Lecture 7 DNA REPLICATION

1. A double helix separate into two single strands and each strand serves as a template on which complementary strand is synthesized.

2. A mechanism is required to separate the strands locally for replication.

3. A mechanism is required to release the strain created by local unwinding.

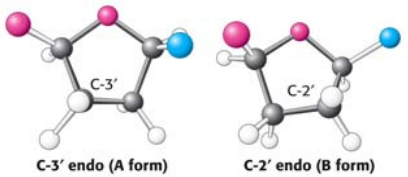
4. A mechanism is required to account for the high fidelity of duplication, 1 per 10^{10} nt.

5. A mechanism is required to account for the speed of replication; 2000 nt per sec in *E.coli*.

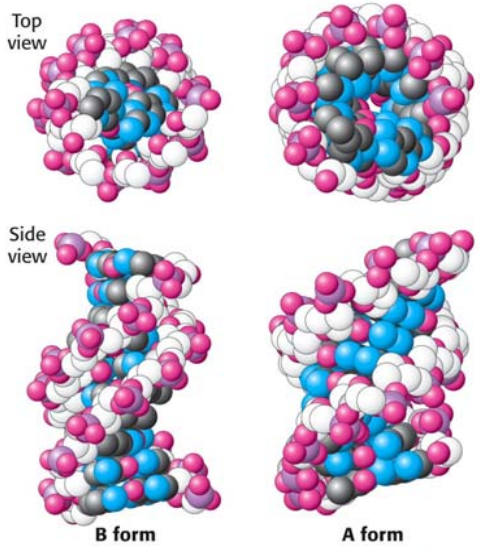
6. Direction of the replication?

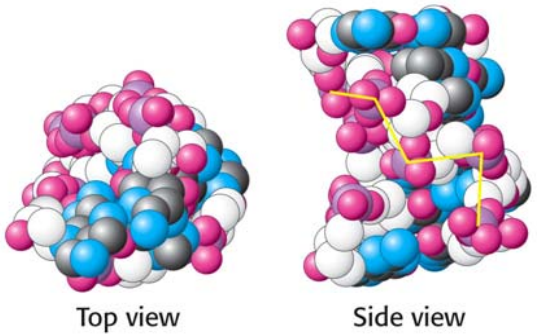
7. How to replicate the ends of linear DNA?

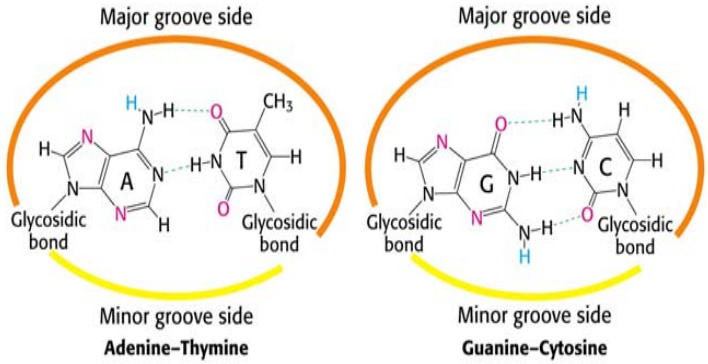
DNA double helix structures

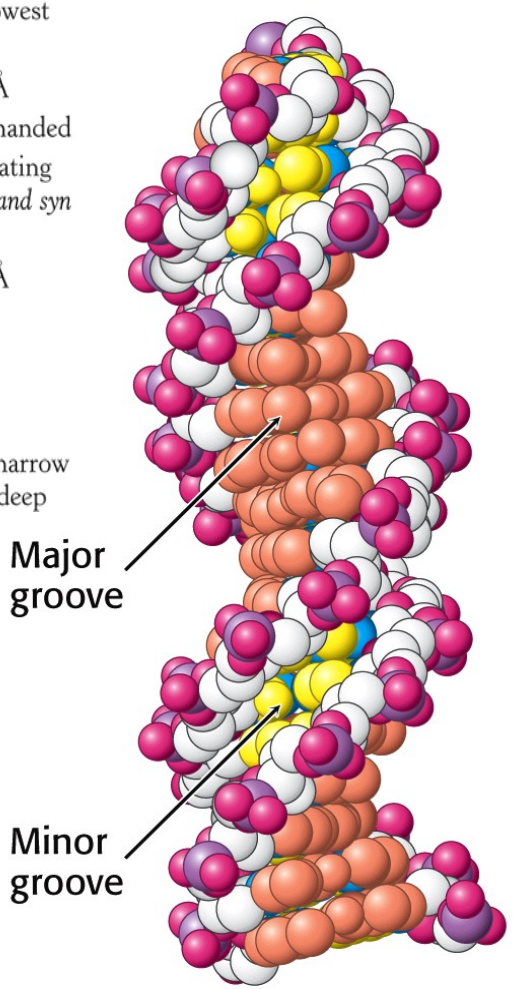


	A	B	Z
Shape	Broadest	Intermediate	Narrowest
Rise per base pair	2.3 Å	3.4 Å	3.8 Å
Helix diameter	25.5 Å	23.7 Å	18.4 Å
Screw sense	Right-handed	Right-handed	Left-handed
Glycosidic bond	*anti*	*anti*	alternating *anti and syn*
Base pairs per turn of helix	11	10.4	12
Pitch per turn of helix	25.3 Å	35.4 Å	45.6 Å
Tilt of base pairs from normal to helix axis	19°	1°	9°
Major groove	Narrow and very deep	Wide and quite deep	Flat
Minor groove	Very broad and shallow	Narrow and quite deep	Very narrow and deep

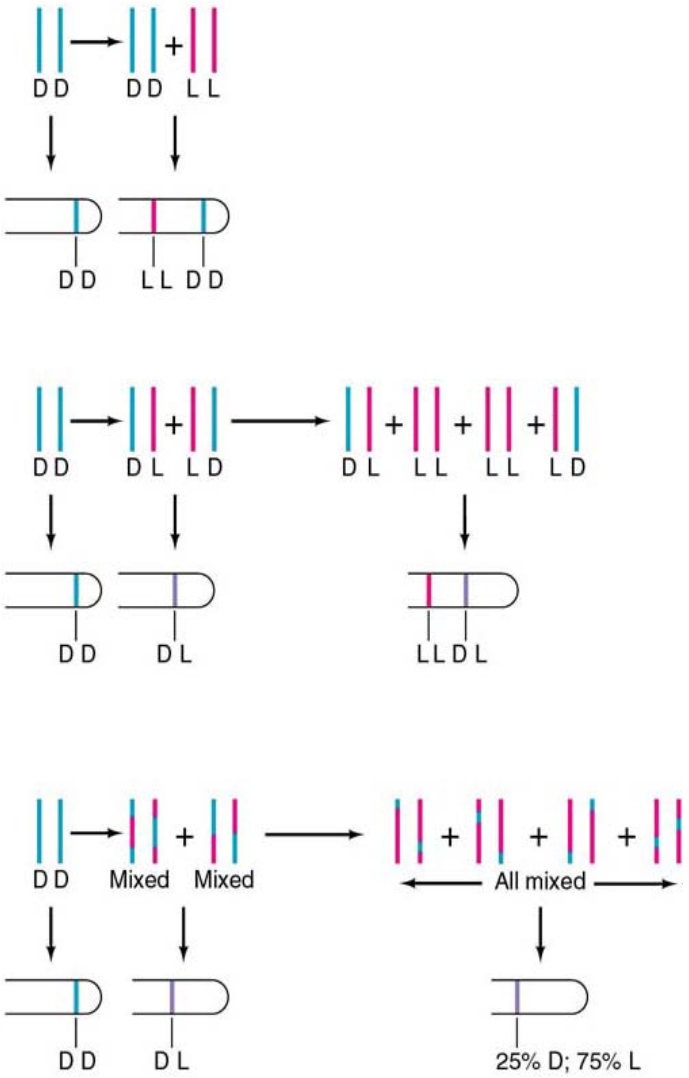




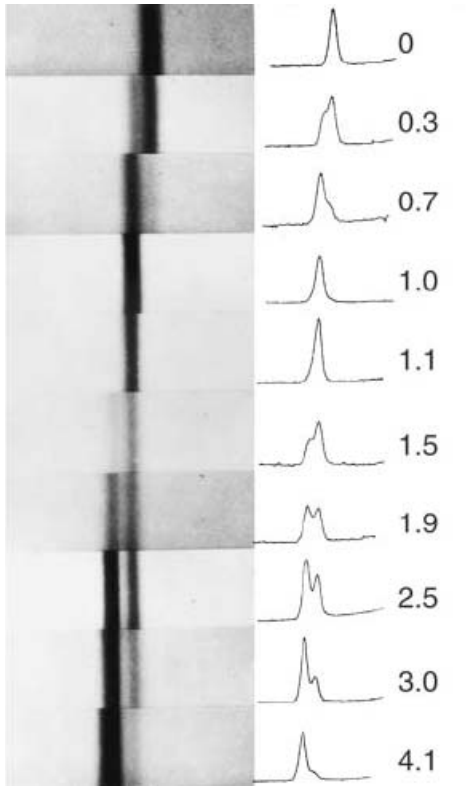


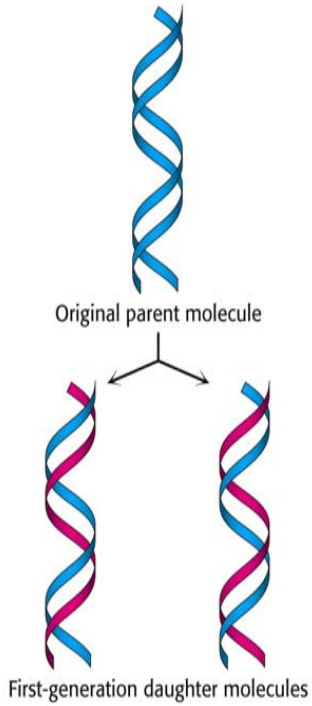


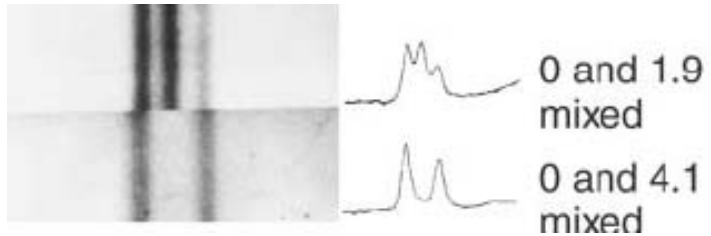
Semiconservative replication



Meselson Stahl Experiment

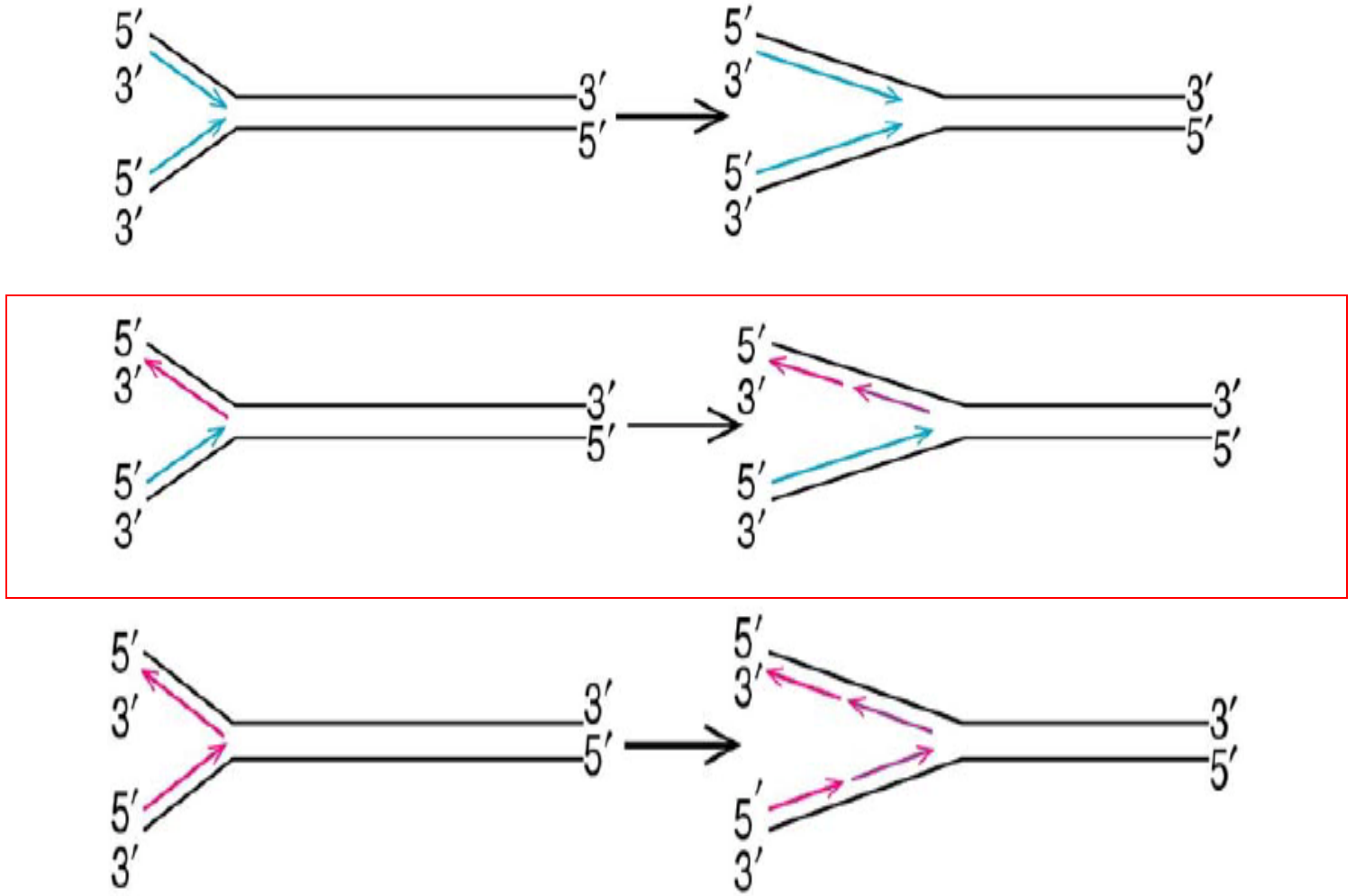






Three replication models: conservative, semiconservative, and random disperse models

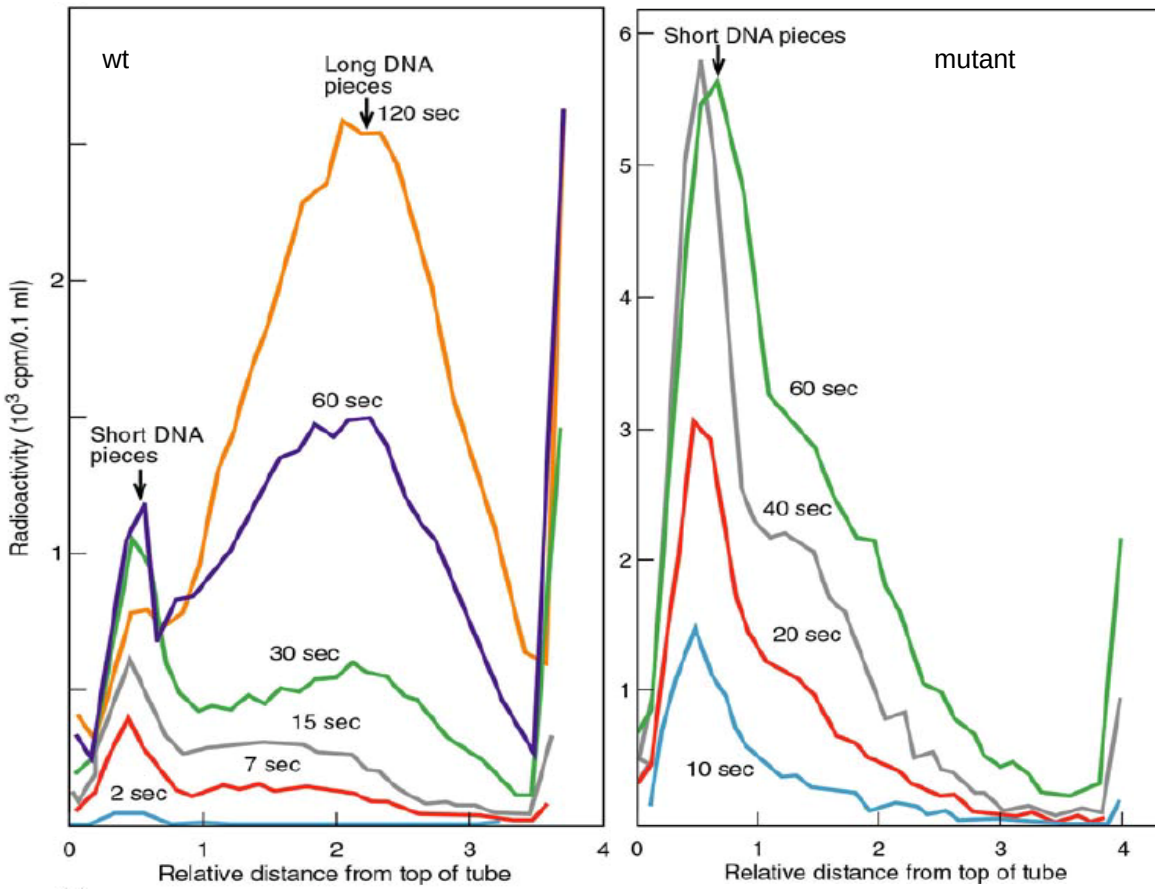
Replication fork



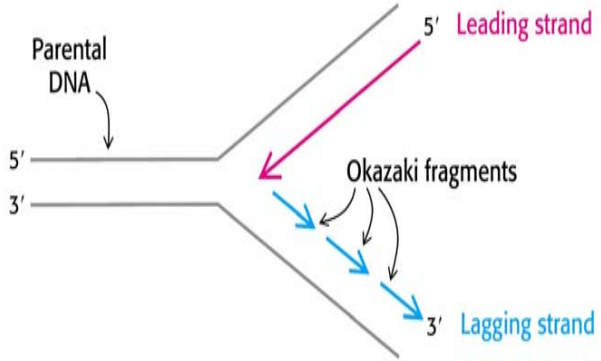
Three models: continuous, semidiscontinuous, and discontinuous.

Replication fork

Reiji Okazaki's predictions and experiments



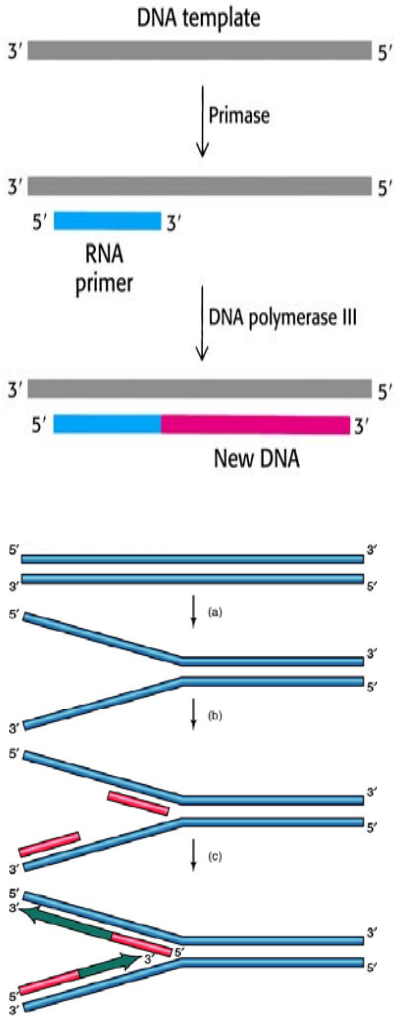
Semidiscontinuous model



Reiji Okazaki's experiments: at least half of the newly synthesized DNA appears as short pieces(1000-2000 nt); if no ligation, short pieces will accumulate. Replication of T4 phage DNA in E. coli, wild type vs. ligase mutant, with ^{3}H-thymidine pulse labeling (when ung- *E. coli* mutant was used, >50% of newly labeled DNA was still in short pieces).

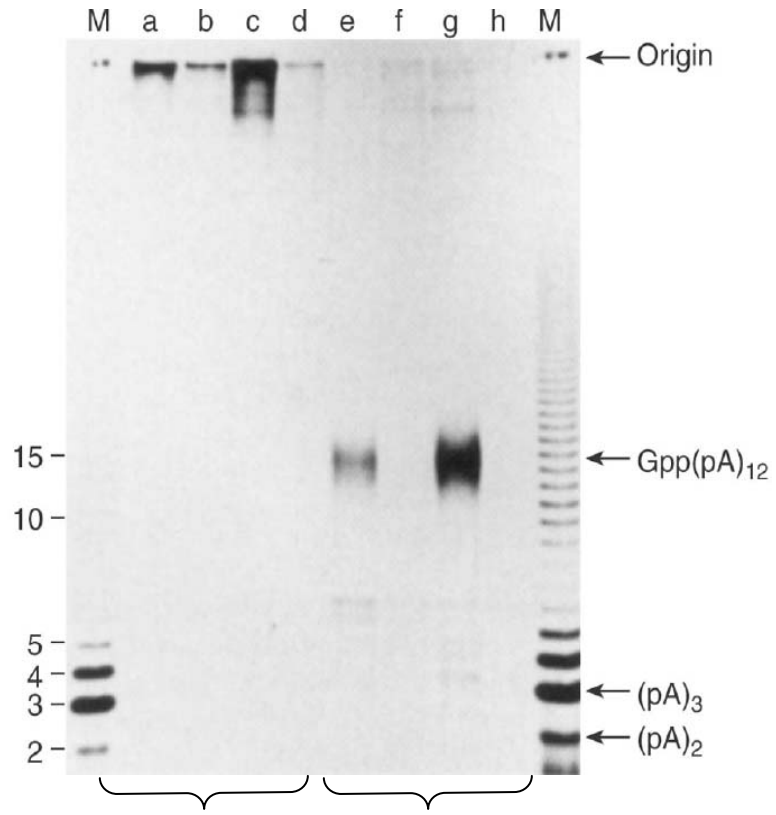
at least half of the newly synthesized DNA appears first as short pieces(1000-2000 nt);

Priming



DNA pol needs s short RNA primer

Tuneko Okazaki's experiment



Use fragment from normal (d,h) and mutant cells (a-c, e-g) labeled the primer with 32P-GTP.

Lanes a,e, no RNase H;

lanes b,f no nuclease activity;

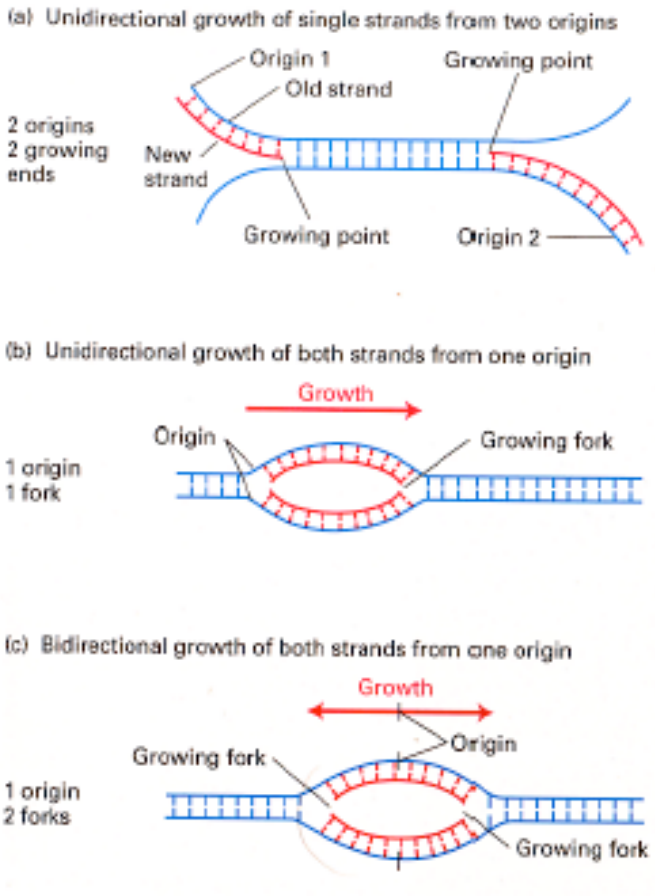
lanes c, g no RNase H and nuclease;

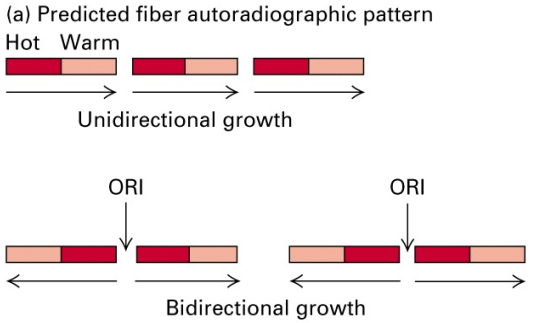
lanes d, h wild type;

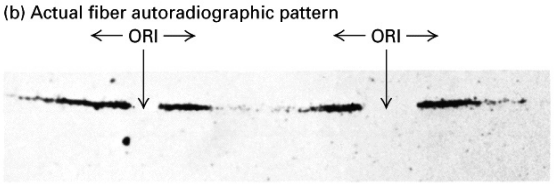
Primer will be removed by a 5’ to 3’ exonuclease activity in DNA polymerase I.

Direction of Replication

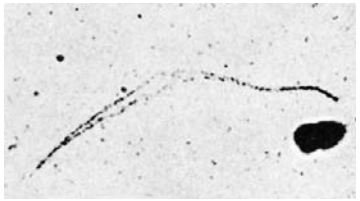
Three possible mechanisms



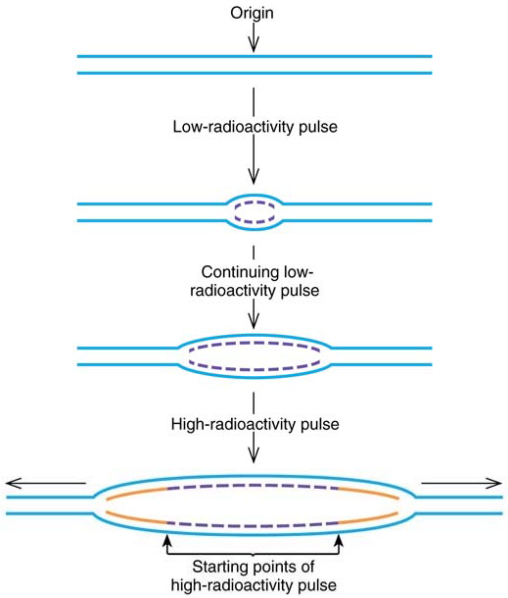




Predicted vs. data (JMB 32, 327, 1968); heavily labeled pulse followed by lightly labeled pulse.

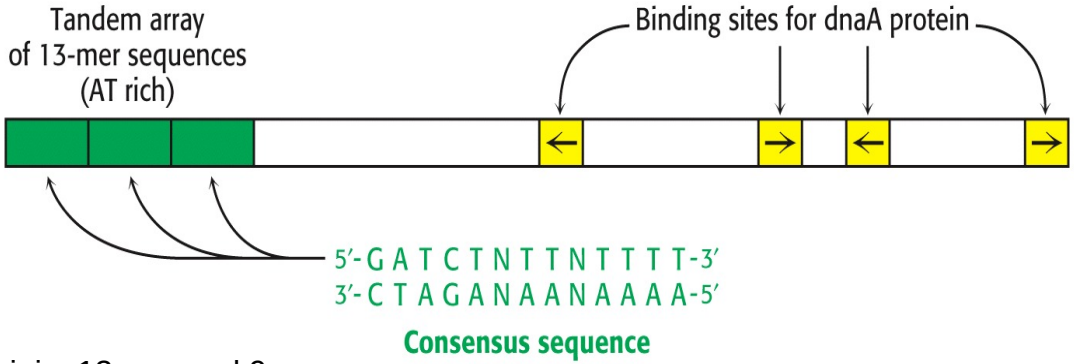


Bidirectional replication in *Bacillus subtilis* DNA (John Cairns, JMB 58, '73): lightly label followed by heavily label;

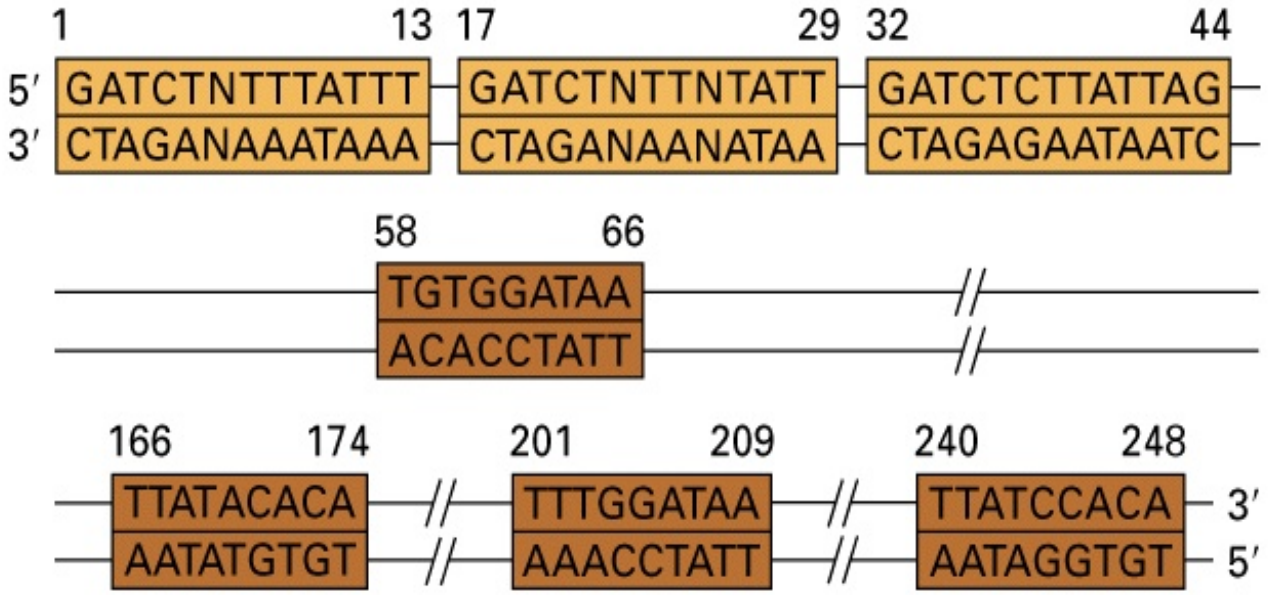


Origin of replication

The consensus sequence of oriC



Minimal bacterial replication origin: 13-mer and 9-mers

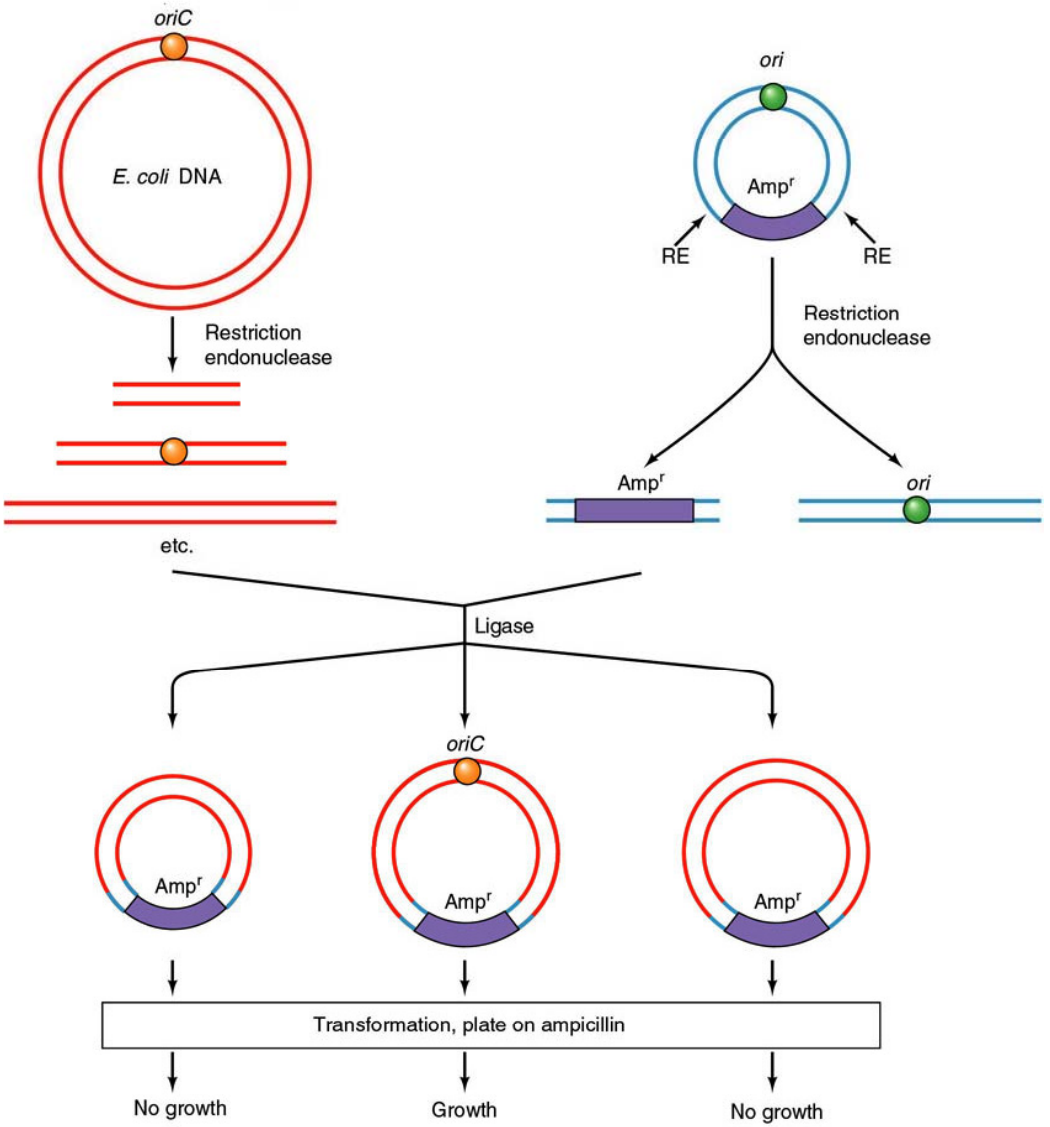


Regardless of organism, replication origins are unique DNA segments with multiple short repeats, recognized by multimeric origin-binding proteins, and usually contain an A-T rich stretch.

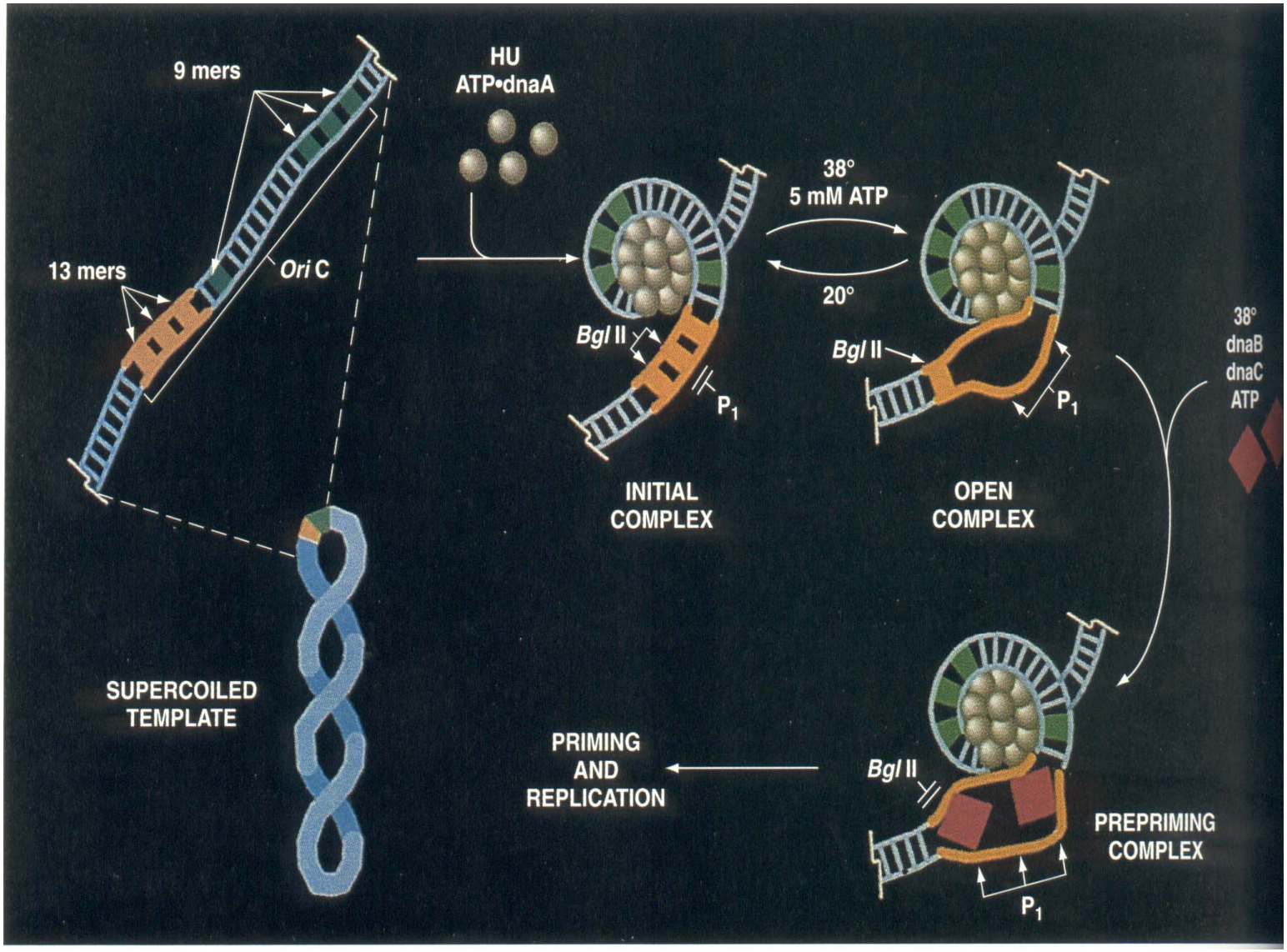
oriC: origin of replication in *E. coli*: *OriC* 245 bp (3 13-nt and dnaA binding sites) in 4.8 m bp genome.

Origin of replication

Construction of an oriC plasmid

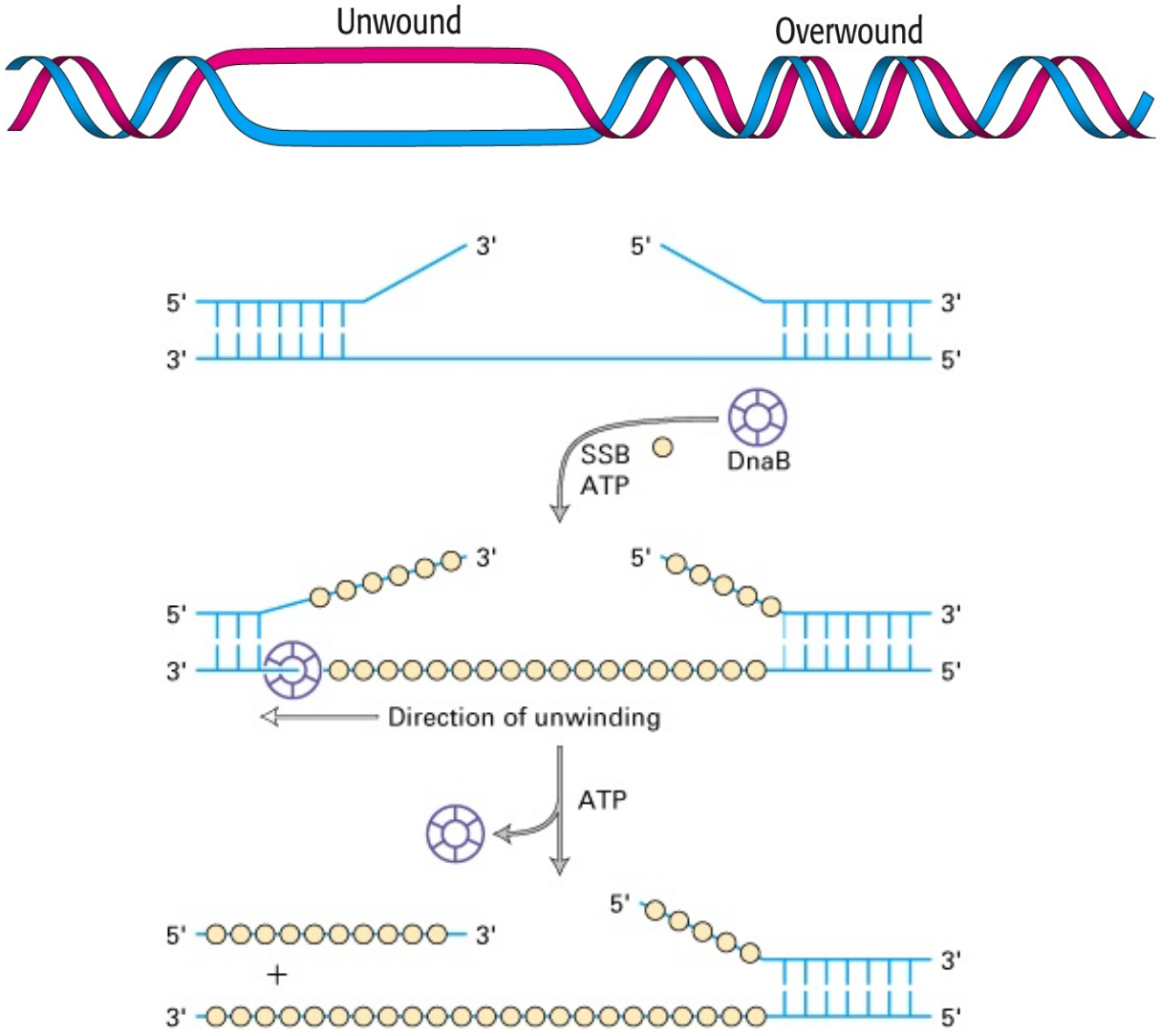


Priming at oriC



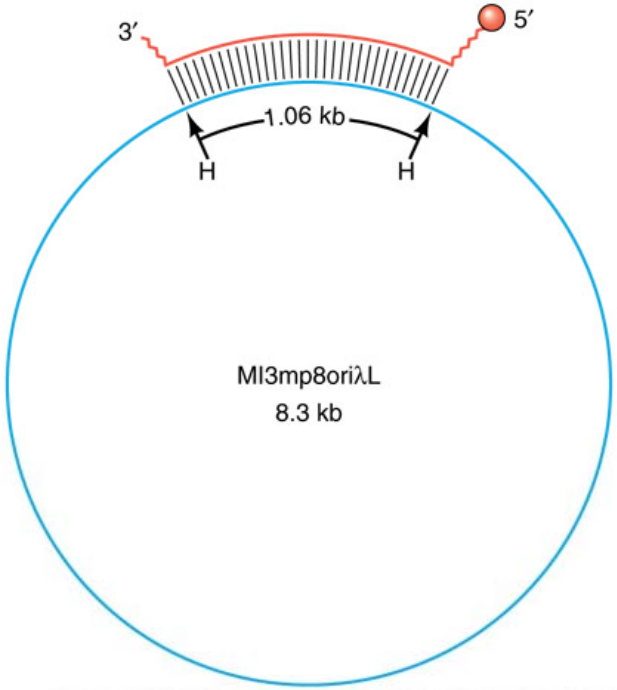
DnaA binds to ATP forming multimers which together with HU bind to the four 9-mers (dnaA boxes), inducing bend and destabilizes the 13-mer repeats and causes local melting, allowing DnaB binding (with DnaC help)

Helicase for unwinding



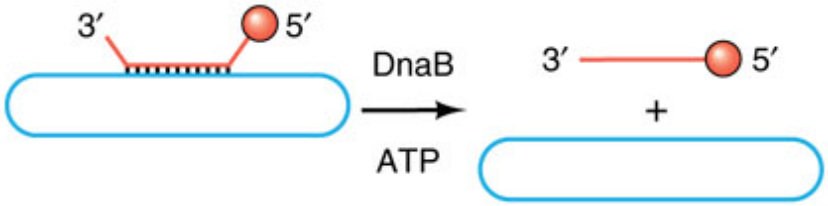
E. coli DnaB helicase: the enzyme will translocate along dsDNA from 3’ to 5’ direction.

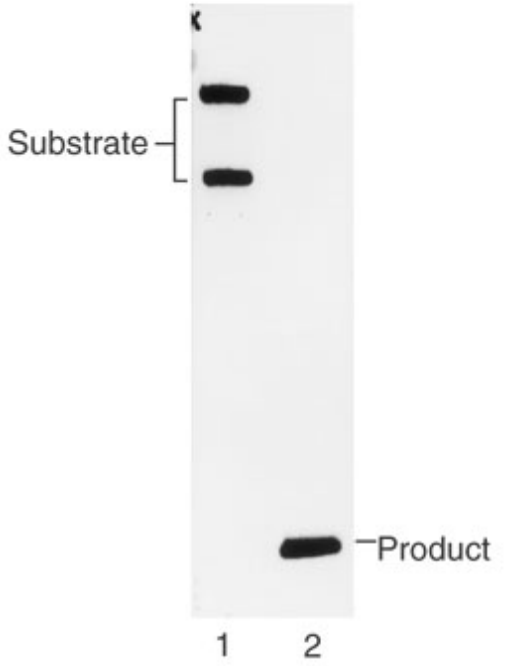
Helicase assay



substrate (labeled 1.06-kb HinCII fragment (red) annealed to M13

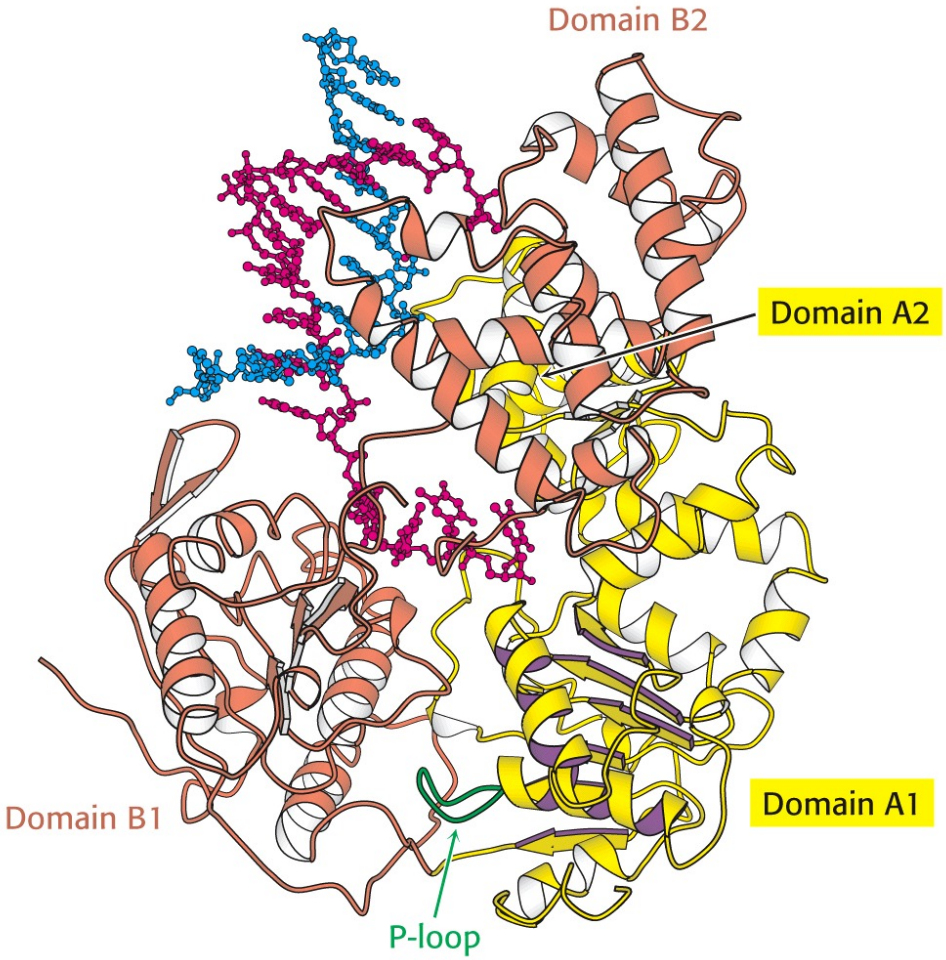
reaction

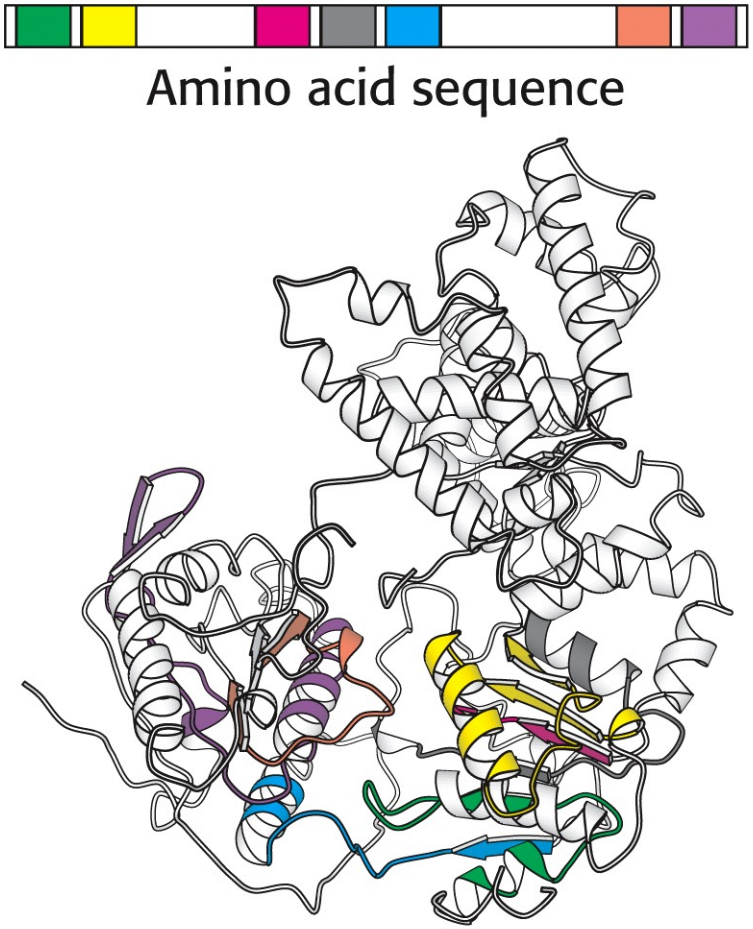




Lane 1, before reaction,
circular and linear form of M13-fragment;
lane 2 after reaction,
JBC 261, 4740, 1986)

Bacterial helicase

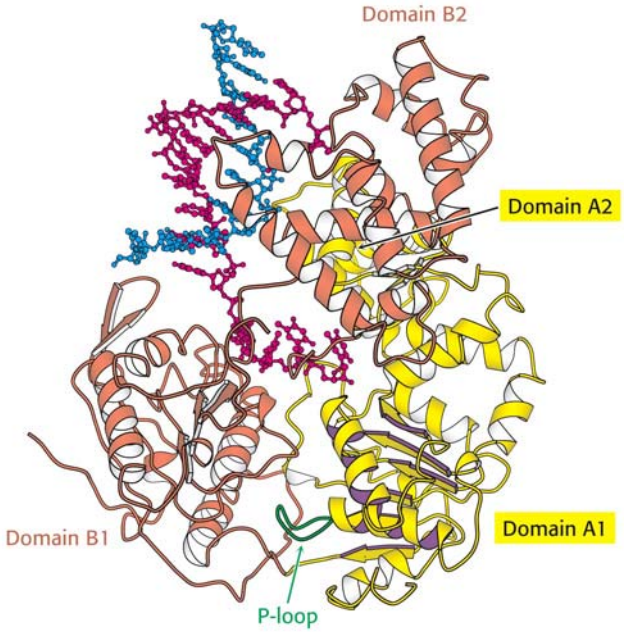




Conserved regions:
A1 and B1 interface and along ATP binding site

Bacterial helicase (PcrA):
A1 with P-loop NTPase fold, B1 similar to A1 without loop

Helicase for unwinding



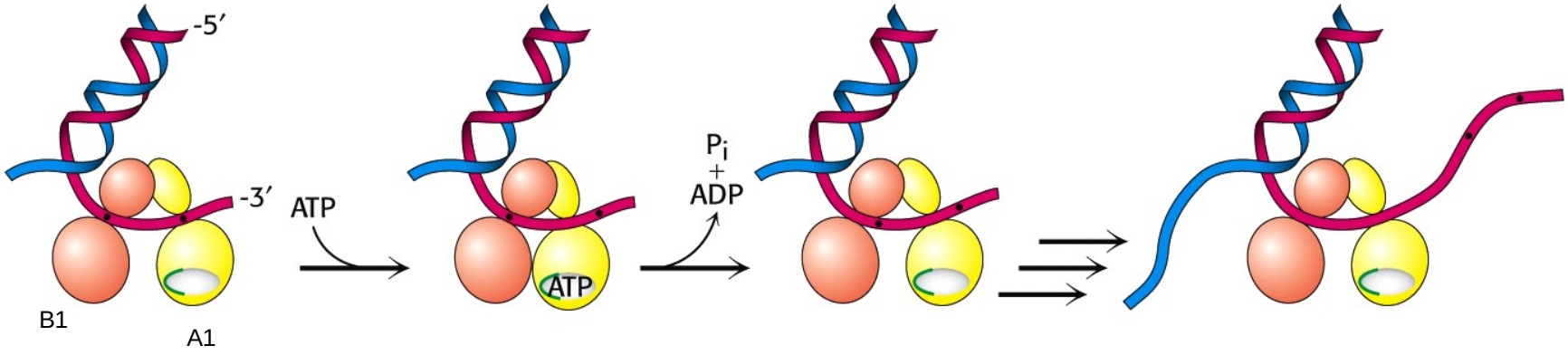
For DNA duplex to replicate, the two strands must be separated from each other,at least locally. Helicase uses ATP energy to perform this strand separation job. Both A1 and B1 bind to ss DNA. ATP hydrolysis leads to cleft closure and sliding of ssDNA. Since A1 has a tighter grip of ssDNA, this causes a net translocation of the enzyme toward the dsDNA

Unwinding mechanism:

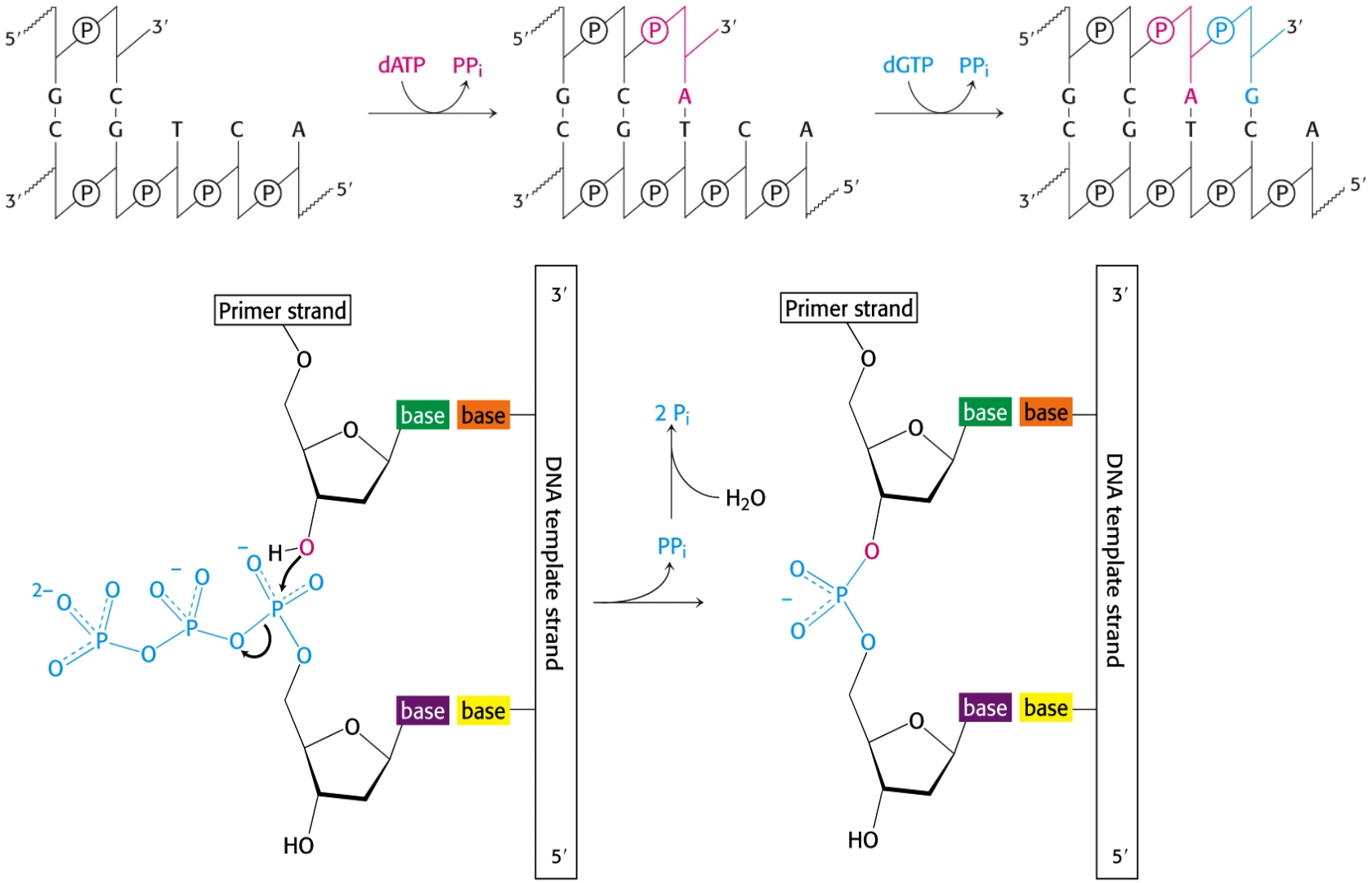
(i) Here the A1 and B1 domain bind to ssDNA.

(ii) Upon ATP binding the cleft between A1 and B1 closes and A1 slides along DNA;

(iii) Upon hydrolysis cleft opens, pulling B1 to A1.

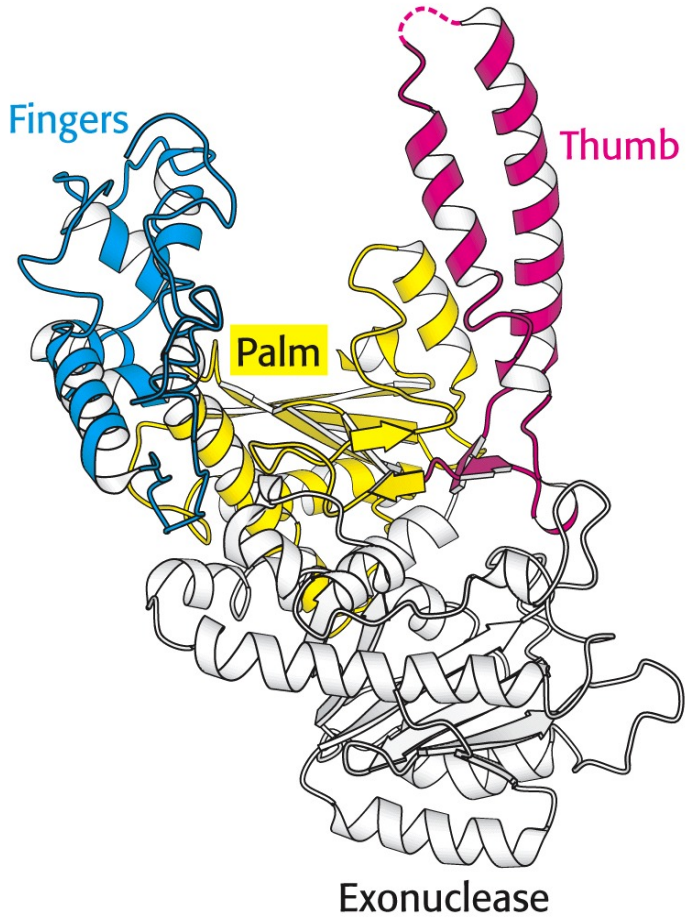


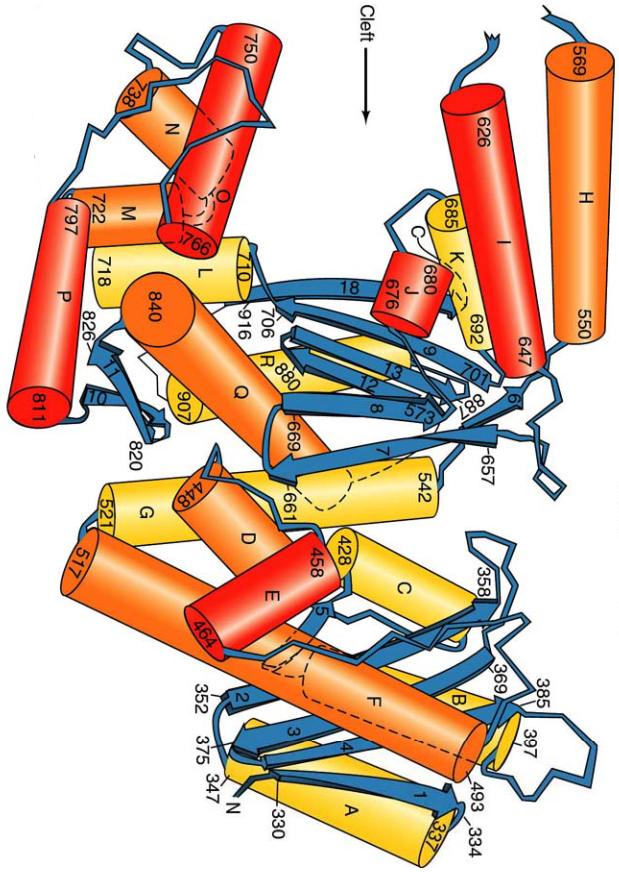
Polymerization



Reaction catalyzed by DNA polymerases and the formation of phosphodiester bond,
DNA polymerase: 5'-p(N)n-3' + dNTP → 5'-p(N)n+1-3'

E. coli DNA polymerase I





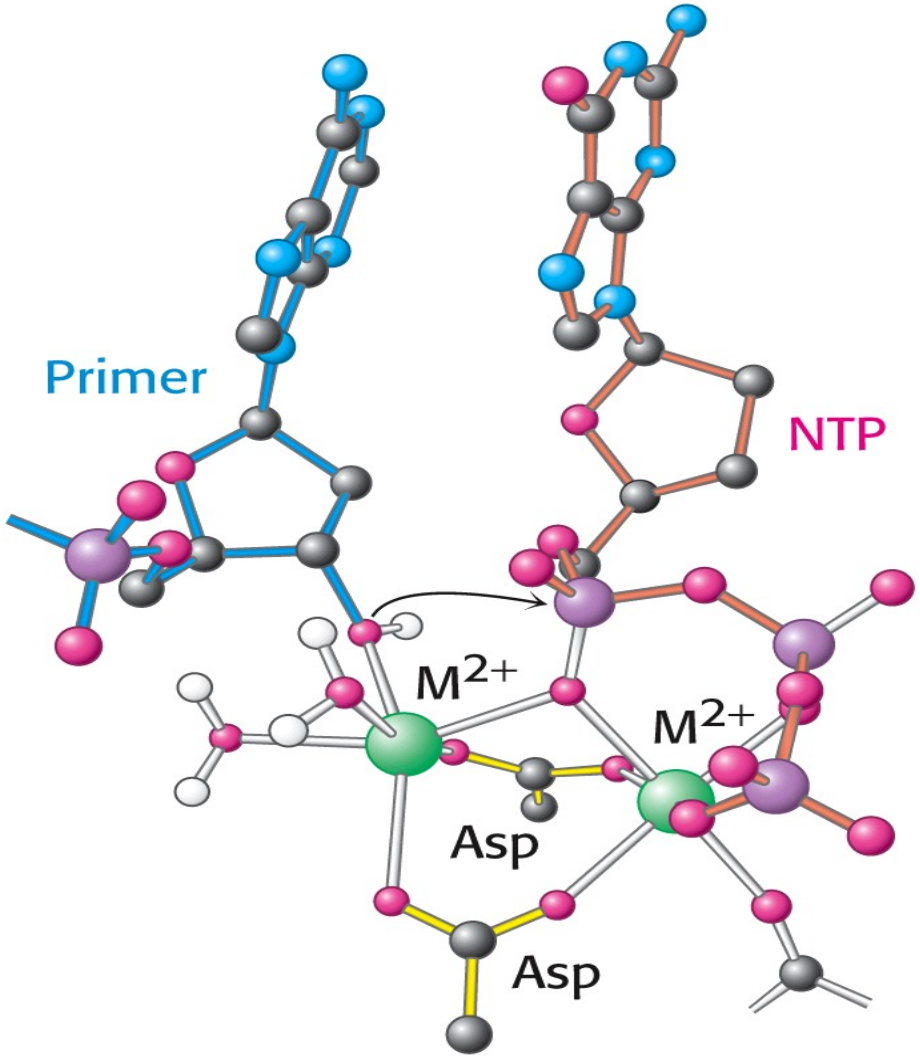
E. coli DNA polymerase I:
102 kD→Klenow fragment (polymerase activity + 3'→5' exonuclease activity) and small domain (5'→3' exonuclease activity); all DNA pol have similar shape, thumb, palm and finger.

DNA pol I= 1 polypeptide (polymerase, 3'-->5' exonuclease proofreading, 5'-->3'exonuclease);

processivity 20, catalytic rate 10 nt/sec

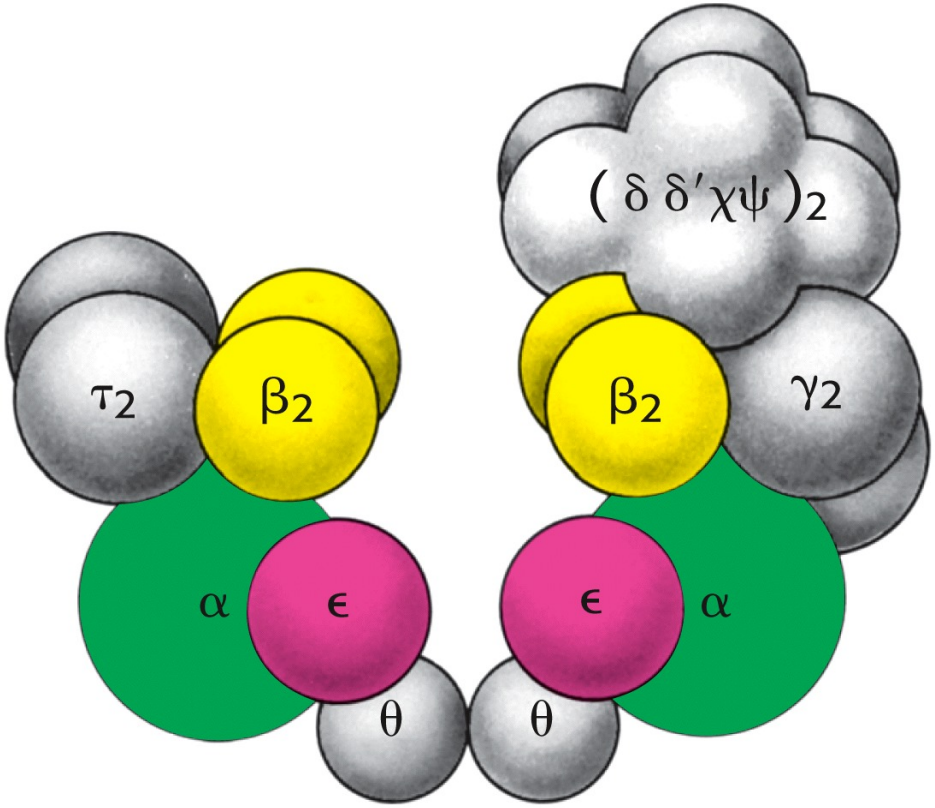
DNA pol III = 10 polypeptides, 900 kD, processivity >5000, rate 1000 nt/sec

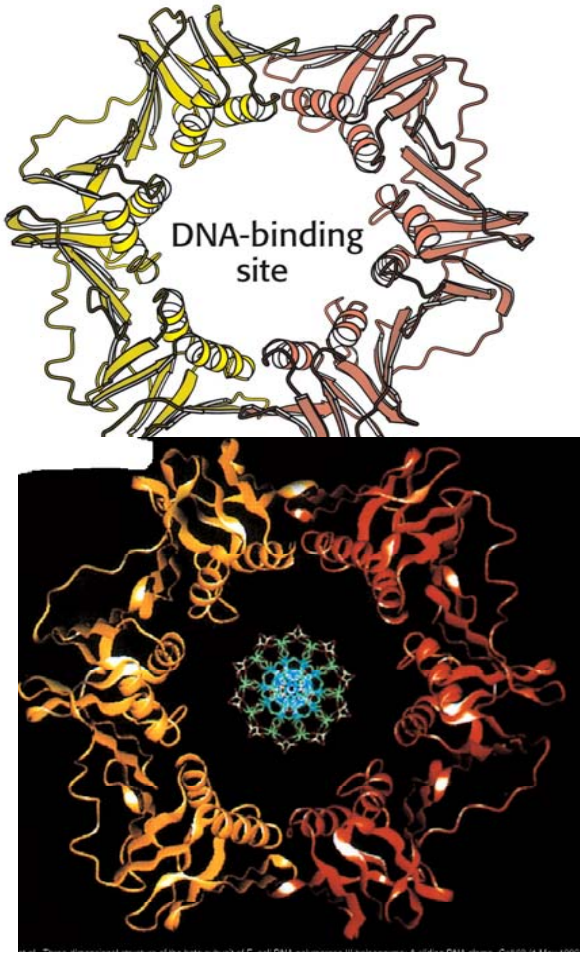
Crucial metals



Two metal ions (Mg or Mn) are crucial to the action of DNA polymerase. One metal coordinates with 3'-OH at the primer whereas the alpha phosphate group from dNTP bridges between two metals

DNA pol III holoenzyme



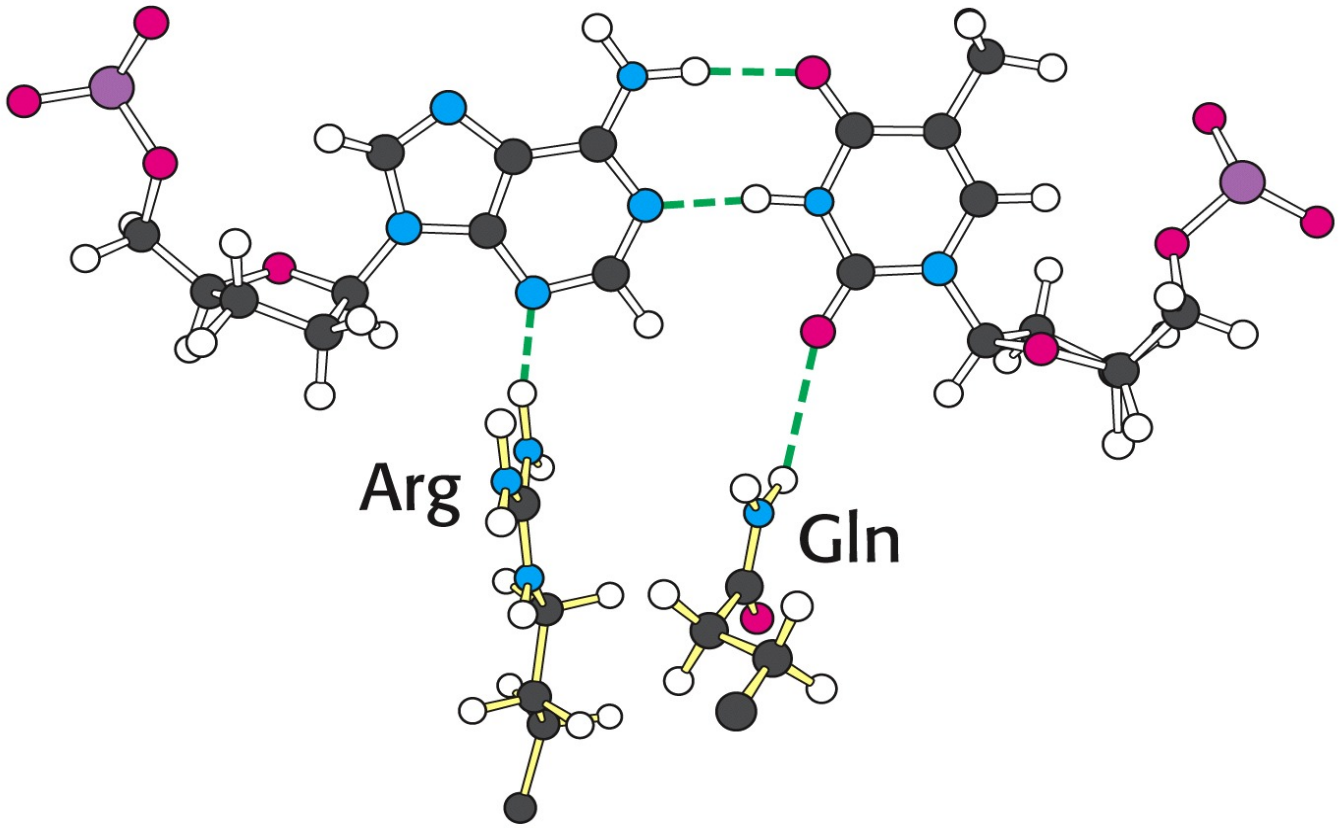


Proposed structure of DNA pol III holoenzyme (900 kD, 10 subunits, asymmetric dimer, one for leading, one for lagging strand (α is polymerase, ε is proofreading 3'→5' exonuclease, β2 and δ2 for processivity). The sliding clamp for processivity is done by β2.

DNA pol I = 1 polypeptide, processivity 20, catalytic rate 10 nt/sec.

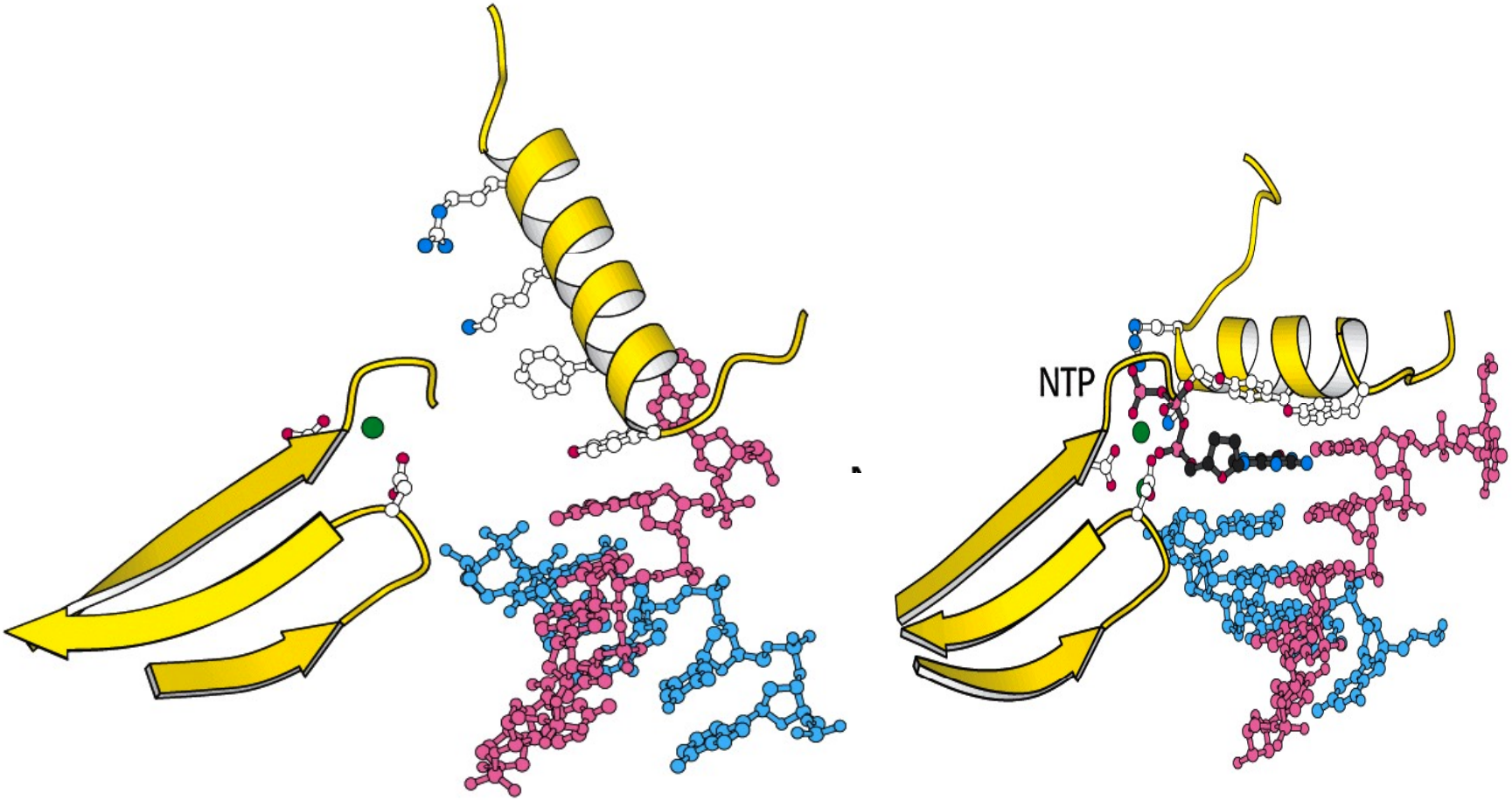
DNA pol III = 10 polypeptide, processivity >5000, rate 1000 nt

Fidelity



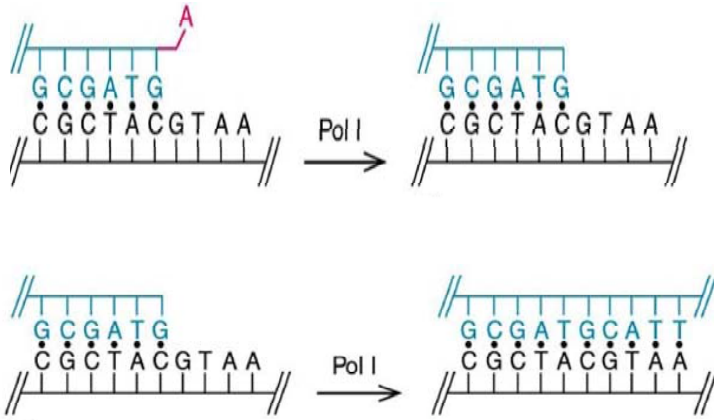
Fidelity of polymerase: R and Q from polymerase serve as a ruler by forming H-bonding at the minor groove of base pair at the active site.

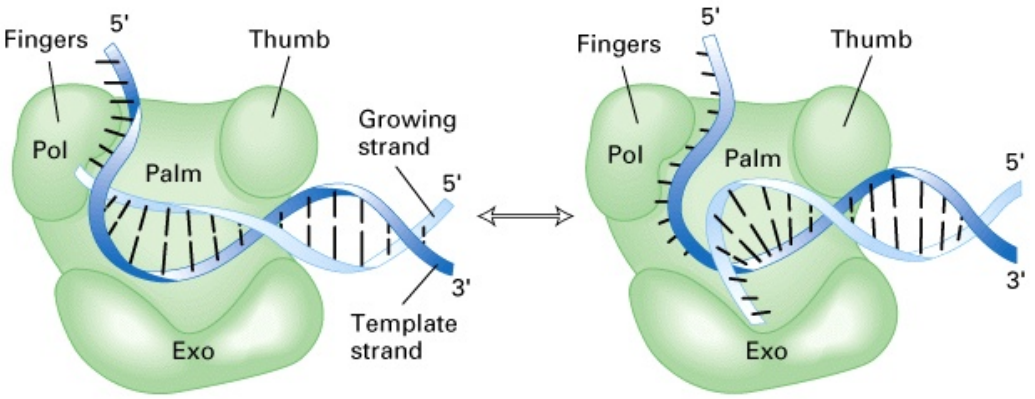
Shape selectivity

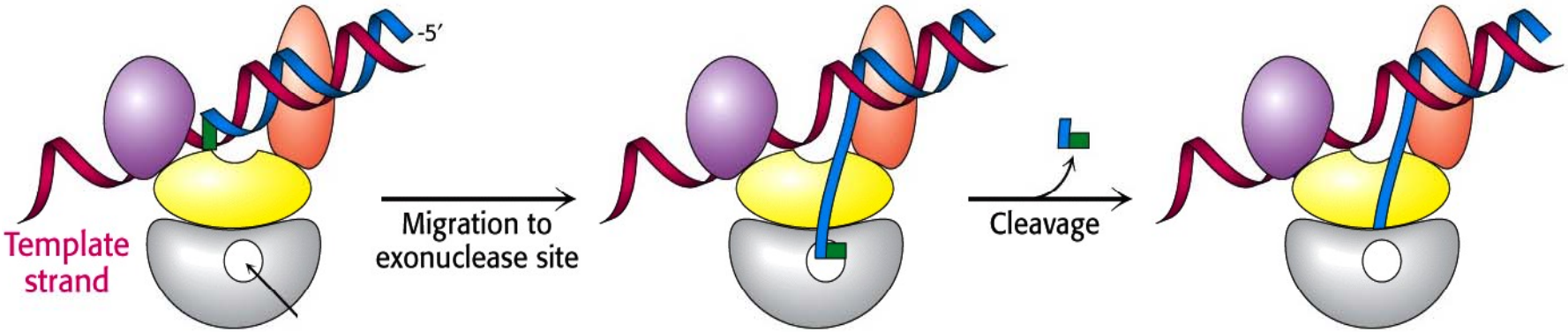


The binding of the correct dNTP induces a conformational change, generating a tight pocket

Proofreading

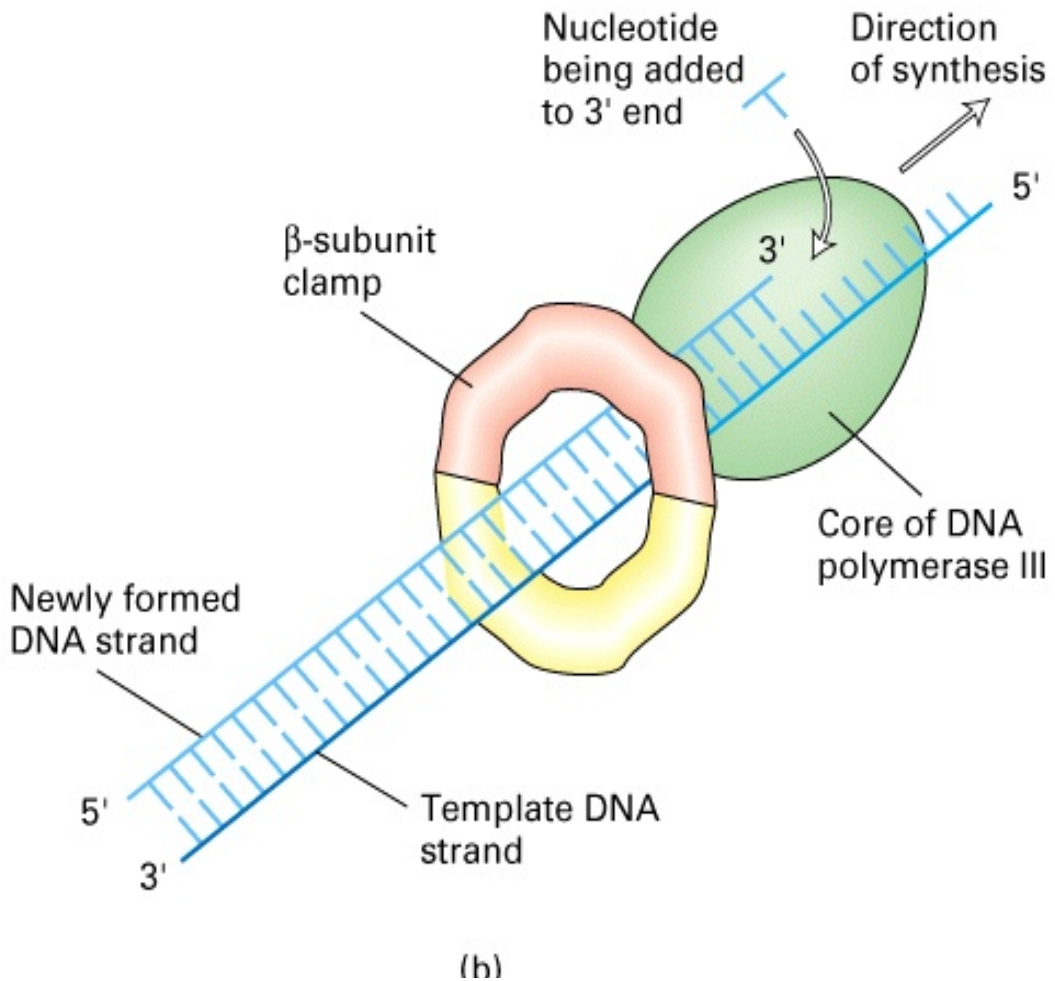


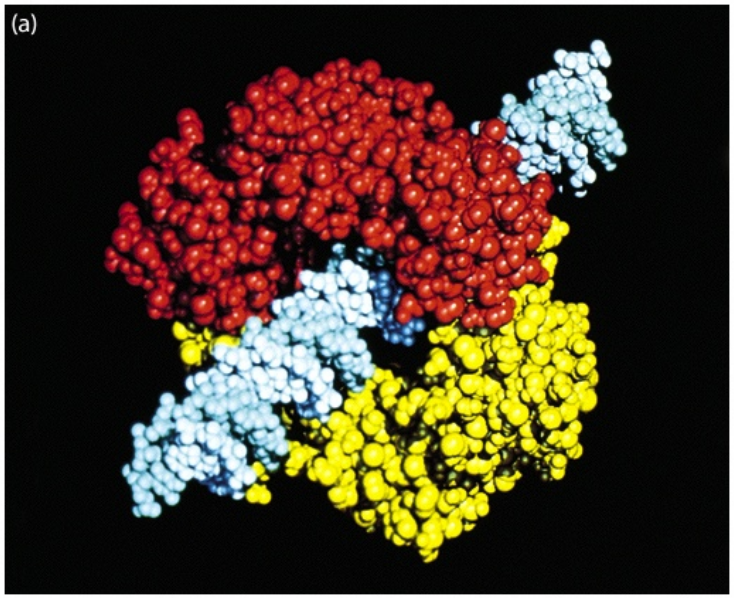




Mismatched base will cause pause or stall and give extra time to excise it.
Mismatched base can leave polymerase site and swing into exonuclease site to be cleaved.
The newly formed duplex in the polymerase site assumes A-form for extensive H-bonding at minor groove.

Processivity



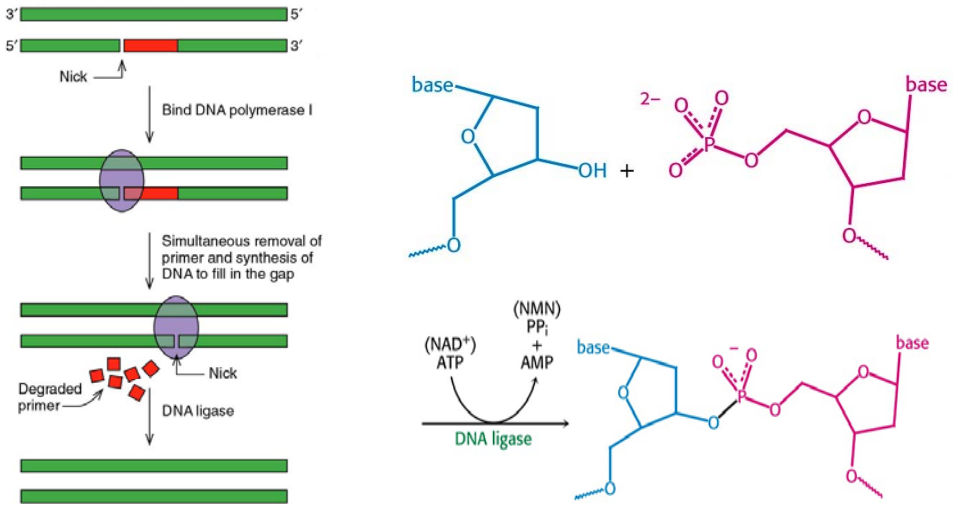


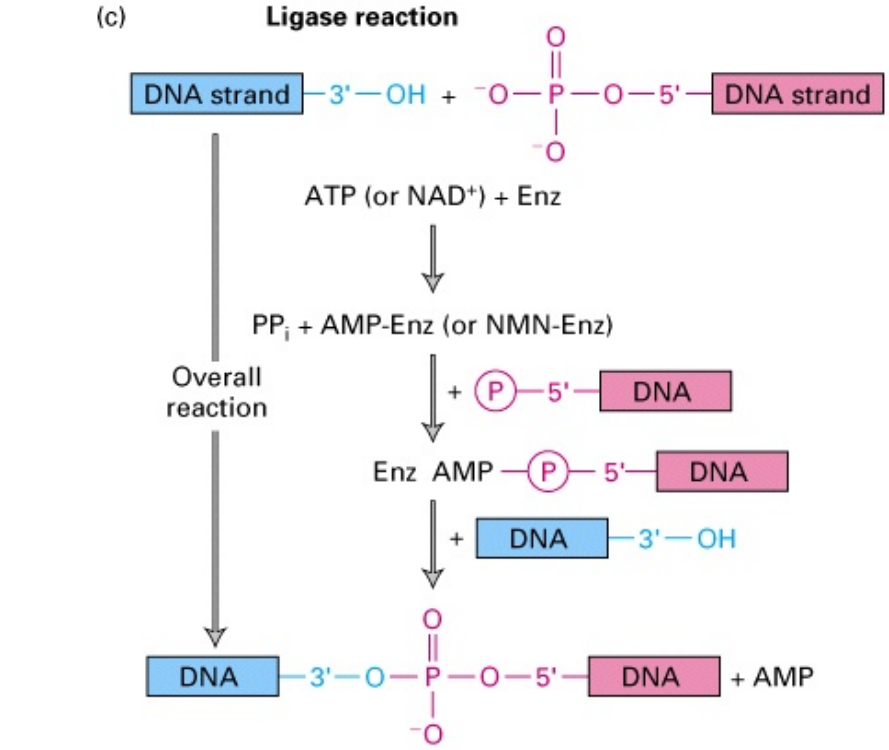
Cell 69, 427, 1992;
84, 643, 1996

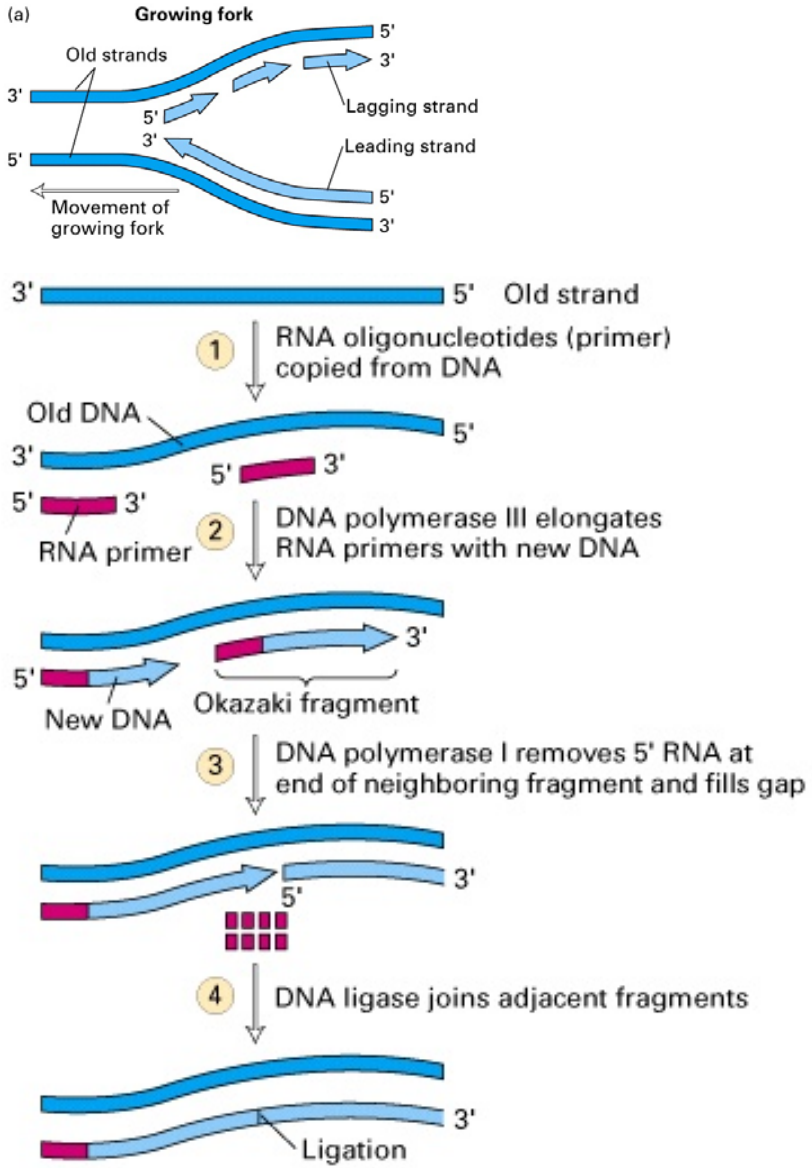
DNA polymerase III has a dimeric structure. is polymerase, β2 and δ2 confer the processivity.

1000 nt added per sec means a sliding of 100 turns of duplex through the central hole of β2 per sec

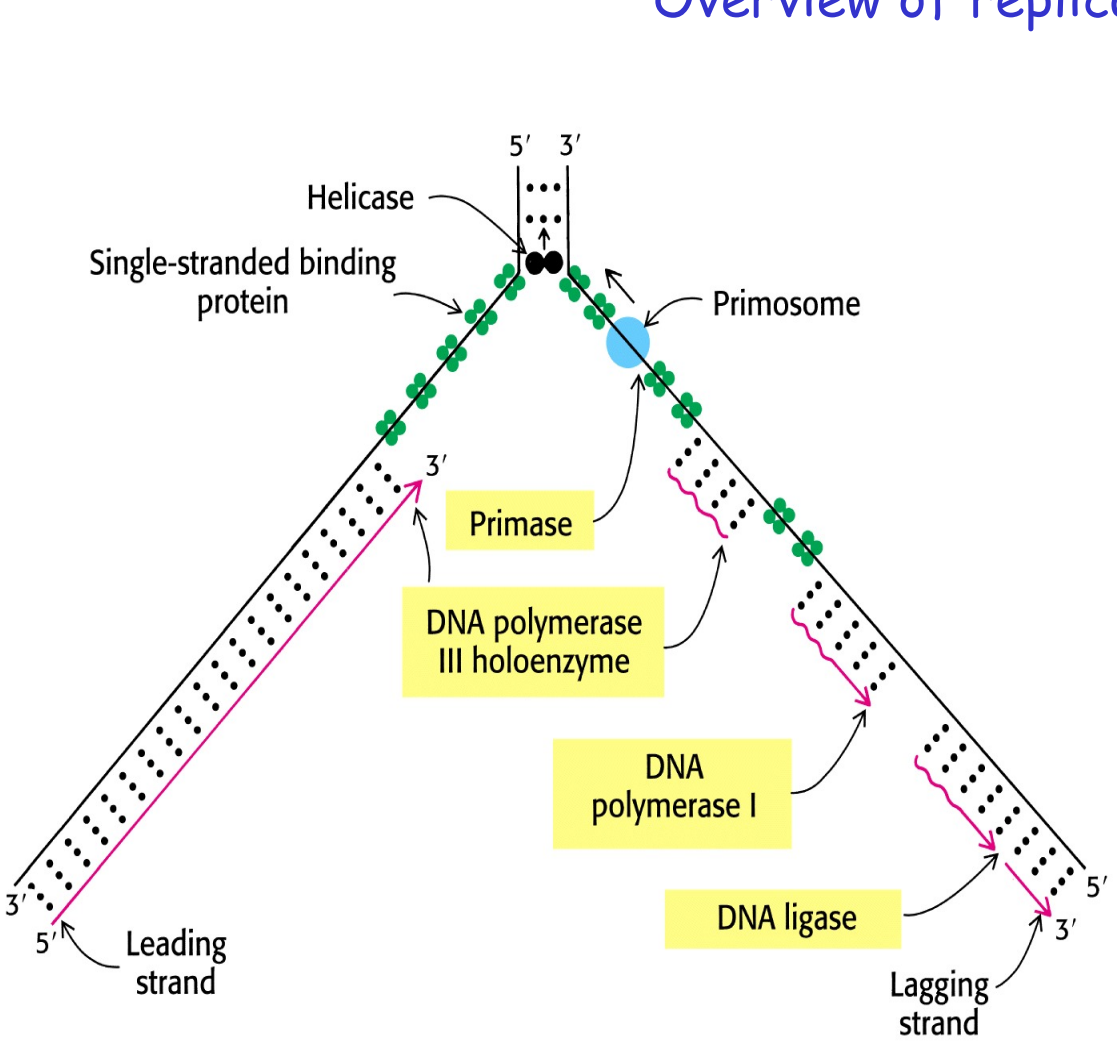
Ligase reaction

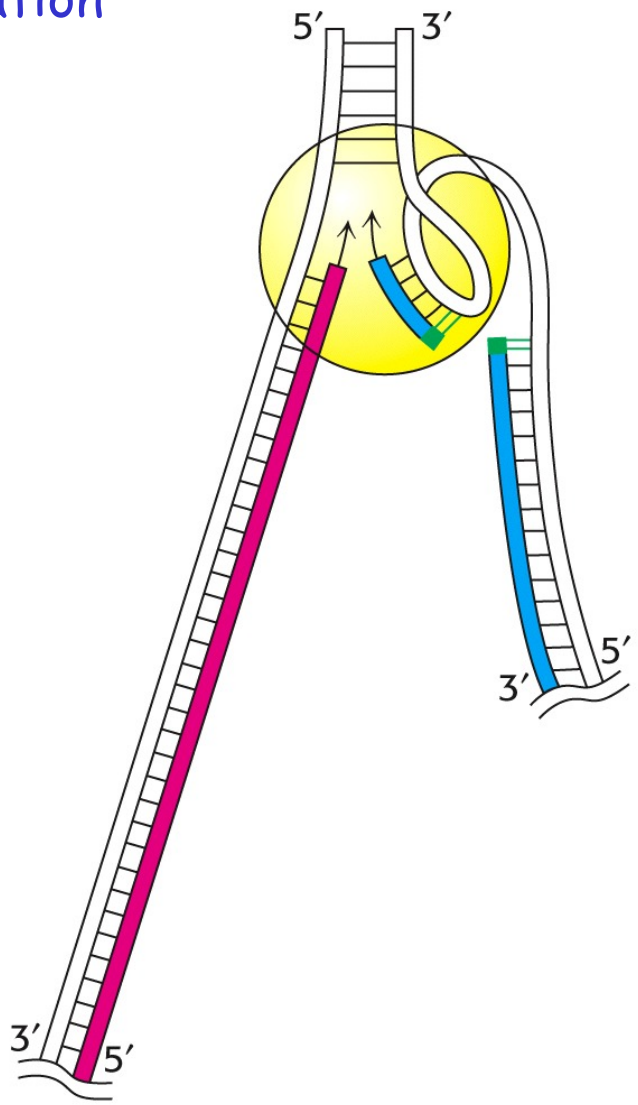






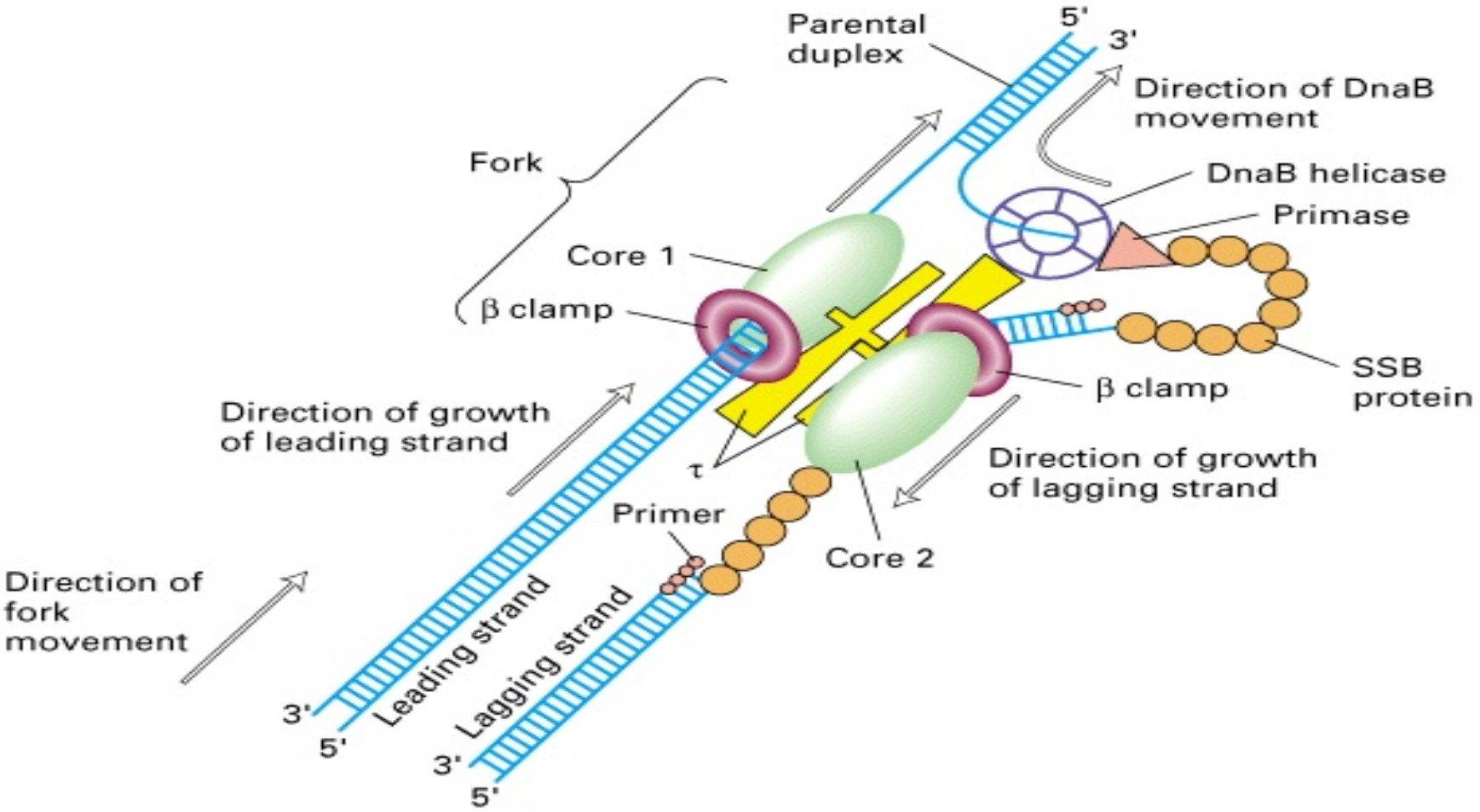
Overview of replication



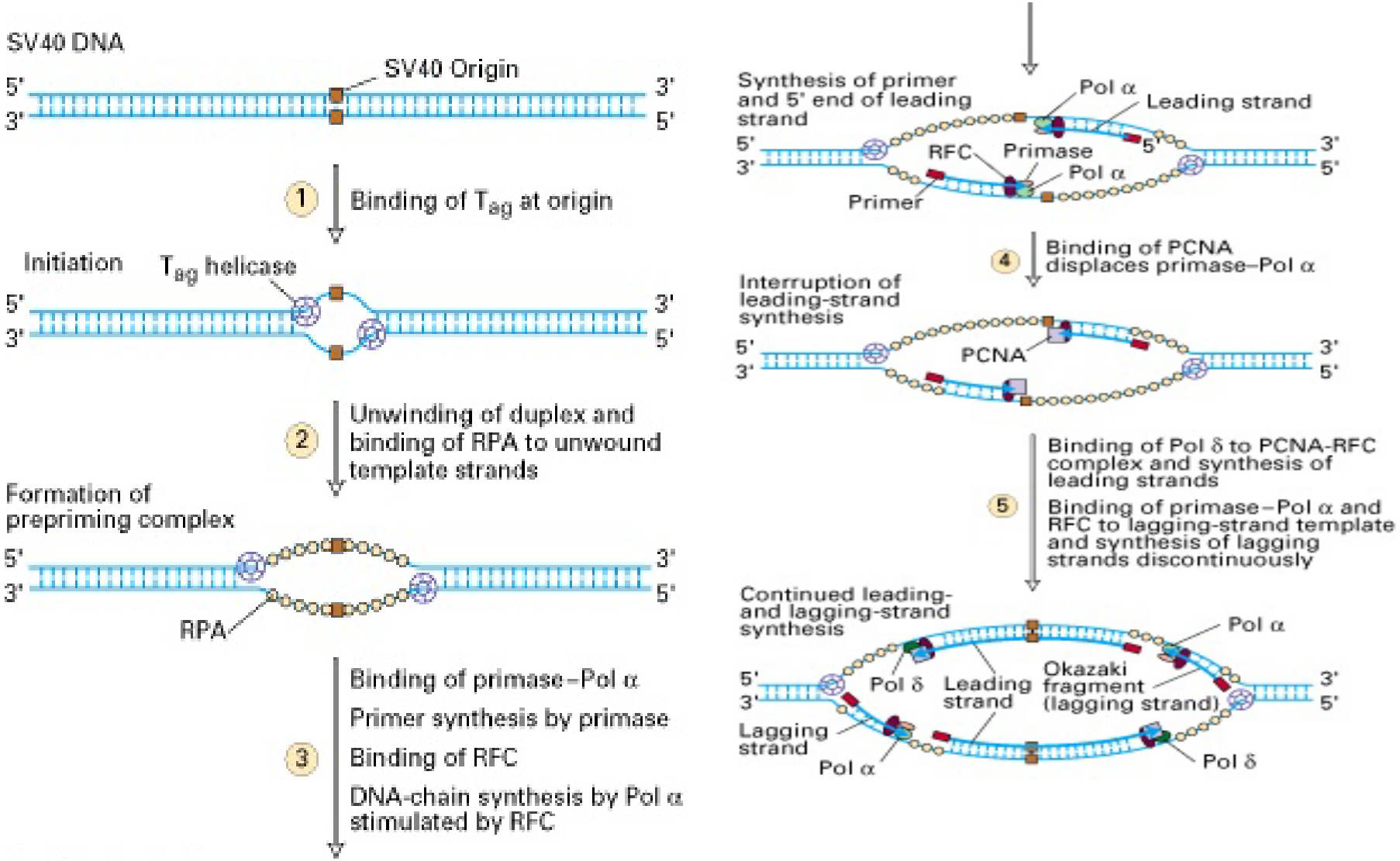


Coordination between the leading and the lagging strands: looping of the template for the lagging strand enables a dimeric DNA pol III holoenzyme to synthesize both daughter strands

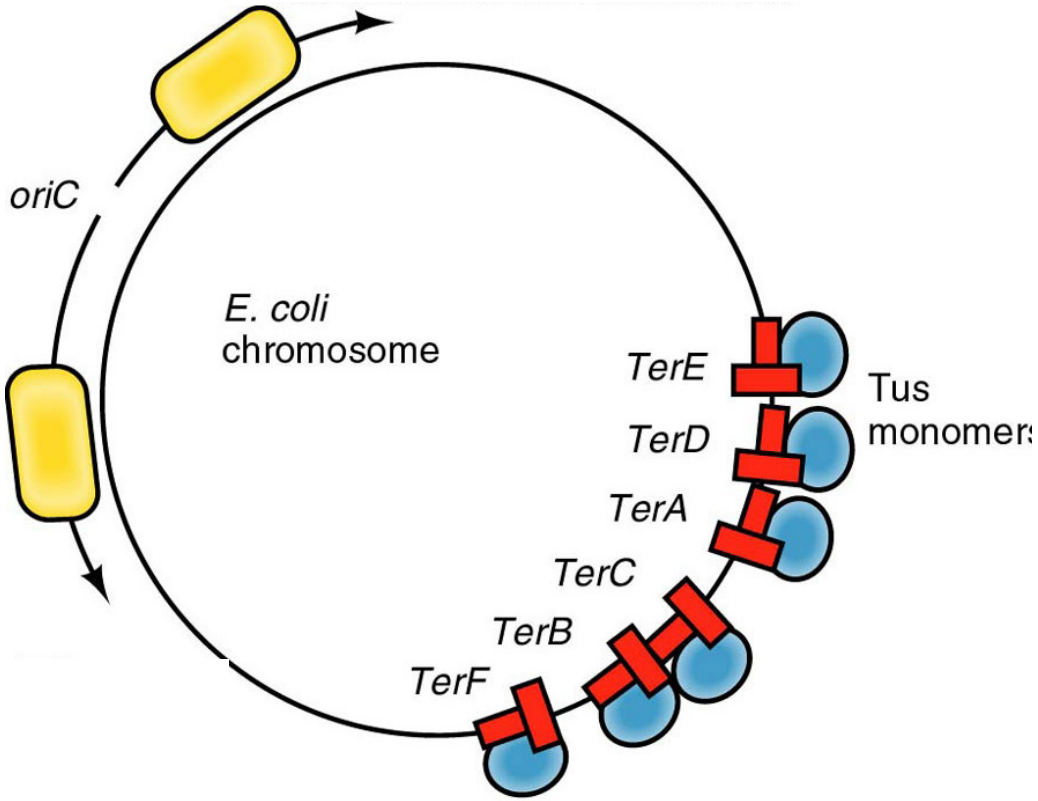
Detailed view of the *E. coli* fork

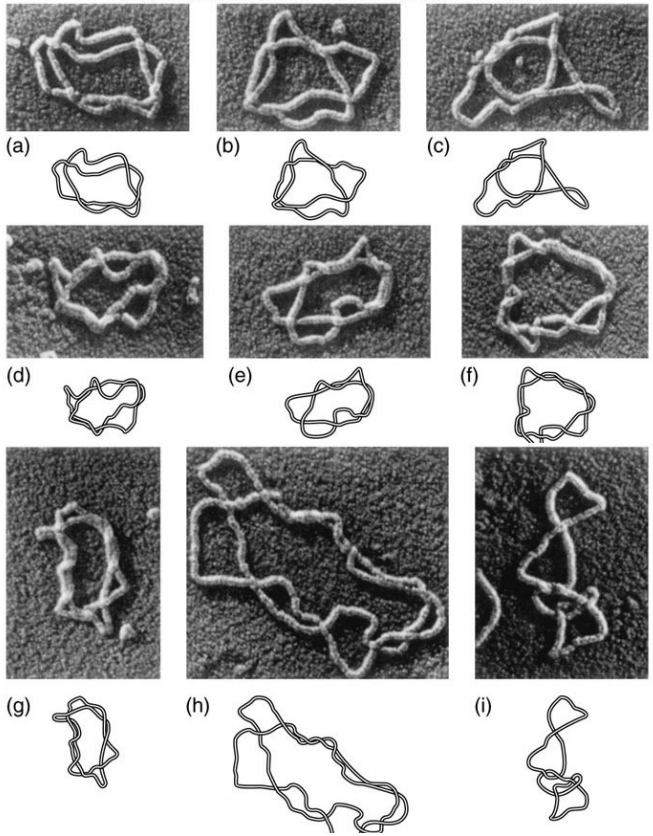


In vitro replication of SV40 DNA



Termination



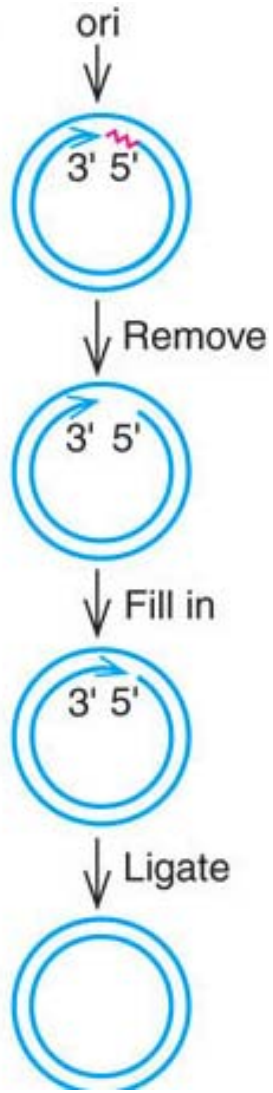


Bacterial termination site: *E. coli* Ter sites (Ter E, D, A stop the ccw fork; Ter F,B,C stop the CW fork; Tus, terminus utilization

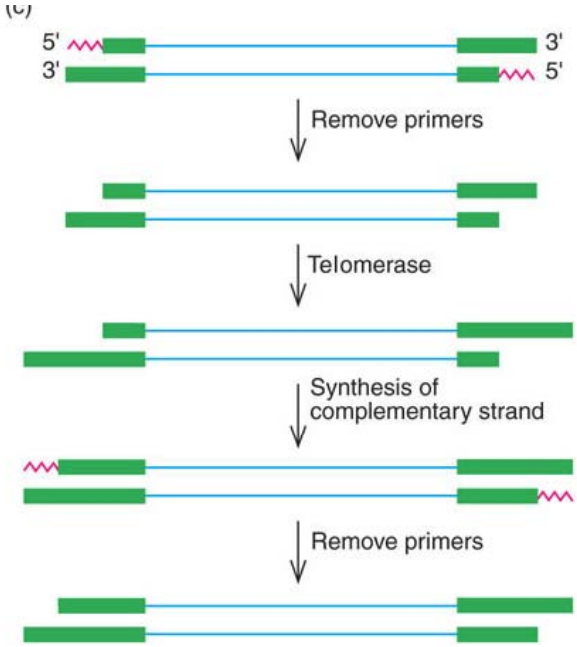
substance, binds to the terminator sites and helps arrest the moving forks).

EM of torus catenanes from replication of pBR322 in mutant *S. typhimurium*

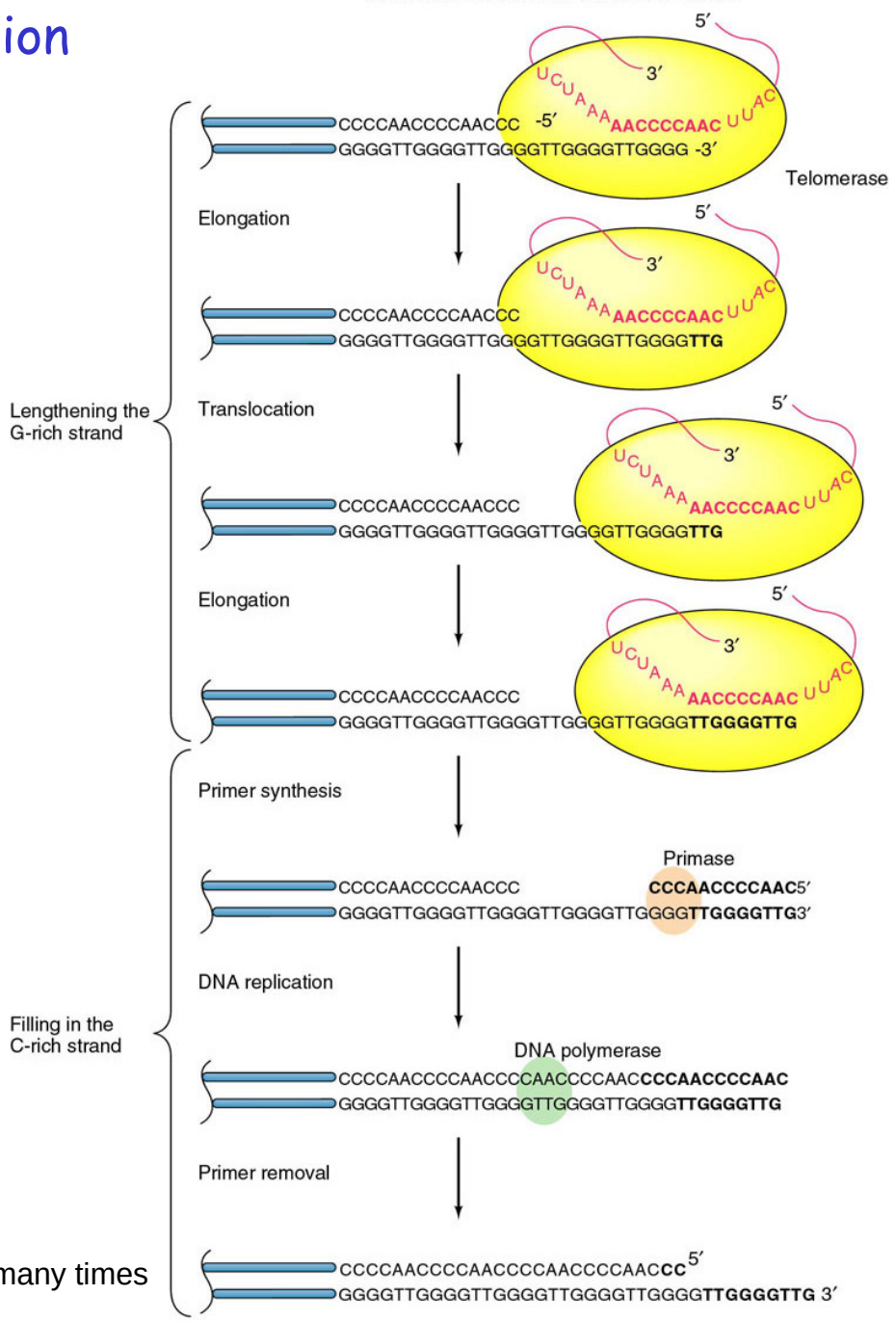
Termination



prokaryote

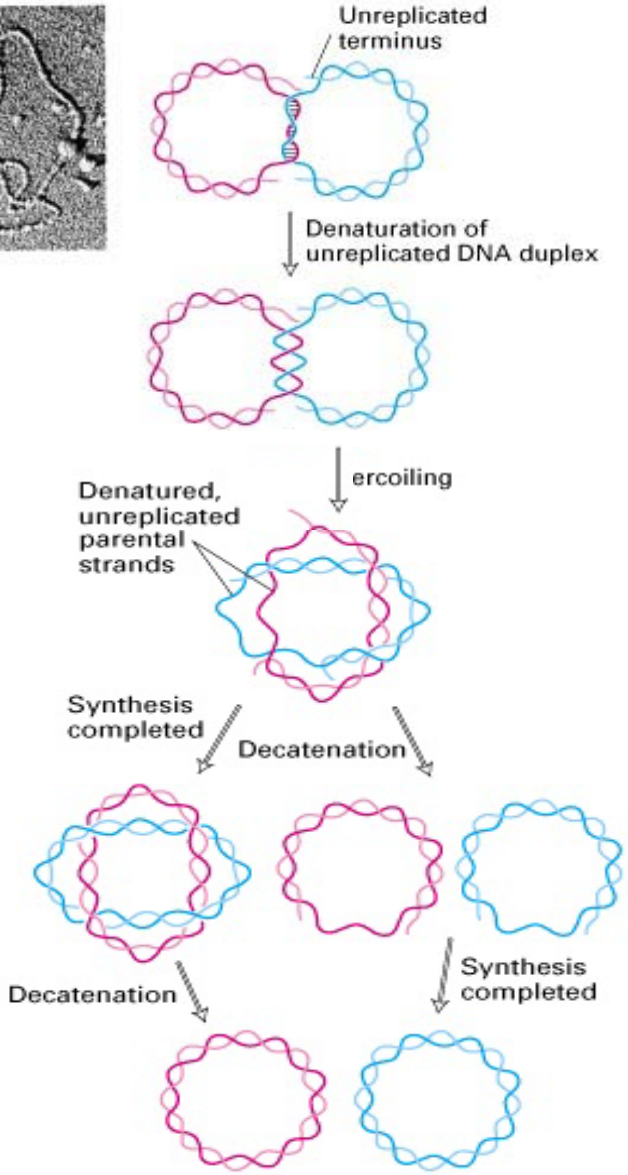


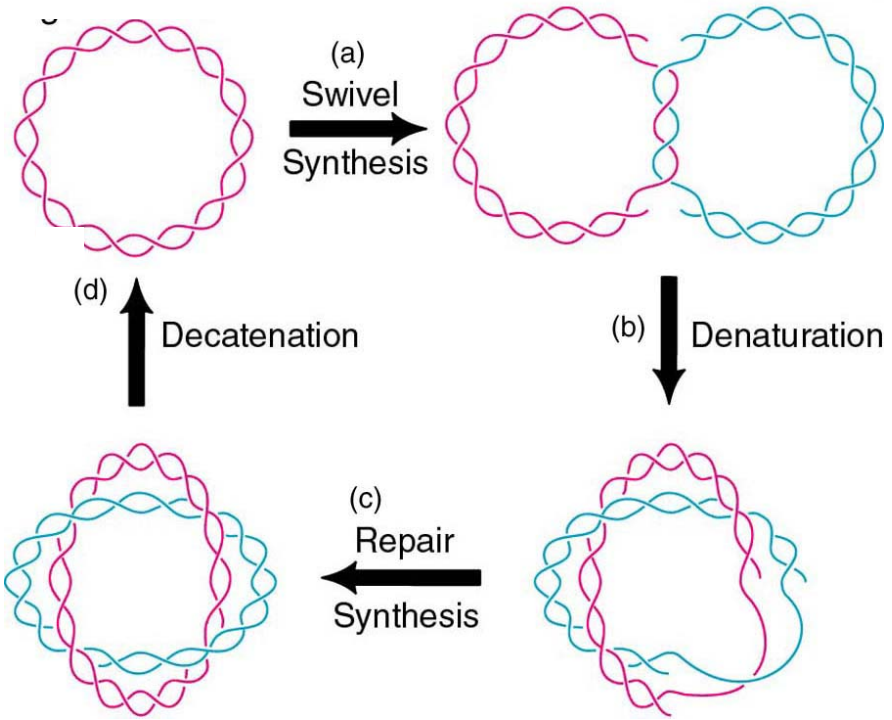
Eukaryote



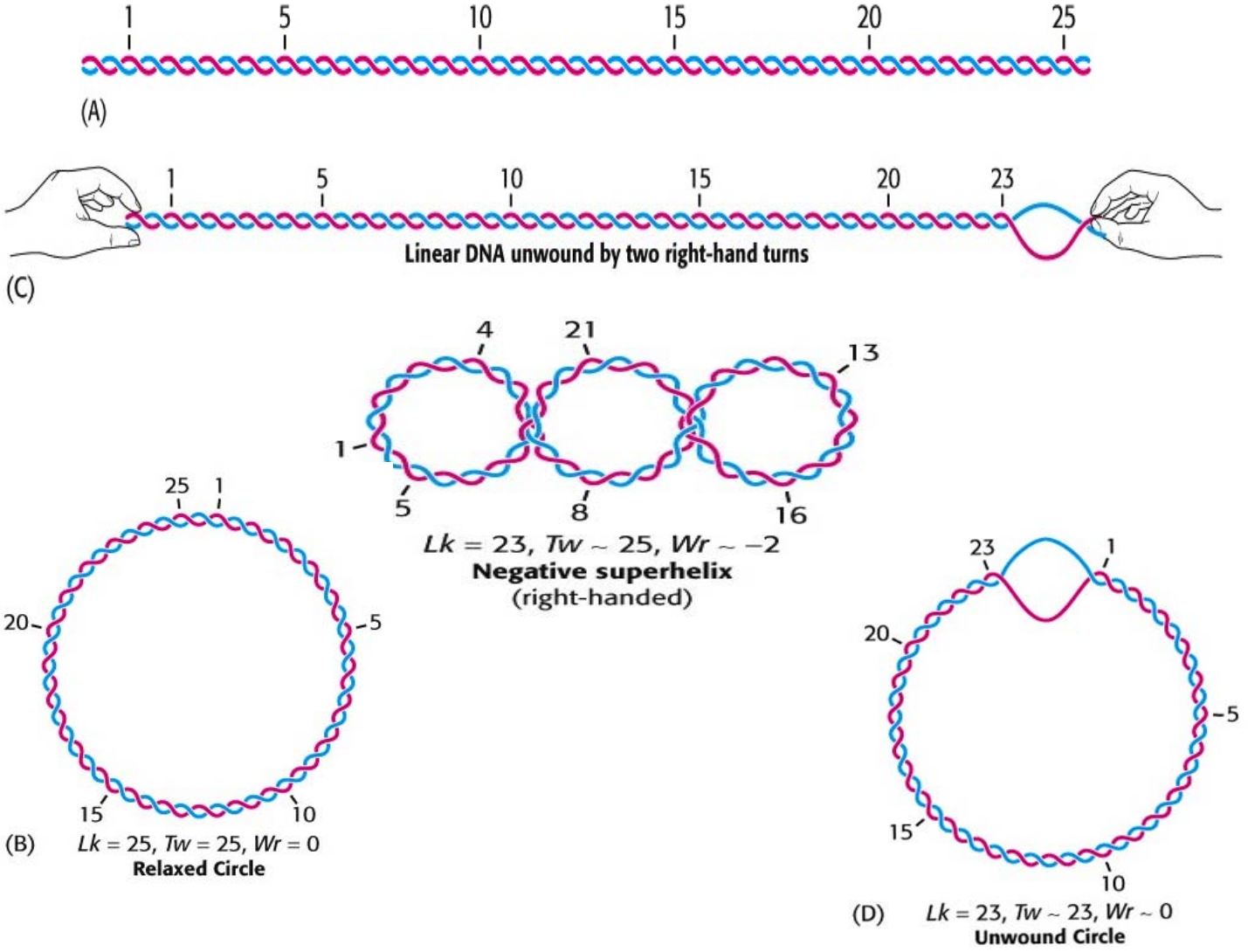
add6 nt (GGGTTG), repeat many times

Termination



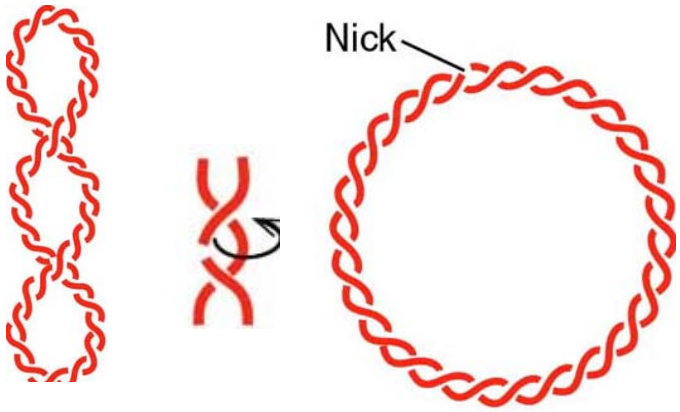


Supercoil

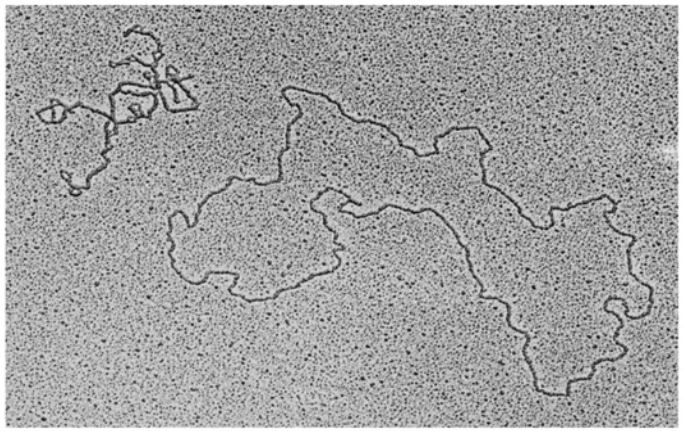


Lk = Tw + Wr (linking number is the sum of twisting number and writhing number)

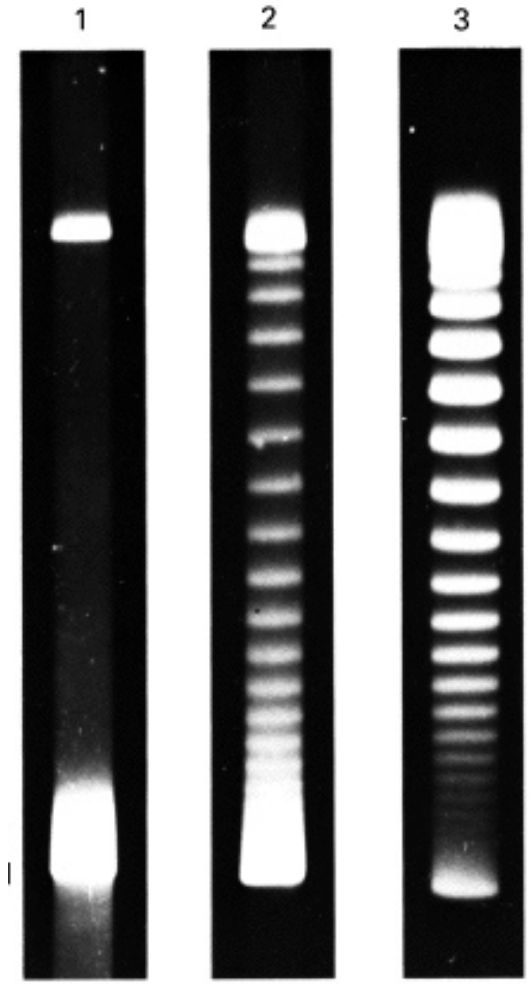
Supercoil



Nicking one strand relaxes supercoiled DNA

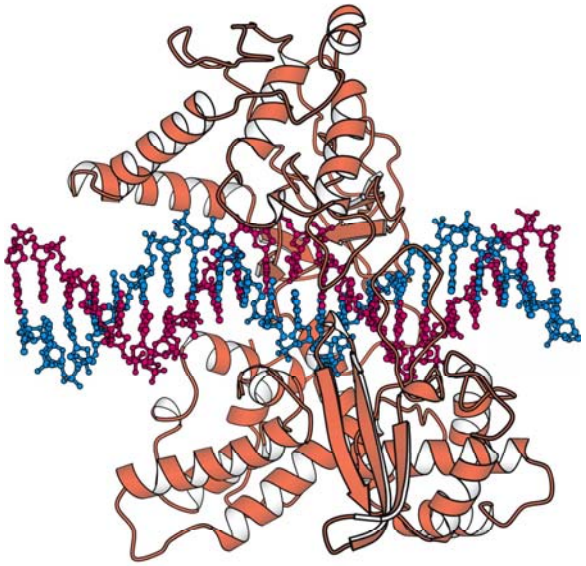


EM picture of two topoisomers (molecules differ in linking numbers) showing relaxed circular and negatively supercoiled DNA

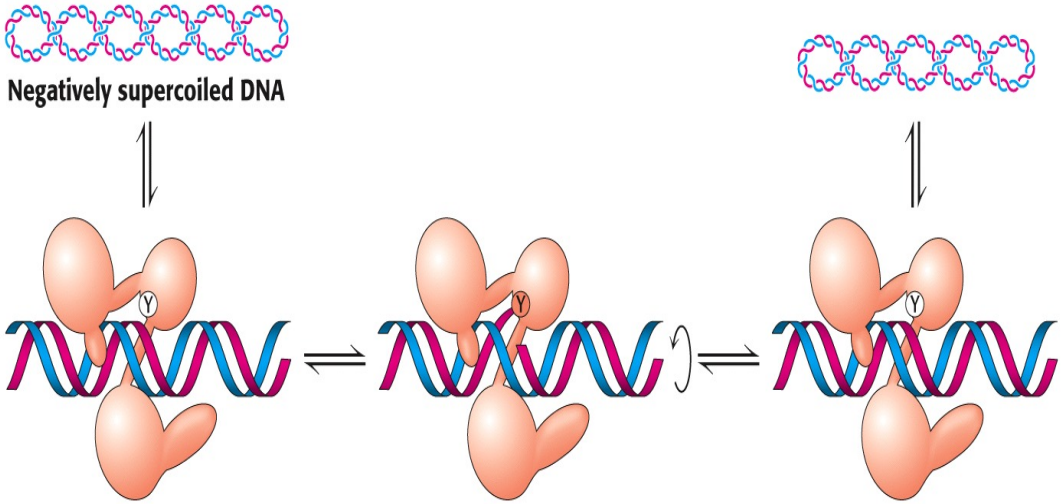


Separation of SV40 DNA topoisomers by gel electrophoresis. Lane 1, relaxed and maximally supercoiled DNA; lane 2 topo I for 3 min; lane 3, topo I for 30 min

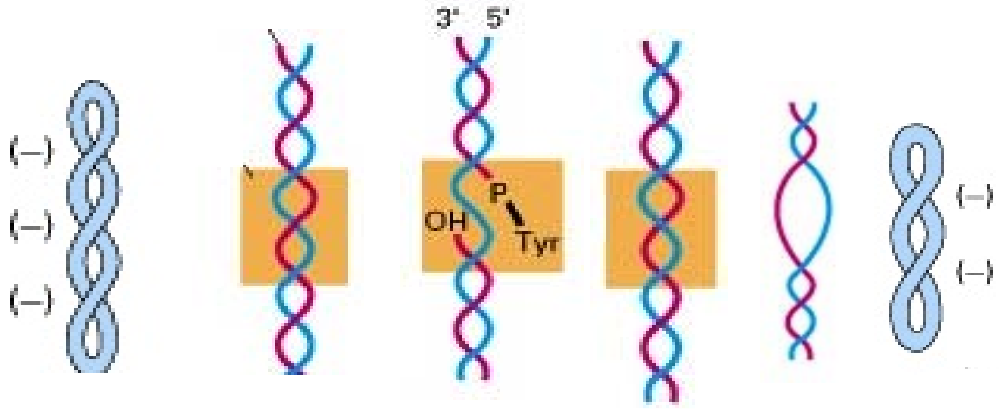
Two types of topoisomerases



Human Topoisomerase I with DNA

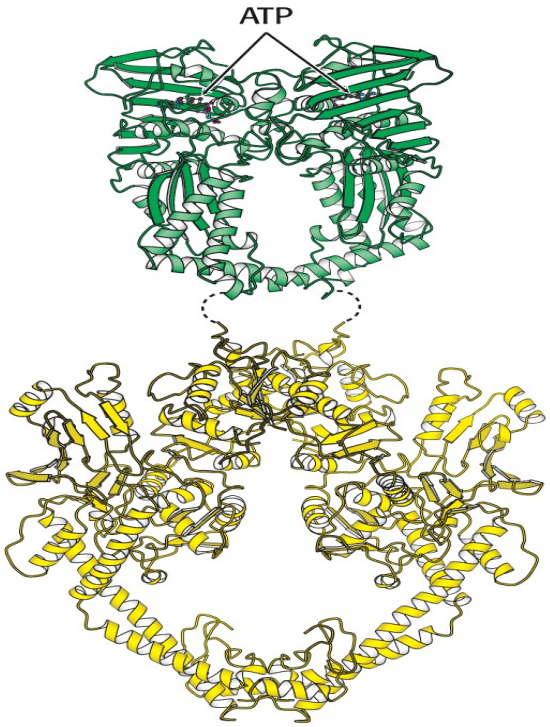


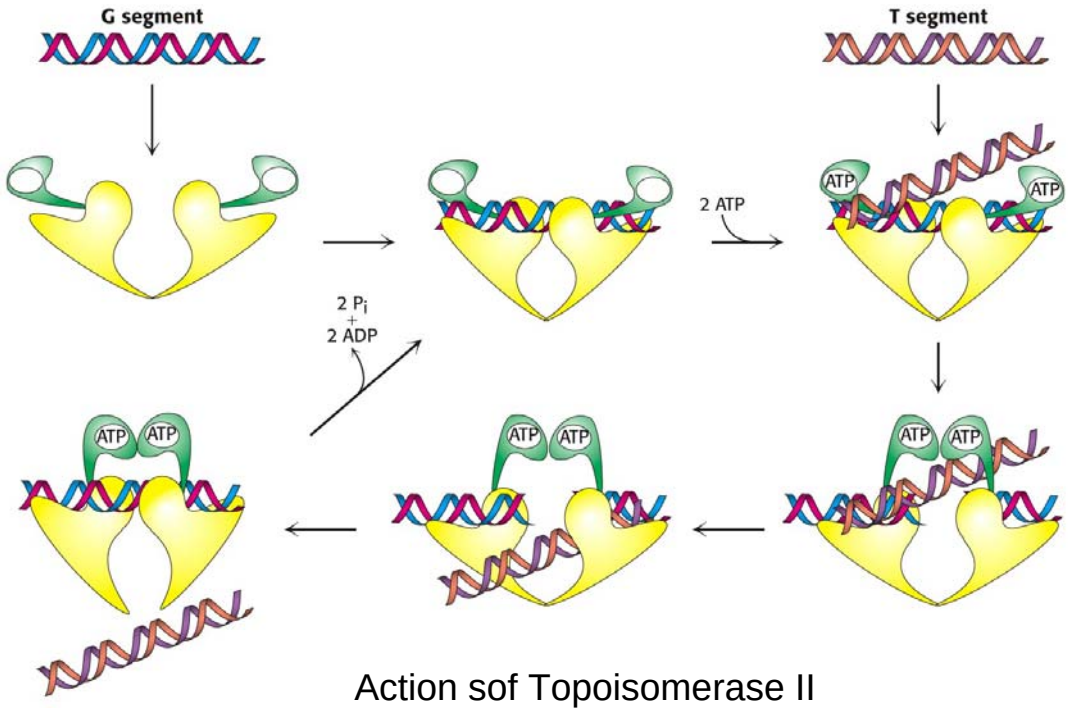
Topo I binds and cleaves one stand. The broken strand will rotate around the other one and rejoin, which leads to partial or complete relaxation of a supercoil



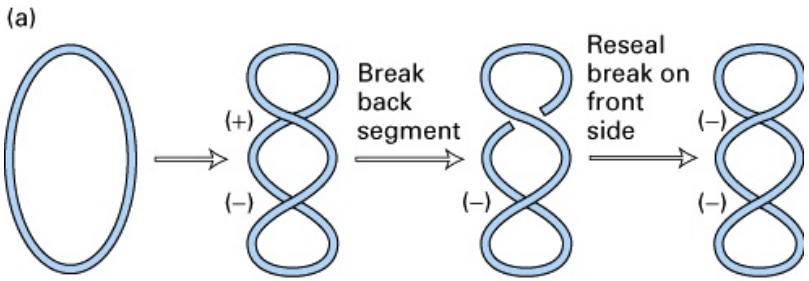
Topo I action, from three negatives to 2 negatives

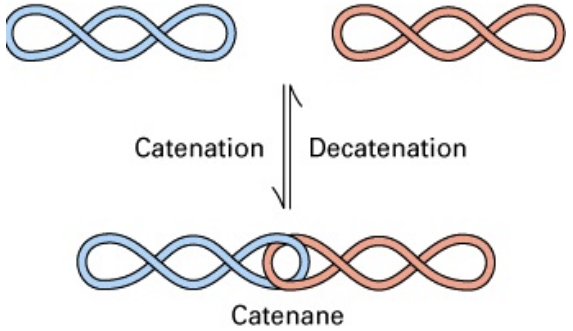
Topo II cleaves both strands and create supercoiling





Action sof Topoisomerase II





1959 "for their discovery of the mechanisms in the biological synthesis of ribonucleic acid and deoxyribonucleic acid"

Severo Ochoa
1905-1993

Arthur Kornberg
1918-

2005

1930-1975
Okazaki