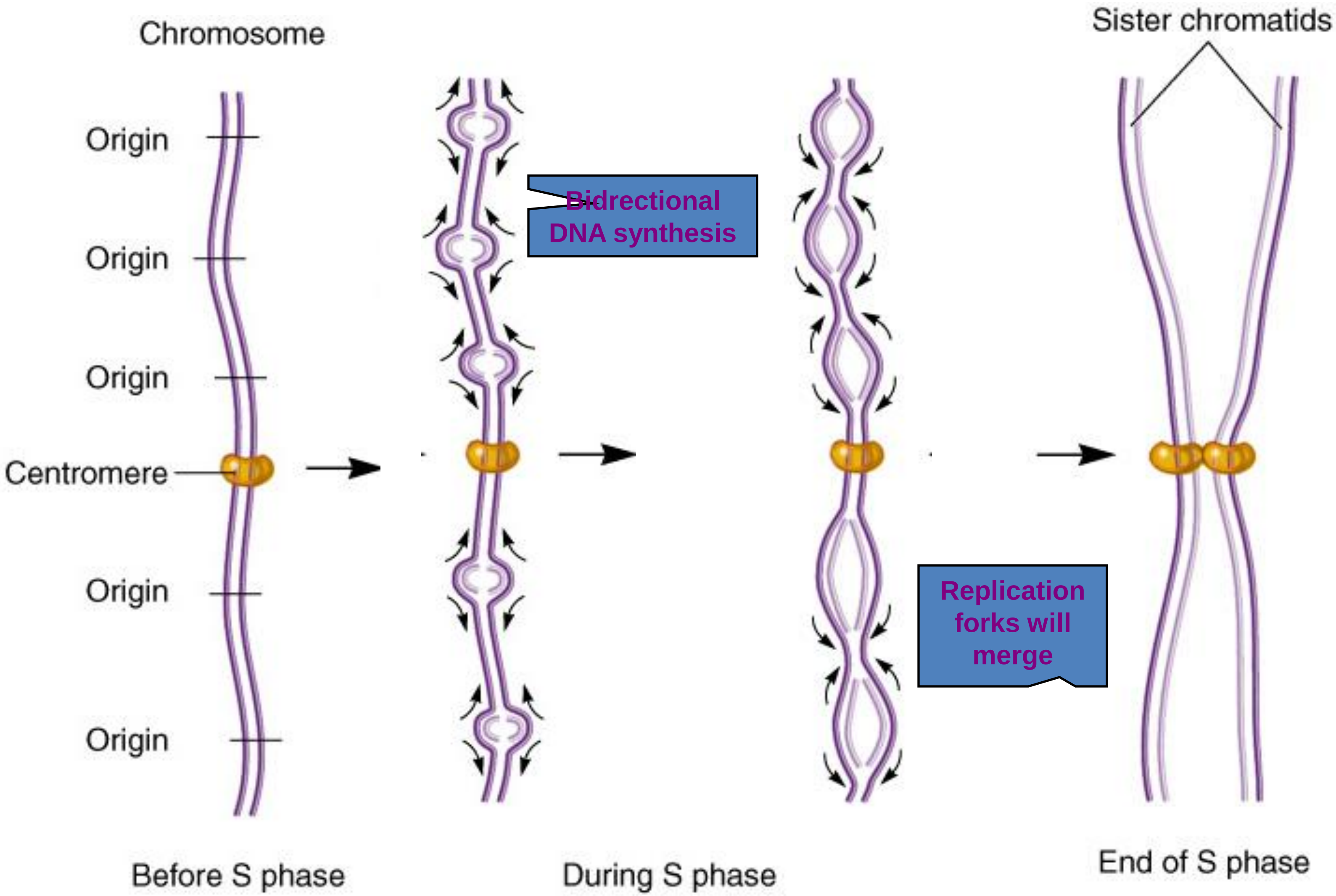
- **The two processes do have extensive similarities**
  - The bacterial enzymes as described earlier have also been found in eukaryotes
- **Nevertheless, DNA replication in eukaryotes is more complex**
  - Large linear chromosomes
  - Tight packaging within nucleosomes
  - More complicated cell cycle regulation

Multiple Origins of Replication

- Eukaryotes have long linear chromosomes
  - They therefore require multiple origins of replication
    - To ensure that the DNA can be replicated in a reasonable time

- In 1968, Huberman and Riggs provided evidence for the multiple origins of replication

- DNA replication proceeds bidirectionally from many origins of replication



(a) DNA replication from multiple origins of replication

Eukaryotes Contain Several Different DNA Polymerases

- **Mammalian cells contain well over a dozen different DNA polymerases**
- **Four: alpha (α), delta (δ), epsilon (ε) and gamma (γ) have the primary function of replicating DNA**
  - **α, δ and ε → Nuclear DNA**
  - **γ → Mitochondrial DNA**

वफ़ादारी, ईमानदारी

DNA polymerase	Function	Structure
	High fidelity replicases	
α	Nuclear replication	350 kD tetramer
δ	Lagging strand	250 kD tetramer
ϵ	Leading strand	350 kD tetramer
γ	Mitochondrial replication	200 kD dimer
	High fidelity repair	
β	Base excision repair	39 kD monomer
	Low fidelity repair	
zeta ζ	Base damage bypass	heteromer
eta η	Thymine dimer bypass	monomer
iota ι	Required in meiosis	monomer
kappa κ	Deletion and base substitution	monomer

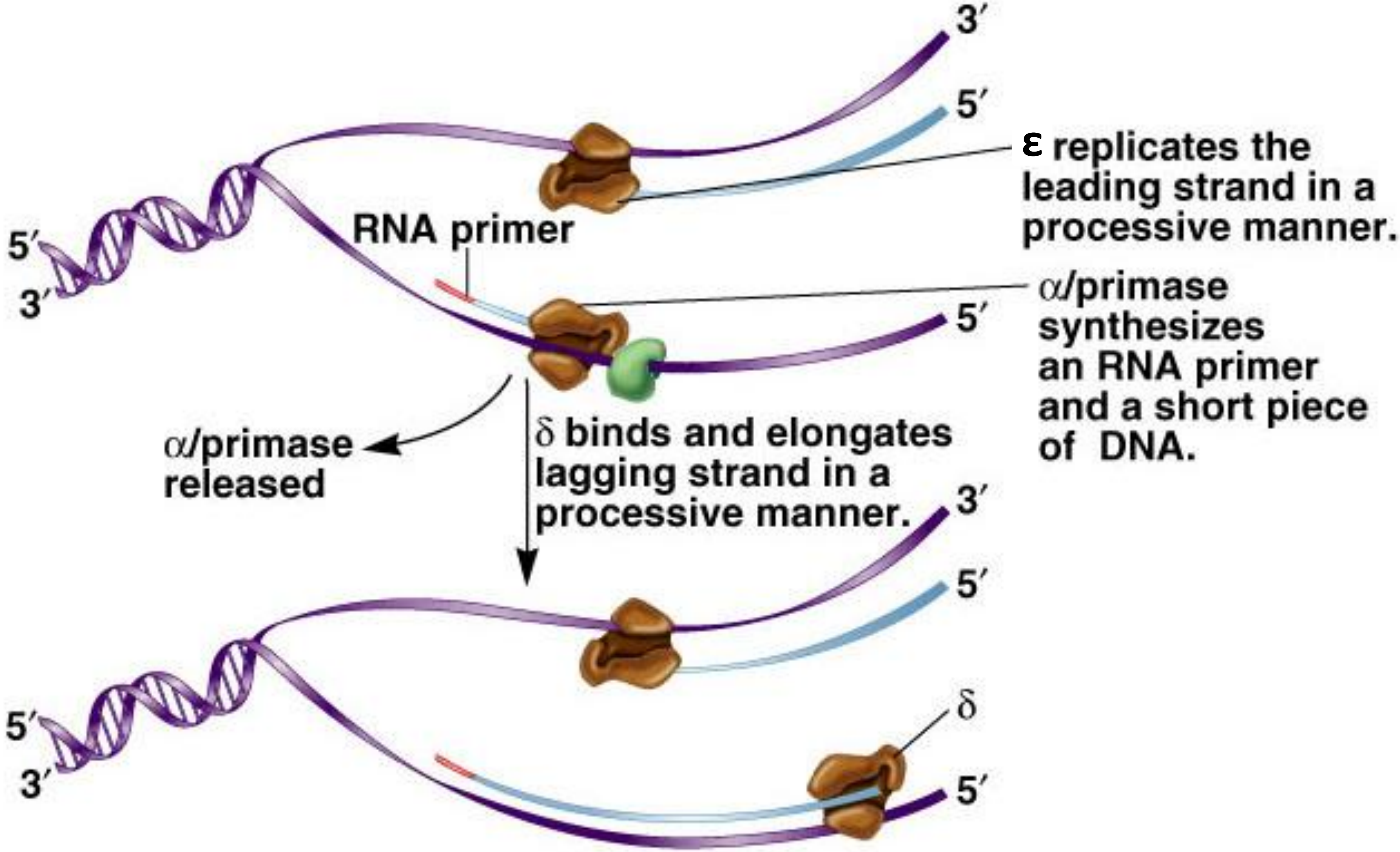
Eukaryotic DNA Polymerases (Mammalian Enzymes)

Polymerase Types*	Function
α, δ, ε	Replication of nondamaged DNA in the cell nucleus during S phase
γ	Replication of mitochondrial DNA
η, κ, ι, ζ (lesion-replicating polymerases)	Replication of damaged DNA
α, β, δ, ε, σ, λ, μ, ϕ, θ	DNA repair or other functions†

†Many DNA polymerases have dual functions. For example, DNA polymerase δ is involved in the replication of normal DNA, and it also plays a role in DNA repair. In cells of the immune system, certain genes that encode antibodies (i.e., immunoglobulin genes) undergo a phenomenon known as hypermutation. This increases the variation in the kinds of antibodies the cells can make. Certain polymerases in this list, such as μ, may play a role in hypermutation of immunoglobulin genes. DNA polymerase σ may play a role in sister chromatid cohesion,

- **DNA pol α is the only polymerase to associate with primase**
  - **The DNA pol α/primase complex synthesizes a short RNA-DNA hybrid**
    - **10 RNA nucleotides (iRNA) followed by 20 to 30 DNA nucleotides (iDNA)**
  - **This is used by DNA pol ε or δ for the processive elongation of the leading and lagging strands respectively**

- **The exchange of DNA pol α for δ or ε is called a polymerase switch.**
  - **It occurs only after the RNA-DNA hybrid is made**


3′
5′
ε replicates the leading strand in a processive manner.
5′
3′
RNA primer
α/primase synthesizes an RNA primer and a short piece of DNA.
5′
α/primase released
δ binds and elongates lagging strand in a processive manner.
3′
5′
5′
3′
δ
5′

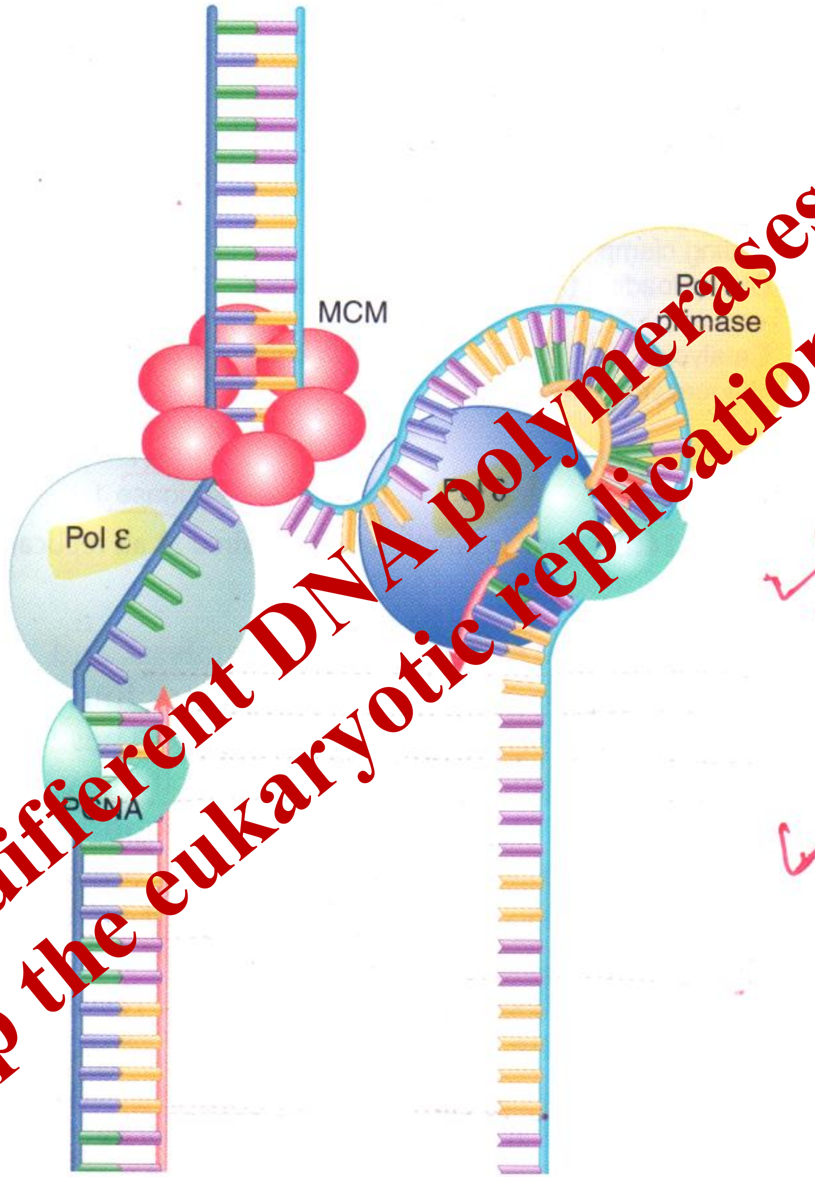
- DNA polymerases also play a role in DNA repair
  - DNA pol β is not involved in DNA replication
  - It plays a role in base-excision repair
    - Removal of incorrect bases from damaged DNA

- Recently, more DNA polymerases have been identified
  - Lesion-replicating polymerases
    - Involved in the replication of damaged DNA
    - They can synthesize a complementary strand over the abnormal region

Function	*E. coli*	Eukaryote	Phage T4
Helicase	DnaB	MCM complex	41
Loading helicase/primase	DnaC	cdc6	59
Single strand maintenance	SSB	RPA	32
Priming	DnaG	Polα/primase	61
Sliding clamp	β	PCNA	45
Clamp loading (ATPase)	γδ complex	RFC	44/62
Catalysis	*Pol III core*	Polδ + Pol ε	43
Holoenzyme dimerization	τ	?	43
RNA removal	*Pol I*	FEN1	43
Ligation	*Ligase*	Ligase 1	T4 ligase

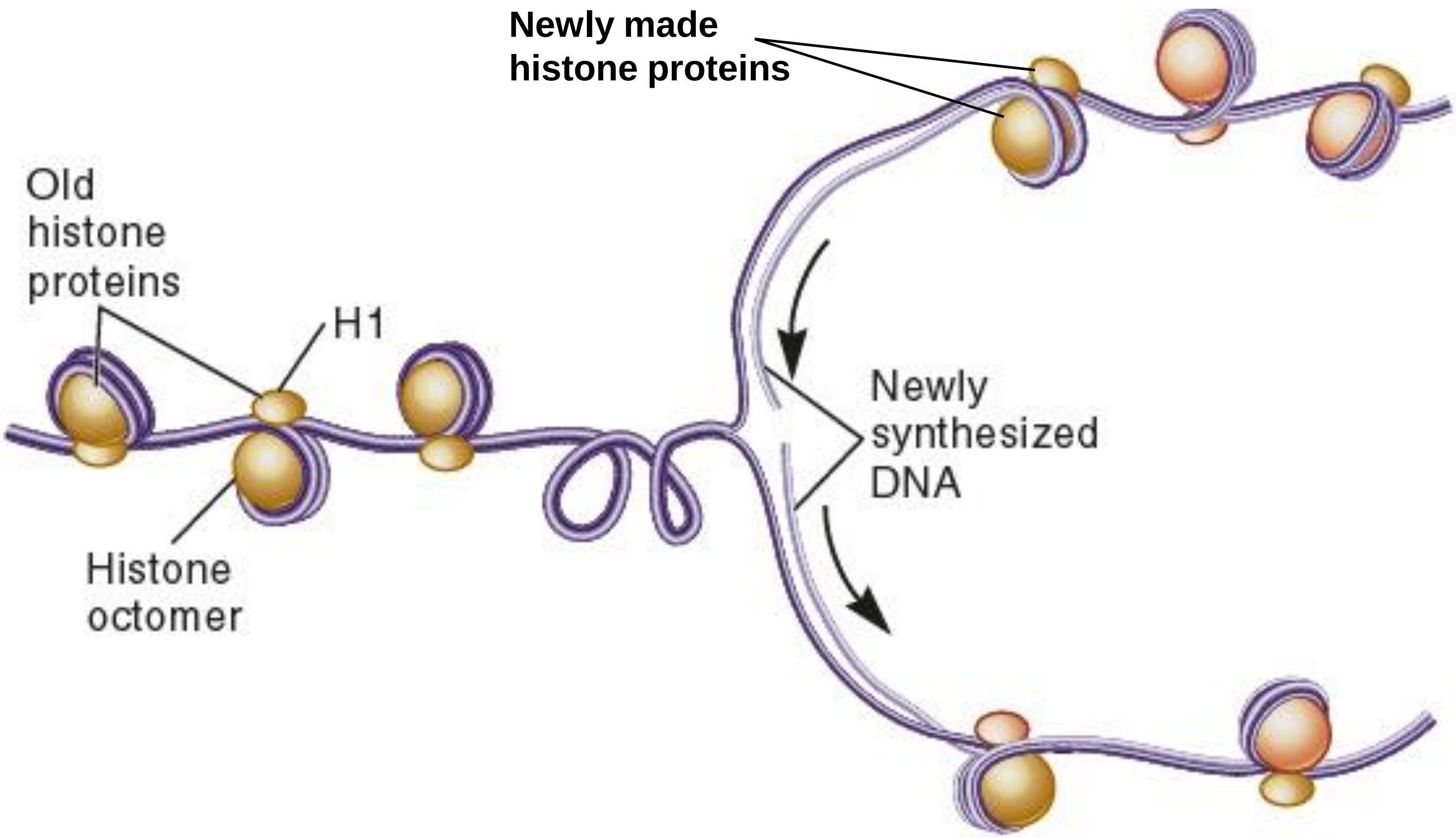
FIGURE 14.27 Similar functions are required at all replication forks.

Three different DNA polymerases make up the eukaryotic replication fork



Nucleosomes and DNA Replication

- Replication doubles the amount of DNA
  - Therefore the cell must synthesize more histones to accommodate this increase
- Synthesis of histones occurs during the S phase
  - Histones assemble into octamer structures
    - They associate with the newly made DNA very near the replication fork
- Thus following DNA replication, each daughter strand has a mixture of “old” and “new” histones



Newly made
histone proteins
Old
histone
proteins
H1
Histone
octomer
Newly
synthesized
DNA