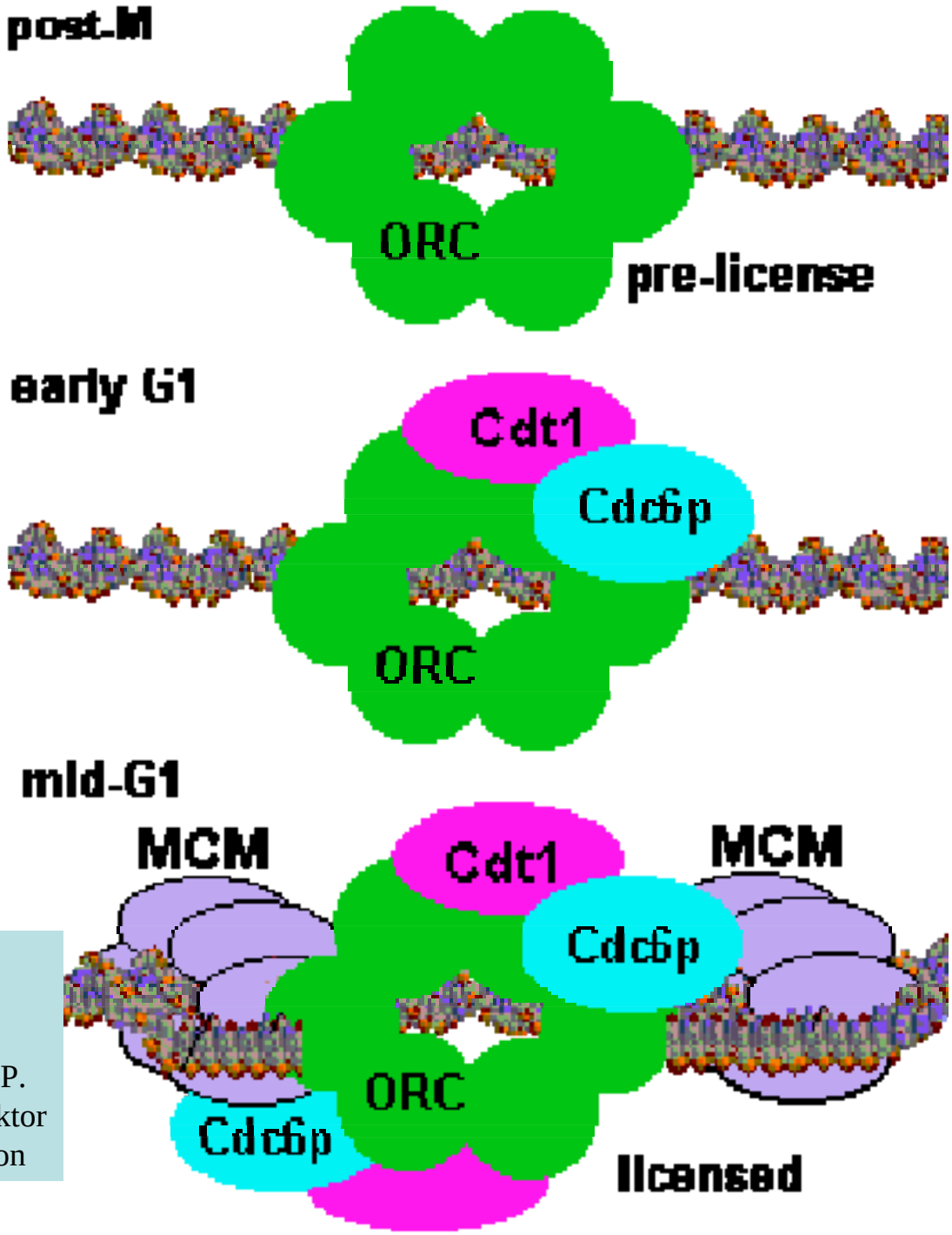
DNA-Replikation

- Ein Prozess in drei Stufen
  1. Initiation
  2. Elongation
  3. Termination

Die **Initiation** der DNA-Replikation bei **Eukaryoten** am ori erfolgt erst nach der „Lizensierung“ durch ORC und weitere Proteine



ORC= Origin recognition complex
Cdk= Cyclin dependent kinase
Cdc= Cell division cycle Protein
Mcm= Minichromosome maintenance P.
Cdt1= Replikationsfaktor/LicensingFaktor
OBR= Origin of bidirectional replication

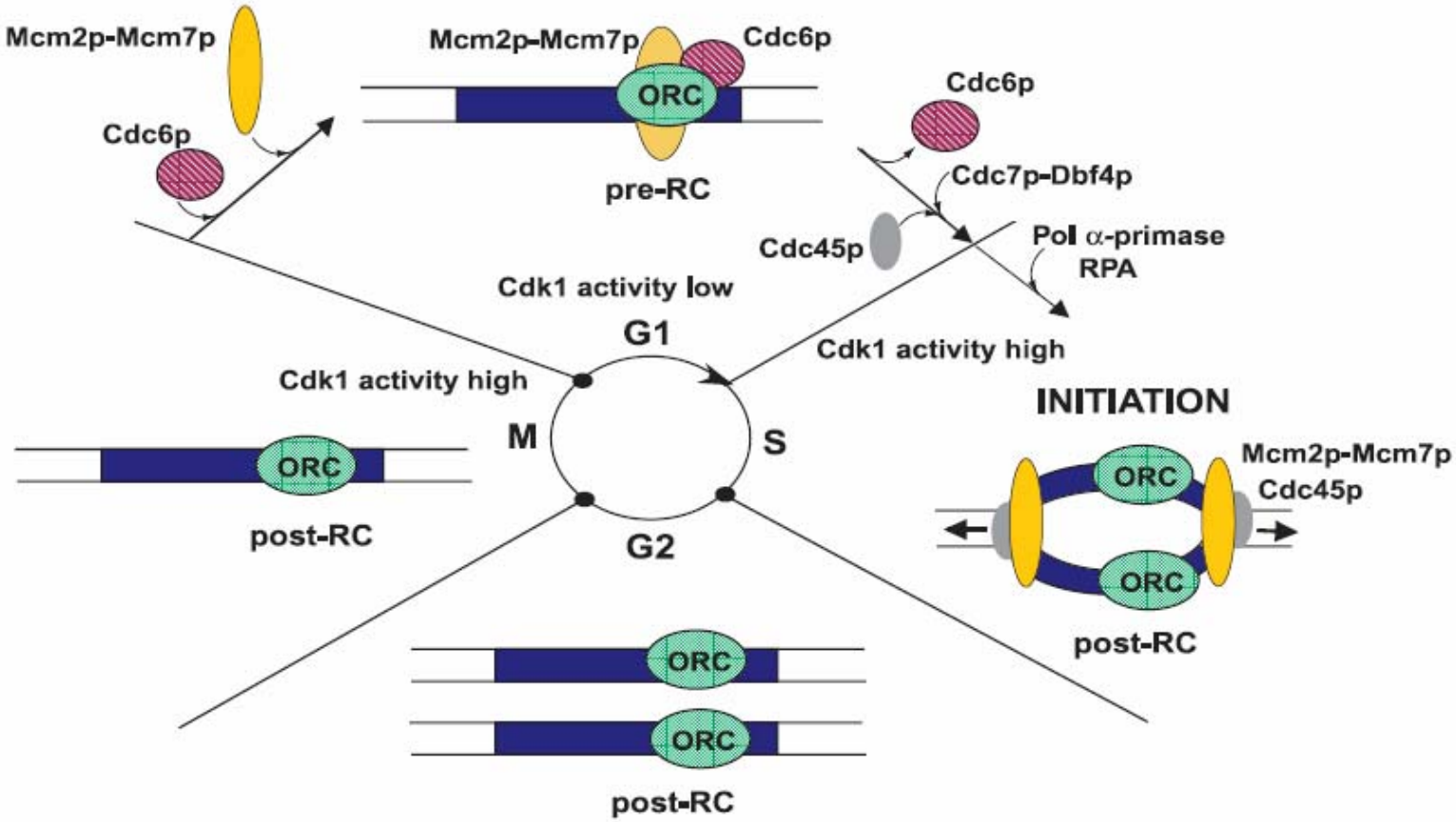
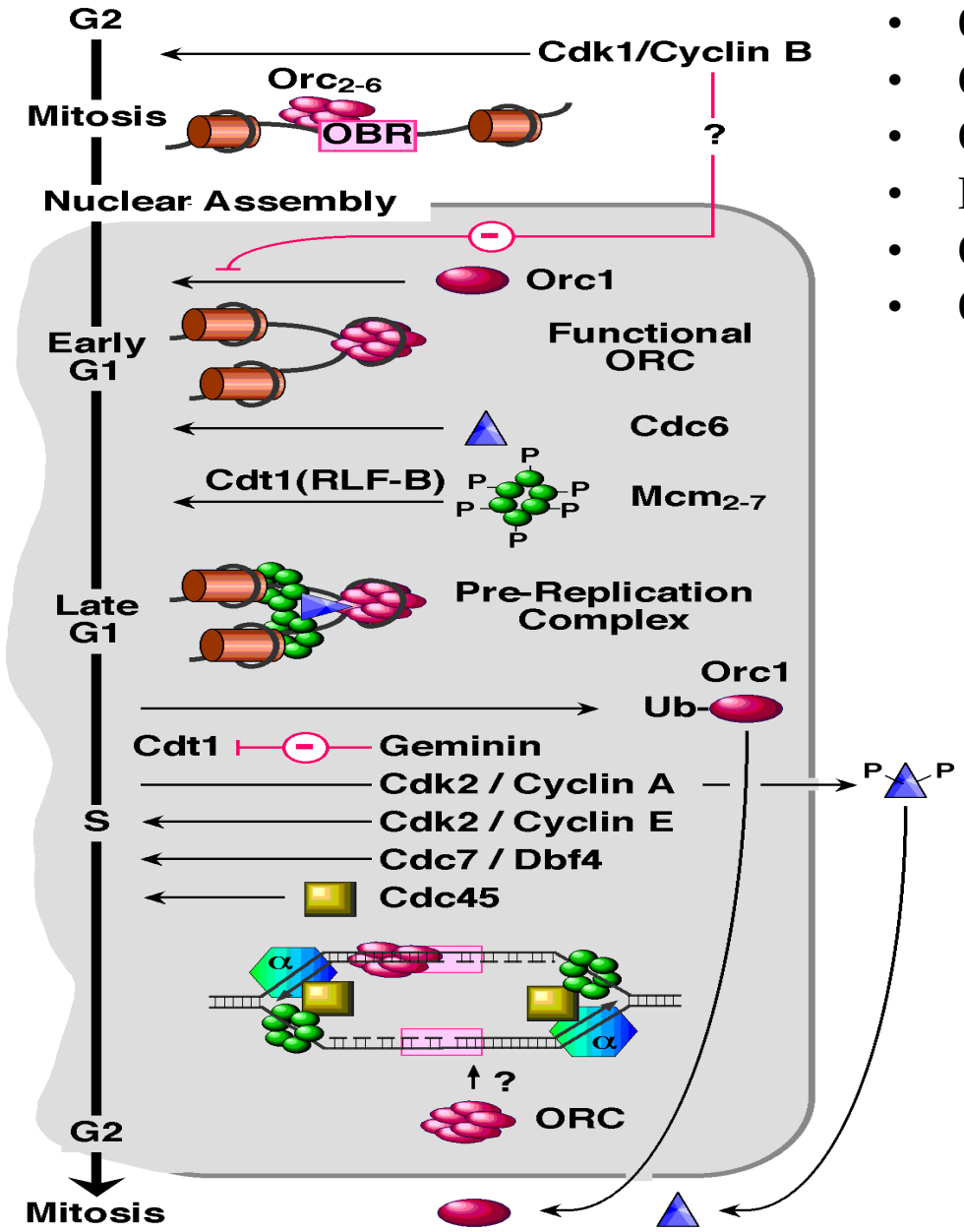


Fig. 1. Events leading to origin activation in budding yeast. Origin recognition complex (ORC) binds to an ARS element in yeast (blue rectangle). For the stepwise assembly of the pre-RC during G_1 phase when Cdk1p activity is low, ORC recruits Cdc6p, which in turn loads Mcm2p-Mcm7p. When Cdk1p activity rises at the G_1/S transition, the pre-RC is disassembled. The post-RC remains stable until the end of mitosis, and owing to high Cdk1p activity the pre-RC cannot re-associate during this time but must await the subsequent G_1 phase.



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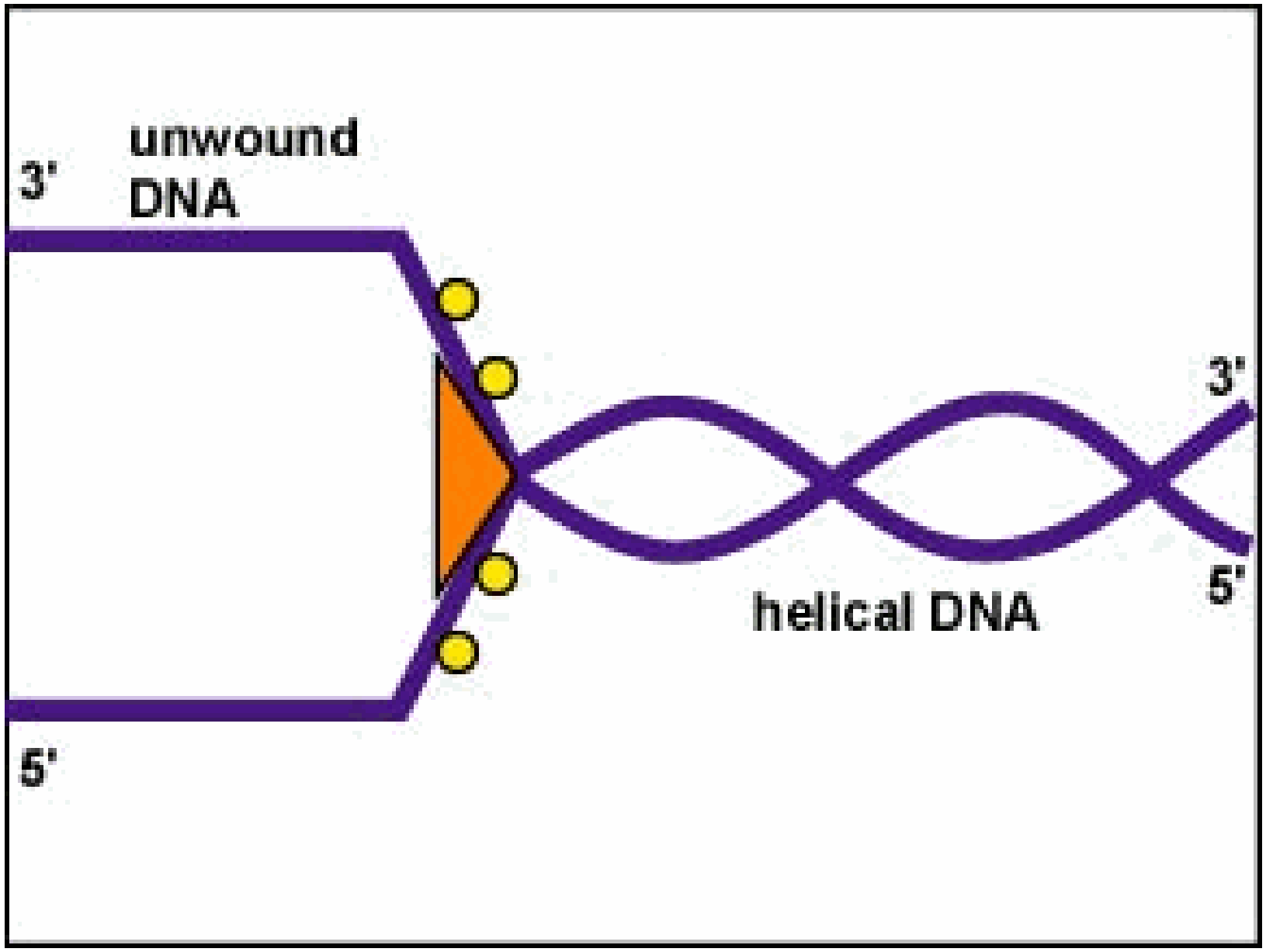
- Cdc= Cell division cycle Protein
- Mcm= Minichromosome maintenance P.
- Cdt1= Replikationfaktor
- OBR= Origin of bidirectional replication

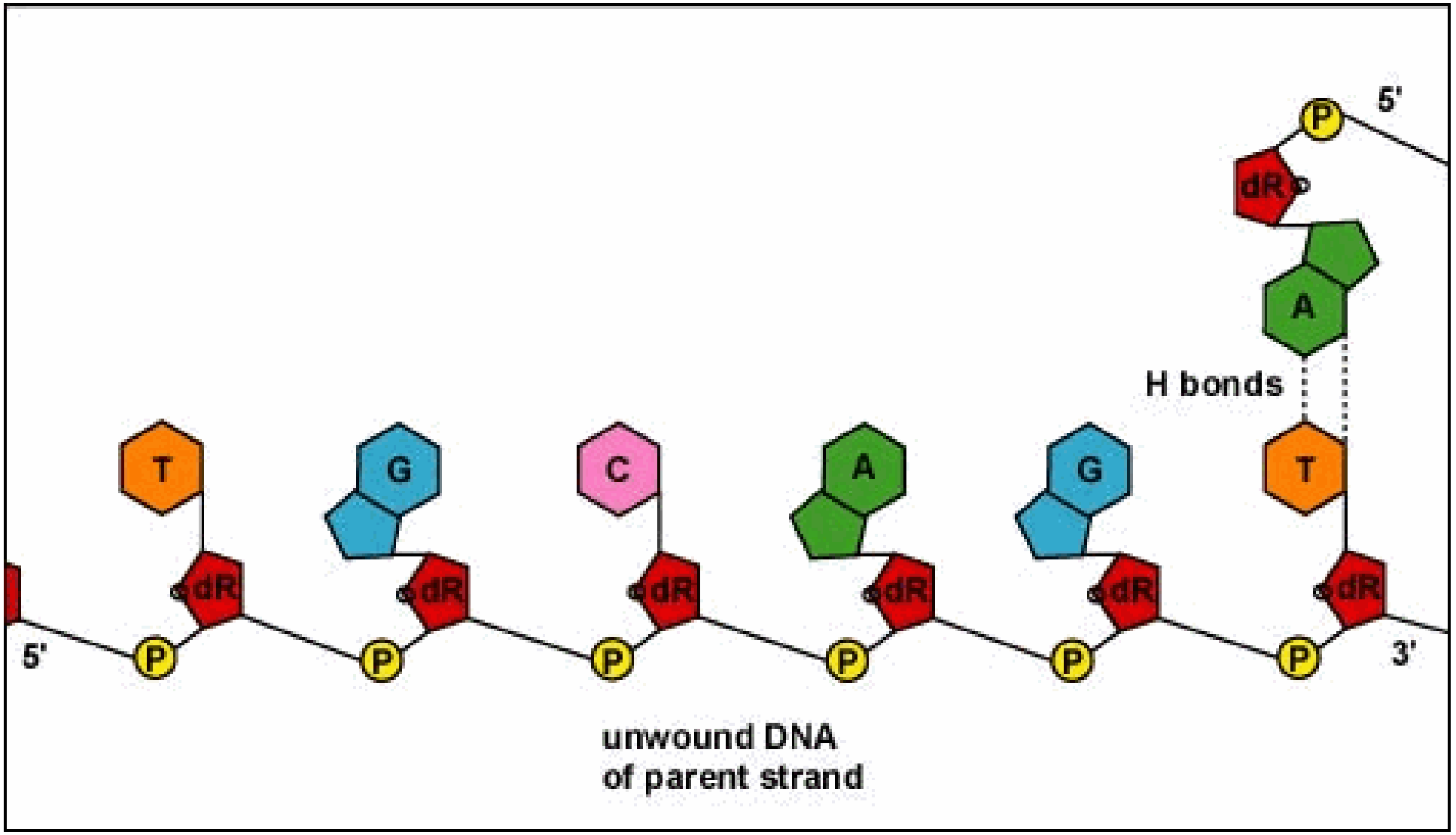
Initiator-Proteine

Table 2. Initiation Proteins

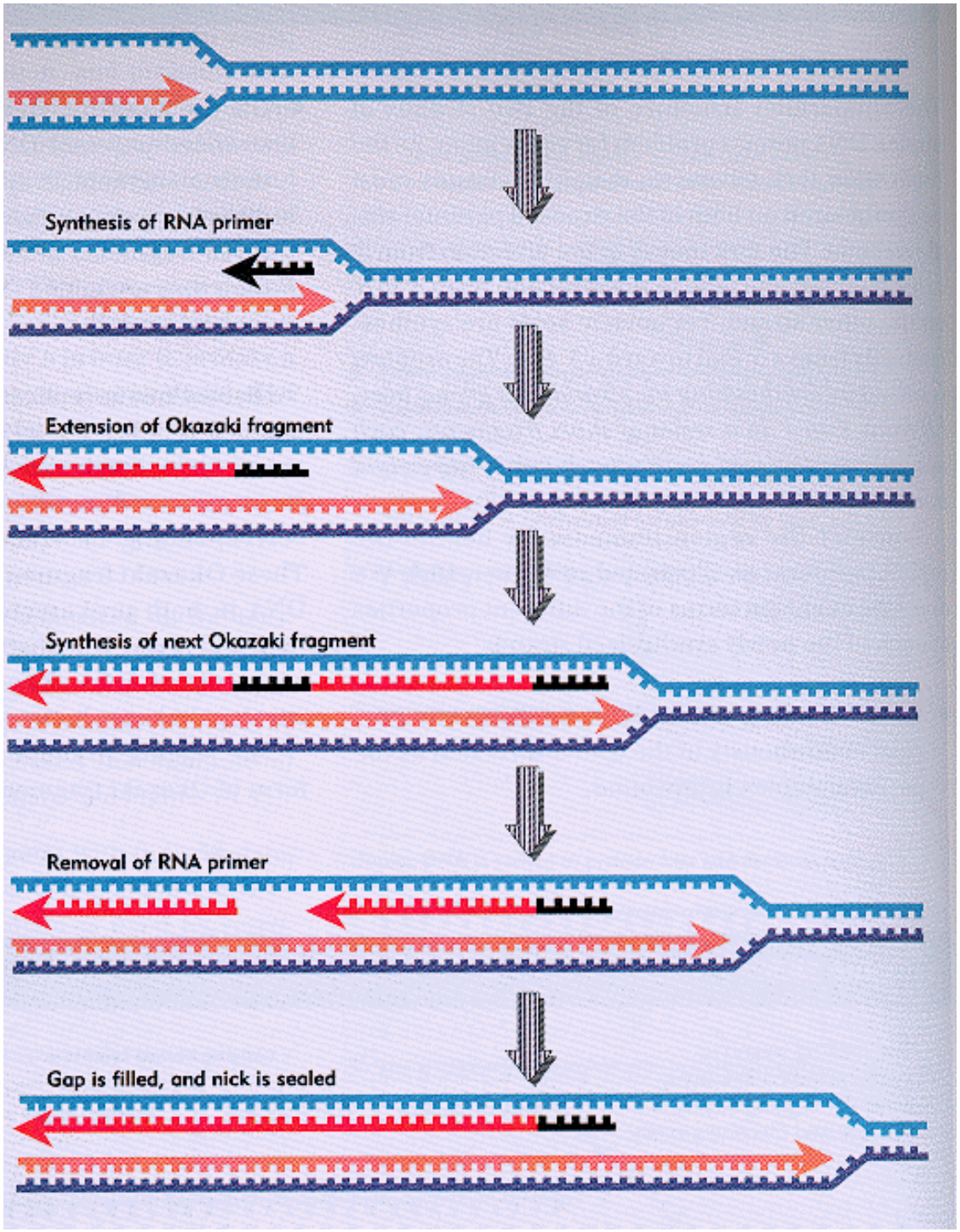
Function	*E. coli*	Phage λ	Phage T4	SV40/Human	Yeast
Initiator protein	DnaA	λ O	none	T antigen	ORC
Loading and remodeling factor(s)	DnaC	λ P, DnaJ, DnaK	gp59	Cellular chaperone?	Cdc6 protein
DNA helicase	DnaB	DnaB	gp41	T antigen	MCM proteins?

Die Elongation („Primer-Extension“)

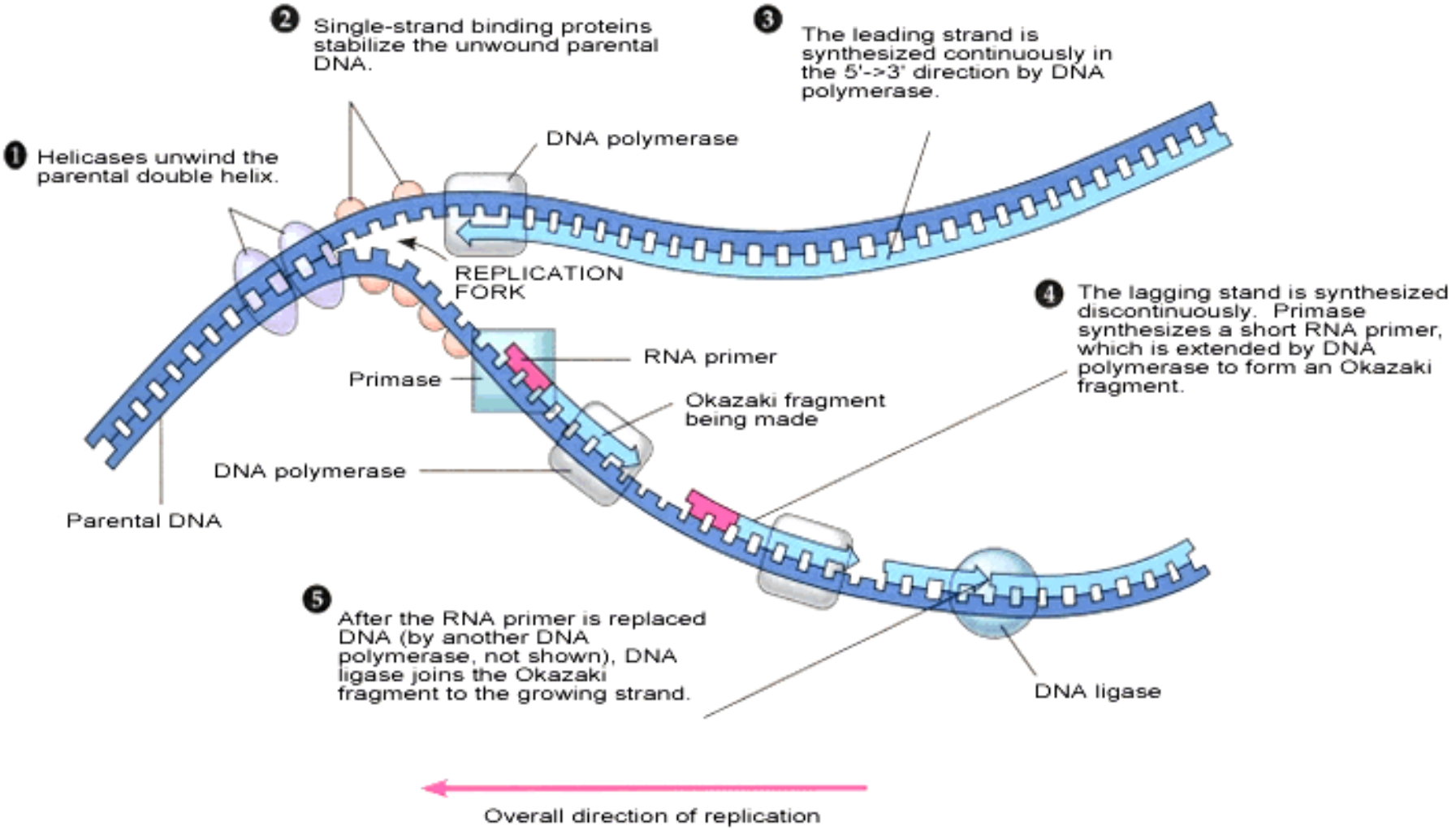



5'
P
dR
A
H bonds
T
G
C
A
G
T
dR
P
5'
3'
unwound DNA
of parent strand

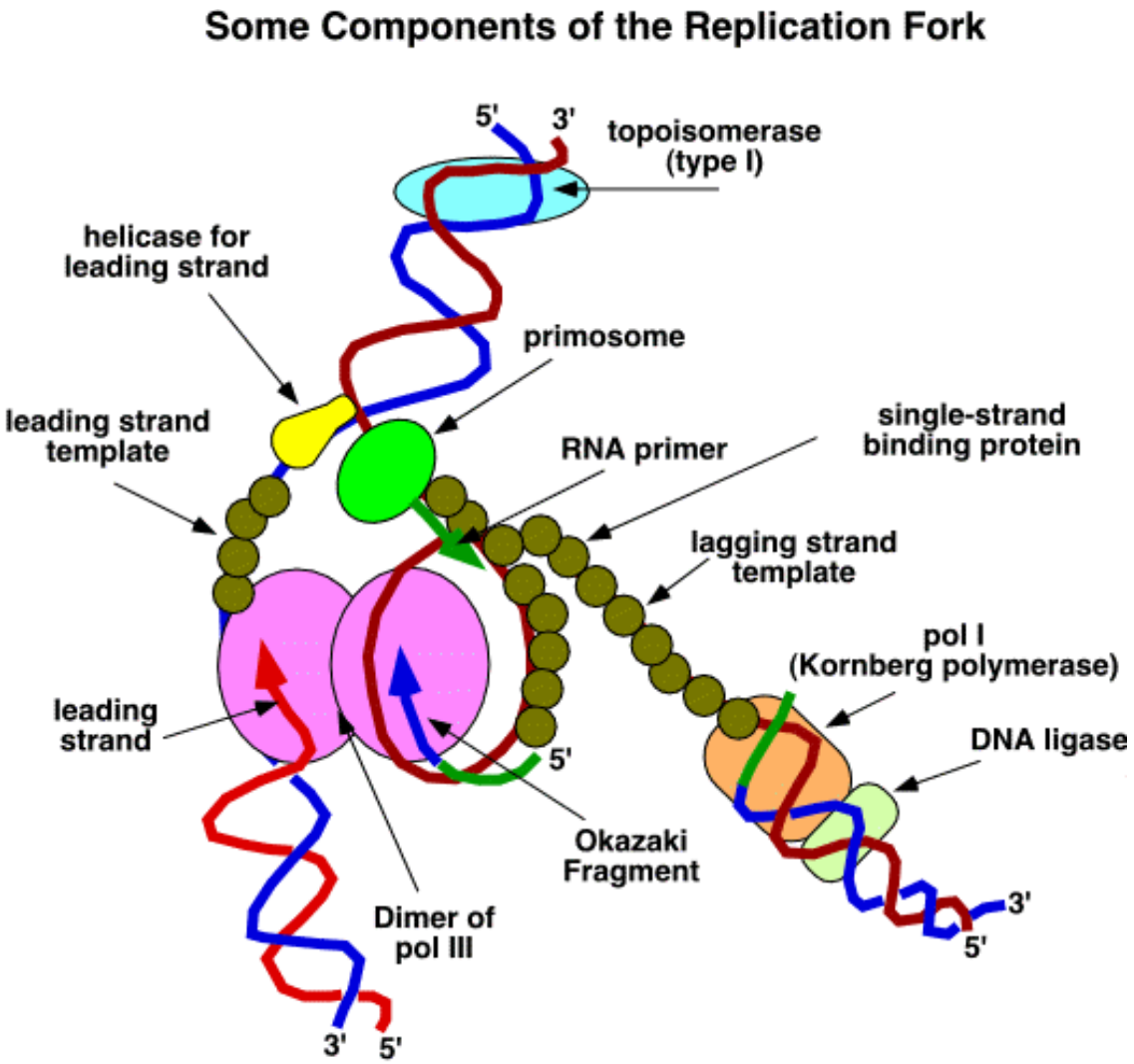
Elongation


Synthesis of RNA primer
Extension of Okazaki fragment
Synthesis of next Okazaki fragment
Removal of RNA primer
Gap is filled, and nick is sealed

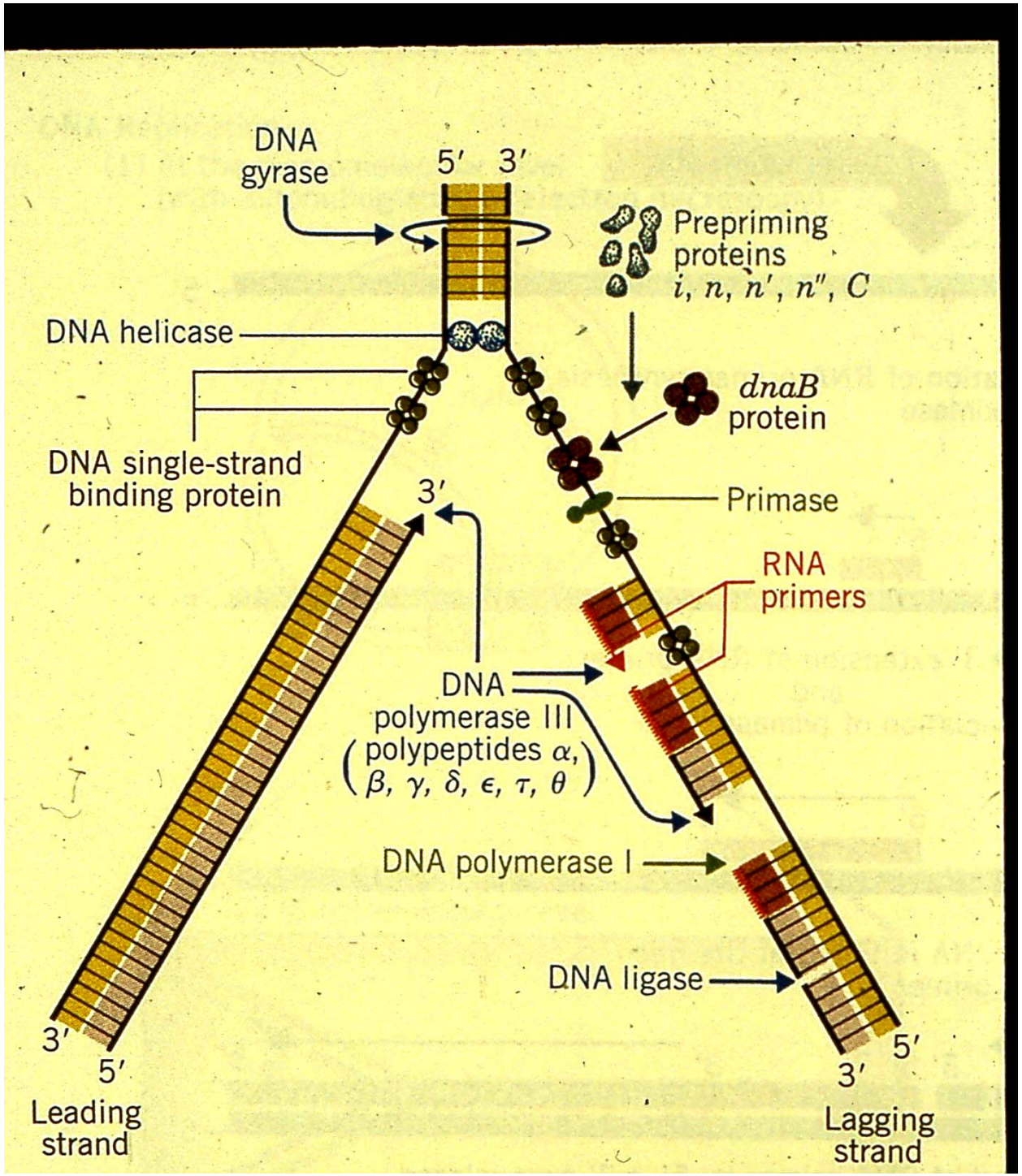
Replikationsgabel



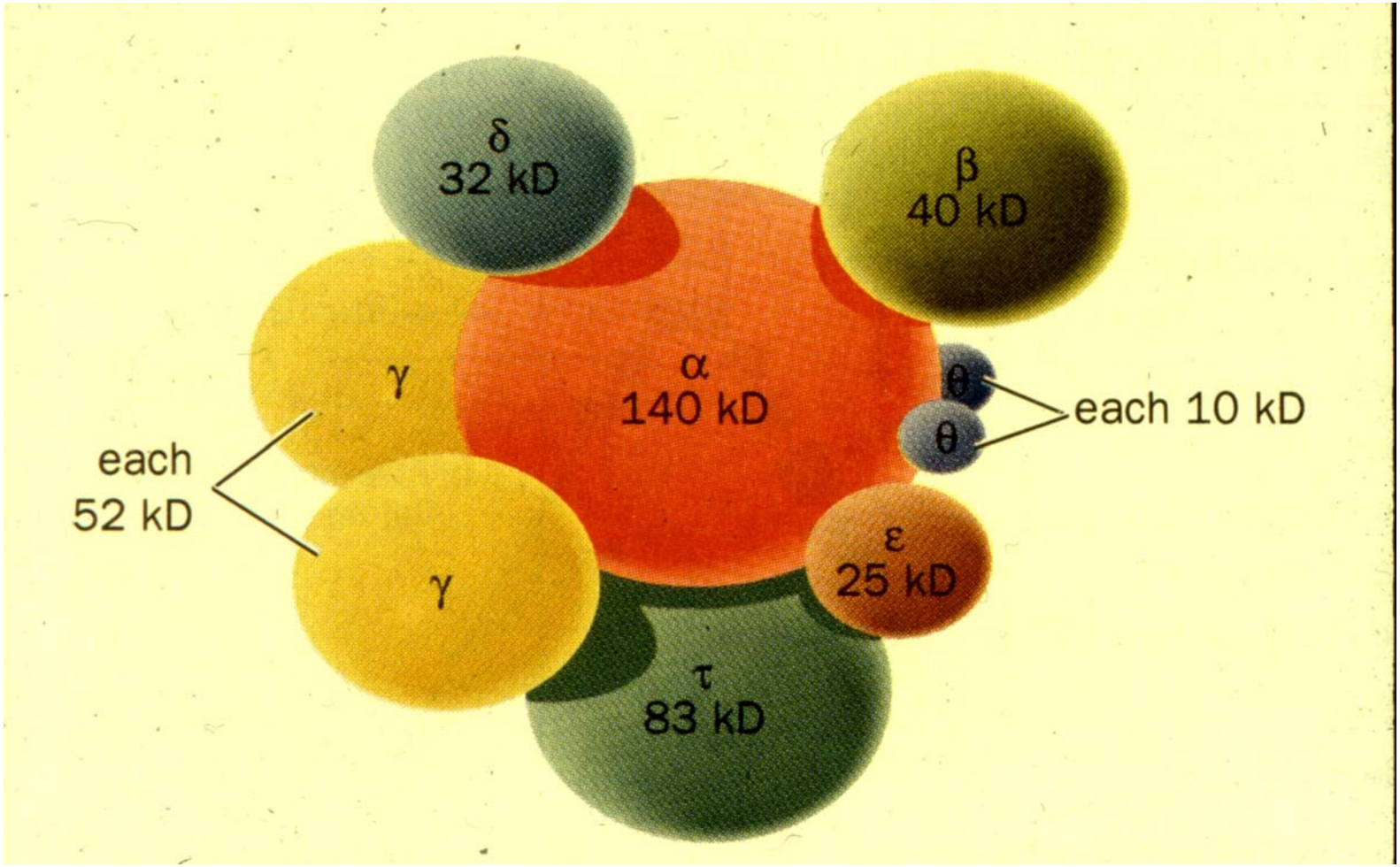
Replikationsgabel bei Prokaryoten



Replikationsgabel
Prokaryoten



DNA-Polymerse III Holoenzym



Beta-Subunit of DNA Pol III

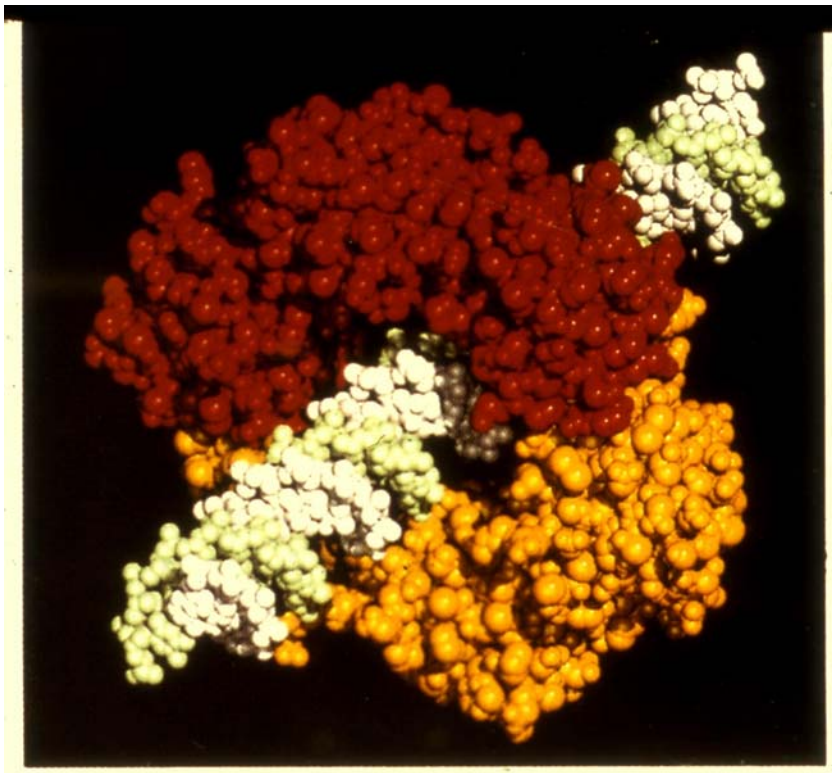
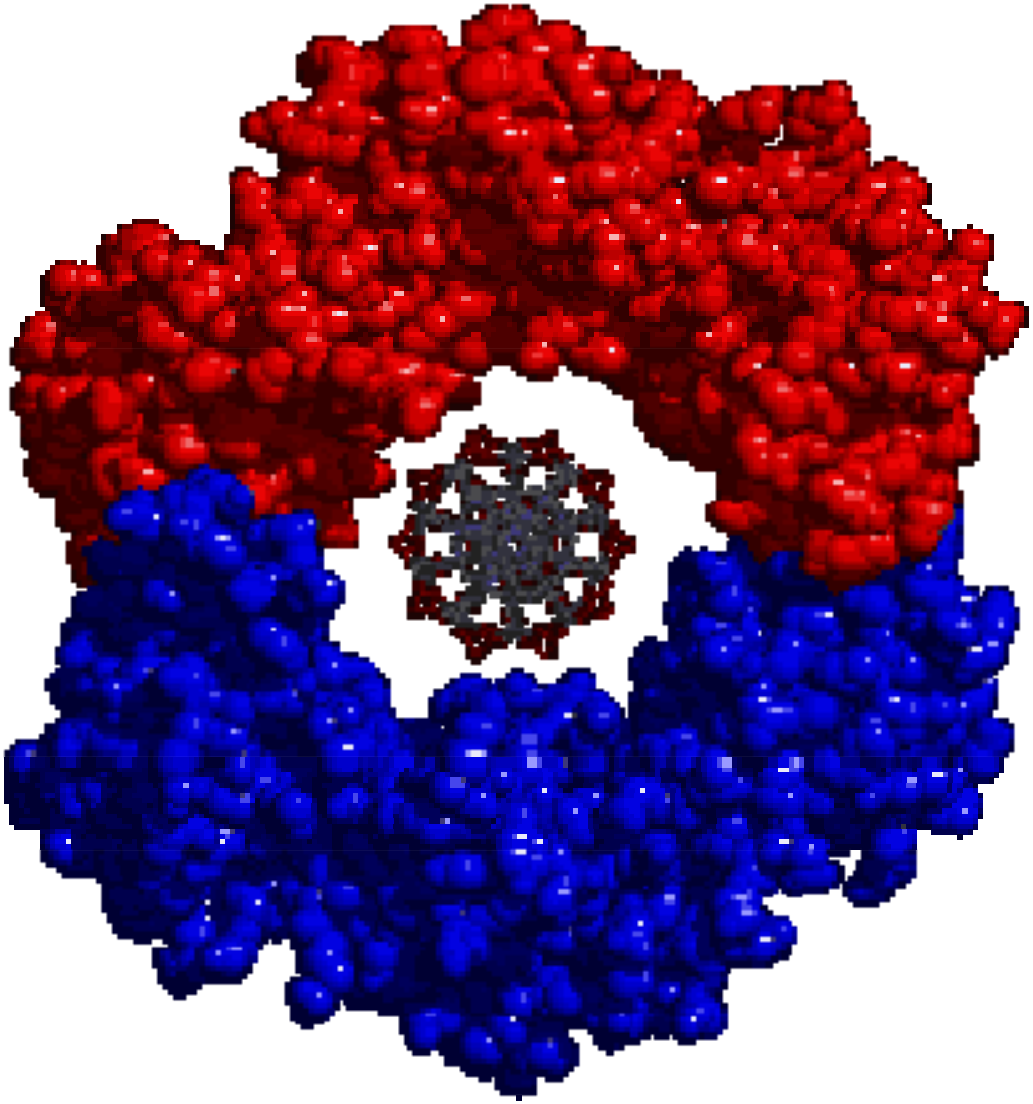


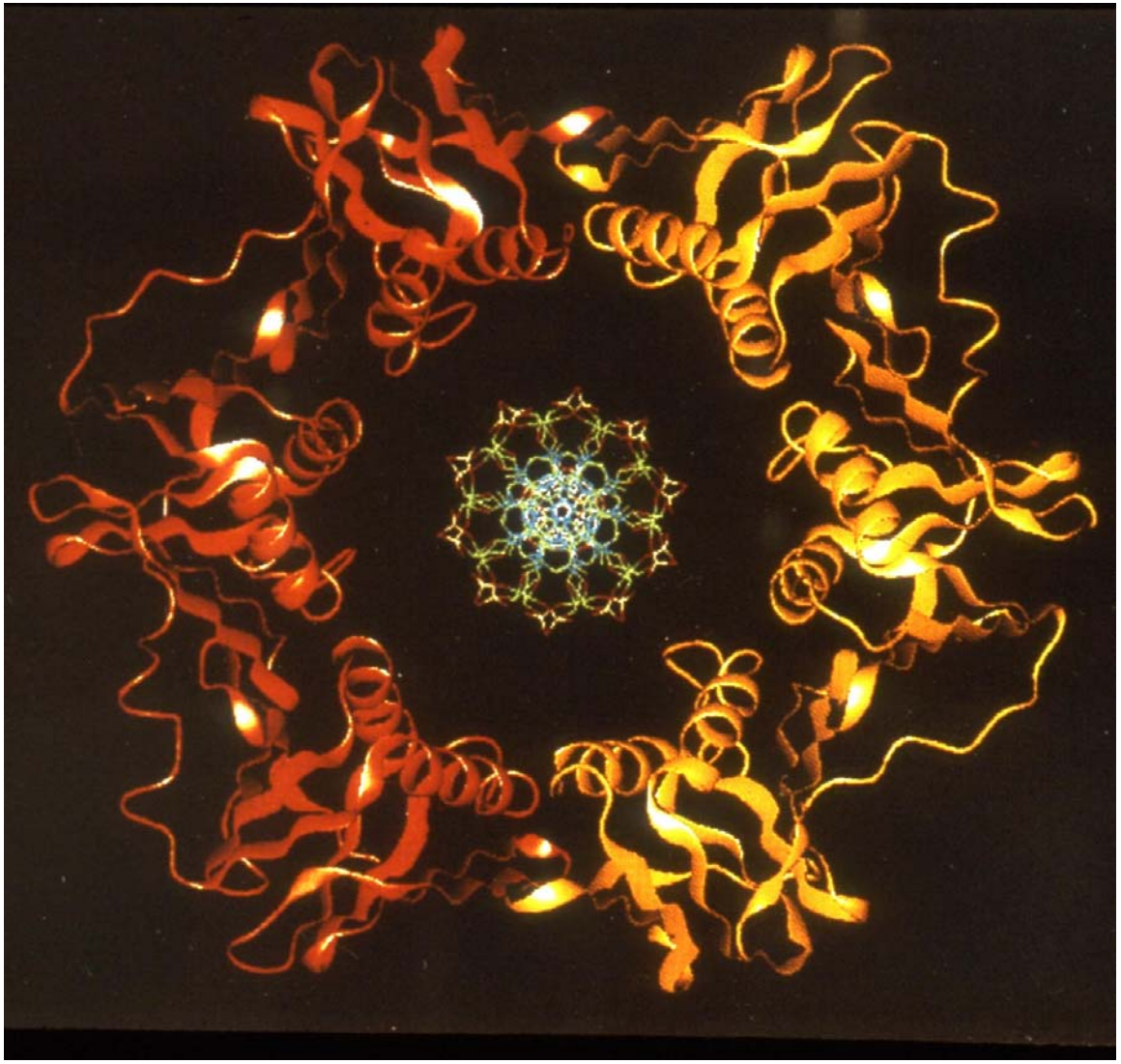
Figure 3. Space-Filling Model of the β Subunit Dimer with B-Form DNA

One monomer is colored red and the other yellow. The radius of the spheres corresponds to the van der Waals radius of the corresponding atom. Hydrogen atoms are not explicitly displayed, but manifest themselves as increased radii for atoms that they are bonded to. The hypothetical model of B-form DNA is as in Figures 1 and 6, and is shown with one strand colored white and the other green. The double helix passes through the hole in the β subunit dimer with no steric repulsions.

Beta Subunit DNA Polymerase



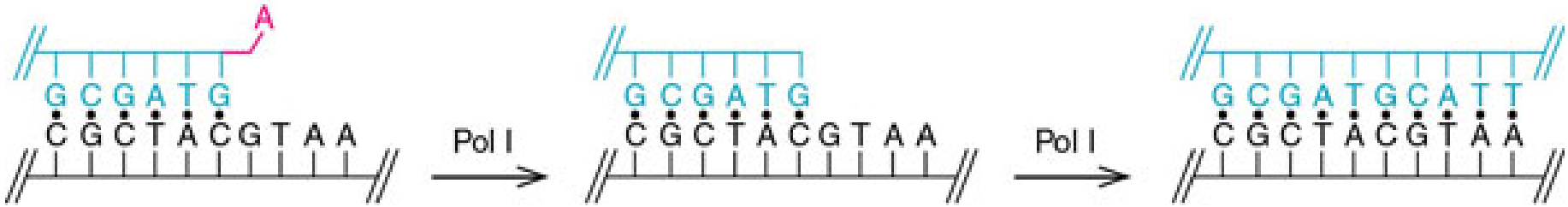
Struktur der beta-Untereinheit der DNA-Polymerase



Protein	Gene	Subassembly	Function
α	*dna*E	Polymerase core	DNA polymerase catalytic subunit
ε	*dna*Q	"	Proofreading exonuclease
θ	*hol*E	"	Unknown
τ	*dna*X	Clamp loader	ATPase of clamp loader
γ	*dna*X	"	ATPase of clamp loader
δ	*hol*A	"	Binds β in clamp loading reaction
δ'	*hol*B	"	Transduces energy from τ/γ to δ
χ	*hol*C	"	Binds SSB in elongation reaction
ψ	*hol*D	"	Links χ to γ
β	*dna*N	Clamp	Clamp, processivity factor
SSB	*ssb*	SSB	Single-stranded DNA binding protein
Primase	*dna*G	Primase	Catalyzes formation of short RNA primers to initiate DNA replication

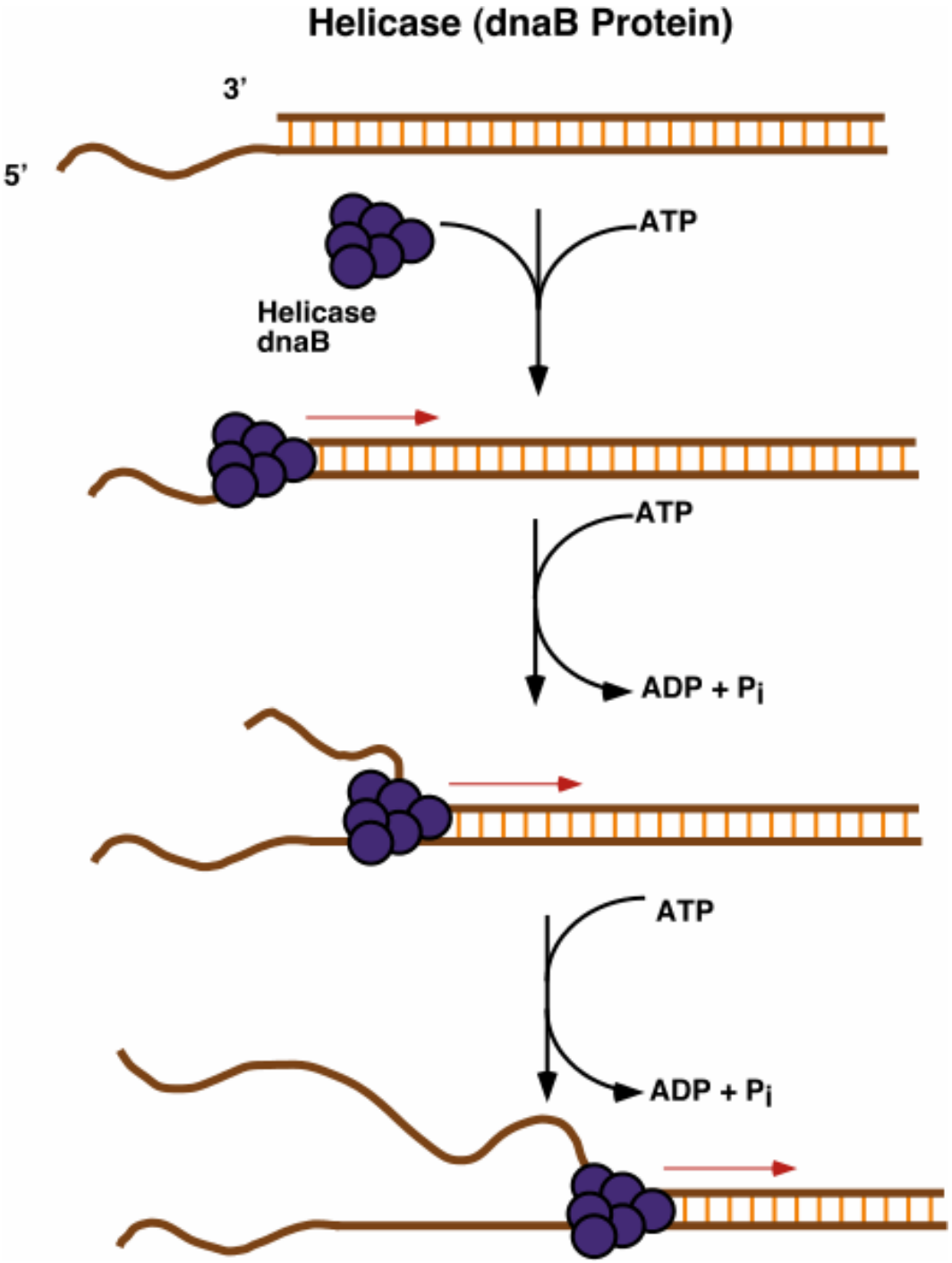
Neben der DNA-Polymerase III spielt die RNA-Polymerase I (Kornberg Enzym) eine wichtige Rolle:
Primer-Entfernung
Auffüllreaktion
Korrekturlesefunktion

Korrekturlesefunktion von Pol I:

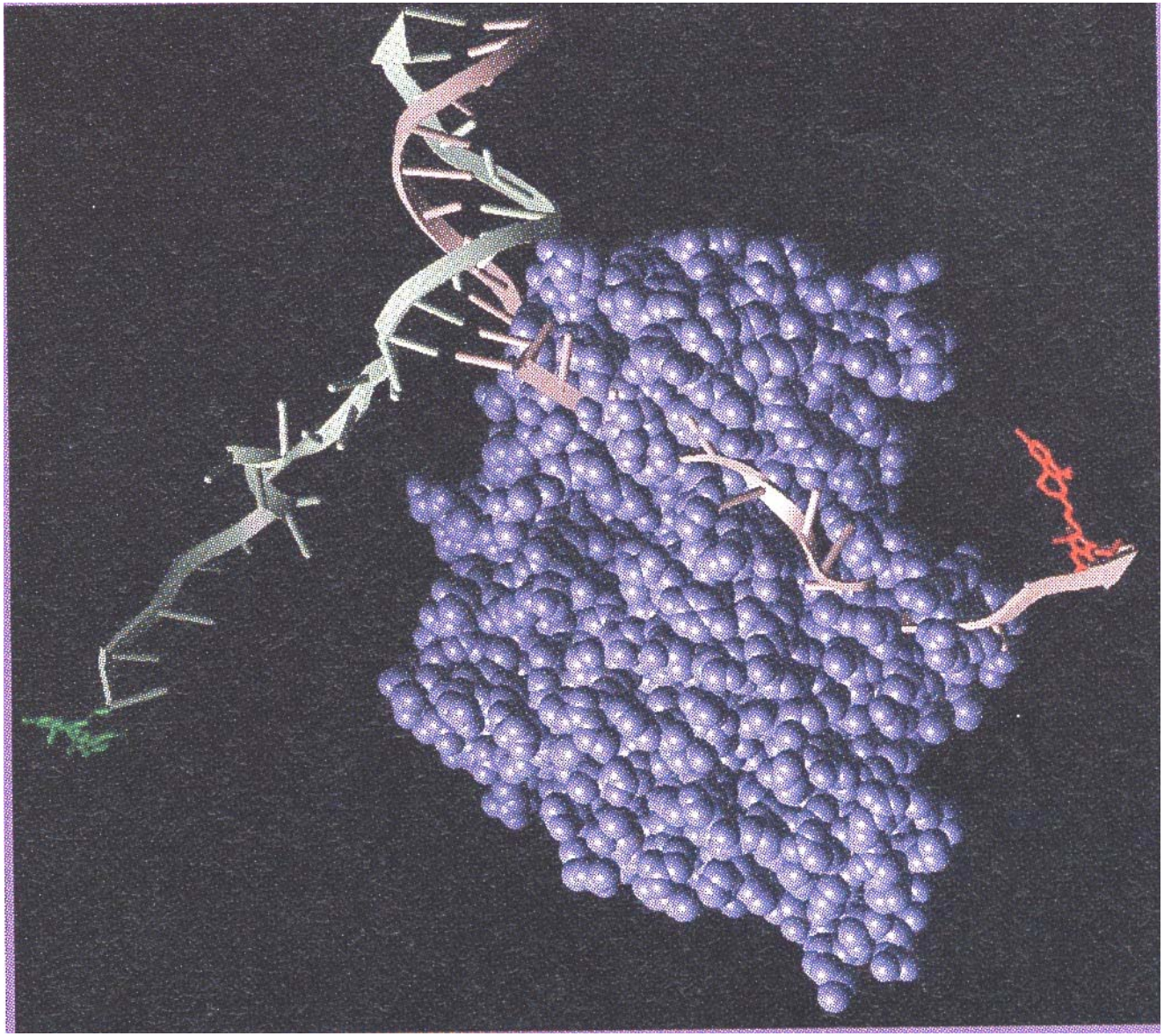


Die Pol I kann durch Subtilisin in zwei Fragmente gespalten werden:
Das große Fragment („Klenow-Fragment“) enthält nur noch die
5´-3´-Synthetase und die 3´-5´-Exonuklease,
aber nicht mehr die 5´-3´- Exonuklease-Aktivität

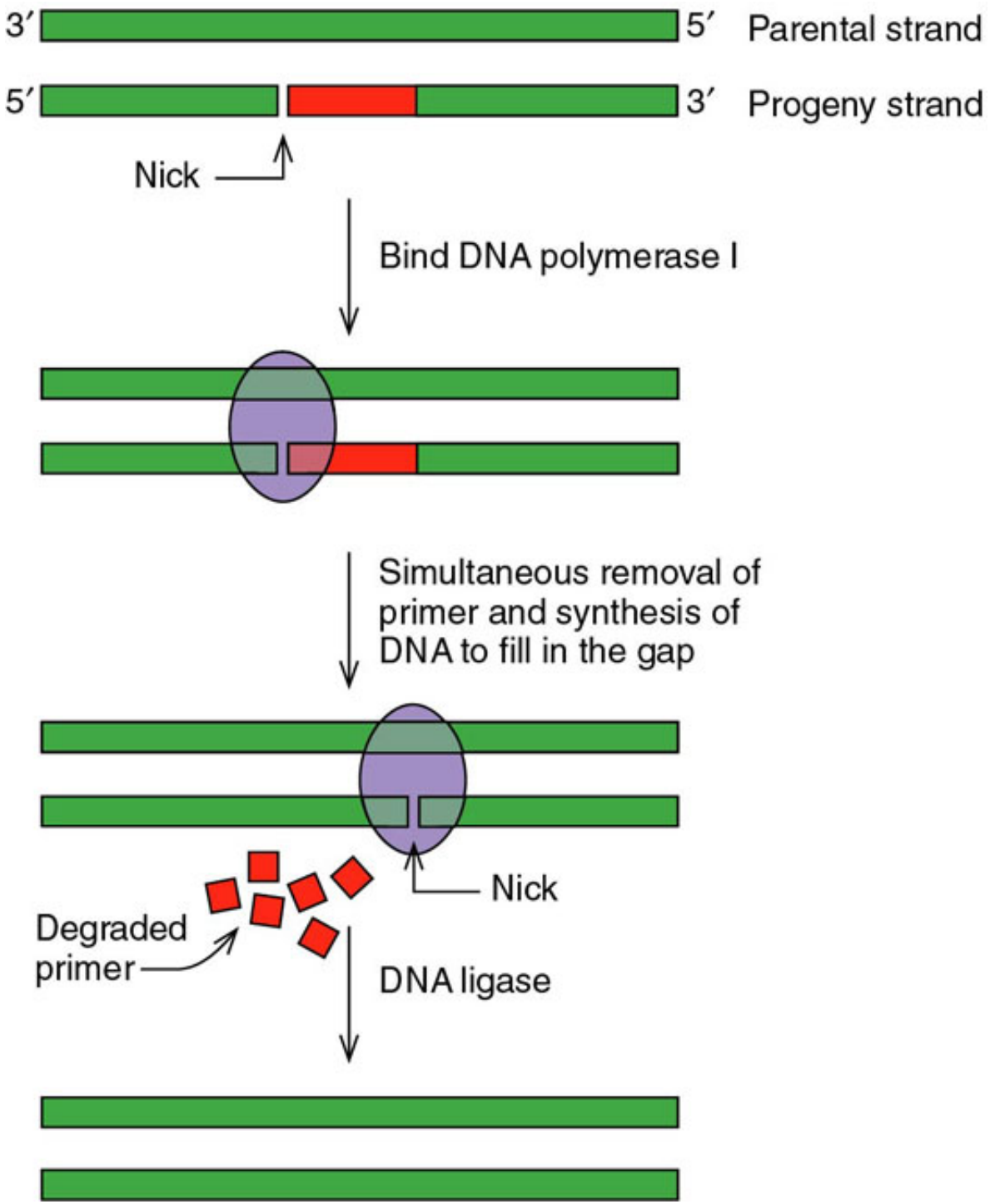
Die Helikase entwindet die Doppelhelix unter ATP-Verbrauch



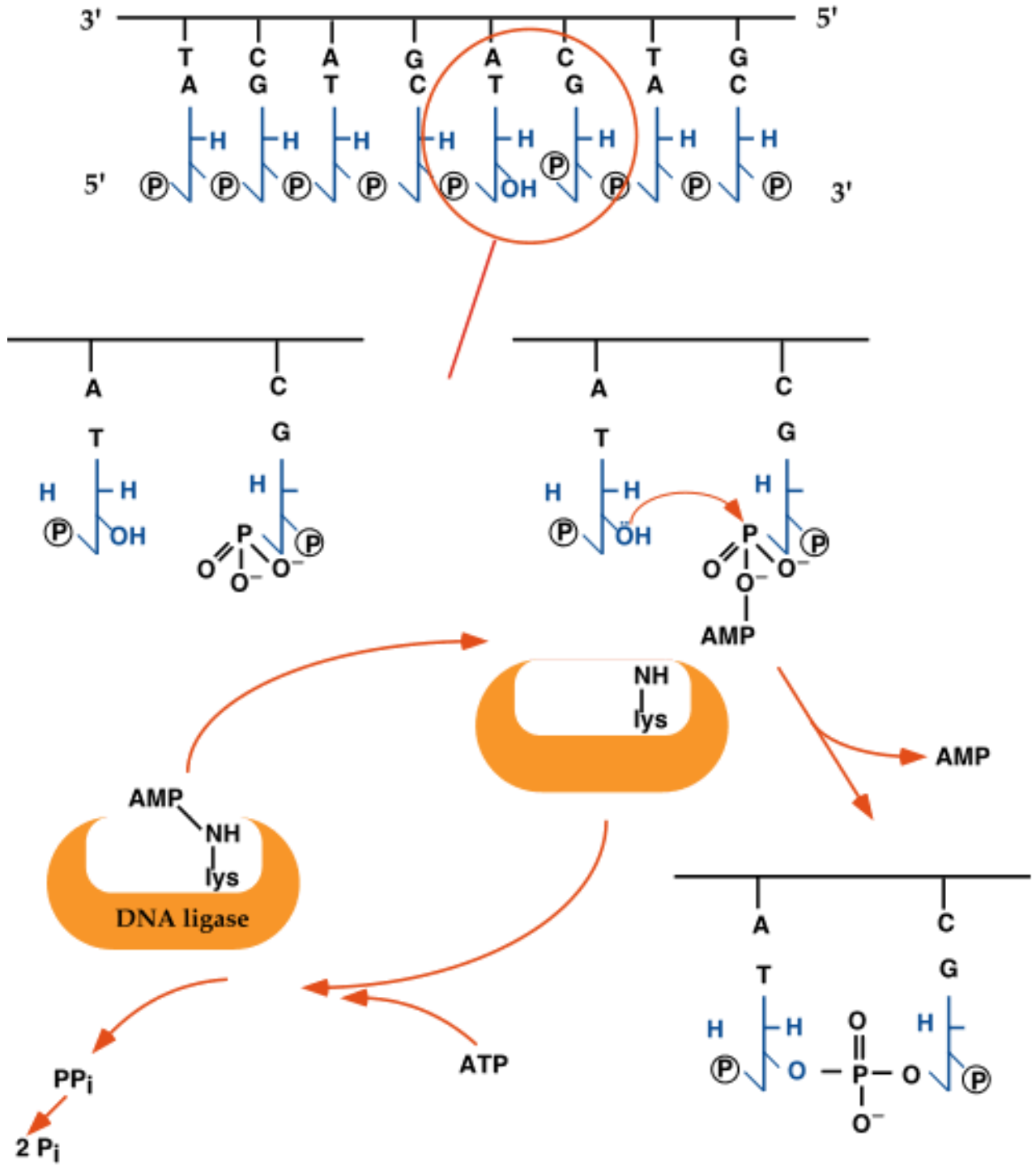
DNA-Helikase



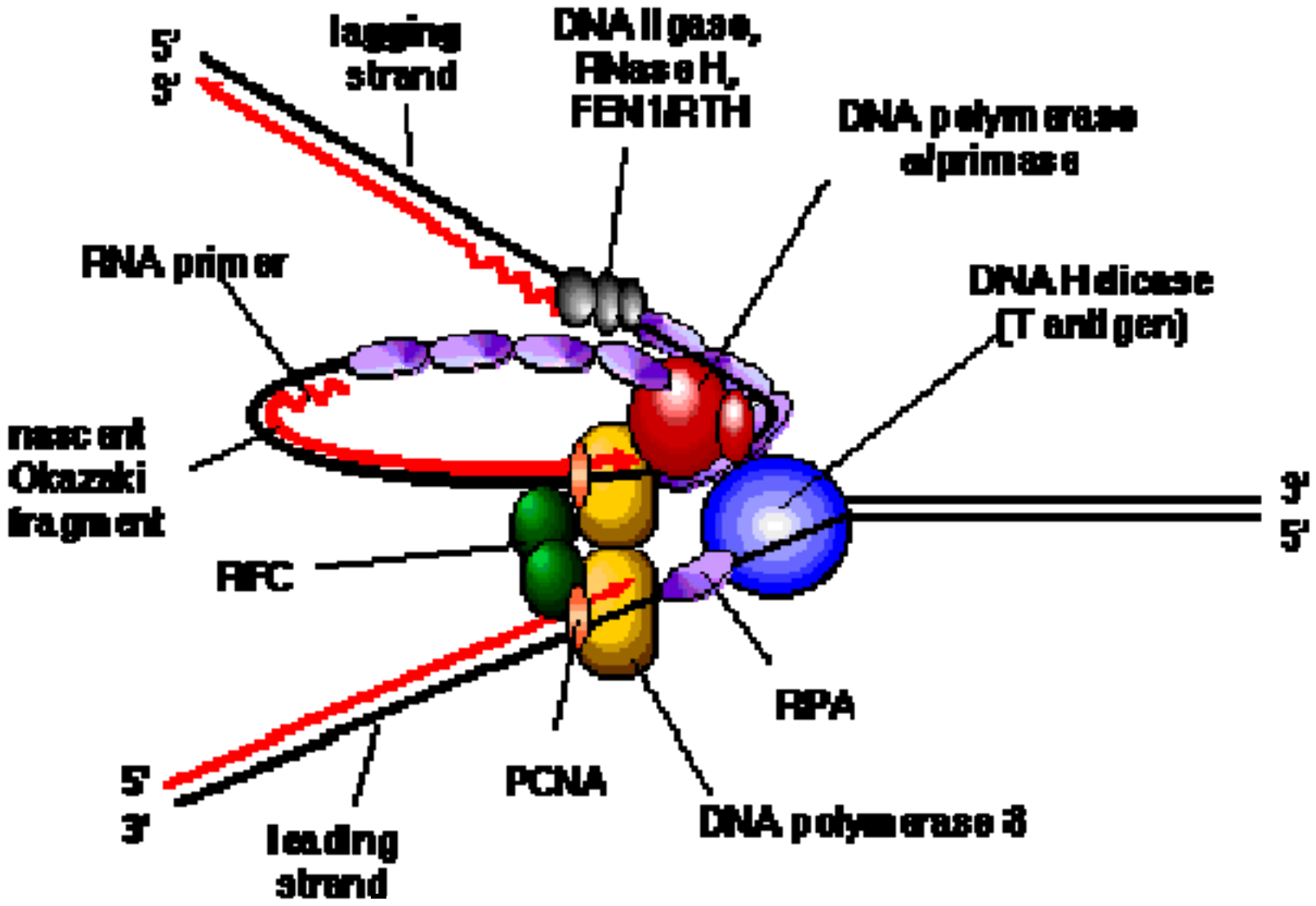
Entfernung der Primer und Schließen der Nicks

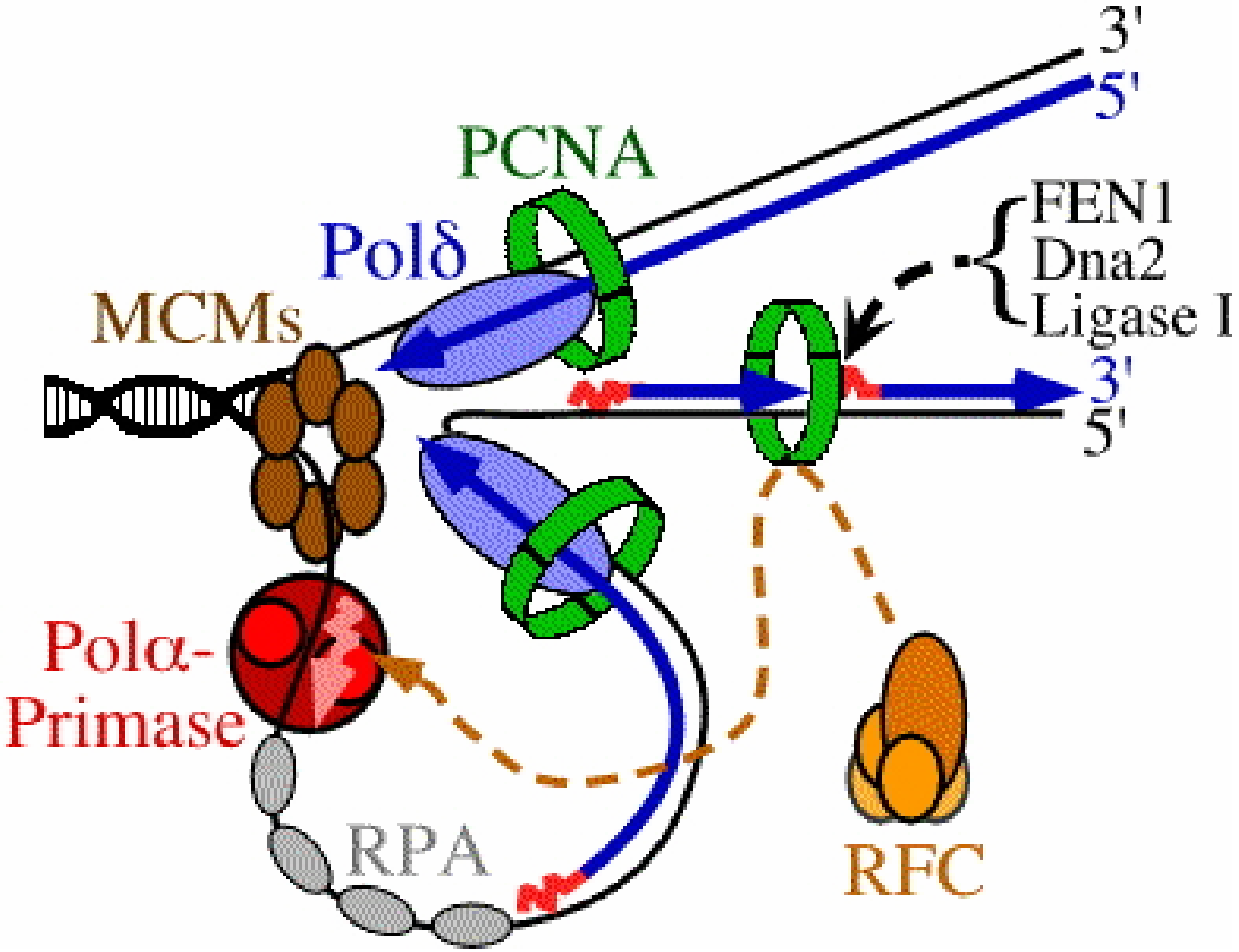


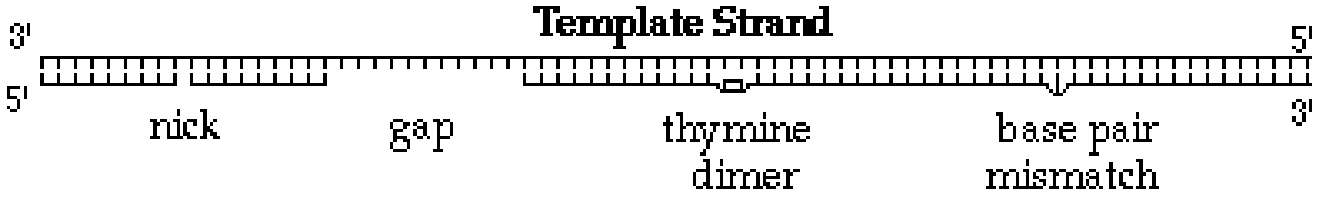
Funktion der DNA-Ligase



Replikationsgabel bei Eukaryoten

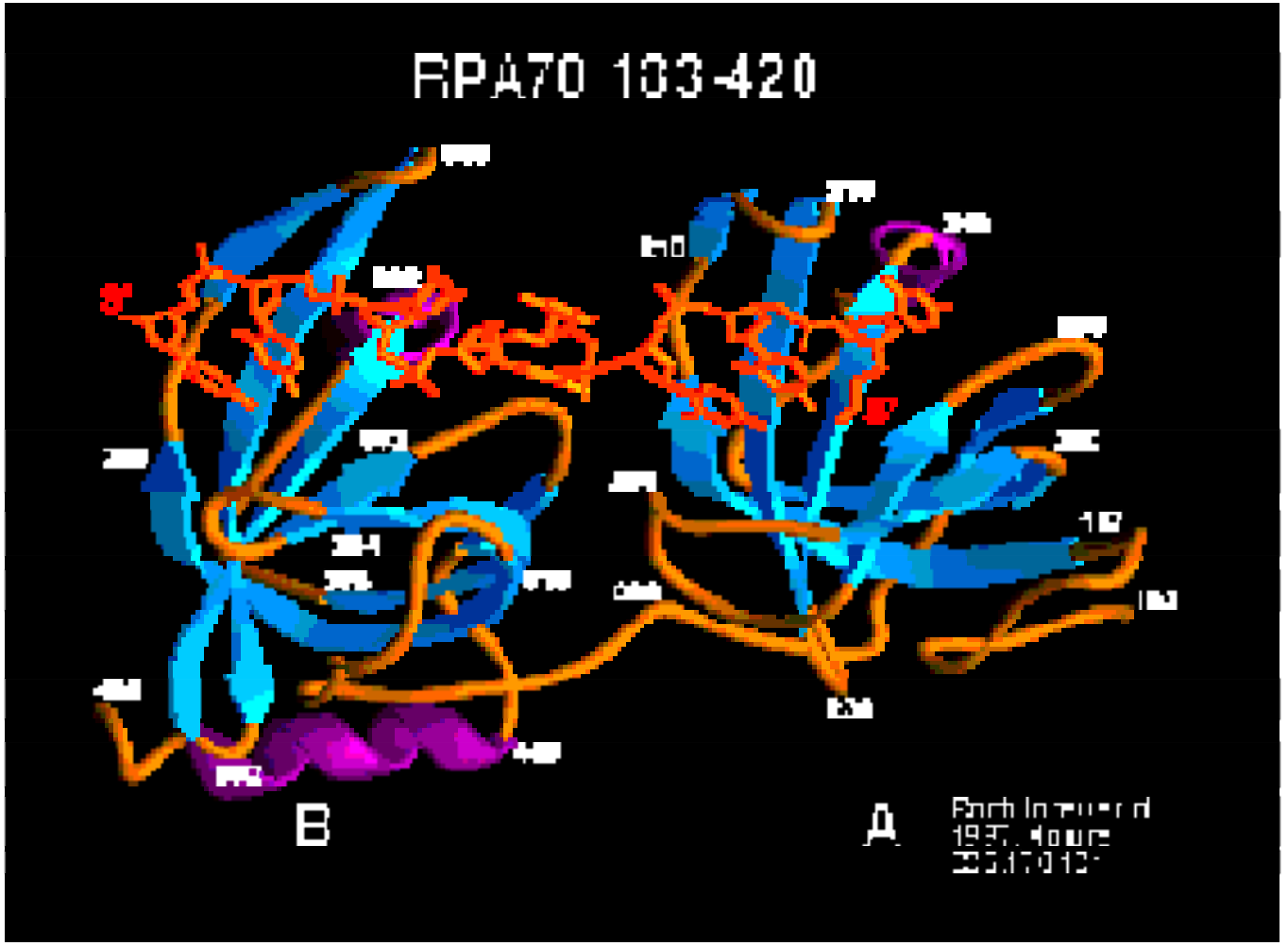



3'
5'
PCNA
Polδ
FEN1
Dna2
Ligase I
MCMs
3'
5'
Polα-
Primase
RPA
RFC

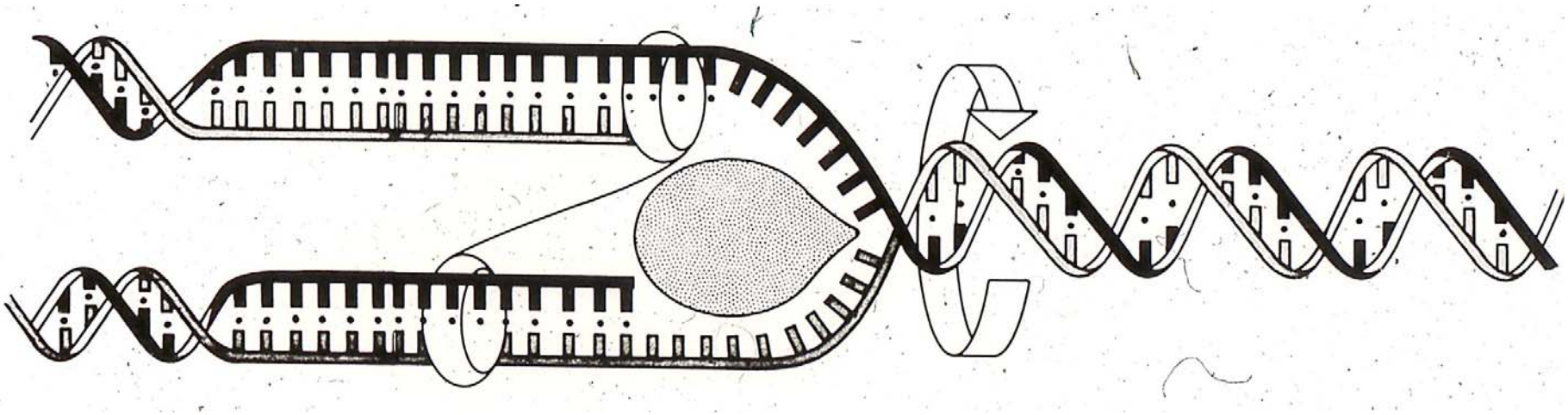


DNA Pol I (polymerase)	Nick translation	Fill-in gap	None	None
Pol I 3'->5' exo	3' mismatch hydrolyzed	3' mismatch hydrolyzed	None	None
Pol I 5'->3' exo	None	None	Removed by nick translation	Removed by nick translation
DNA ligase	Seals nick	None	None	None
DNA Pol III	None	Fill-in gap	None	None
SSB	None	Binds tightly	None	None
primase	None	Makes an RNA primer	None	None
helicase	None	Loads at 5' end	None	None
Eukaryotic Pol α	3' mismatch hydrolyzed	Synthesis on lagging strand	None	None
Eukaryotic Pol δ	3' mismatch hydrolyzed	Synthesis on leading strand	None	None
photolyase	None	None	Removes dimer leaving a gap	None
UvrABC endonuclease	None	None	Removes mismatch leaving a gap	None

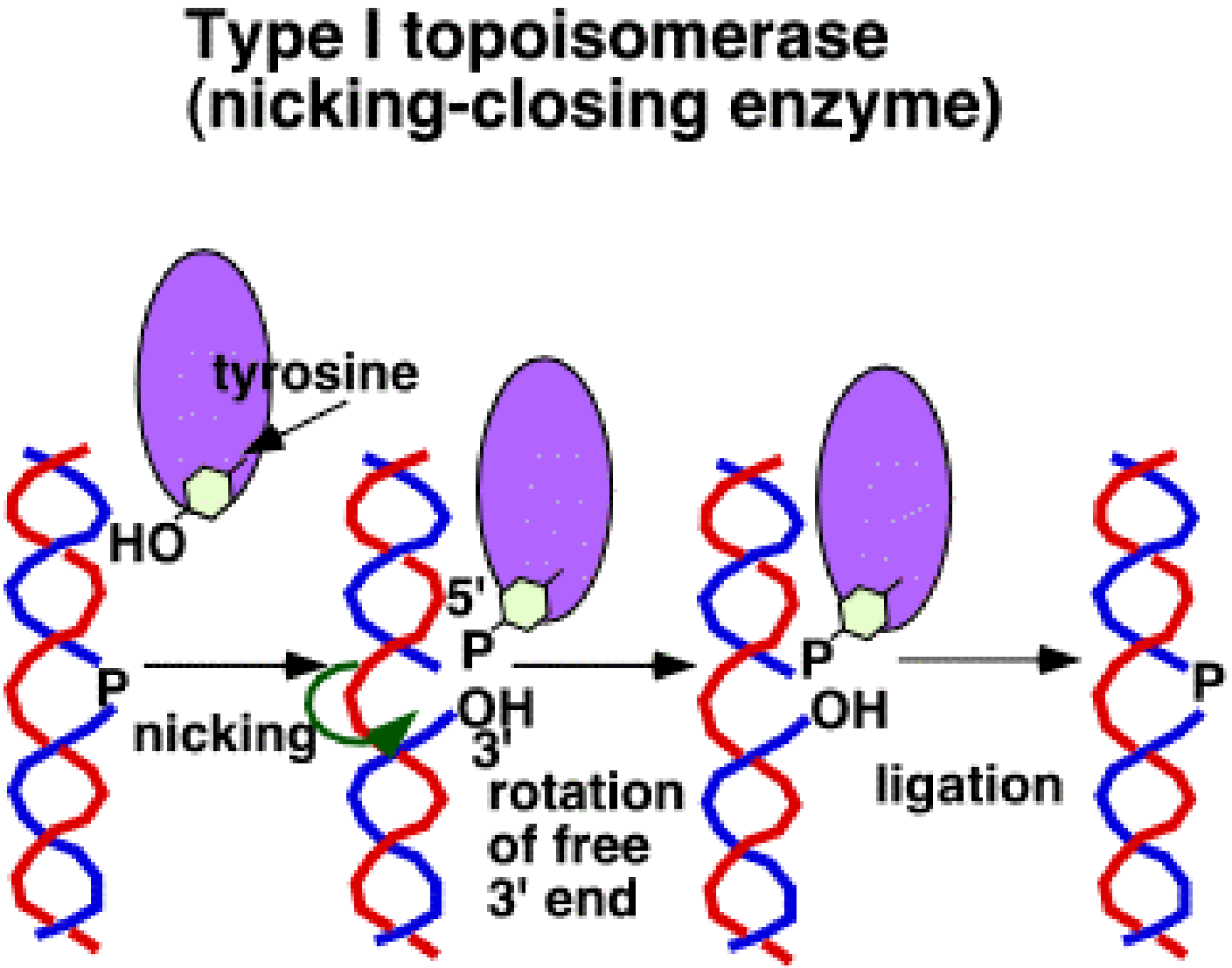
Struktur RPA



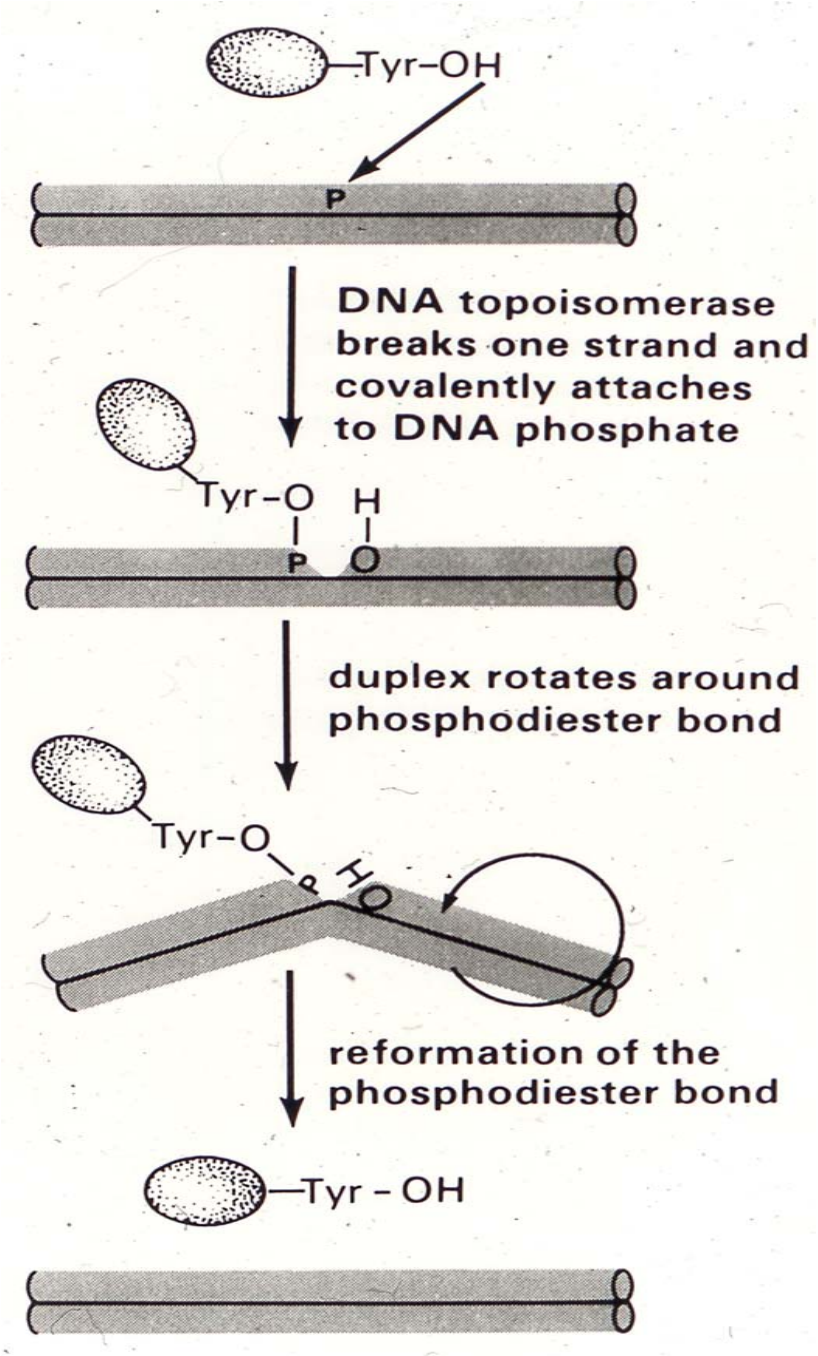
Superhelikaler Stress durch Entwindung der Doppelhelix



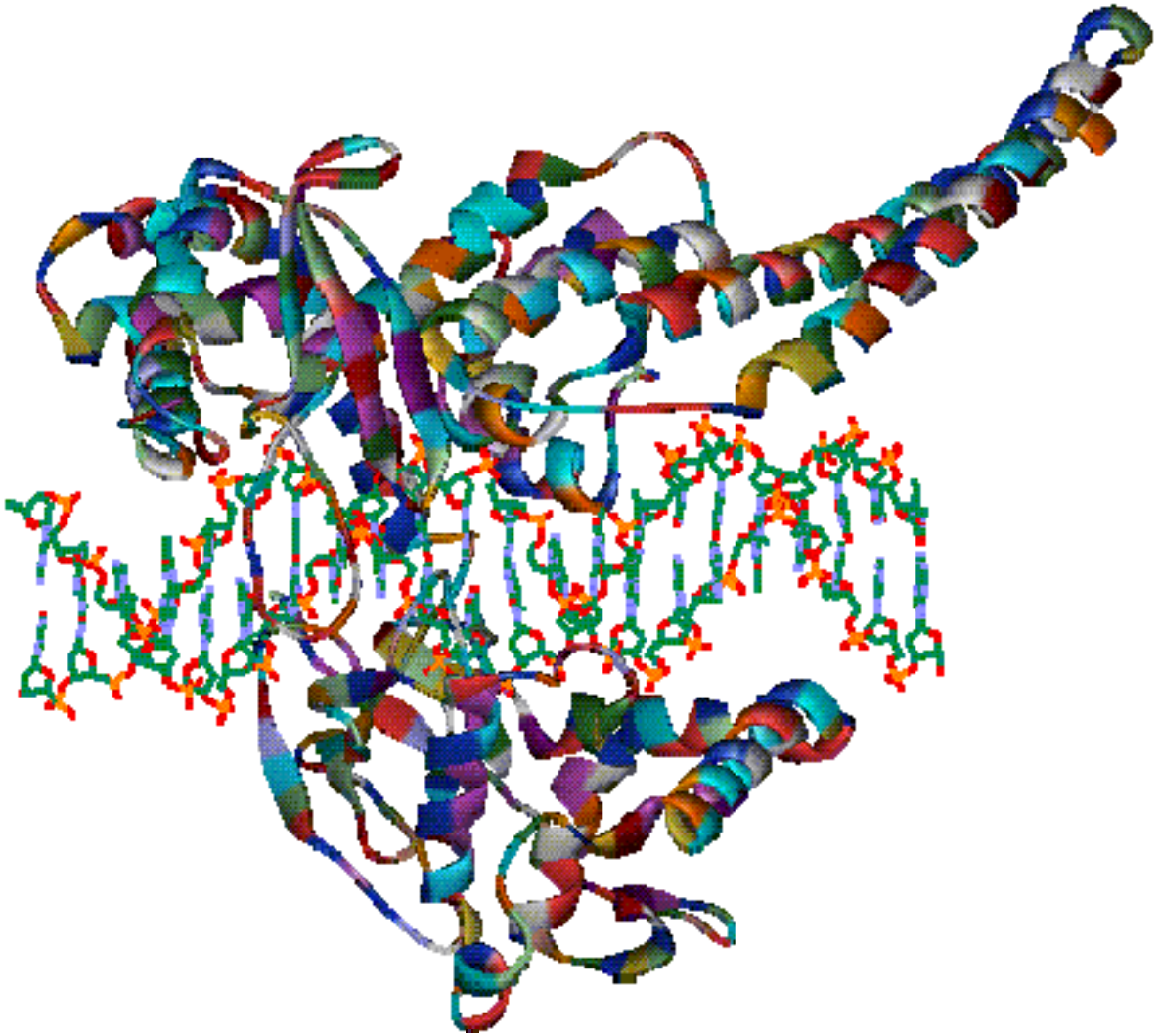
Abbau des superhelikalen Stresses durch Topoisomerasen



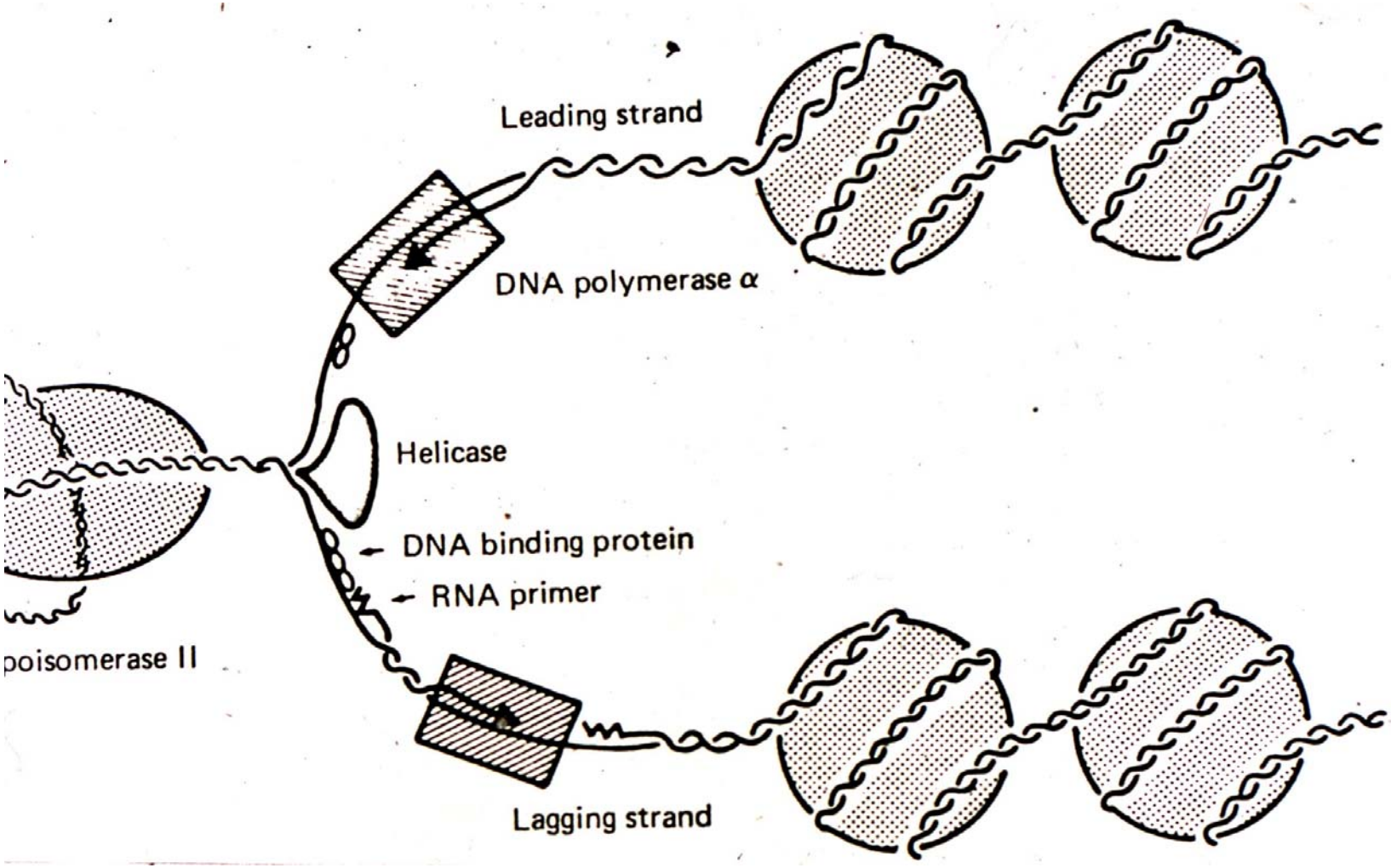
DNA-Topoisomerase



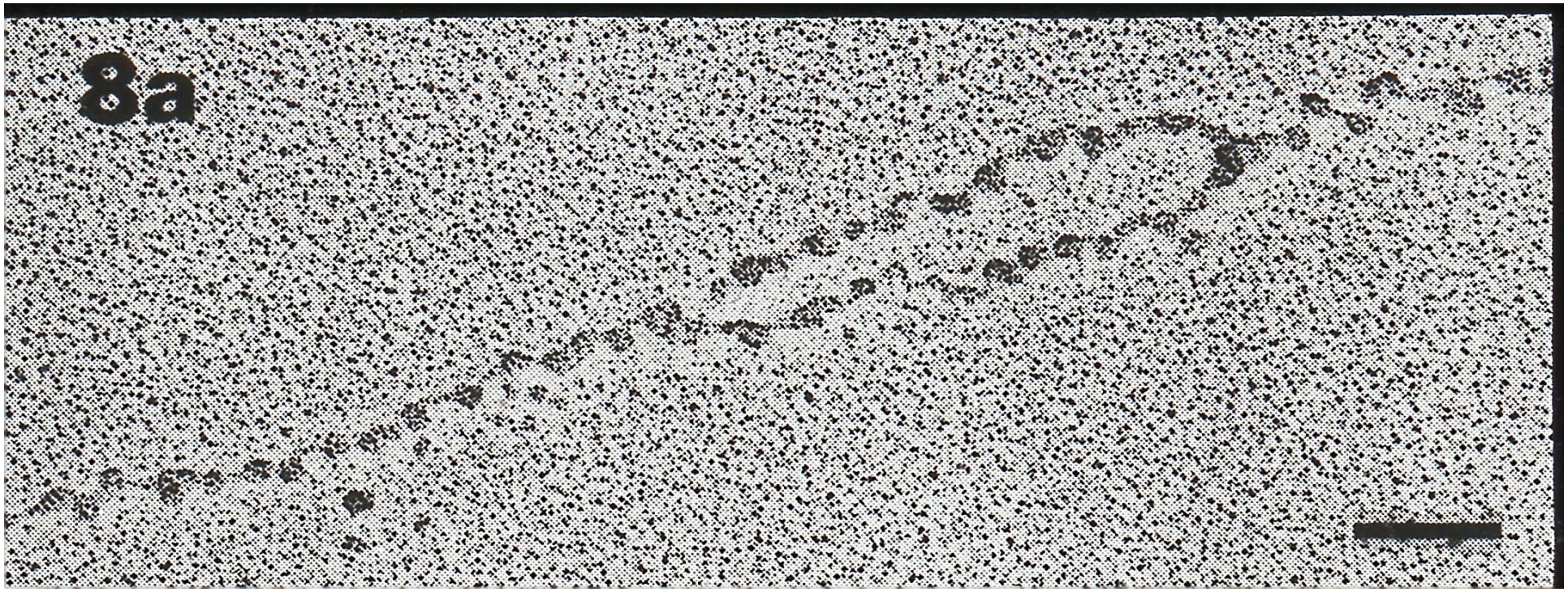
Struktur Topoisomerase

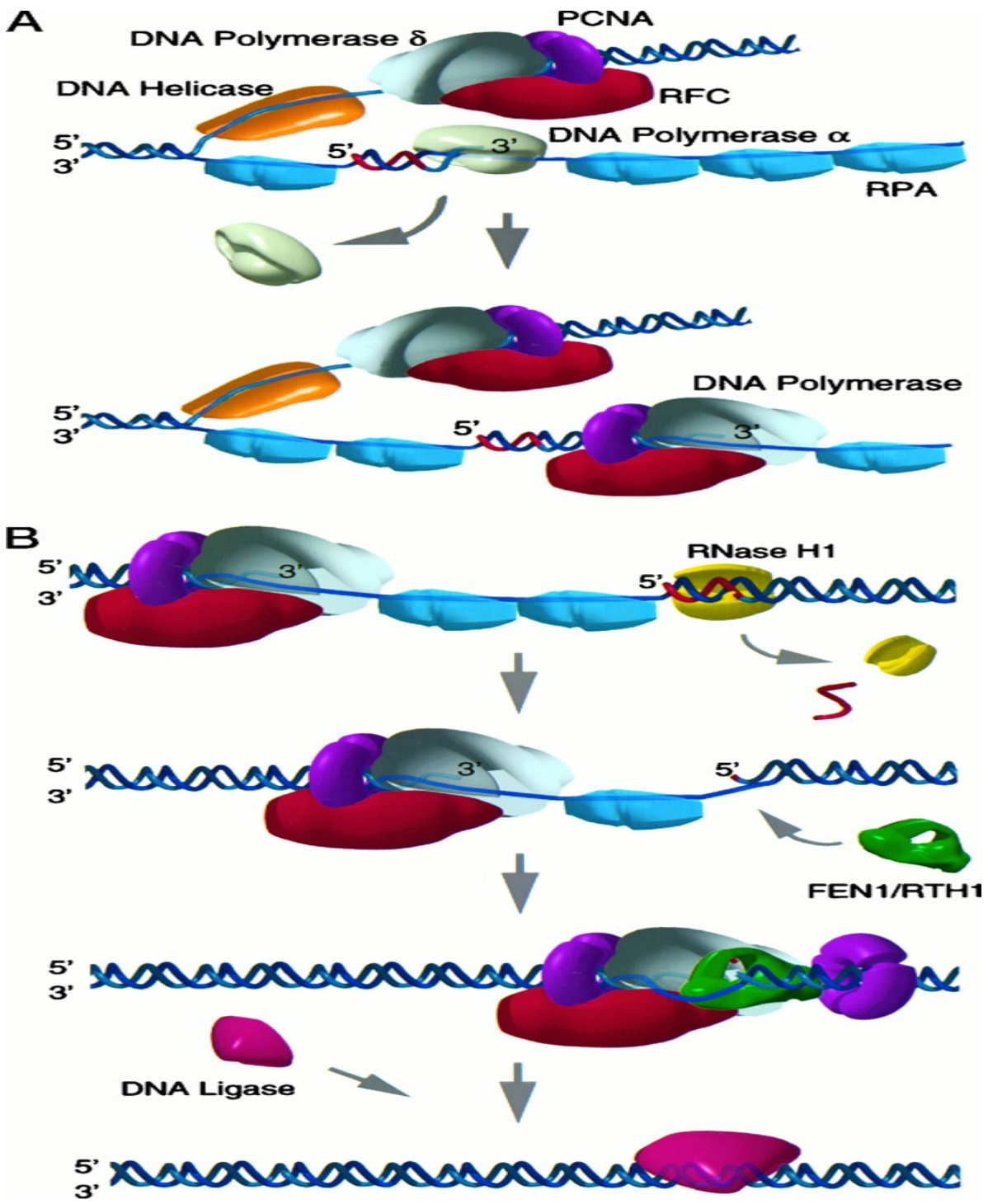


Replikation findet im Chromatin statt



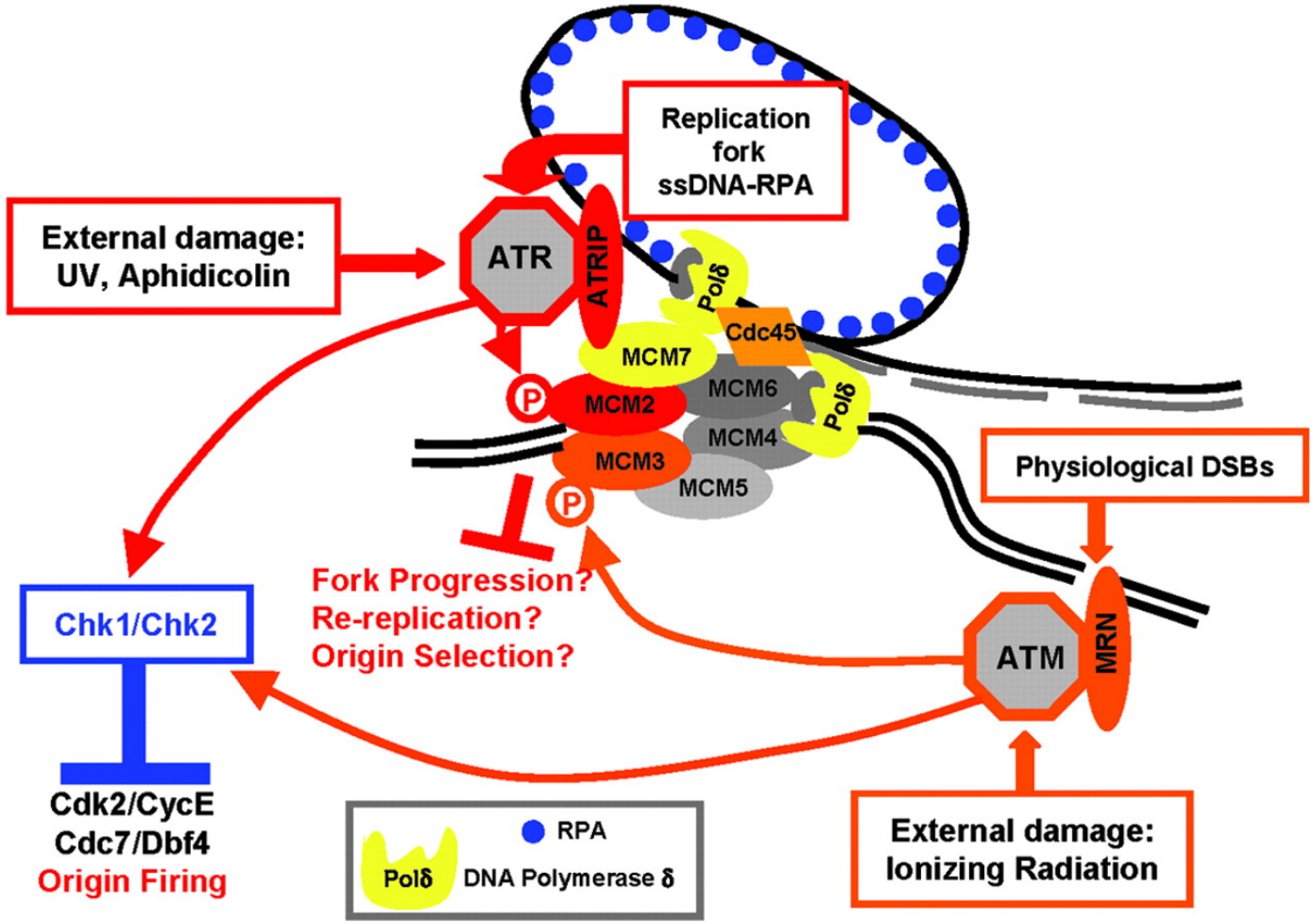
Bei Eukaryoten ist die DNA während der Replikation als Chromatin verpackt



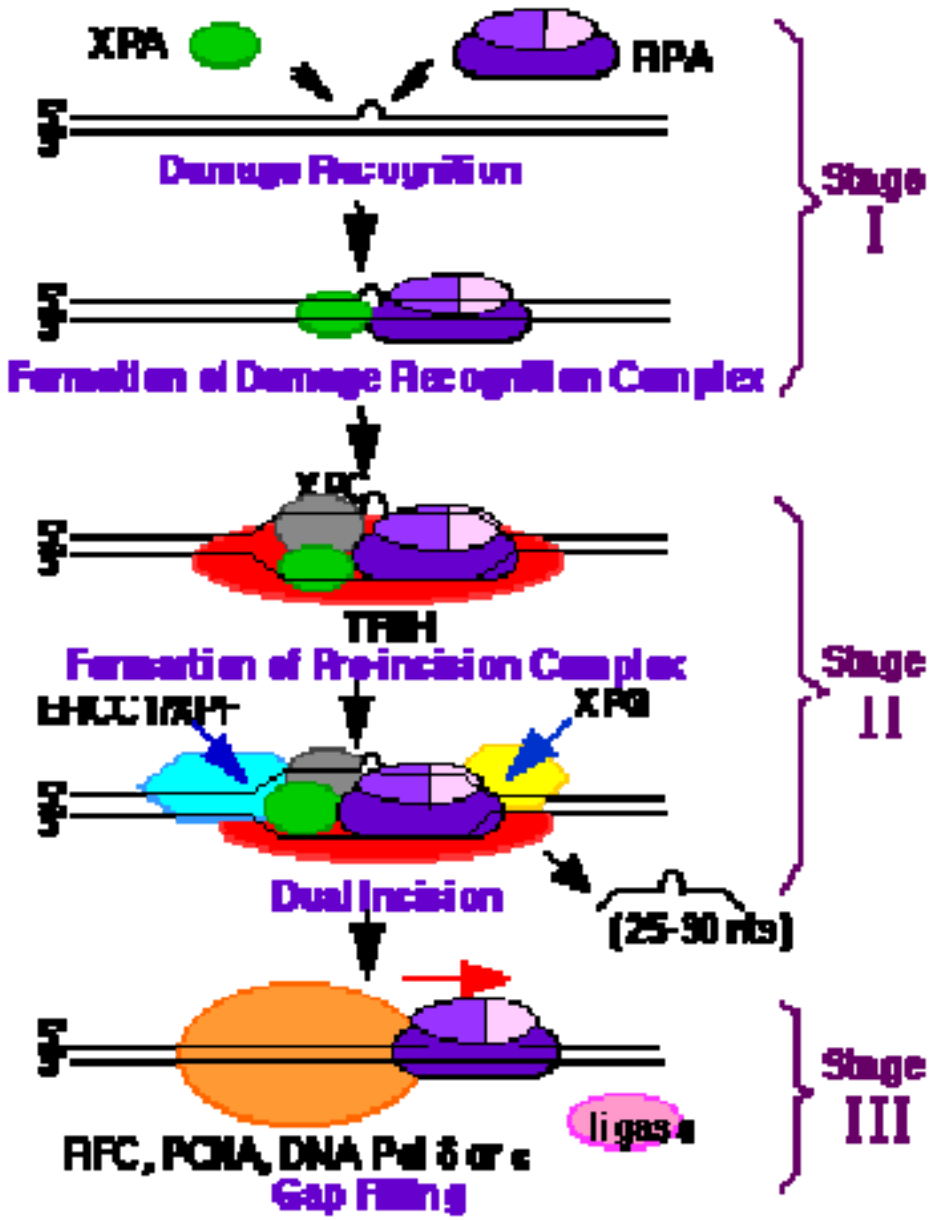

A
PCNA
DNA Polymerase δ
DNA Helicase
RFC
5'
3'
5'
3'
DNA Polymerase α
RPA
DNA Polymerase
5'
3'
5'
3'
B
RNase H1
5'
3'
3'
5'
S
5'
3'
3'
5'
FEN1/RTH1
5'
3'
DNA Ligase
5'
3'

Protein	Funktion 1	Literaturlink
DNA-Polymerase α	DNA-Synthese „lagging strand“	
DNA-Polymerase δ	DNA-Synthese „leading stand“	
ORC	Erkennung/Aktivierung Ori: Assembly of preRC	
Cdt	Licensing protein; oncogene	
RFC	Clamp loader of PCNA	http://cat.inist.fr/?aModele=afficheN&cpsidt=15405201
RPA	Single stranded binding protein	http://nar.oxfordjournals.org/cgi/content/full/34/15/4126
MCM	Origin assambly factor; später Helikase	http://www-rcf.usc.edu/~forsburg/MCM.html
PCNA (=Cyclin)	Processivity factor of DNA-Pol delta (stimul. Repl.>10x)	http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=176740
RNAse HI	Primerentfernung	http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=84926
FEN1	„flap endonuclease“; Entfernung letztes Primernukleotid und 5‘ „flaps“	http://cat.inist.fr/?aModele=afficheN&cpsidt=1189753

Fig. 1. A schematic view of the signaling pathways inhibiting DNA replication. ssDNA-RPA intermediates and DSBs arise as a consequence of external insults (irradiation and polymerase inhibitors) or during normal replication



Shechter, David and Gautier, Jean (2004) Proc. Natl. Acad. Sci. USA 101, 10845-10846



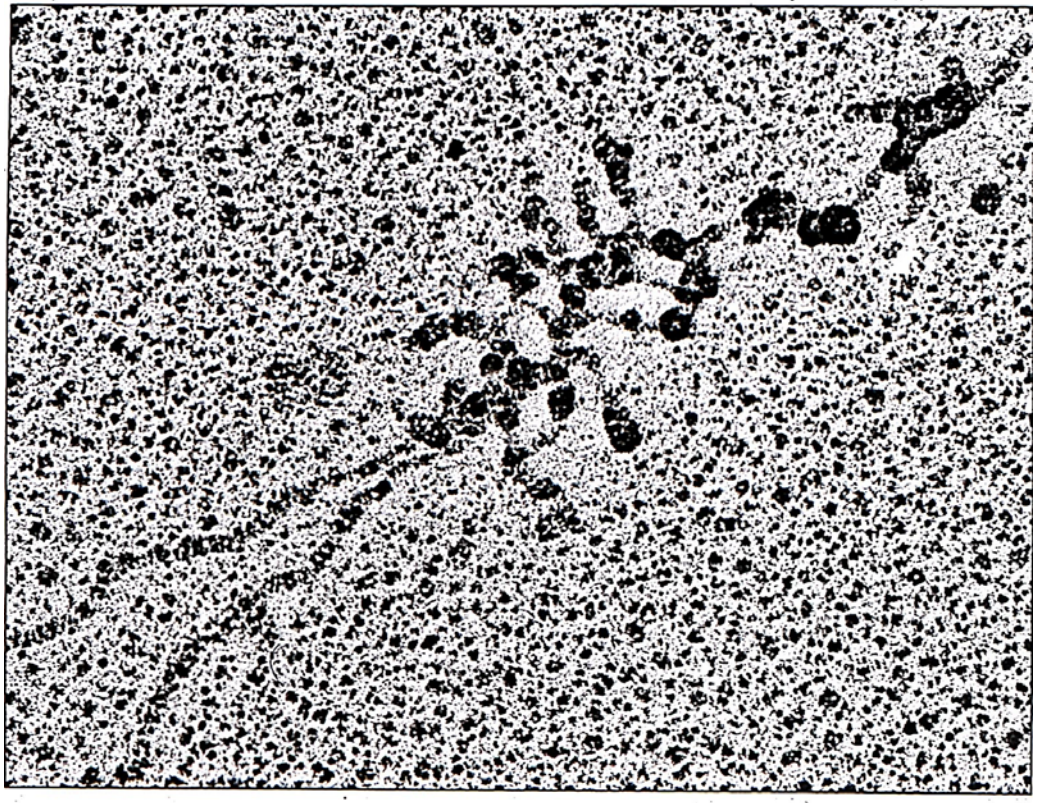
Model of Nucleotide Excision Repair (NER)

Transkription und Replikation

ASM News

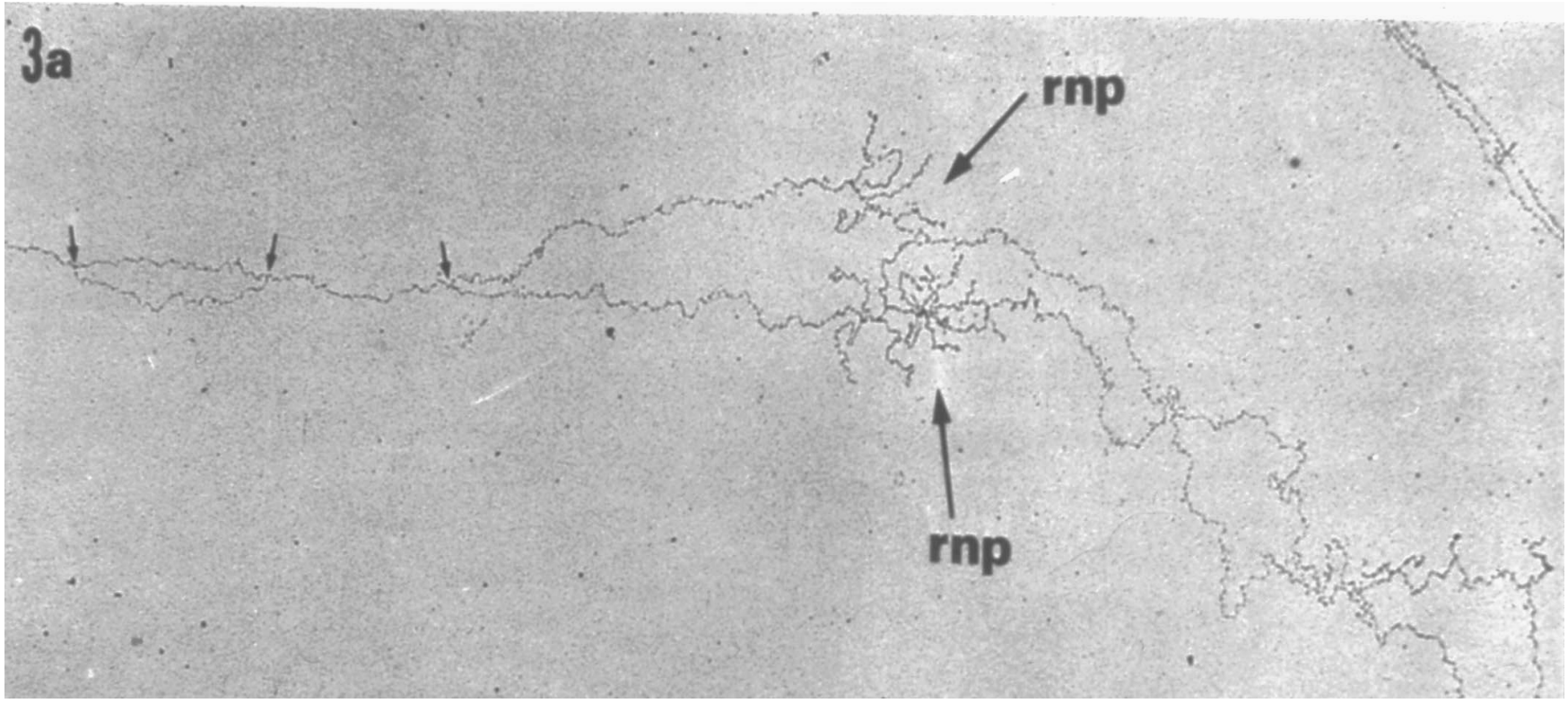
Volume 59
Number 9
September 1993

American Society for Microbiology

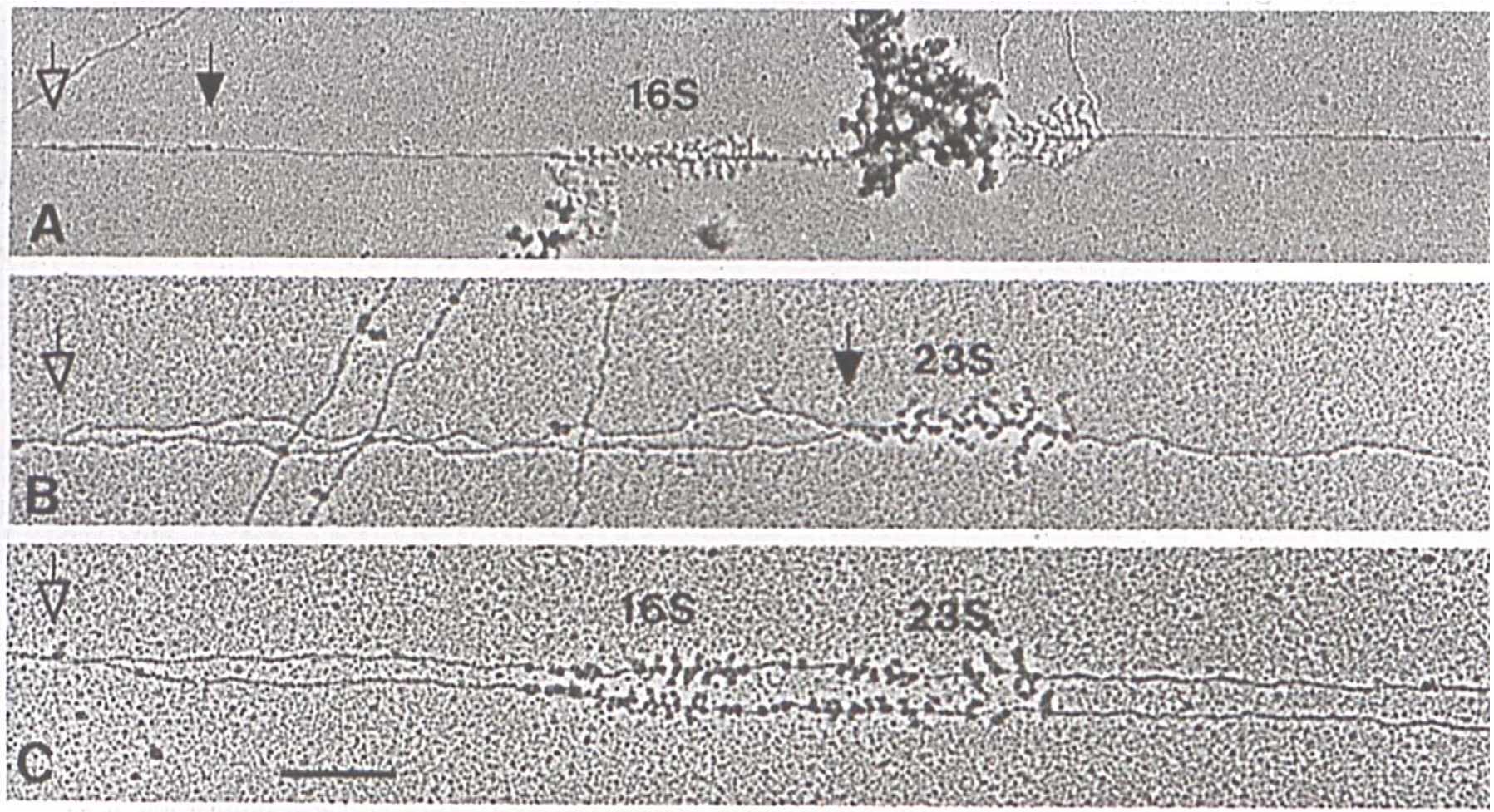


Right of Way in Replication and Transcription

Transkription und Replikation, gleichsinnig

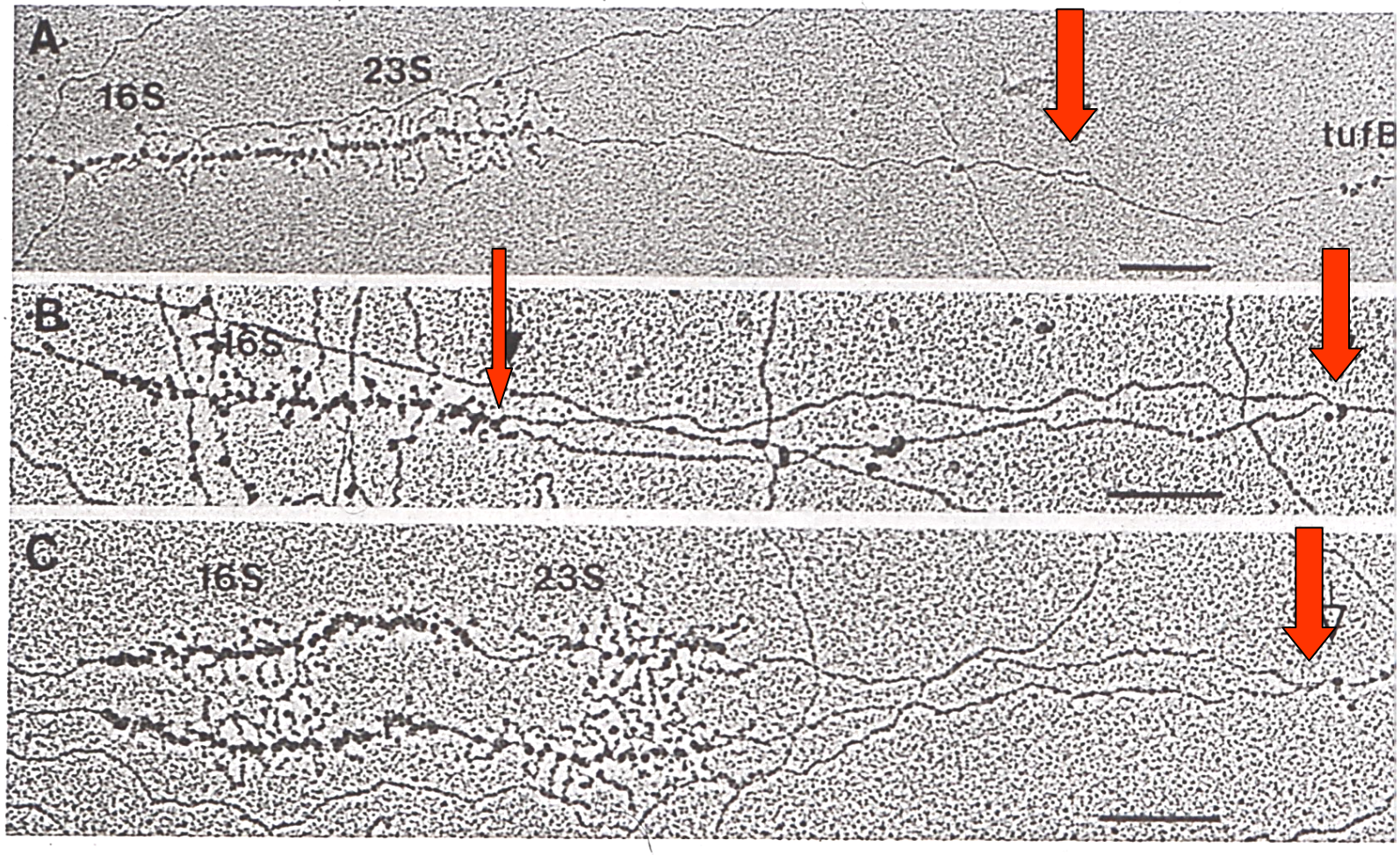


Replikation und Transkription, gleiche Richtung



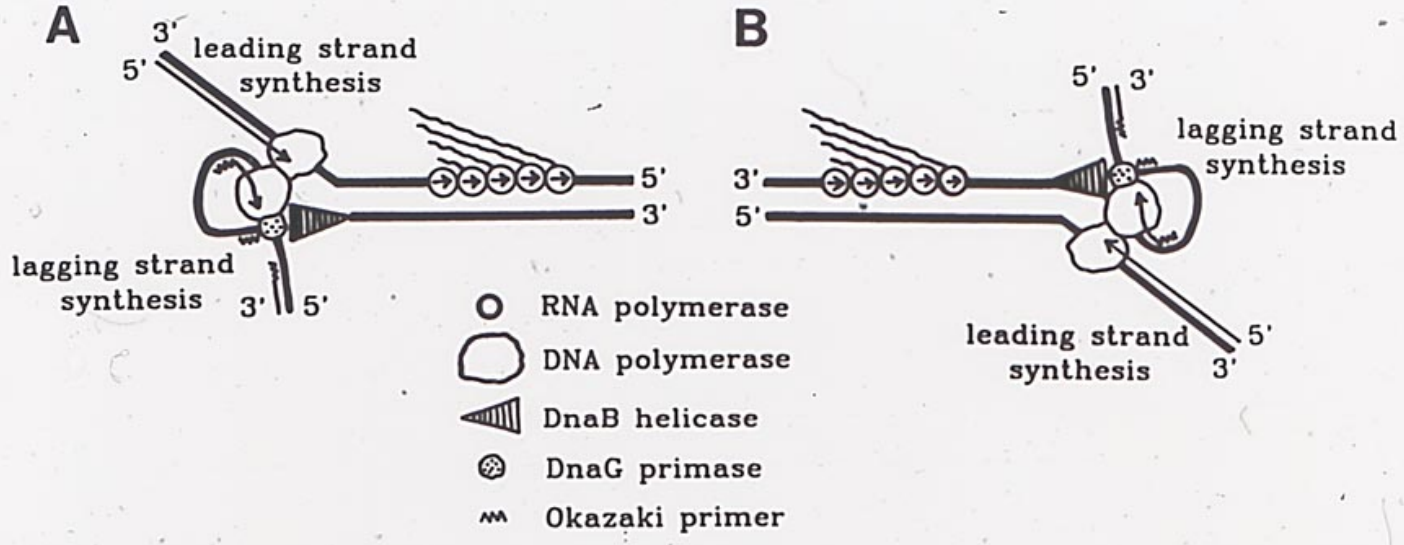
Replikation und Transkription, gegenläufige Richtung, die Transkription wird durch Replikation unterbrochen

Figure 5. CF95: Micrographs of Oppositely Oriented Replication and Transcription



Replikation und Transkription

Figure 1. Relative Orientation of Replication and Transcription Affects the Subunits Impacted When DNA and RNA Polymerases Collide

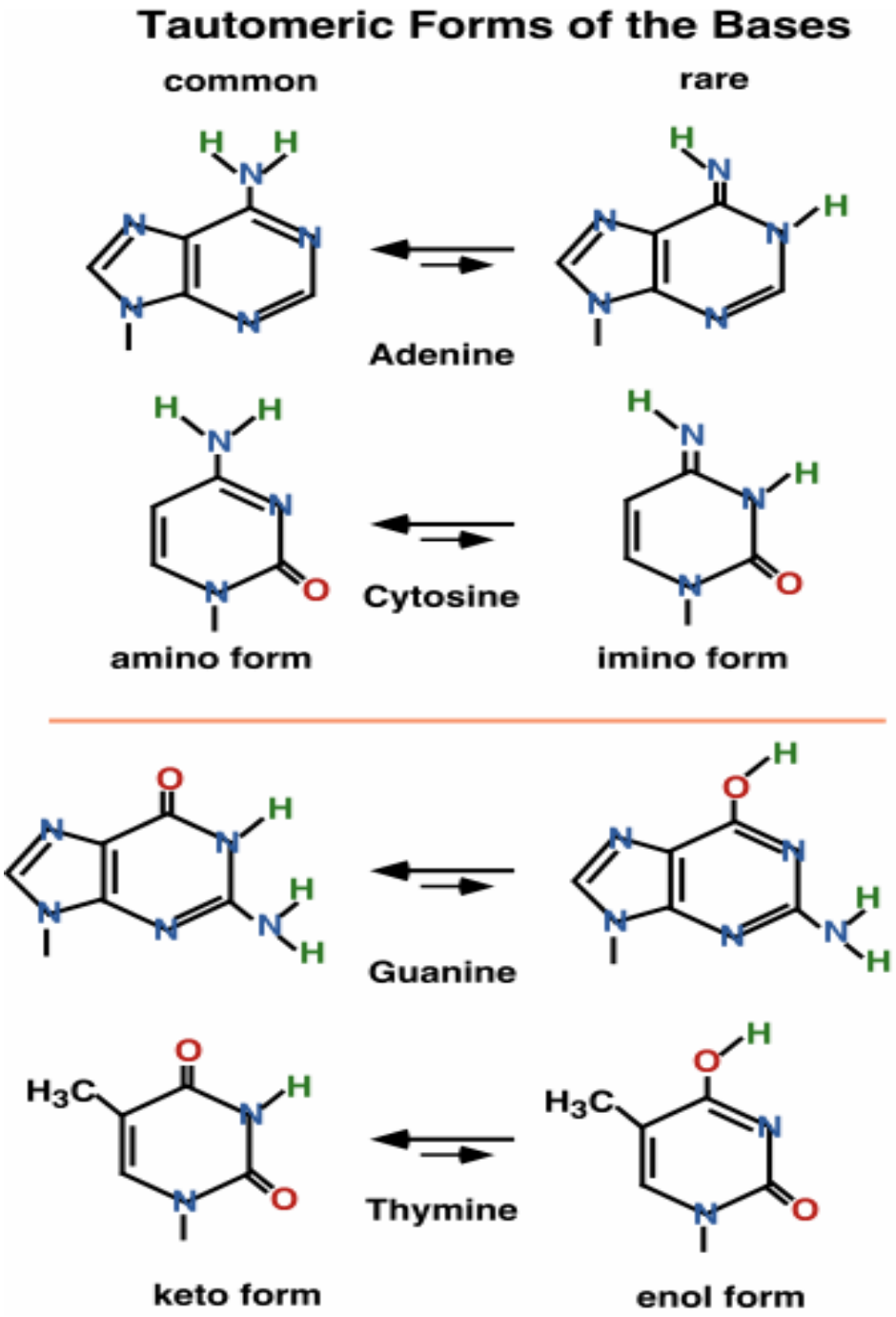


Replication forks are drawn in the asymmetric dimer configuration for coupled leading- and lagging-strand DNA synthesis. Thick lines, parental strands of the DNA duplex; thin lines, newly synthesized DNA. RNA and DNA polymerases move along the particular DNA strand which serves as the template for their activities in a 3′-to-5′ direction. (A) Replication fork and RNA polymerases moving in the same direction. The leading-strand DNA polymerase is on the same DNA strand as the RNA polymerases. (B) Replication fork and RNA polymerases moving in opposite directions. The leading-strand DNA polymerase and the RNA polymerases are on opposite DNA strands. The DnaB helicase, moving in a 5′-to-3′ direction, is on the same strand as the RNA polymerases.

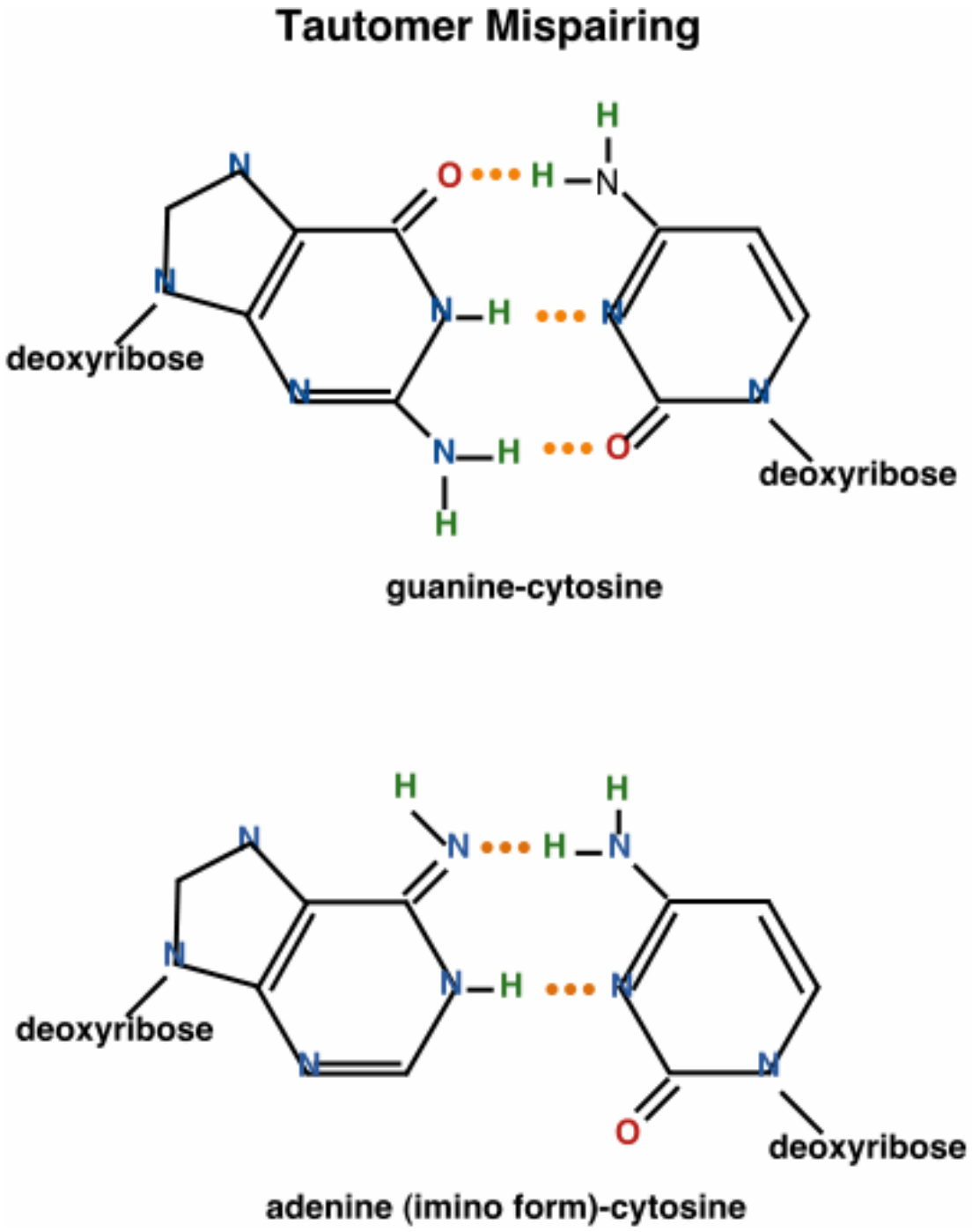
Fehler bei der Replikation

....und ihre Reparatur

Tautomerie der Nukleobasen

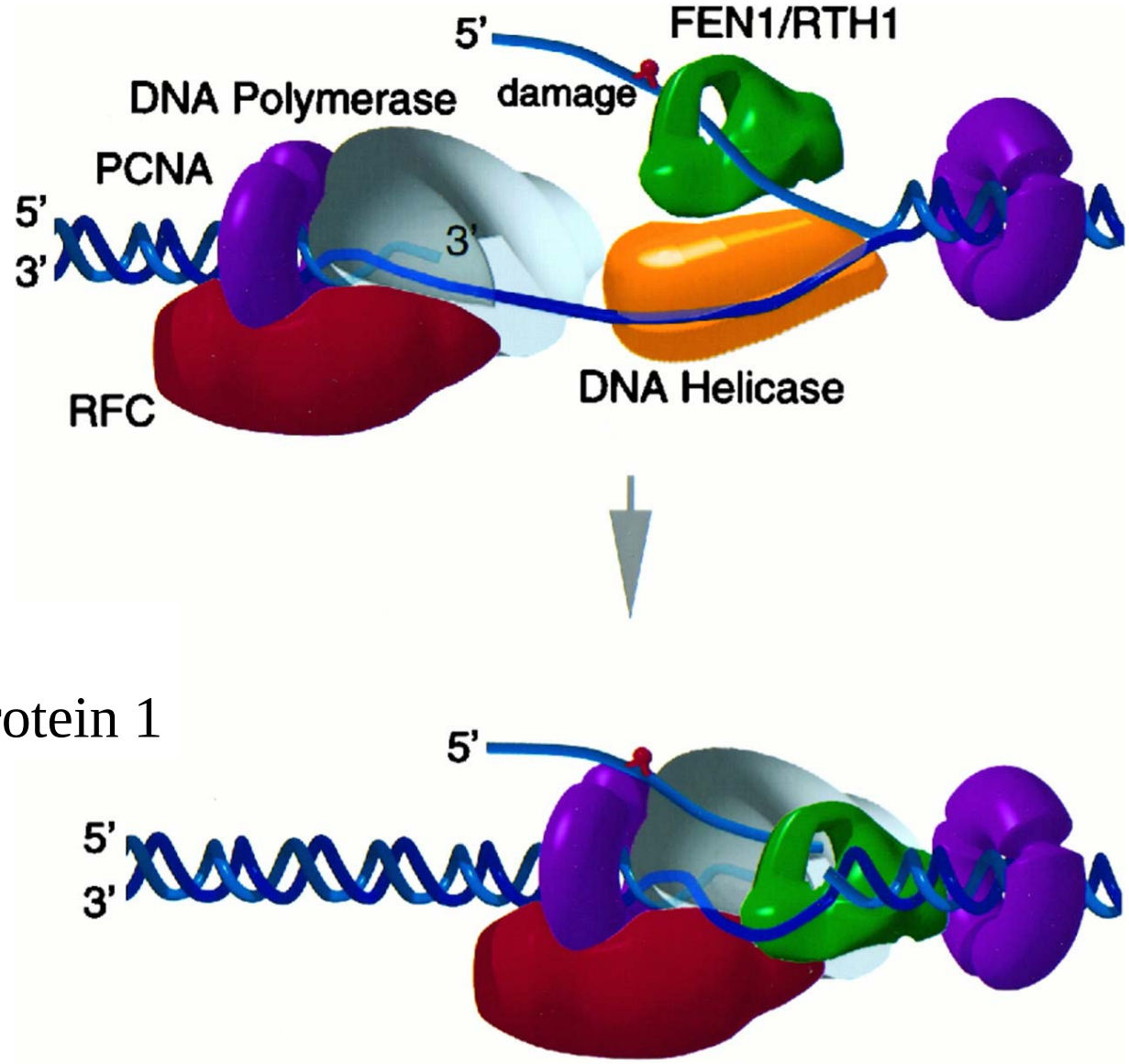


Replikationsfehler durch tautomere Formen der Nukleobasen



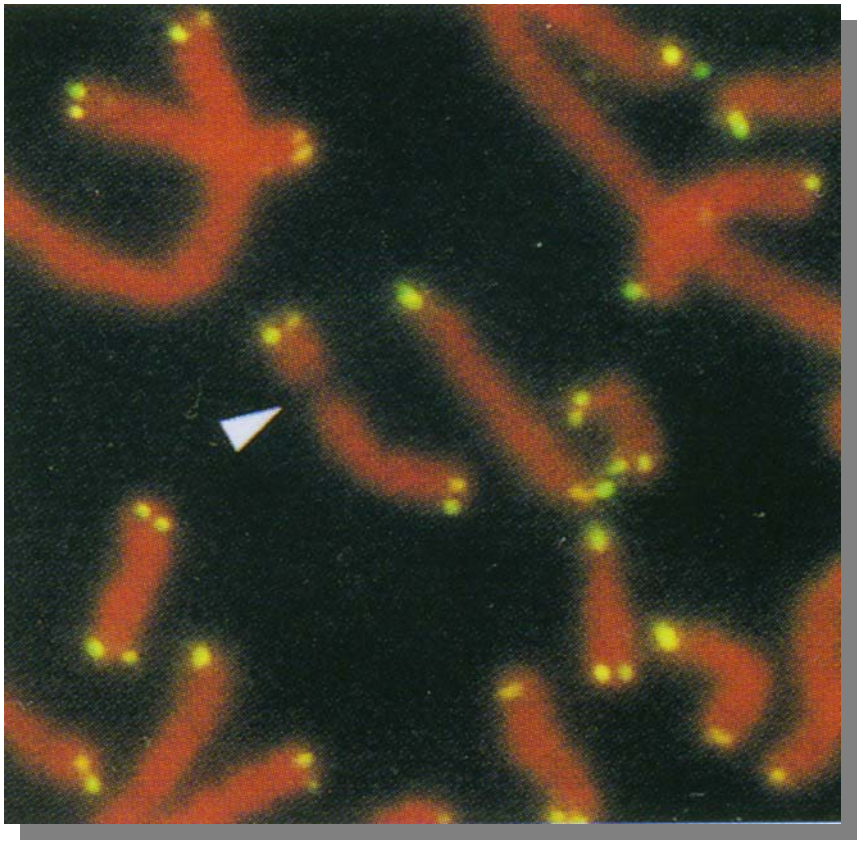
Reparatur von Replikations-fehlern

FEN1=
flap structure specific protein 1



Replikation der Telomere

Speziell gebaute Chromosomen-Enden (Telomere)
sowie eigens dafür vorgesehene Replikationsenzyme
(Telomerase) sorgen dafür, dass die Verluste kompensiert werden

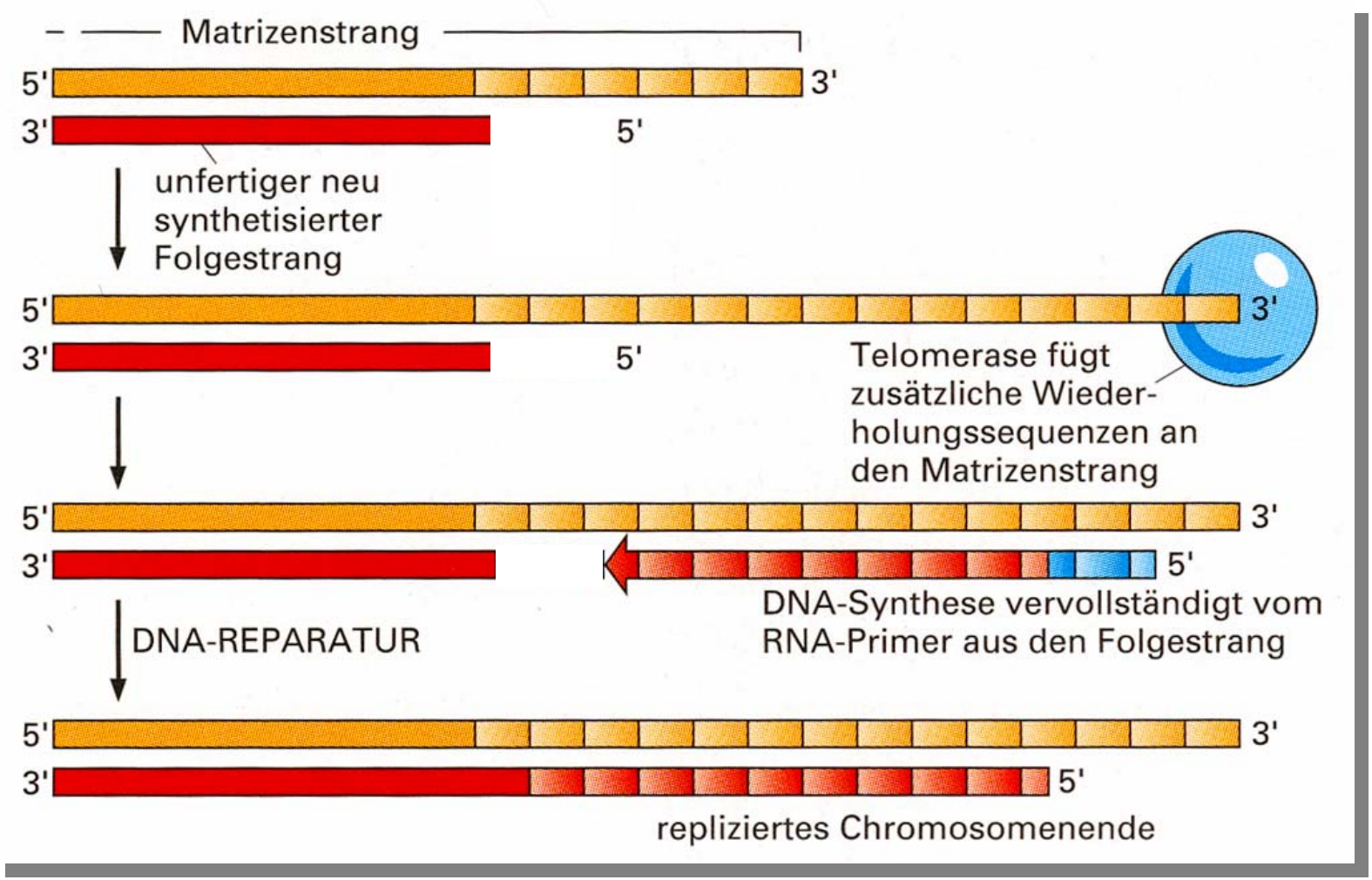


• die Telomer-DNA der meisten Tiere und Pflanzen enthält kurze, tandem-repetitive Sequenzen

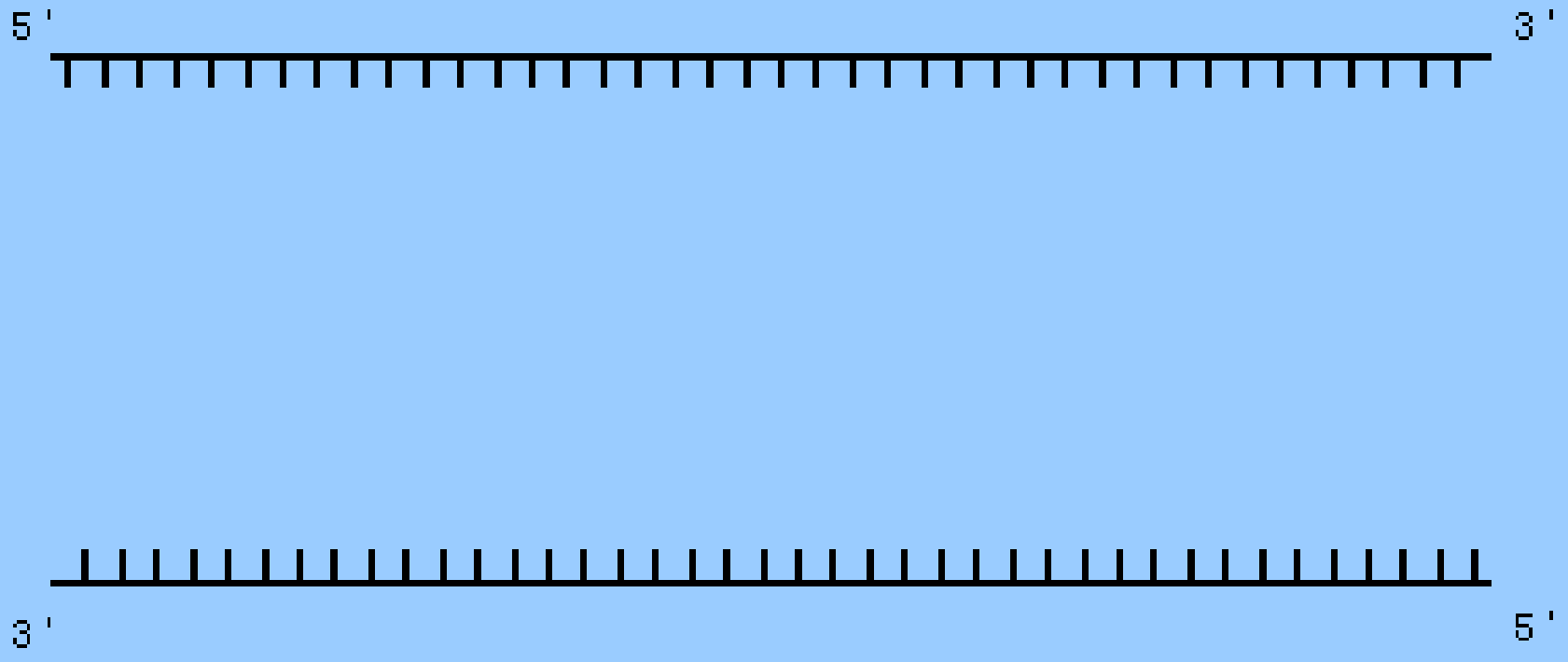
z. B. $(TTAGGG)_n$ beim Menschen

(Ausnahme: Dipteren wie Drosophila,
sowie wenige Pflanzenarten)

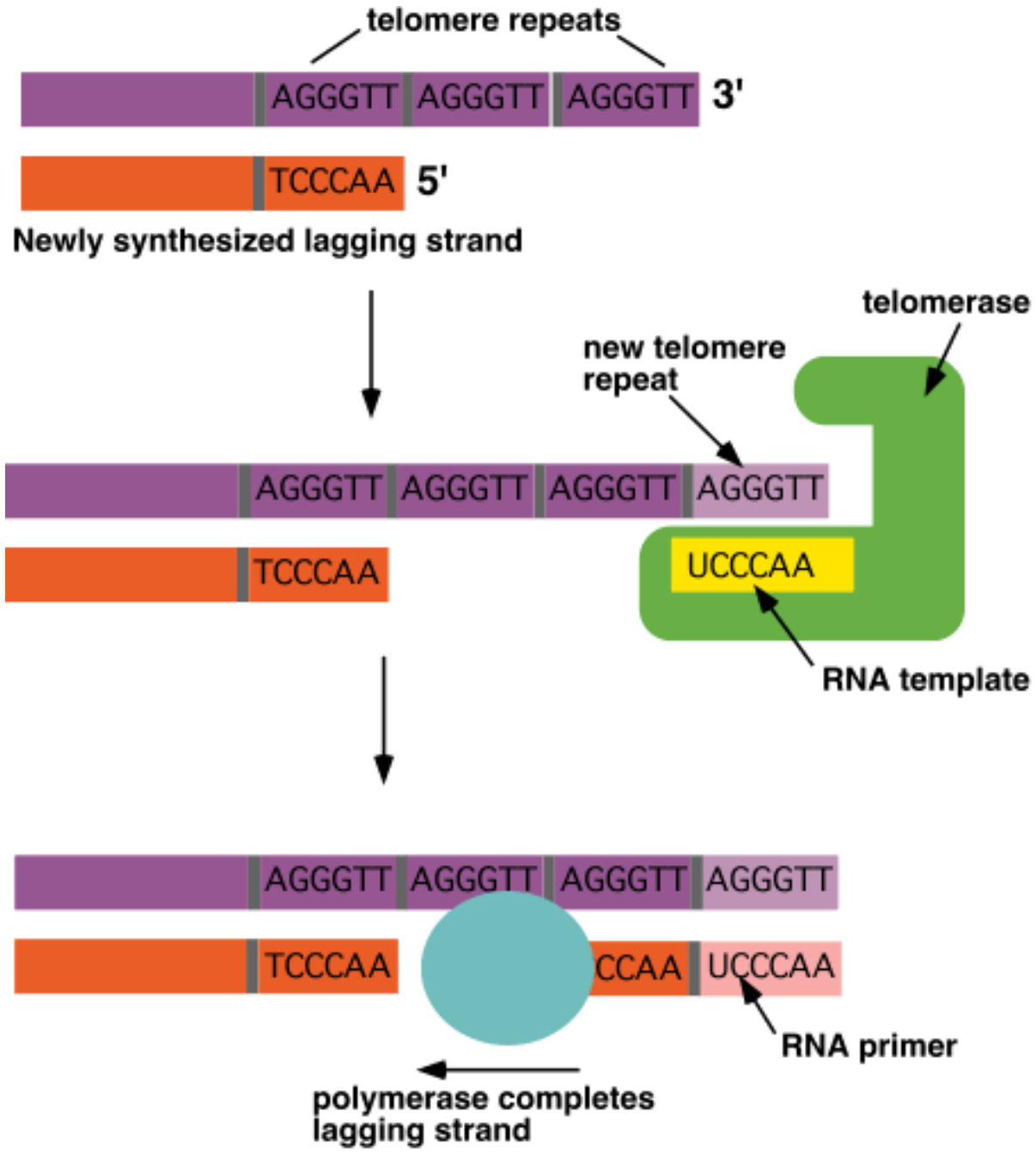
Die Telomerase verlängert den überhängenden 3‘-Strang



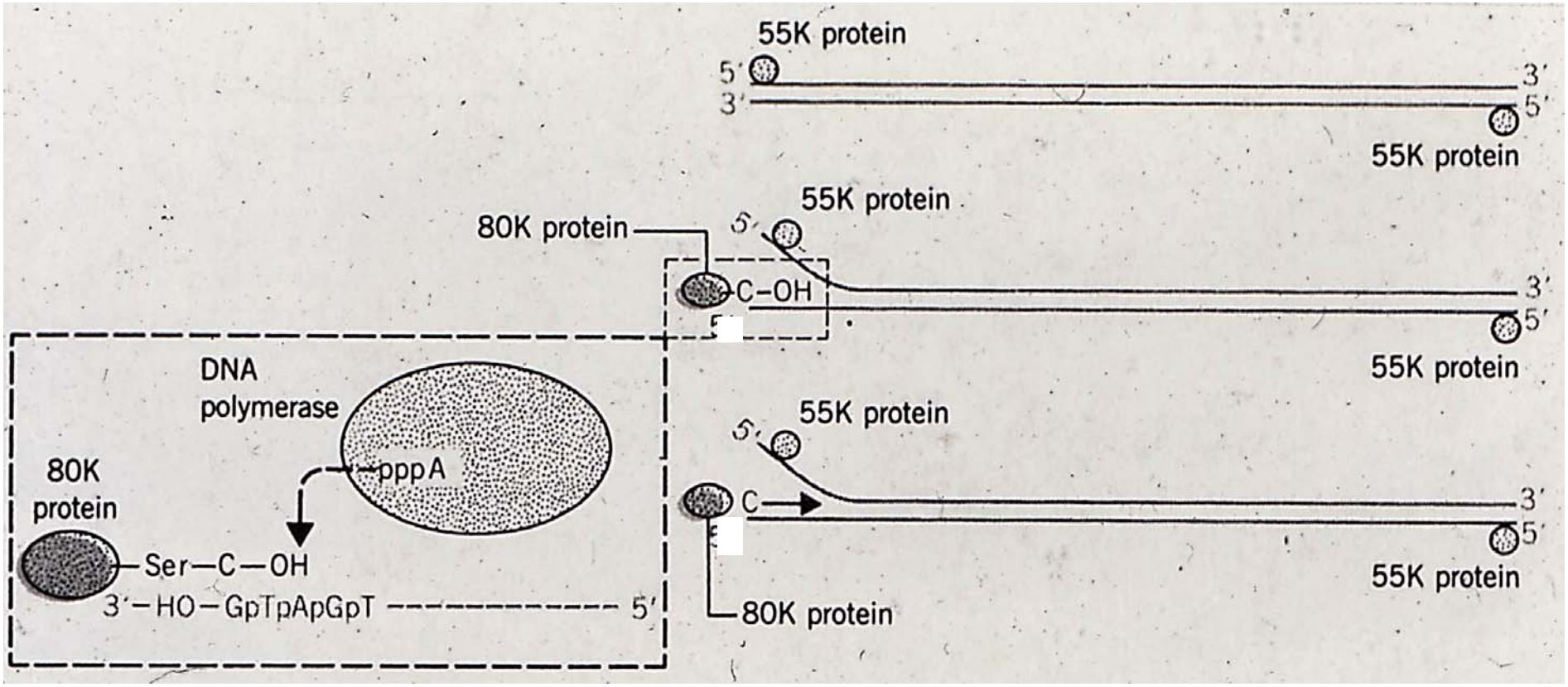
Replication of the lagging strand of a linear chromosome encounters a problem at the 3' end

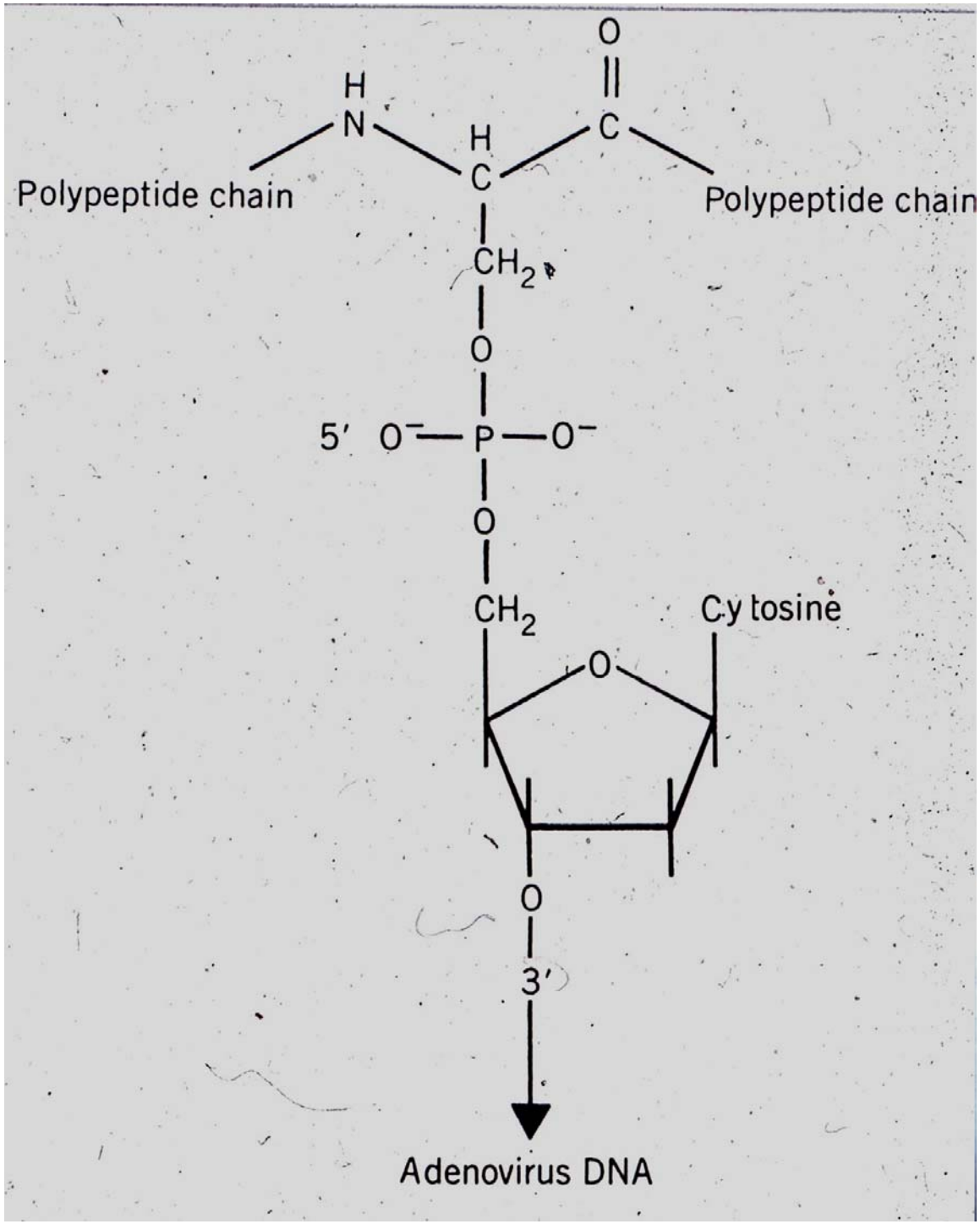


Telomer-replikation



Replikation linearer Virus-Genome
Beispiel Adenovirus

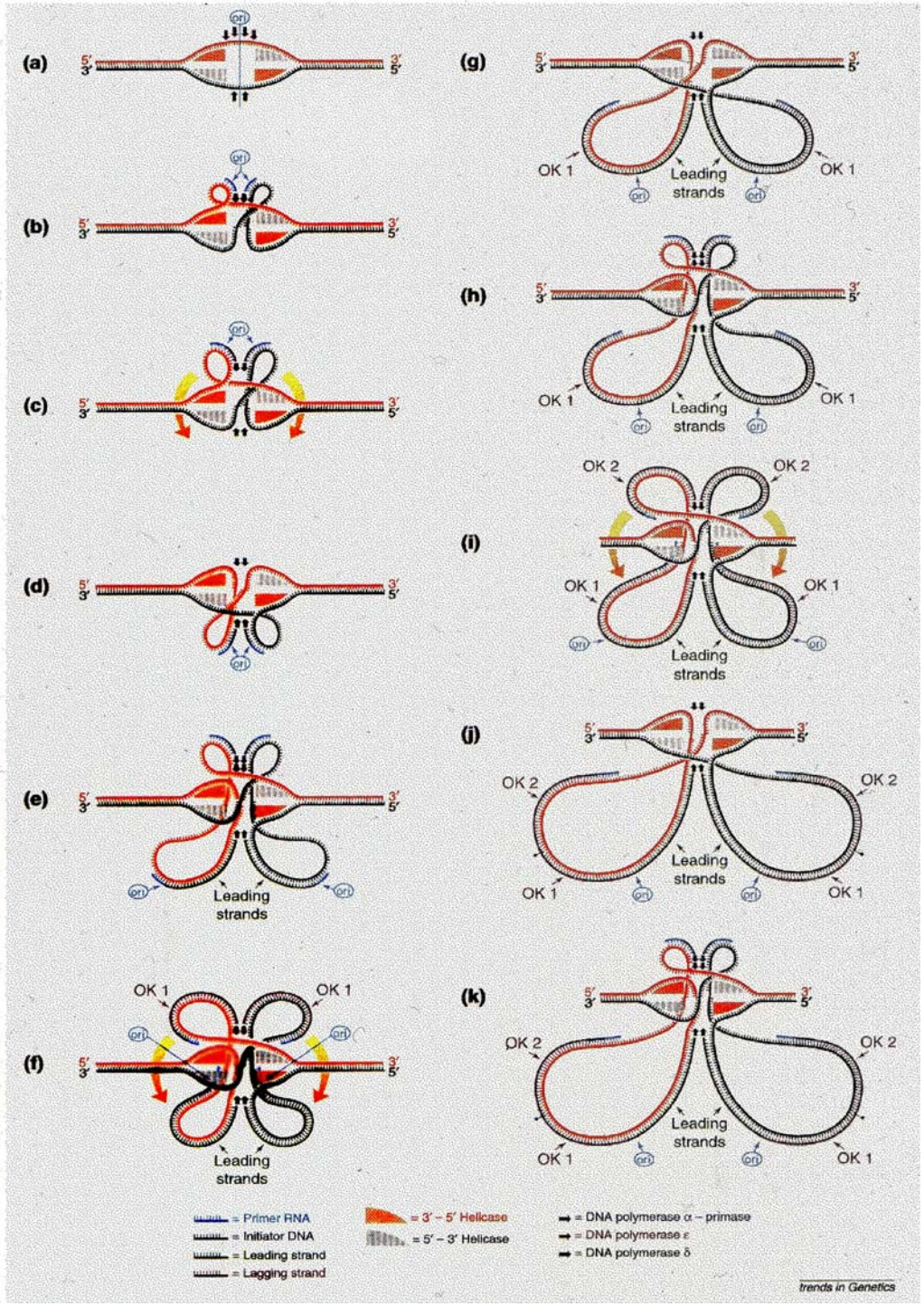



O
H
N
H
C
C
Polypeptide chain
Polypeptide chain
CH2
O
5′ O⁻ P O⁻
O
CH2
Cytosine
O
O
3′
Adenovirus DNA

„Stationäre“ Replikation

- Es gibt Hinweise darauf, dass nicht das Replisom, sondern die DNA sich bei der Replikation bewegt:

  „die Fabrik steht fest und das Fließband bewegt sich“


(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)
(k)
ori
5′
3′
OK 1
OK 2
Leading strands
= Primer RNA
= Initiator DNA
= Leading strand
= Lagging strand
= 3′ – 5′ Helicase
= 5′ – 3′ Helicase
= DNA polymerase α – primase
= DNA polymerase ε
= DNA polymerase δ
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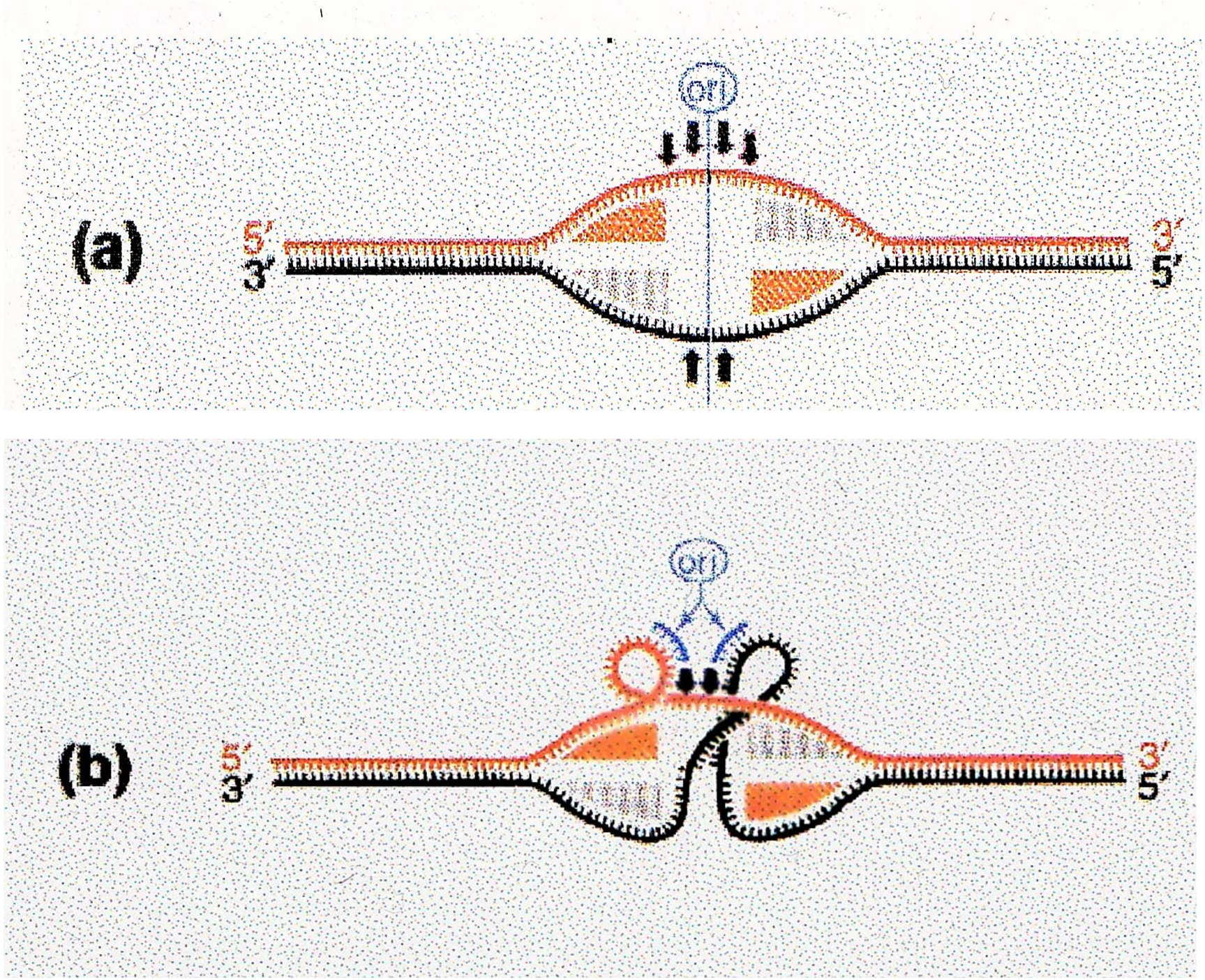
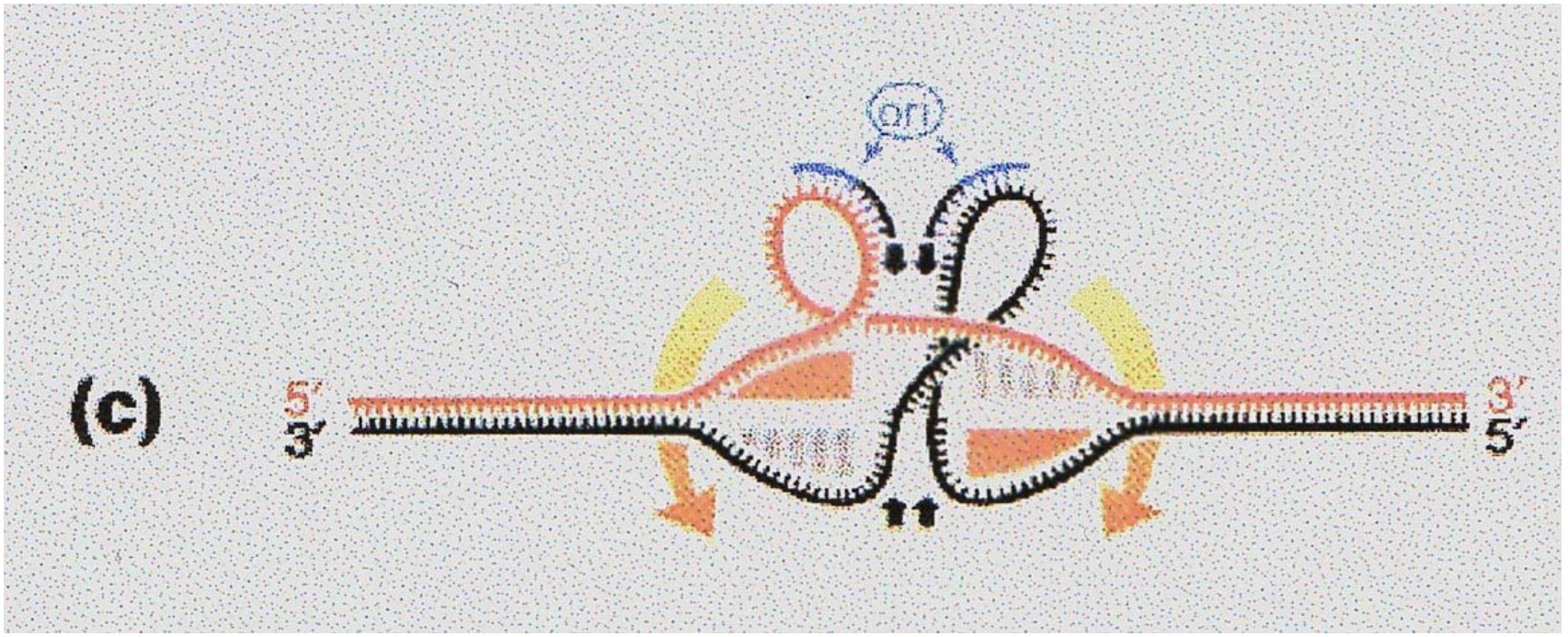
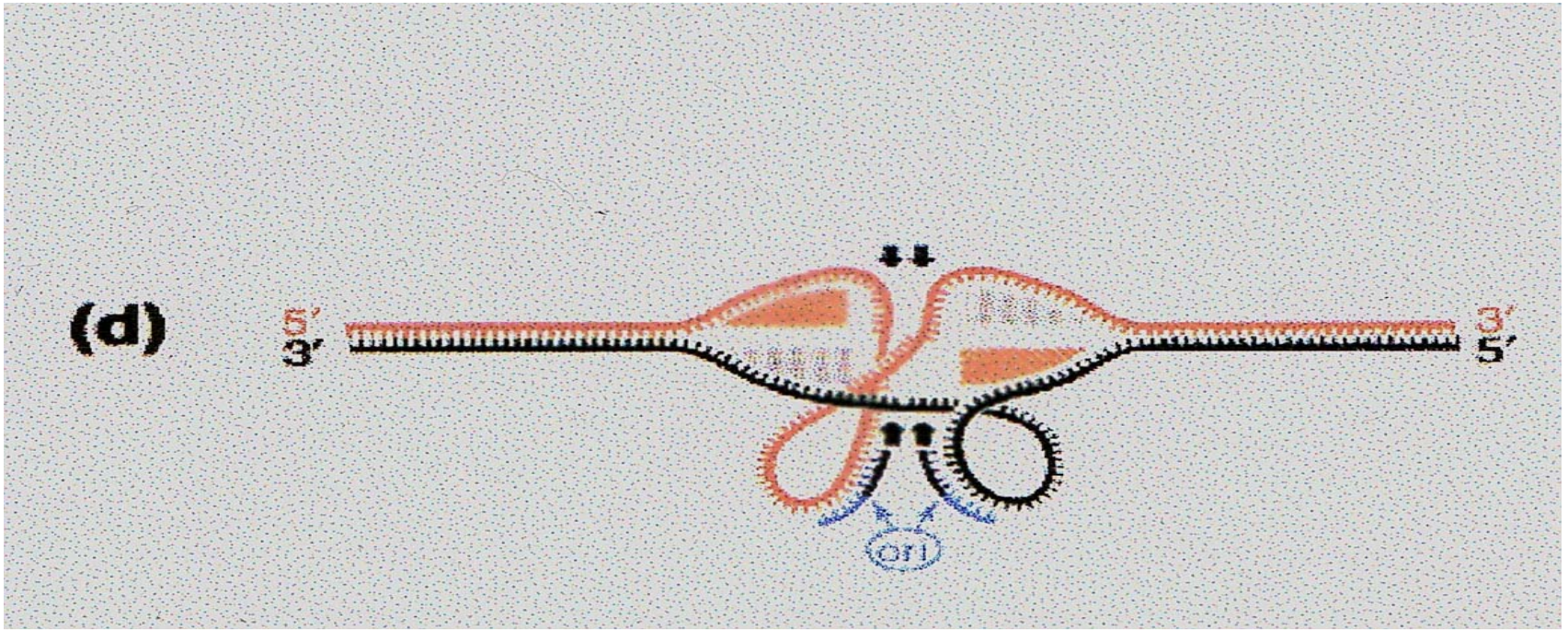
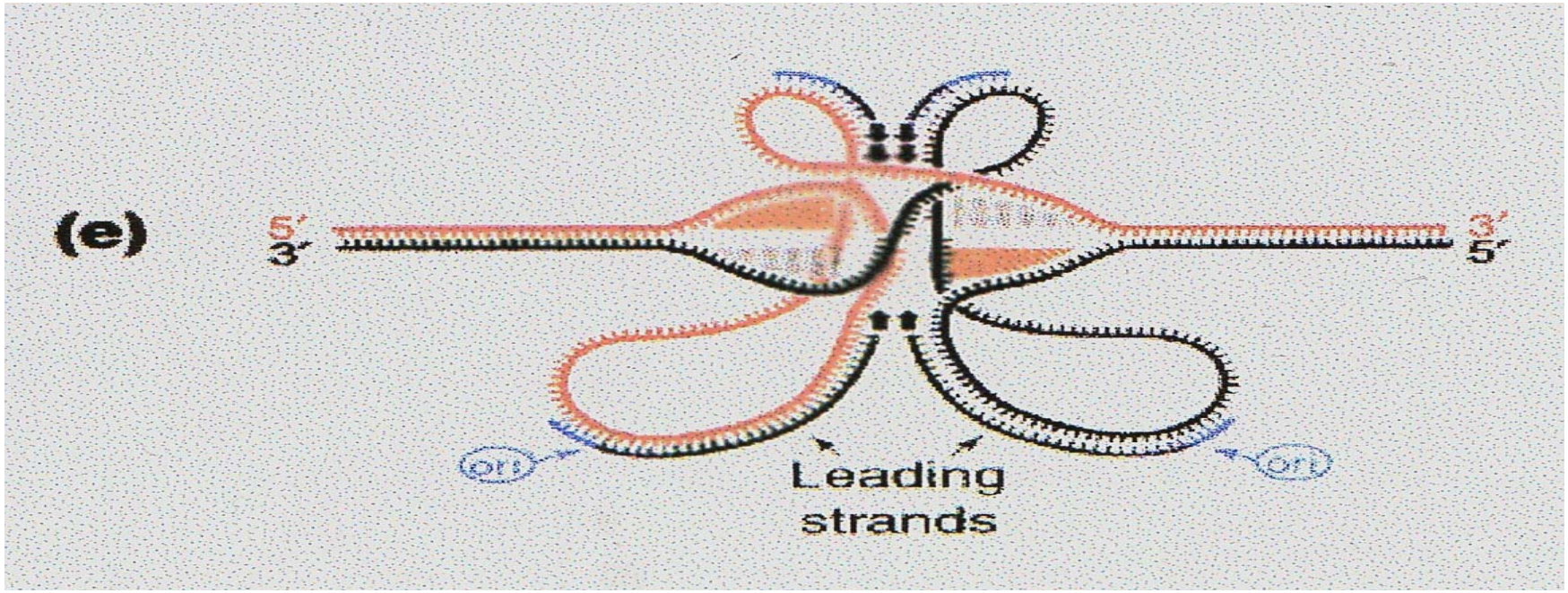
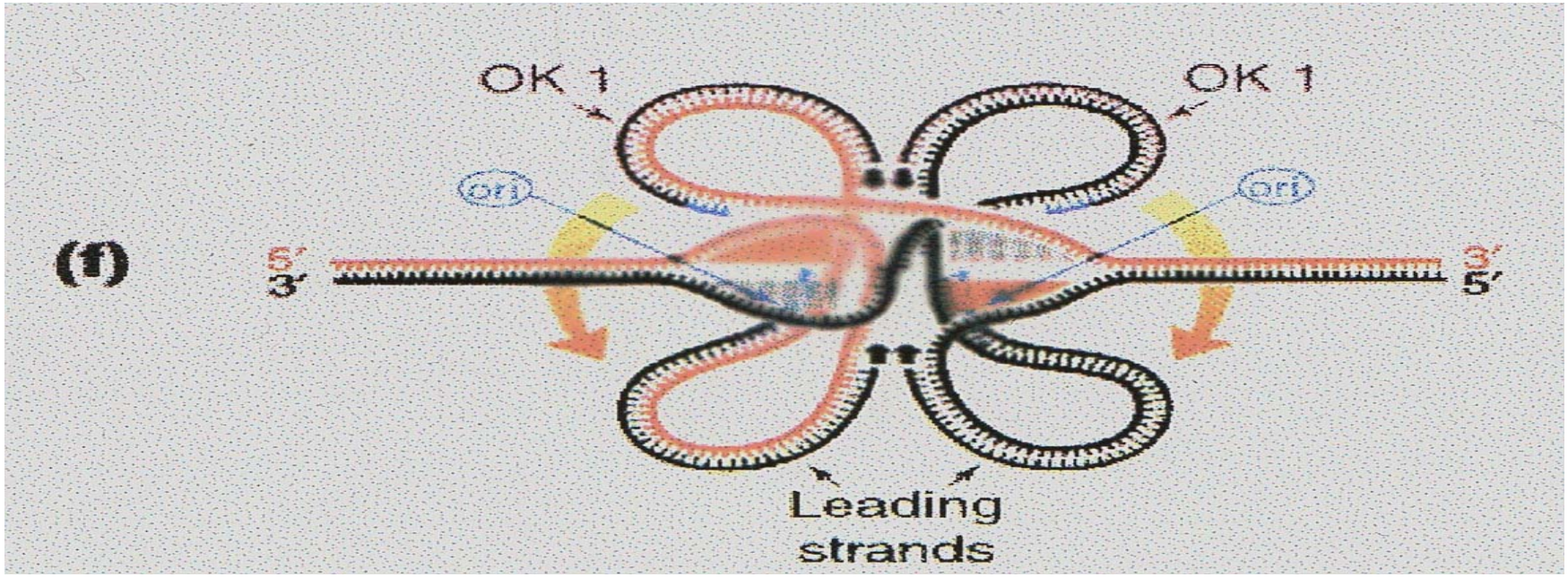
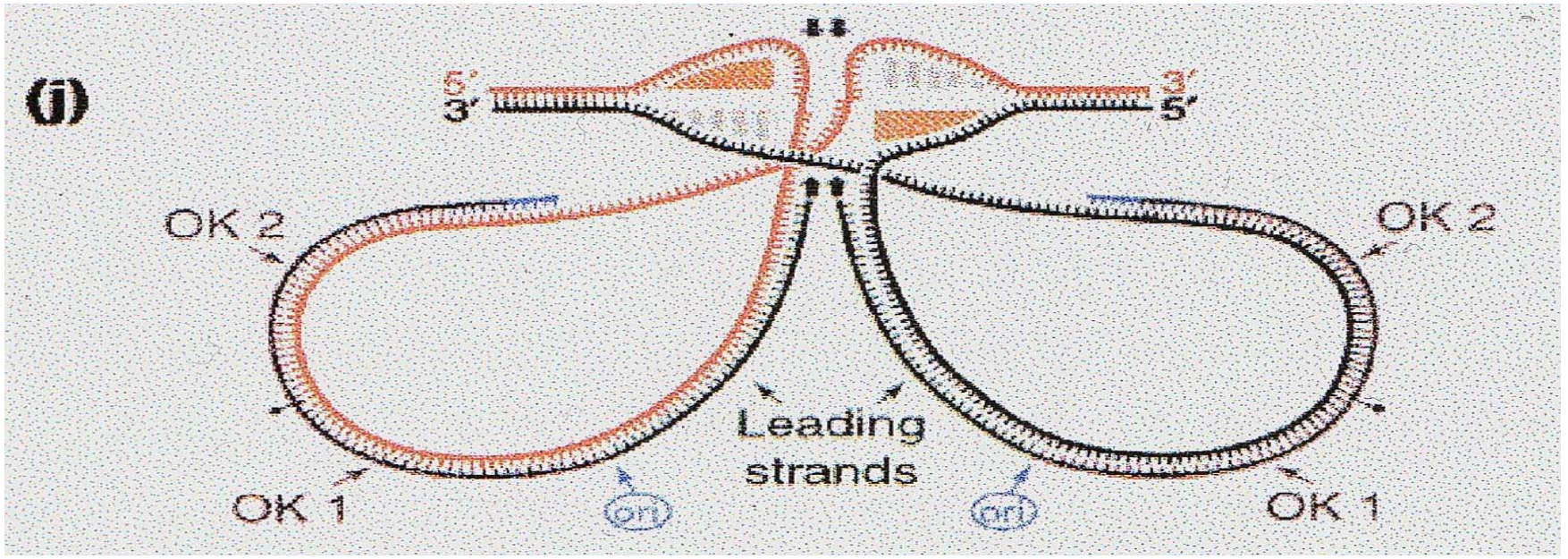

(a)
ori
5′
3′
3′
5′
(b)
ori
5′
3′
3′
5′

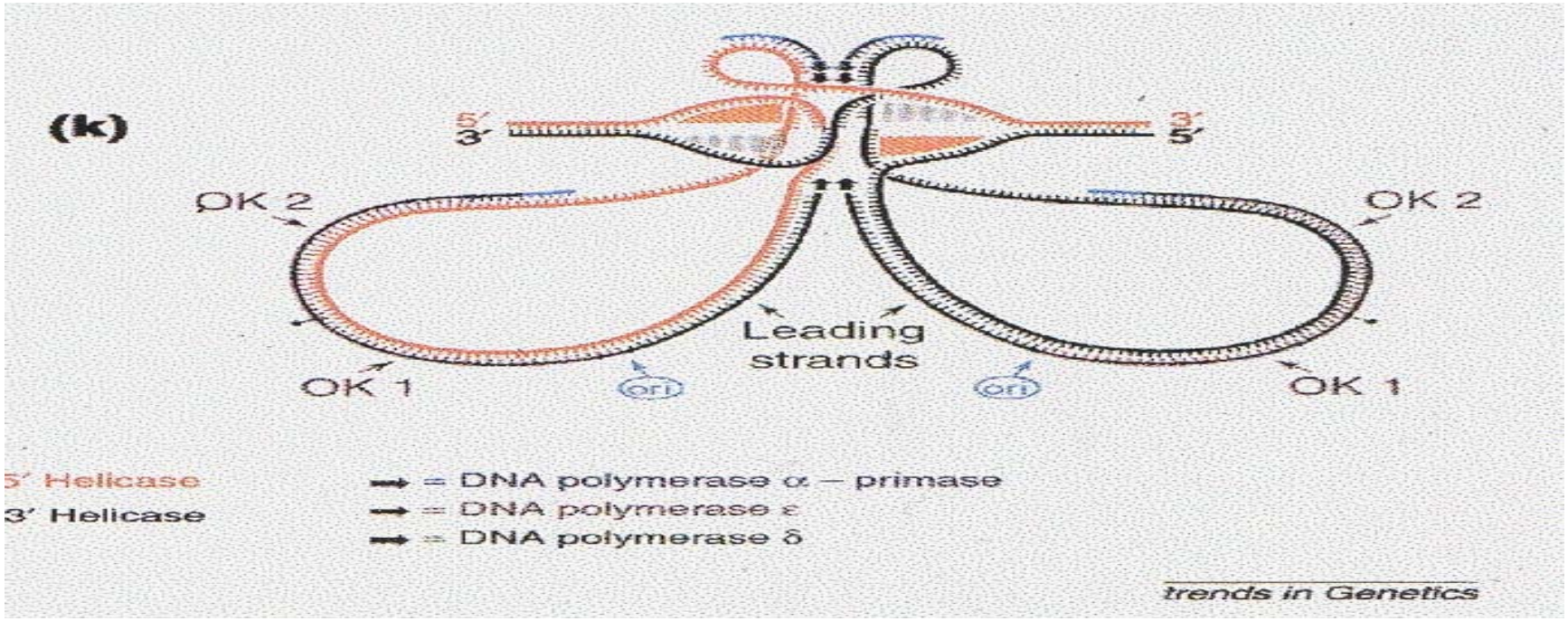

(c)
ori
5'
3'
3'
5'


(d)
5'
3'
3'
5'
ori


(e)
5′
3′
3′
5′
ori
ori
Leading
strands


OK 1
OK 1
(f)
ori
ori
5′
3′
3′
5′
Leading
strands


(j)
5′
3′
3′
5′
OK 2
OK 2
Leading
strands
OK 1
ori
ori
OK 1


(k)
5′
3′
3′
5′
OK 2
OK 2
Leading
strands
OK 1
ori
ori
OK 1
5′ Helicase
3′ Helicase
= DNA polymerase α – primase
= DNA polymerase ε
= DNA polymerase δ
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