DNA REPLICATION, DNA MUTABILITY & DNA REPAIR

Course: Molecular Biology (02022312)

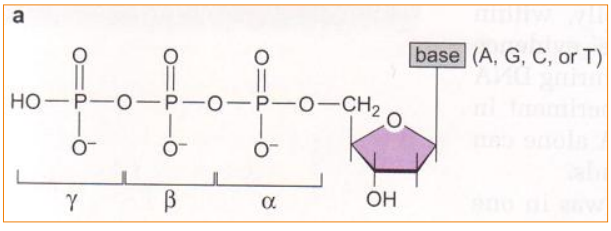
Instructor: Dr. M A Srour

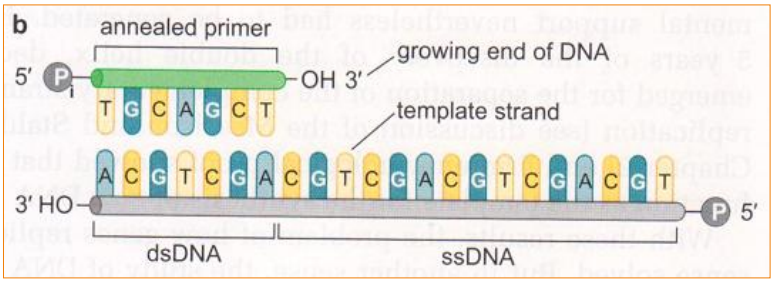
Textbook:
Watson J, Baker TA, Bell SP, Gann A, Levine M, Losick R (2008). Molecular Biology of the Gene, 6th ed. Chap 8 / pp.195-256; Chap 9 /pp.257-82

Lec # 07 Wed 29.02.2012

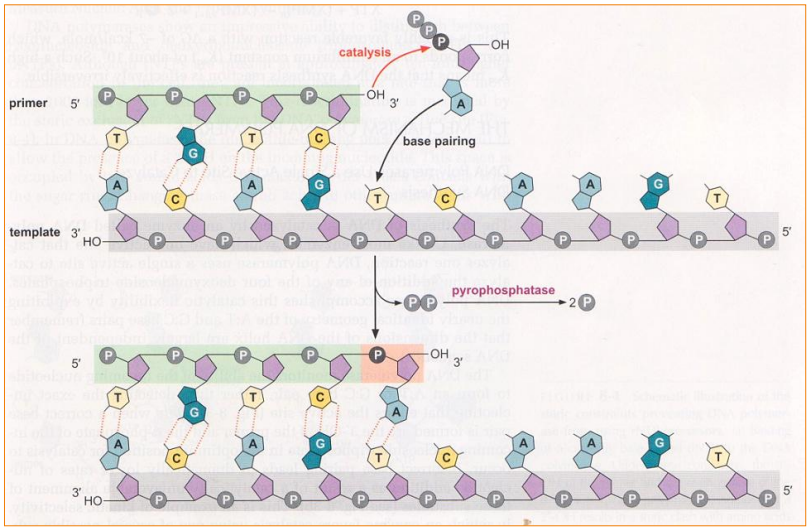
(Chapter 8)

DNA synthesis requires (a) Deoxynucleoside triphosphates & (b) a primer: template junction





DNA is synthesized by extending the 3' end of the primer



What is the driving force for DNA synthesis?

- XTP + (XMP)n → (XMP)n+1 + P~P

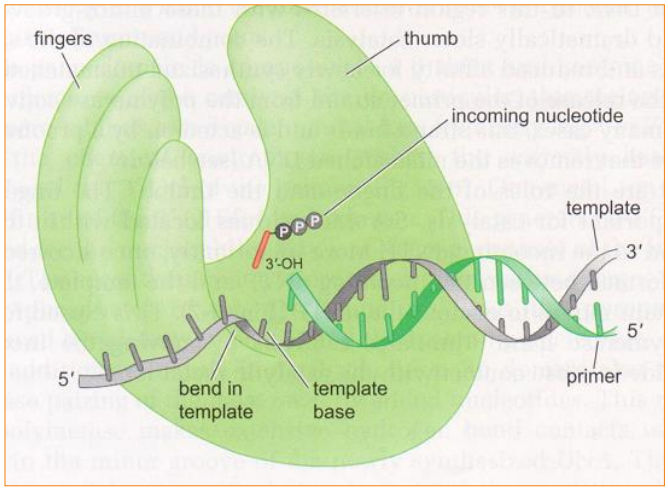
  (ΔG -3.5kCal/mole)

- XTP + (XMP)n → (XMP)n+1 + $2P_i$

  (ΔG -7kCal/mole)

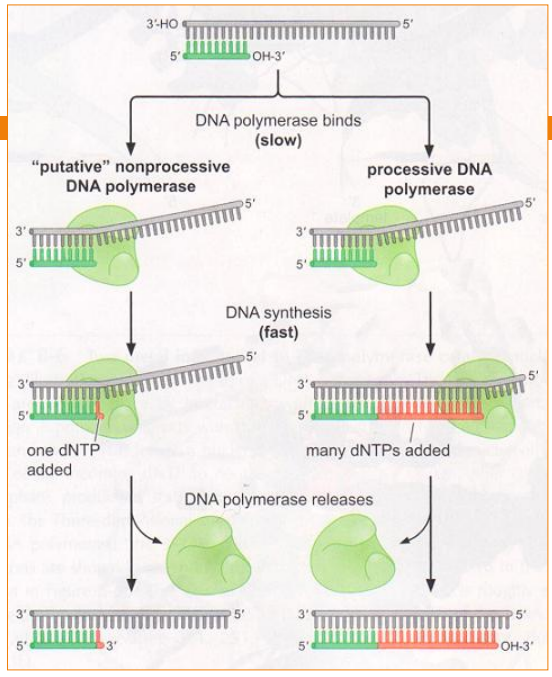
DNA polymerase resemble a Hand that Grips the Primer: Template junction

Three domains of DNA Pol:
- Thumb,
- Fingers,
- Palm

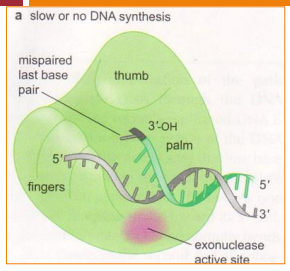


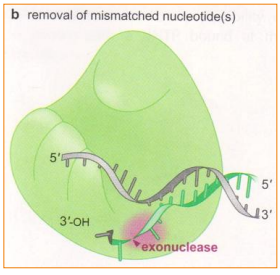
DNA Polymerases are processive enzymes

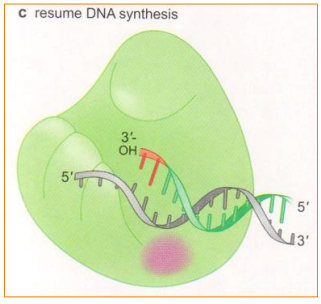
- Processivity: average number of nts added each time the enzyme binds a primer:template junction
- Catalysis rate: can be up to 1000 nt/second
- Processivity: depending on the specific enzyme, it can range from few nts to >50,000 bases added per binding event



Exonucleases proofread newly synthesized DNA

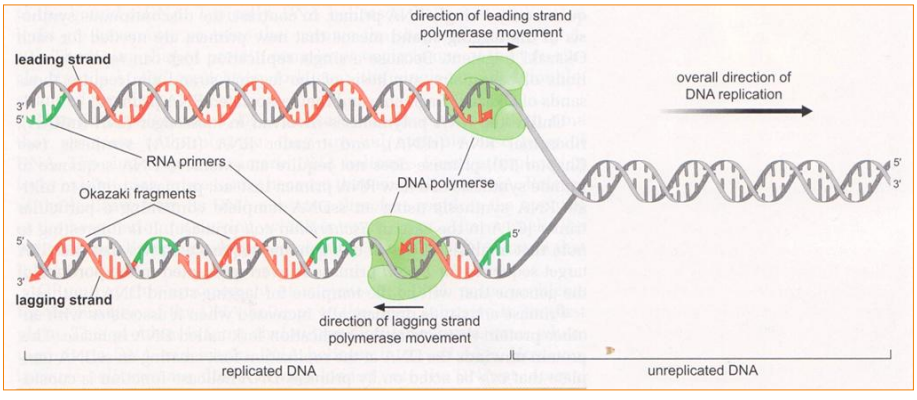






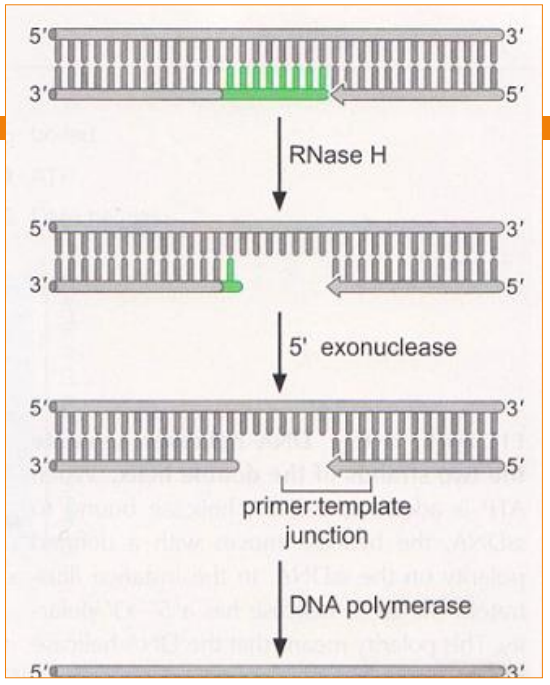
DNA Pol inserts 1 incorrect nt every 10^5 nts
Proofreading exonucleases decrease the error rate to 1 in every 10^7 nts
Postreplication mismath repair process reduces error rate to 1 in 10^{10},

Replication fork: both strands of DNA are synthesized together at same replication fork



Okazaki fragments length: 1000-2000 bp in bacteria and 100-400 nts in eukaryotes.
RNA primers: 5-10 nts.

RNA primers must be removed to complete DNA replication



DNA polymerases are specialized for different roles in the cell

TABLE 8-2 Activities and Functions of DNA Polymerases

Prokaryotic (*E. coli*)	Number of Subunits	Function
Pol I	1	RNA primer removal, DNA repair
Pol II (Din A)	1	DNA repair
Pol III core	3	Chromosome replication
Pol III holoenzyme	9	Chromosome replication
Pol IV (Din B)	1	DNA repair, translesion synthesis (TLS)
Pol V (UmuC, $UmuD'_2C$)	3	TLS

Only Pol I & III have proofreading activity and function in DNA replication

DNA polymerases are specialized for different roles in the cell

Eukaryotic	Number of Subunits	Function
Pol α	4	Primer synthesis during DNA replication
Pol β	1	Base excision repair
Pol γ	3	Mitochondrial DNA replication and repair
Pol δ	2–3	Lagging-strand DNA synthesis; nucleotide and base excision repair
Pol ε	4	Leading-strand DNA synthesis; nucleotide and base excision repair
Pol θ	1	DNA repair of cross-links
Pol ζ	1	TLS
Pol λ	1	Meiosis-associated DNA repair
Pol μ	1	Somatic hypermutation
Pol κ	1	TLS
Pol η	1	Relatively accurate TLS past *cis-syn* cyclobutane dimers
Pol ι	1	TLS, somatic hypermutation
Rev1	1	TLS

Class activity??

- Both the leading and lagging strands requires primase to initiate DNA synthesis, but the frequency of primase function on the two strands is dramatically different. WHY??
- Why the primase adds an RNA primer, not a DNA primer??