Organelle Genomes

- Usually (not always) circular molecules of DNA
- Multiple copies of the genome in each organelle
- Variable dimension
- Organelle genomes encode some, but not all, of the proteins used in the organelle.

An early eukaryote was parasitized by or ingested some aerobic prokaryotes. The cells were protected and produced energy for the eukaryote.

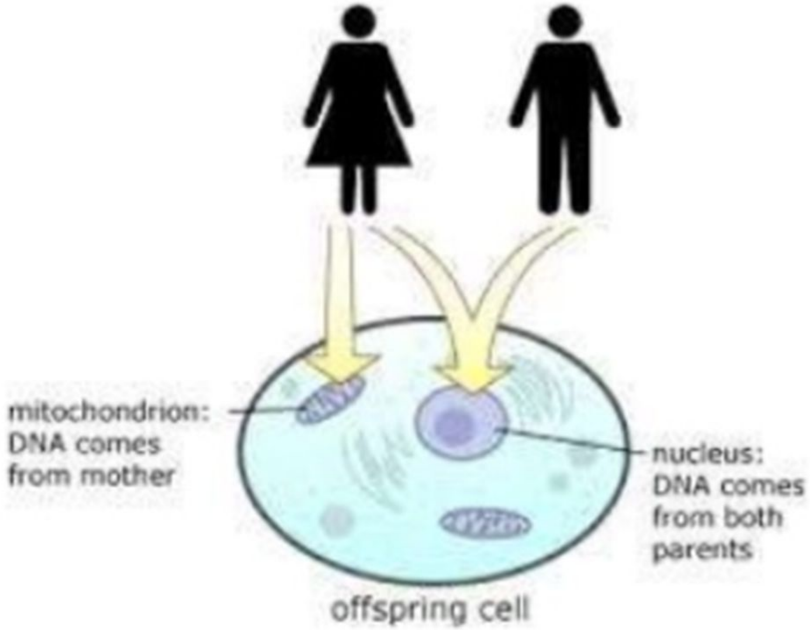
Over millions of years, the aerobic prokaryotes became mitochondria, no longer able to live on their own.

Some eukaryotes also formed symbiotic relationships with cyanobacteria, which contain photosynthetic pigments.

The aerobic prokaryotes became mitochondria in all eukaryotic cells.

The cyanobacteria became chloroplasts in protist or plant cells.

In humans, mitochondrial DNA can be assessed as the smallest chromosome coding for 37 genes and containing approximately 16,600 base pairs.
Human mitochondrial DNA was the first significant part of the human genome to be sequenced.
In most species, including humans, mtDNA is inherited solely from the mother



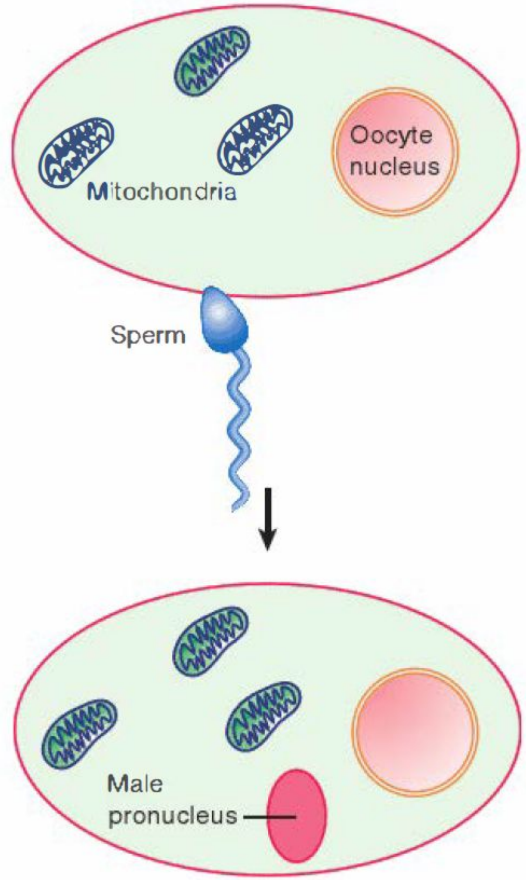
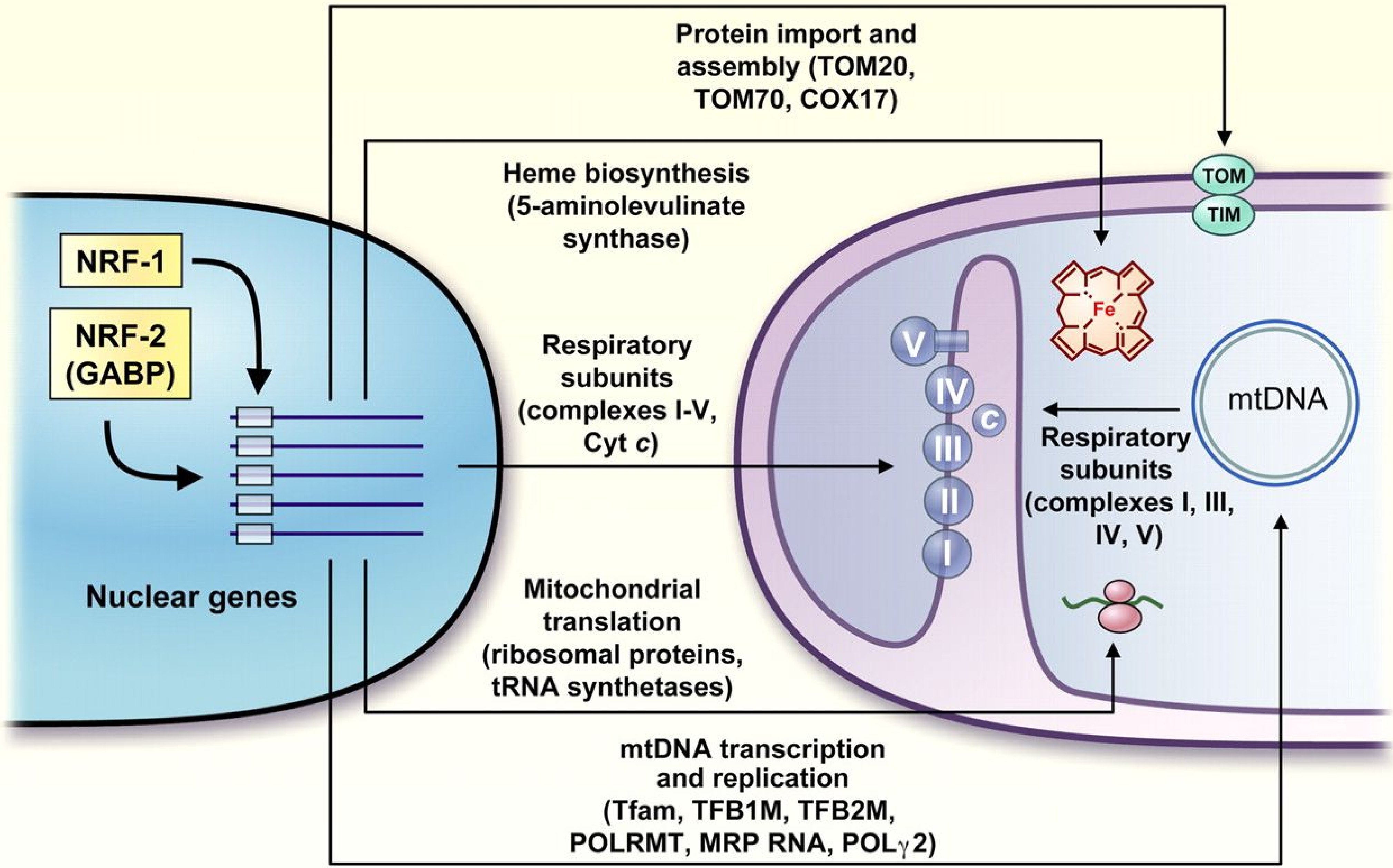


FIGURE 5.10 DNA from the sperm enters the oocyte to form the male pronucleus in the fertilized egg, but all the mitochondria are provided by the oocyte.


Protein import and assembly (TOM20, TOM70, COX17)
Heme biosynthesis (5-aminolevulinate synthase)
NRF-1
NRF-2 (GABP)
Respiratory subunits (complexes I-V, Cyt c)
Nuclear genes
Mitochondrial translation (ribosomal proteins, tRNA synthetases)
mtDNA transcription and replication (Tfam, TFB1M, TFB2M, POLRMT, MRP RNA, POLγ2)
TOM
TIM
Fe
V
IV
c
III
II
I
mtDNA
Respiratory subunits (complexes I, III, IV, V)

Species	Size (kb)	Protein-coding genes	RNA-coding genes
Fungi	19–100	8–14	10–28
Protists	6–100	3–62	2–29
Plants	186–366	27–34	21–30
Animals	16–17	13	4–24

FIGURE 5.11 Mitochondrial genomes have genes encoding (mostly complex I–IV) proteins, rRNAs, and tRNAs.

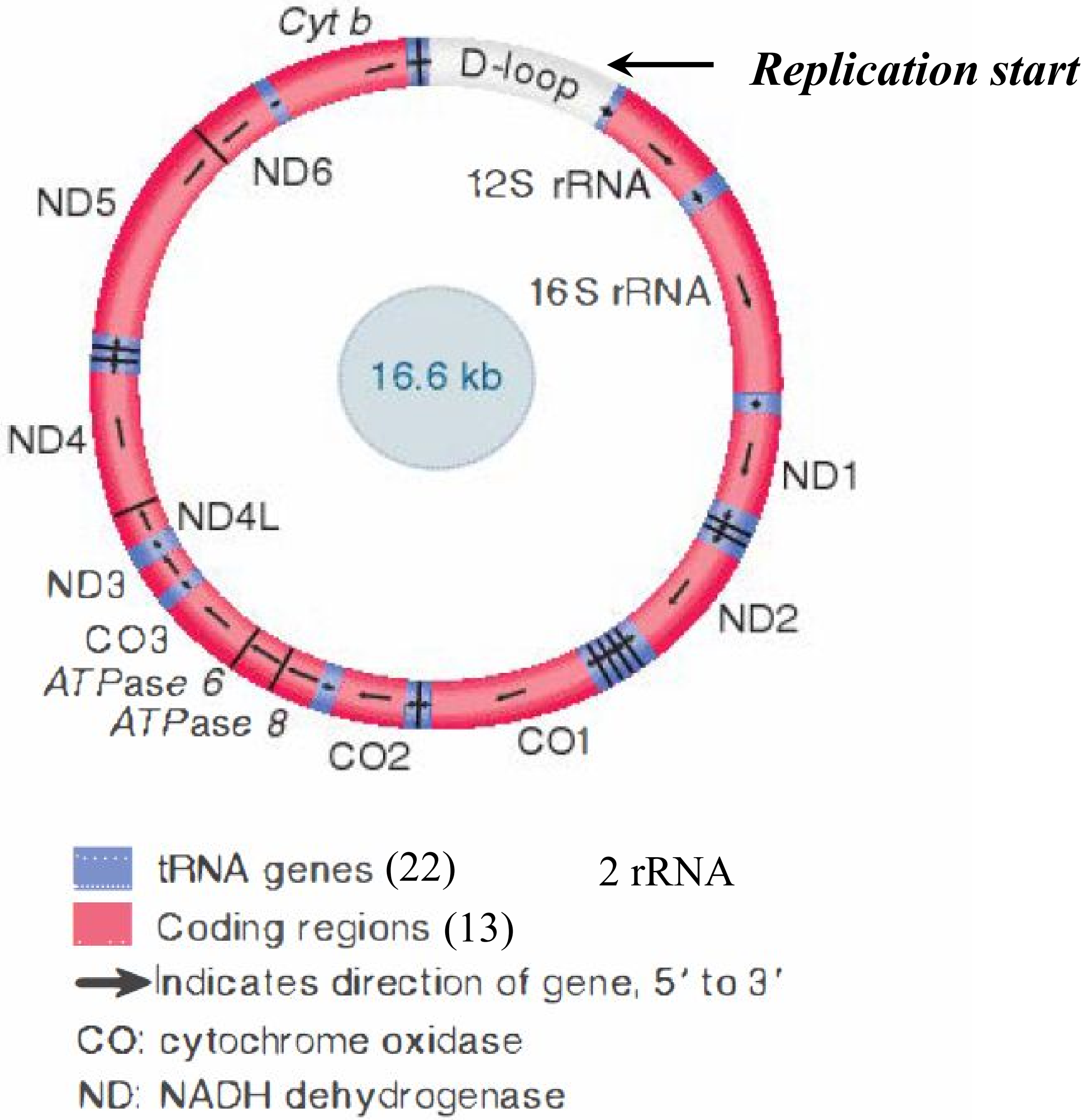


FIGURE 5.12 Human mitochondrial DNA has 22 tRNA genes, 2 rRNA genes, and 13 protein-coding regions. Fourteen of the 15 protein-coding or rRNA-coding regions are transcribed in the same direction. Fourteen of the tRNA genes are expressed in the clockwise direction and 8 are read counterclockwise.

5x longer than animal cell mtDNA

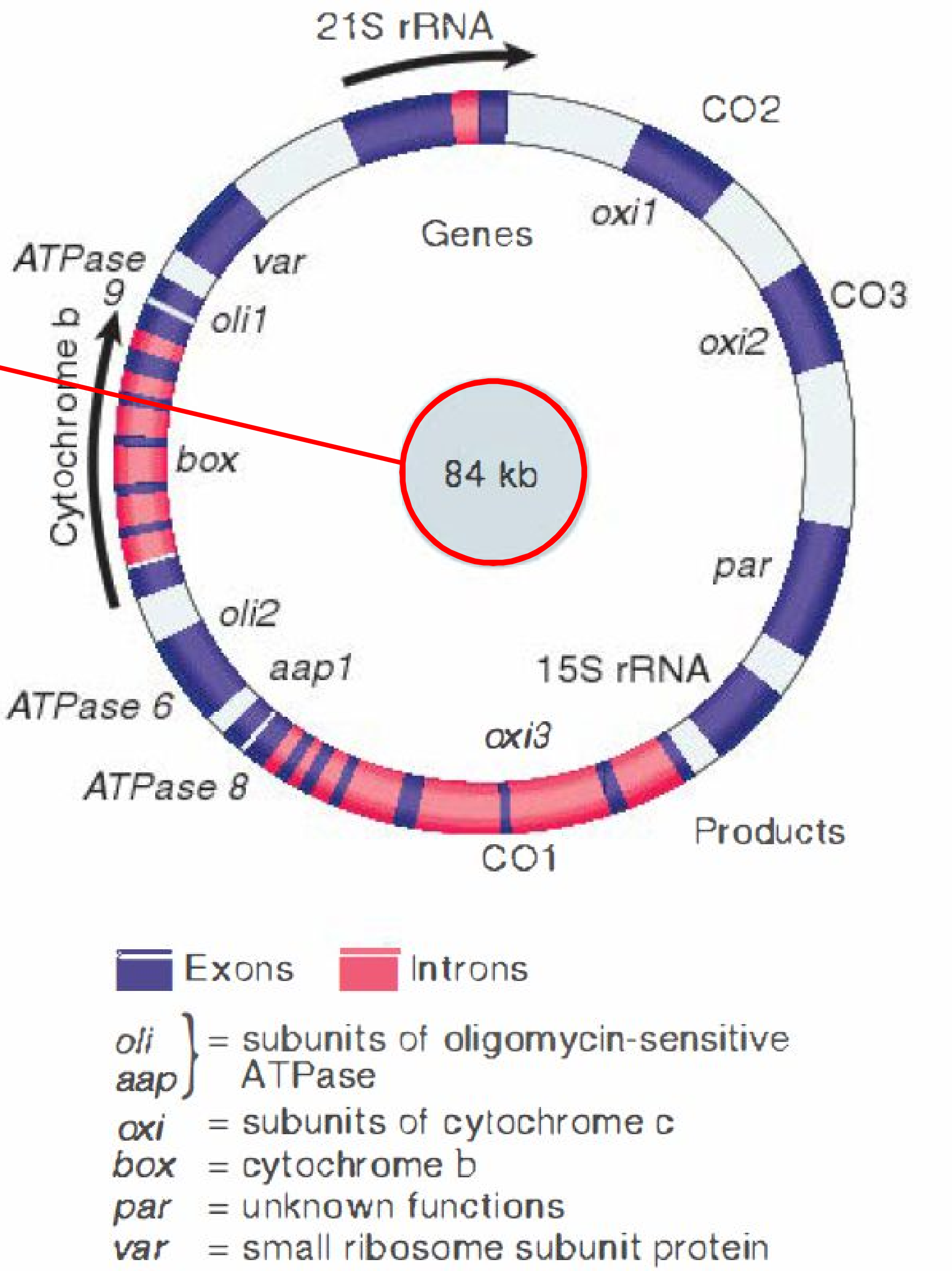


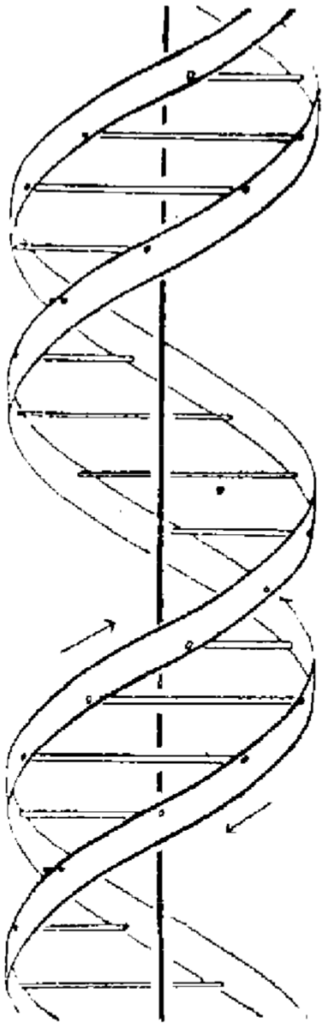
FIGURE 5.13 The mitochondrial genome of *S. cerevisiae* contains both interrupted and uninterrupted protein-coding genes, rRNA genes, and tRNA genes (positions not indicated). Arrows indicate direction of transcription.

Genes	Types
RNA-coding	
16S rRNA	1
23S rRNA	1
4.5S rRNA	1
5S rRNA	1
tRNA	30–32
Gene Expression	
r-proteins	20–21
RNA polymerase	3
Others	2
Chloroplast functions	
Rubisco and thylakoids	31–32
NADH dehydrogenase	11
Total	105–113

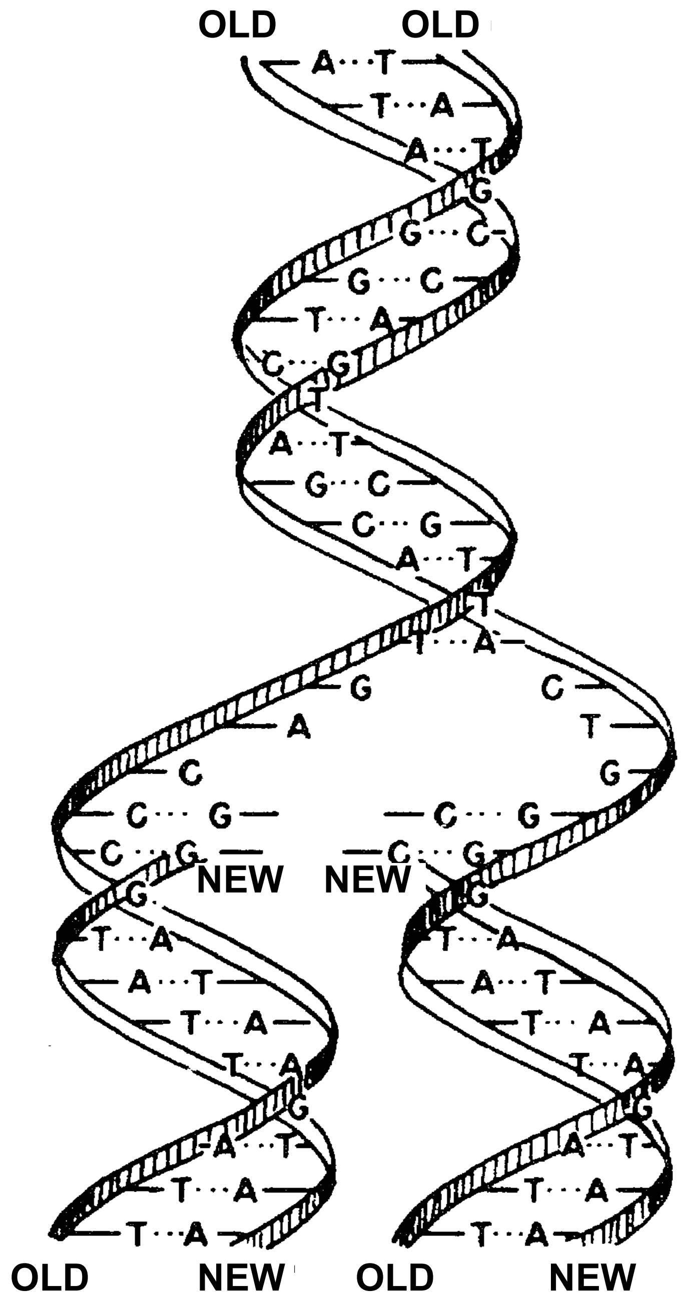
FIGURE 5.14 The chloroplast genome in land plants encodes 4 rRNAs, 30 tRNAs, and ~60 proteins.

DNA Replication

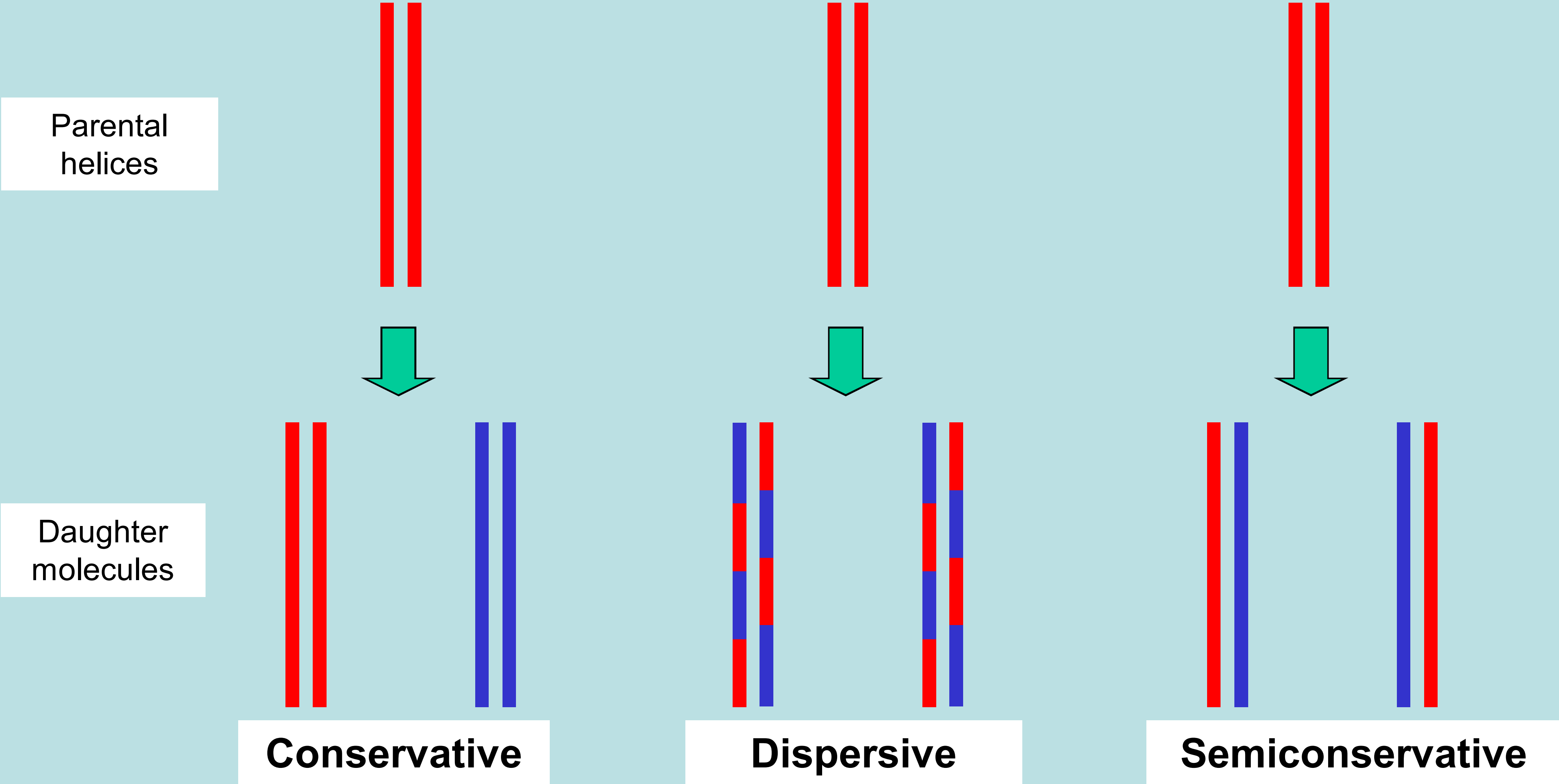
The Watson & Crick double helix model suggest a replication mechanism

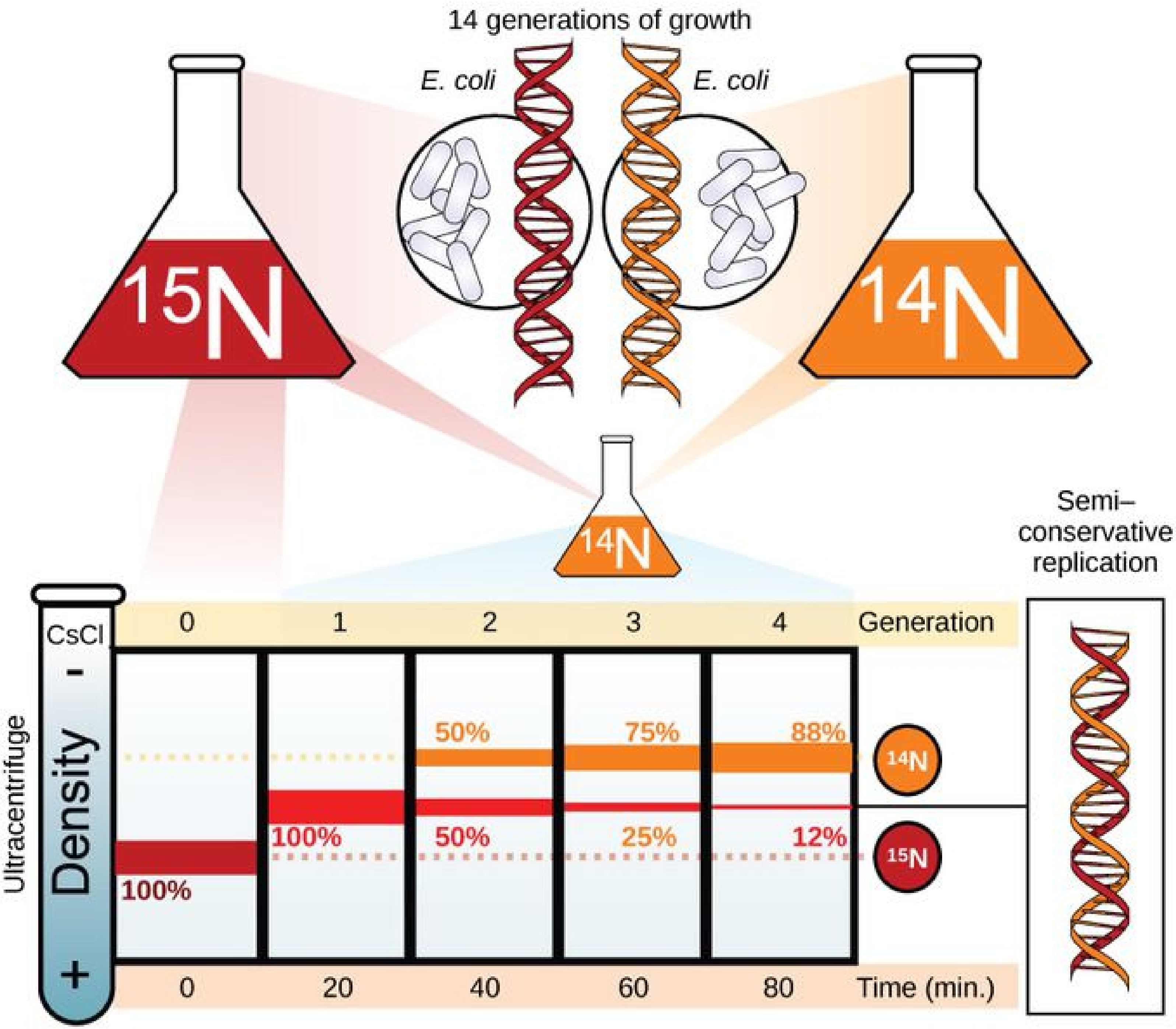


This figure is purely diagrammatic. The two ribbons symbolize the two phosphate—sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

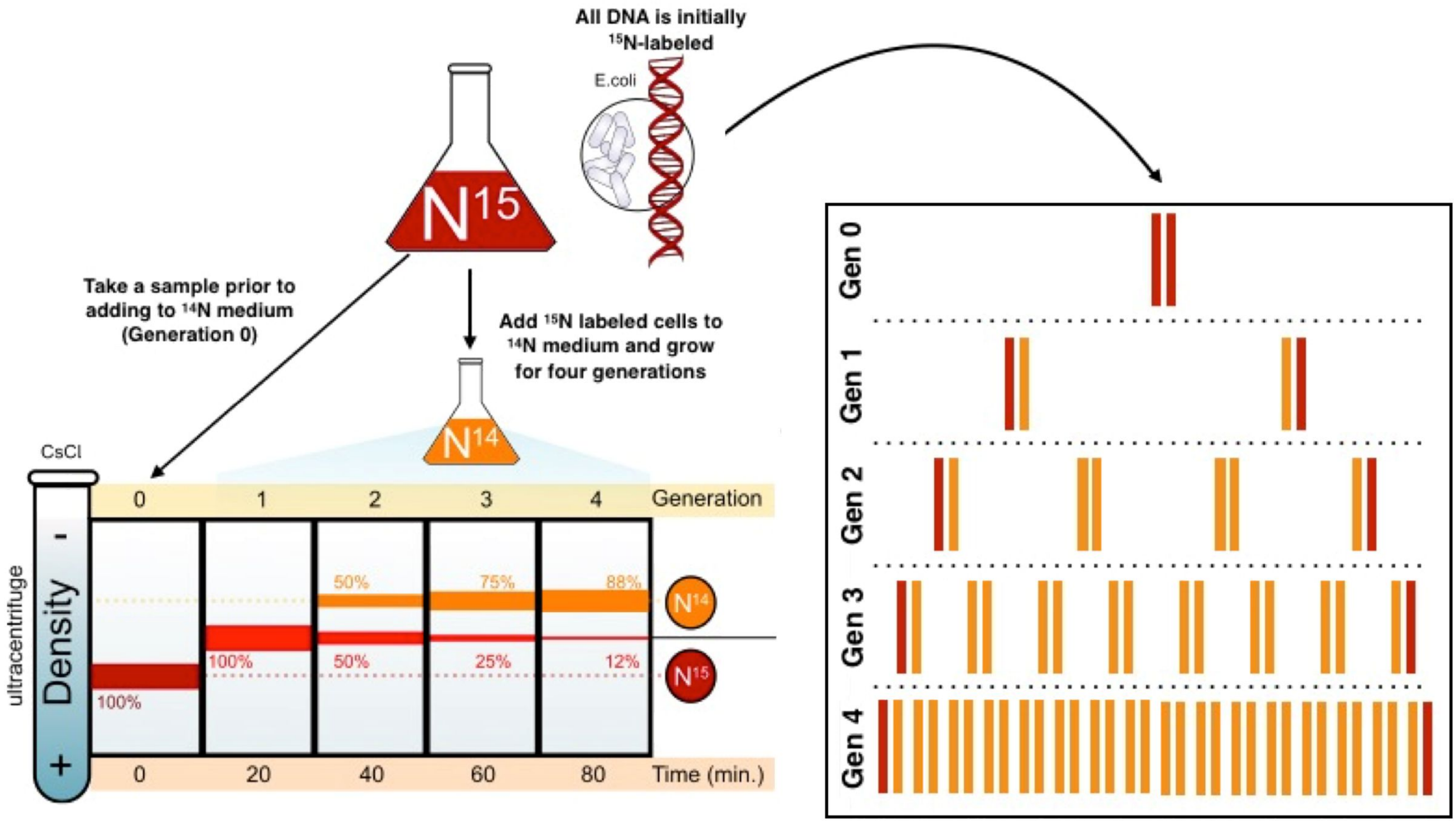


Replication mechanisms





14 generations of growth
E. coli
E. coli
15N
14N
14N
CsCl
Ultracentrifuge
Density
-
+
0
1
2
3
4
Generation
50%
75%
88%
14N
100%
100%
50%
25%
12%
15N
0
20
40
60
80
Time (min.)
Semi–conservative replication



All DNA is initially ^{15}N-labeled
E.coli
Take a sample prior to adding to ^{14}N medium (Generation 0)
Add ^{15}N labeled cells to ^{14}N medium and grow for four generations
N15
N14
CsCl
ultracentrifuge
Density
Generation
0
1
2
3
4
50%
75%
88%
100%
100%
50%
25%
12%
Time (min.)
0
20
40
60
80
Gen 0
Gen 1
Gen 2
Gen 3
Gen 4

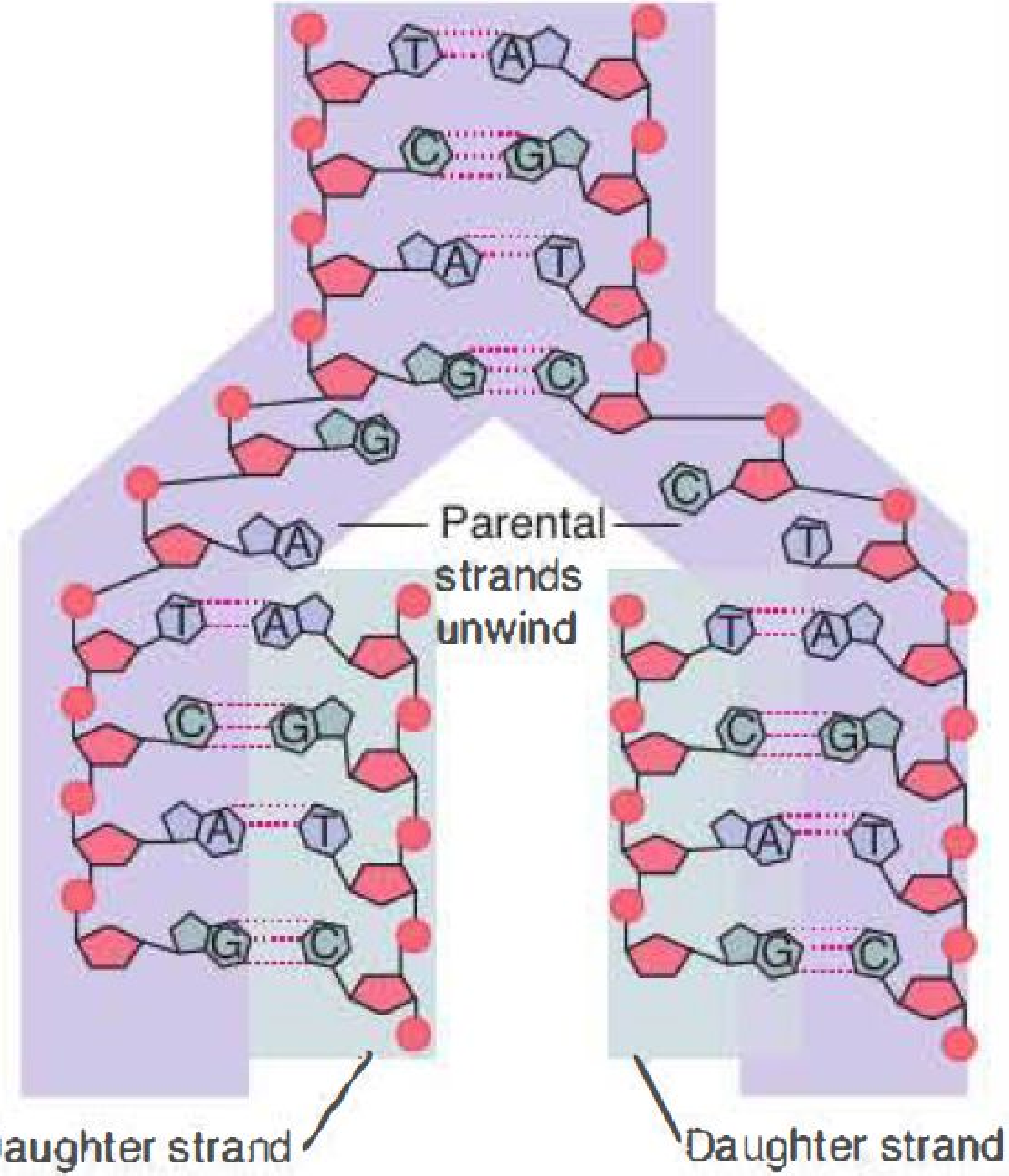


FIGURE 1.14 Base pairing provides the mechanism for replicating DNA.

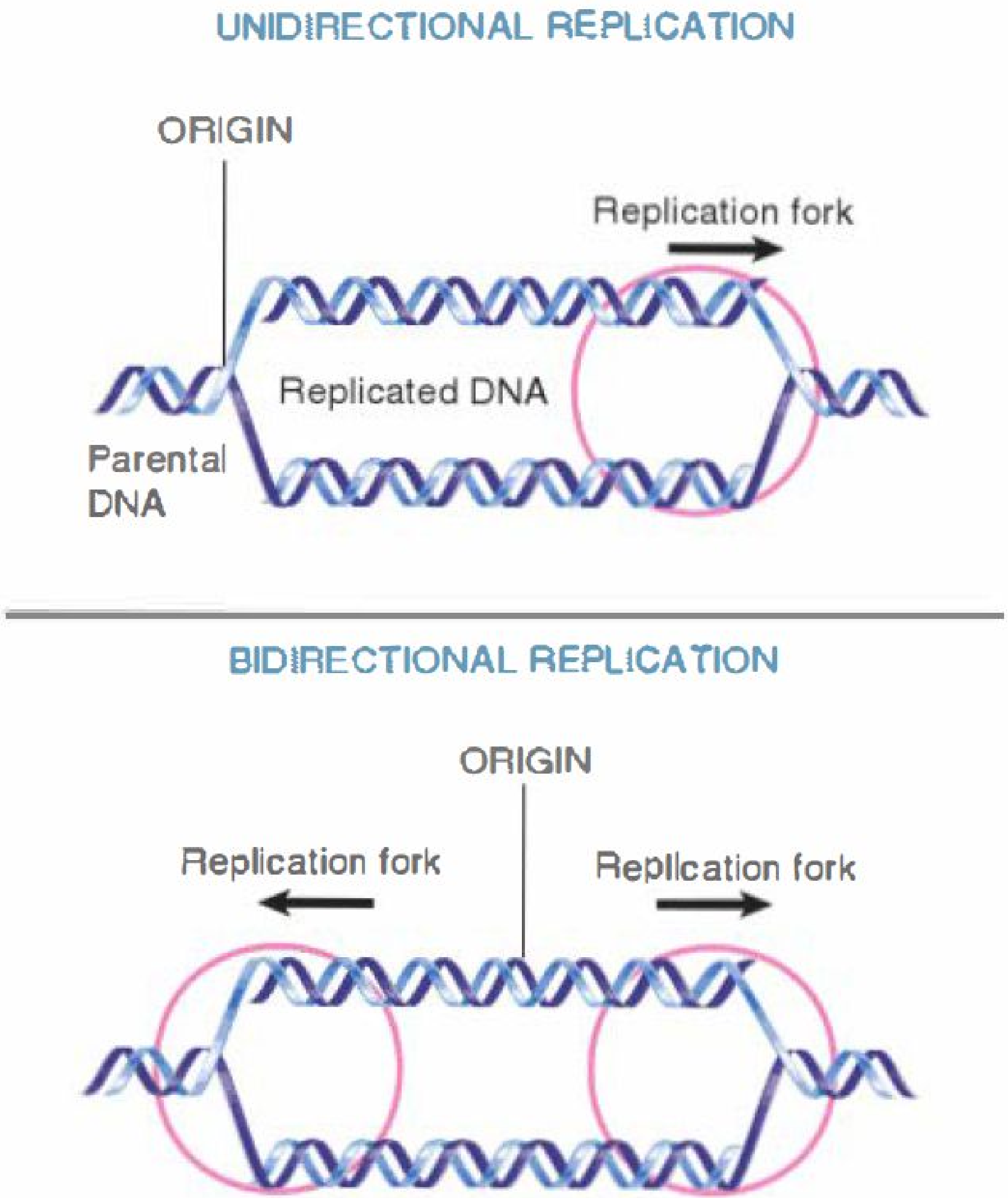
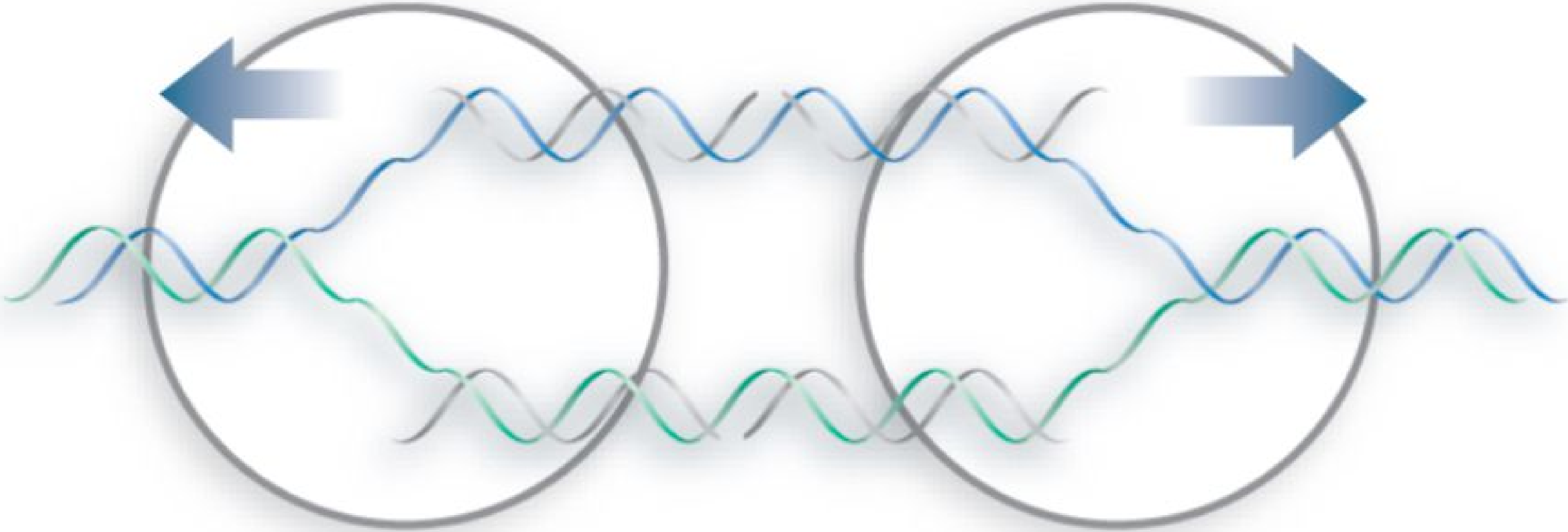


FIGURE 12.3 Replicons may be unidirectional or bidirectional, depending on whether one or two replication forks are formed at the origin.

Replication is usually bidirectional



Leftward replication fork

Rightward replication fork

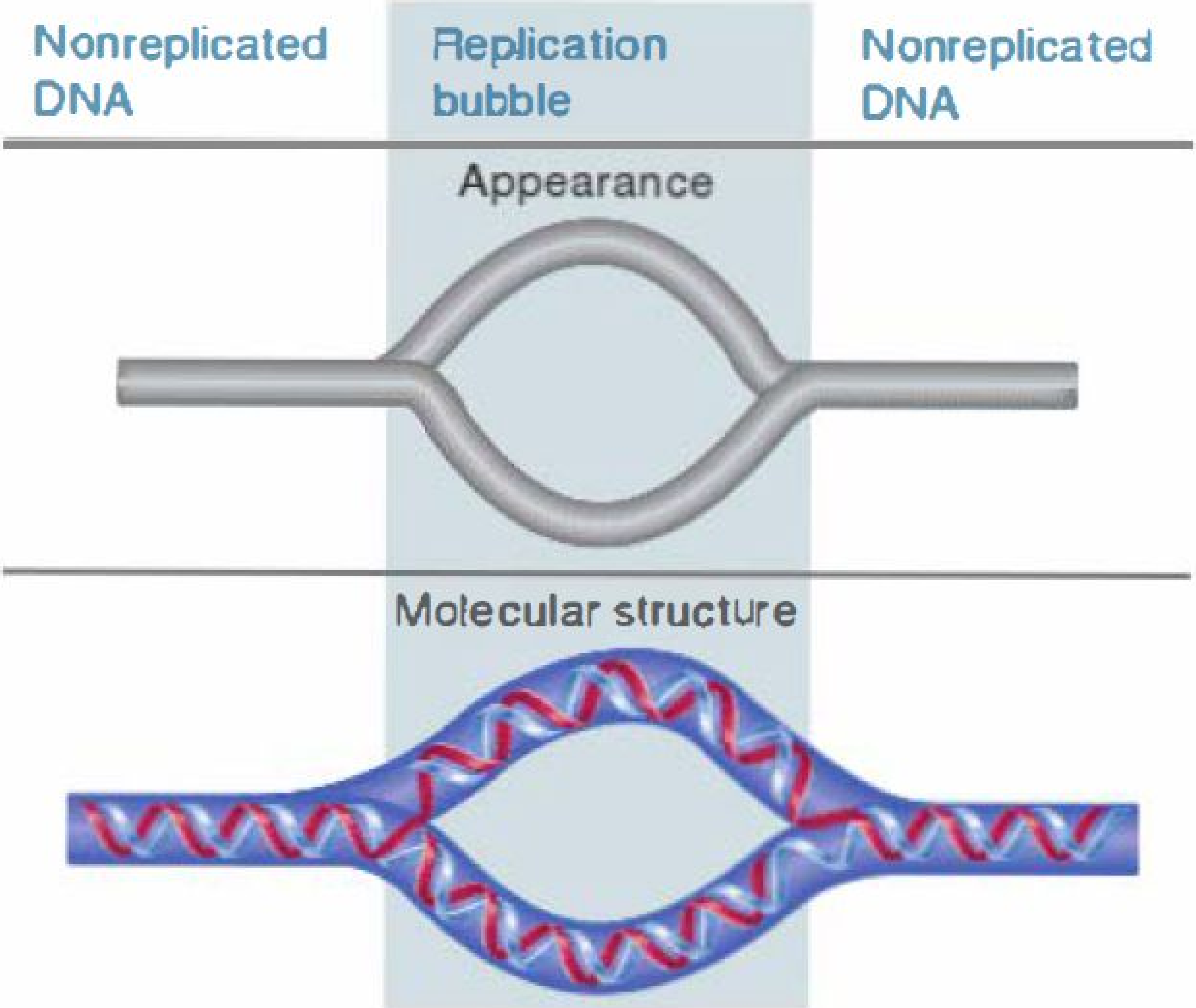


FIGURE 12.2 Replicated DNA is seen as a replication bubble flanked by nonreplicated DNA.

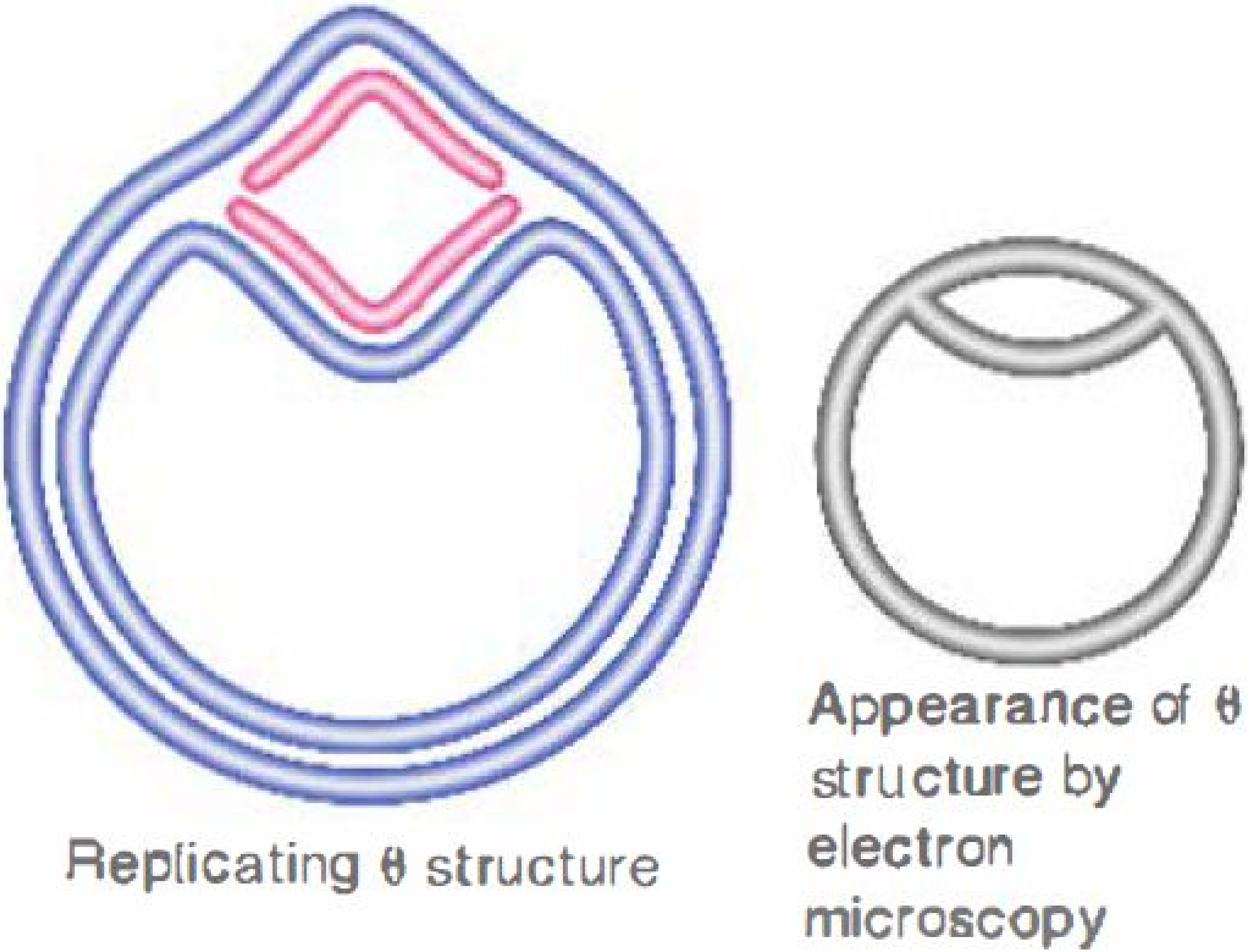
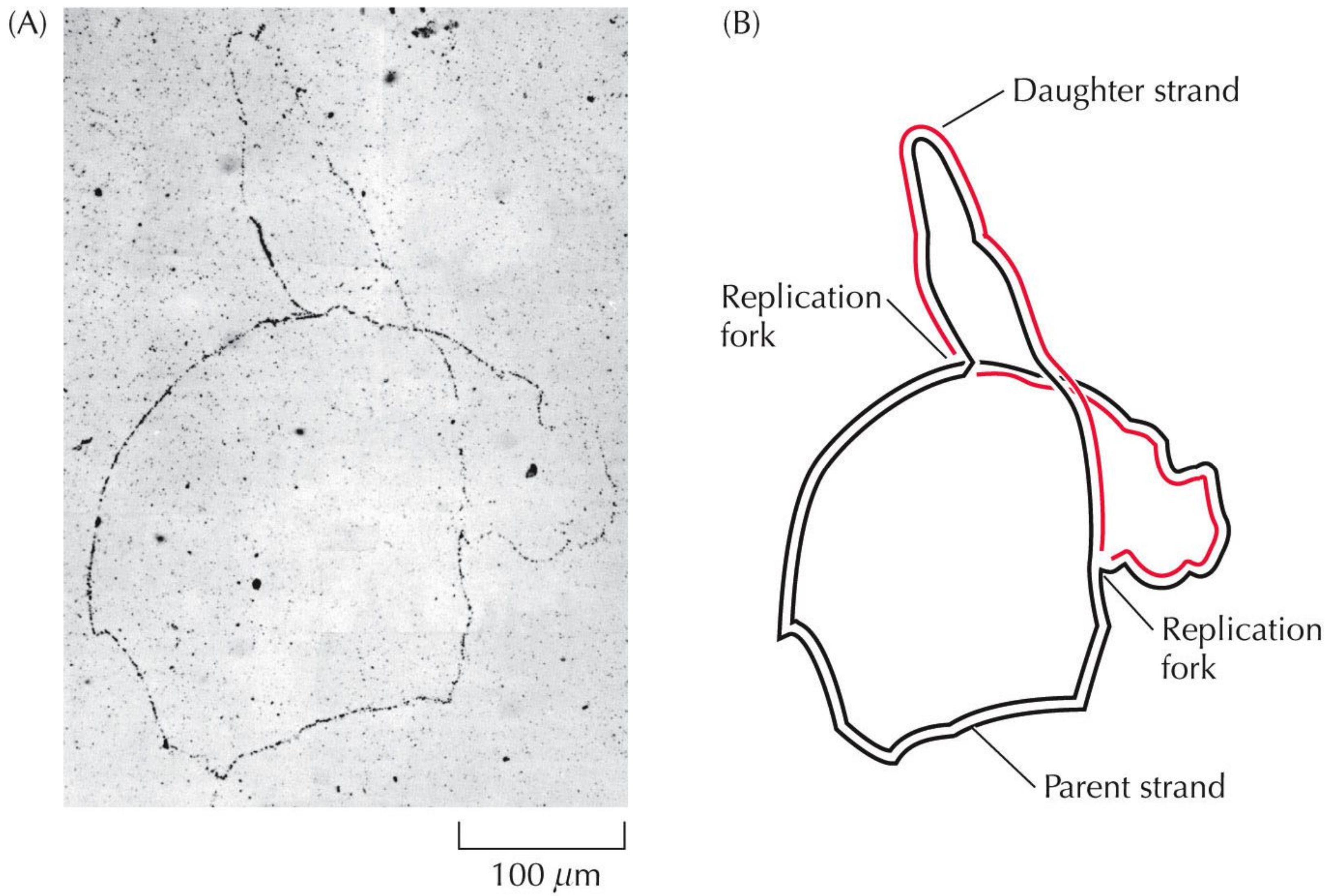


FIGURE 12.4 A replication bubble forms a θ structure in circular DNA.


(A)
100 μm
(B)
Daughter strand
Replication fork
Replication fork
Parent strand

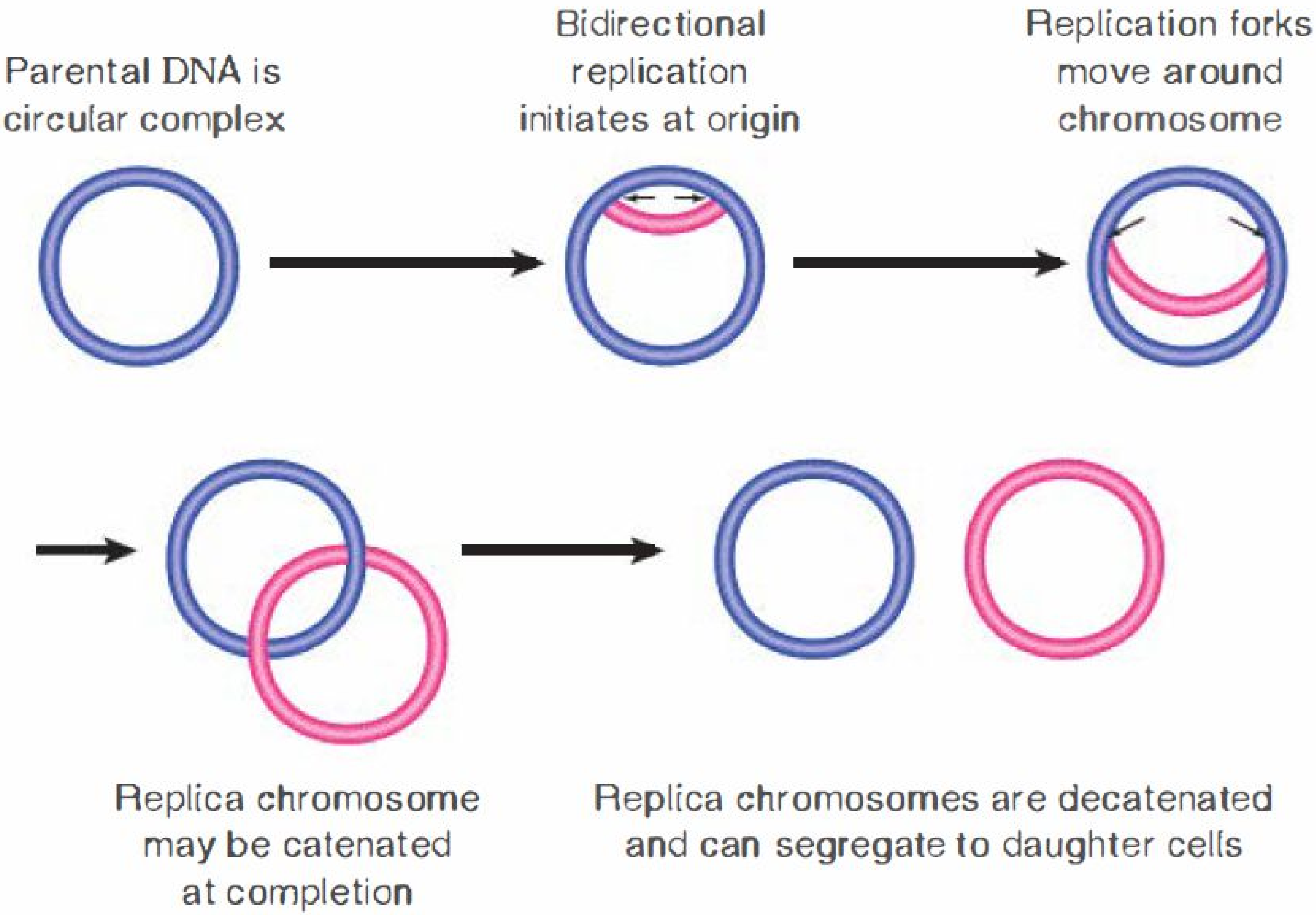
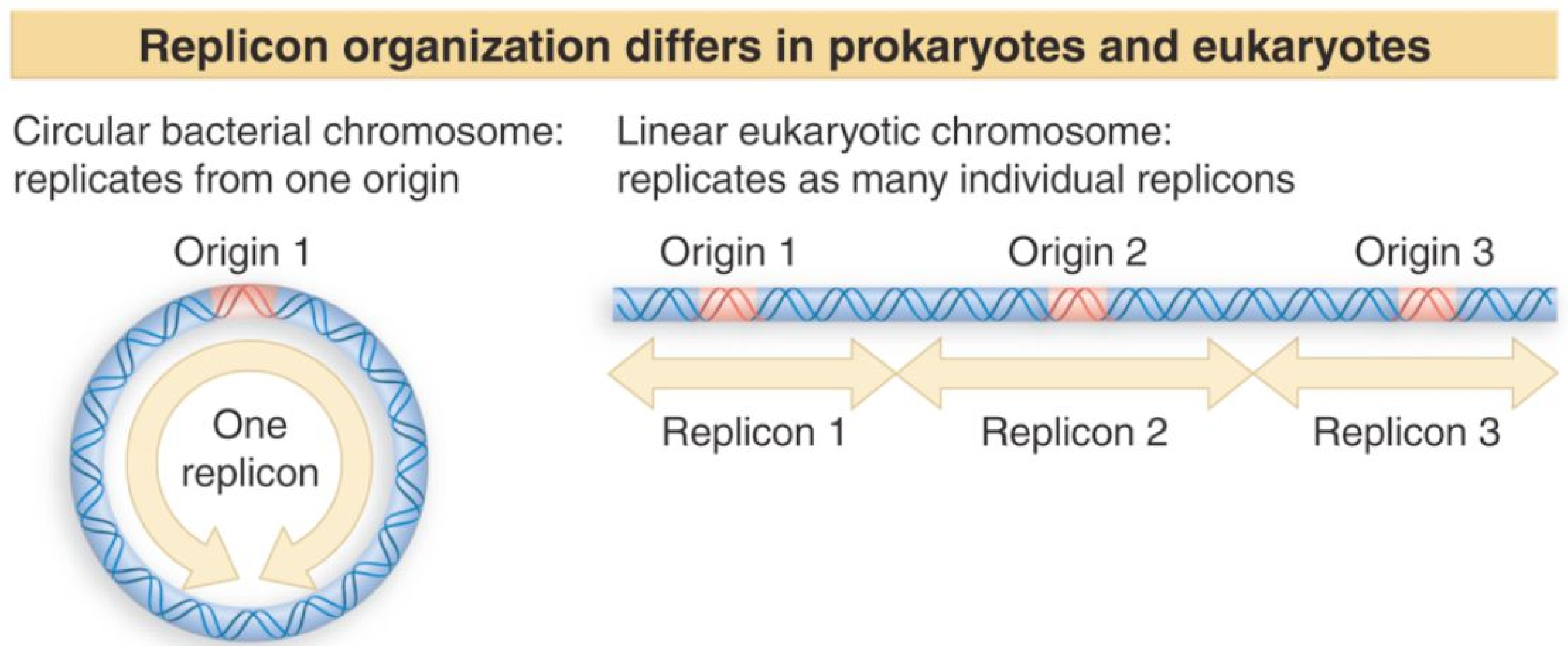


FIGURE 12.5 Bidirectional replication of a circular bacterial chromosome is initiated at a single origin. The replication forks move around the chromosome. If the replicated chromosomes are catenated, they must be disentangled before they can segregate to daughter cells.

"A replicon is a DNA molecule or RNA molecule, or a region of DNA or RNA, that replicates from a single origin of replication."



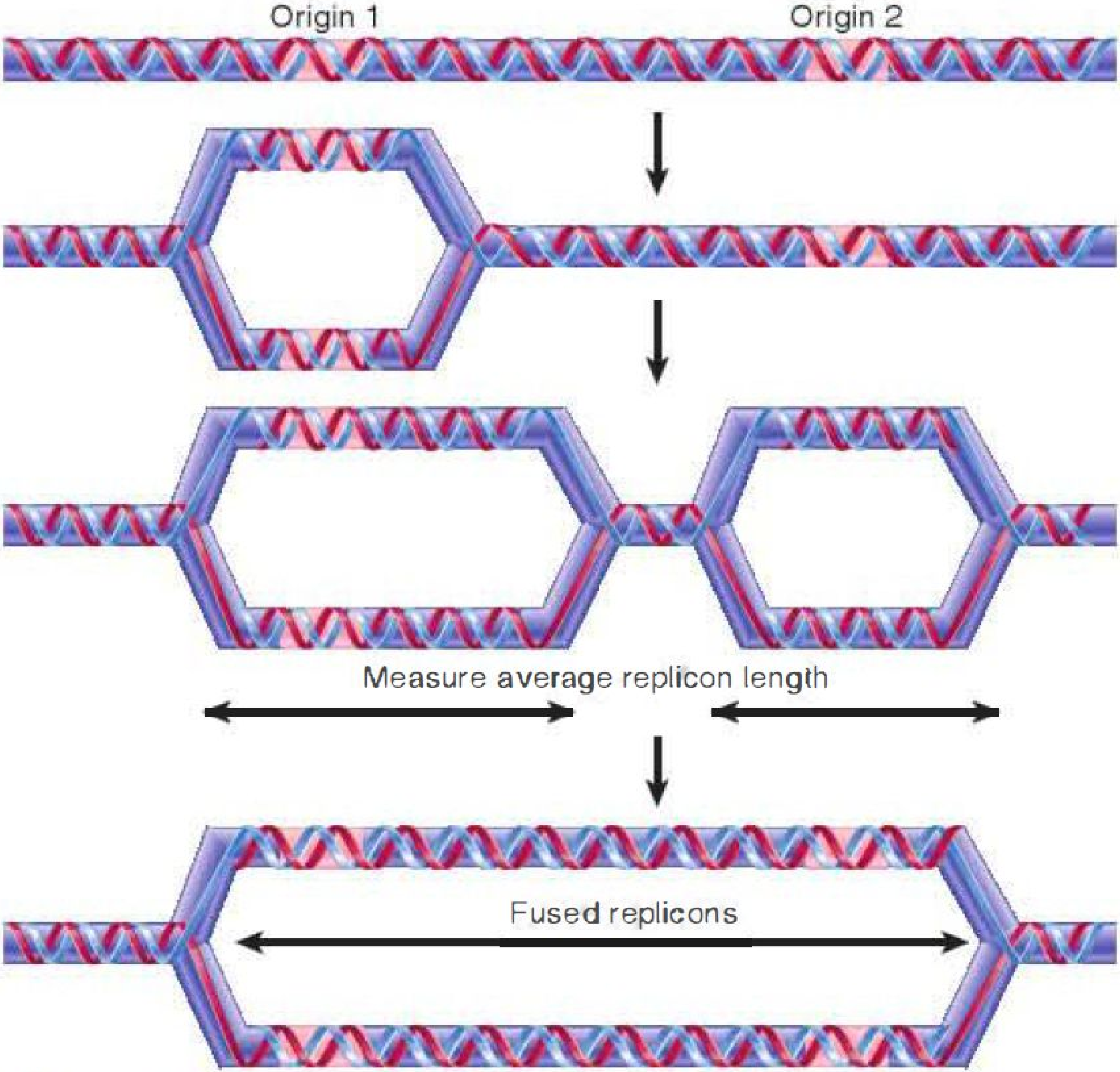


FIGURE 12.10 A eukaryotic chromosome contains multiple origins of replication that ultimately merge during replication.

Genome replicons in different organisms

Organism	N° of replicons	Average length	Replication fork speed
E. coli	1	4200 kb	50.000 bp/min
Yeast	500	40 kb	3.600 bp/min
Drosophila	3.500	40 kb	2.600 bp/min
Xenopus	15.000	200 kb	500 bp/min
Mouse	25.000	150 kb	2.200 bp/min
Vicia faba	35.000	300 kb	

Replicons in Drosophila DNA

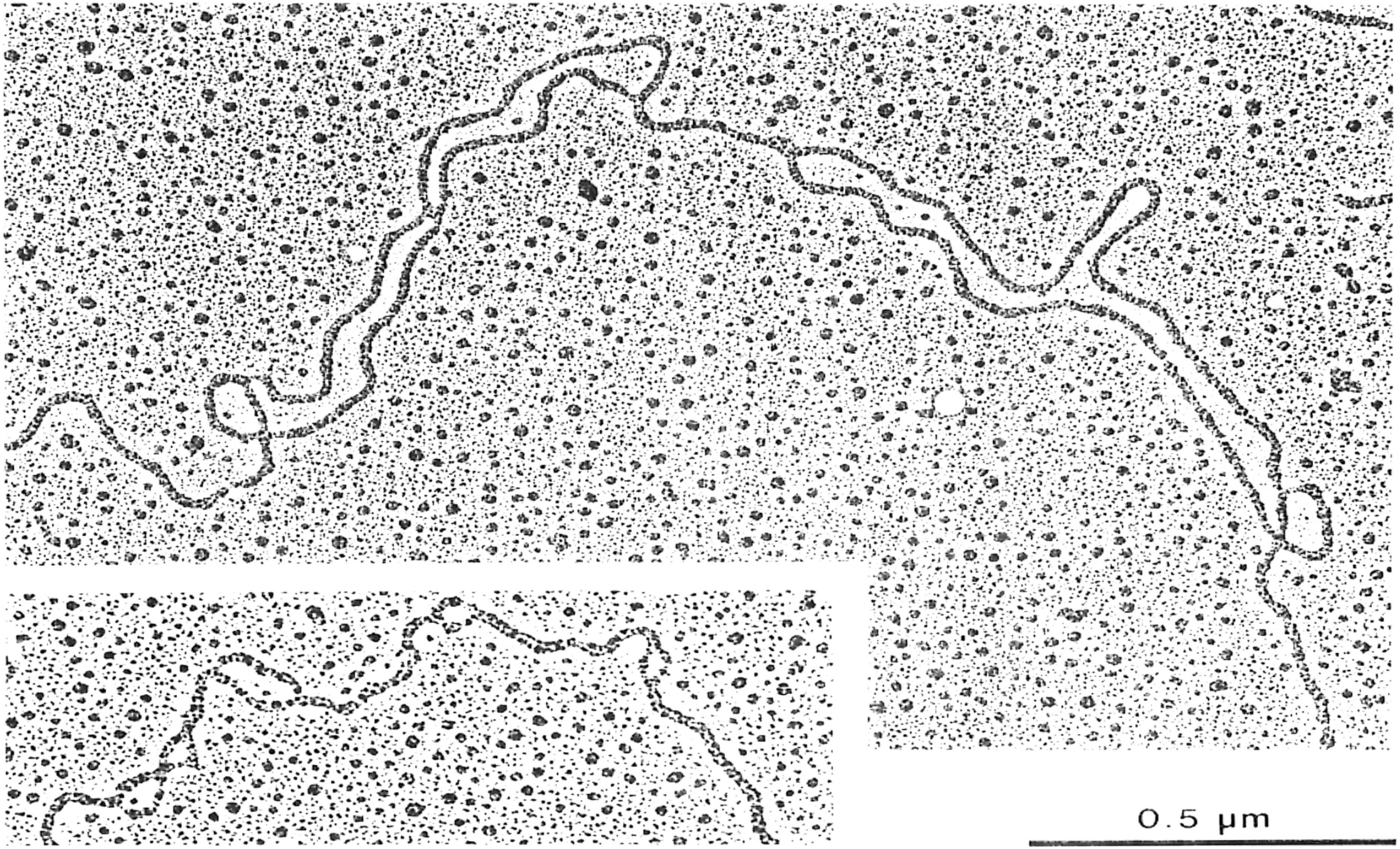
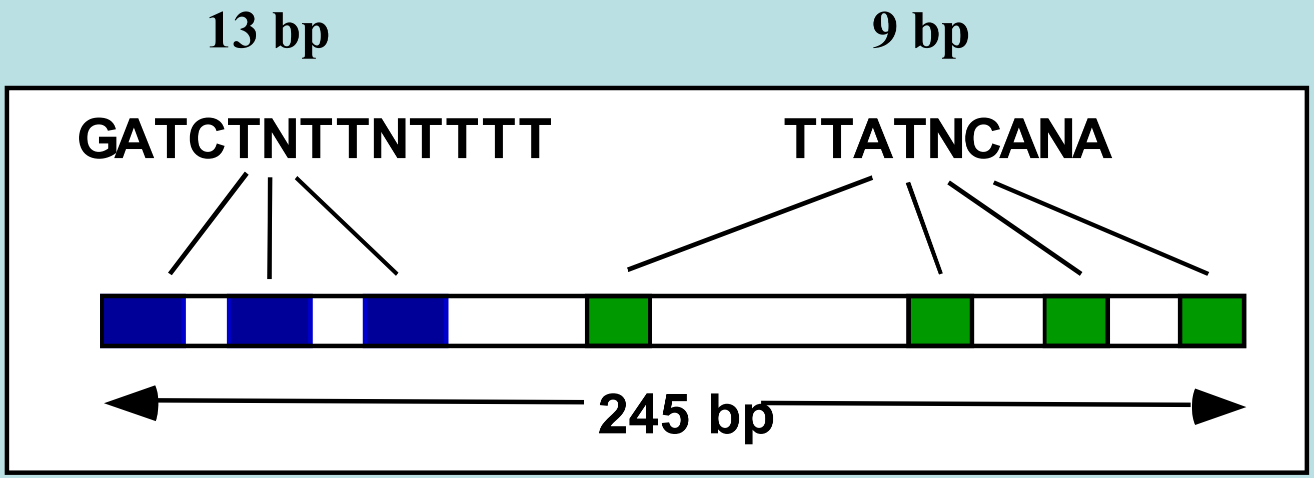


Figure 8. Electron Micrographs of Replicating DNA from Drosophila melanogaster Cleavage Nuclei
Examples of long eyes and a cluster of microbubbles are shown. Bar 0.5 μm.

Replication origins in E.coli (Ori)
And yeast ARS



Replication origin in E.coli (OriC)

---ATTTATPuTTTA---
---TAAATAPyAAAT---

11 bp

Yeast
A.R.S.
(about 50 bp)

"Autonomous Replication Sequence"

ARS consensus sequence

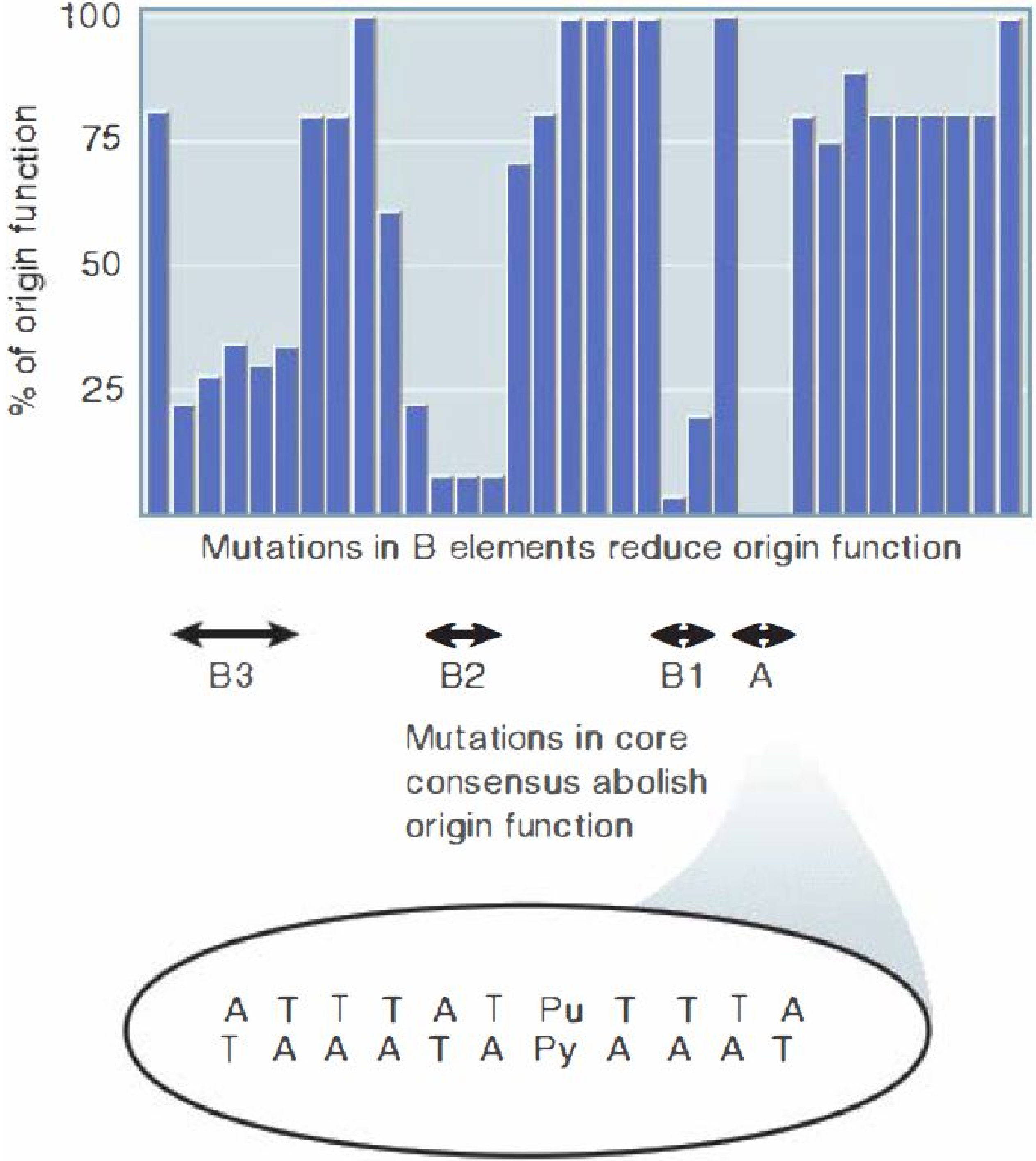
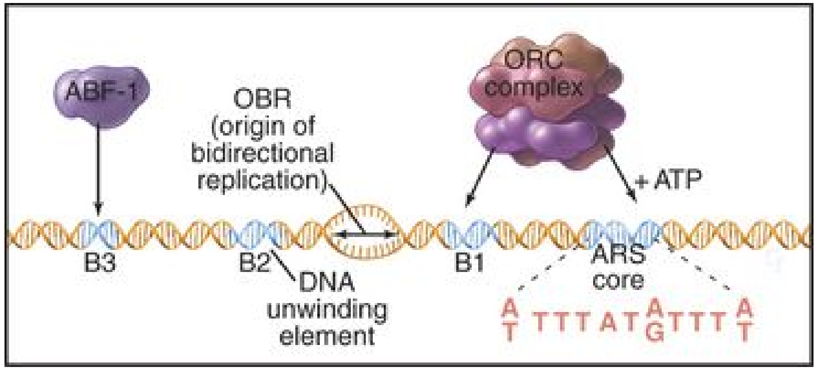
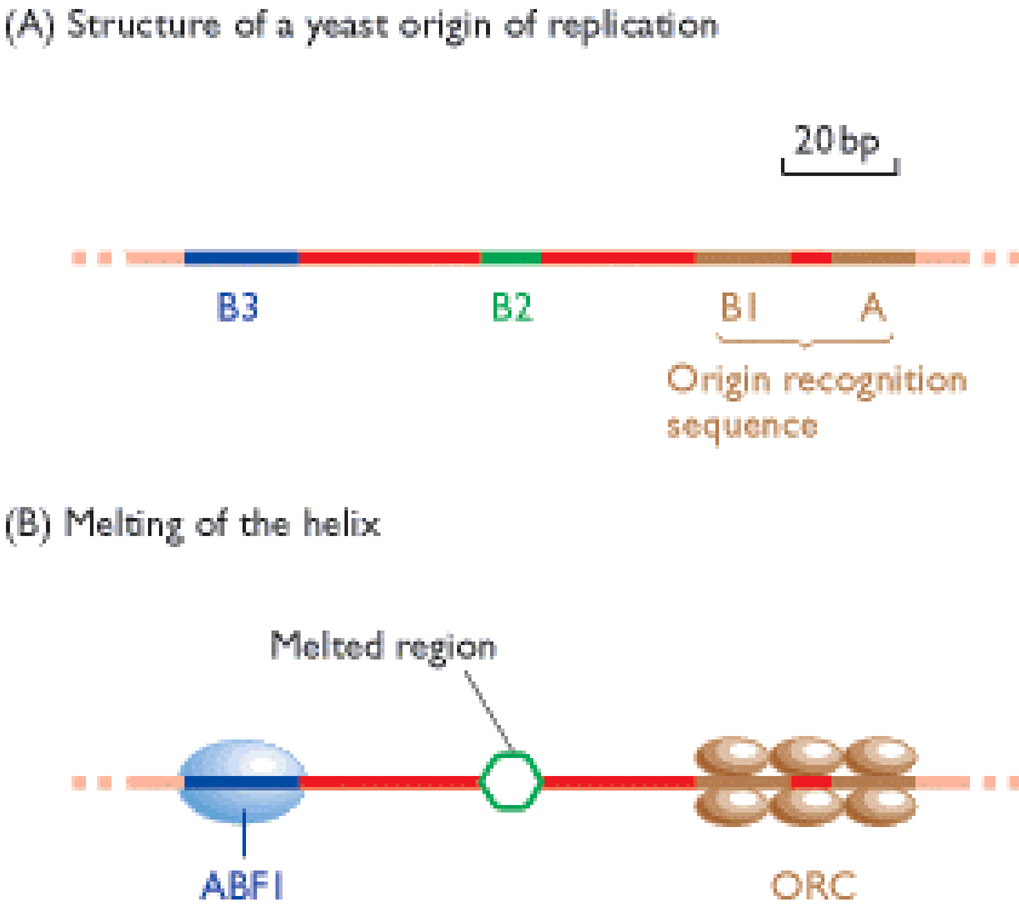


FIGURE 12.12 An *ARS* extends for ~50 bp and includes a consensus sequence (A) and additional elements (B1–B3).

Yeast ARS element





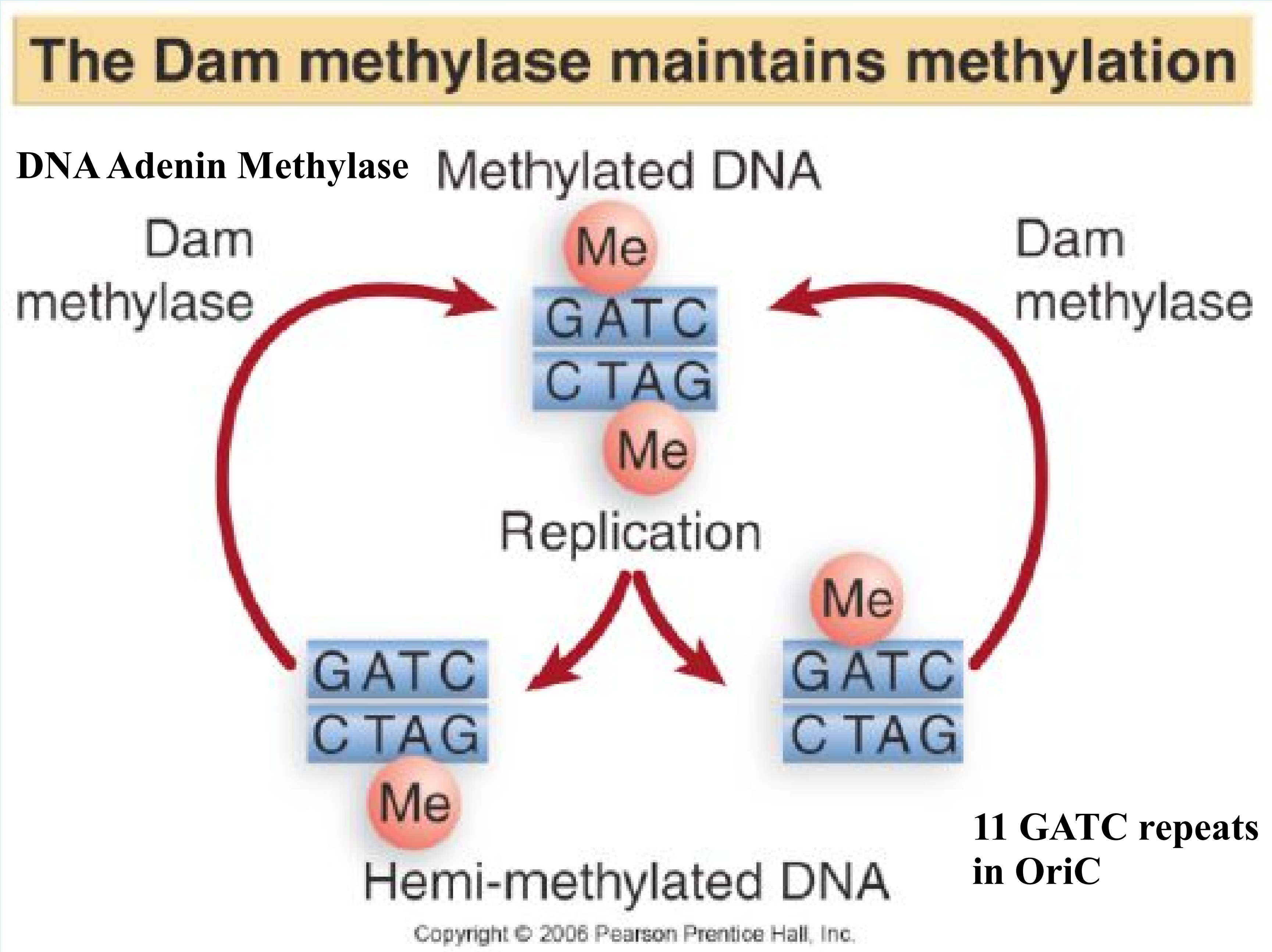
ARS Binding Factor 1

Origin Recognition Complex

Regulation of replication

Whether a cell has only one chromosome (as in most prokaryotes) or has many chromosomes (as in eukaryotes), the entire genome must be replicated precisely, once for every cell division. What is the regulation mechanism for DNA replication?

In prokaryotes



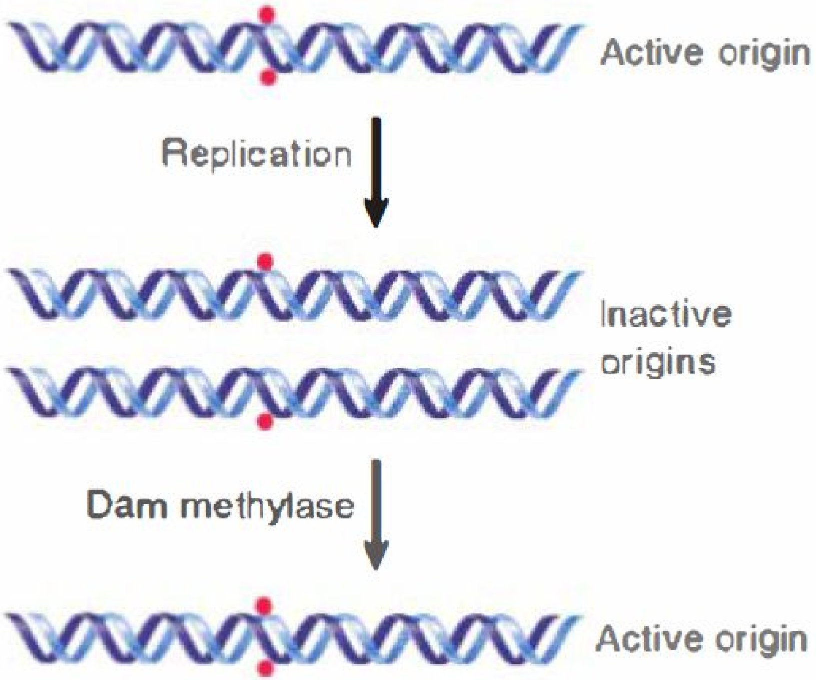
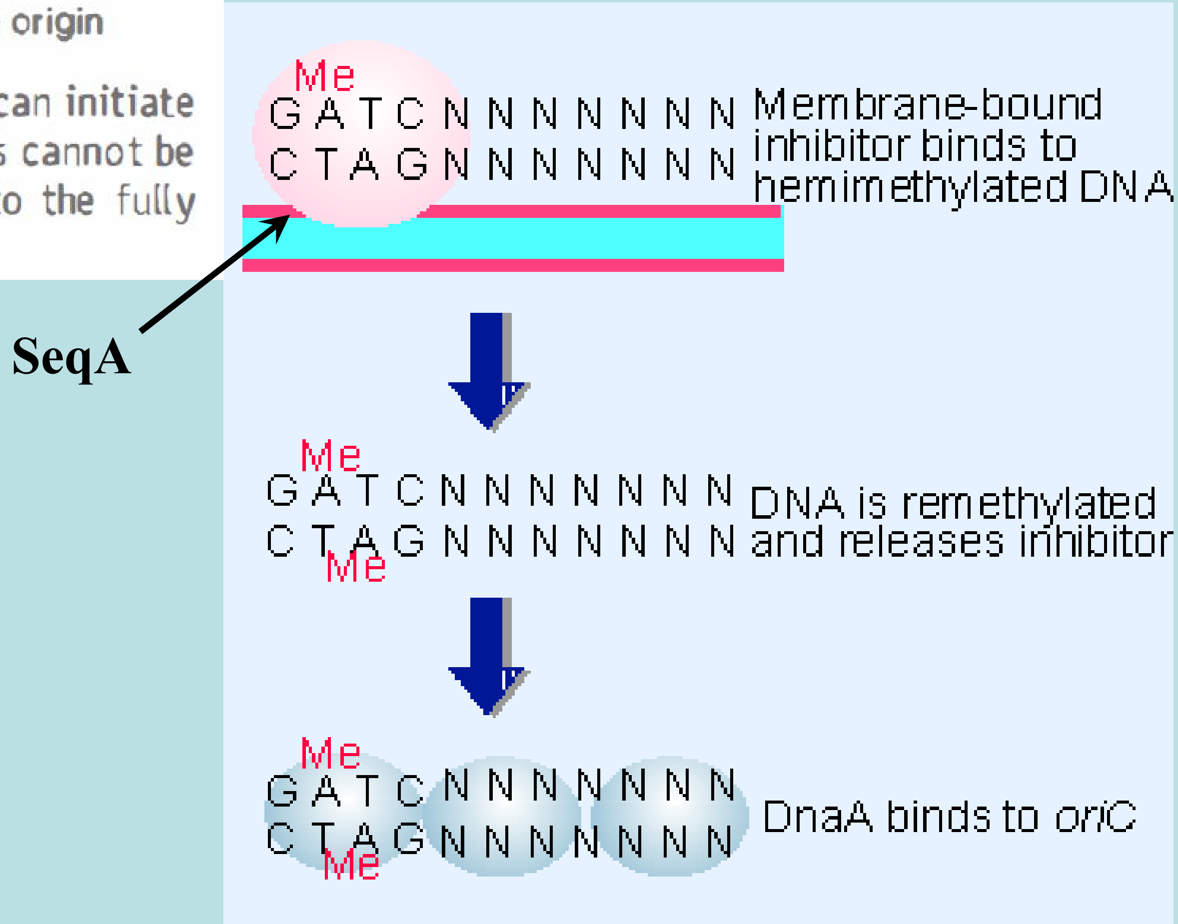


FIGURE 12.7 Only fully methylated origins can initiate replication; hemimethylated daughter origins cannot be used again until they have been restored to the fully methylated state.



In eukaryotes

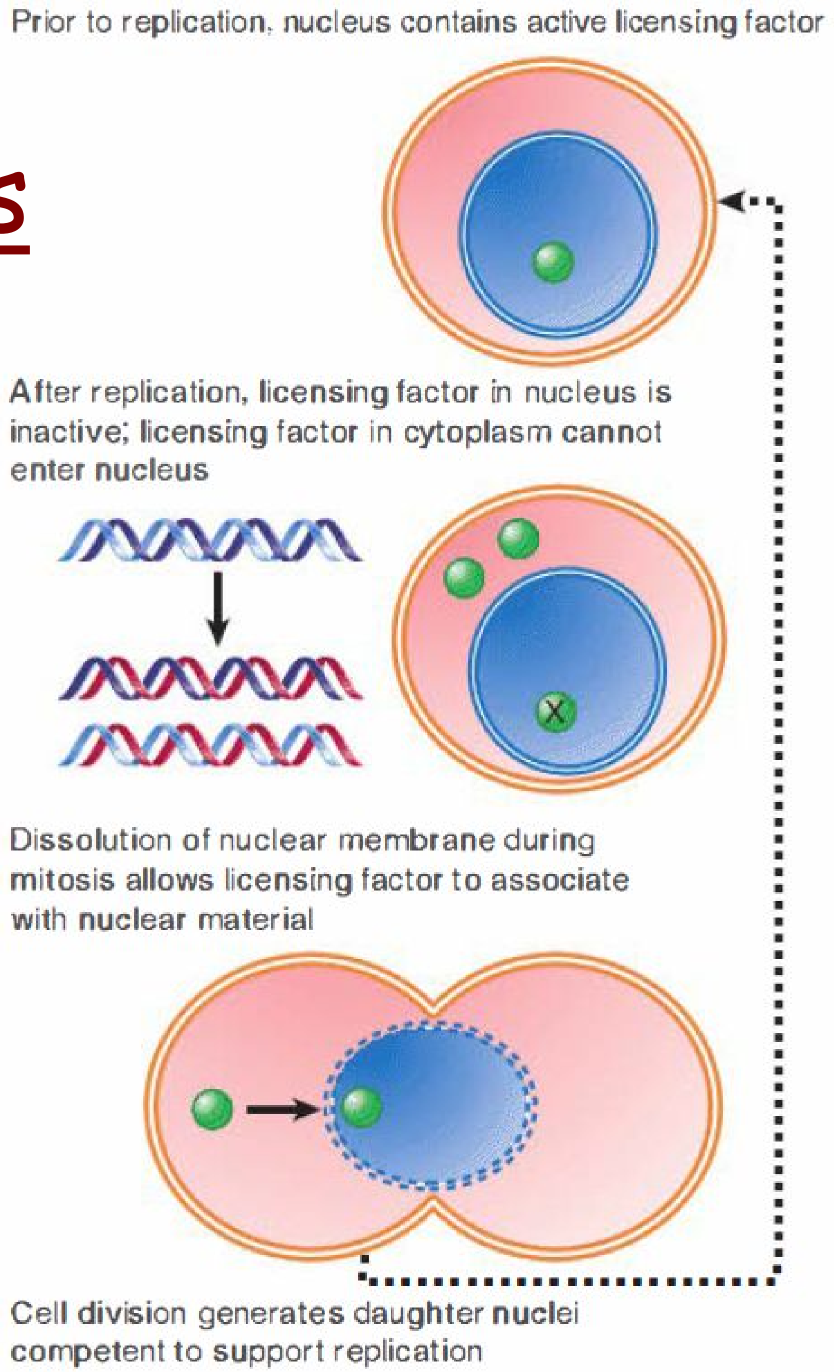
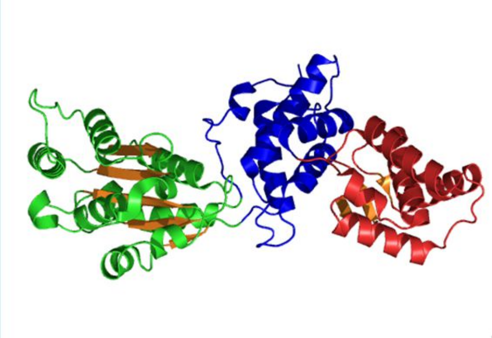
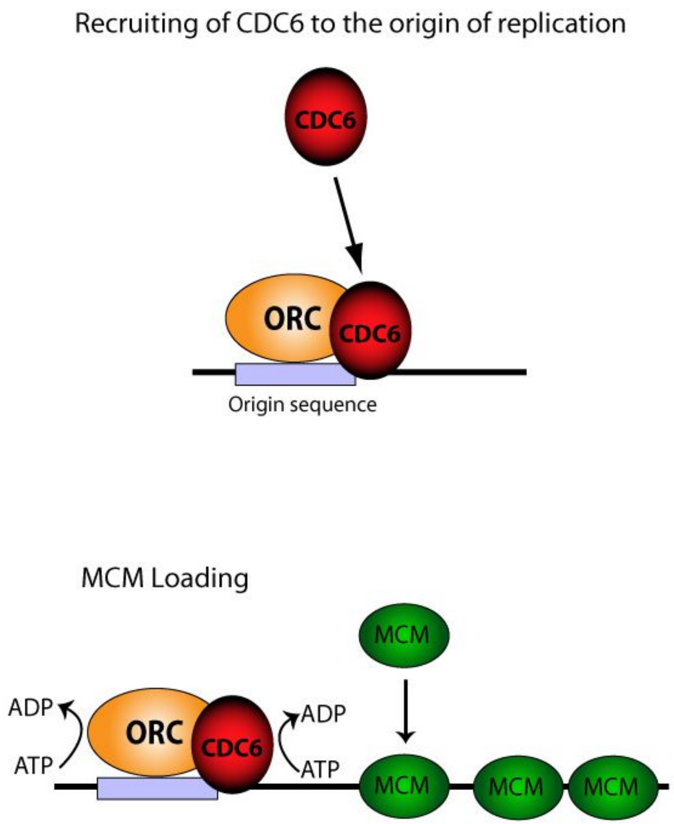


FIGURE 12.14 Licensing factor in the nucleus is inactivated after replication. A new supply of licensing factor can enter only when the nuclear membrane breaks down at mitosis.



Cell Division Cycle 6

Licensing factor elements



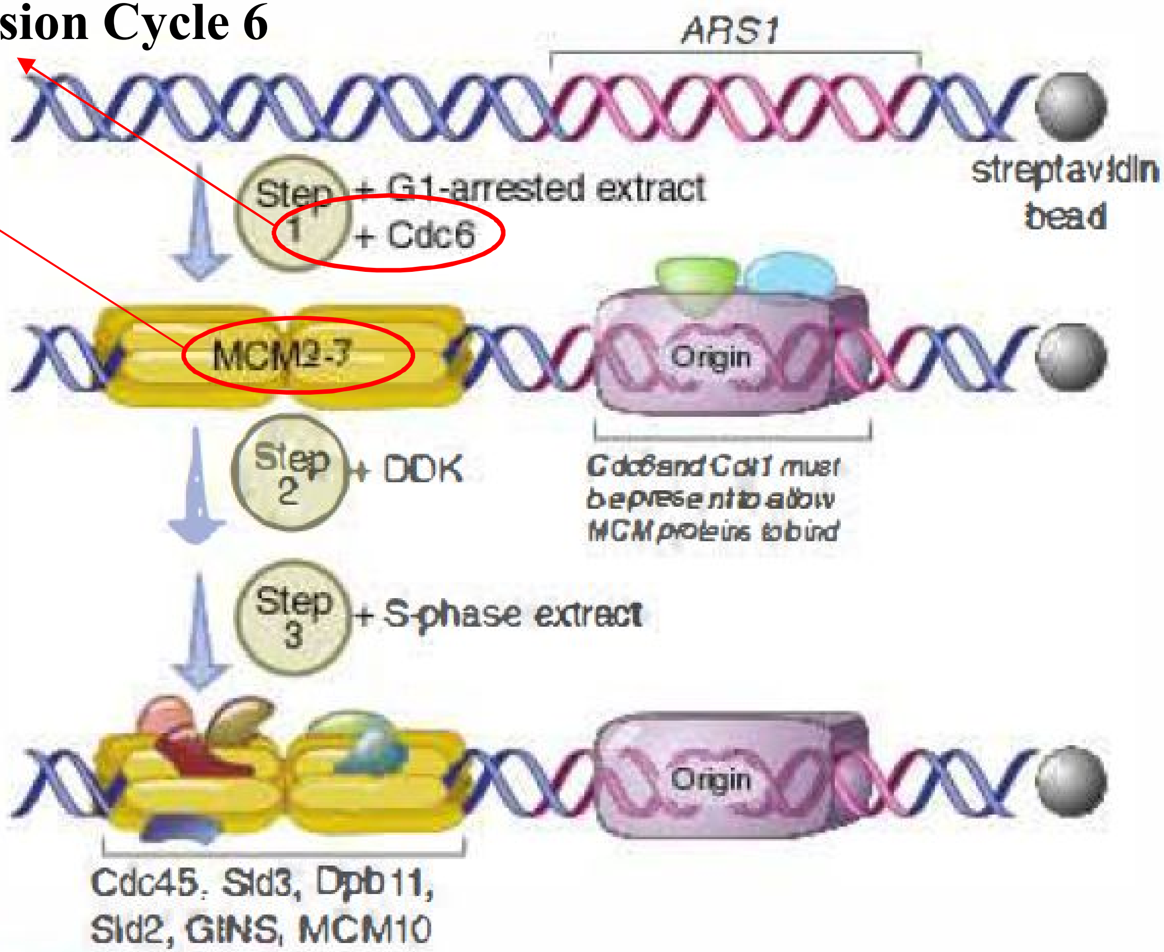


FIGURE 12.15 Proteins at the origin control susceptibility to initiation. Adapted from R. C. Heller, et al., *Cell* 146 (2011): 80-91.

MCM= Mini Chromosome Maintenance → helicase

Circular replicons

E.coli chromosome is made by a single bidirectional replicon

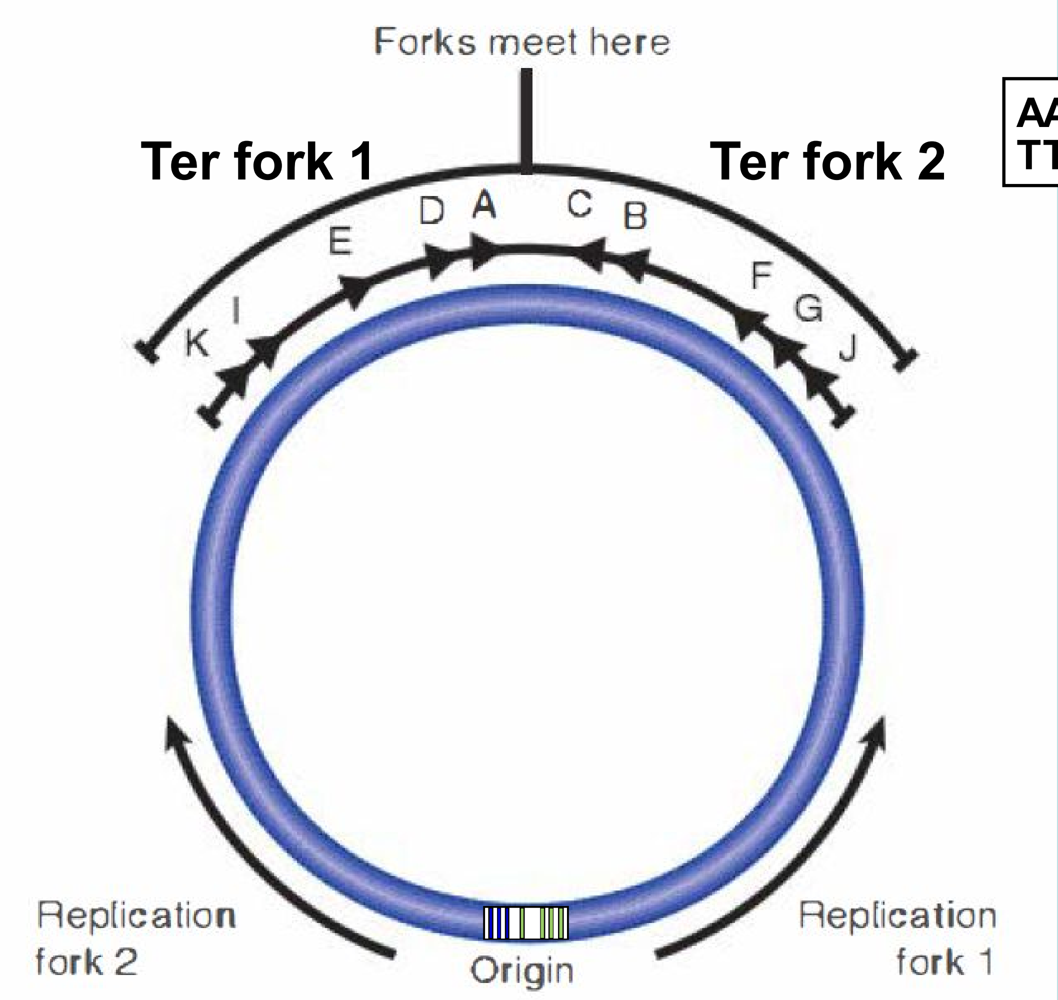
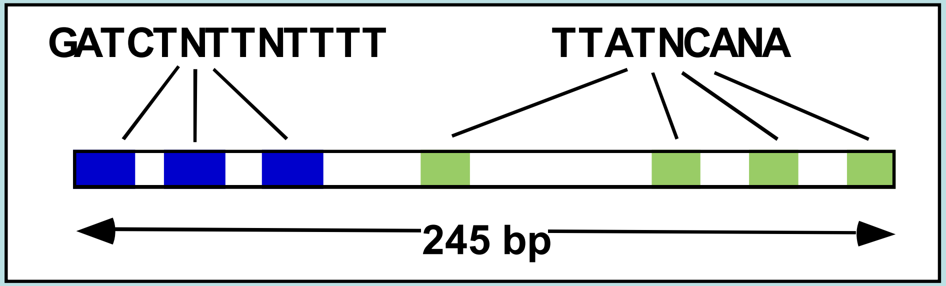
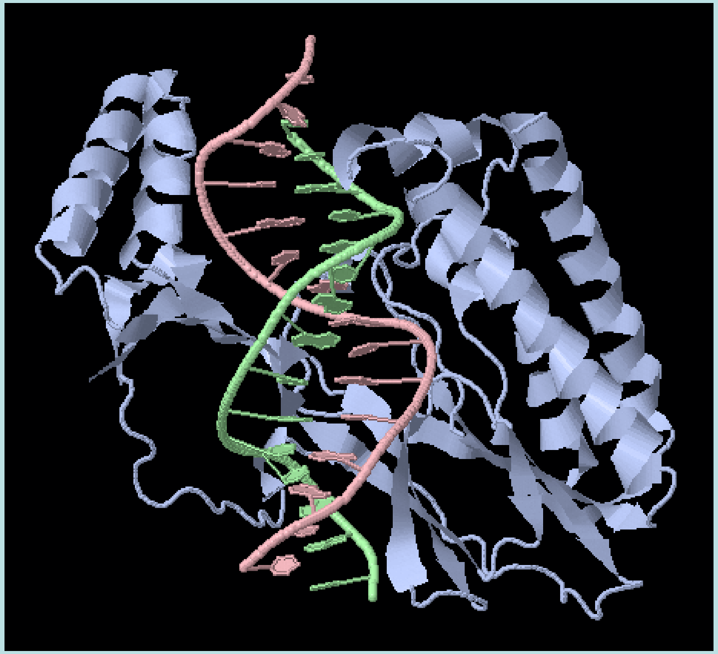


FIGURE 13.30 Replication termini in *E. coli* are located in a region between two sets of *ter* sites.



Ter Site (23 bp)

AATTAGTATGTTGTAACTAAAGT
TTAATCATACAACATTGATTTCA



Replication Terminator Protein (Tus)

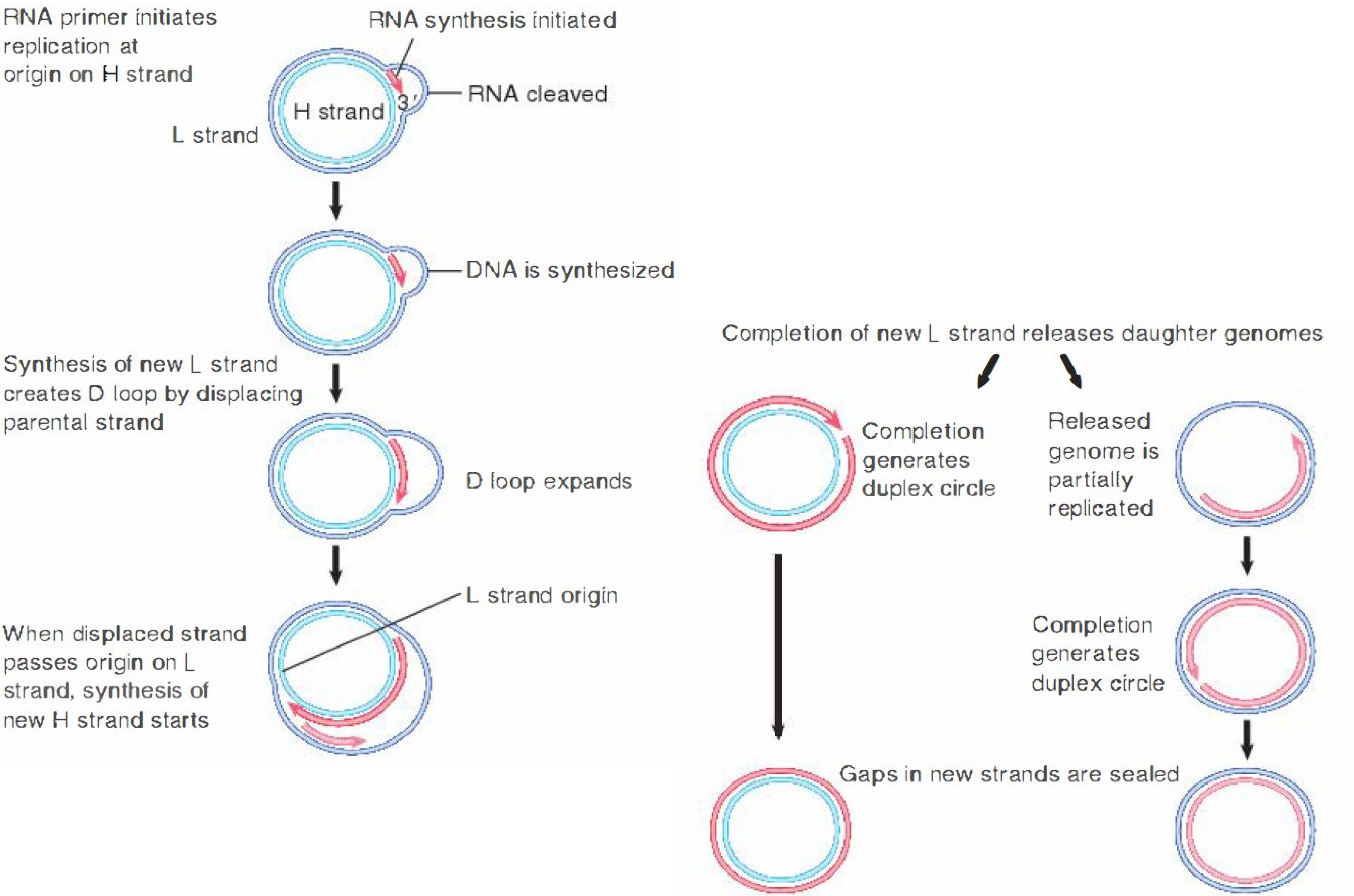


FIGURE 14.22 The D loop maintains an opening in mammalian mitochondrial DNA, which has separate origins for the replication of each strand.

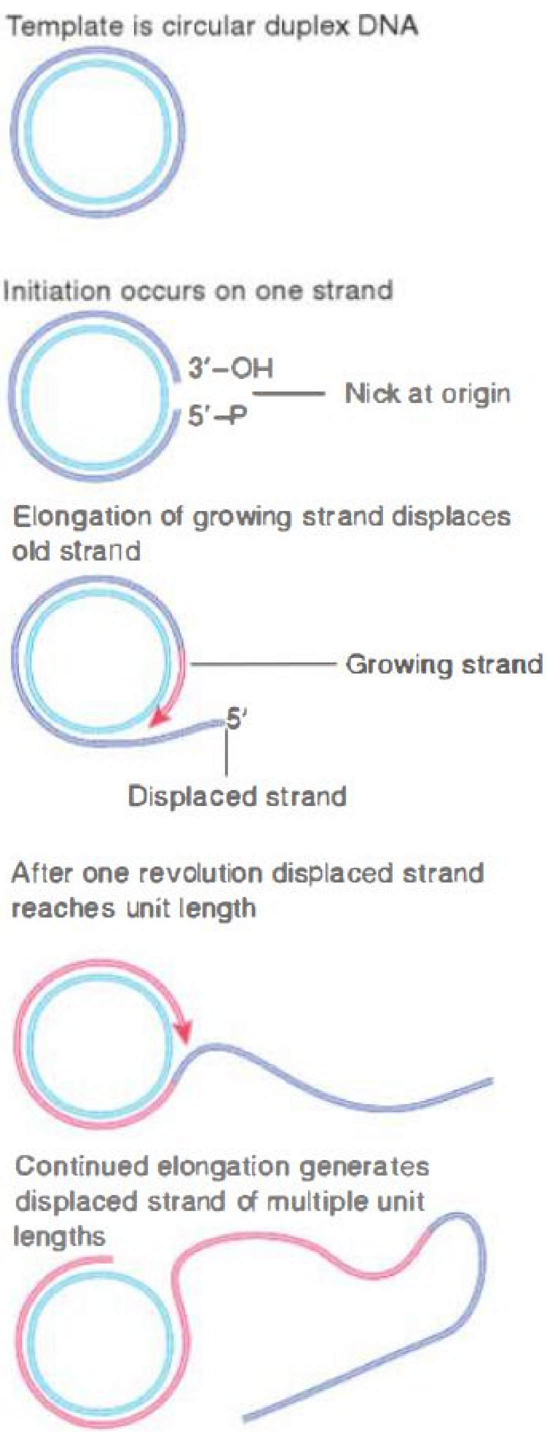


FIGURE 14.5 The rolling circle generates a multimeric single-stranded tail.

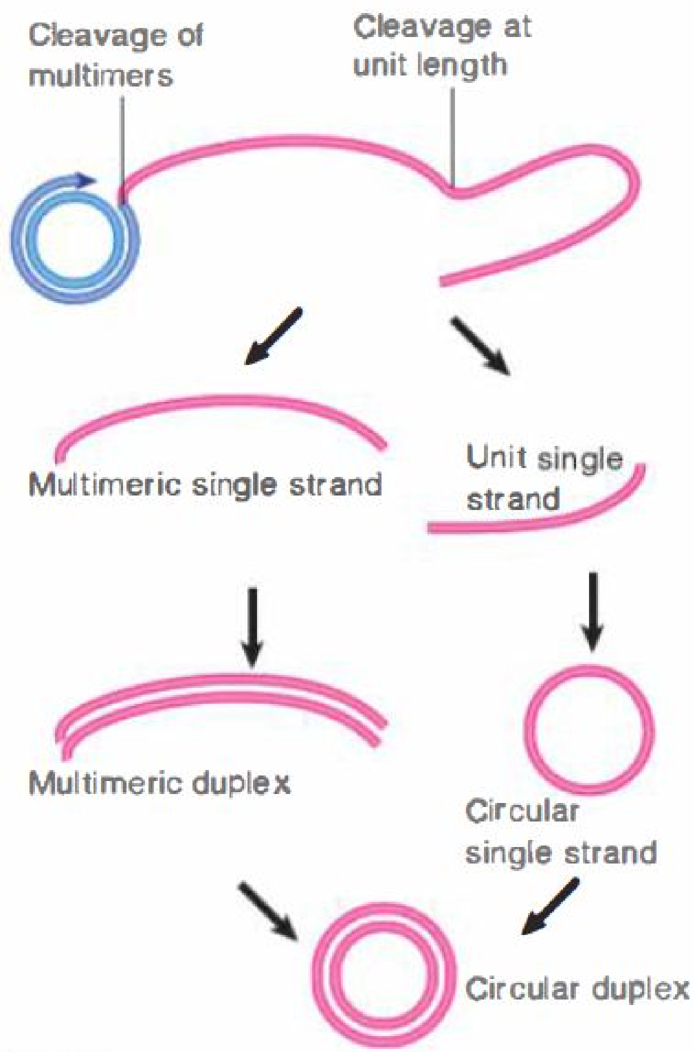


FIGURE 14.7 The fate of the displaced tail determines the types of products generated by rolling circles. Cleavage at unit length generates monomers, which can be converted to duplex and circular forms. Cleavage of multimers generates a series of tandemly repeated copies of the original unit. Note that the conversion to double-stranded form could occur earlier, before the tail is cleaved from the rolling circle.

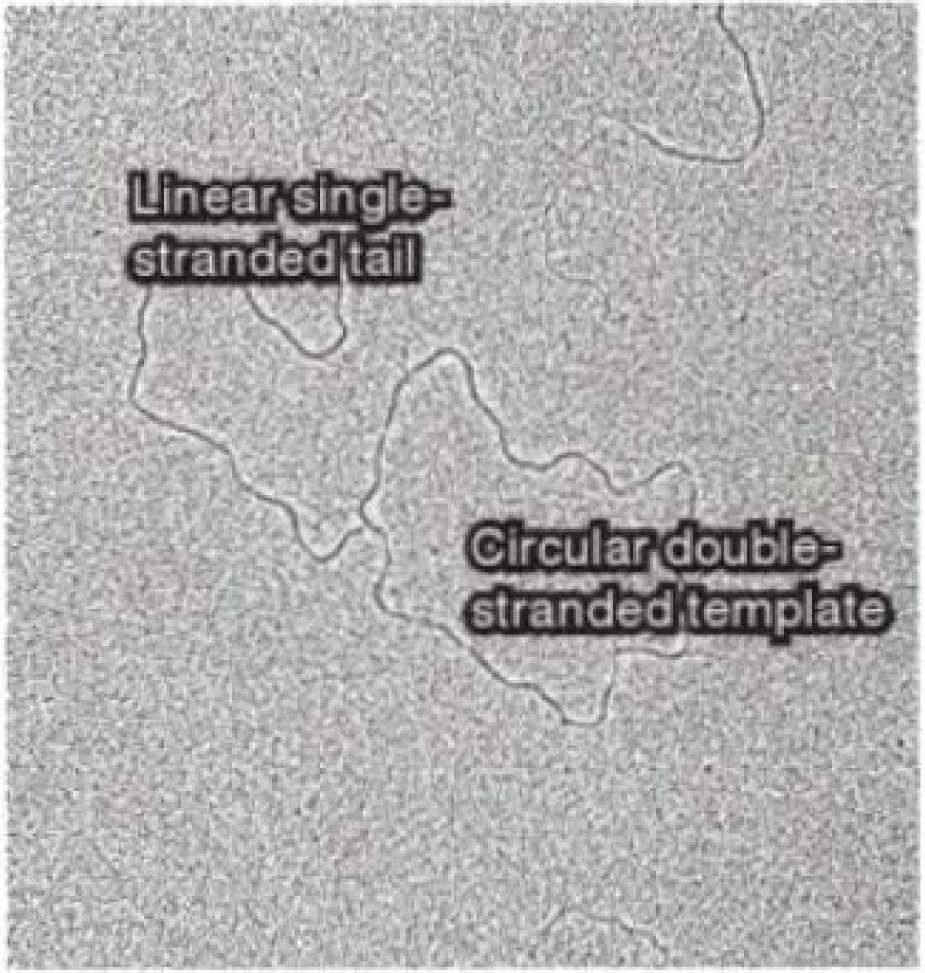


FIGURE 14.6 A rolling circle appears as a circular molecule with a linear tail by electron microscopy. Photo courtesy of Ross B. Inman, Institute of Molecular Virology, Bock Laboratory and Department of Biochemistry, University of Wisconsin, Madison, Wisconsin, USA.

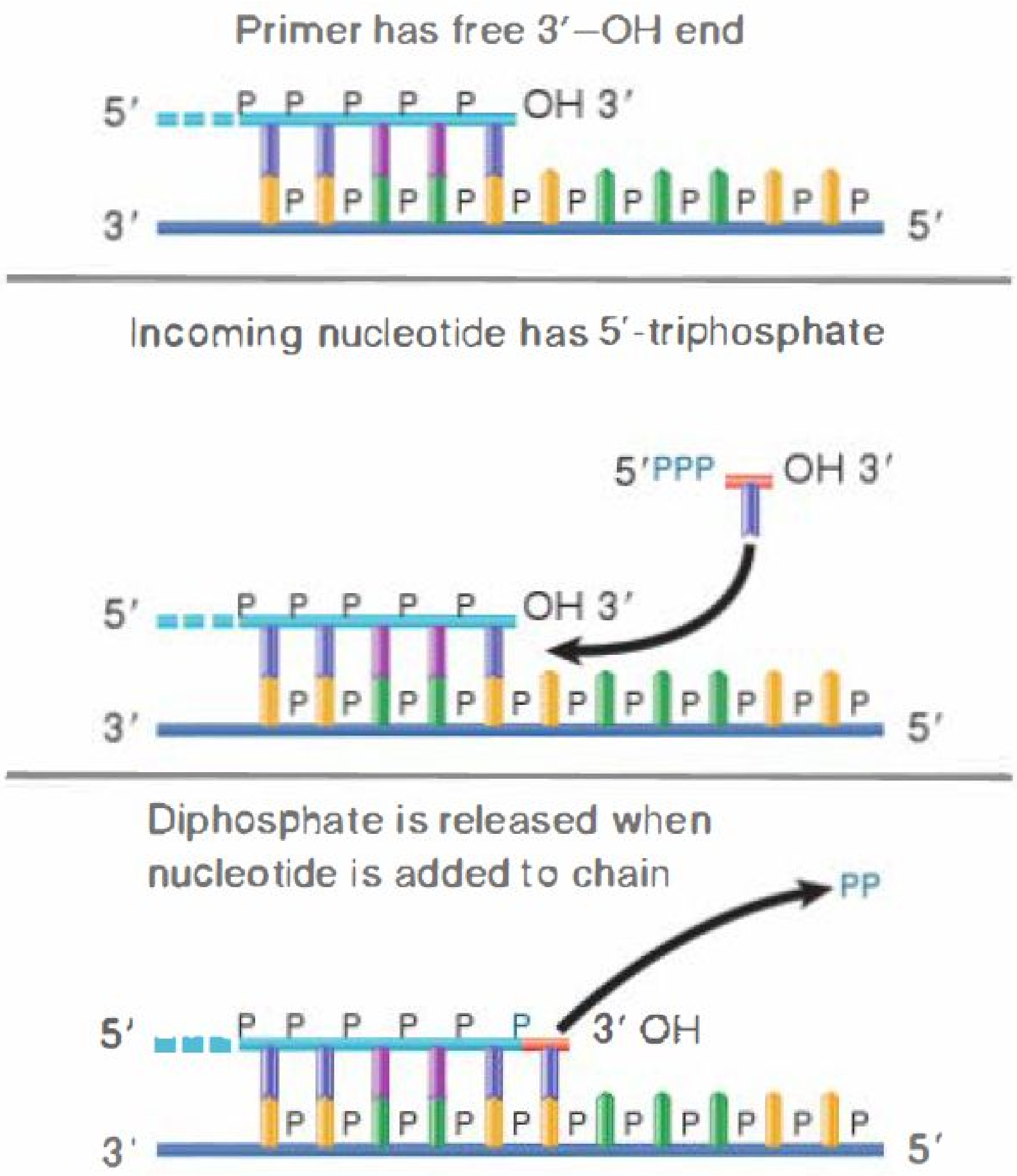


FIGURE 13.4 DNA is synthesized by adding nucleotides to the 3′–OH end of the growing chain, so that the new chain grows in the 5′ to 3′ direction. The precursor for DNA synthesis is a nucleoside triphosphate, which loses the terminal two phosphate groups in the reaction.

The ends of linear DNA are a problem for Replication

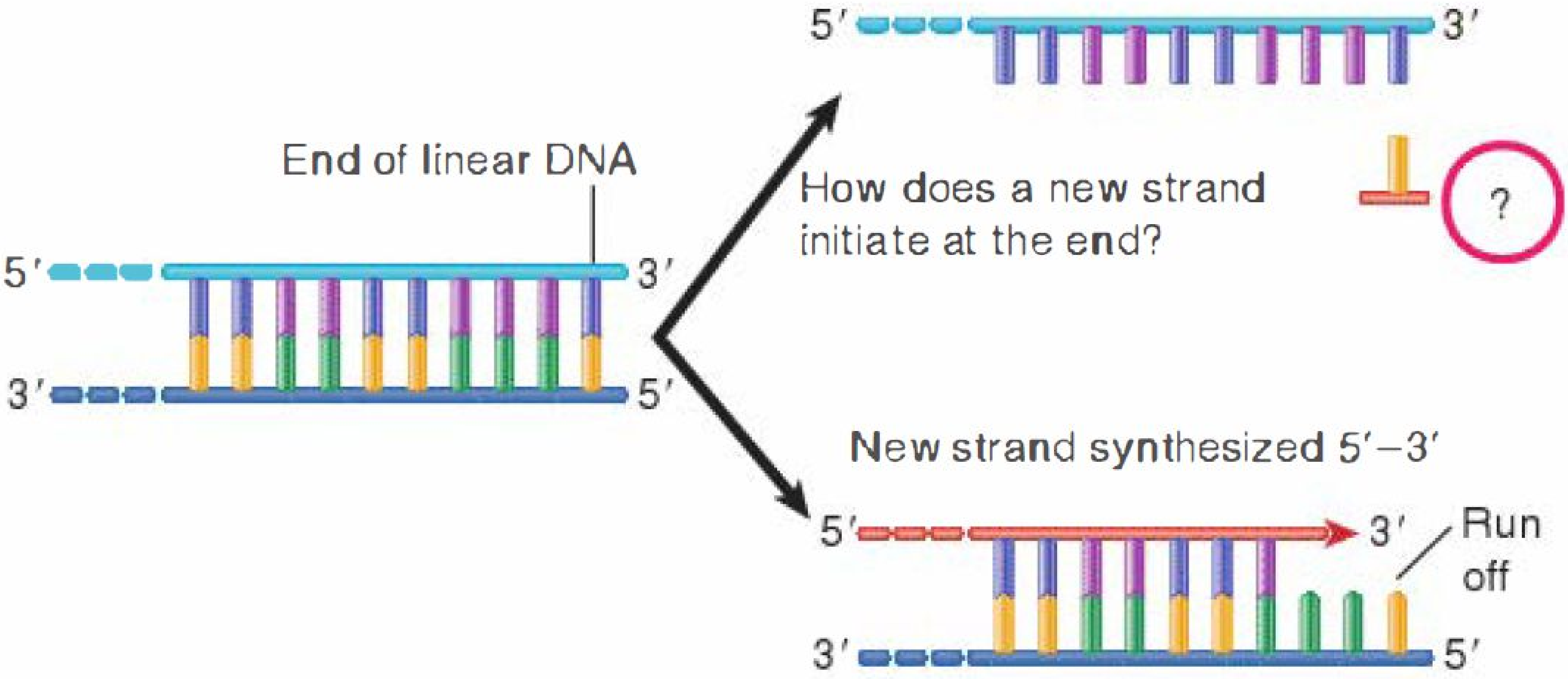


FIGURE 14.1 Replication could run off the 3′ end of a newly synthesized linear strand, but could it initiate at a 5′ end?

Special arrangements must be made to replicate the DNA strand with a 5' end.

- The problem may be circumvented by converting a linear replicon into a circular or multimeric molecule (E.G. Phages such as T4 or lambda use such mechanisms).
- The DNA may form an unusual structure-for example, by creating a hairpin at the terminus.
- A protein may intervene to make initiation possible at the actual terminus.
- Instead of being precisely determined, the end may be variable. (telomeres)

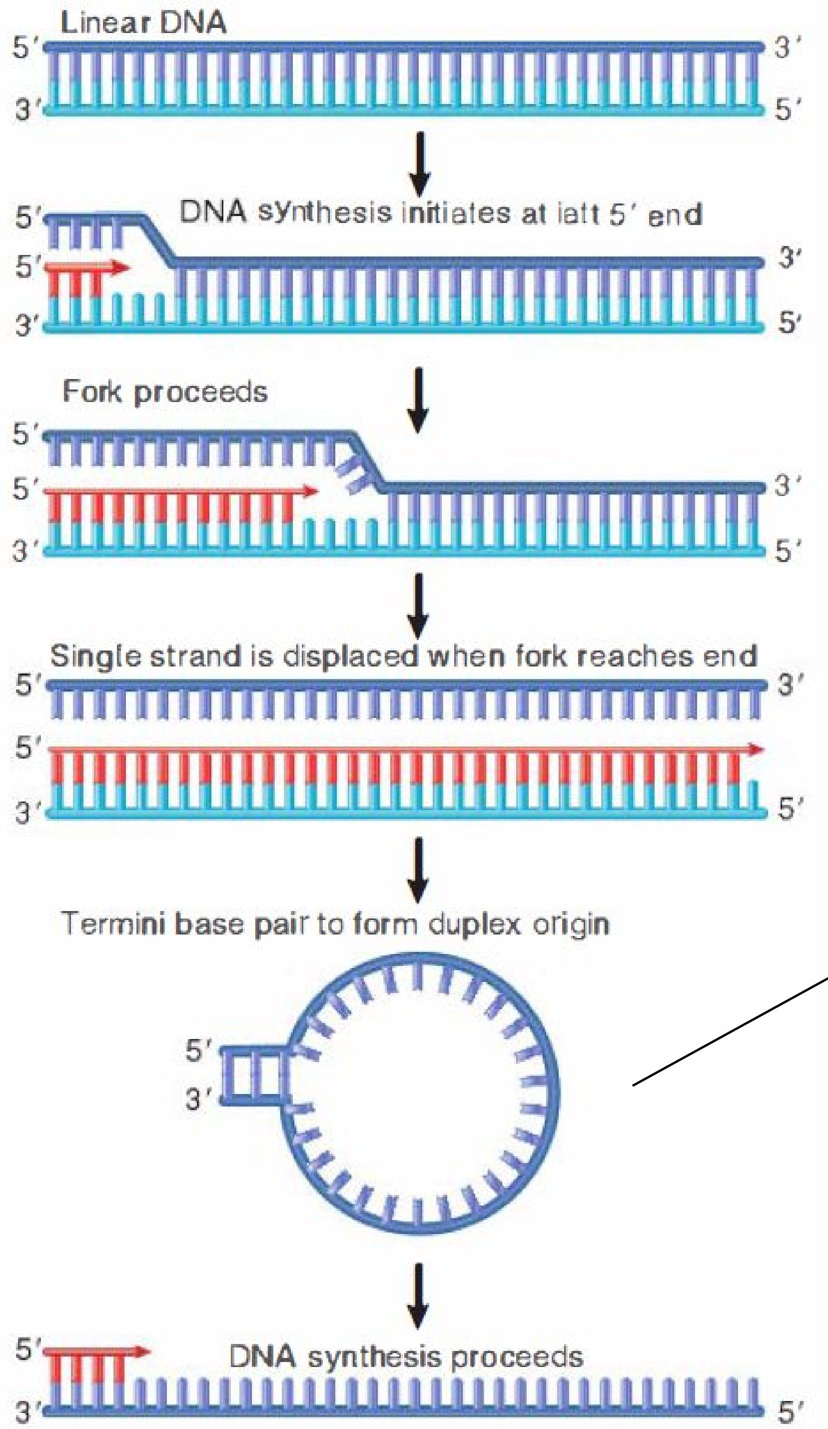


FIGURE 14.2 Adenovirus DNA replication is initiated separately at the two ends of the molecule and proceeds by strand displacement.

Conversion of linear DNA into a pseudocircular DNA

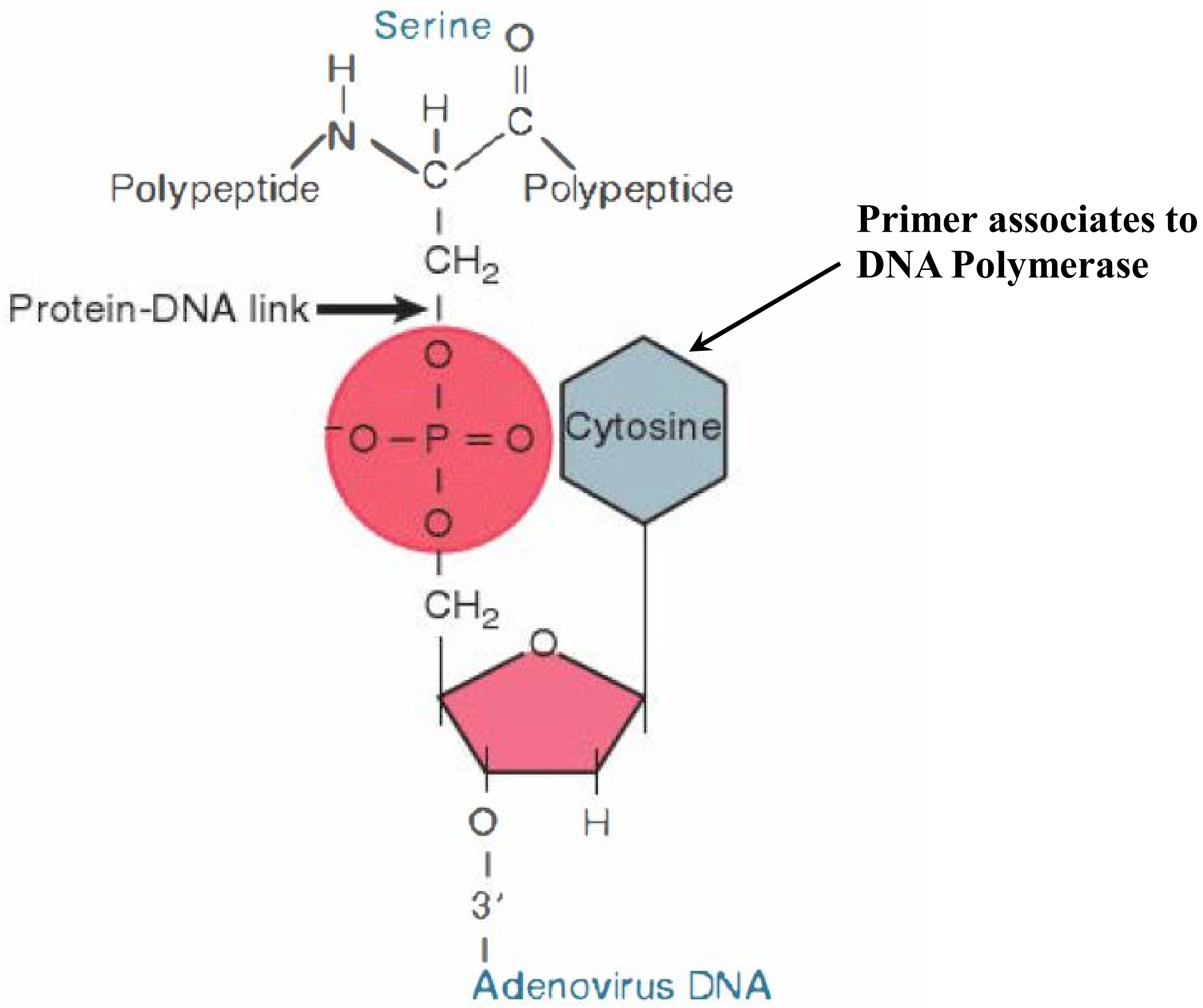


FIGURE 14.3 The 5′ terminal phosphate at each end of adenovirus DNA is covalently linked to serine in the 55-kD Ad-binding protein.

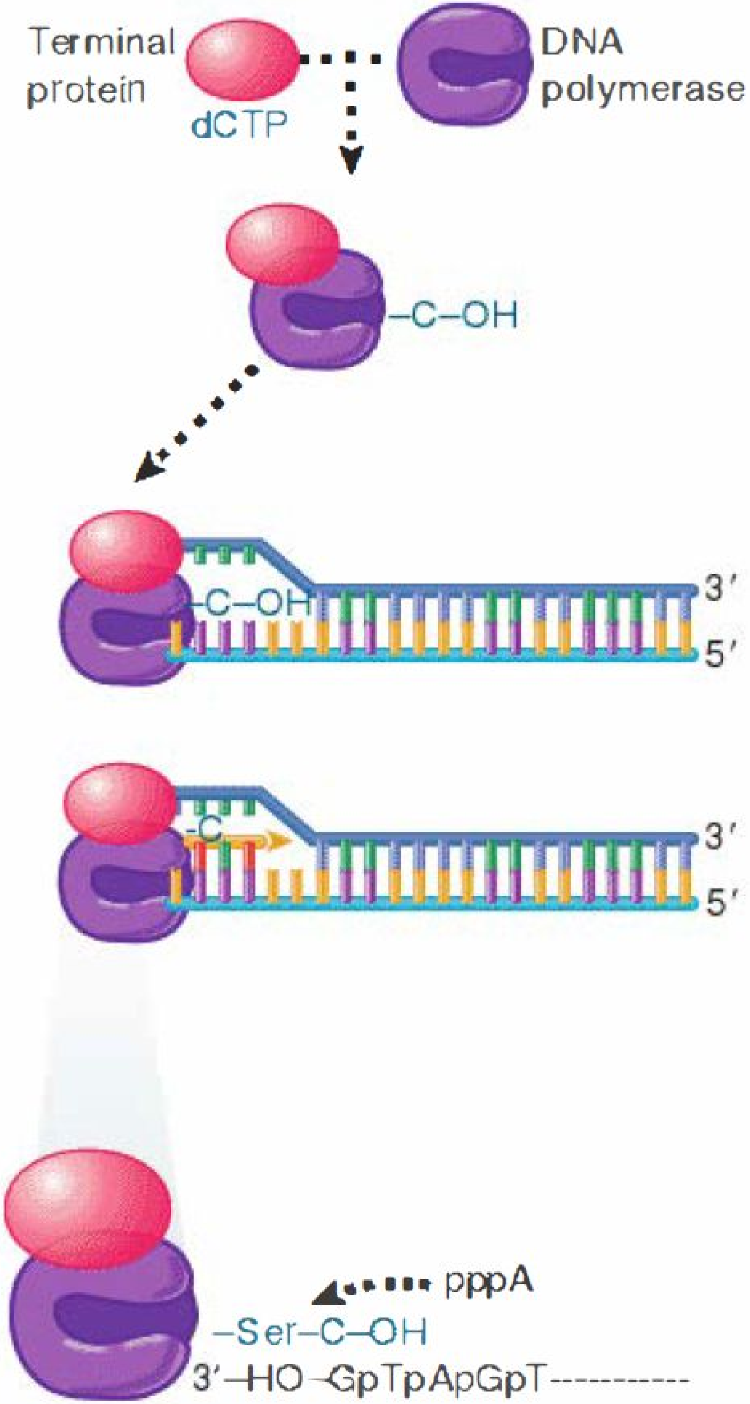


FIGURE 14.4 Adenovirus terminal protein binds to the 5′ end of DNA and provides a C-OH end to prime synthesis of a new DNA strand.

Prokaryotic replication

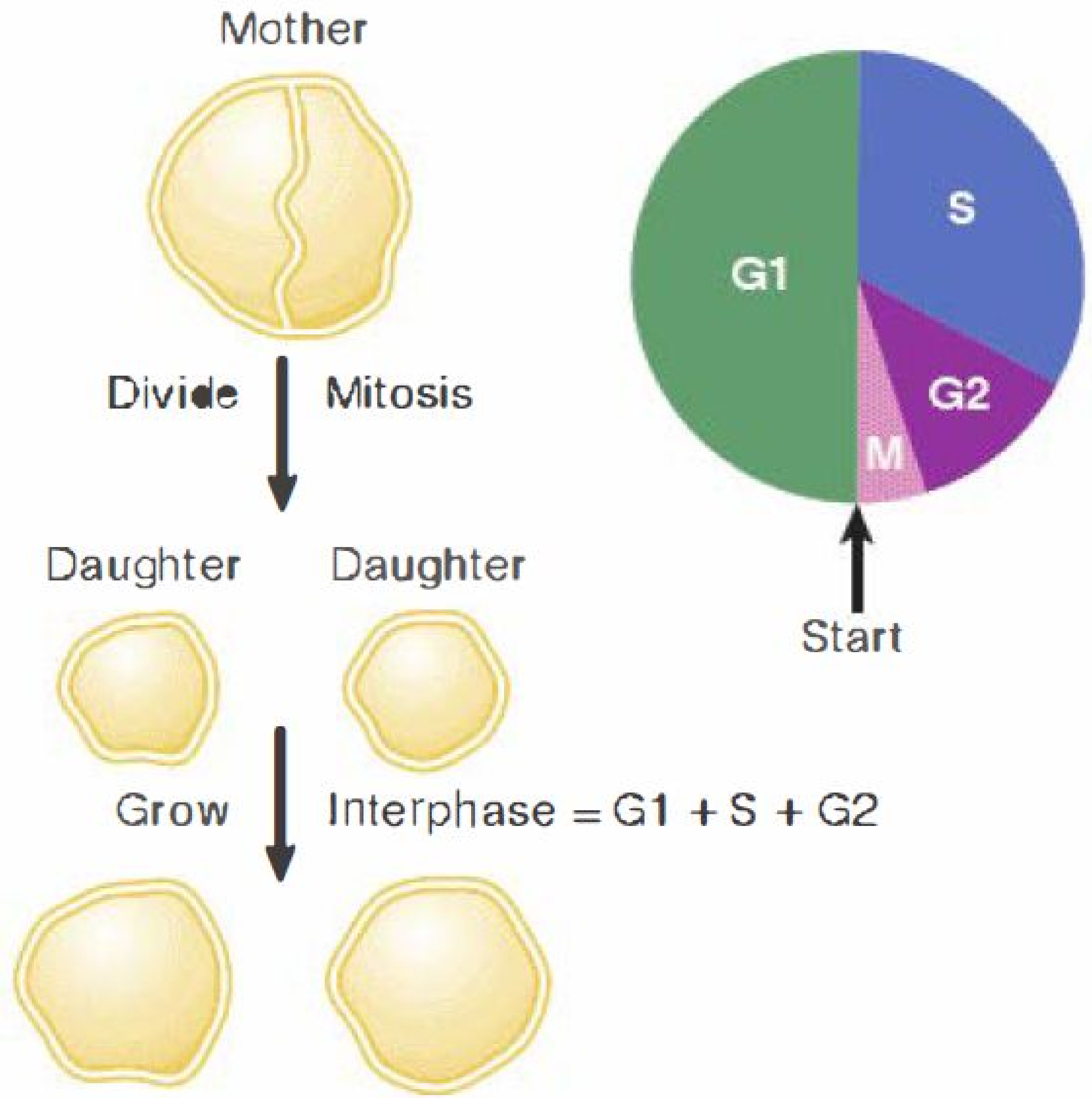


FIGURE 11.1 A growing cell alternates between cell division of a mother cell into two daughter cells and growth back to the original size.

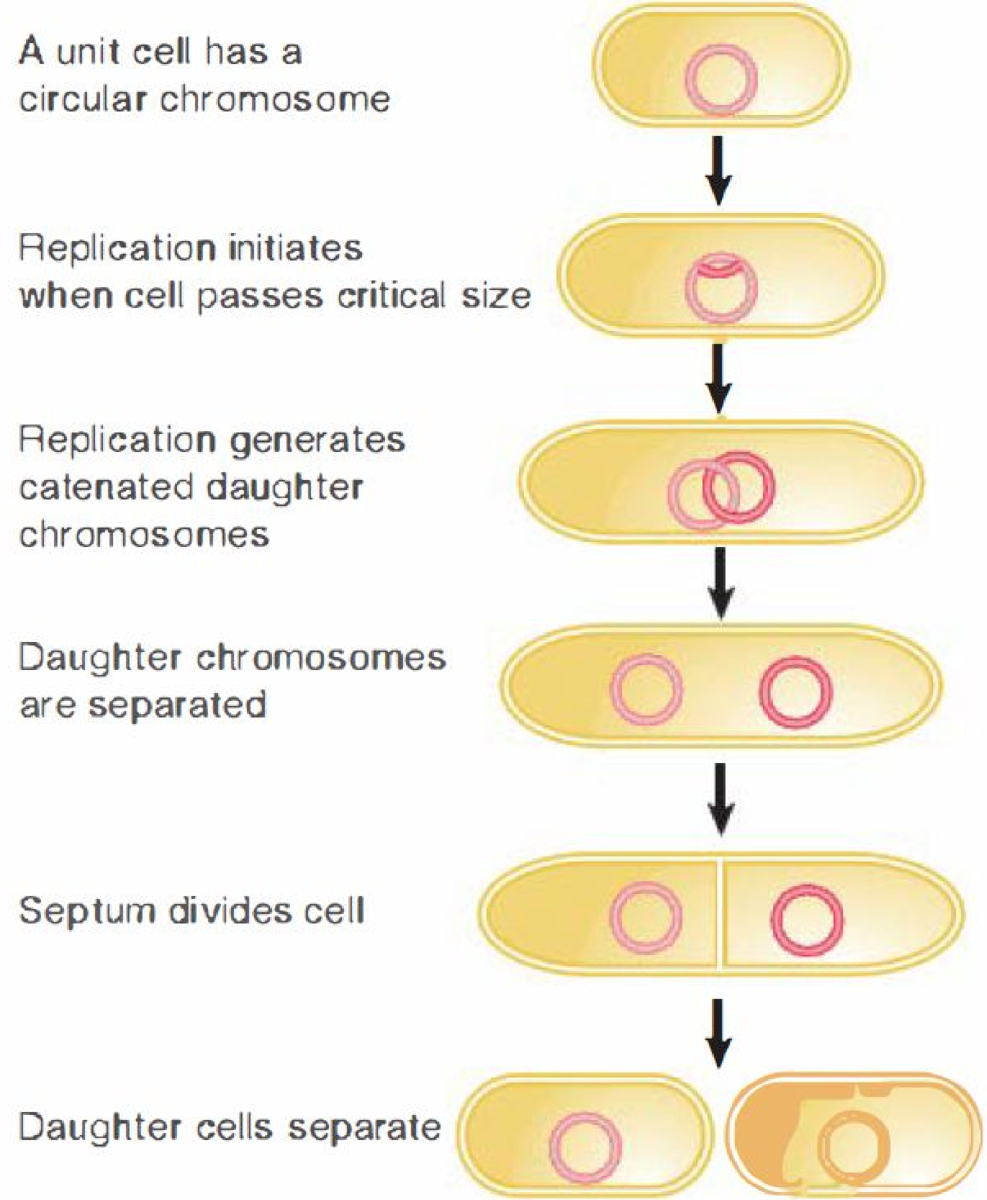


FIGURE 11.2 Replication initiates at the bacterial origin when a cell passes a critical threshold of size. Completion of replication produces daughter chromosomes that may be linked by recombination or that may be catenated. They are separated and moved to opposite sides of the septum before the bacterium is divided into two.

Bacterial replication is connected to the cell cycle

- The doubling time of *E. coli* can vary over a range of up to 10X, depending on growth conditions. It requires 40 minutes to replicate the bacterial chromosome (at normal temperature).
- *Escherichia coli* growth rates can range from doubling times as fast as 18 minutes to slower than 180 minutes.
- If the doubling time is -60 minutes, a replication cycle is initiated before the division resulting from the previous replication cycle. The start of these "new" replication forks creates a multiforked chromosome.
- The completion of a replication cycle is connected with division of the cell.

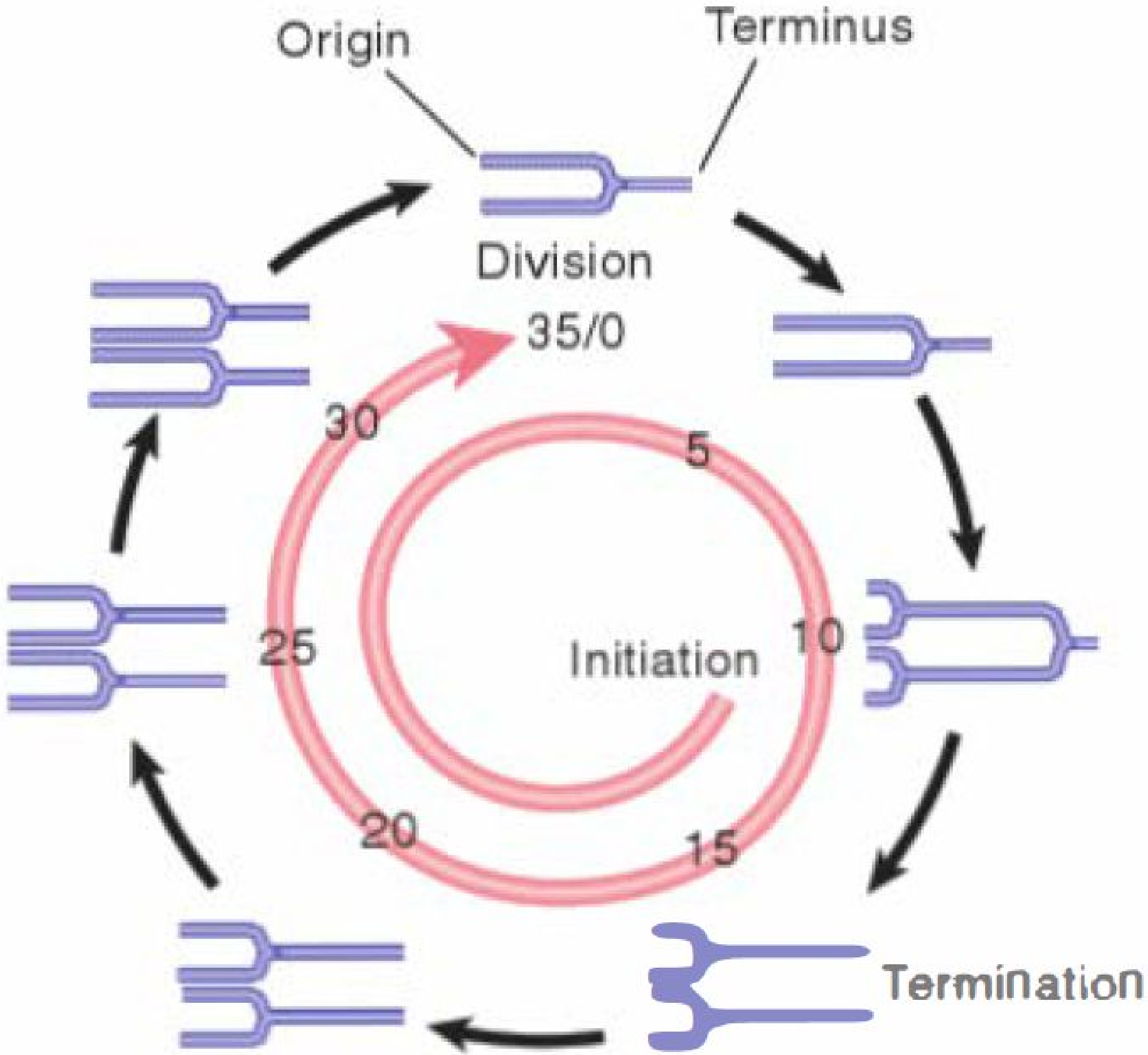
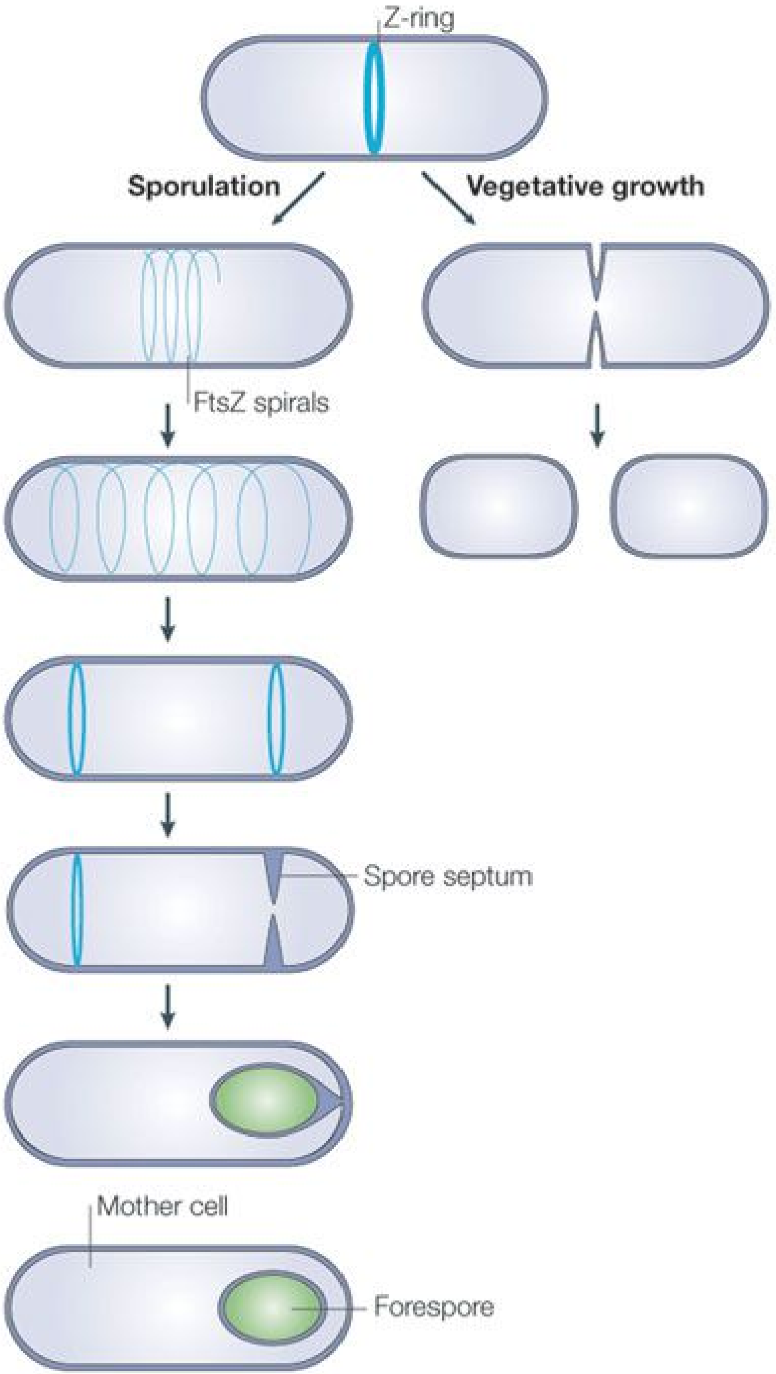


FIGURE 11.3 The fixed interval of 60 minutes between initiation of replication and cell division produces multiforked chromosomes in rapidly growing cells. Note that only the replication forks moving in one direction are shown; the chromosome actually is replicated symmetrically by two sets of forks moving in opposite directions on circular chromosomes.



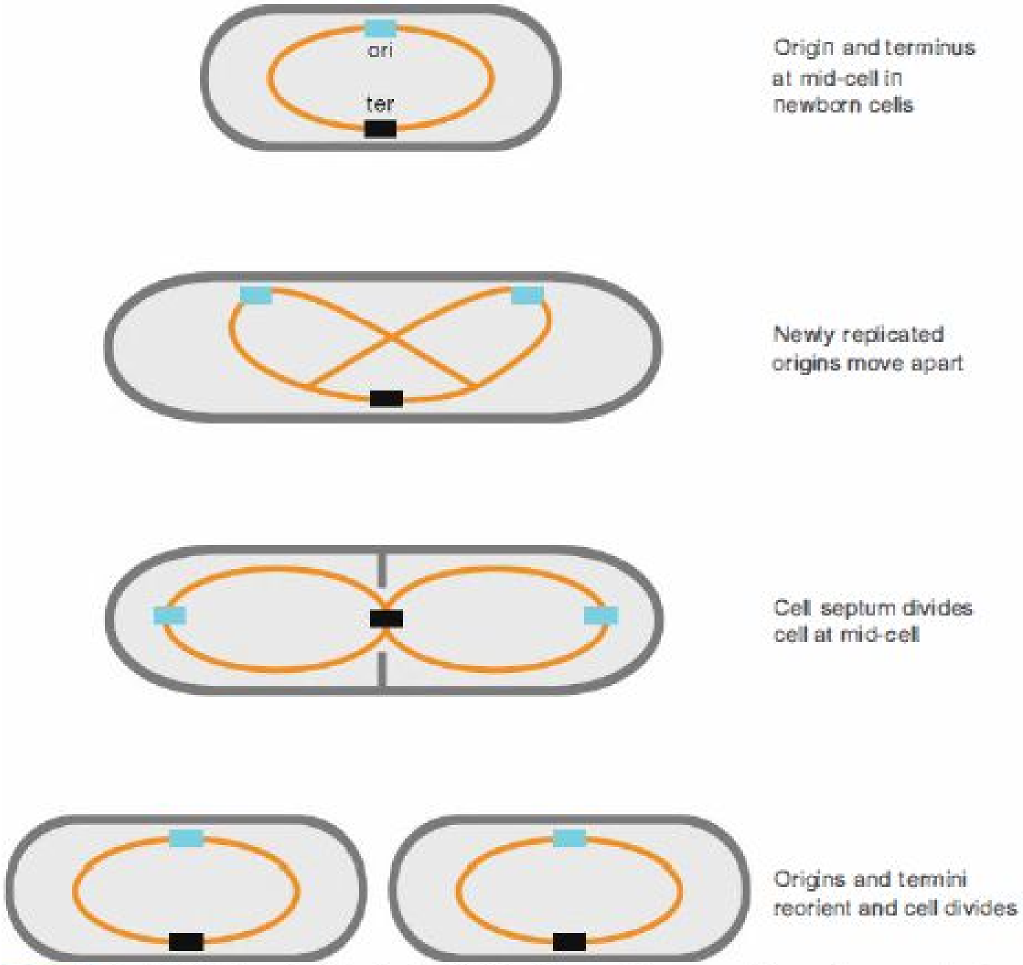


FIGURE 11.4 Attachment of bacterial DNA to the membrane could provide a mechanism for segregation.