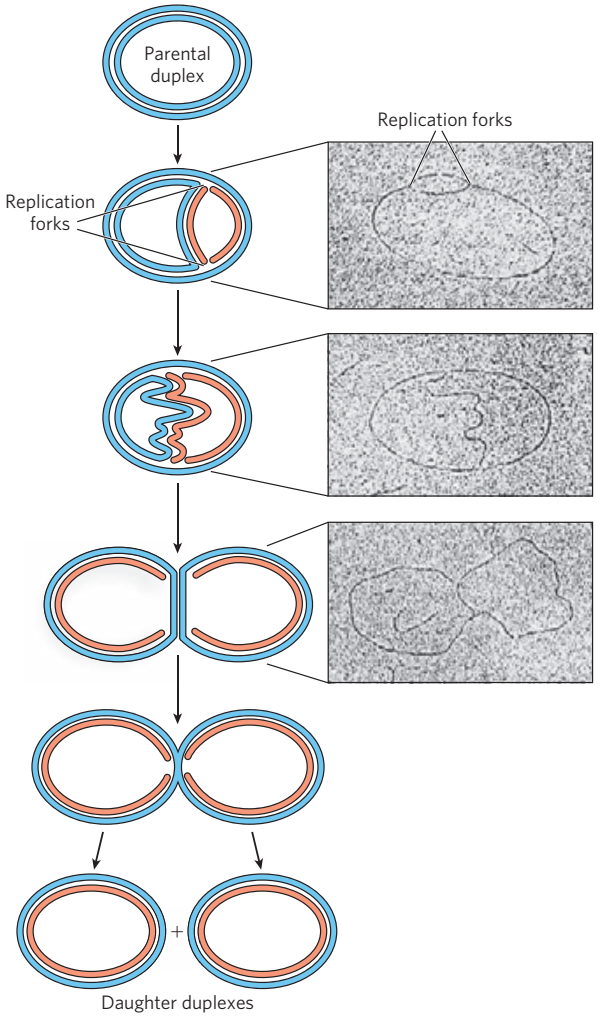
DNA Metabolism

DNA Replication

DNA Replication: Rules

- DNA replication is semiconservative: Each DNA strand serves as a template for the synthesis of a new strand, producing two new DNA molecules, each with one new strand and one old strand.
- Replication begins at an origin and usually proceeds Bidirectionally: formation of replication forks

DNA Replication: E. Coli



- **replication forks:** where parent DNA is being unwound and the separated strands quickly replicated.
- The technique revealed that in this system the replication loops always initiate at a unique point, which was termed an **origin.**

DNA Replication: E. Coli

- A new strand of DNA is always synthesized in the 5’➔3’ direction, with the free 3’-OH as the point at which the DNA is elongated (**Leading Strand**); Because the two DNA strands are antiparallel, the strand serving as the template is read from its 3’ end toward its 5’ end.
- The strand on direction 3’➔5’: is synthesized in short pieces called **Okazaki fragment** (from the scientist who discovered it). This strand is called **Lagging** strand.

DNA Replication: E. Coli

- **DNA is degraded by Nucleases (or DNAases):**

➢ **Exonucleases:** degrade nucleic acids from one end of the molecule. Many operate in only the 5'→3' or the 3'→5'direction, removing nucleotides only from the 5'or the 3' end, respectively, of one strand of a double-stranded nucleic acid or of a single- stranded DNA.

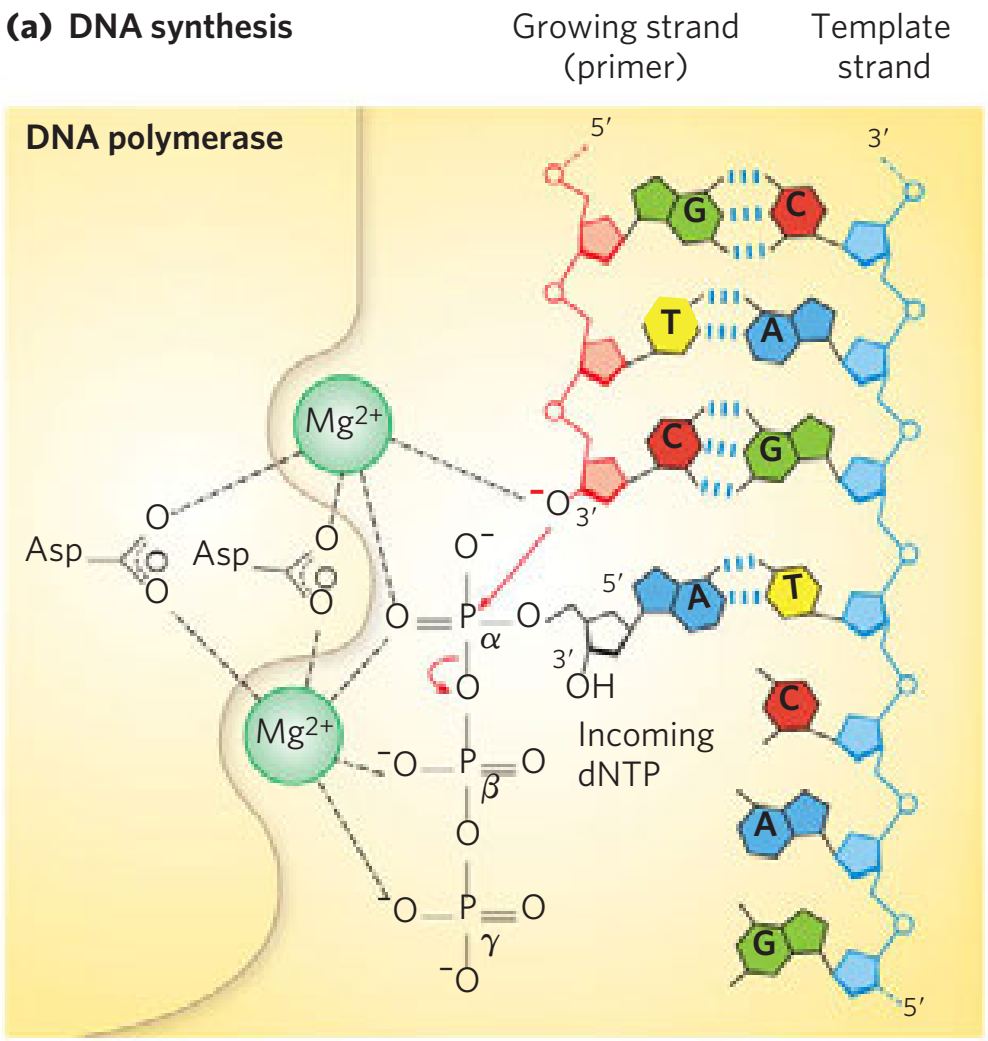
➢ **Endonucleases** can begin to degrade at specific internal sites in a nucleic acid strand or molecule, reducing it to smaller and smaller fragments.

- *A few exonucleases and endonucleases degrade only single- stranded DNA.*

DNA Replication: E. Coli

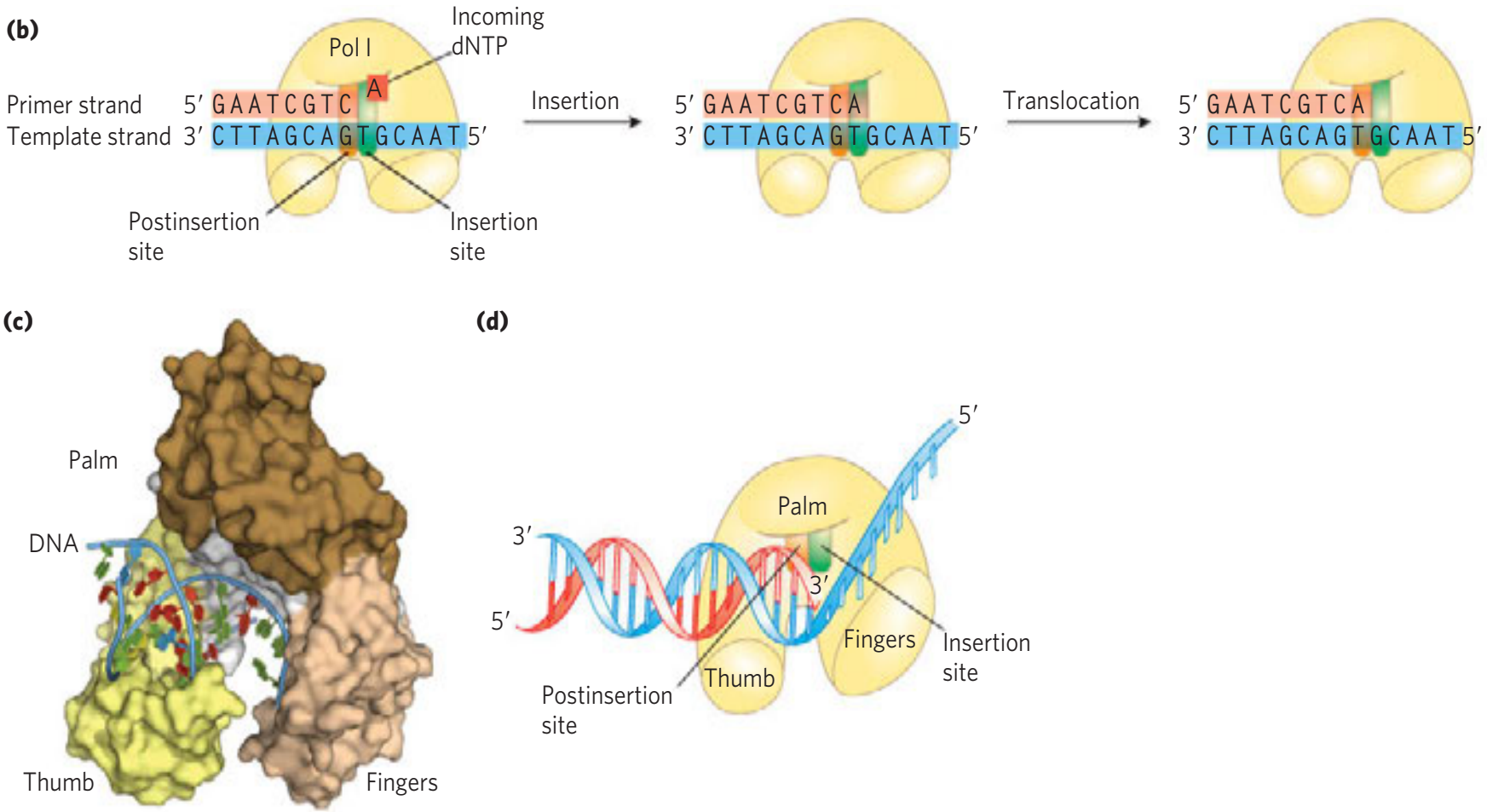
- DNA is synthesized by DNA polymerases
- The fundamental reaction is a phosphoryl group transfer. The nucleophile is the 3’-hydroxyl group of the nucleotide at the 3’ end of the growing strand. Nucleophilic attack occurs at the α phosphorus of the incoming deoxynucleoside 5’-triphosphate. Inorganic pyrophosphate is released in the reaction.

DNA Replication: E. Coli



The Mg^{2+} facilitates the attack of the 3’-hydroxyl group of the primer on the α phosphate of the nucleotide triphosphate; the other Mg^{2+} ion facilitates displacement of the pyrophosphate. Both ions stabilize the structure of the pentacovalent transition state. RNA polymerases use a similar mechanism.

DNA Replication: E. Coli



DNA Replication: E. Coli

- **the polymerases require a primer.** A primer is a strand segment (complementary to the template) with a free 3’-hydroxyl group to which a nucleotide can be added; the free 3’ end of the primer is called the **primer terminus.** In other words, part of the new strand must already be in place: all DNA polymerases can only add nucleotides to a pre-existing strand. Many primers are oligonucleotides of RNA rather than DNA, and specialized enzymes synthesize primers when and where they are required.

DNA Replication: E. Coli

DNA polymerases in E. Coli

	DNA polymerase		
	I	II	III
Structural gene*	*polA*	*polB*	*polC (dnaE)*
Subunits (number of different types)	1	7	≥10
M_r	103,000	88,000†	791,500
3′→5′ Exonuclease (proofreading)	Yes	Yes	Yes
5′→3′ Exonuclease	Yes	No	No
Polymerization rate (nucleotides/s)	10–20	40	250–1,000
Processivity (nucleotides added before polymerase dissociates)	3–200	1,500	≥500,000

DNA Replication: E. Coli

- **DNA replicase system** or **replisome:** 20 or more different enzymes and proteins, each performing a specific task during DNA replication.

➢ Helicases: separation of the two parent strands

➢ Toposisomerases

➢ DNA binding proteins

➢ Primases: synthesis of primers

➢ DNA Ligases: closing the nick remained after primer removing by polymerases

DNA Replication: E. Coli

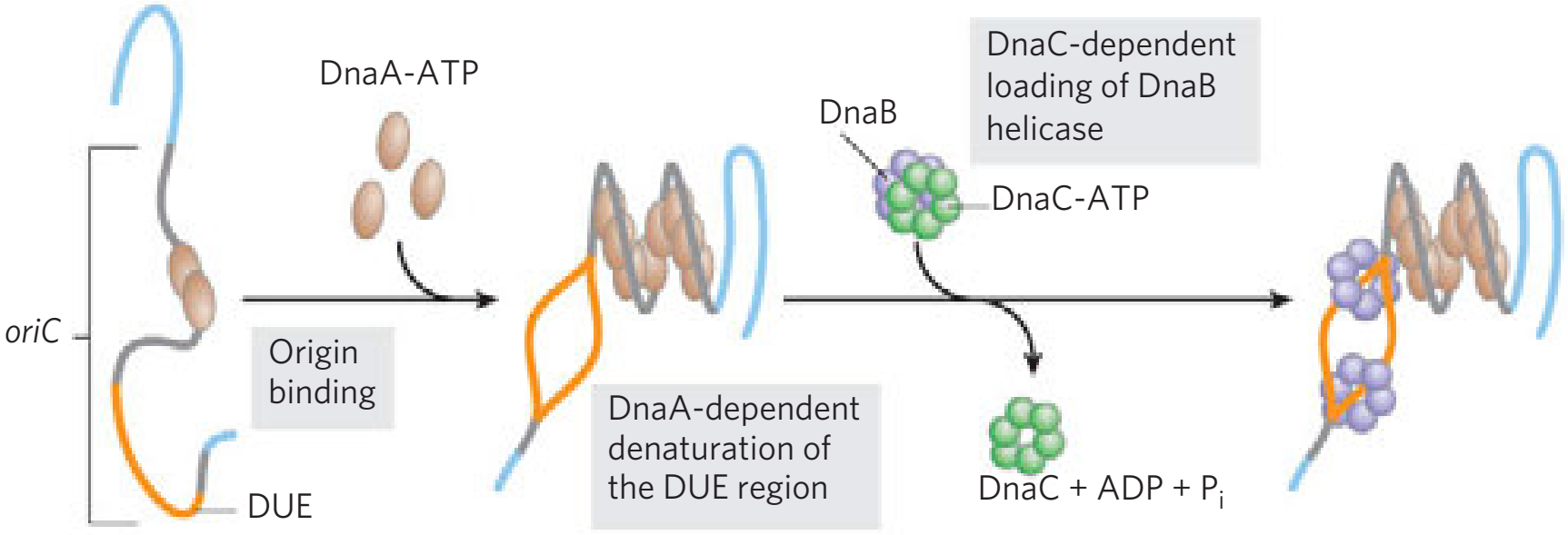
Proteins necessary for replication

Protein	M_r	Number of subunits	Function
DnaA protein	52,000	1	Recognizes ori sequence; opens duplex at specific sites in origin
DnaB protein (helicase)	300,000	6*	Unwinds DNA
DnaC protein	174,000	6*	Required for DnaB binding at origin
HU	19,000	2	Histonelike protein; DNA-binding protein; stimulates initiation
FIS	22,500	2*	DNA-binding protein; stimulates initiation
IHF	22,000	2	DNA-binding protein; stimulates initiation
Primase (DnaG protein)	60,000	1	Synthesizes RNA primers
Single-stranded DNA-binding protein (SSB)	75,600	4*	Binds single-stranded DNA
DNA gyrase (DNA topoisomerase II)	400,000	4	Relieves torsional strain generated by DNA unwinding
Dam methylase	32,000	1	Methylates (5′)GATC sequences at *oriC*

Replication Mechanism in E. Coli

Initiation_1: oriC (245 bp) binding for DnaA + region called DUE (DNA unwinding element).

- 8 DnaA + IHF+ FIS: form an helical complex in oriC.
- DnaB+ SSB: formation of the replication forks.



Replication Mechanism in E. Coli

Initiation_2

- DNA polymerase III is loaded onto the DNA (ATP hydrolysis on DnaA which is released from the OriC and vice versa)
- **Dam Methylase:** methylate sequence (5')-GATC and subsequently interaction with bacteria plasma membrane

Replication Mechanism in E. Coli

Elongation leading strand:

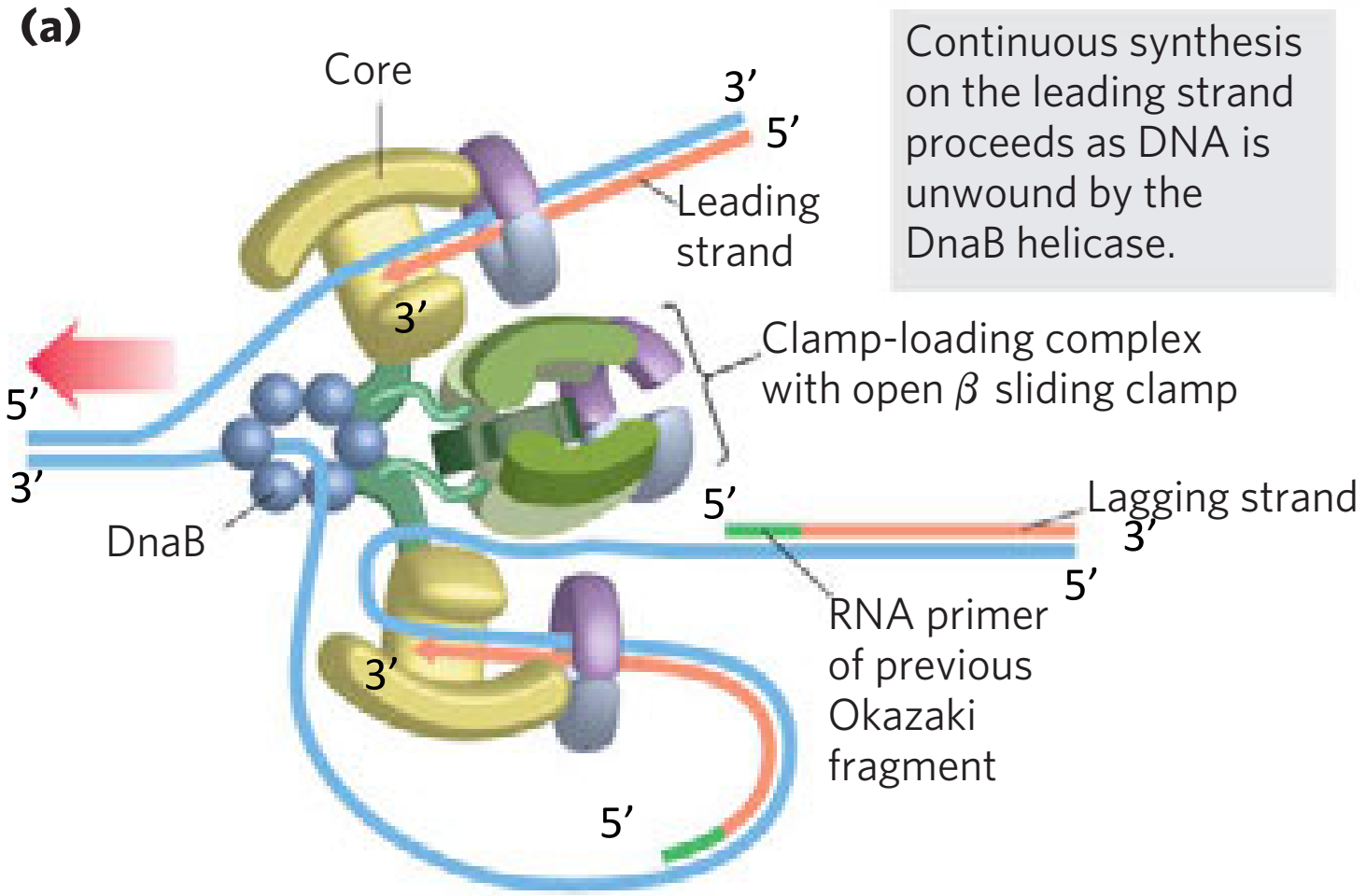
- synthesis by primase (DnaG protein) of a short (10 to 60 nucleotide) RNA primer at the replication origin. DnaG interacts with DnaB helicase to carry out this reaction, and the primer is synthesized in the direction opposite to that in which the DnaB helicase is moving.
- Deoxyribonucleotides are added to this primer by a DNA polymerase III complex linked to the DnaB helicase tethered to the opposite DNA strand. Leading strand synthesis then proceeds continuously, keeping pace with the unwinding of DNA at the replication fork.

Replication Mechanism in E. Coli

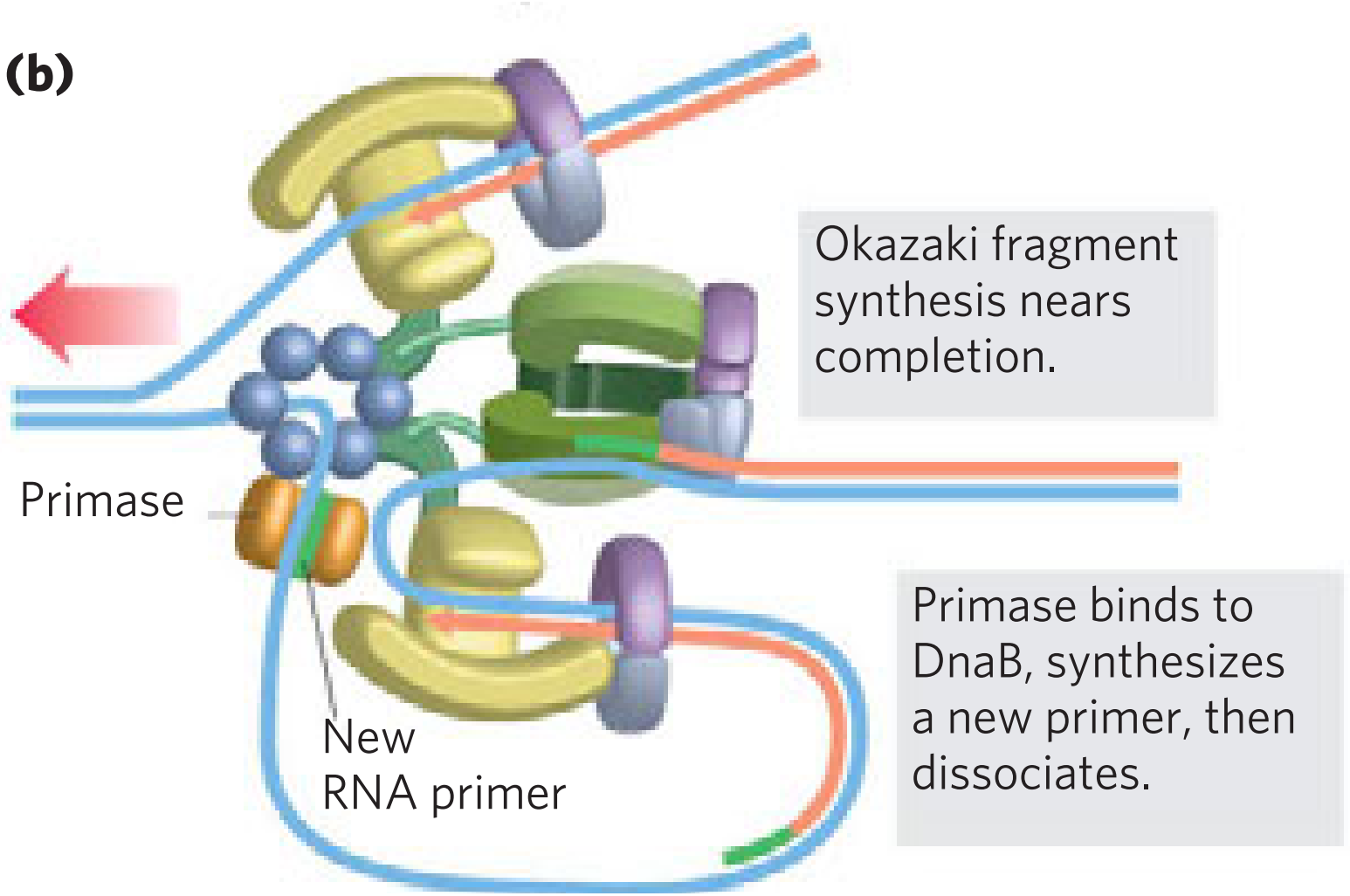
Elongation lagging strand:

- an RNA primer is synthesized by primase and, as in leading strand synthesis, DNA poly-merase III binds to the RNA primer and adds deoxyribonucleotides in direction 5’➔3’;
- the entire complex responsible for coordinated DNA synthesis at a replication fork is known as the **replisome**.

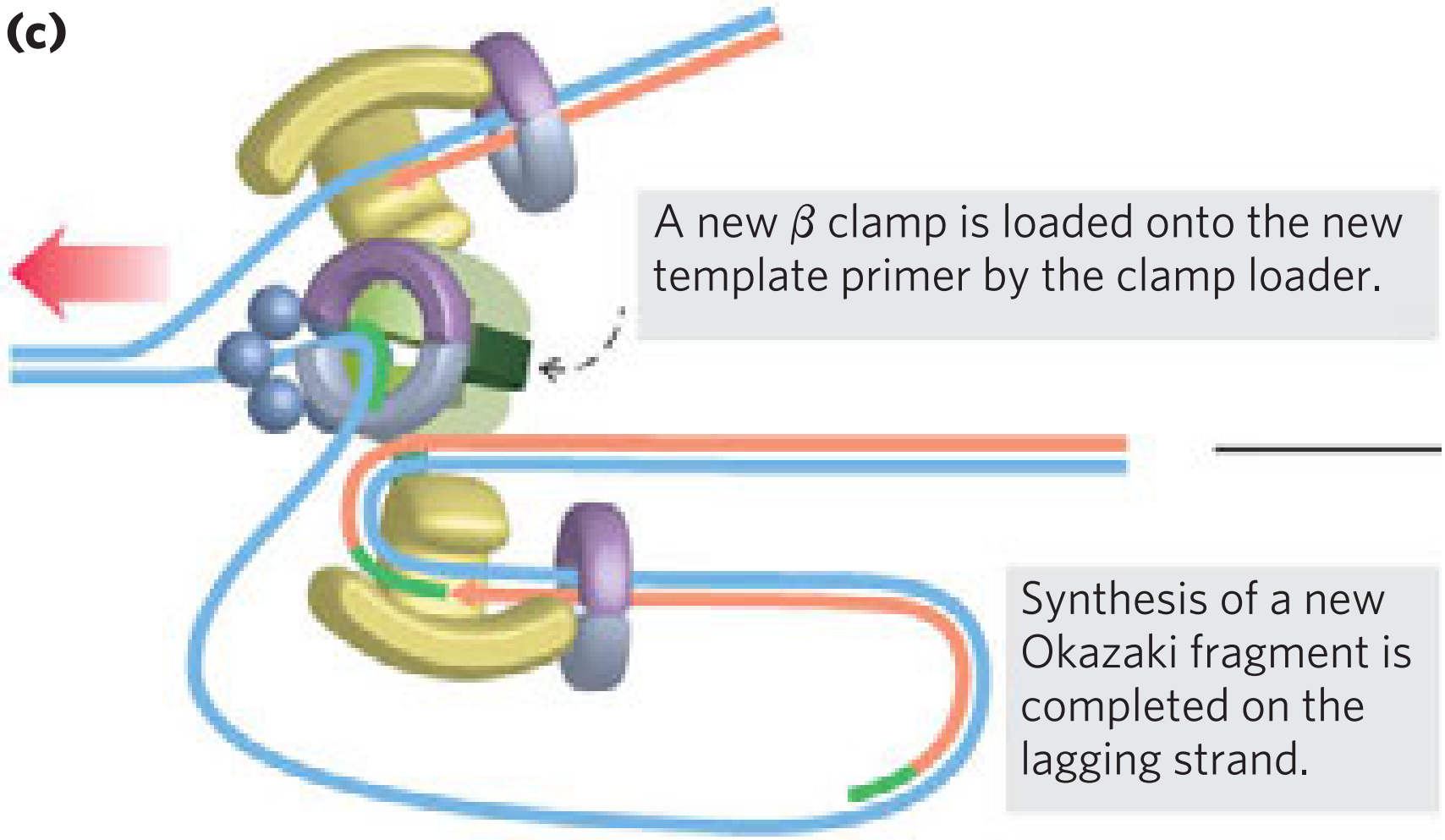
Replication Mechanism in E. Coli



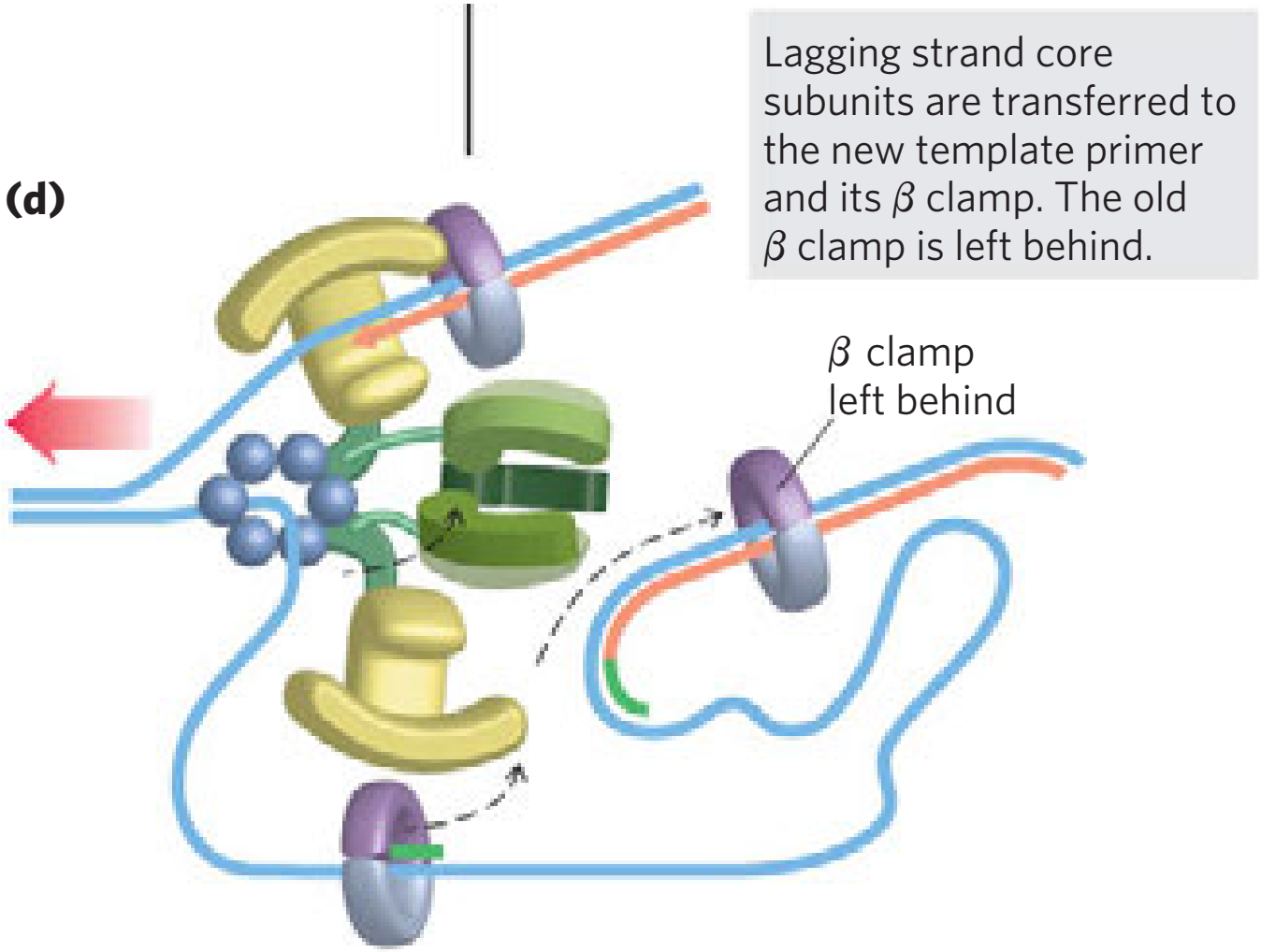
Replication Mechanism in E. Coli



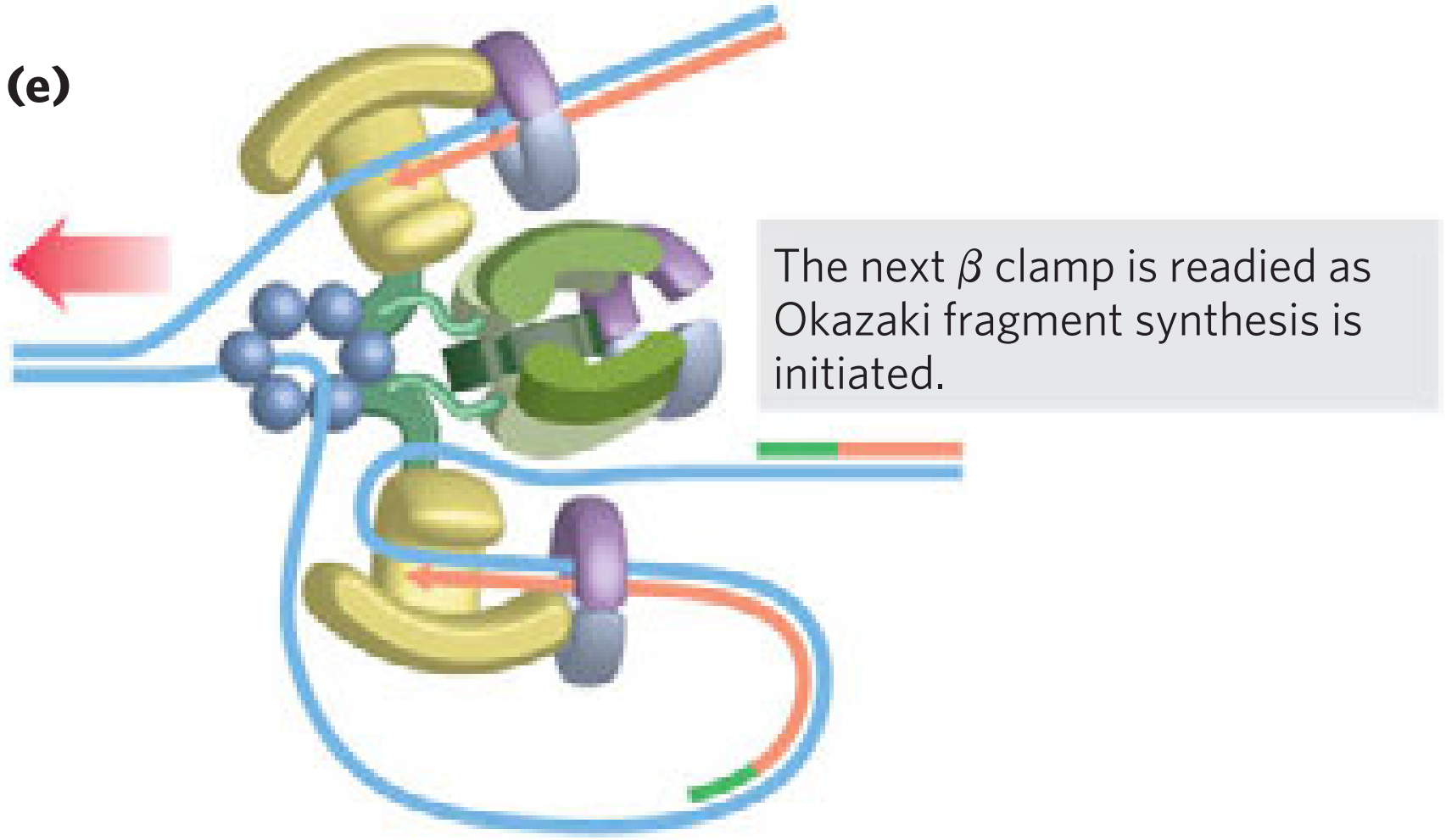
Replication Mechanism in E. Coli



Replication Mechanism in E. Coli



Replication Mechanism in E. Coli



Clamp is ATP dependent

Replication Mechanism in E. Coli

TABLE 25–4 Proteins of the *E. coli* Replisome

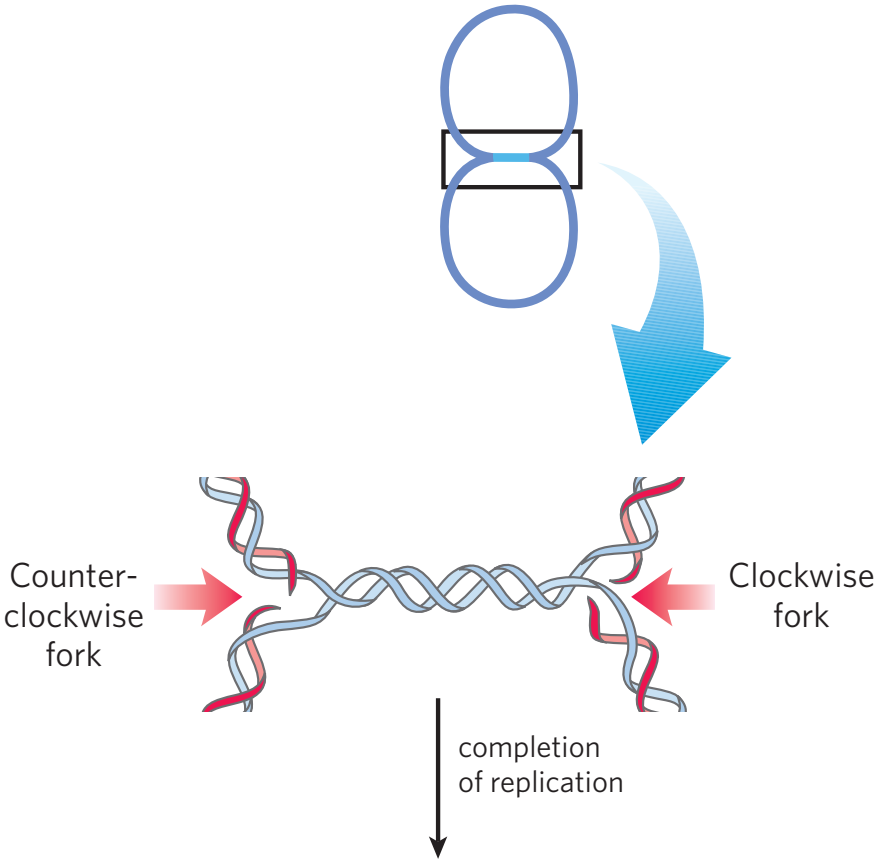
Protein	M_r	Number of subunits	Function
SSB	75,600	4	Binding to single-stranded DNA
DnaB protein (helicase)	300,000	6	DNA unwinding; primosome constituent
Primase (DnaG protein)	60,000	1	RNA primer synthesis; primosome constituent
DNA polymerase III	791,500	17	New strand elongation
DNA polymerase I	103,000	1	Filling of gaps; excision of primers
DNA ligase	74,000	1	Ligation
DNA gyrase (DNA topoisomerase II)	400,000	4	Supercoiling

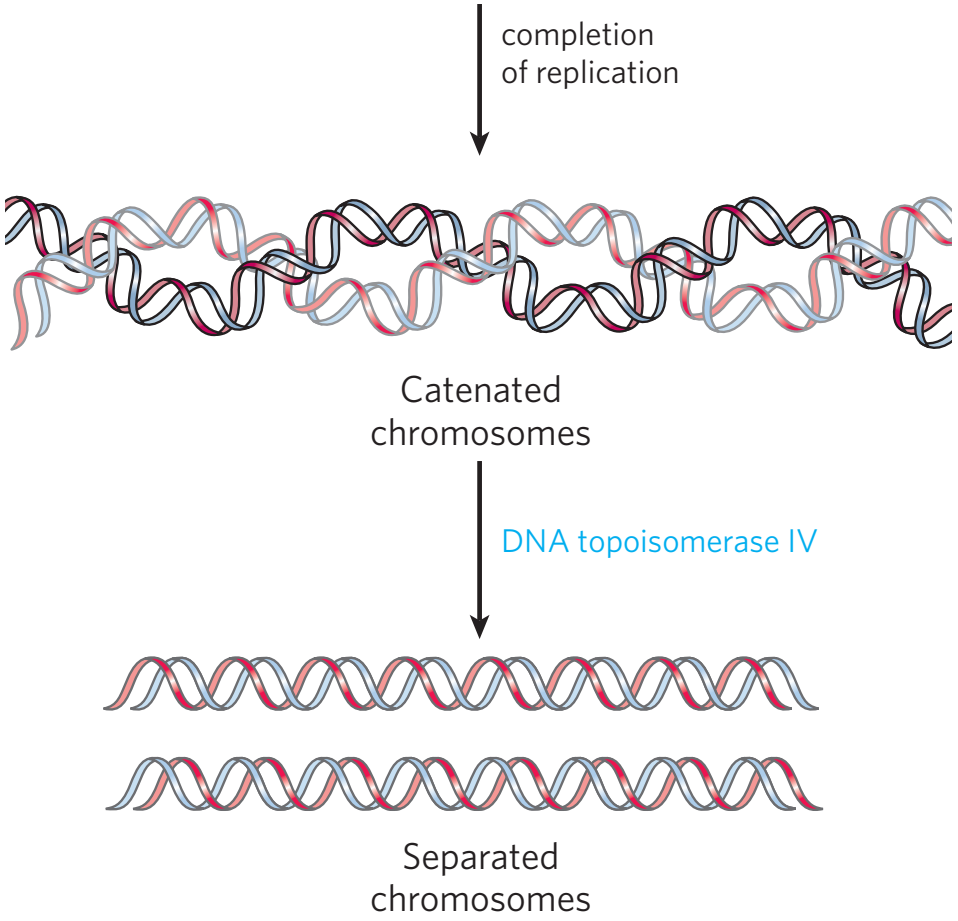
Replication Mechanism in E. Coli

Termination:

- Terminus region called Ter+ Tus (terminus utilization substance), this complex is preset only in one strand.
- replication forks halt when they meet each other:

  one fork stop when interact with the Ter+Tus complex, then the other fork stop when meet the first already halted fork.
- The final few hundred base pairs of DNA between these large protein complexes are then replicated completing two topologically interlinked circular chromosomes(catenases).


Counter-
clockwise
fork
Clockwise
fork
completion
of replication


completion
of replication
Catenated
chromosomes
DNA topoisomerase IV
Separated
chromosomes

Replication in Eukaryotic Cells

- The DNA molecules in eukaryotic cells are considerably larger than those in bacteria and are organized into complex nucleoprotein structures (chromatin);
- eukaryotic replication is regulated and coordi-nated with the cell cycle
- Origins of replication have a well-characterized structure in some lower eukaryotes, but they are much less defined in higher eukaryotes. In vertebrates, a variety of A=T-rich sequences may be used for replication initiation, and the sites may vary from one cell division to the next.

Replication in Eukaryotic Cells

- Regulation ensures that all cellular DNA is replicated once per cell cycle. Much of this regulation involves proteins **called cyclins** and the cyclin-dependent kinases (CDKs) with which they form complexes. The cyclins are rapidly destroyed by **ubiquitin-dependent proteolysis at the end of the M phase (mitosis)**, and the absence of cyclins allows the establishment of **prereplicative complexes (pre-RCs)** on replication initiation sites.

Replication in Eukaryotic Cells

- Speed of replication is 1/20(th) than e prokaryotes, and is bidirectional from many origins.
- The initiation of replication is similar to that eukaryotes through ORC (origin recognition complex).
- Eukaryotes have several types of DNA polymerases (nuclear and mitochondrial DNA)

Replication in Eukaryotic Cells

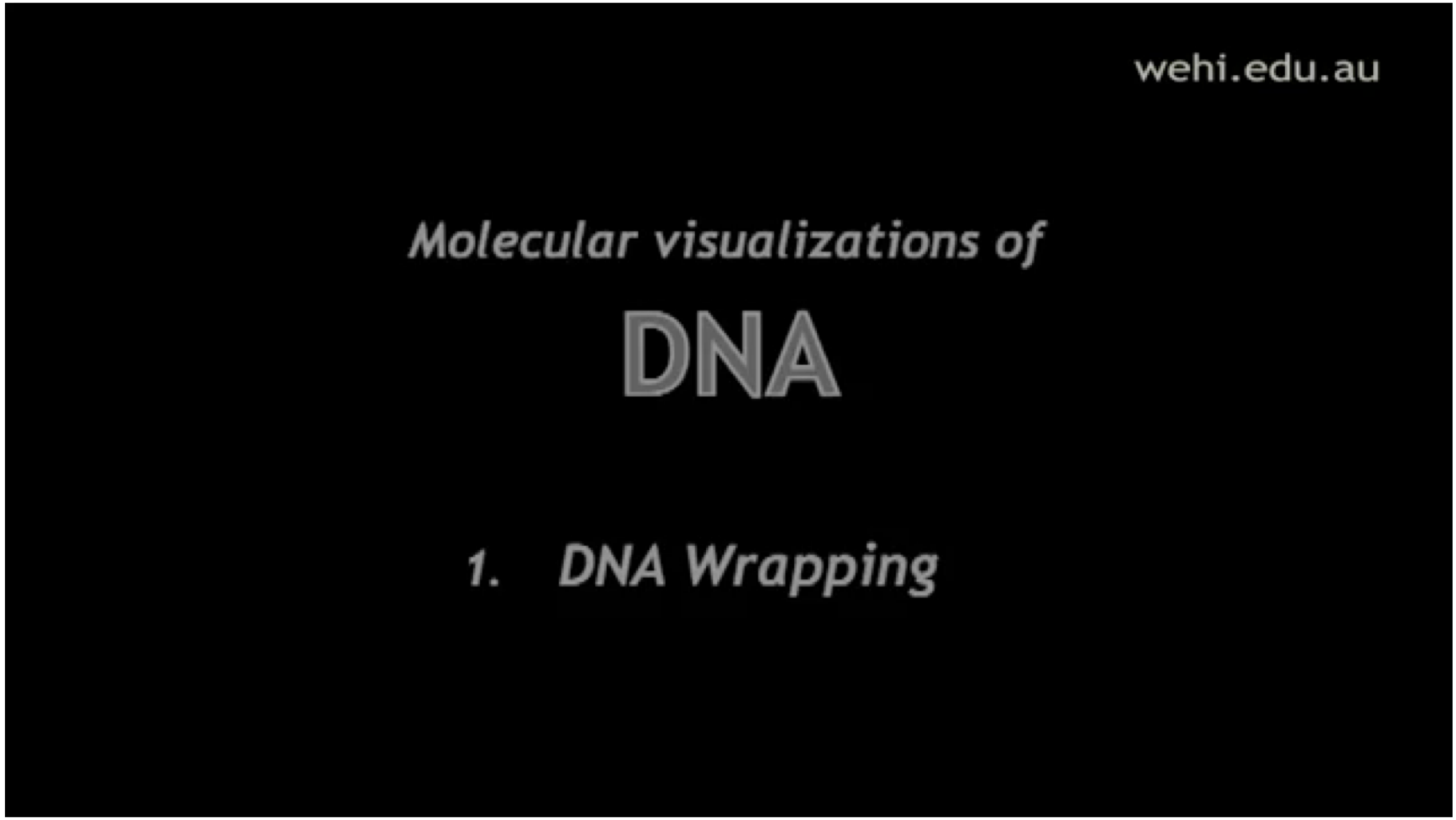
- **DNA polymerase α:** is typically a multisubunit enzyme with similar structure and properties in all eukaryotic cells. One subunit has a primase activity, and the largest subunit (Mr = 180,000) contains the polymerization activity, is believed to function only in the synthesis of short primers (either RNA or DNA) for Okazaki fragments on the lagging strand.

Replication in Eukaryotic Cells

- **DNA polymerase δ: forms** complex with proliferating cell nuclear antigen (PCNA; *Mr*= 29,000). PCNA is remarkably similar to that of the β subunit of *E. coli* DNA polymerase III. DNA polymerase δ has a 3'→ 5' proofreading exonuclease activity and seems to carry out both leading and lagging strand synthesis in a complex comparable to the dimeric bacterial DNA polymerase III.

Replication in Eukaryotic Cells

- **DNA polymerase ε**: replaces DNA polymerase δ in some situations, such as in DNA repair. DNA polymerase ε may also function at the replication fork, perhaps playing a role analogous to that of the bacterial DNA polymerase I, removing the primers of Okazaki fragments on the lagging strand.
- Termination occurs through the **telomers**.


wehi.edu.au
Molecular visualizations of
DNA
1. DNA Wrapping