SOS in Biochemistry, Jiwaji University, Gwalior
M.Sc. II Semester (2019-20)
Paper BCH 201: Fundamentals of Molecular Biology (Unit 1)

ENZYMOLOGY

of

DNA REPLICATION - IV

Reverse Transcriptase

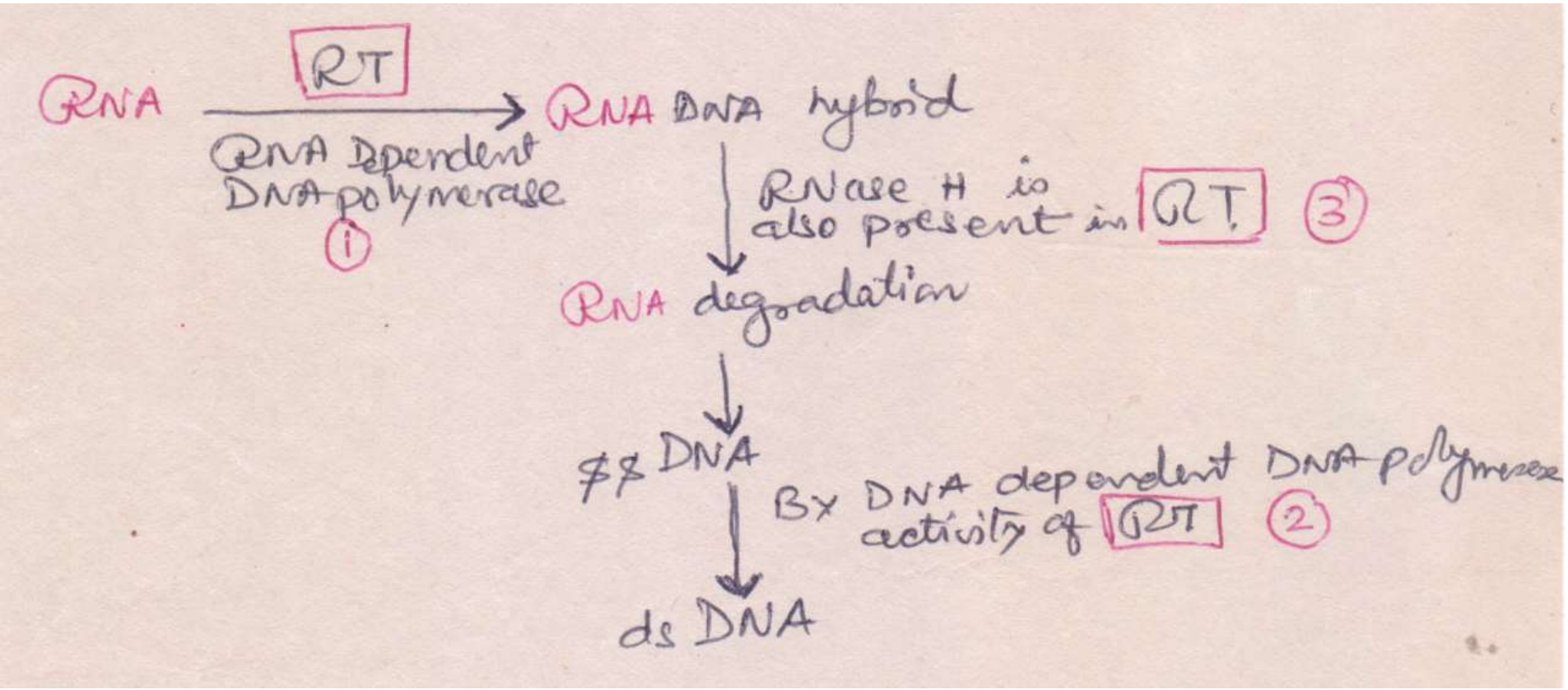
Reverse Transcriptase
(RNA-dependent DNA polymerase)

- discovered by Temin & Mizutani (1970) in Rous Sarcoma virus (Nature, 226: 1211 (1970))

  &

  by Baltimore, D. (1970) in Rauscher Mouse leukemia virus (Nature, 226: 1209, 1970))

- Spiegelman et al in 1970 (Nature, 228: 430) confirmed the presence in other viruses.


RNA
RT
RNA Dependent DNA polymerase
(1)
RNA DNA hybrid
RNase H is also present in RT
(3)
RNA degradation
ss DNA
By DNA dependent DNA polymerase activity of RT
(2)
ds DNA

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	Consists of two polypeptide chains that carry both a polymerase activity & a powerful RNase H activity High activity of RNase H suppress the yield of cDNA and also restrict its length	Has a single polypeptide chain (Mr = 84 kD) and has a polymerase activity but a comparatively weak RNase H activity 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
2	Work efficiently at 42°C RNAs rich in secondary structures are copied more efficiently than the murine	Work efficiently at 37°C Murine RT gets inactivated at 42°C
3	Can be contaminated with an endonuclease that cleaves DNA	Free of contamination of any endonuclease
4	pH Optimum = 8.3 (Change of pH by 0.2 with change	pH Optimum = 7.6 the rate of synthesis of cDNA)

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.

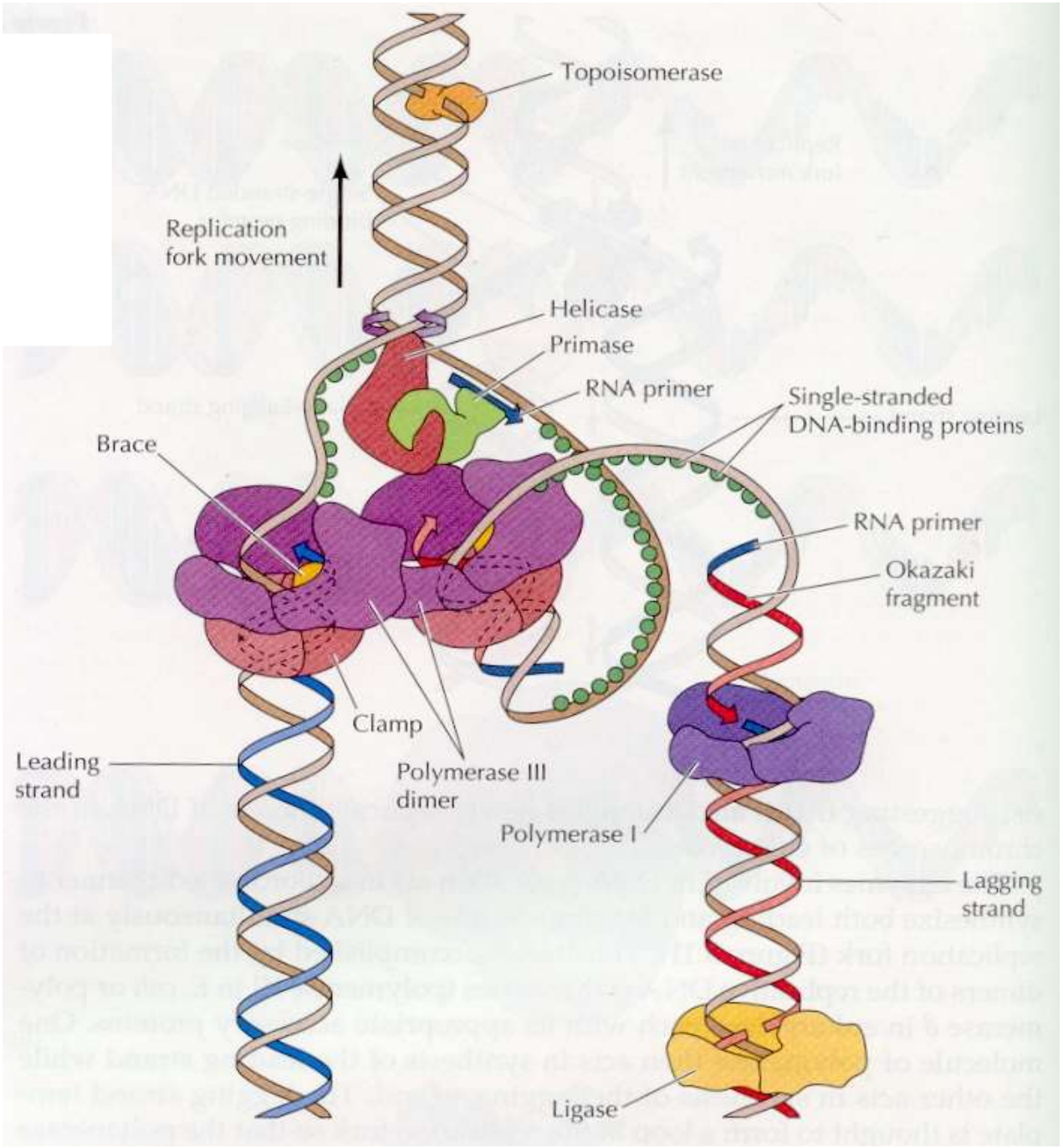
Single Stranded Binding Protein

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

	E. coli	Eukaryotes
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	• stabilizes single stranded regions during replication, recombination and repair • Directs priming of to origin of M13-related genome • Associate with PriB in primosome complex (Possible priming function)	• Stabilizes single stranded regions during replication, recombination > repair • Interacts with pol α: primase to prevent non specific priming events • Interacts with transcription factors, repair protein XP-A & several helicases (possible specific roles in transcription & repair)
Properties	ss DNA-Specific but no sequence specificity; cooperative binding	• ss DNA specific, partial sequence specificity. • Activity modulated by phosphorylation
Genes	ssb	RP-A1, RP-A2, RP-A3

DNA Topoisomerases

(EC 5.99)


Topoisomerase
Replication fork movement
Helicase
Primase
RNA primer
Single-stranded DNA-binding proteins
Brace
RNA primer
Okazaki fragment
Clamp
Leading strand
Polymerase III dimer
Polymerase I
Lagging strand
Ligase

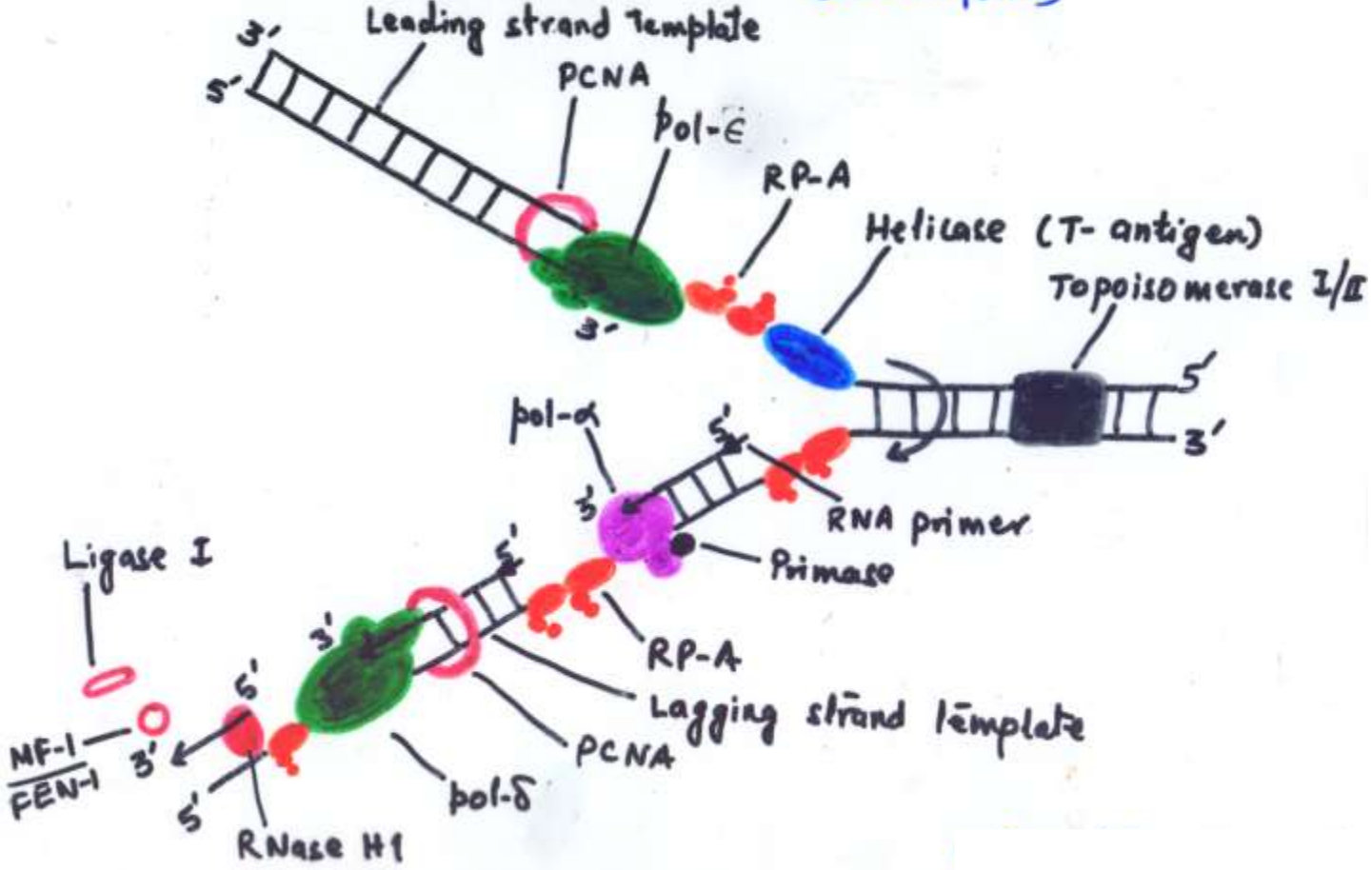
TOPOISOMERASES

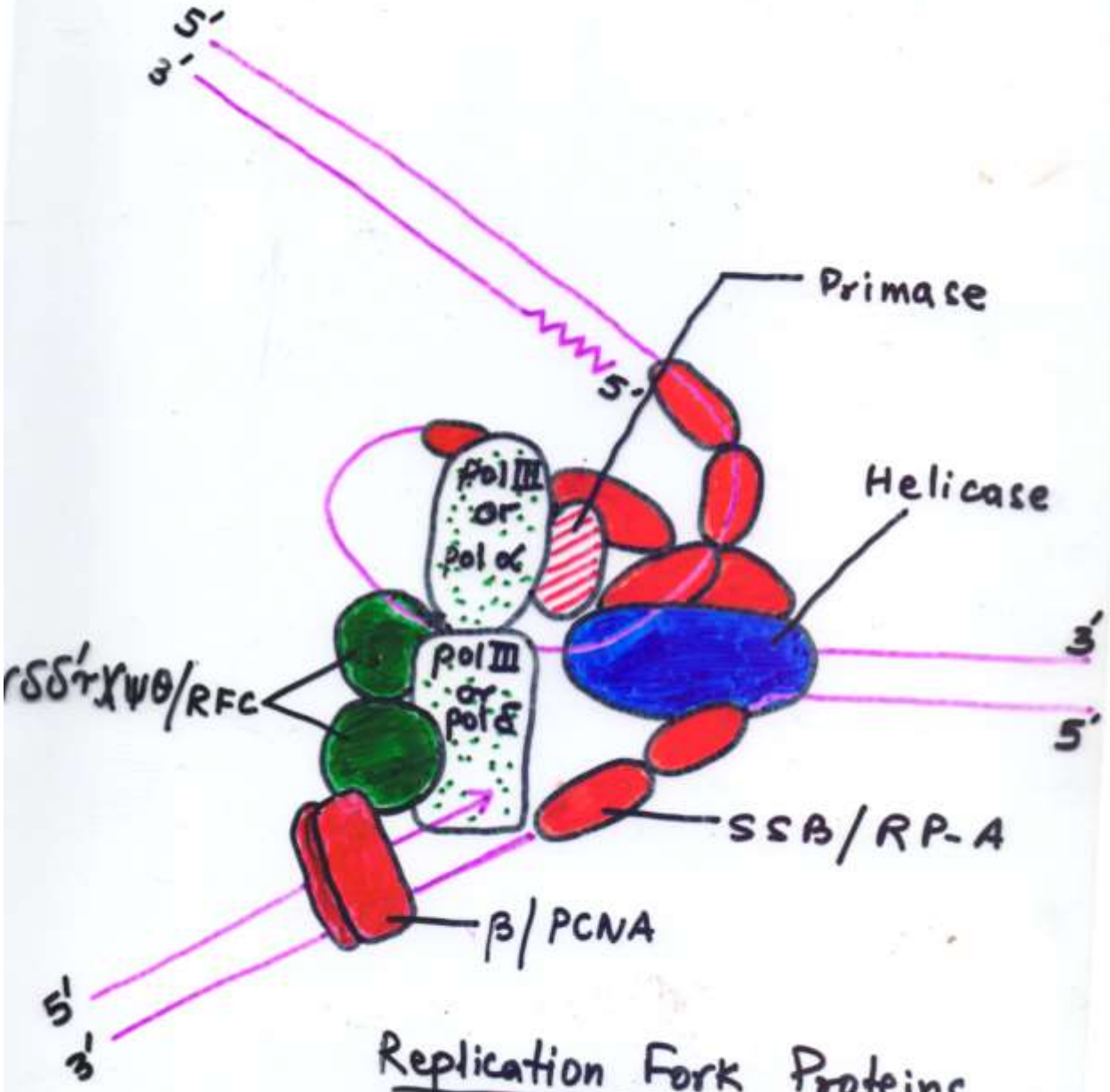
	PROKARYOTIC		EUKARYOTIC	
	Type I	Type II	Type I	Type II
1. MW (Kd)	100	400	100	309
2. Sub unit	Monomer	Tetramer (A_2B_2)	?	Dimer (Subunit MW 132 kD)
3. DNA strand cleaved	one	two	one	two
4. Gene	Top A	gyr A (A-chain, MW 105 kD activity inhibited by nalidixic acid & oxolinic acid) gyr B (B-chain MW 95 kD activity inhibited by coumermycin & novobiocin)	?	?
5. Covalent intermediate with DNA	At 3'-P	Sub unit A tyrosine covalent bond 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 (ω protein) Topo III	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