Sequential actions of

DNA Replication

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Sequential actions

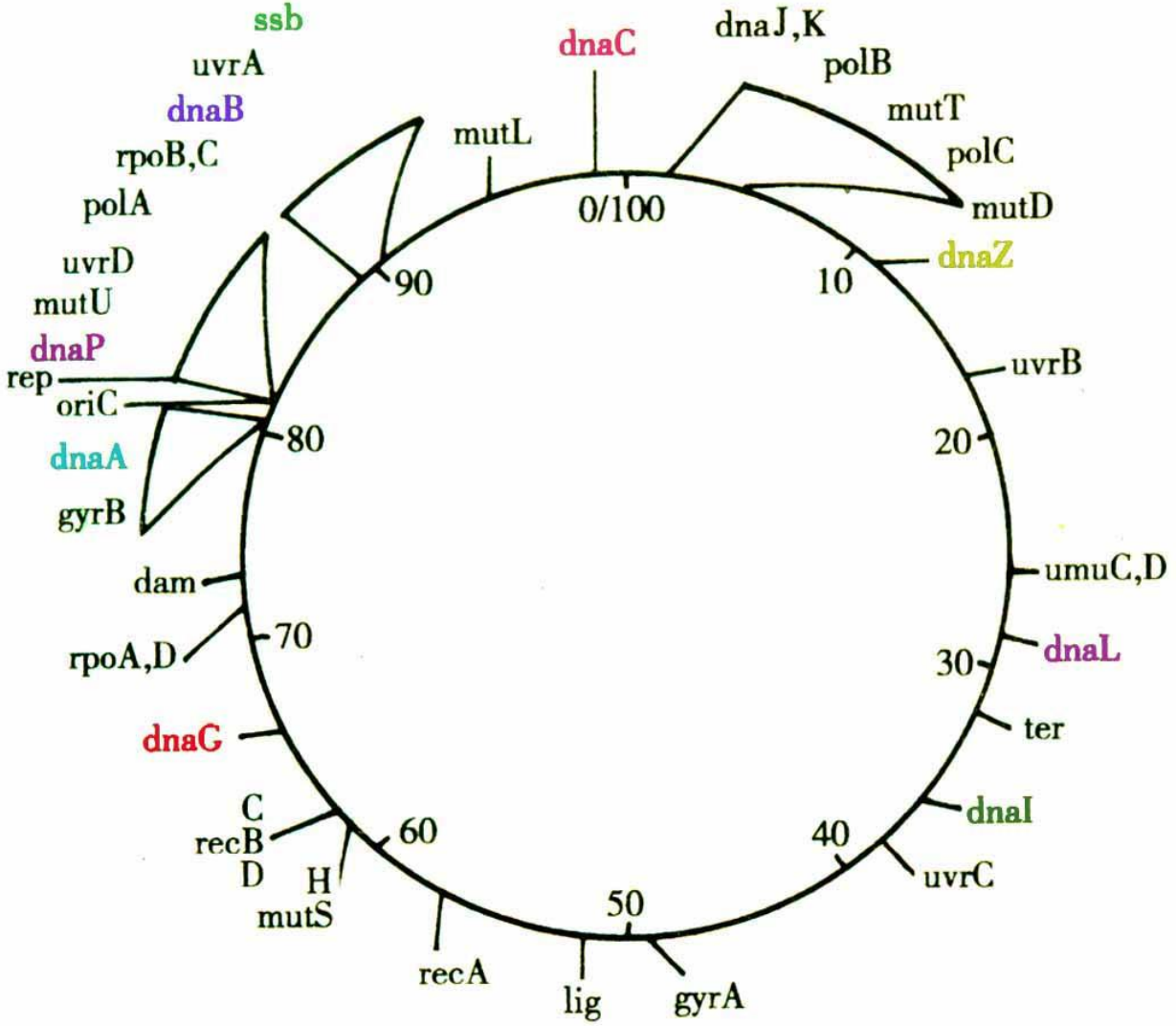
- **Initiation:** recognize the starting point, separate dsDNA, primer synthesis, …
- **Elongation:** add dNTPs to the existing strand, form phosphoester bonds, correct the mismatch bases, extending the DNA strand, …
- **Termination:** stop the replication

Replication of prokaryotes

a. Initiation

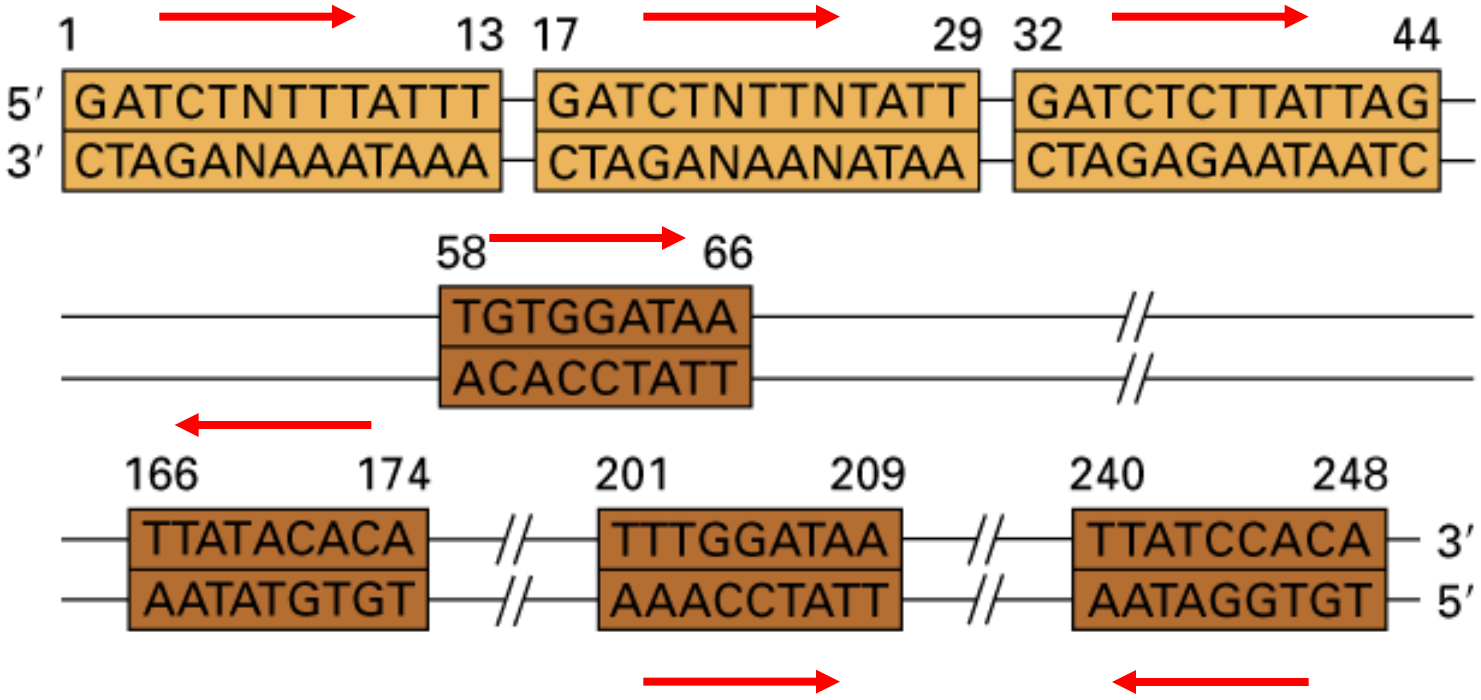
- **The replication starts at a particular point called origin.**
- **The origin of *E. coli*, ori C, is at the location of 82.**
- **The structure of the origin is 248 bp long and AT-rich.**

Genome of *E. coli*

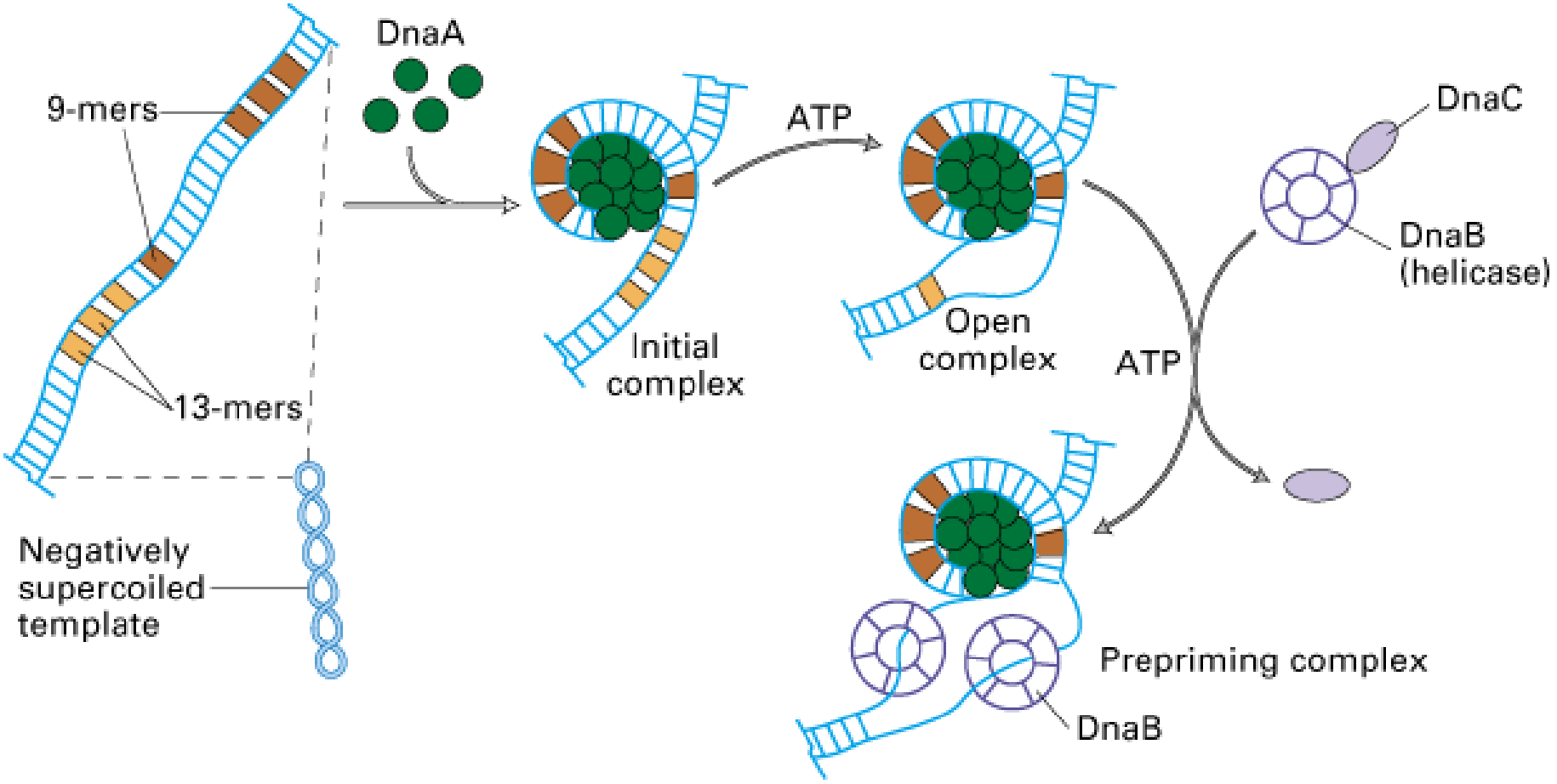


Structure of ori C

- **Three 13 bp consensus sequences**
- **Two pairs of anti-consensus repeats**



Formation of preprimosome



Formation of replication fork

- **DnaA recognizes ori C.**
- **DnaB and DnaC join the DNA-DnaA complex, open the local AT-rich region, and move on the template downstream further to separate enough space.**
- **DnaA is replaced gradually.**
- **SSB protein binds the complex to stabilize ssDNA.**

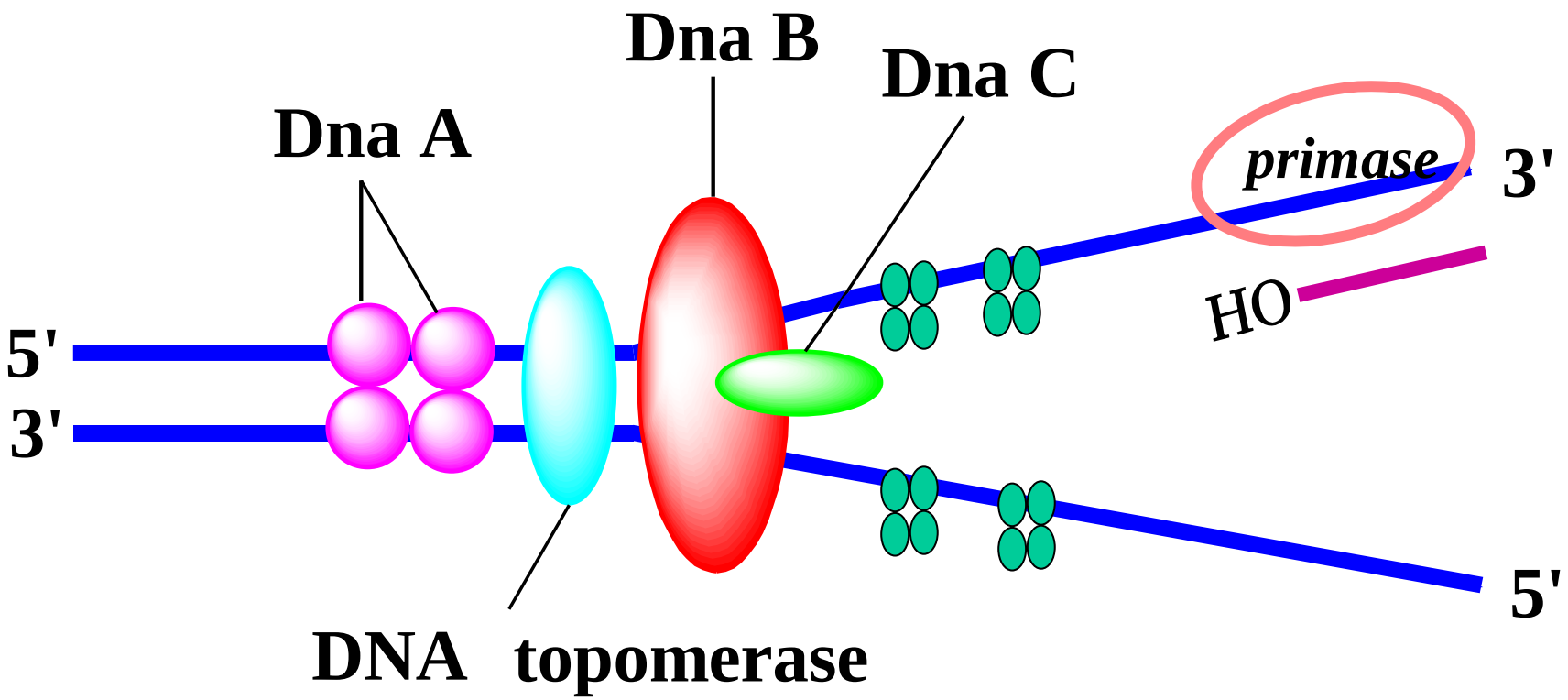
Primer synthesis

- **Primase joins and forms a complex called primosome.**
- **Primase starts the synthesis of primers on the ssDNA template using NTP as the substrates in the 5′- 3′ direction at the expense of ATP.**
- **The short RNA fragments provide free 3′-OH groups for DNA elongation.**

Releasing supercoil constraint

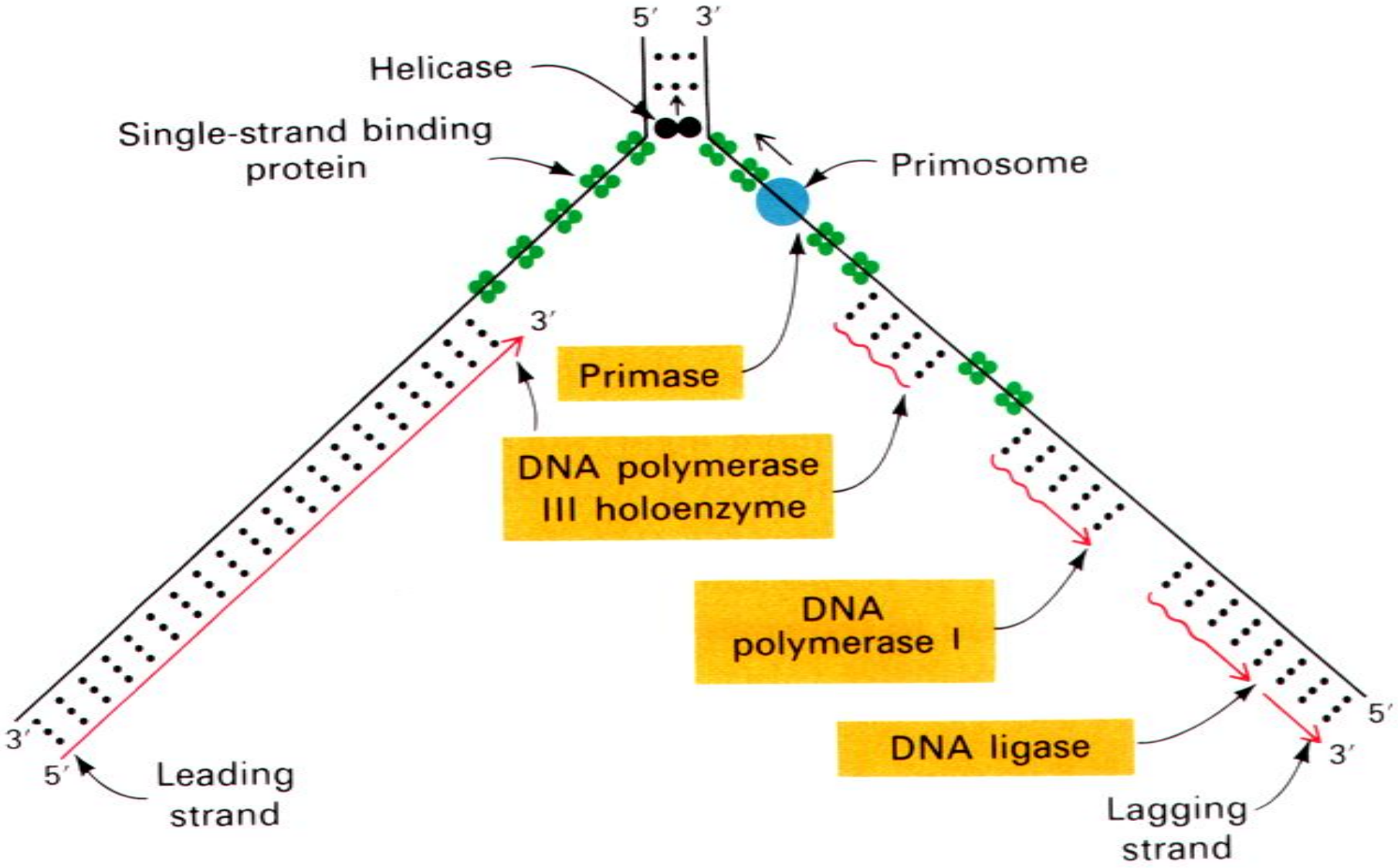
- **The supercoil constraints are generated ahead of the replication forks.**
- **Topoisomerase binds to the dsDNA region just before the replication forks to release the supercoil constraint.**
- **The negatively supercoiled DNA serves as a better template than the positively supercoiled DNA.**

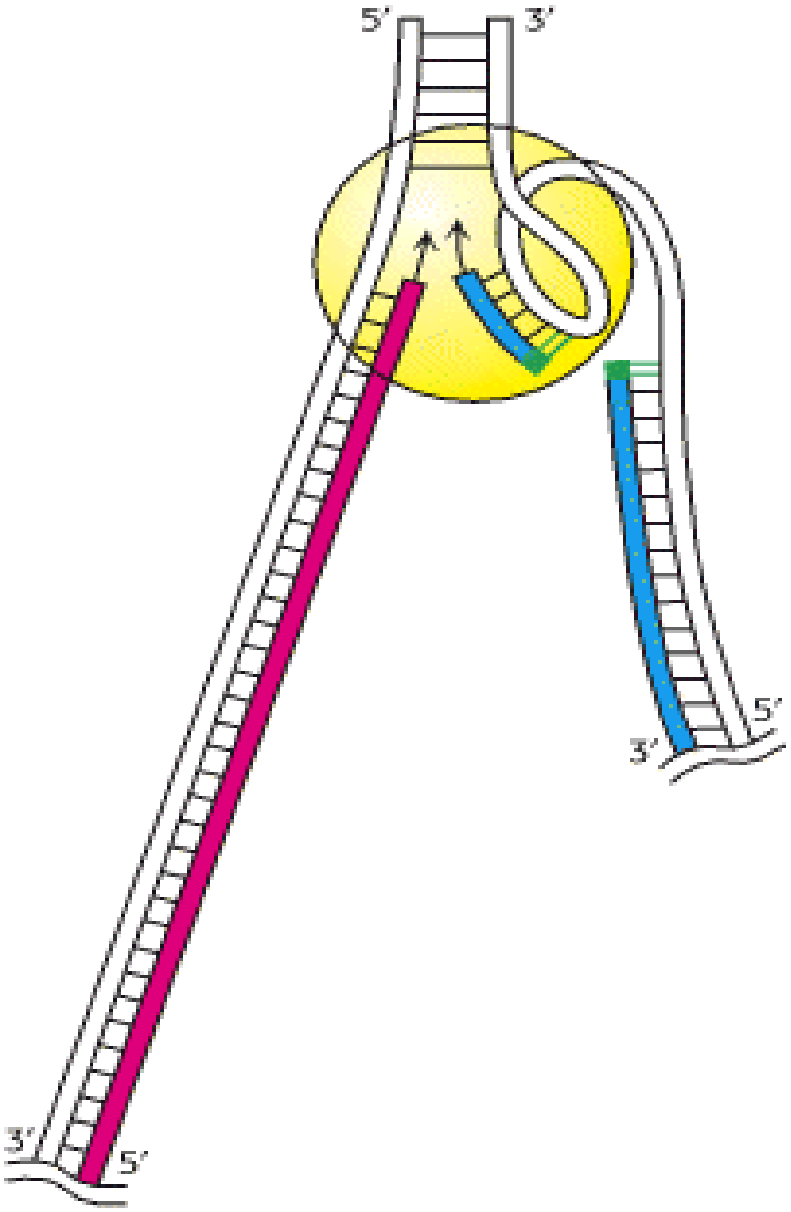
Primosome complex



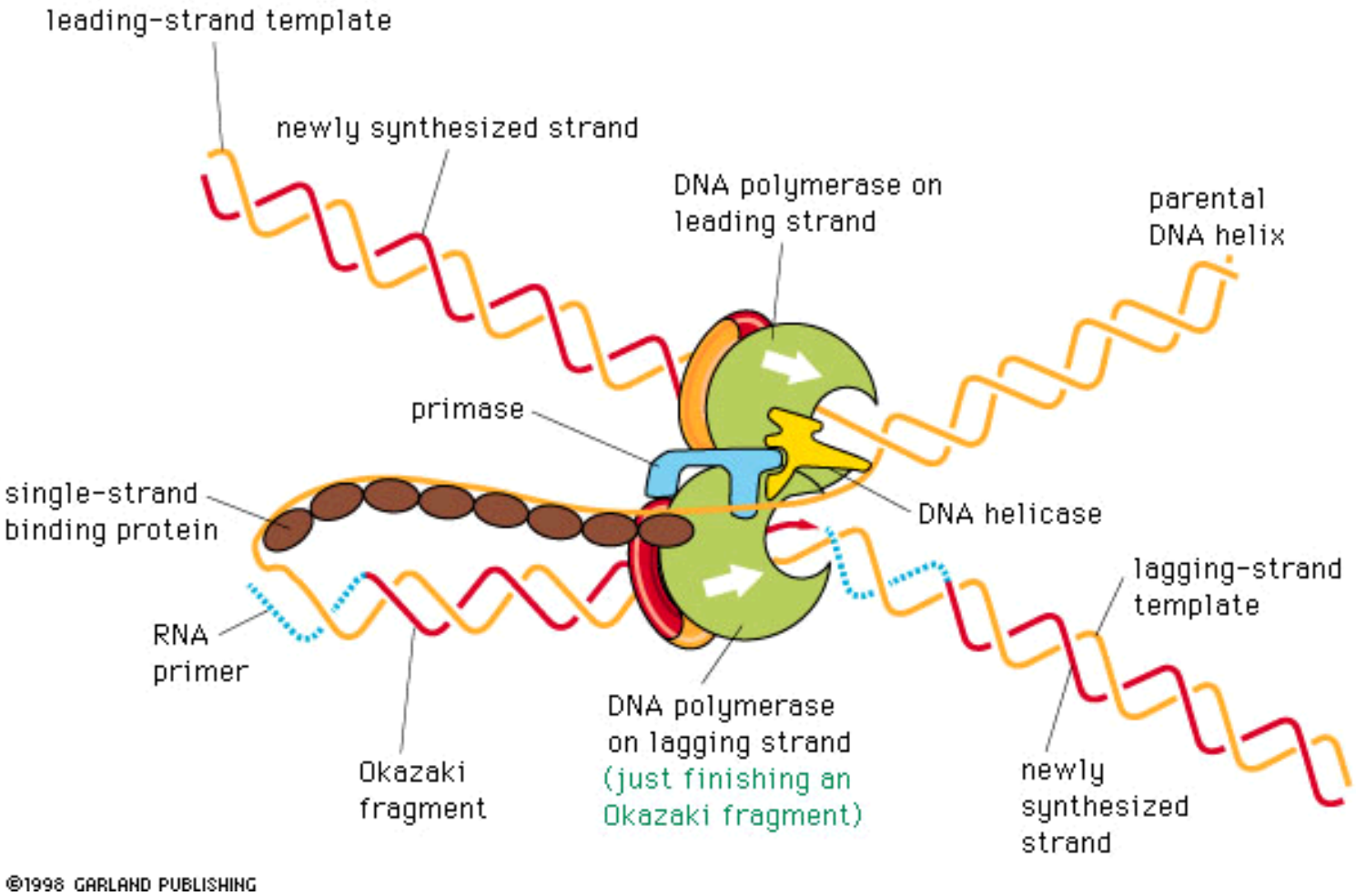
b. Elongation

- **dNTPs are continuously connected to the primer or the nascent DNA chain by DNA-pol III.**
- **The core enzymes (α、ε、and θ) catalyze the synthesis of leading and lagging strands, respectively.**
- **The nature of the chain elongation is the series formation of the phosphodiester bonds.**


5′
3′
Helicase
Single-strand binding protein
Primosome
3′
Primase
DNA polymerase III holoenzyme
DNA polymerase I
DNA ligase
3′
5′
5′
Leading strand
Lagging strand



- **The synthesis direction of the leading strand is the same as that of the replication fork.**
- **The synthesis direction of the latest Okazaki fragment is also the same as that of the replication fork.**


leading-strand template
newly synthesized strand
DNA polymerase on leading strand
parental DNA helix
primase
single-strand binding protein
DNA helicase
lagging-strand template
RNA primer
Okazaki fragment
DNA polymerase on lagging strand (just finishing an Okazaki fragment)
newly synthesized strand
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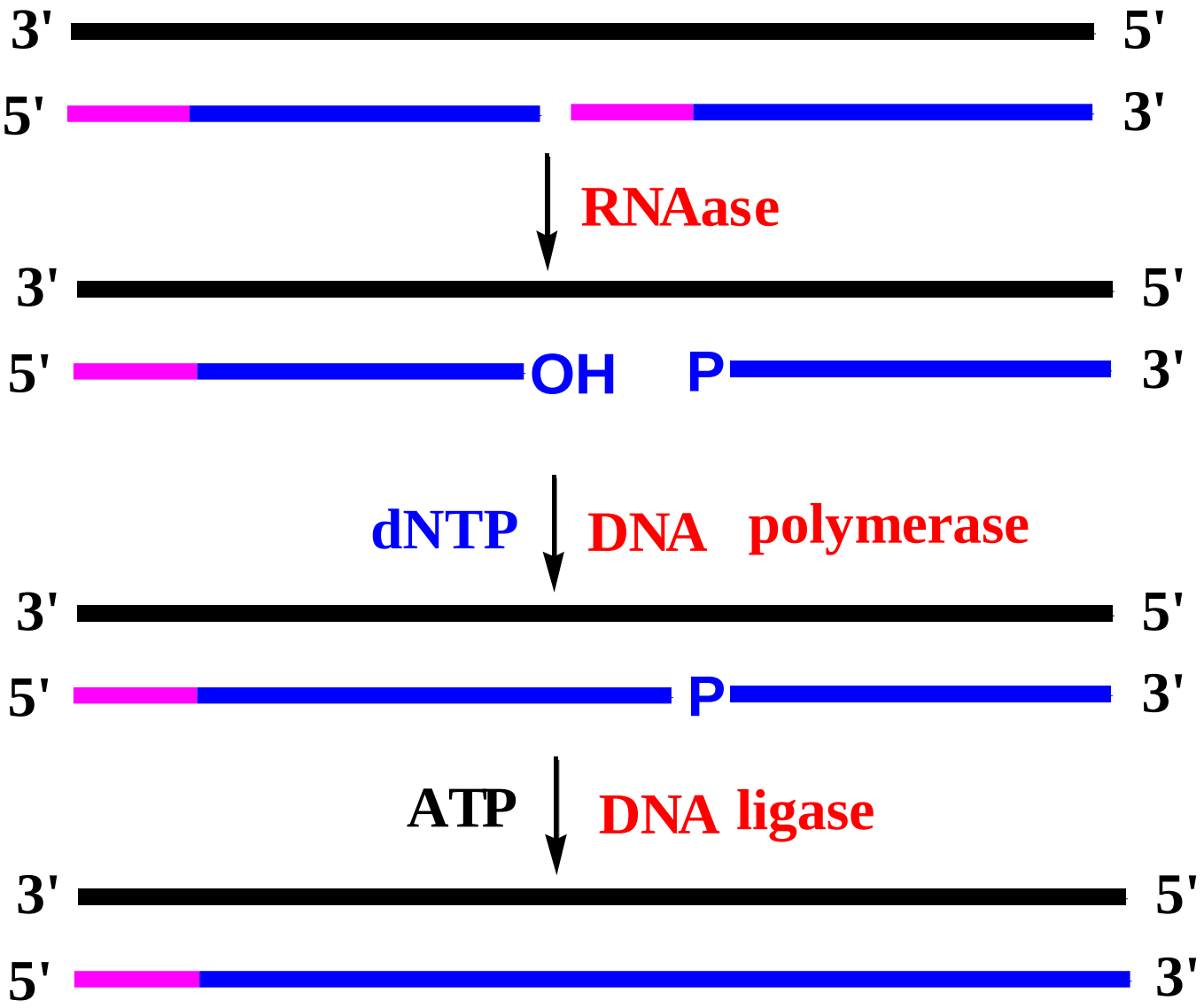
Lagging strand synthesis

- **Primers on Okazaki fragments are digested by RNase.**
- **The gaps are filled by DNA-pol I in the 5′→3′ direction.**
- **The nick between the 5′ end of one fragment and the 3′ end of the next fragment is sealed by ligase.**


3'
5'
5'
3'
RNAase
3'
5'
5'
OH
P
3'
dNTP
DNA
polymerase
3'
5'
5'
P
3'
ATP
DNA ligase
3'
5'
5'
3'

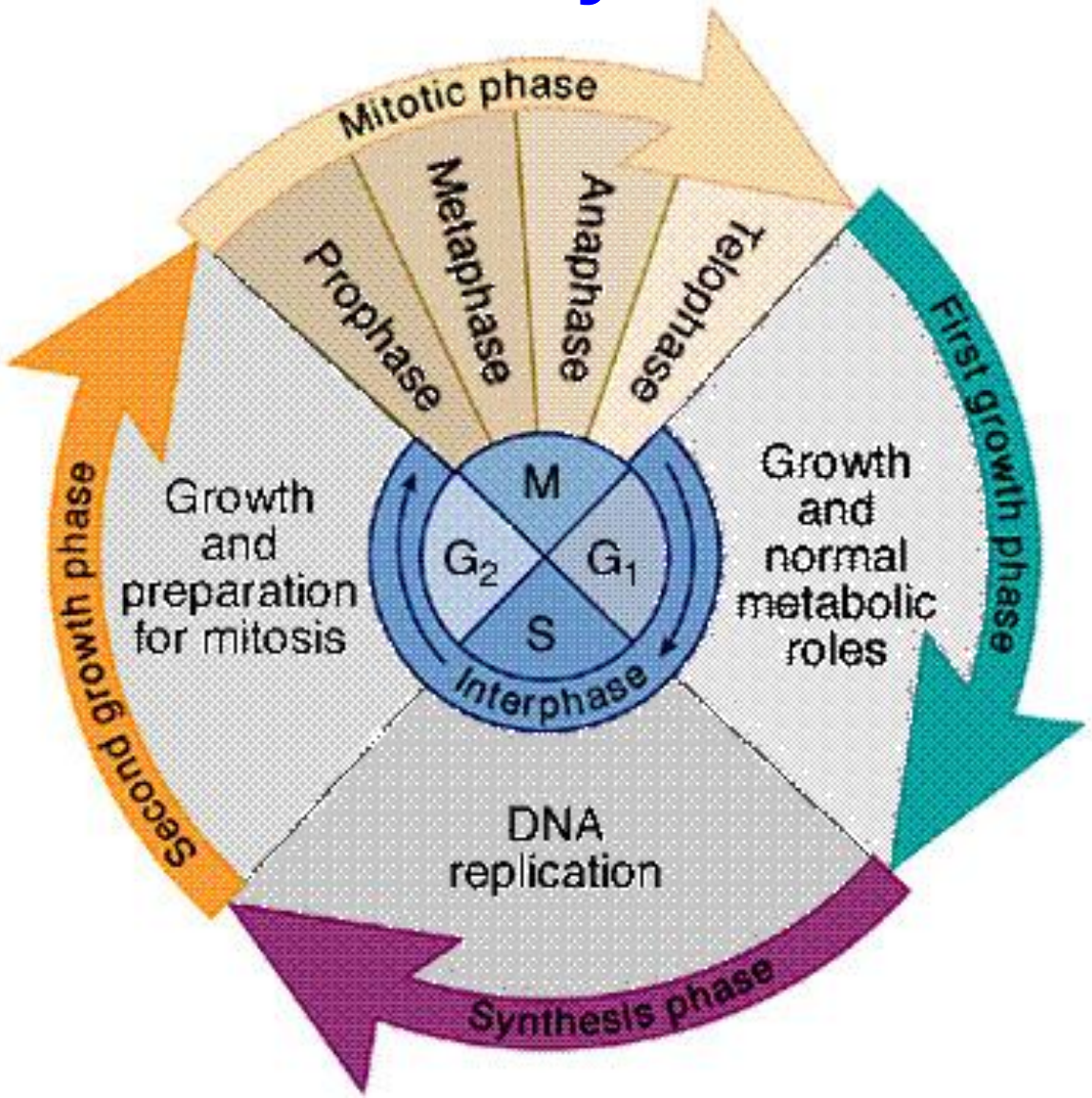
c. Termination

- **The replication of *E. coli* is bidirectional from one origin, and the two replication forks must meet at one point called ter at 32.**
- **All the primers will be removed, and all the fragments will be connected by DNA-pol I and ligase.**

Replication of Eukaryotes

- **DNA replication is closely related with cell cycle.**
- **Multiple origins on one chromosome, and replications are activated in a sequential order rather than simultaneously.**

Cell cycle


Mitotic phase
Prophase
Metaphase
Anaphase
Telophase
First growth phase
Growth and normal metabolic roles
DNA replication
Synthesis phase
Second growth phase
Growth and preparation for mitosis
M
G2
G1
S
Interphase

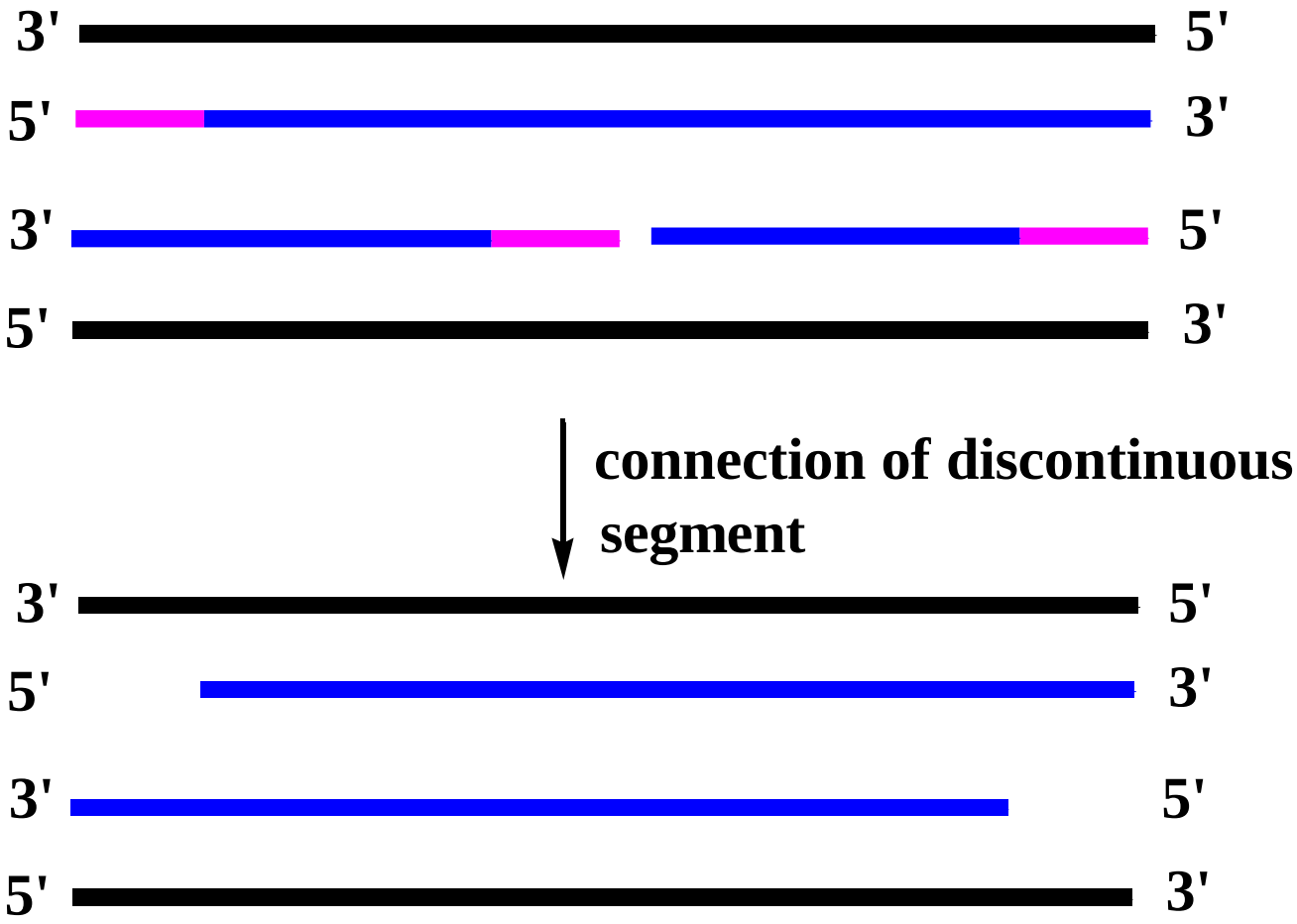
Initiation

- **The eukaryotic origins are shorter than that of *E. coli*.**
- **Requires DNA-pol α (primase activity) and DNA-pol δ (polymerase activity and helicase activity).**
- **Needs topoisomerase and replication factors (RF) to assist.**

b. Elongation

- **DNA replication and nucleosome assembling occur simultaneously.**
- **Overall replication speed is compatible with that of prokaryotes.**

c. Termination

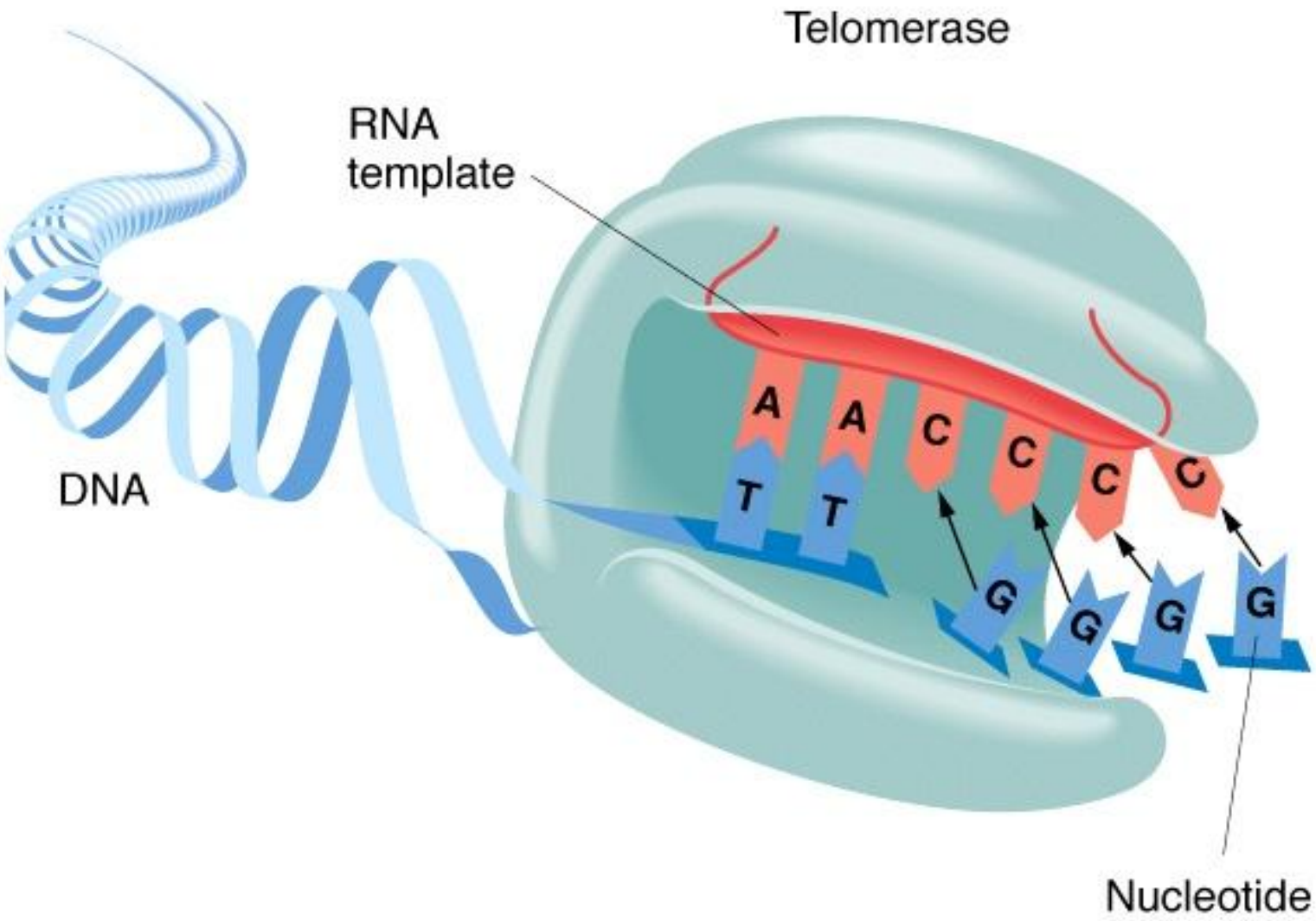


Telomere

- **The terminal structure of eukaryotic DNA of chromosomes is called telomere.**
- **Telomere is composed of terminal DNA sequence and protein.**
- **The sequence of typical telomeres is rich in T and G.**
- **The telomere structure is crucial to keep the termini of chromosomes in the cell from becoming entangled and sticking to each other.**

Telomerase

- **The eukaryotic cells use telomerase to maintain the integrity of DNA telomere.**
- **The telomerase is composed of**
  - **telomerase RNA**
  - **telomerase association protein**
  - **telomerase reverse transcriptase**
- **It is able to synthesize DNA using RNA as the template.**


Telomerase
RNA
template
DNA
A
A
C
C
C
C
T
T
G
G
G
G
Nucleotide

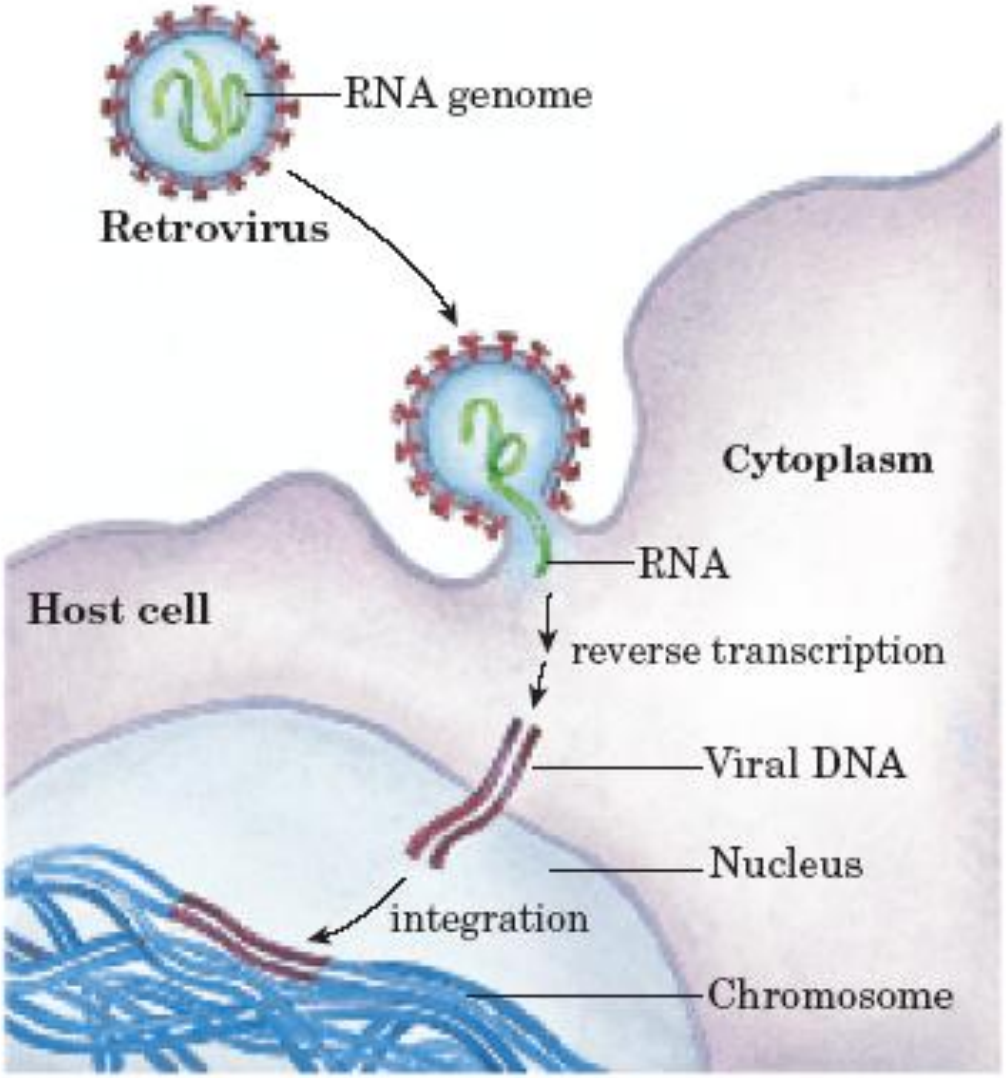
Significance of Telomerase

- **Telomerase may play important roles is cancer cell biology and in cell aging.**

Other Replication Modes

Reverse transcription

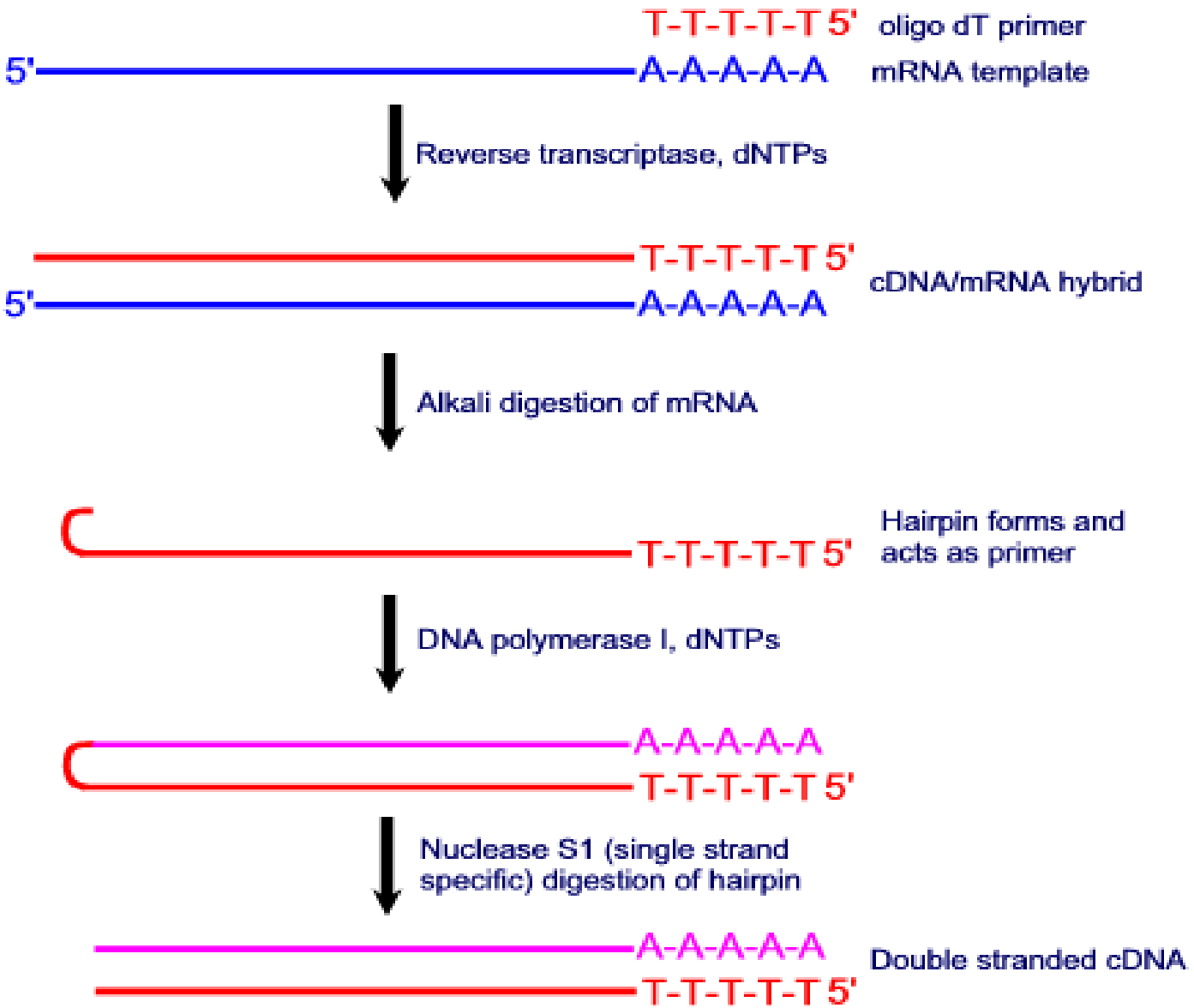
- **The genetic information carrier of some biological systems is ssRNA instead of dsDNA (such as ssRNA viruses).**
- **The information flow is from RNA to DNA, opposite to the normal process.**
- **This special replication mode is called reverse transcription.**


RNA genome
Retrovirus
Cytoplasm
RNA
Host cell
reverse transcription
Viral DNA
Nucleus
integration
Chromosome

Reverse transcription is a process in which ssRNA is used as the template to synthesize dsDNA.

Process of Reverse transcription

- **Synthesis of ssDNA complementary to ssRNA, forming a RNA-DNA hybrid.**
- **Hydrolysis of ssRNA in the RNA-DNA hybrid by RNase activity of reverse transcriptase, leaving ssDNA.**
- **Synthesis of the second ssDNA using the left ssDNA as the template, forming a DNA-DNA duplex.**


T-T-T-T-T 5'
oligo dT primer
5'
A-A-A-A-A
mRNA template
Reverse transcriptase, dNTPs
T-T-T-T-T 5'
cDNA/mRNA hybrid
5'
A-A-A-A-A
Alkali digestion of mRNA
Hairpin forms and
acts as primer
T-T-T-T-T 5'
DNA polymerase I, dNTPs
A-A-A-A-A
T-T-T-T-T 5'
Nuclease S1 (single strand
specific) digestion of hairpin
A-A-A-A-A
Double stranded cDNA
T-T-T-T-T 5'

Reverse transcriptase

Reverse transcriptase is the enzyme for the reverse transcription. It has activity of three kinds of enzymes:

- **RNA-dependent DNA polymerase**
- **RNase**
- **DNA-dependent DNA polymerase**

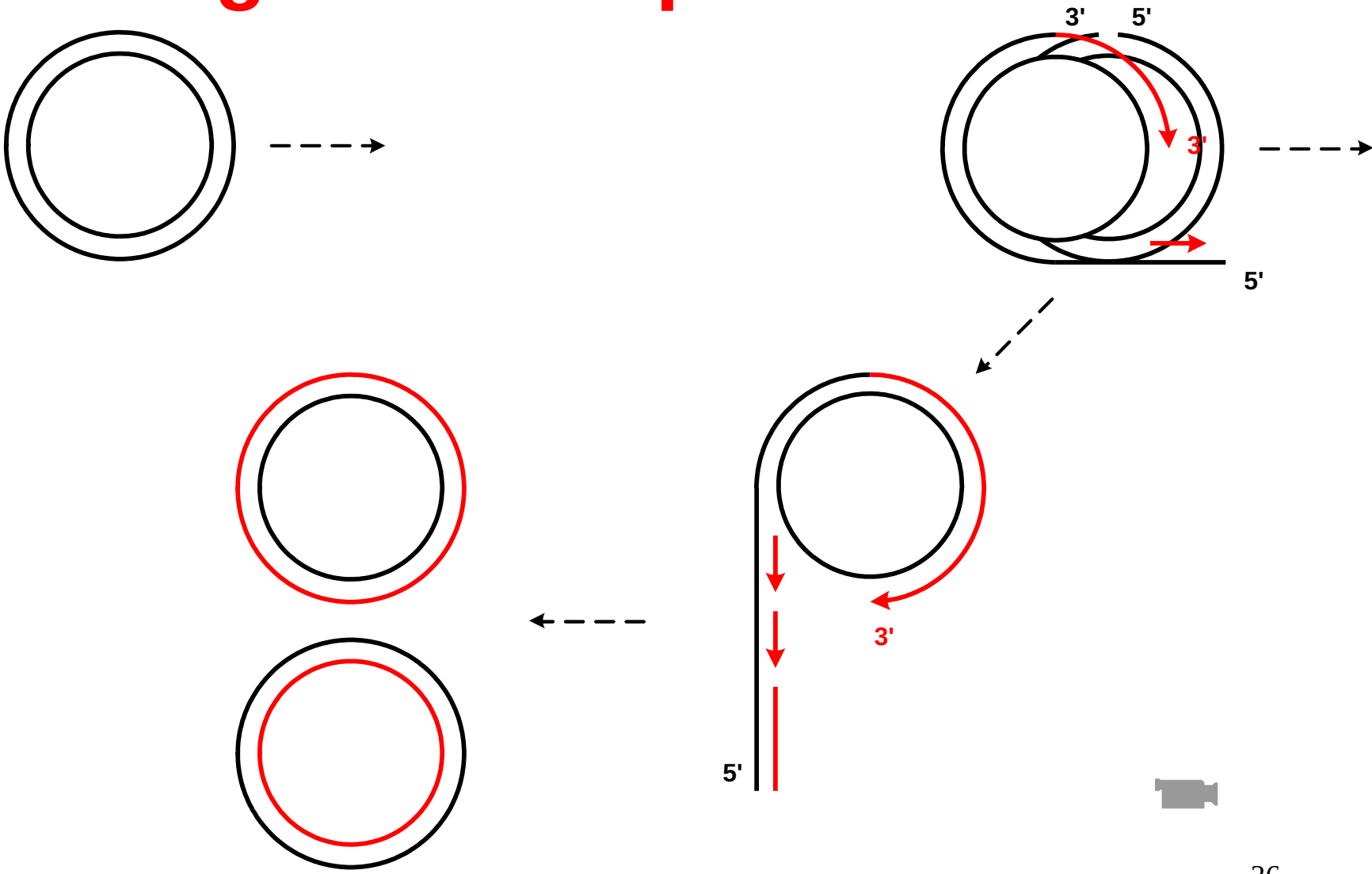
Significance of RT

- **An important discovery in life science and molecular biology**
- **RNA plays a key role just like DNA in the genetic information transfer and gene expression process.**
- **RNA could be the molecule developed earlier than DNA in evolution.**
- **RT is the supplementary to the central dogma.**

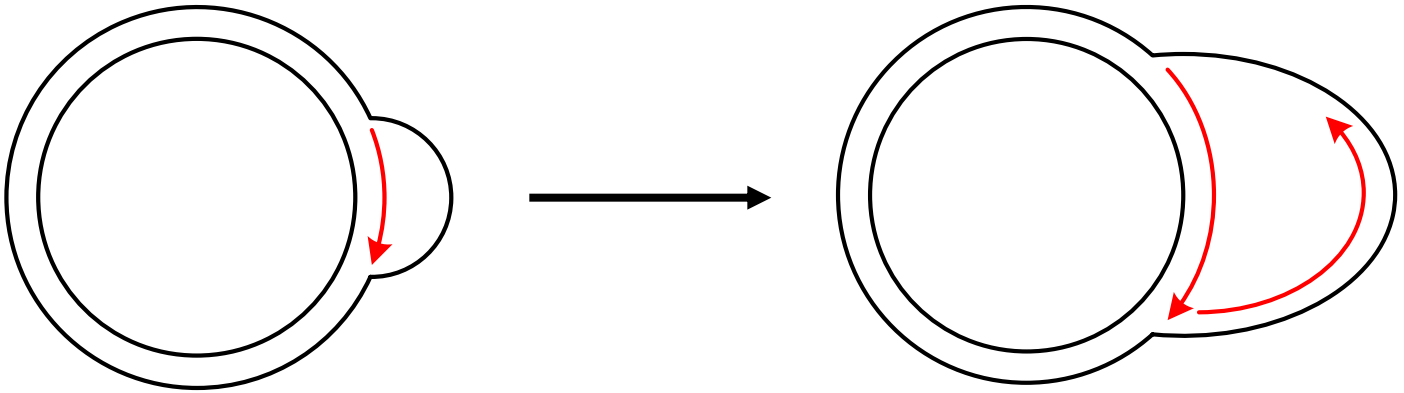
Significance of RT

- **This discovery enriches the understanding about the cancer-causing theory of viruses. (cancer genes in RT viruses, and HIV having RT function)**
- **Reverse transcriptase has become a extremely important tool in molecular biology to select the target genes.**

Rolling Circle Replication



D-loop Replication



- **Students will get an overview on the DNA replication process *in-vivo*.**
- **Students would comprehend the basic differences in prokaryotic and eukaryotic DNA replication process in details**
- **They will also get an insight into the understanding of the various enzymes and its function in the process**

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