ZOO 203 – MOLECULAR BIOLOGY
UNIT 1: DNA REPLICATION
(PART – II)

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THE MECHANISM OF DNA POLYMERASE

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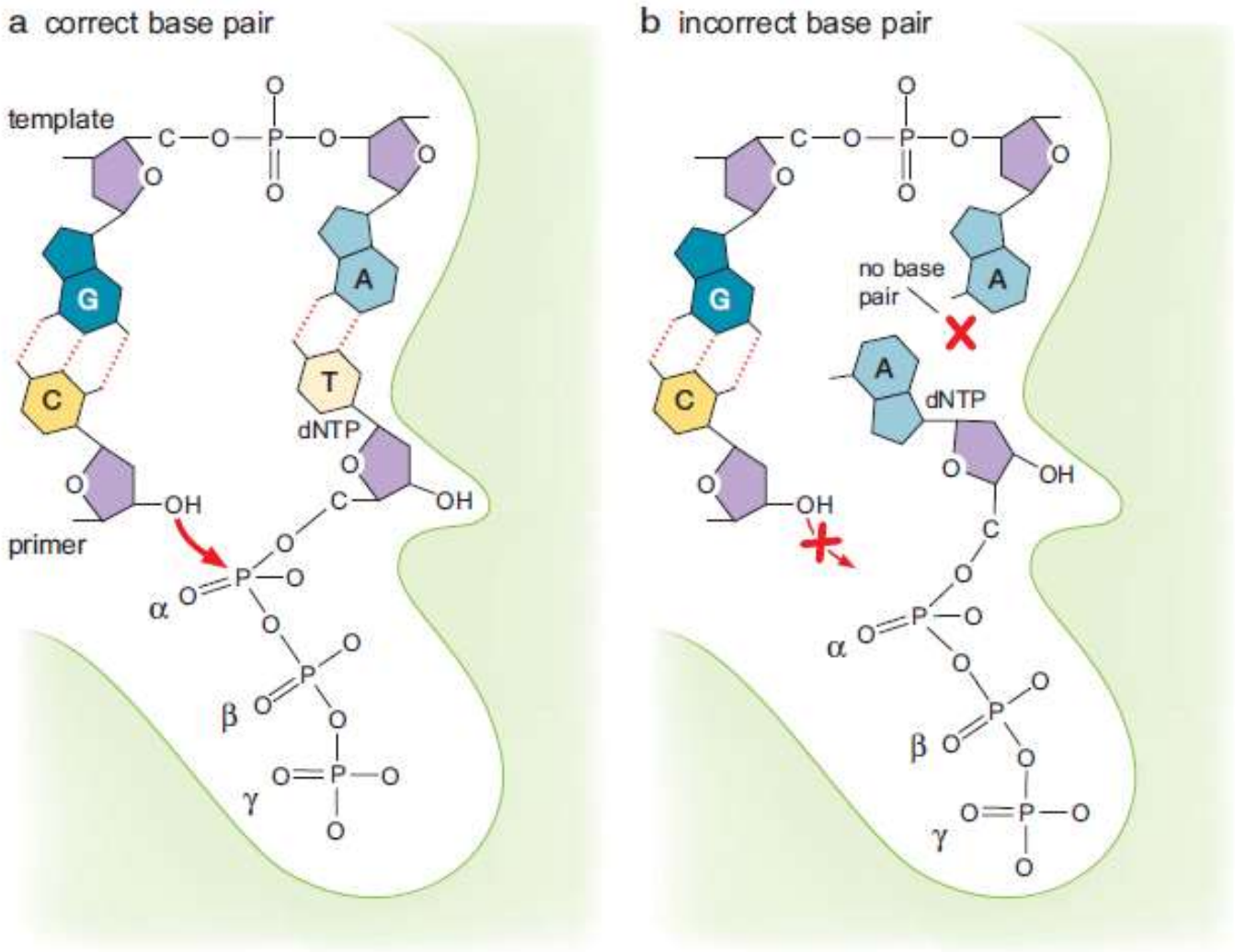
- DNA polymerase uses **a single active site** to catalyze the addition of any of the four deoxynucleoside triphosphates
- It accomplishes this catalytic flexibility by exploiting the **nearly identical geometry** of the **A:T** and **G:C** base pairs
- It monitors the ability of the incoming nucleotide to form an A:T or G:C base pair, rather than detecting the exact nucleotide that enters the active site
- Only when a correct base pair is formed, then the **3'-OH of the primer** and the **α-phosphate of the incoming nucleoside triphosphate** are kept in the optimum position for catalysis to occur.
- Incorrect base pairing leads to dramatically lower rates of nucleotide addition as a result of a catalytically unfavorable alignment of these substrates

Correctly paired bases are required for DNA-polymerase-catalyzed nucleotide addition



How does DNA Polymerase discriminate between dNTPs from rNTPs?

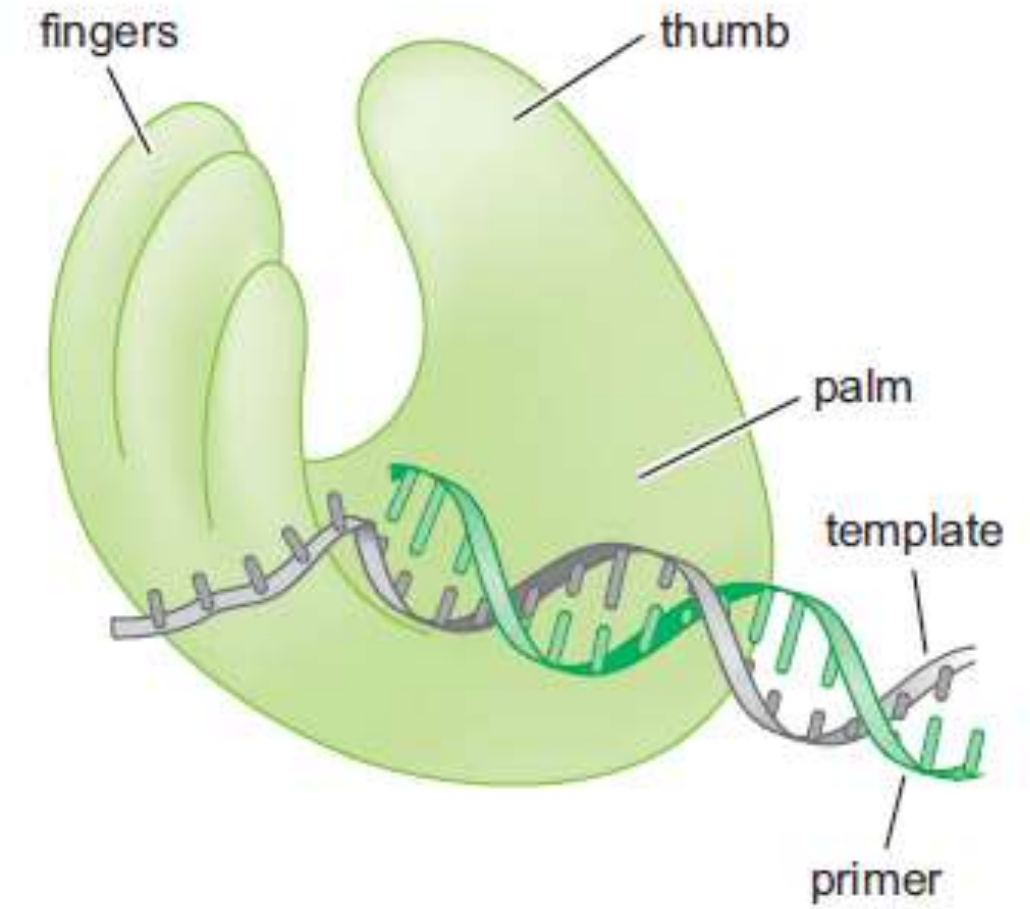
- DNA polymerases show an impressive ability to distinguish between ribonucleoside and deoxyribonucleoside triphosphates (rNTPs and dNTPs).
- Although rNTPs are present at approximately 10-fold higher concentration in the cell, they are incorporated at a rate that is more than 1000-fold lower than dNTPs.
- This **discrimination** is mediated by the **steric exclusion** of rNTPs from the DNA polymerase active site.
- In DNA polymerase, **the nucleotide-binding pocket cannot accommodate a 2'-OH on the in-coming nucleotide**. This space is occupied by **two amino acids** that make **van der Waals** contacts with the **sugar ring**.
- Changing these amino acids to other amino acids with smaller side chains (e.g., by changing a glutamate to an alanine) results in a DNA polymerase with significantly reduced discrimination between dNTPs and rNTPs.

DNA Polymerases Resemble a Hand That Grips the Primer:Template Junction

- Based on the hand analogy, the three domains of the polymerase are called the thumb, fingers, and palm.

- The DNA (substrate) sits in a large cleft that resembles a partially closed right hand.

3D structure of DNA polymerase resembles a right hand



ROLE OF PALM DOMAIN

- The **palm domain** is composed of a **β sheet** and contains the primary elements of the catalytic site.
- This region of DNA polymerase binds **two divalent metal ions** (typically Mg2+ or Zn2+) that alter the chemical environment around the correctly base-paired dNTP and the **3'-OH of the primer** (Fig. 9-6).

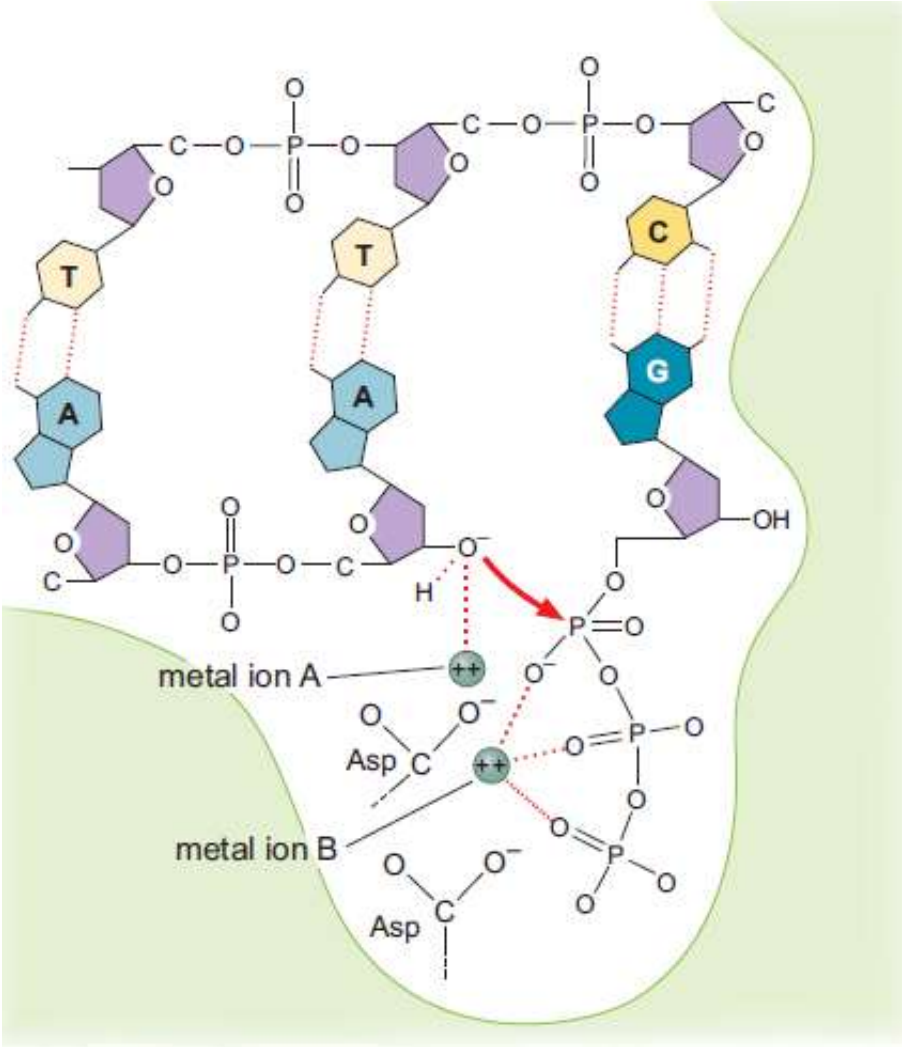
- **One metal ion** reduces the affinity of the 3'-OH for its hydrogen. This generates a 3'O- that is primed for the nucleophilic attack of the α-phosphate of the incoming dNTP. The

- **Second metal ion** coordinates the negative charges of the β-phosphate and γ-phosphate of the dNTP and stabilizes the pyrophosphate produced by joining the primer and the incoming nucleotide.

Two metal ions bound to DNA polymerase catalyze nucleotide addition

The active site of a DNA polymerase.

- The two metal ions (shown in **green**) are held in place by interactions with two highly conserved **aspartate** residues.
- **Metal ion A** primarily interacts with the 3'-OH, resulting in reduced association between the O and the H. This leaves a nucleophilic 3'O- .
- **Metal ion B** interacts with the triphosphates of the incoming dNTP to neutralize their negative charge.
- After catalysis, the pyrophosphate product is stabilized through similar interactions with metal ion B (not shown).



ROLE OF PALM DOMAIN

- In addition to catalysis, the palm domain also monitors the base pairing of the most recently added nucleotides
- This region of the polymerase makes extensive hydrogen-bond contacts with base pairs in the minor groove of the newly synthesized DNA.
- These contacts are not base specific but only form if the recently added nucleotides (whichever they may be) are correctly base-paired.
- Mismatched DNA in this region interferes with these minor-groove contacts and dramatically slows catalysis.

ROLE OF FINGERS

- Several residues located within the fingers bind to the incoming dNTP.
- once a correct base pair is formed between the incoming dNTP and the template, the finger domain moves to enclose the dNTP.
- This closed form of the polymerase “hand” stimulates catalysis by moving the incoming nucleotide into close contact with the catalytic metal ions.
- The finger domain also associates with the template region, leading to a nearly 90^0 turn of the phosphodiester backbone between the first and second bases of the template.
- This bend serves to expose only the first template base after the primer at the catalytic site and avoids any confusion concerning which template base should pair with the next nucleotide to be added.

ROLE OF THE THUMB

- Instead of involving intimately in catalysis, the thumb interacts with the DNA that has been most recently synthesized.

This serves two purposes

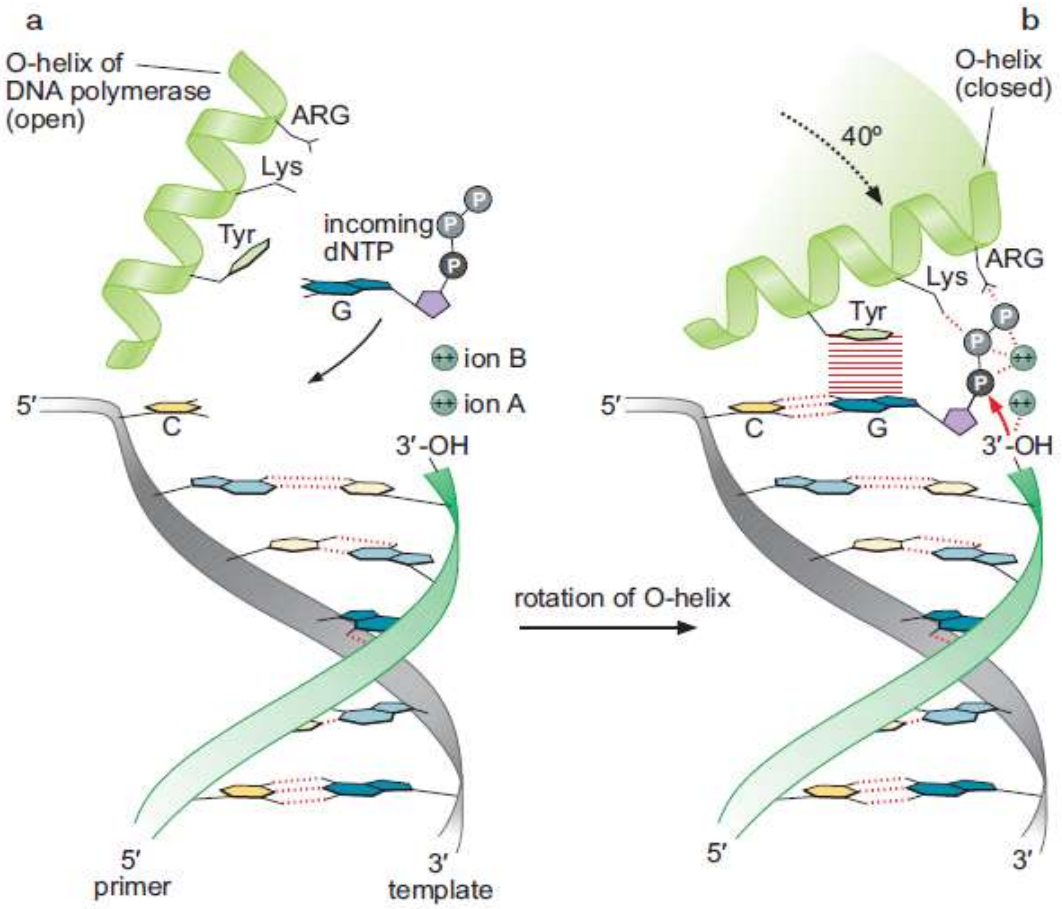
- First, it maintains the correct position of the primer and the active site.
- Second, the thumb helps to maintain a strong association between the DNA polymerase and its substrate.

  This association contributes to the ability of the DNA polymerase to add many dNTPs each time it binds a primer: template junction.

DNA polymerase "grips the template and the incoming nucleotide when a correct base pair is made

- The primary change is a 40^0 rotation of one of the helices in the **finger domain** called the O-helix.
- In the open conformation, this helix is distant from the incoming nucleotide. When the polymerase is in the **closed conformation**, this helix moves and makes several important interactions with the incoming dNTP.
- A **tyrosine** makes stacking interactions with the base of the dNTP, and two charged residues associate with the triphosphate.
- The combination of these interactions positions the dNTP for catalysis mediated by the two metal ions bound to the DNA polymerase.

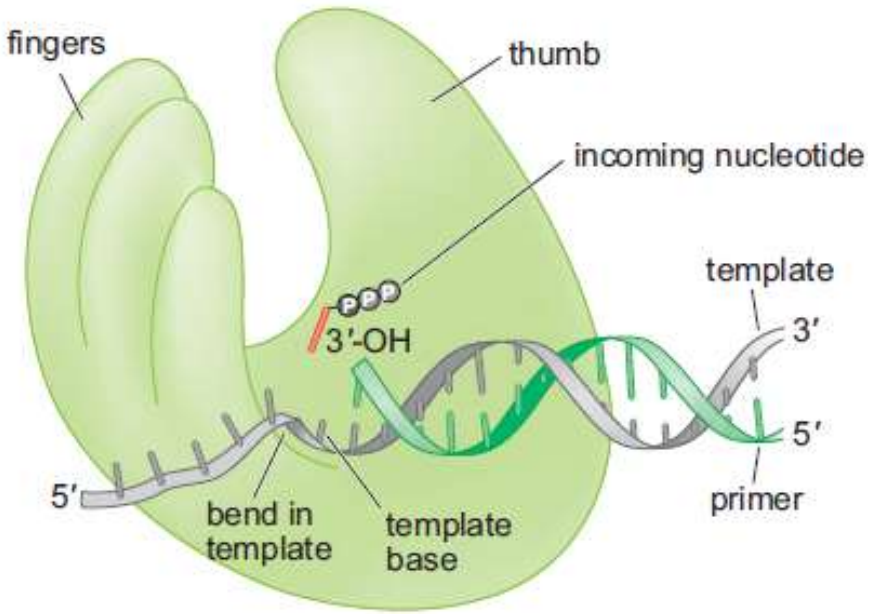
An illustration of the changes in DNA polymerase structure after the incoming nucleotide base-pairs correctly to the template DNA.



(Based on Doublie´ S. et al. 1998. Nature 391: 251–258, Fig. 5. # 1998.)

Illustration of the path of the template DNA through the DNA polymerase

- The recently replicated DNA is associated with the **palm** region of the DNA polymerase.
- At the active site, the first base of the single-stranded region of the template is in a position expected for dsDNA.
- As one follows the template strand toward its 5’ end, the phosphodiester backbone abruptly bends 90^0.
- This results in the second and all subsequent single-stranded bases being placed in a position that prevents any possibility of base pairing with a dNTP bound at the active site.



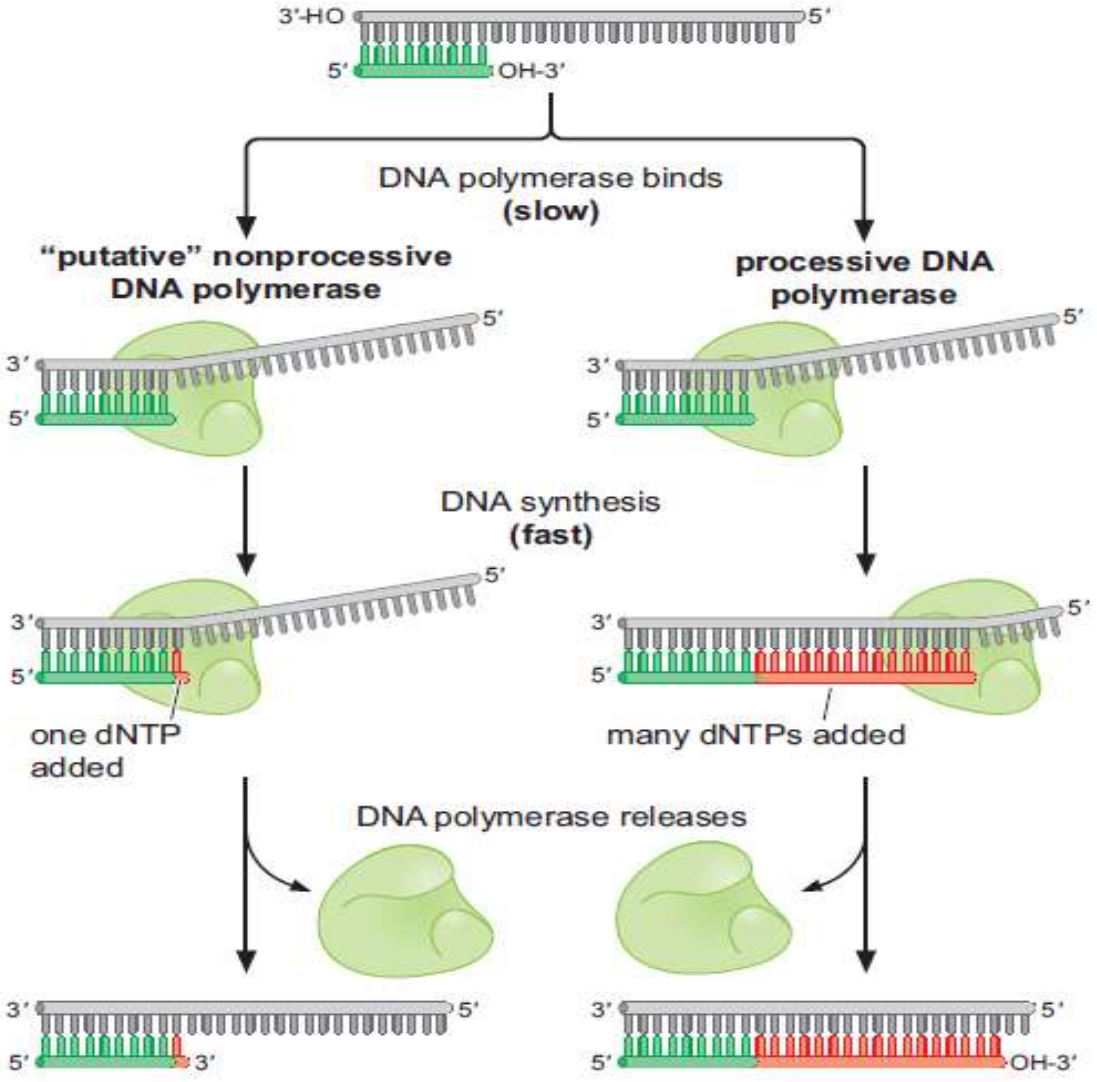
DNA Polymerases Are Processive Enzymes

- Processivity is a characteristic of enzymes that operate on polymeric substrates.
- In the case of DNA polymerases, the **degree of processivity** is defined as the average number of nucleotides added each time the enzyme binds a primer:template junction.
- A highly processive polymerase increases the overall rate of DNA synthesis by as much as 1000-fold compared with a nonprocessive enzyme.

Processivity

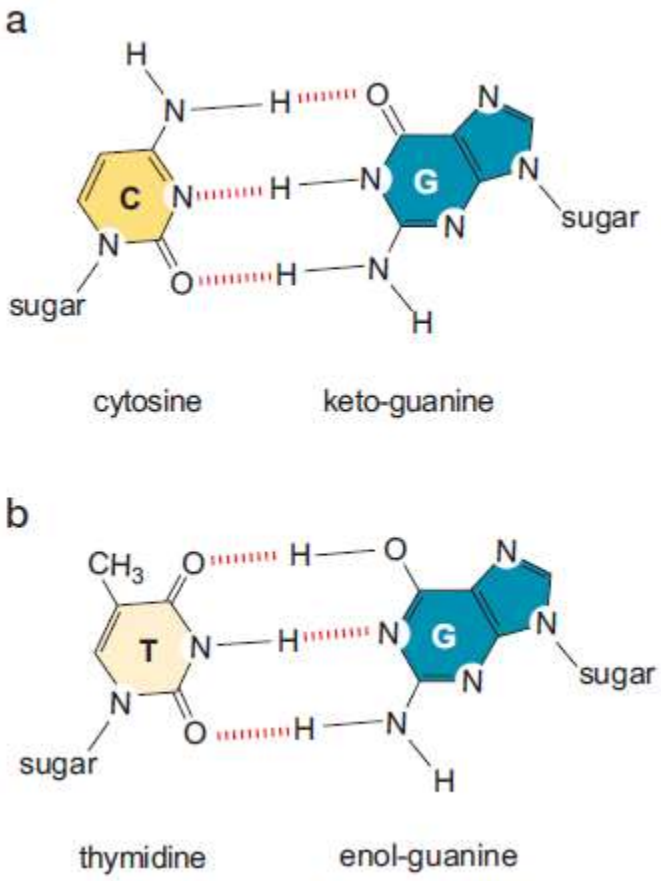
- **Processivity** is facilitated by **sliding of DNA polymerases along the DNA template.**
- Once bound to a primer:template junction, DNA polymerase interacts tightly with much of the double-stranded portion of the DNA in a sequence-nonspecific manner.
- These interactions include **electrostatic interactions** between **the phosphate backbone and the thumb domain** and **interactions between the minor groove of the DNA and the palm domain**.
- The sequence-independent nature of these interactions permits the easy movement of the DNA even after it binds to polymerase.
- **Each time a nucleotide is added to the primer strand, the DNA partially releases from the polymerase**. (The hydrogen bonds with the minor groove are broken, but the electrostatic interactions with the thumb are maintained.)
- **The DNA then rapidly rebinds to the polymerase in a position that is shifted by 1 bp using the same sequence-nonspecific mechanism**.
- Further increases in processivity are achieved through interactions between the DNA polymerase and accessory proteins.

Illustration showing the difference between processive and nonprocessive DNA polymerase



PROOFREADING EXONUCLEASE ACTIVITY

- Errors in nucleotide selection by DNA polymerase arise only about once per 100,000 nucleotides due to flickering of the bases into the "wrong" tautomeric form (imino or enol).
- These alternate forms of the bases permit incorrect base pairs to be correctly positioned for catalysis.
- When the nucleotide returns to its "correct" state, the incorporated nucleotide is mismatched with the template and must be eliminated
- Removal of these incorrectly base-paired nucleotides is mediated by a **proofreading exonuclease** enzymes that degrade DNA starting from a 3' DNA end (i.e., from the growing end of the new DNA strand).
- The removal of mismatched nucleotides is facilitated by the reduced ability of DNA polymerase to add a nucleotide adjacent to an incorrectly base paired primer.
- Mispaired DNA alters the geometry between the 3'-OH and the incoming nucleotide because of **poor interactions with the palm region**. This altered geometry reduces the rate of nucleotide addition in much the same way that addition of an incorrectly paired dNTP reduces catalysis.

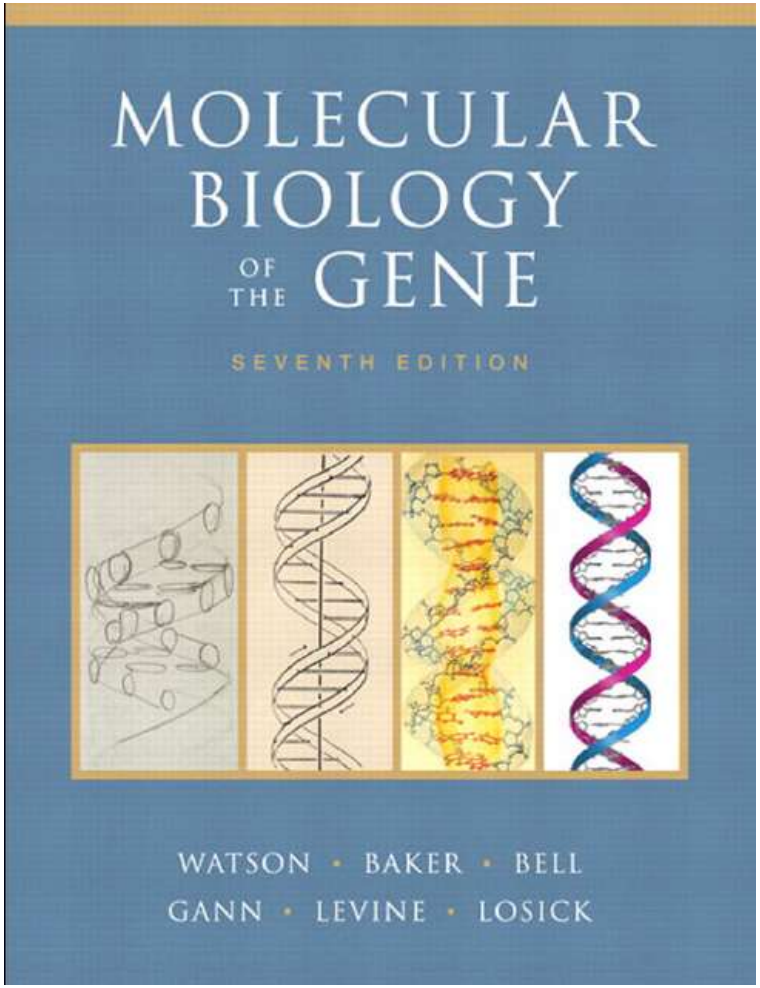


The tautomeric shift of guanine results in mispairing with thymidine

In processive DNA synthesis, proofreading occurs without releasing the DNA from the polymerase

- When a **mismatched base pair is present in the polymerase** active site, the **primer:template junction is destabilized**, creating several base pairs of unpaired DNA.
- The DNA polymerase active site binds such a mismatched template **poorly,** but the exonuclease active site has a 10-fold higher affinity for single-stranded 3’ ends.
- Thus, the newly unpaired 3’ end moves from the polymerase **active site** to **the exonuclease active site**. The incorrect nucleotide is removed by the exonuclease (an additional nucleotide may also be removed).
- The **removal of the mismatched base** allows the **primer:template junction to re-form and rebind the polymerase active site**, enabling DNA synthesis to continue.

REFERENCE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