

SOS in Biochemistry, Jiwaji University, Gwalior

M.Sc. II Semester (2019-20)

Paper BCH 205: Fundamentals of Molecular Biology (Unit 1)

ENZYMOMOLOGY

of

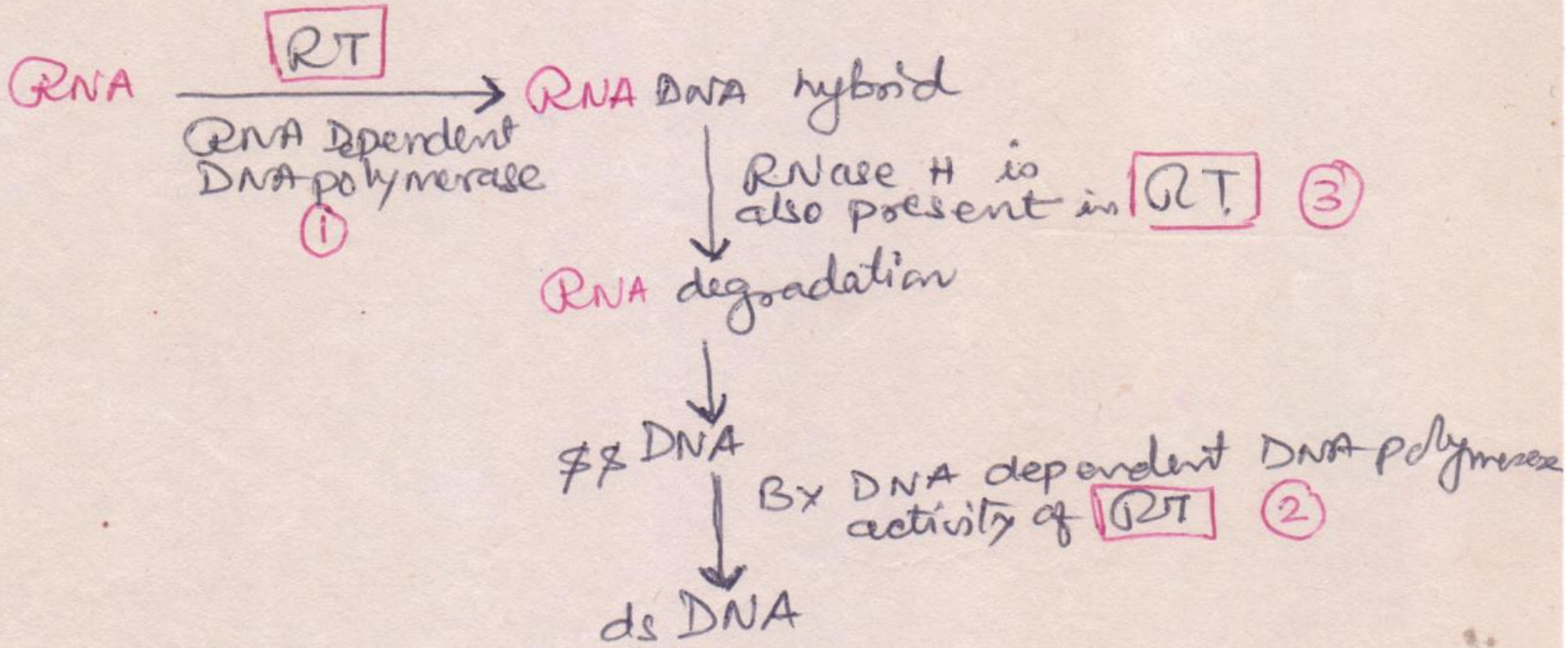
DNA REPLICATION - IV

Reverse Transcriptase

Reverse Transcriptase

(RNA-dependent DNA Polymerase)

- discovered by Temin & Mizutani (1970) in Rous Sarcoma virus (Nature, 226:1211 (1970))
♀
by Baltimore, D. (1970) in Rouscher Mouse leukemia virus (~~226:1209~~ Nature, 226:1209, 1970)
- Spiegelman et al in 1970 (Nature, 228:430) confirmed its presence in other viruses.



USES OF REVERSE TRANSCRIPTASES

1. RT is used chiefly to transcribe mRNA into ds cDNA which can further be used for cDNA cloning
2. RT can also be used with either ssDNA or RNA templates to make probes for use in hybridization experiments
3. RT can be used for labeling the termini of DNA fragments with protruding 5' termini (filling reaction)
4. RT can also be used to sequence DNAs by the dideoxy chain termination reaction (Sanger's Method) when other enzymes (eg., Klenow fragment & PolI or Sequenase) yield unsatisfactory results

Forms of Reverse Transcriptases

(Commercially available for cDNA preparation)

Two Forms

1. Avian RT

(A preparation made from purified avian myeloblastome virus (AMV))

2. Murine RT

(An enzyme isolated from a strain of E. coli that expresses a cloned copy of the reverse transcriptase gene of the Moloney murine leukemia virus)

TYPES OF REVERSE TRANSCRIPTASES

	Avian RT	Murine RT
1	<p>Consists of two polypeptide chains that carry both a polymerase activity & a powerful RNase H activity</p> <p>High activity of RNase H suppress the yield of cDNA and also restrict its length</p>	<p>Has a single polypeptide chain ($M_r = 84$ kD) and has a polymerase activity but a comparatively weak RNase H activity</p> <p>Enzyme with weak RNase H activity will degrade mRNA under cDNA synthesis at a Very low rate, hence cDNA from long mRNA can be easily synthesized</p>
2	<p>Work efficiently at 42°C</p> <p>RNAs rich in secondary structures are copied more efficiently than the murine</p>	<p>Work efficiently at 37°C</p> <p>Murine RT gets inactivated at 42°C</p>
3	<p>Can be contaminated with an endonuclease that cleaves DNA</p>	<p>Free of contamination of any endonuclease</p>
4	<p>pH Optimum = 8.3</p> <p>(Change of pH by 0.2 with change</p>	<p>pH Optimum = 7.6</p> <p>the rate of synthesis of cDNA)</p>

Thus, the murine enzyme, with its weaker complement of endogenous degradative activities, is a safer choice when attempting to obtain full length cDNA copies of mRNA longer than 2-3 kb in length.

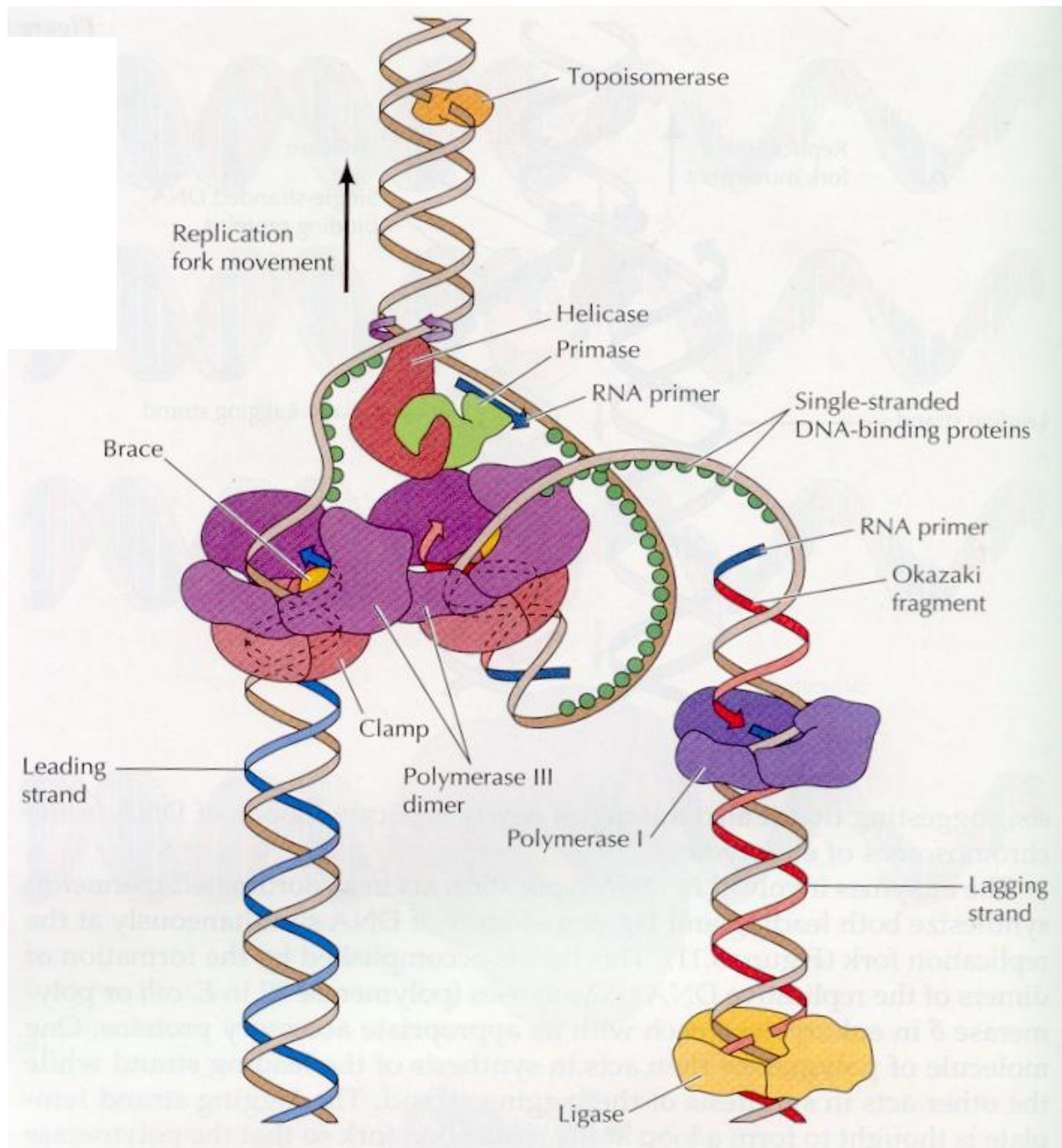
Single Stranded Binding Protein

Properties of Single-stranded DNA binding proteins in E. coli and Eukaryotes

	<u>E. coli</u>	<u>Eukaryotes</u>
Protein	SSB (Single stranded binding protein)	RP-A (Replication protein A) or HSSB (human SSB)
Structure	Homotetramer of 19 kDa subunits	Heterotrimer of 70 kDa, 32 kDa & 15 kDa subunits
Function	<ul style="list-style-type: none"> • stabilizes single stranded regions during replication, recombination and repair • Directs priming of to origin of M13-related genome • Associate with Pri B in primosome complex (possible priming function) 	<ul style="list-style-type: none"> • stabilizes single stranded regions during replication, recombination & repair • Interacts with pold: primase to prevent non specific priming events • Interacts with transcription factors, repair proteins RP-A & several helicases (possible specific roles in transcription & repair)
Properties	ssDNA-specific but no sequence specificity; cooperative binding	<ul style="list-style-type: none"> • ssDNA specific, partial sequence specificity. • Activity modulated by phosphorylation
Genes	ssb	RP-A1, RP-A2, RPA3

DNA Topoisomerases

(EC 5.99)

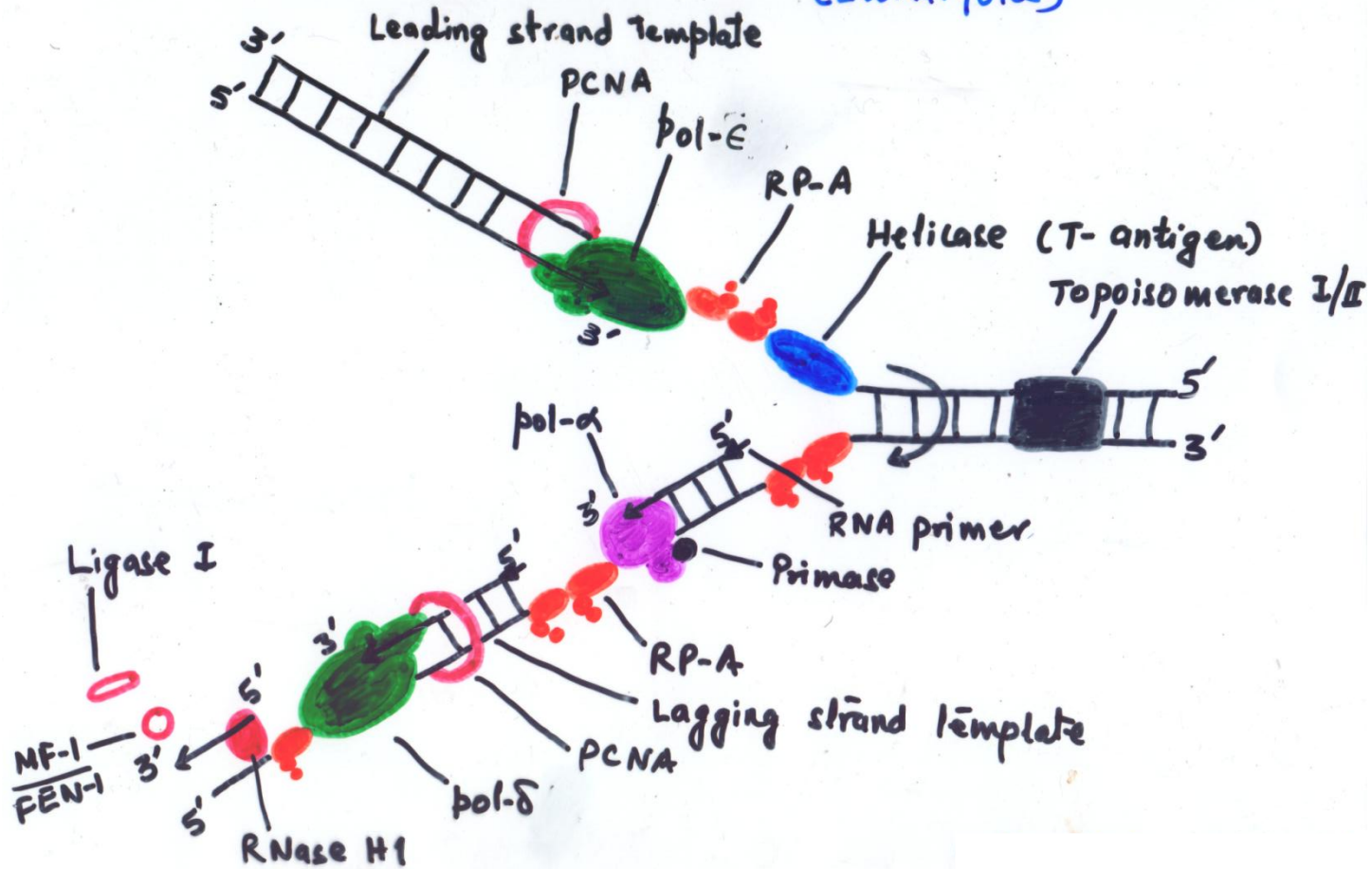


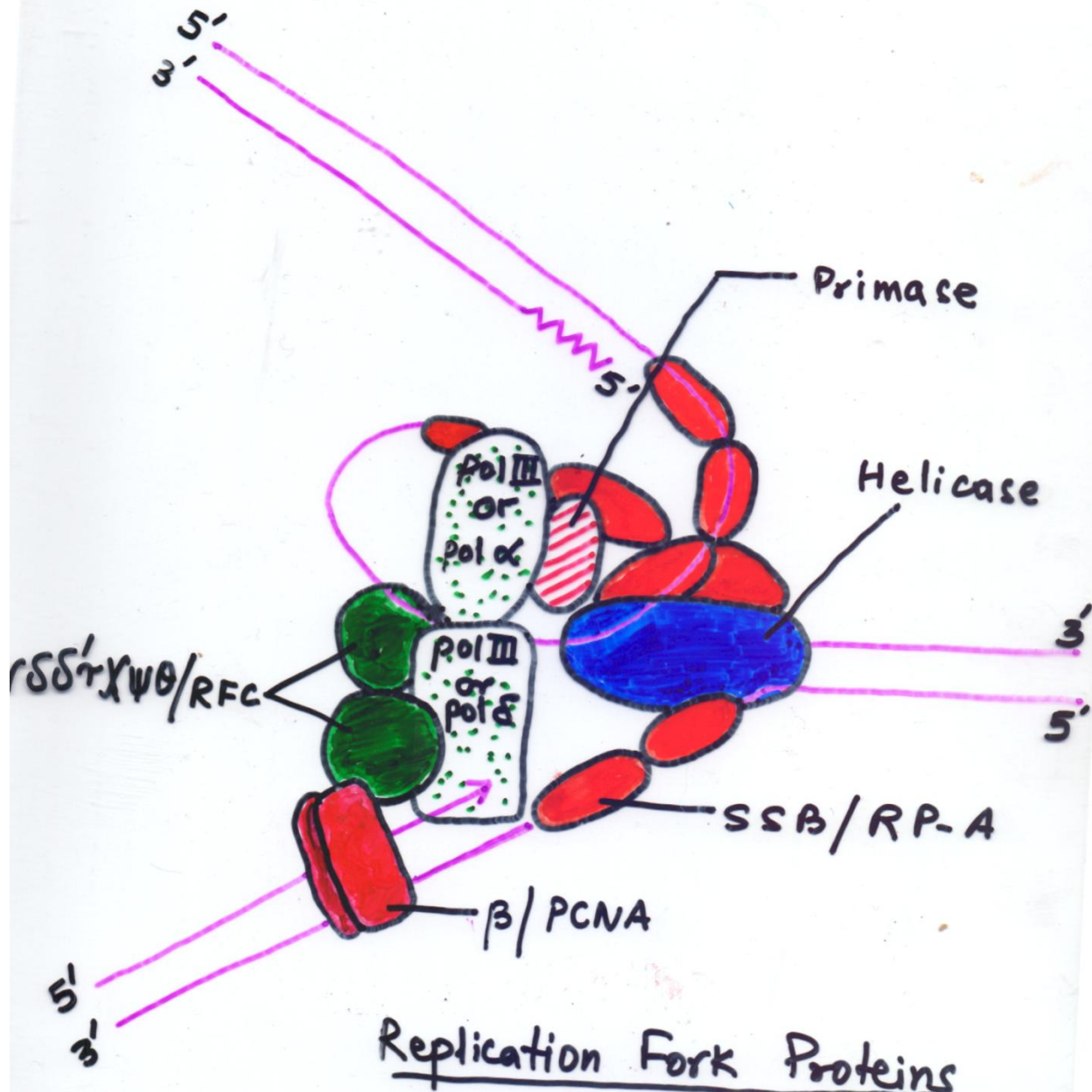
TOPOISOMERASES

	PROKARYOTIC		EUKARYOTIC	
	Type I	Type II	Type I	Type II
1. MW (Kd)	100	400	100	309
2. Sub units	MONOMER	Tetramer (A ₂ B ₂)	?	Dimer (subunit- mw 172Kd)
3. DNA strand cleaved	one	two	one	two
4. Gene	Top A	gyr. A <small>(A-chain, mw 105Kd) activity inhibited by nalidixic acid + enoxacin acid)</small> gyr. B <small>(β-chain, mw 45Kd) activity inhibited by coumestrolin + novobiocin)</small>	?	?
5. Covalent intermediate with DNA	AT 3'-P	subunit A tyrosine covalent bond to 5'-P	AT 3'-P	AT 5'-P
6. Relaxation	Mg ⁺⁺ Required; ATP independent	ATP independent	NO Mg ⁺⁺ Requirement ATP independent	ATP dependent
7. ATP Requirement	NO	Yes	NO	Yes
8. DNA dependent ATPase	NO	Yes	NO	Yes
9. Negative Supercoiling	None	ATP dependent	None	None
10. Functions	Catalyzes relaxation of -ve supercoiled DNA	Introduces -ve supercoiling (gyrase) Decatenates linked circle (topo IV)	Relaxes both -ve & +ve supercoiled DNA (Topo I) Relaxes -ve super coiling weakly no decatenation (Topo III)	Relaxes +ve & -ve super coiled DNA (Topo II & IV)
11. Examples	Topo I (w protein) Topo II	Gyrase (Topo II) Topo IV	Topo I Topo III	Topo II Topo III

Organization of Replication fork in Eukaryotes

Semi-discontinuous DNA Synthesis (Eukaryotes)





Replication Fork Proteins

Model for a dimeric DNA polymerase at the replication fork