DNA and RNA Polymerases

Introduction - Definitions

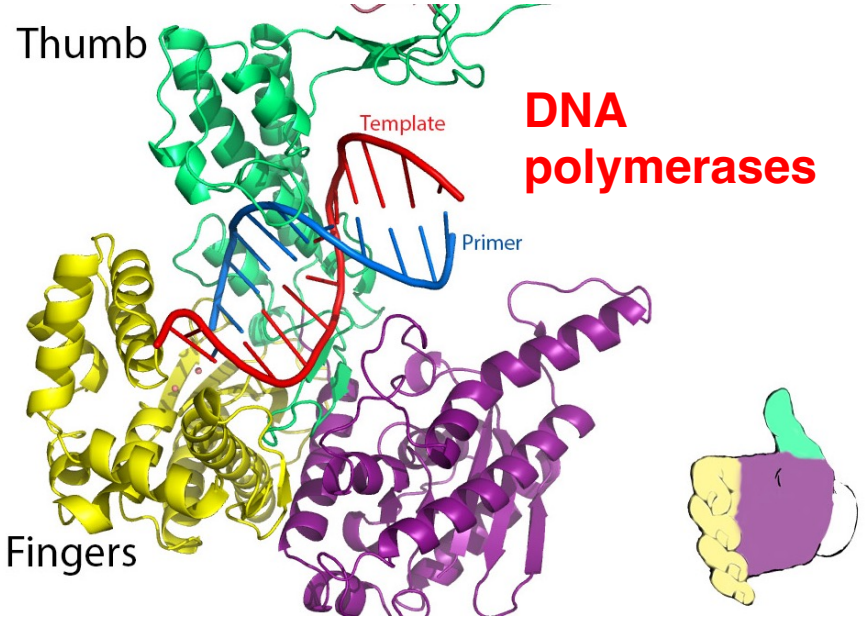
1) Enzymatic activities and different families of DNA and RNA polymerases

2) Two metal ions catalysis model for DNA and RNA polymerases

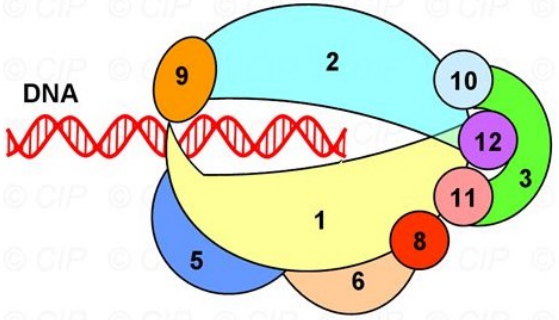
3) Sugar discrimination by DNA and RNA polymerases

4) Fidelity of nucleotide incorporation and editing/proofreading mechanisms

5) Speed and processivity: clamps & processivity factors



DNA polymerases



RNA polymerases

Learning outcomes:
What you need to know/understand after this unit

Basic mechanisms of polymerization by DNA/RNA polymerases

Understand the structure of a Polymerase active site; Visualize the different substrates of the reaction and the elements of the active site involved in catalysis/ 2 metal ions catalysis

Understand the active site components of DNA/RNA polymerases that confer specificity for the proper nucleotide to be incorporated: Base and Sugar

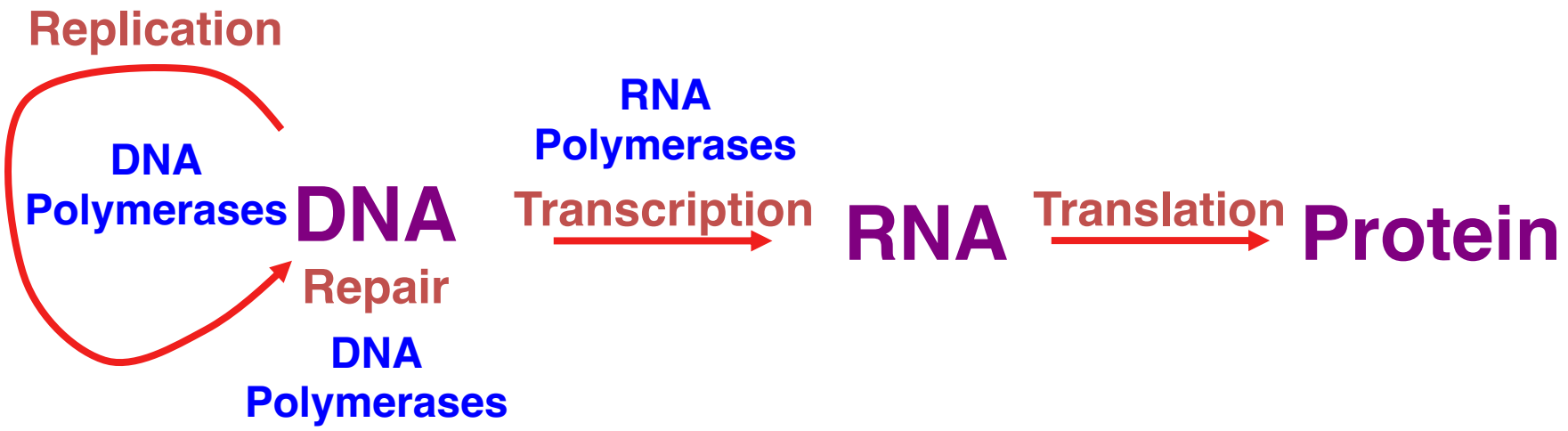
Understand how editing activities confer higher fidelity to DNA/RNA polymerases

Understand the mechanisms used to increase processivity of these polymerases

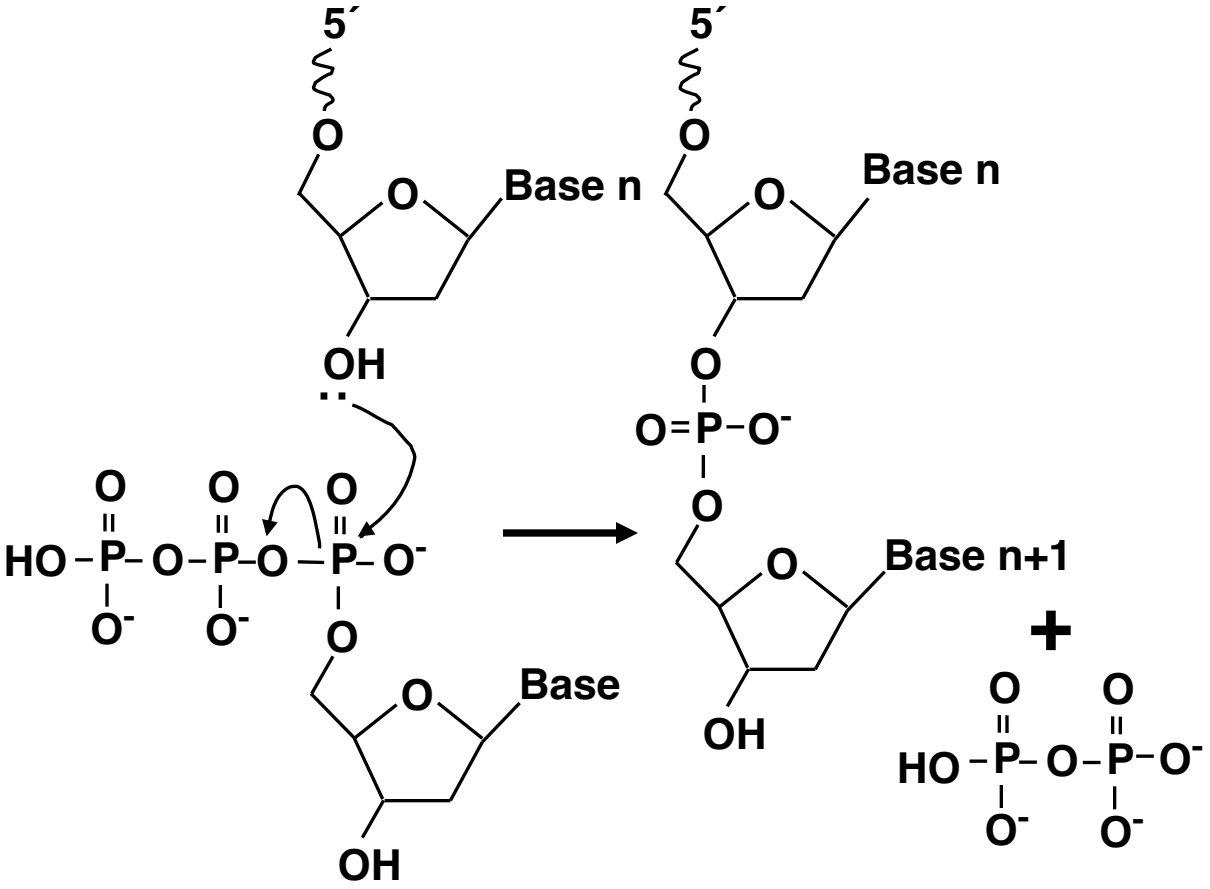
Nucleic Acid Polymerases

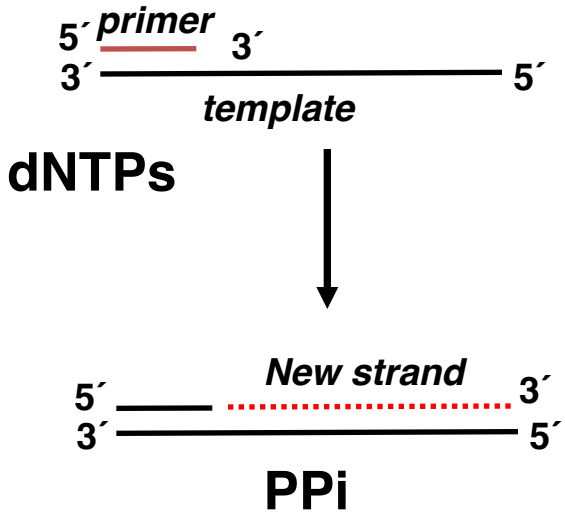
Why study them together and separately from the processes they are involved?

- **Common mechanisms for nucleic acids polymerization**
- **Comparison of substrate specificity**
- **Common principles for fidelity and proofreading**
- **Sets the stage for the next chapters (Replication, Repair, Transcription) to integrate their functions in the cellular context**



Fundamental properties of DNA Polymerases



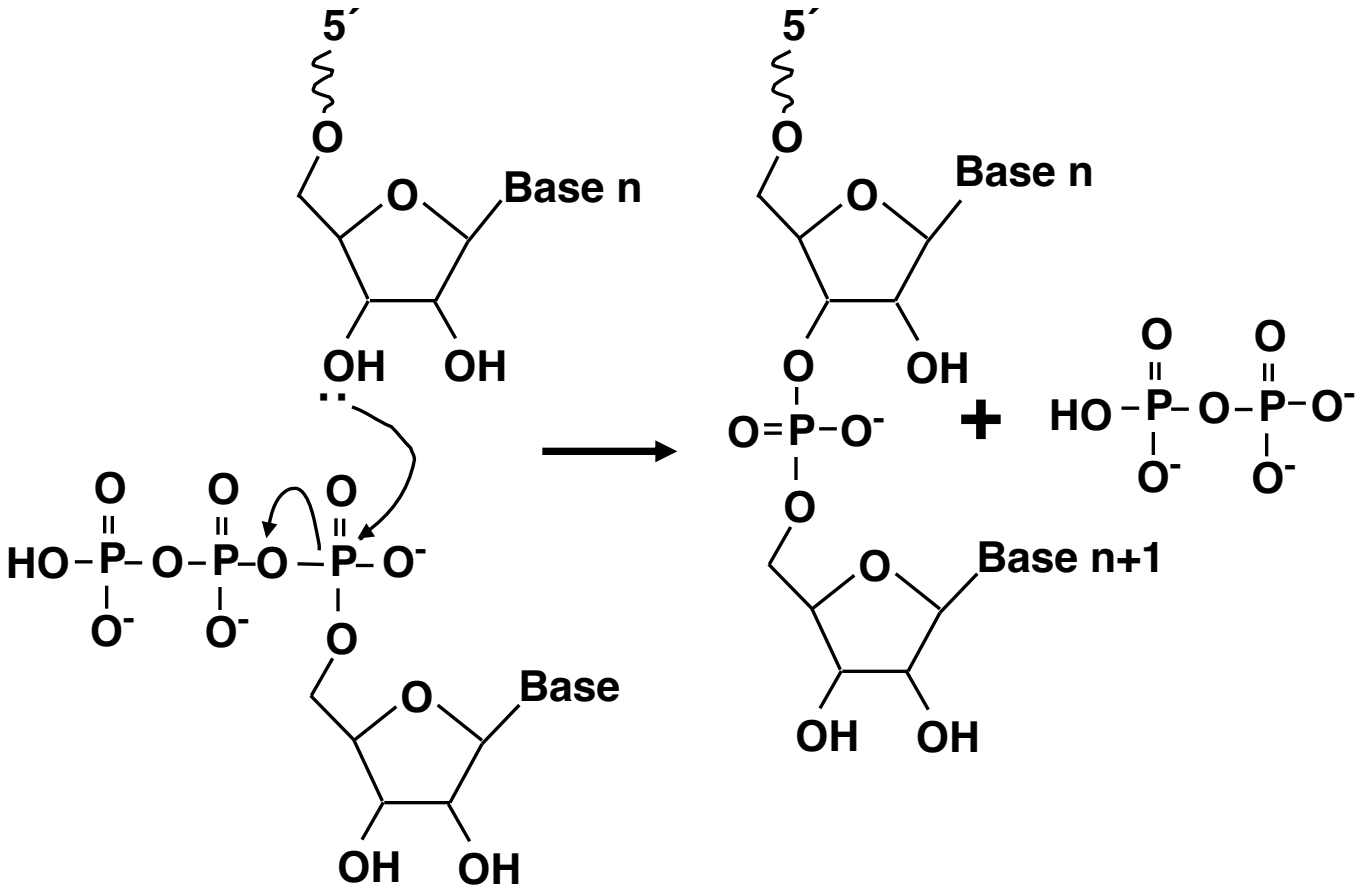


1) Catalyze the polymerization of deoxyribonucleotides in the 5´->3´ direction: $(dNMP)_n + dNTP \rightarrow (dNMP)_{n+1} + PP_i$

2) Require a template (DNA, sometimes RNA=Reverse Transcriptases)

3) Require a primer: DNA or RNA

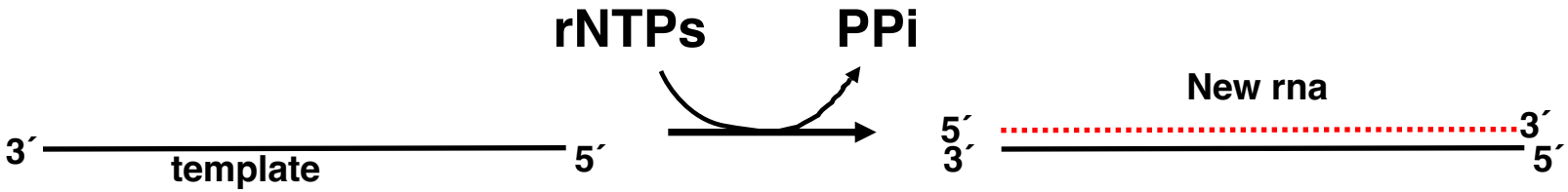
Fundamental properties of RNA Polymerases



1) Catalyze the polymerization of ribonucleotides in the 5´->3´ direction:
$(rNMP)_n + rNTP \rightarrow (rNMP)_{n+1} + PP_i$

2) Require a template (usually DNA)

3) Do not require a primer



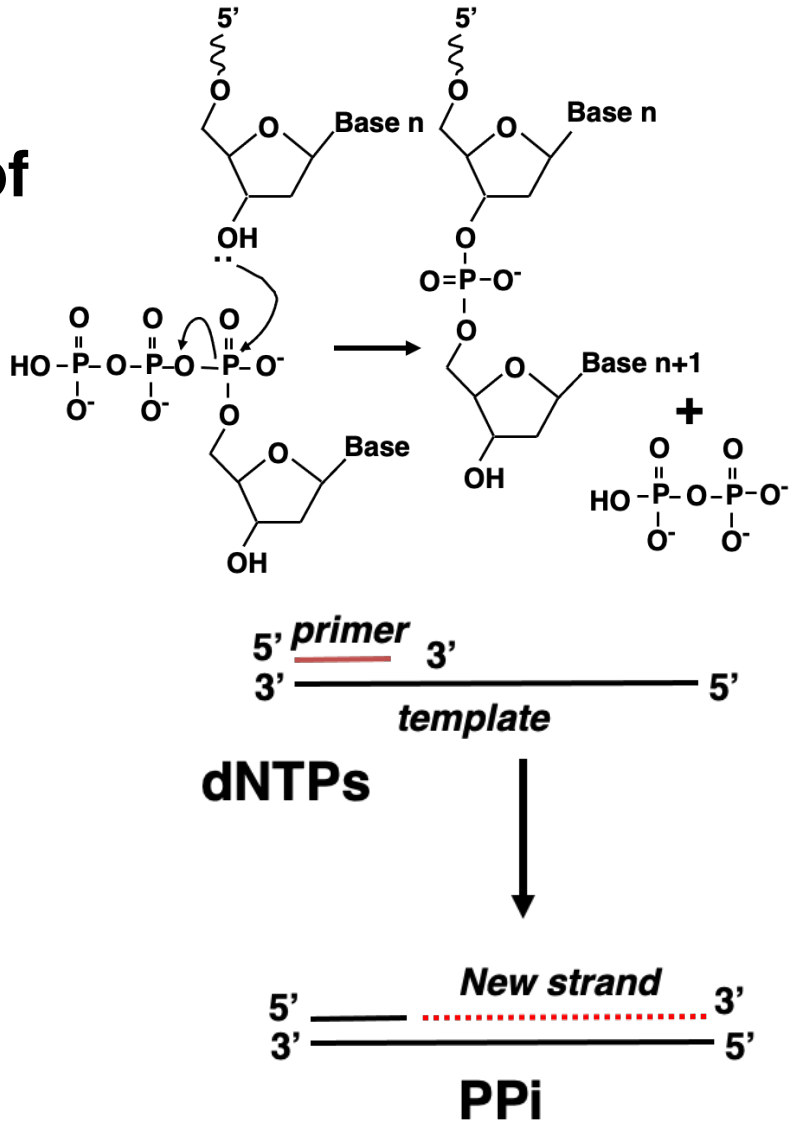
Why is DNA/RNA polymerization mostly occurring in the forward reaction considering all chemical reactions are reversible?

A: Because of the forward motion of the polymerase on the template

B: Because of the (d)NTP hydrolysis reactions

C: Because of the PPi produced is hydrolyzed in vivo by pyrophosphatases

D: Because of the PPi produced is hydrolyzed in the active site of the polymerase

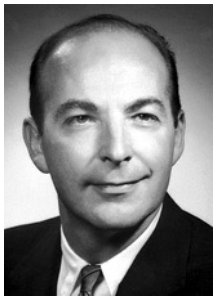


Different Families of DNA polymerases

A Family	bacteriophage T7		DNA Replication
	Bacteria	Pol. I	DNA Repair/Replication
B Family	Eukaryotes	Pol.α	DNA Replication
		Pol.δ	DNA Replication
		Pol.ε	DNA Replication
		Pol.ζ	Translesion / bypass
	Bacteria	Pol. II	
C Family	Bacteria	Pol. III	DNA Replication
Y Family	Eukaryotes	Pol.η Pol.κ	Translesion/ bypass
	Bacteria	Pol.IV, V	
Reverse Transcriptases	Retrovirus	Telomerase	Chromosomes Ext.
		RTs	Viral Replication
D Family		Pol.D	
X Family		Pols β,λ,μ,δ	

Not Covered

Pol I:
Nobel Prize
A.Kornberg
1959



Families need not to be memorized

= Nobel Prize 2009
Blackburn, Szostak, Greider

= Nobel Prize 1975
Baltimore, Temin

RNA polymerases

Single subunit RNAPs
(= 1 protein)

- **Bacteriophages (e.g. T7)**
- **Mitochondria**
- **Chloroplasts**

Multisubunit RNAPs

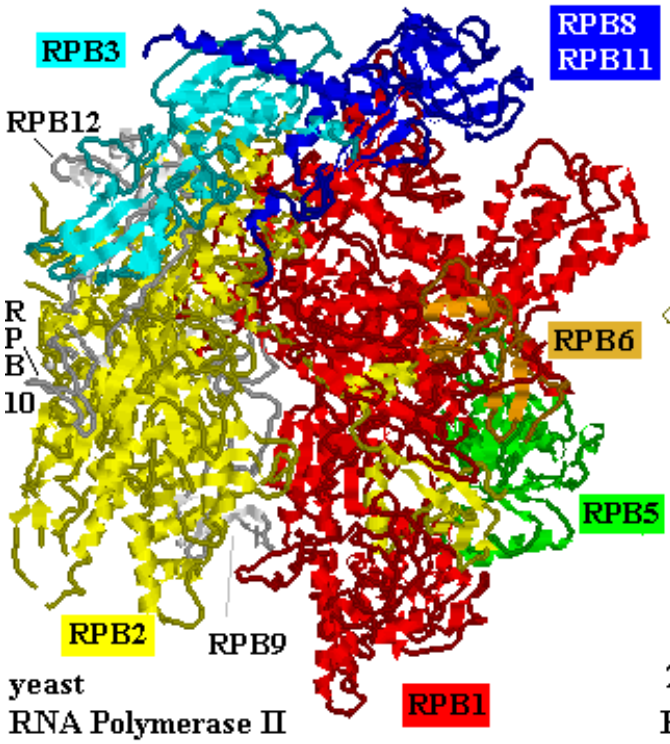
(= multiple proteins)

Crab Claw shape

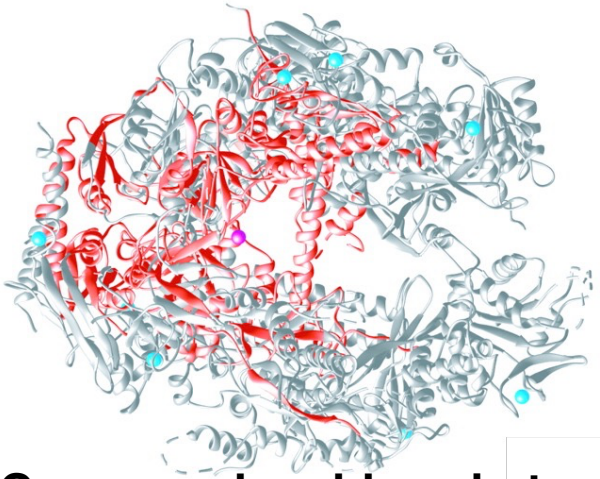
- **Bacteria** $\alpha_2\beta\beta'\omega$ **core**
  (sigma -> specificity)
- **Eukaryotes**

similar architecture to bacteria

but many more subunits and 3 major RNA Pols: RNA Pol I,II,III

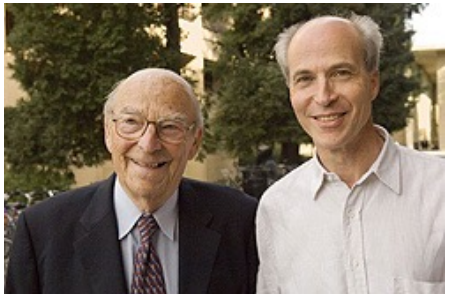


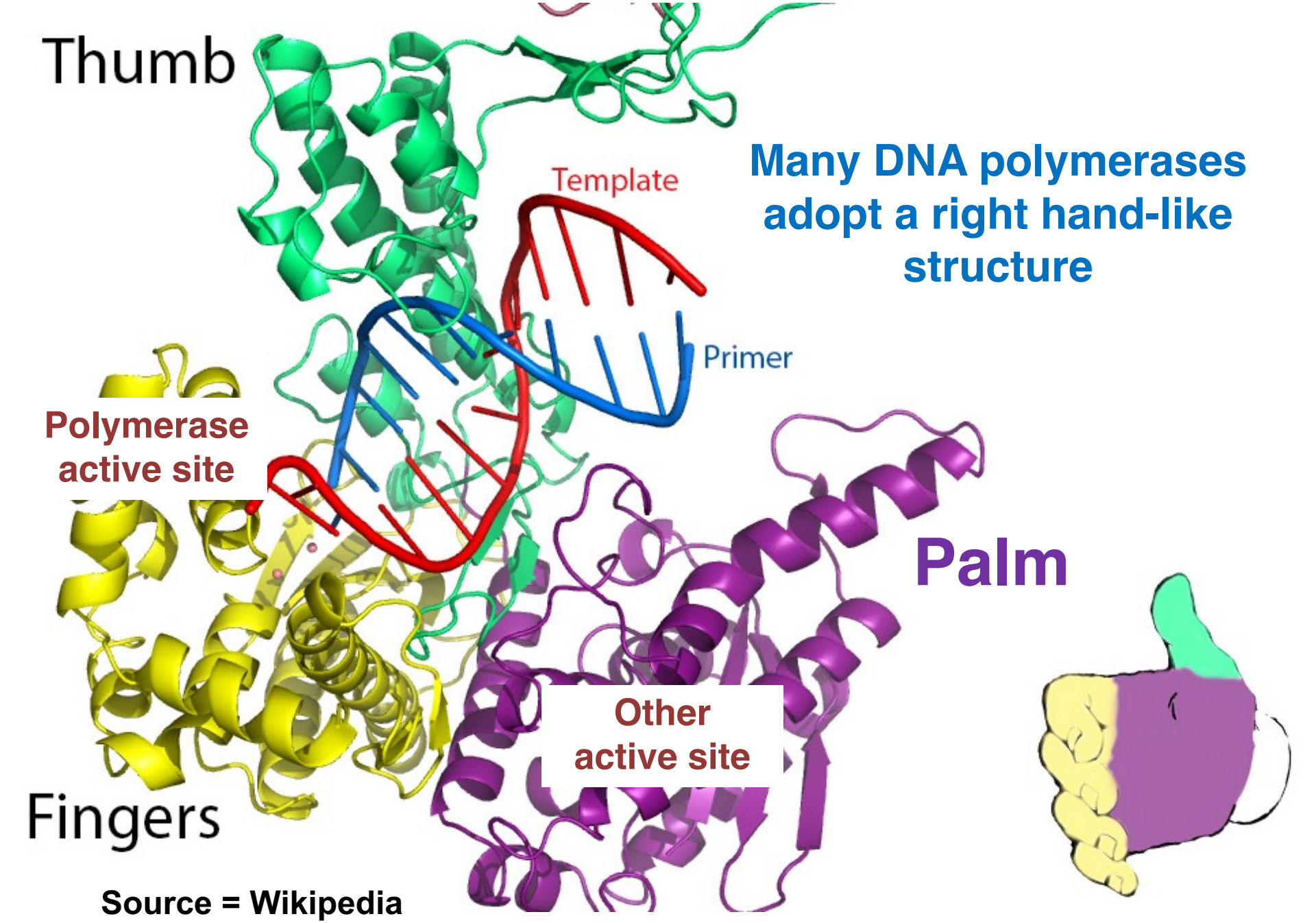
Eukaryotic=yeast RNAPII **Prokaryotic: $\alpha_2\beta\beta'\omega$**



Conserved residues between euk. and bacterial RNA Pol (red)

Pol.II Structure = 2006 Chemistry Nobel Prize Roger Kornberg.
When the apple does not fall far from the tree..

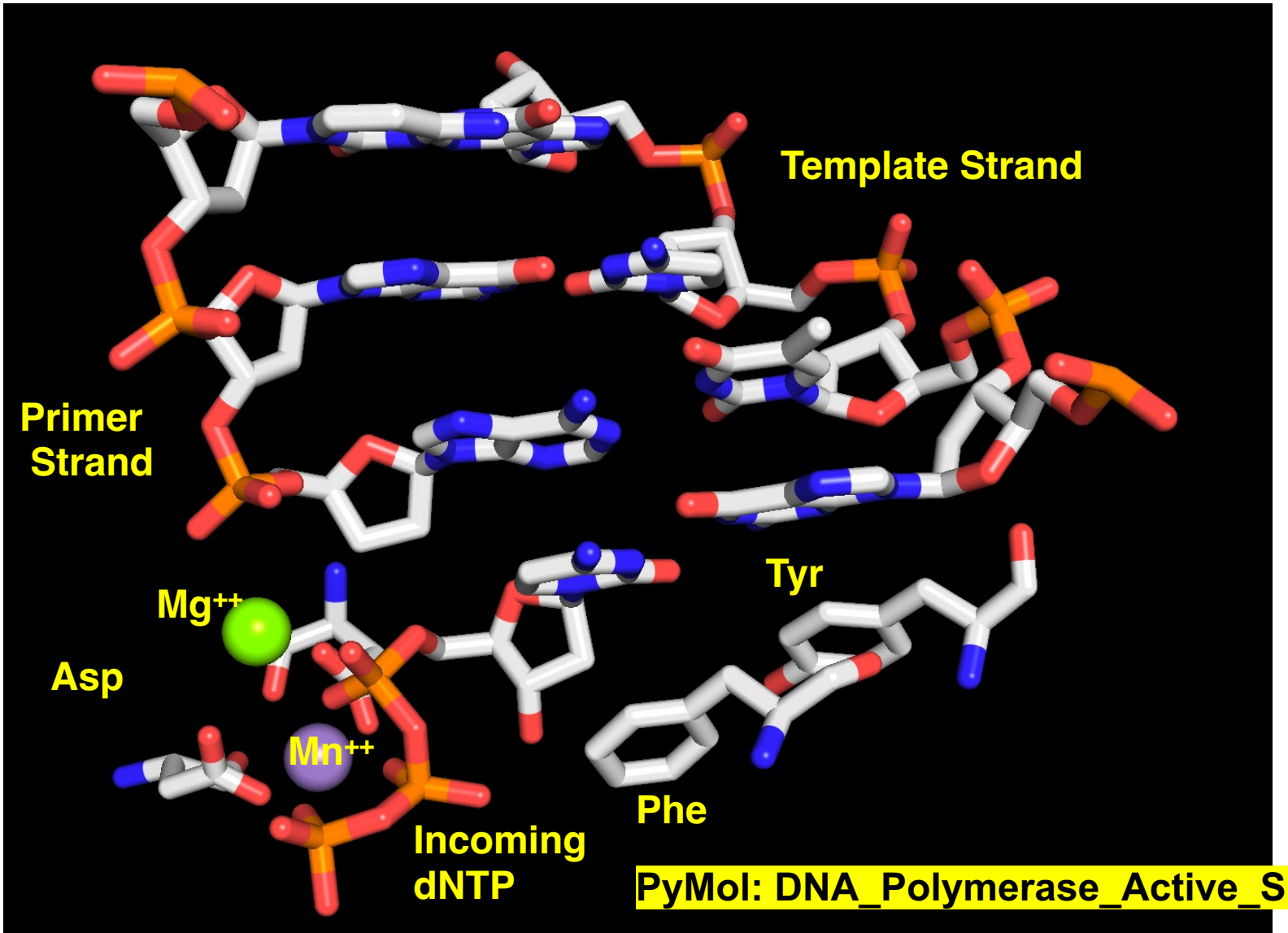




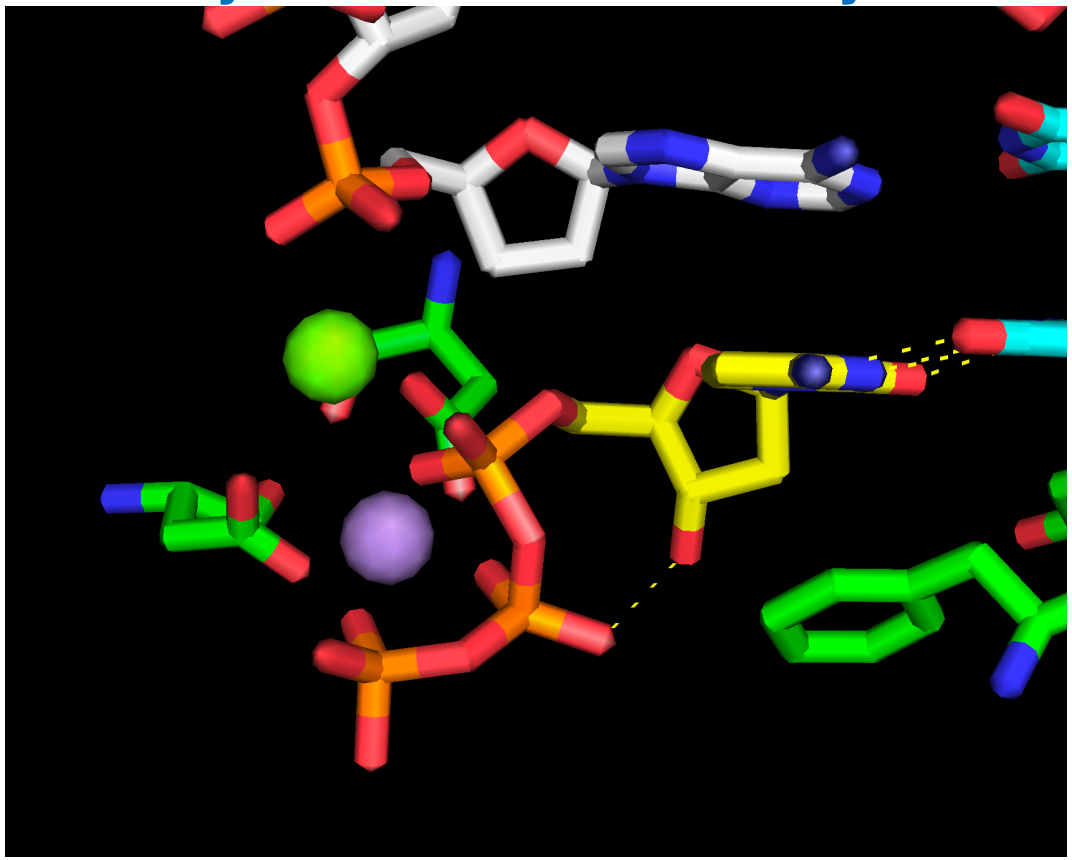
Many DNA polymerases adopt a right hand-like structure

Source = Wikipedia

The active site of a DNA Polymerase shows two metals ions at the vicinity of the substrates and reactive groups



Why is chemistry/polymerization not happening in this crystal? This is an enzyme after all…



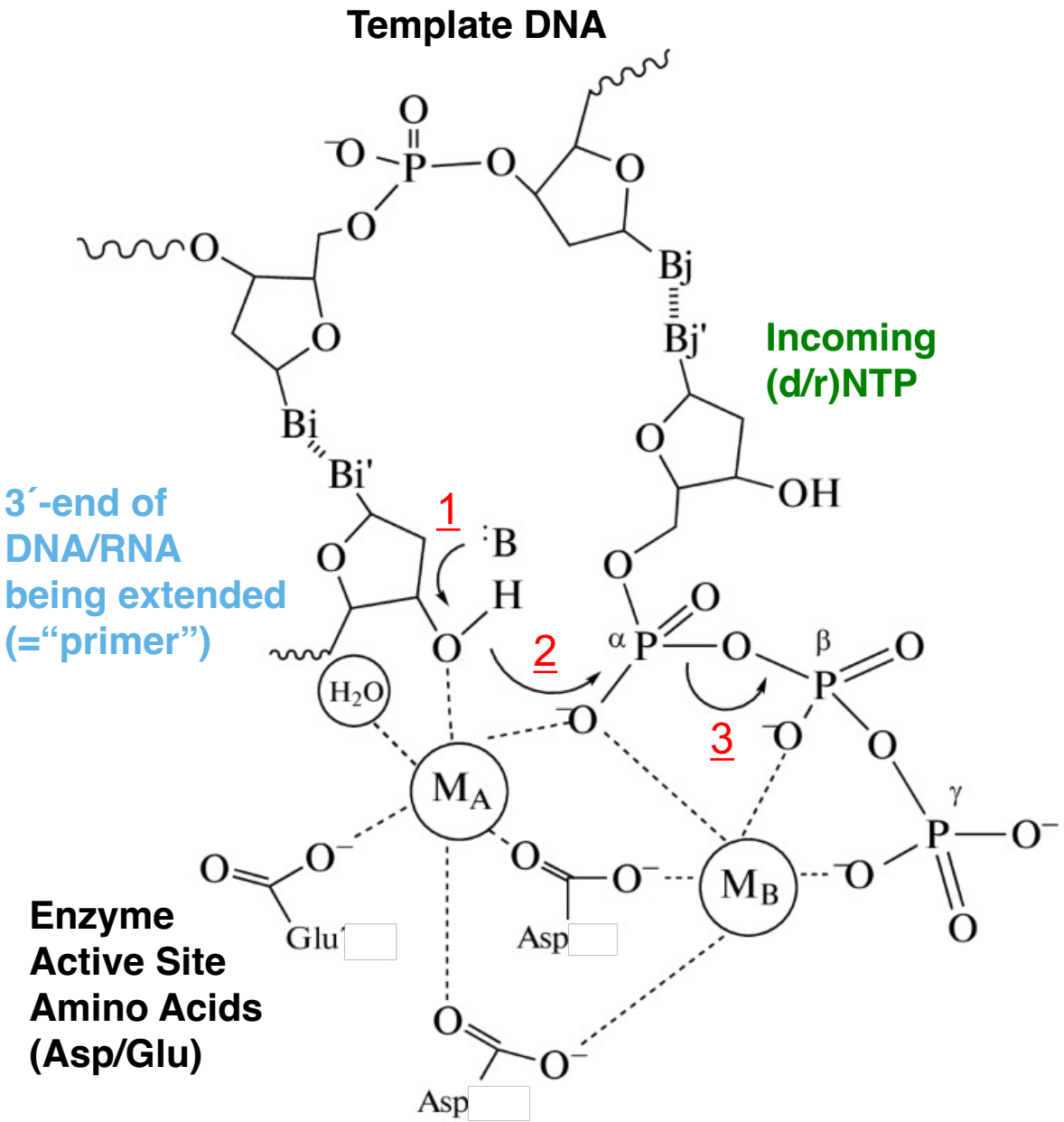
A: a dNTP with the incorrect base has been added to the reaction

B: It's a crystal – chemical reactions can´t occur in the solid state

C: The leaving group (β/γ-phosphates of incoming dNTP) is not functional

D: The nucleophile is absent (no 3´-hydroxyl at 3´-end of primer

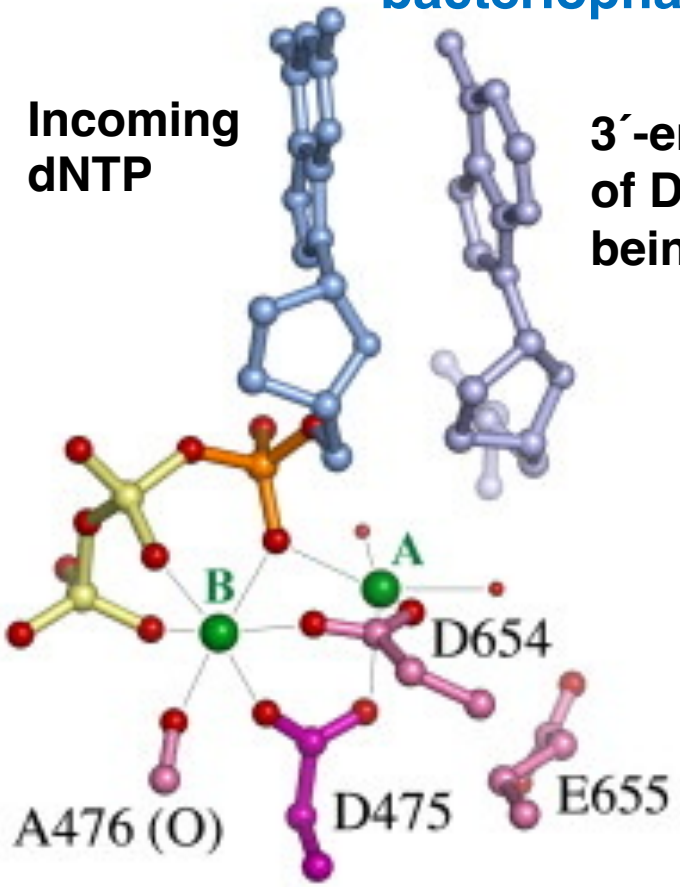
Two metals ion mechanism for DNA Polymerases (and RNA Polymerases)



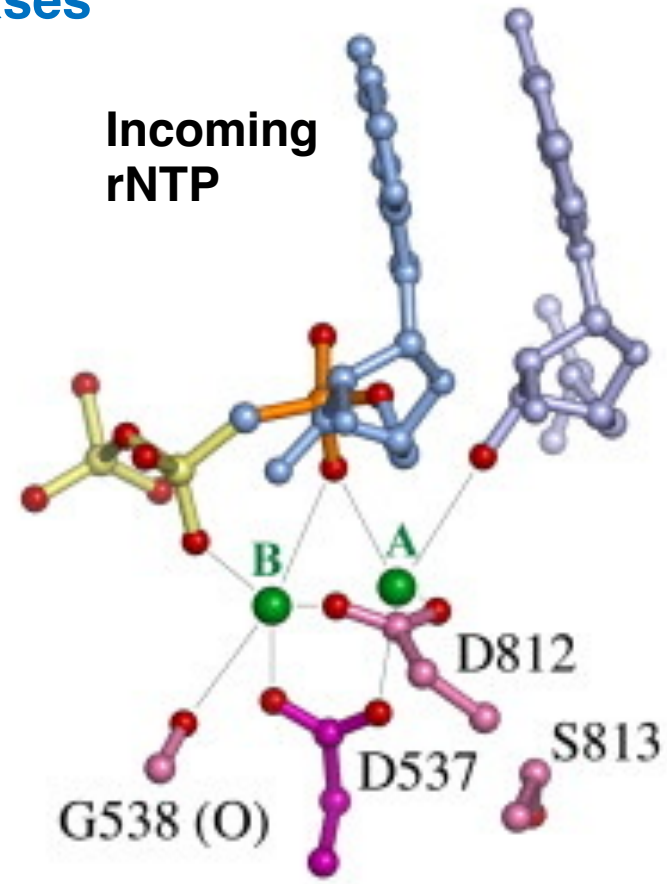
- M: typically Mg^{++} or Mn^{++}
- M_A=lowers pKa of 3´O to activate the 3´OH for the attack on the α phosphate of the incoming (r/d)NTP
- M_B can be stably associated -with the enzyme or come with the incoming (d/r)NTP
- M_B=plays the dual role of stabilizing the negative charge that builds up on the leaving oxygen and binding the β and γ phosphates of the incoming NTP
- M_A and M_B stabilize both the structure and charge of the pentavalent transition state

DNA and RNA polymerase active sites are very similar

Two metal ions (A/B) in the active site of both DNA and RNA polymerases: example of bacteriophage T7 polymerases



T7 DNA Polymerase
Active Site



T7 RNA Polymerase
Active Site

What primer sequence could DNA polymerase use to synthesize a new DNA strand from the following template?

5’ – GTTCACGATTAACCCGAC – 3’

A: GTTCAC

B: CAAGTC

C: CCCGAC

D: GTCGGG

E: CCCGAC

Sugar Discrimination is particularly critical for DNA polymerases because of cellular NTPs concentrations: [ribo] >>[deoxy]

[rNTPs] >> [dTNPs] → this is a challenge for DNA polymerases

	[μM]		[μM]
rATP	3000	dATP	16
rGTP	700	dGTP	12
rCTP	500	dCTP	14
UTP	1700	dTTP	30

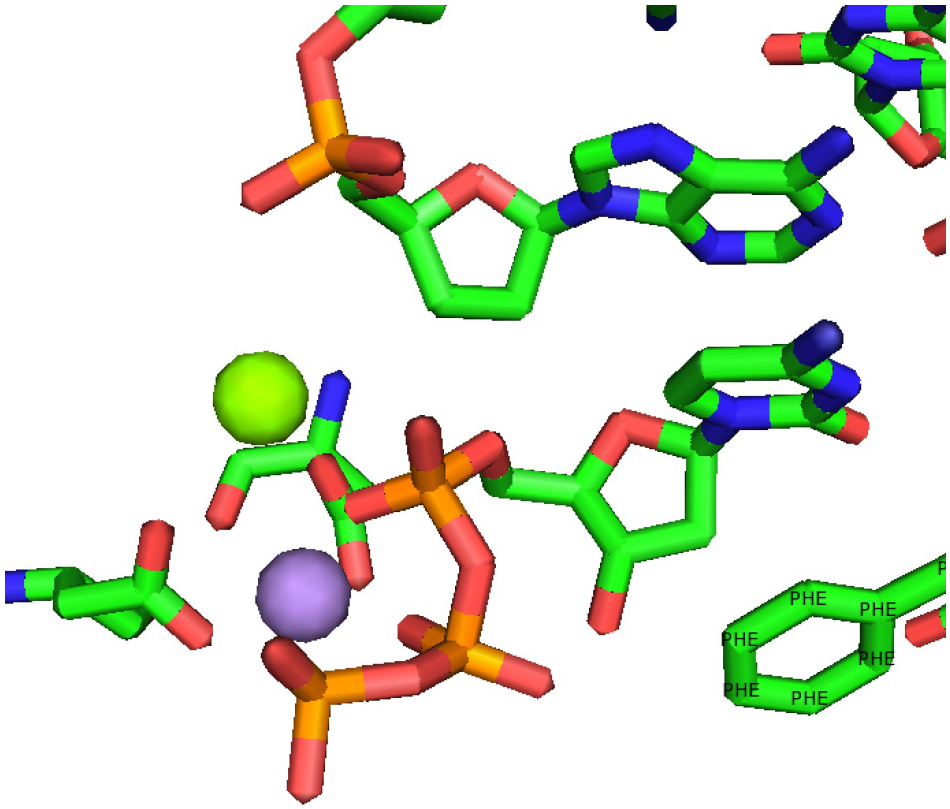
Why would it be problematic if an rNTP is incorporated instead of a dNTP?

Concentrations from: *S. cerevisiae*, log phase

From Cerritelli & Crouch Trends Biochem. Sci. 2016

- **Cells need to limit/avoid misincorporation of ribonucleotides into DNA (leads to strand breaks/replication blocks)**

How is this Phenylalanine side chain involved in selecting dNTPs over rNTPs?



A: It makes a stacking interaction with dNTPs that are not possible with rNTPs

B: It makes a H-bond with the 2´-H of dNTPs

C: It prevents rNTPs from entering the active site because a 2´-OH would not fit

D: It is allosterically activated by dNTPs but not rNTPs

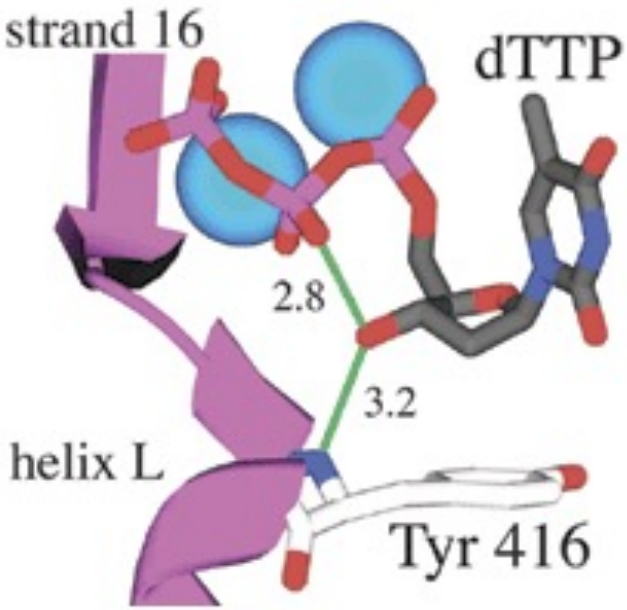
PyMol: DNA_Polymerase_Active_Site.pse

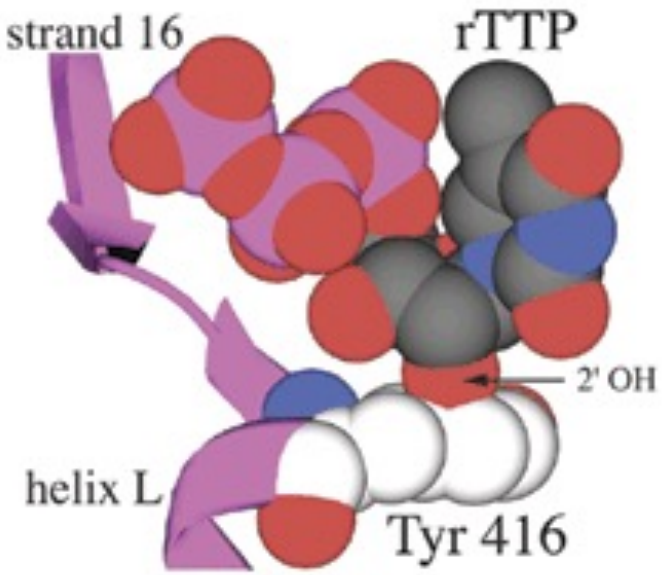
Discrimination for deoxynucleotides vs ribonucleotides by DNA polymerases:

To assess conservation, we can create a *sequence alignment*
Comparison of amino acid sequences in polymerases active site

D = Catalytic Asp
Conserved
in DNA and RNA Pol.

DNA Polymerases D---- (Y/F) --- **use dNTPs**
RNA Polymerases D---- (D/G) --- **use rNTPs**



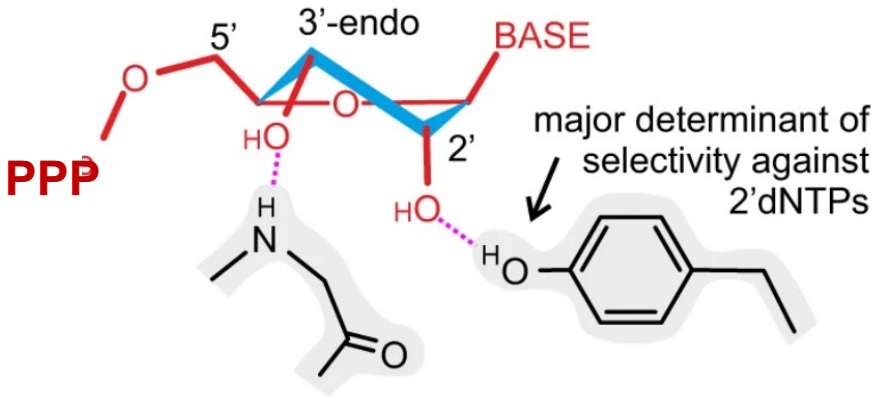


Steric Clash with the Y416 residue if a 2´-OH is present

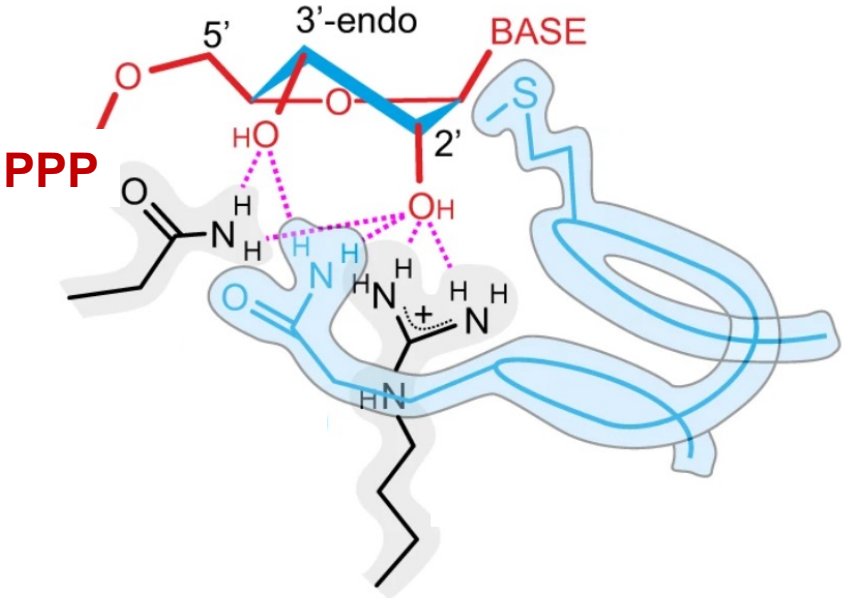
Biochemistry, 41 10256–10261

The aromatic/large side chain found in DNA polymerases close to the dNTP binding site provides a steric gate against riboNTP entry

Sugar Discrimination in RNA polymerases:
Not as critical as for DNA polymerases but typically occurs through recognition of 2´-OH of riboses by side chains in the active of RNAP



Bacteriophage T7 and mitochondrial RNAPs

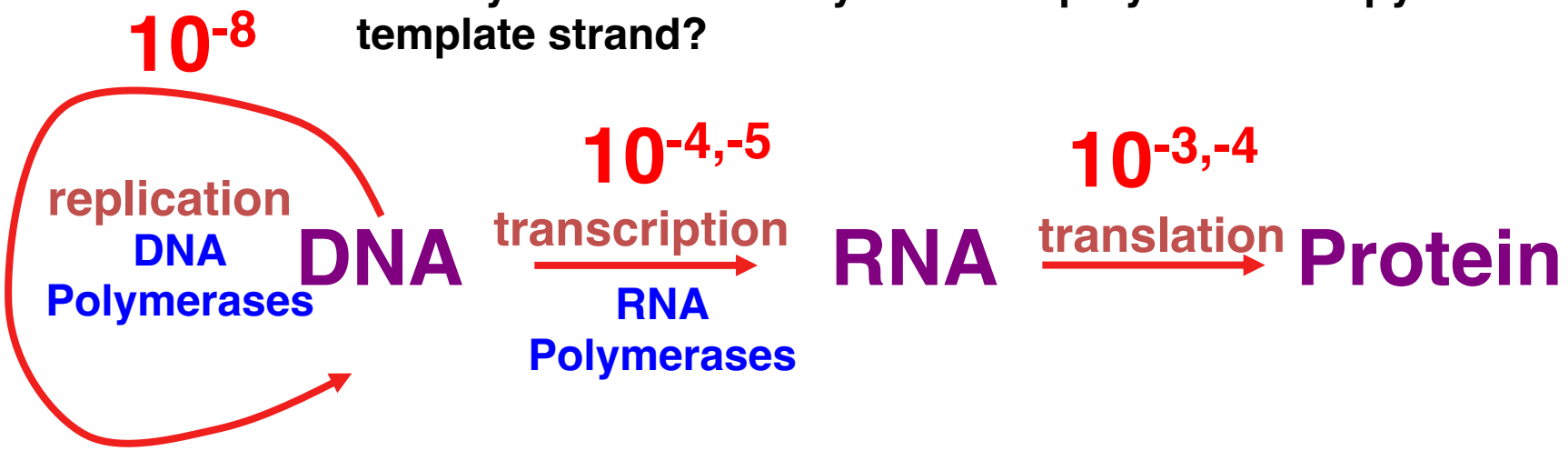


Multisubunit RNAPs

Mäkinen et al.
Nat. Comm. 2021

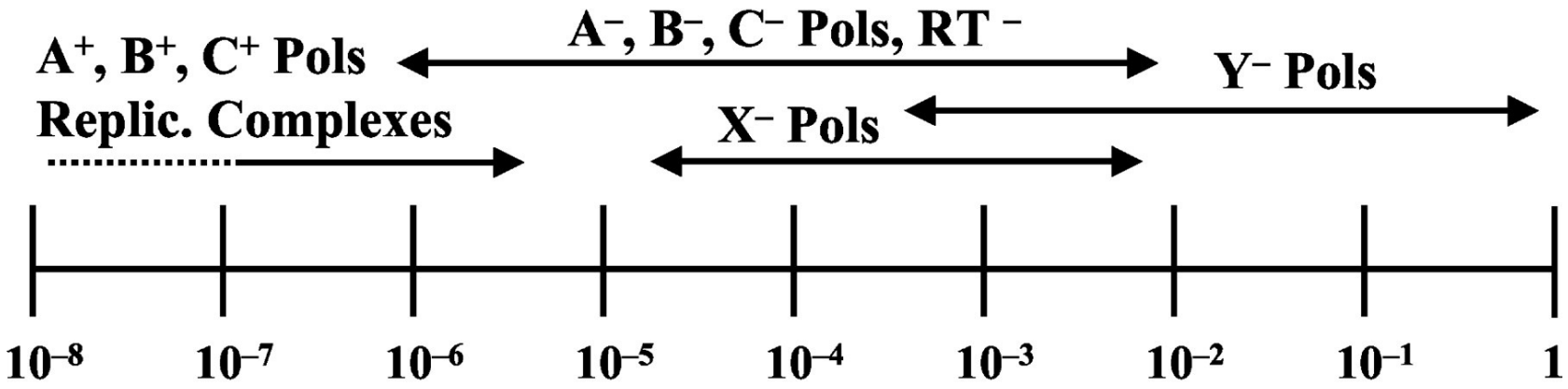
Fidelity and Error Rates in DNA and in RNA polymerases

Fidelity: How accurately does the polymerase copy the template strand?



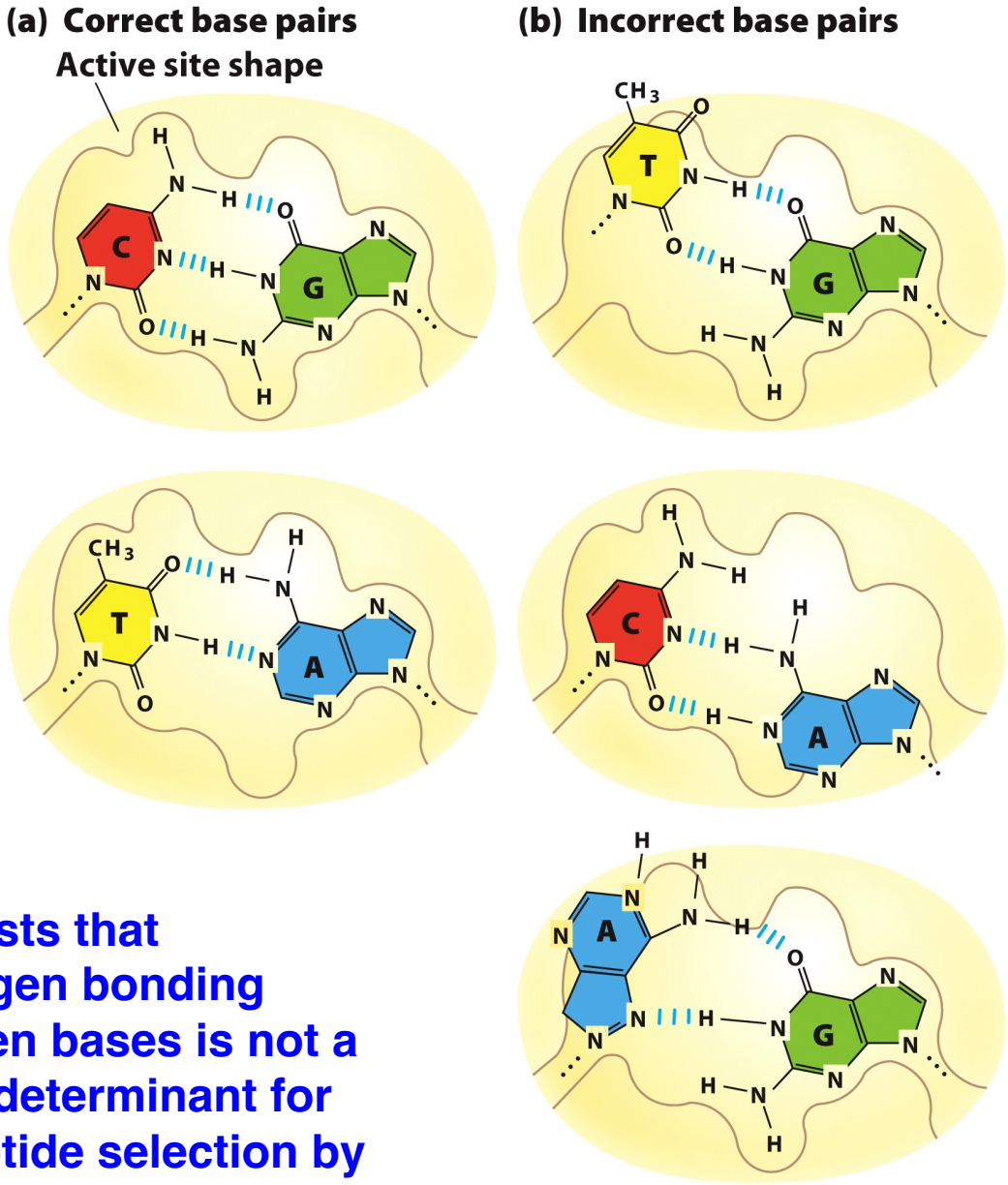
Different families of polymerases have different fidelities

Base Substitution Error Rates for DNA Polymerases



Thomas A. Kunkel J. Biol. Chem. 2004;279:16895-16898

How do Replicative DNA Polymerases select correct nucleotides from incorrect nucleotides ?



Sequence doesn't matter as long as it forms a Watson-Crick base pair

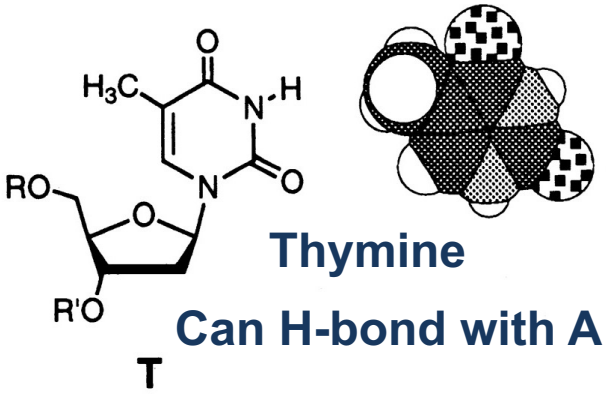
Nucleotides forming Watson-Crick base pairs fit the active site

Nucleotides forming non Watson-Crick base pairs do not fit the active site and are ejected

Suggests that Hydrogen bonding between bases is not a major determinant for nucleotide selection by DNA polymerases

PyMol: DNA_Polymerase_Active_Site_KK_surface.pse

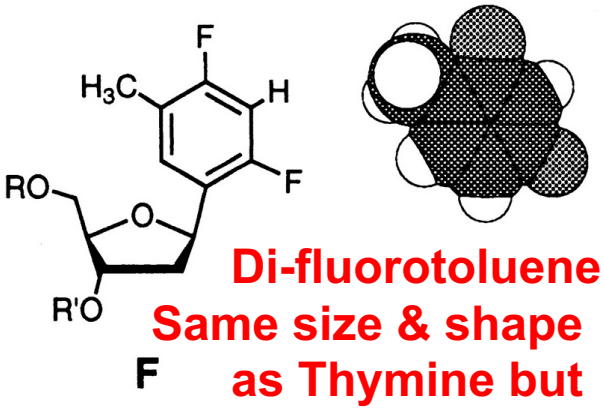
How do Replicative DNA Polymerases select the proper nucleotide: Testing the importance of H-bonding in base pairs for the fidelity of nucleotide incorporation



Thymine

Can H-bond with A

T



F

Di-fluorotoluene
Same size & shape as Thymine but cannot H-bond with A

Template = 24 nt

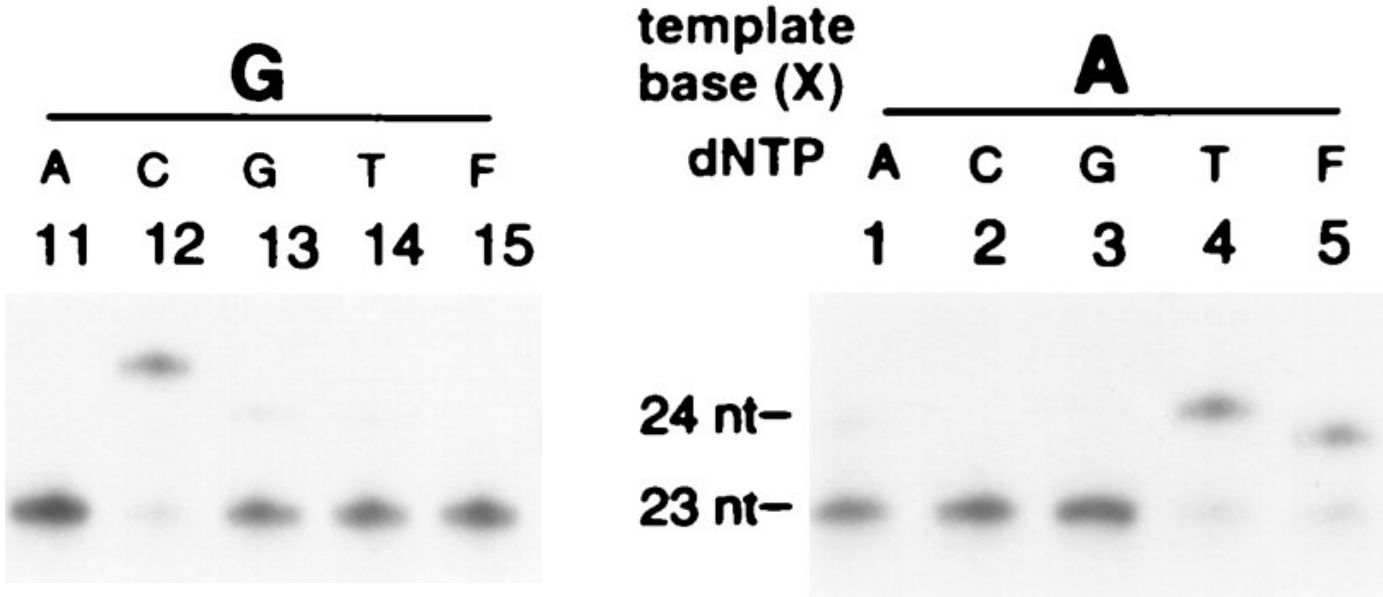
5´ X ——— 3´

5´

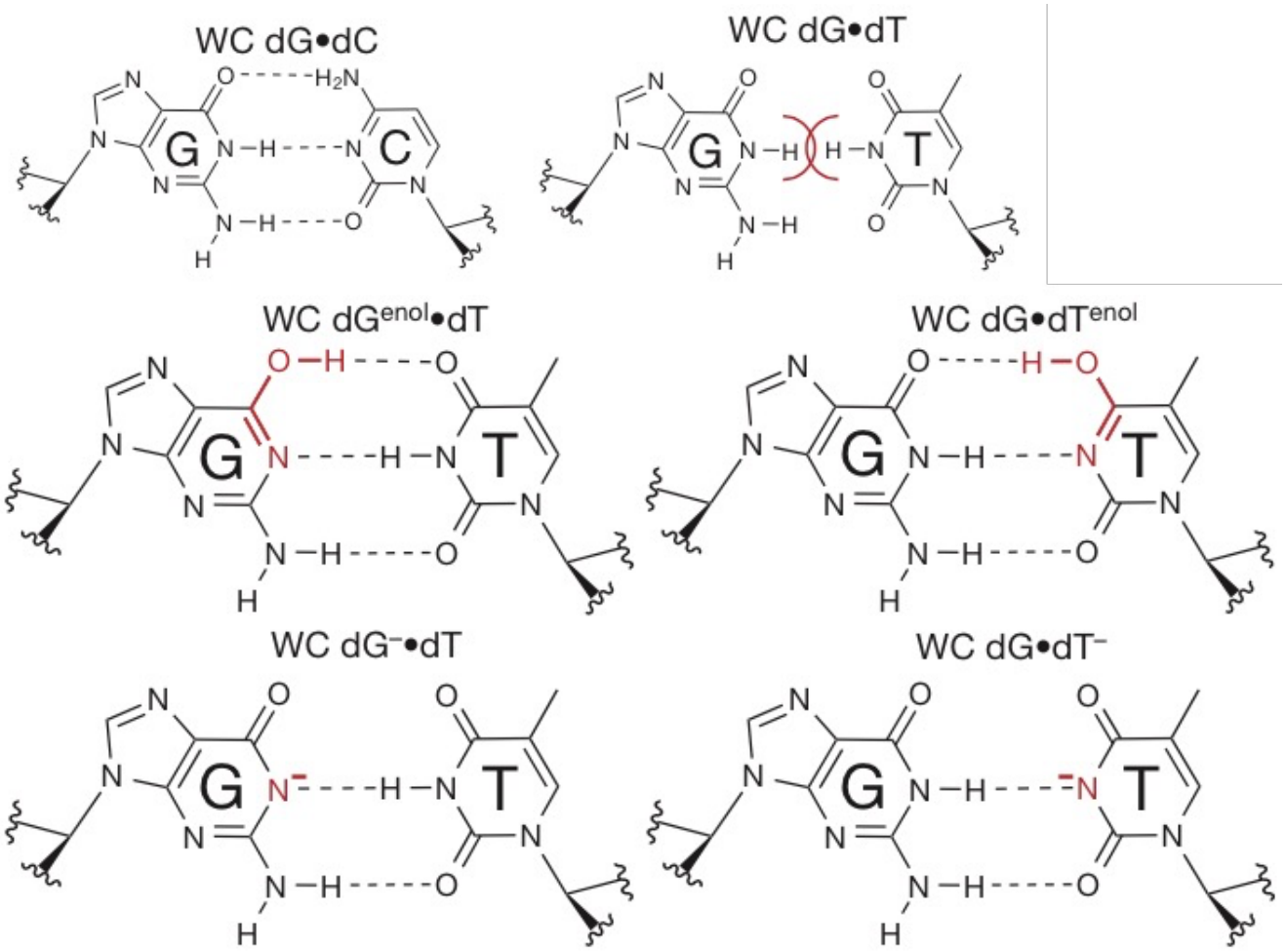
Labeled primer = 23 nt
After extension = 24 nt

A,C,G,T or F ?

Electrophoresis Direction (smaller molecules migrate faster)



Formation of mismatch base pairs in the active site of DNA polymerase can be due to bases isomerization Examples of G-T base pairs

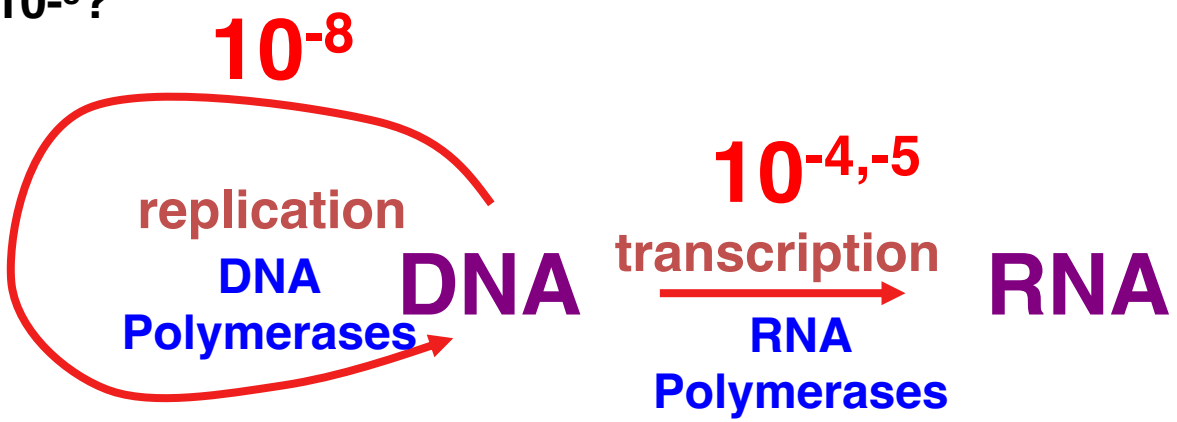


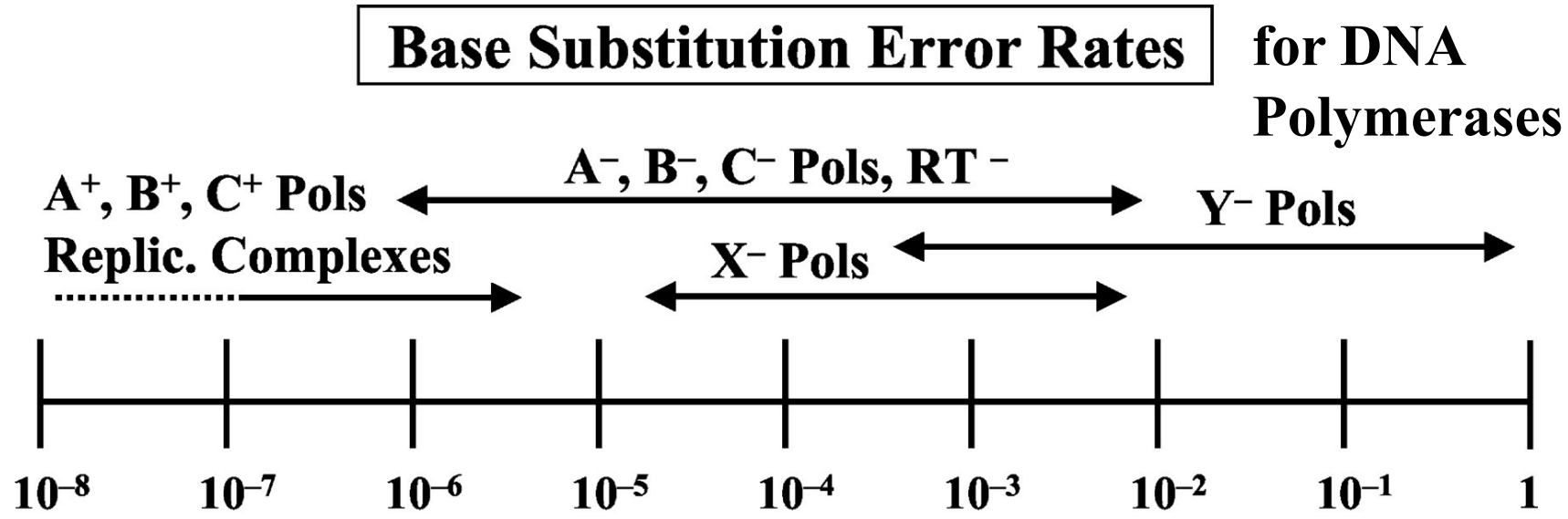
Kimsey et al.
Nature 2015

These G^{enol}•T, G•T^{enol}, G^{-}•T, G•T^{-} form with a probability of 10^{-3} to 10^{-5} and fit the active site of DNA polymerase resulting in misincorporation/mutations

These G^{enol}•T, G•T^{enol}, G^{-}•T, G•T^{-} form with a probability of 10^{-3} to 10^{-5} and fit the active site of DNA polymerase resulting in misincorporation/mutations…

how do we get to 10^{-8}?





Thomas A. Kunkel J. Biol. Chem. 2004;279:16895-16898

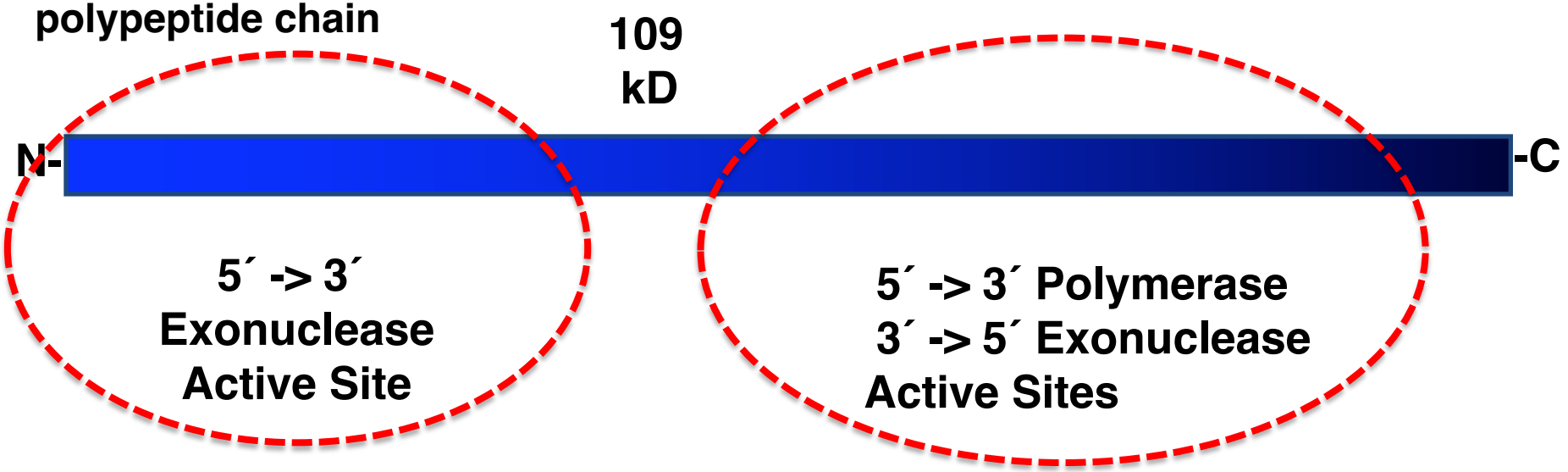
Proofreading activity of DNA Polymerases first discovered in Pol.I

- Pol.I= First DNA polymerase discovered in the late '50s by Arthur Kornberg (1959 Nobel Prize in Medicine)

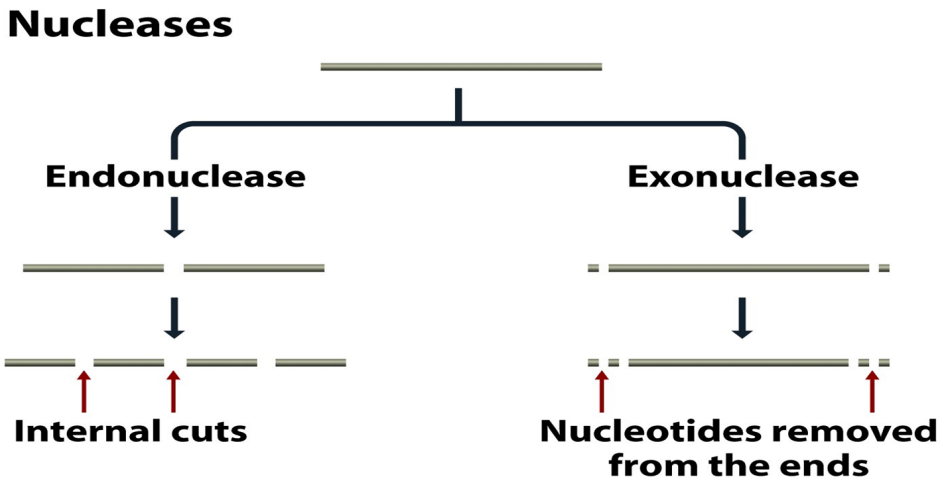
Critiques of the original 1957 papers :

- *"It is very doubtful that the authors are entitled to speak of the enzymatic synthesis of DNA"*
- *"Polymerase is a poor name"*

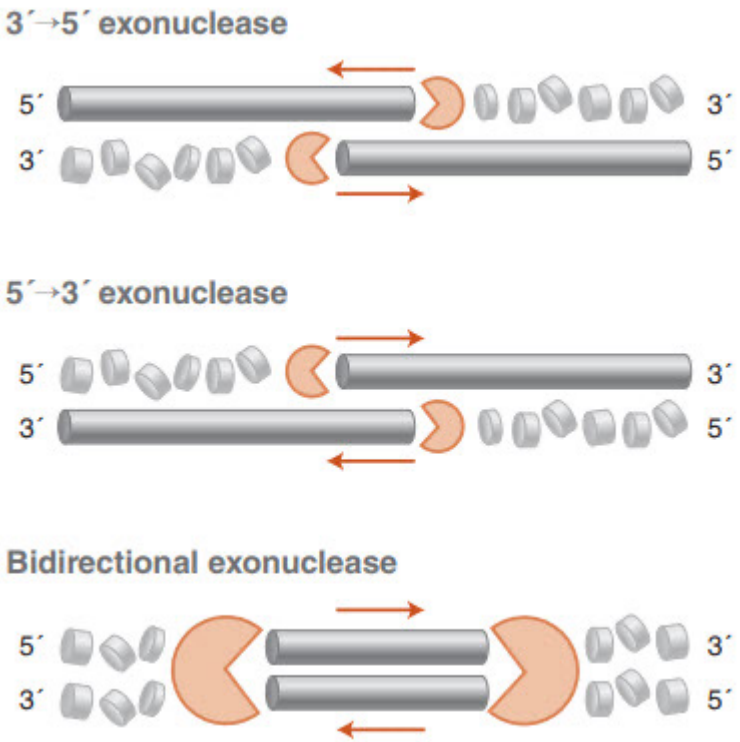
• 3 Enzymatic activities associated to three distinct active sites on a single polypeptide chain



Nuclease terminology

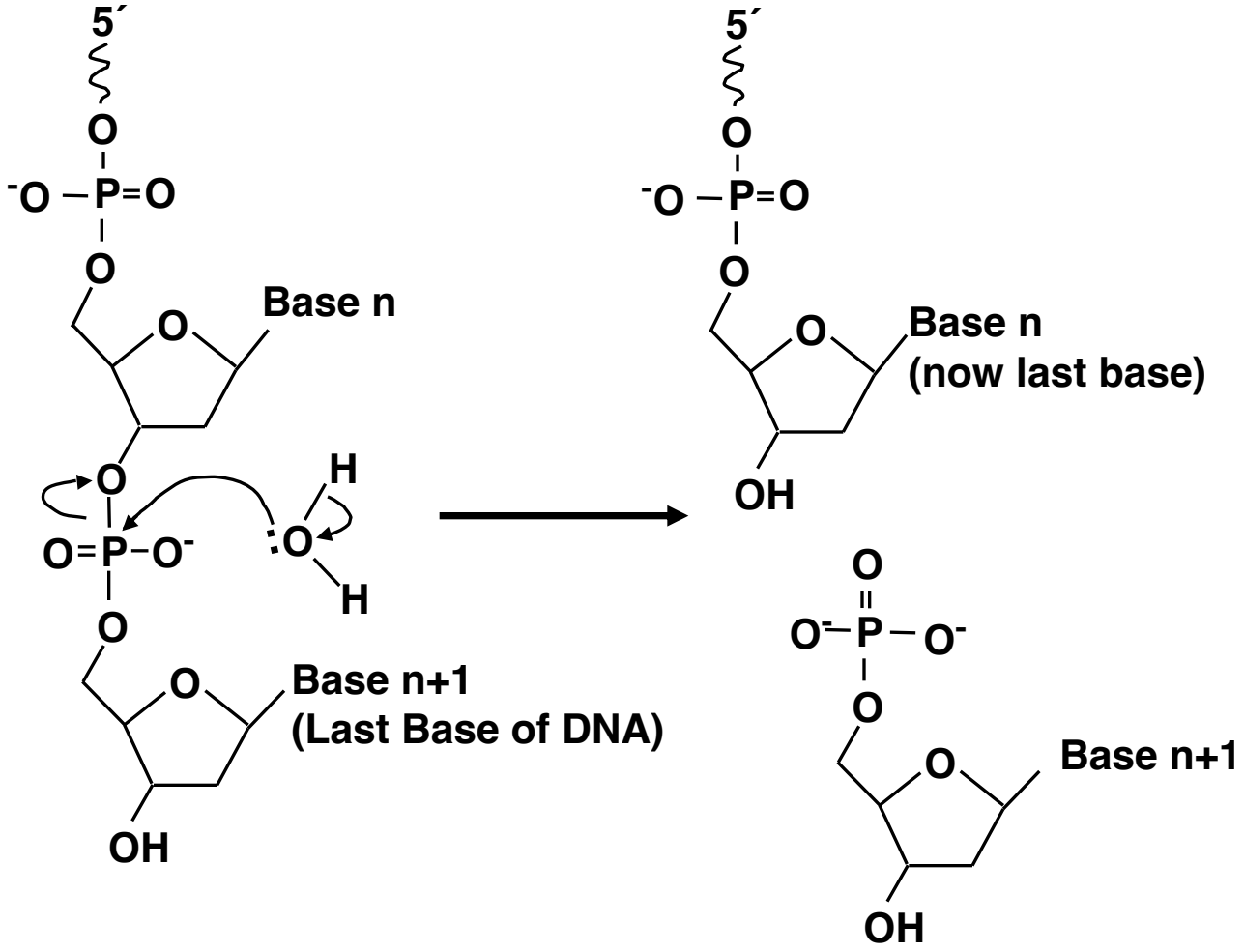


Exonuclease directionality



3´-> 5´ exonuclease activity of DNA Polymerase I (similar for other DNA Polymerases)

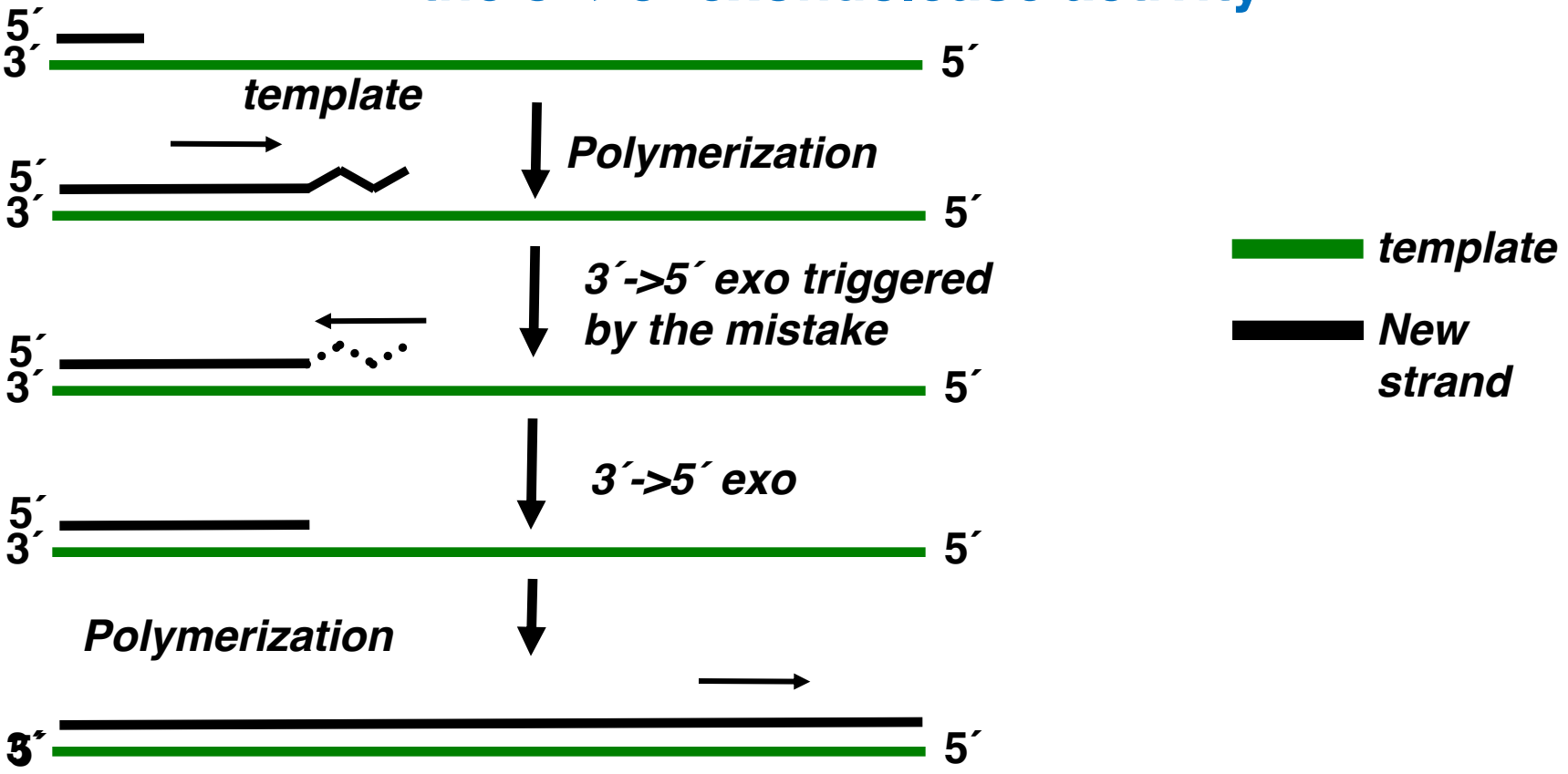
Editing newly polymerized sequences



This reaction *is not* the reversal of the 5´->3´ polymerization!

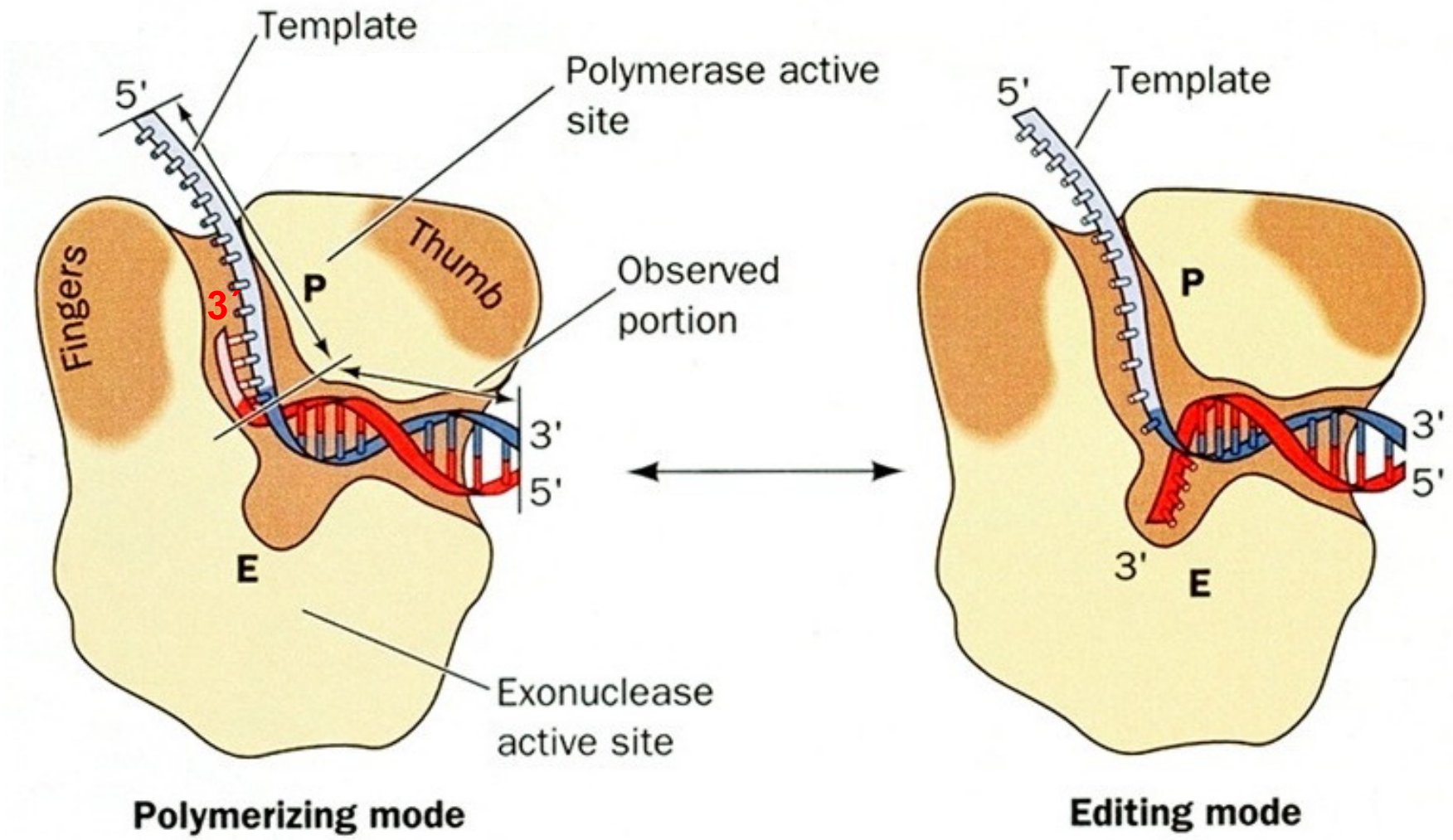
This reaction happens in a different active site (not the same active site where polymerization happens)!

Editing of newly synthesized DNA by the 3´->5´ exonuclease activity

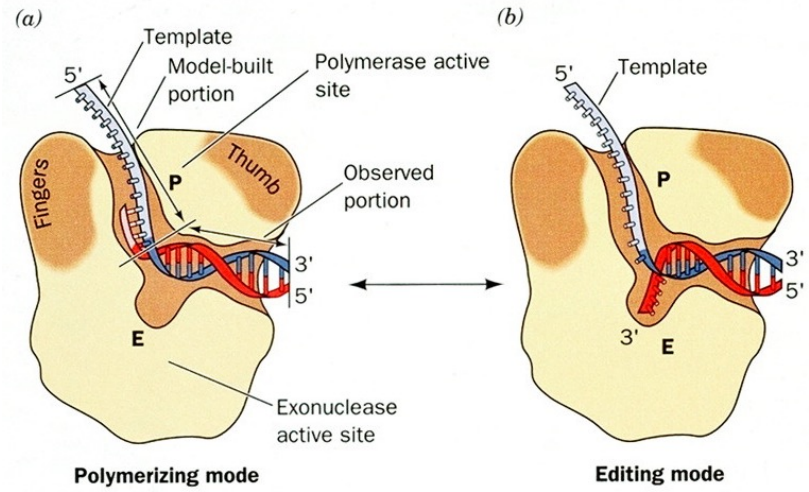


Editing of mistakes requires a switch between polymerization mode and editing mode

Switch between Polymerizing and Editing Modes in DNA Polymerase: Structural Basis for "Proofreading"



What structural transition mediates the switch of the primer strand into the editing site?



A: The mismatch induces a dissociation of the primer strand from the template allowing more flexibility and entry into the editing site

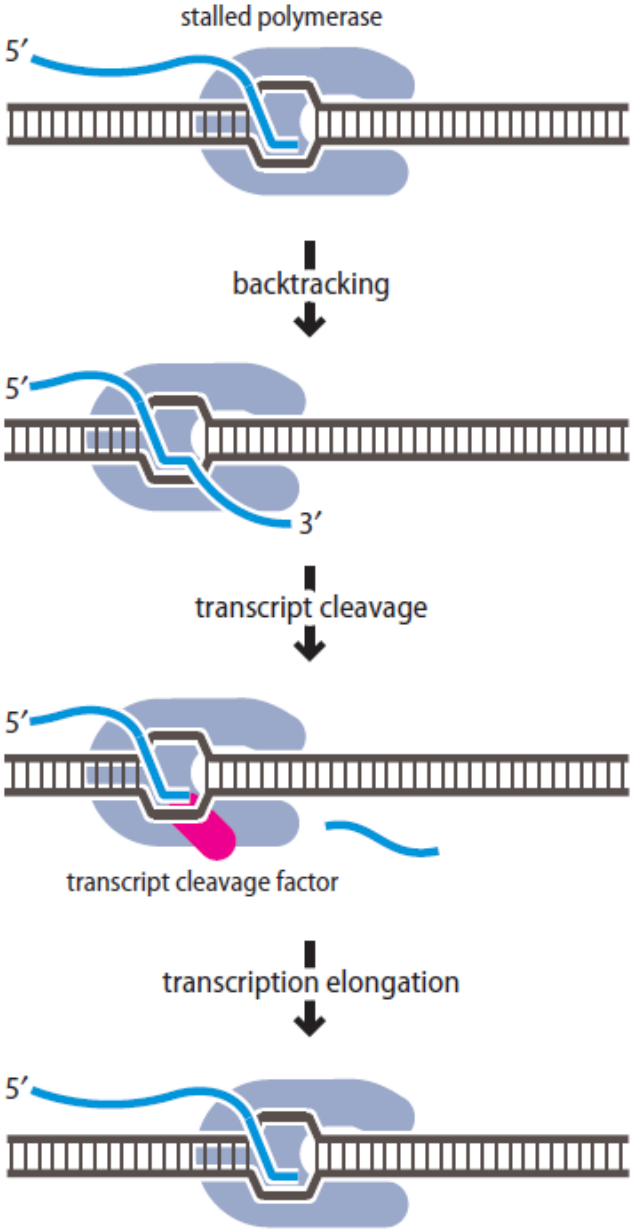
B: The mismatch induces a conformational change in the polymerase active site that ejects the primer strand

C: An ATP-dependent step mediates the switch between the polymerase and editing active site

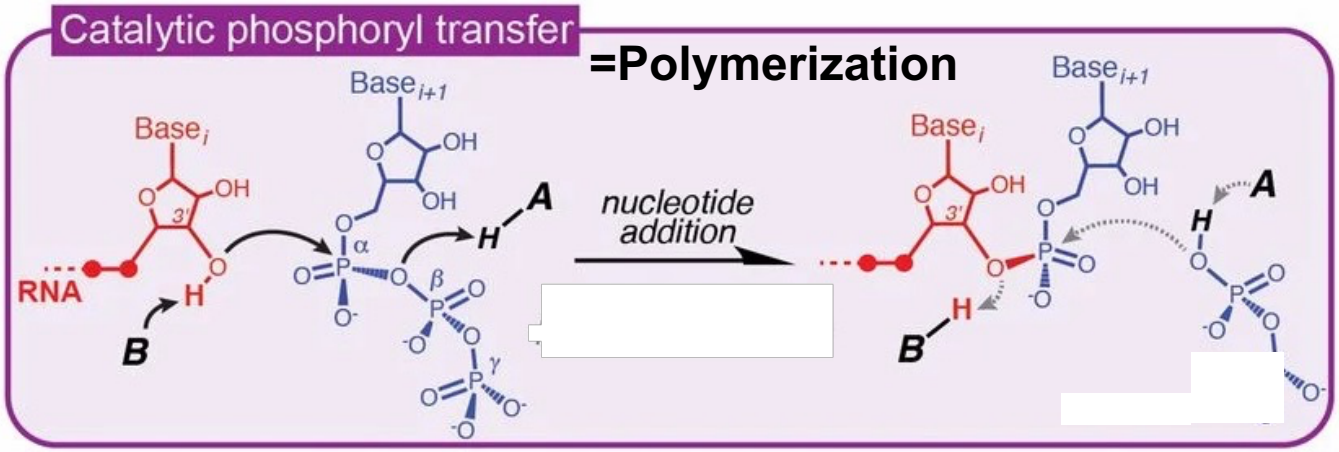
D: The mismatch does not fit in the polymerase active site and results in the ejection of the primer strand from the polymerase and into the editing site

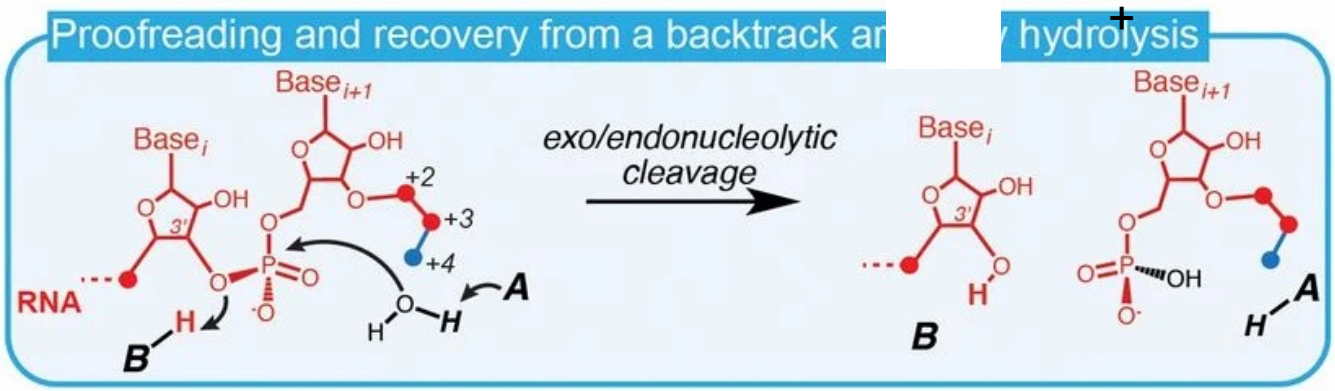
Fidelity is a bit less important for RNA polymerases than for DNA polymerases

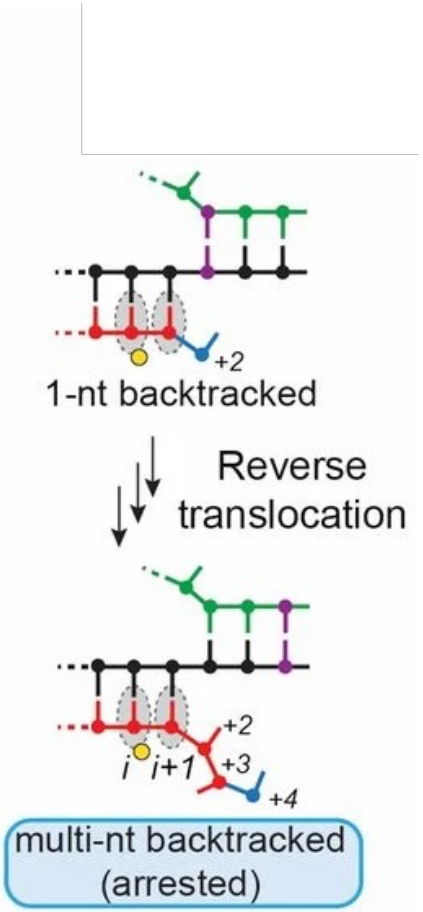
- **RNA polymerases do not have 3´-5´ exonuclease domains**
- **But there is still some proofreading (Error Rate ≈$10^{-4,-5}$)**
- **Proofreading by RNA polymerases involves backtracking and cleavage of nucleotide(s) no longer base paired to the template**



Catalytic mechanism uses a water molecule to hydrolyze the nucleotide (you do not need to know this for an exam)







Michanina et al. PNAS 2017

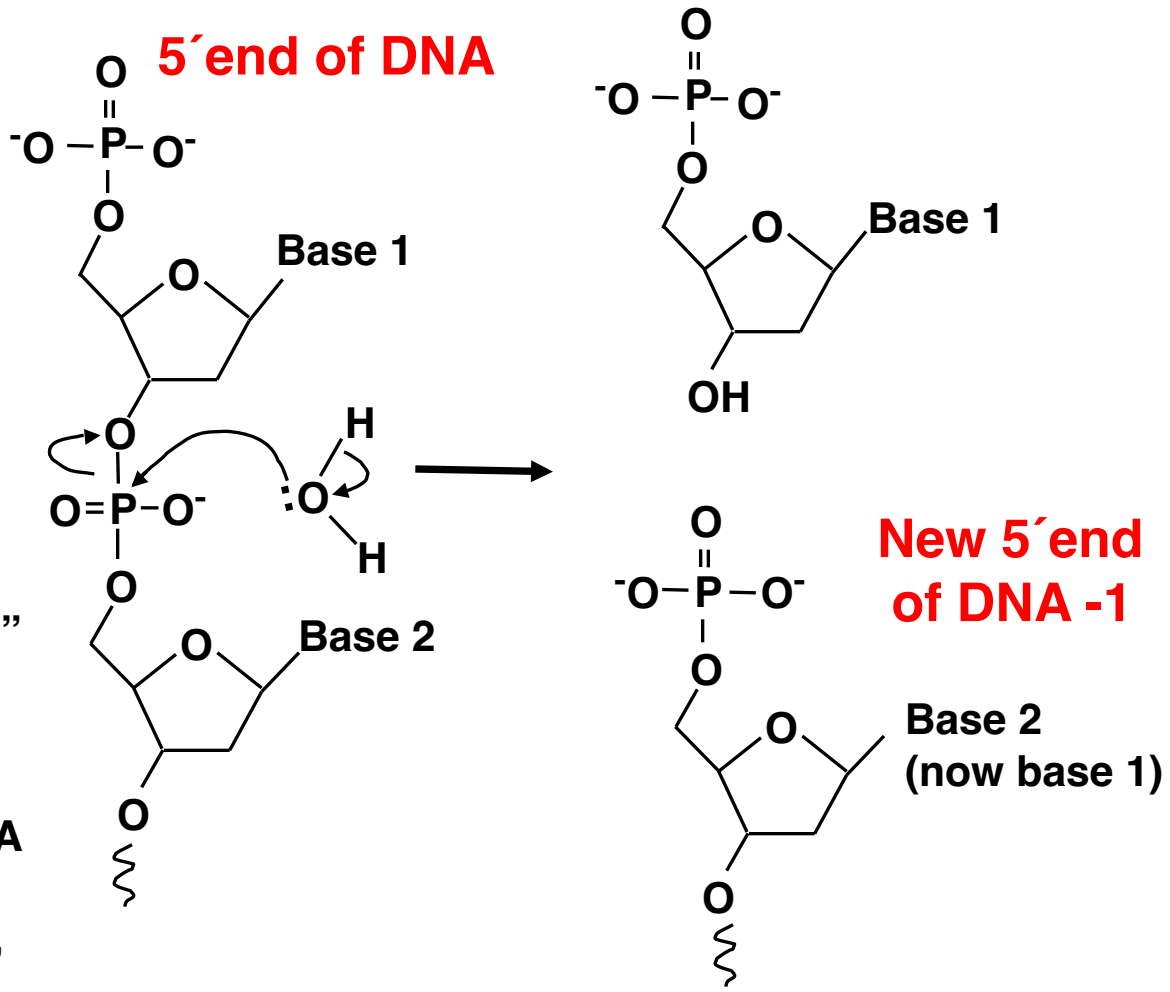
3rd activity of DNA Pol. I: 5´→3´ exonuclease activity

Performed by a different domain of DNA Pol. I

Biological Activity:

-Allows the replacement of damaged or abnormal DNA sequences by "Nick translation" (important for DNA Repair Chapter)

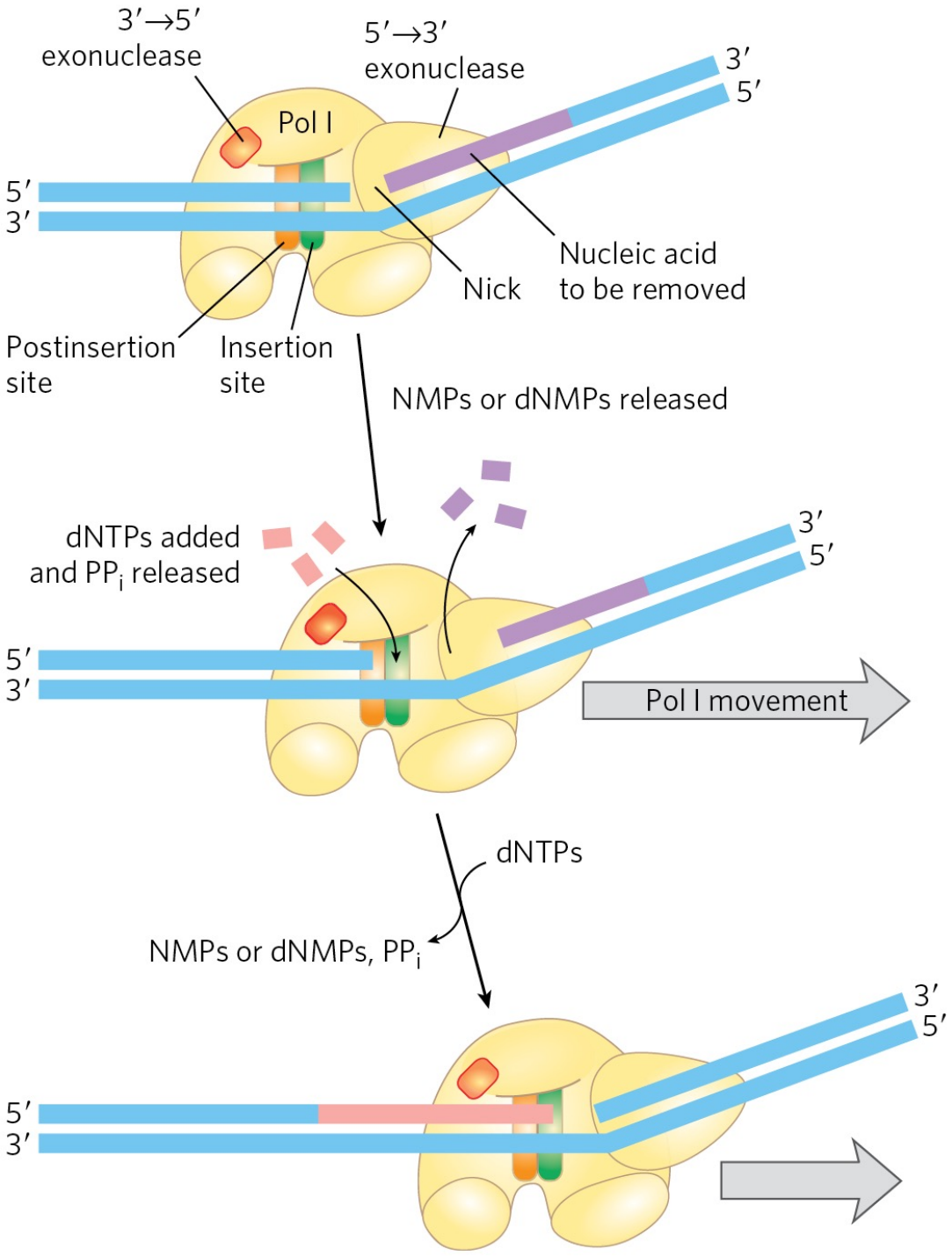
-Also allows the removal of RNA sequences embedded in DNA (removal of replication primers, we'll see this in the DNA replication chapter)



Nick translation

DNA Pol. I combines 5´→3´ exonuclease and 5´→3´ polymerase activities simultaneously to remove nucleotides of one strand and replace them with newly synthesized nucleotides

Note: “translation” in nick translation is unrelated to the process of protein translation



Summary of Catalytic Activities found in DNA and RNA Polymerases

DNA Polymerases

5′→3′ Polymerase

3′→5′ exonuclease = editing/proofreading

Most DNA Pols have 3′→5′ exo activity catalyzed by an active site distinct from the polymerase active site

DNA Pol.I = same protein

DNA Pol.III = different protein subunit than the polymerase

5′→3′ exonuclease

only present DNA Pol. I;
Absent in DNA Pol.III or Pol.$\alpha/\delta/\varepsilon$..
=hydrolyze RNA primers; damaged DNA

RNA Polymerases

5′→3′ Polymerase

No 3′→5′ exonuclease;
Proofreading
achieved in the
polymerase
active site by backtracking

No 5′→3′ exonuclease

• **Speed** = **overall rate of nucleotides polymerization per unit of time**

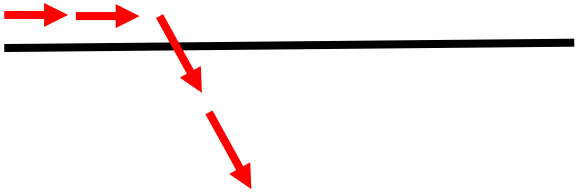
In vivo Speed of DNA polymerases ≈ 1000 nt / second

In vivo Speed of RNA polymerases ≈ 50 nt / second

• **Processivity** = Ability of an enzyme (Polymerase) to remain attached to its substrate (template) and perform multiple rounds of catalysis (=nucleotides addition) before dissociating from the template

• **Speed =/= Processivity!**

• A poorly processive enzyme is called **Distributive**



• High Processivity is a necessity for DNA polymerases involved in DNA replication but is mechanistically problematic because of the need to translocate on the template after each round of nucleotide addition, providing opportunities for dissociation

Some Processivity Numbers:

Bacterial RNA Polymerase = 10,000

DNA Polymerase III >5000

DNA Polymerase δ > 13,000

For what type of polymerases is processivity more important/problematic?

A: It is more problematic for DNA polymerases because they interact only with 2 nucleic acids strands vs. 3 for RNA polymerases

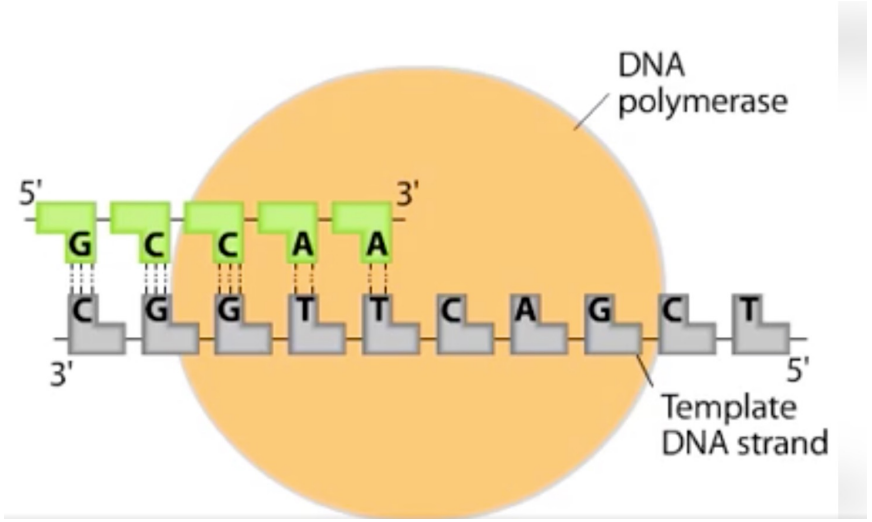
B: It is more important for RNA polymerases because transcription stops would lead to truncated mRNAs and shorter proteins

C: It is more important for DNA polymerases so that they can replicate genomic DNA without interruptions

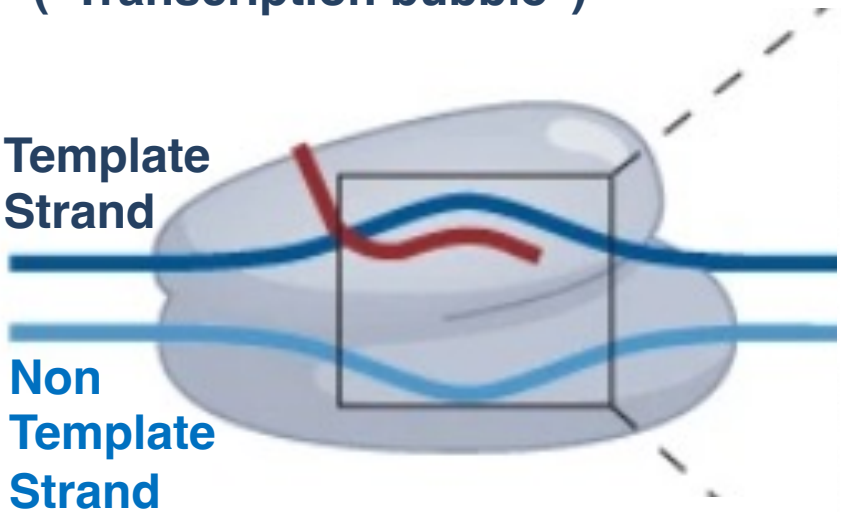
D: It is more problematic for RNA polymerases because of the tendency of RNA to break, leading to RNA polymerase dissociation

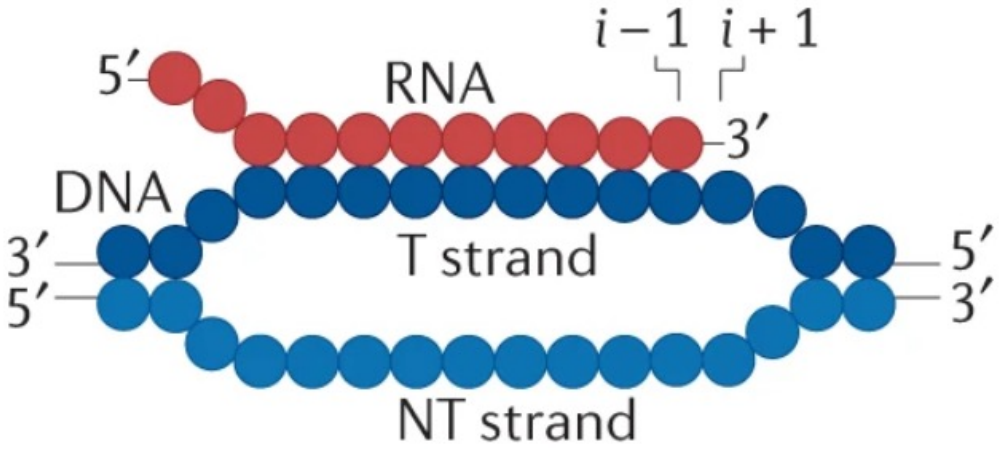
DNA and RNA Polymerases do not interact the same way with the DNA used for polymerization

DNA Polymerase:
does not interact with the non-template strand

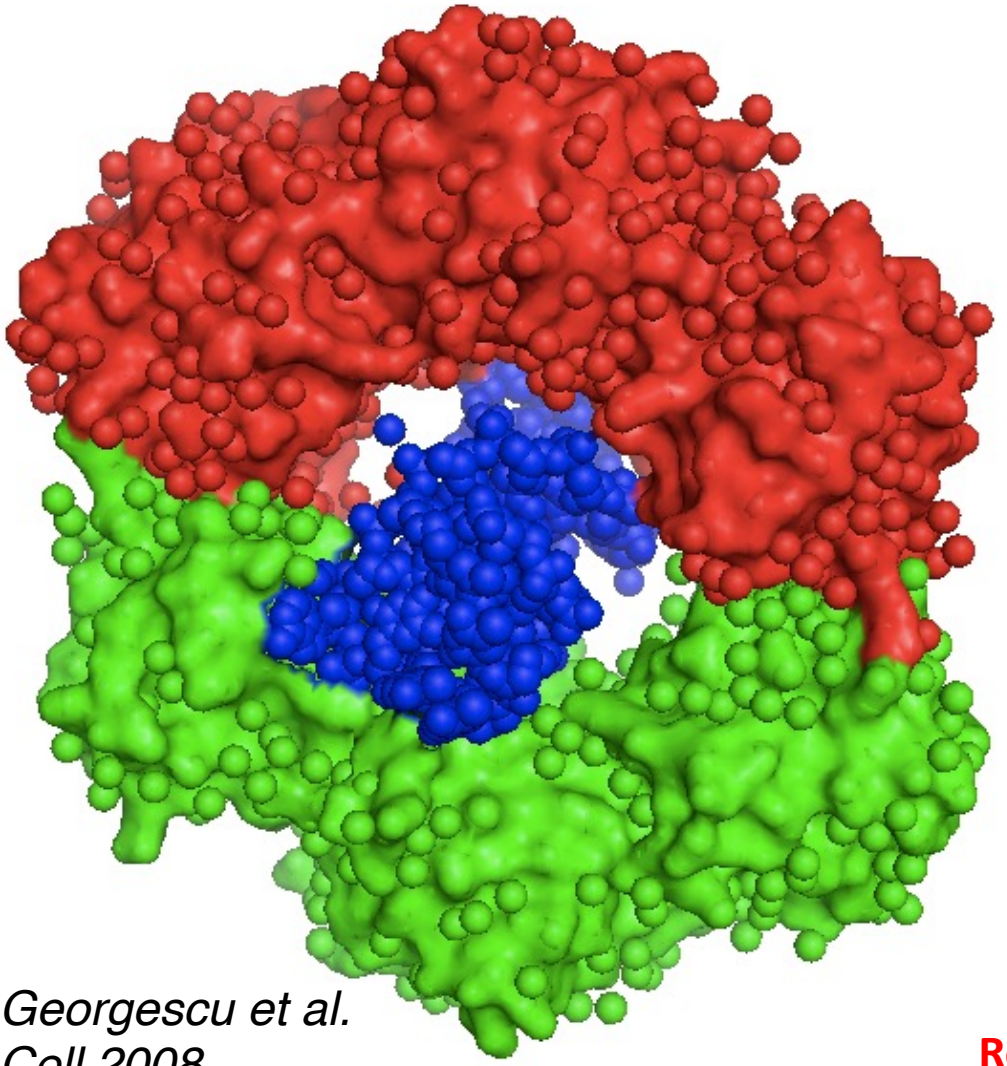


RNA Polymerase:
Maintains contact with the non-template strand ("Transcription bubble")





Sliding Clamps increase processivity of DNA polymerases

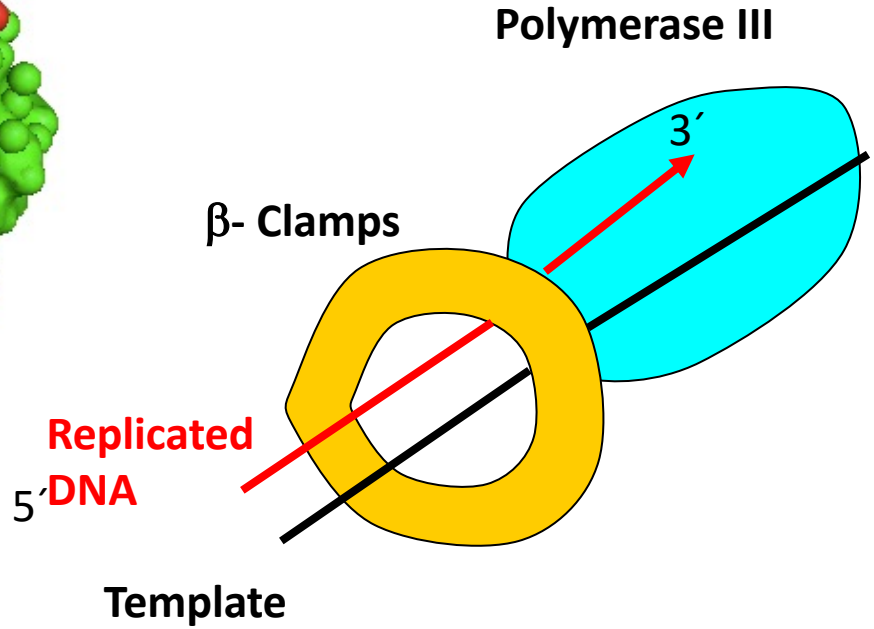


Georgescu et al.
Cell 2008

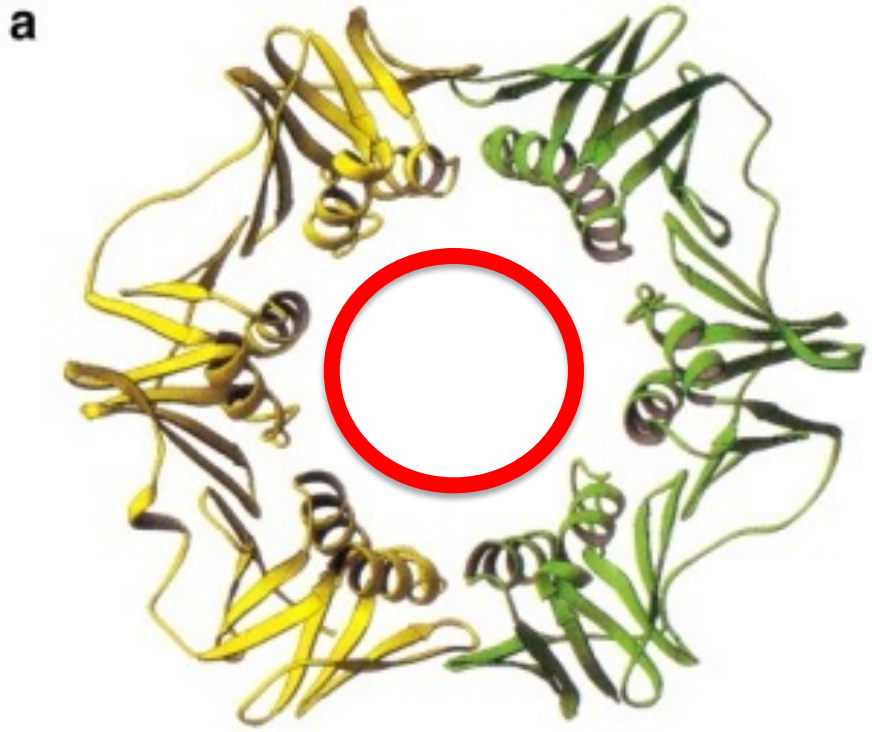
homodimer of two proteins (green and red) wrapped around dsDNA (blue)

DNA Polymerase III Core is poorly processive by itself: This is an issue in the context of replication in vivo

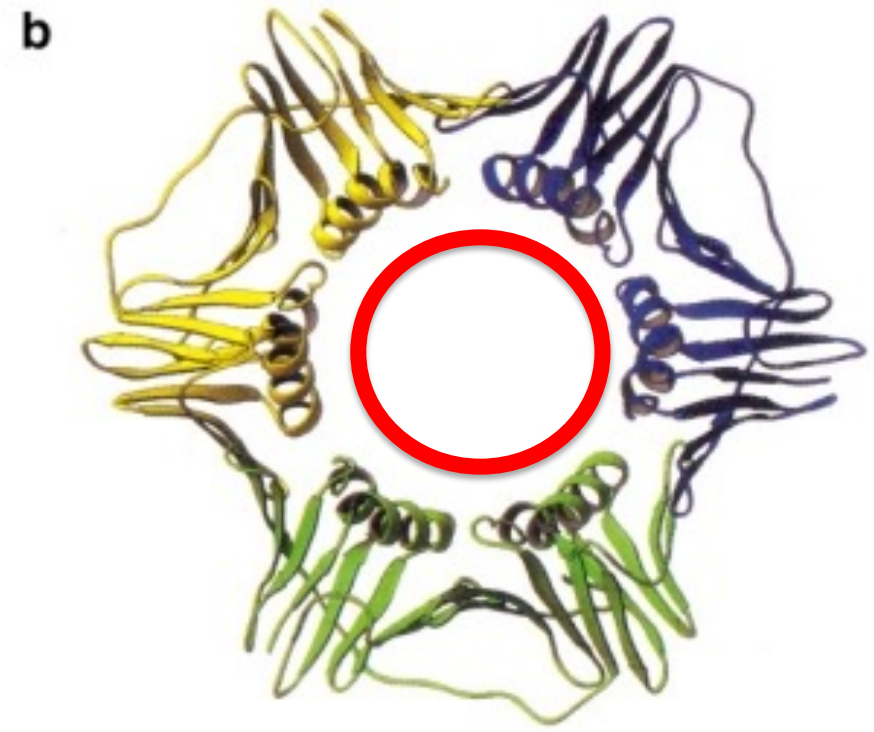
β-clamps wrapped around DNA also interact with DNA Pol.III and force the DNA Polymerase to maintain contact with the DNA template thus enhancing processivity



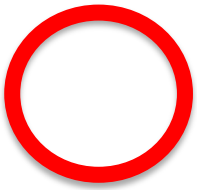
Many Eukaryotic DNA polymerases use a similar "clamp" molecule = PCNA to increase processivity or help load the polymerase onto the DNA



β- Clamps = Dimer
(Bacterial DNA Polymerases)



PCNA /Trimer
= (Proliferating Cells Nuclear Antigen)
used by Eukaryotic DNA Polymerases)



= B-DNA diameter

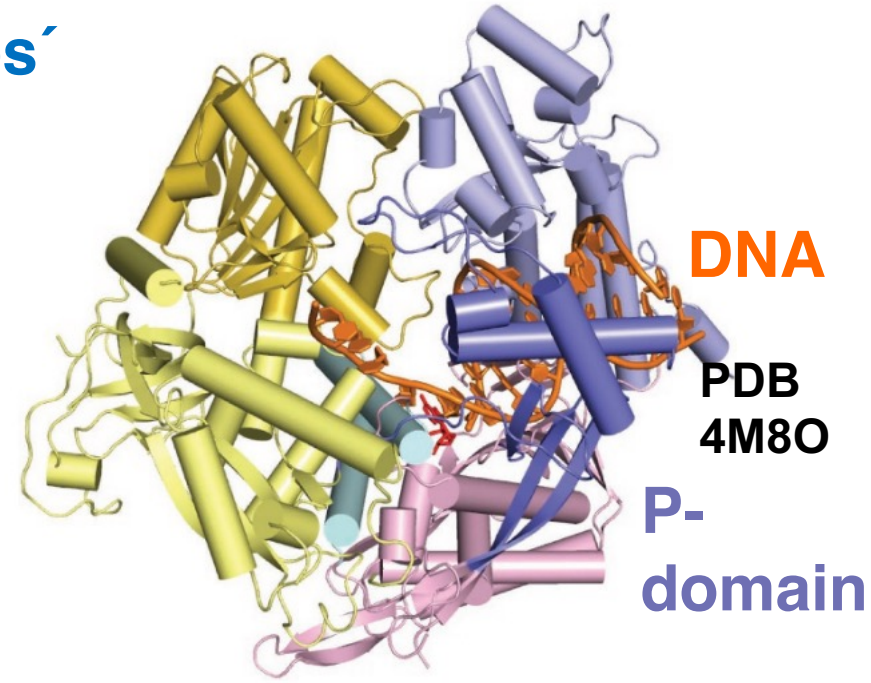
Breyer & Matthews Protein Sci. 2001 Sep; 10(9): 1699–1711.

Some DNA Polymerases can be processive without 'clamps´

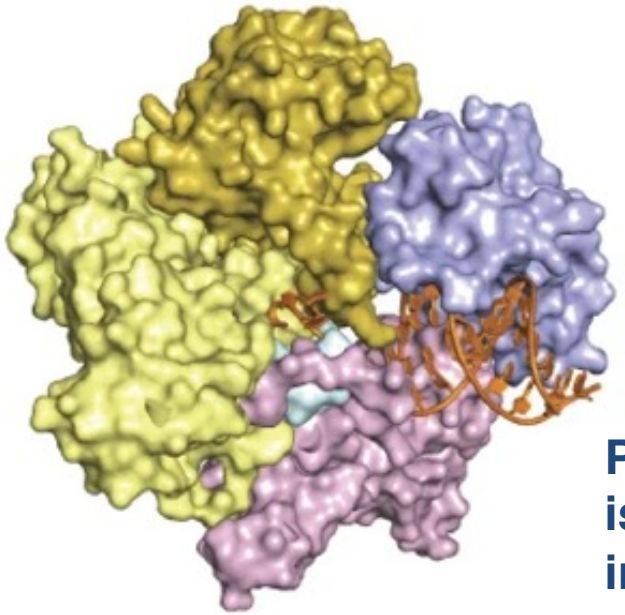
- **Eukaryotic Pol Ɛ is highly processive without PCNA**

Processivity is due to the presence of an additional protein domain, the P-domain, which encircles the DNA =forces the protein to remain bound to the DNA template

Pol ε



Pol δ



Hogg et al. Nature Structural & Molecular Biology (2014)