Before starting the course...

From theory to practice...
From patient to the genetic lab...
Then back to the patient

✓ Basic Genetic Knowledge

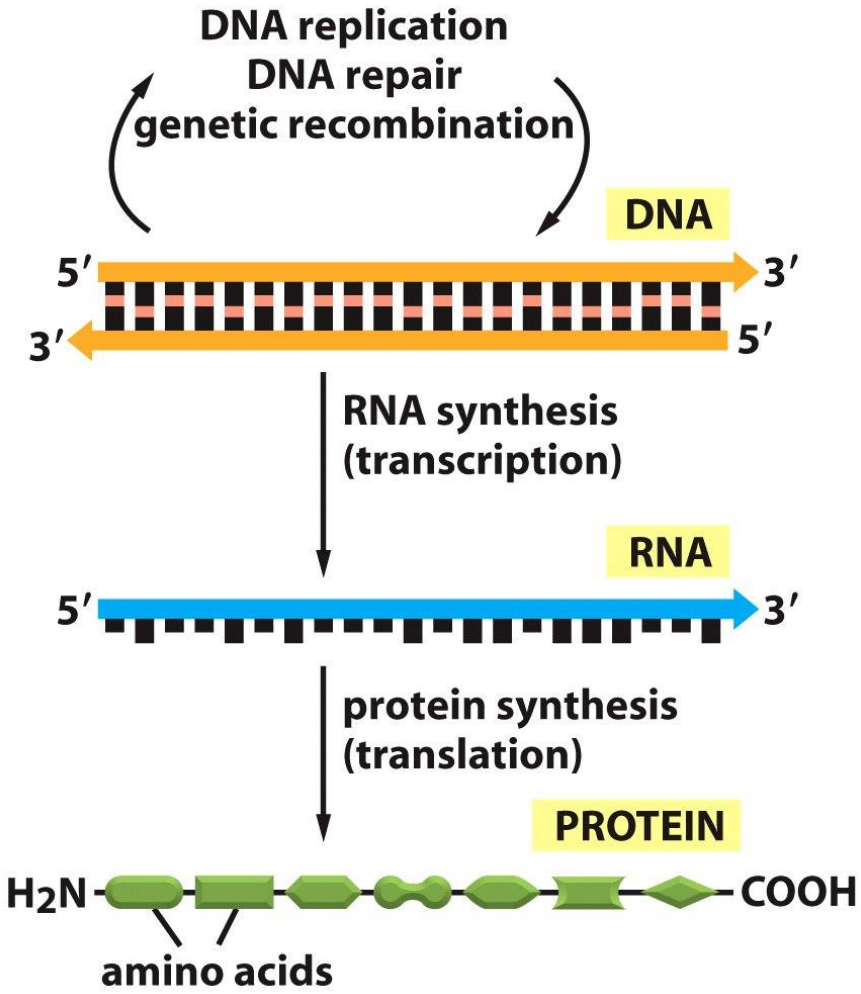


Figure 6-2 *Molecular Biology of the Cell* (© Garland Science 2008)

DNA
Genetics&Epigenetics

RNA (mRNA, miRNA)
Gene expression, seq

PROTEINS
- **expression**
- **localization**
- **activity**

EVALUATE

WHAT?

WHO?

WHEN?

WHY?

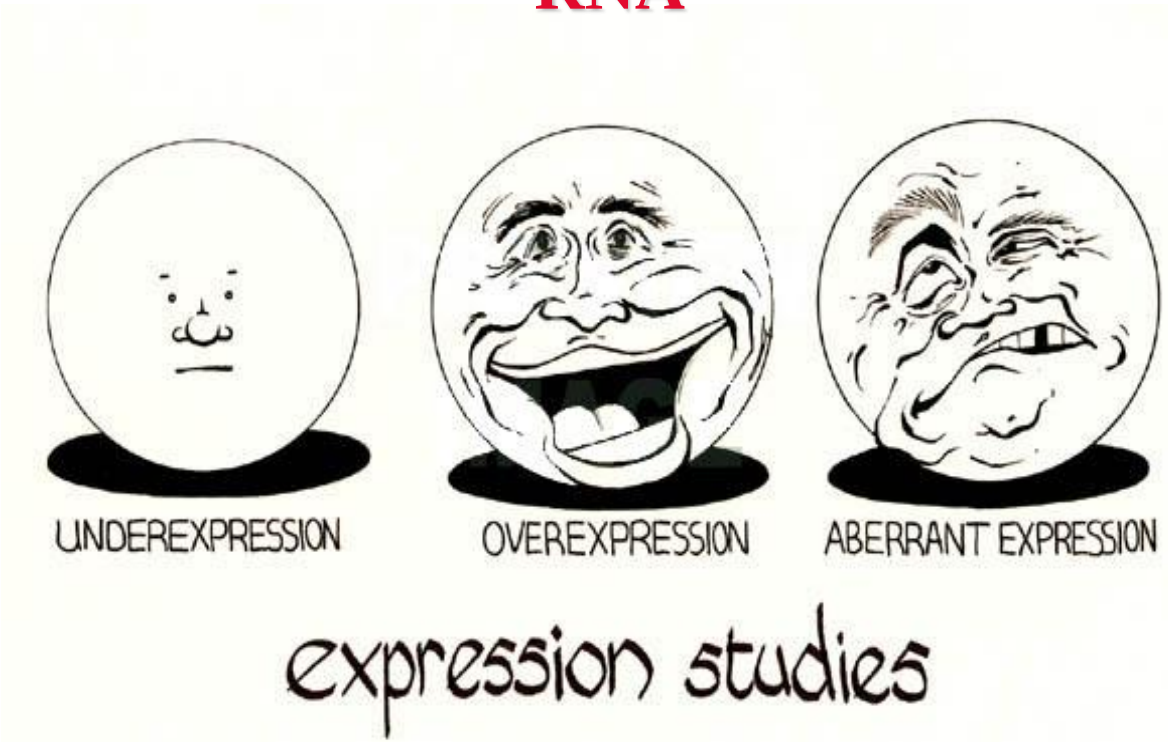
WHERE?

HOW?

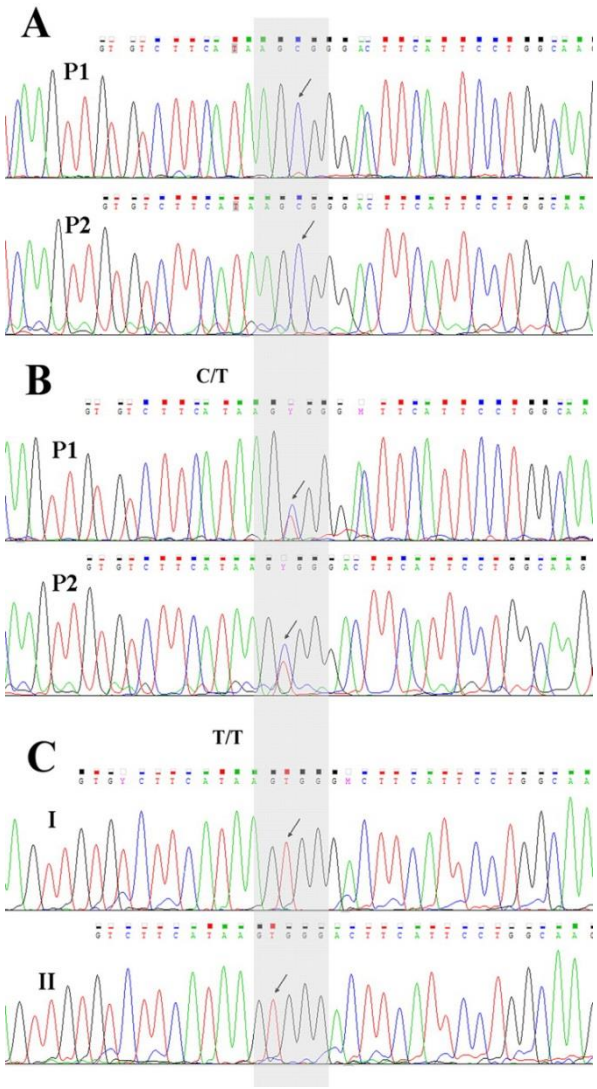
✓ Basic Genetic Knowledge

CHANGES (genetic and epigenetic) may affect phenotype!

RNA



DNA



Genetic studies

✓ From the Patient to the Laboratory

- BIOSPECIMENS (TRANSPORT&BioBanking)
- METHODS

- BIOSPECIMENS (TRANSPORT&BioBanking)

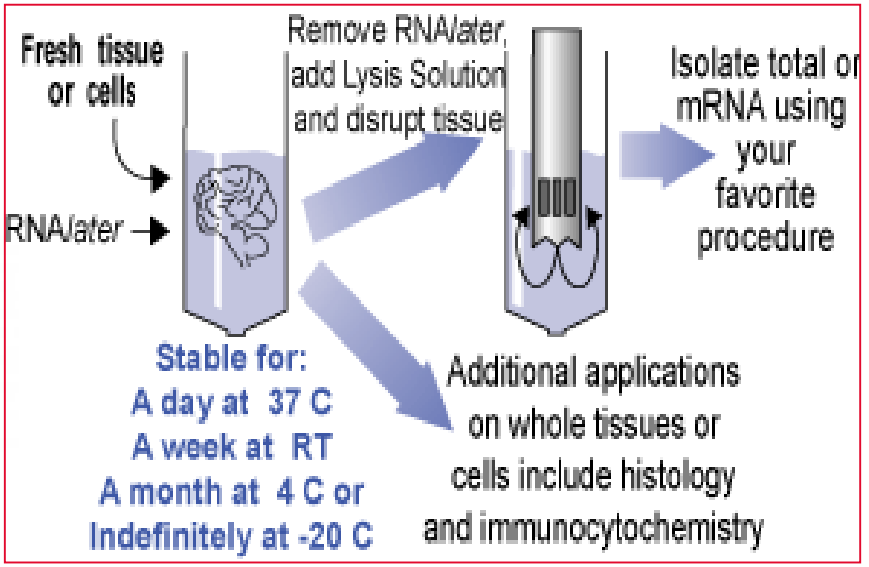
A. fresh tissue - Liquid nitrogen/*RNA stabilizers*

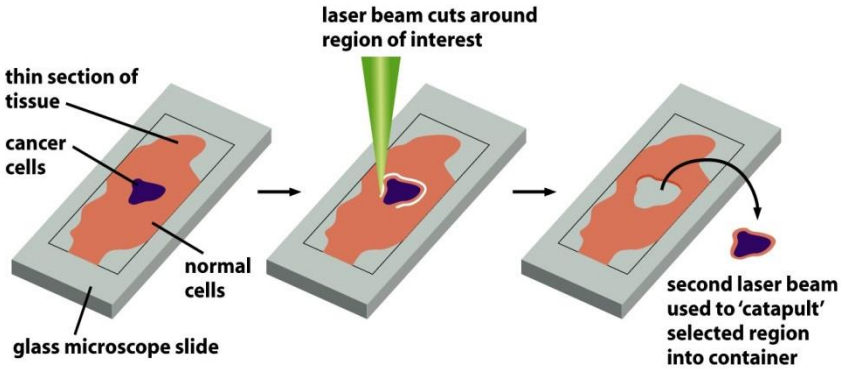
Primary Culture Media (NA)

- biopsies (Endoscopic, Needle, etc.)
- surgical specimens
- blood (EDTA)
- stool, *etc*

B. archived tissues

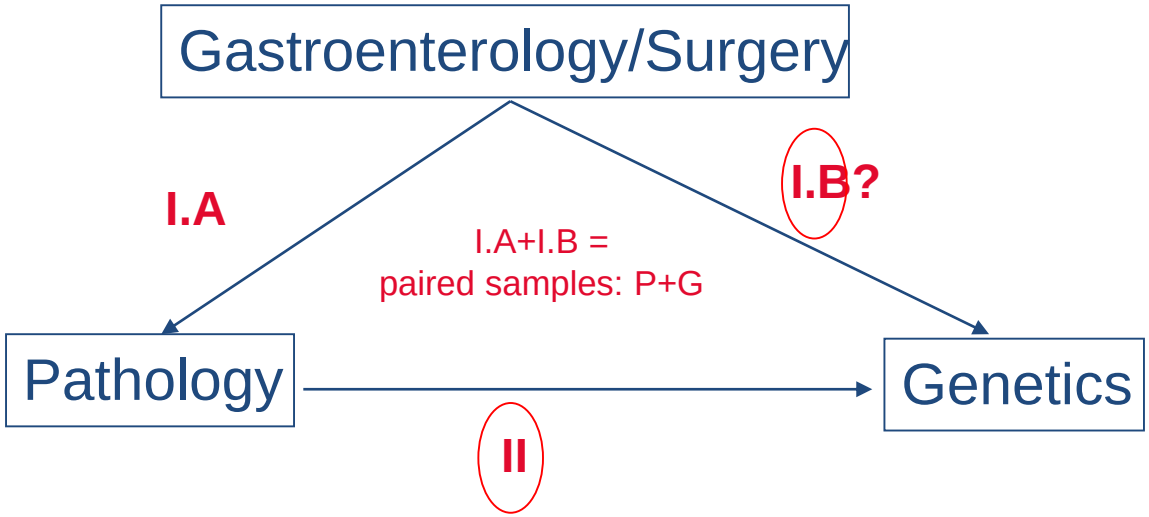
- paraffin-embedded tissues (Dept. of Pathology)
- biobanks





Laser dissection microscopy

- BIOSPECIMENS – *research workflow*



THE BEST PROTOCOL:
“I.B results” validated by “II results”!!!

YES/NO - IDENTICAL PATTERN?

- BIOSPECIMENS - research

SAMPLE SET (pre-/malignant, normal tissue, blood, etc) /patient

TO COMPARE!!!

ALWAYS COLLECT BLOOD SAMPLES!!!

1. *de novo* or *inherited* mutations! *2.* Further GWAS

DNA-blood	DNA-malignant tissue	DNA-premalignant tissue	DNA-normal
Mutation +	Mutation +	Mutation +	Mutation +
Mutation -	Mutation +	Mutation ?+/-	Mutation -

TISSUE SAMPLES

Gene expression level/s => pattern

RNA-malignant tissue → **"Z" gene pattern?**

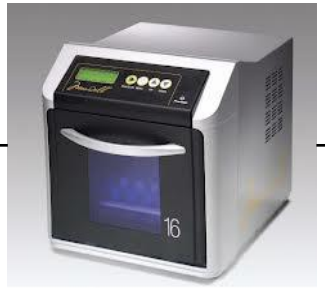
RNA-premalignant tissue

RNA-normal

- METHODS

DNA

Purification



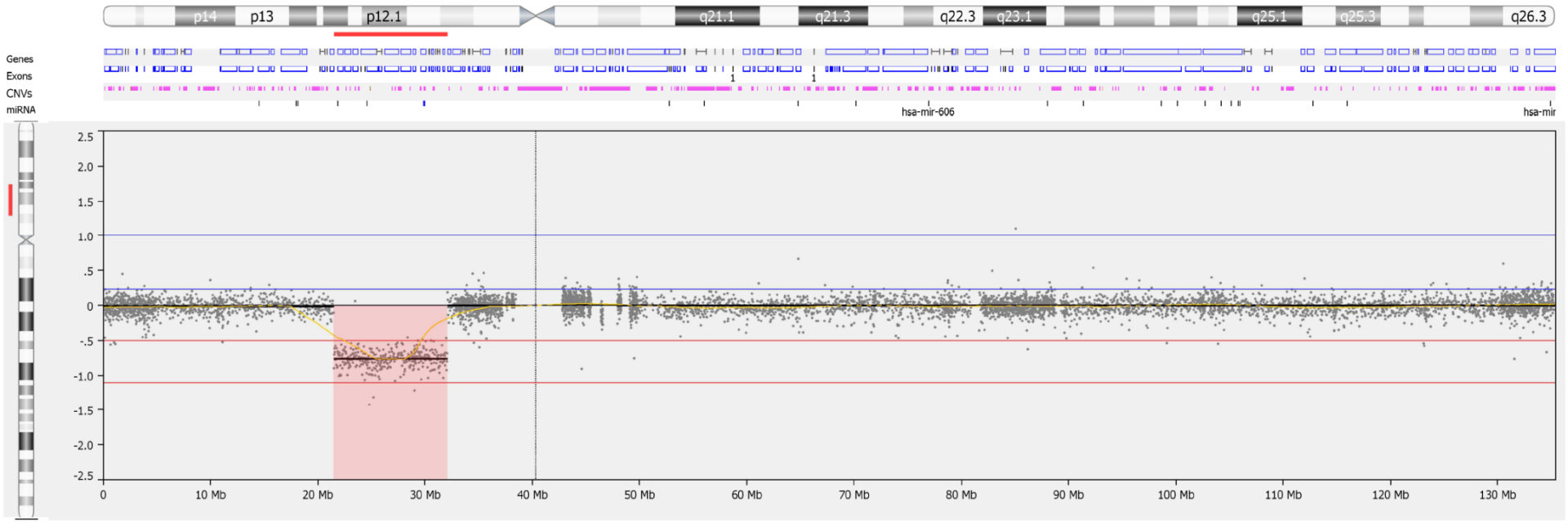
MICROARRAY - Screening:
SNPs, LOH, **CNV**

Real-time PCR:
SNPs, INDEL,
promoter methylation status

Capillary sequencing, MLPA

NimbleGen MS 200 Microarray Scanner

MICROARRAY – Nexus – Nimblegen – Roche/Agilent



Bolovan: Chromosome 10

- METHODS

RNA

I. Sample collection, transportation and storage.

II. Procedures for Quality Control of RNA Samples.

III. Reverse Transcription (RT) and cDNA Synthesis.

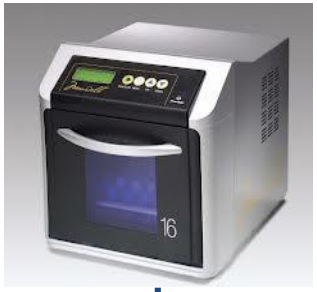
IV. Screening of gene expression signatures (microarray-based methods).

V. Validation - Quantitative Real-Time PCR.

- METHODS

RNA

Purification



MICROARRAY
- Gene expression

SCREENING

Quality Control of RNA Samples
RNA integrity number

Real-time PCR:
Gene expression
Array/assay

VALIDATION

TOPICS – 4 courses:

- the structure of DNA;
- basic genetic mechanisms, how the genetic information of the cell is:
  - maintained
  - replicated
  - expressed
  - occasionally improved.

Most of the genetic testing methods are PCR-based

PCR – Polymerase chain reaction – next laboratory....

... but PCR

is a procedure that mimics the cellular process of DNA replication

Previous Course

I. DNA and CHROMOSOMES:

- **I.A.** The Structure and Function of DNA
- **I.B.** Chromosomal DNA and Its Packaging in the Chromatin Fiber
- **I.C.** The Global Structure of Chromosomes

Course structure

II. DNA Replication and Repair:

- **II.A.** The Maintenance of DNA Sequences
- **II.B.** DNA Replication Mechanisms
- **II.C.** The Initiation and Completion of DNA Replication in Chromosomes
- **II.D.** DNA Repair

REPLICATION

The process of making an **identical copy (!?)** of a section of a double-stranded DNA, using **existing DNA as a template** for the synthesis of new DNA strands.

In humans and other eukaryotes, replication occurs in the cell nucleus.

Before a cell can produce two genetically identical daughter cells.

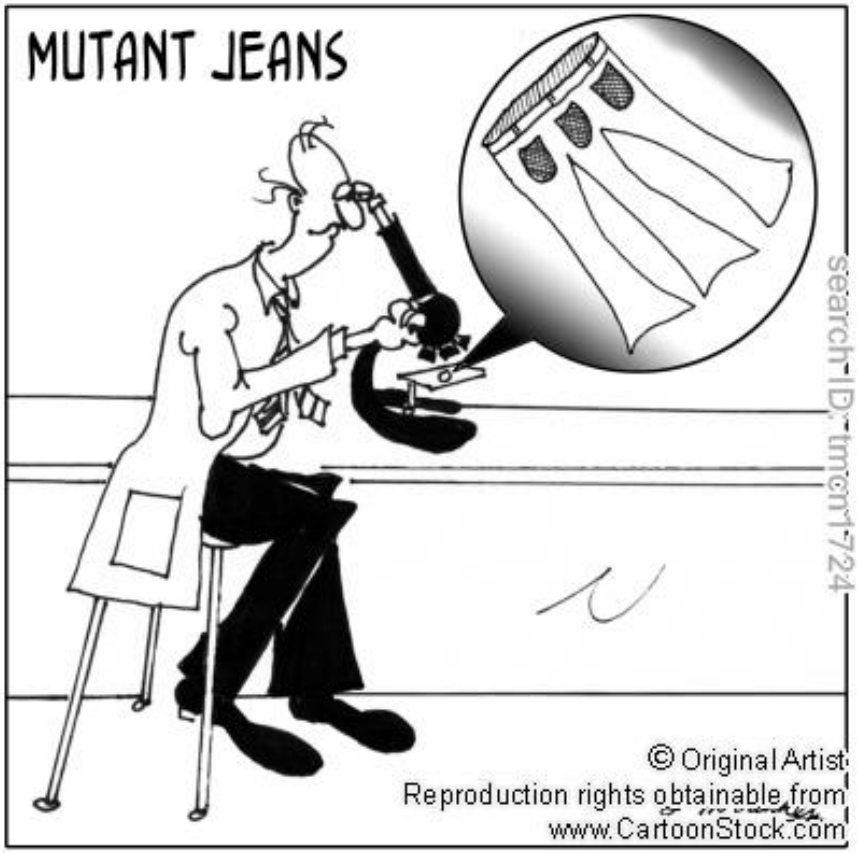
genetic changes *vs* genetic stability

A SPECIES

- long-term survival
- enhanced by occasional genetic changes

AN INDIVIDUAL

- survival - genetic stability



II.A. The Maintenance of DNA Sequences

DNA Damaging:

- chemicals and radiation from the environment

- thermal accidents

- reactive molecules

DNA Repair *(when possible):*

- detection systems

- repair systems

APOPTOSIS

BUT SOME DNA damages remain unfixed!!!

II.A. The Maintenance of DNA Sequences

Mutation – damaging/silent

- permanent change in the DNA

<= DNA-maintenance processes fail

Mutation rate – in E. Coli

- 1 nucleotide change/10^9 nucleotides/cell/ generation.

Mutation rate – germ-line – in mammals

- 1 nucleotide change/10^9 nucleotides each time DNA is replicated

II.A. The Maintenance of DNA Sequences

- Many Mutations in Proteins Are Deleterious and Are Eliminated by Natural Selection

But

- Low Mutation Rates Are Necessary for Life as We Know It

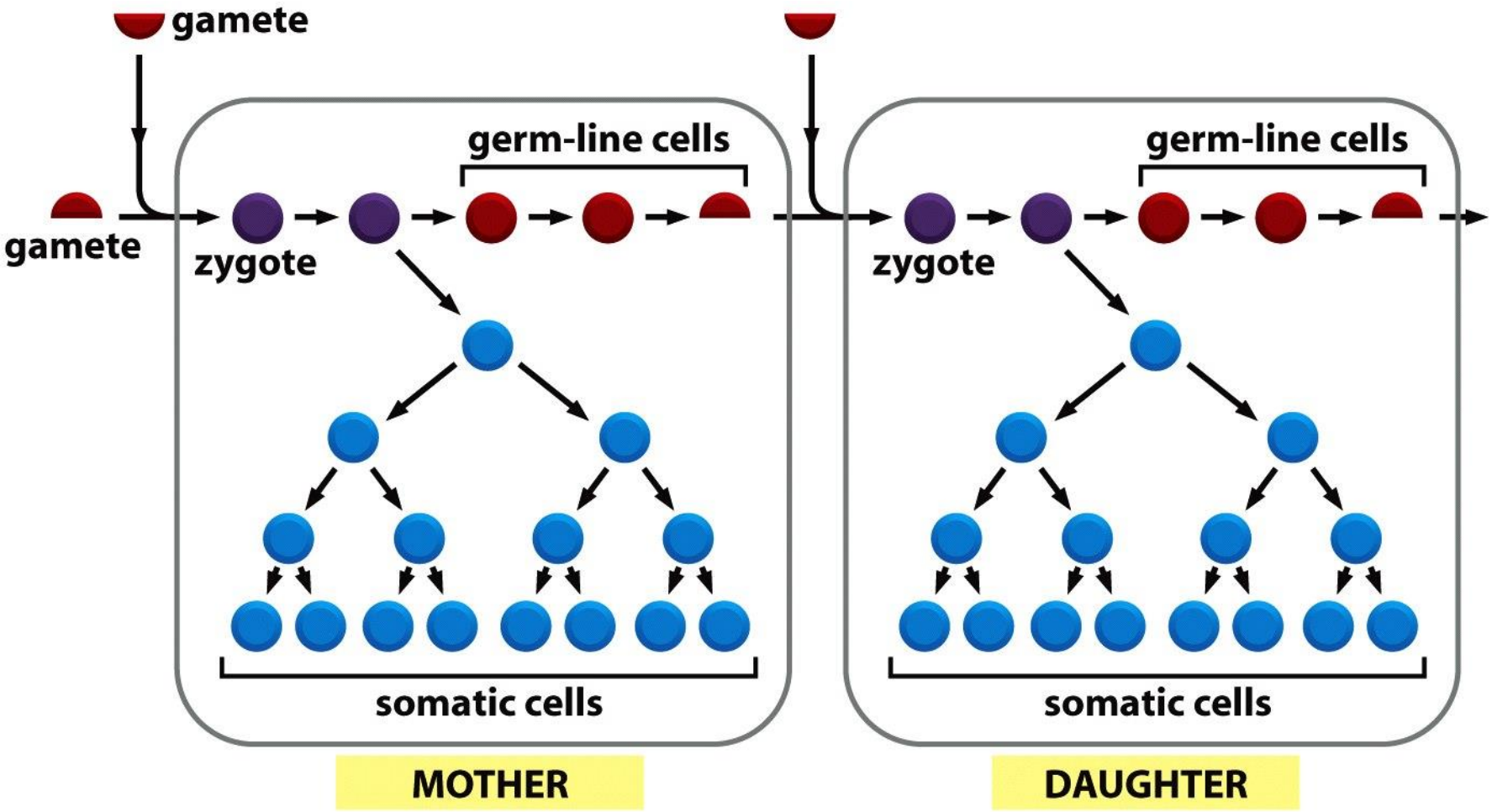


Figure 5-1 *Molecular Biology of the Cell* (© Garland Science 2008)

II.A. The Maintenance of DNA Sequences

GERM CELLS must be protected against high rates of mutation to maintain the species;

SOMATIC CELLS of multicellular organisms must be protected from genetic change to safeguard each individual.

II.B. DNA Replication Mechanisms

- duplicating DNA - 1000 nucleotides per second

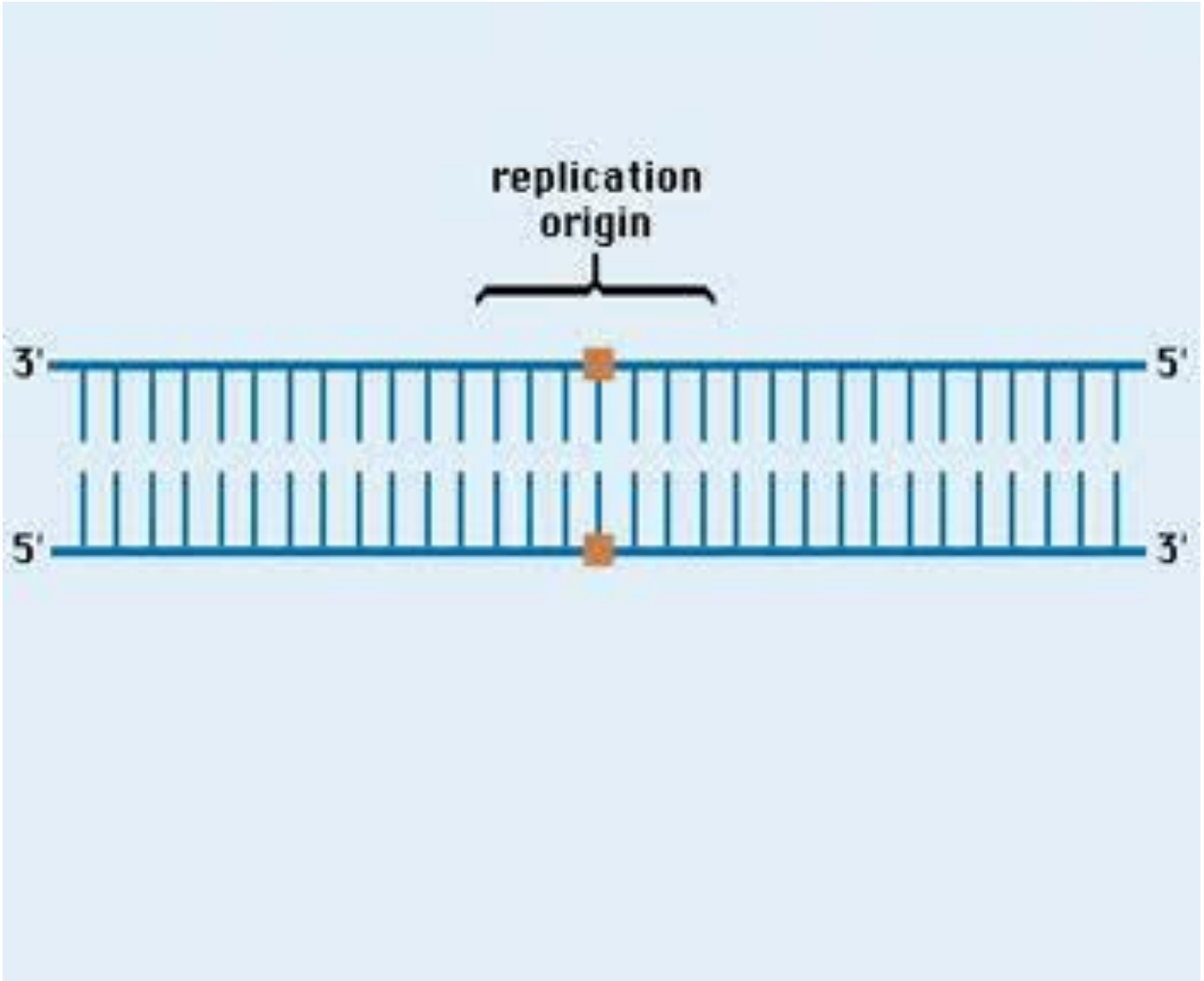
- DNA templating

- complementary base-pairing (A with T, and G with C)

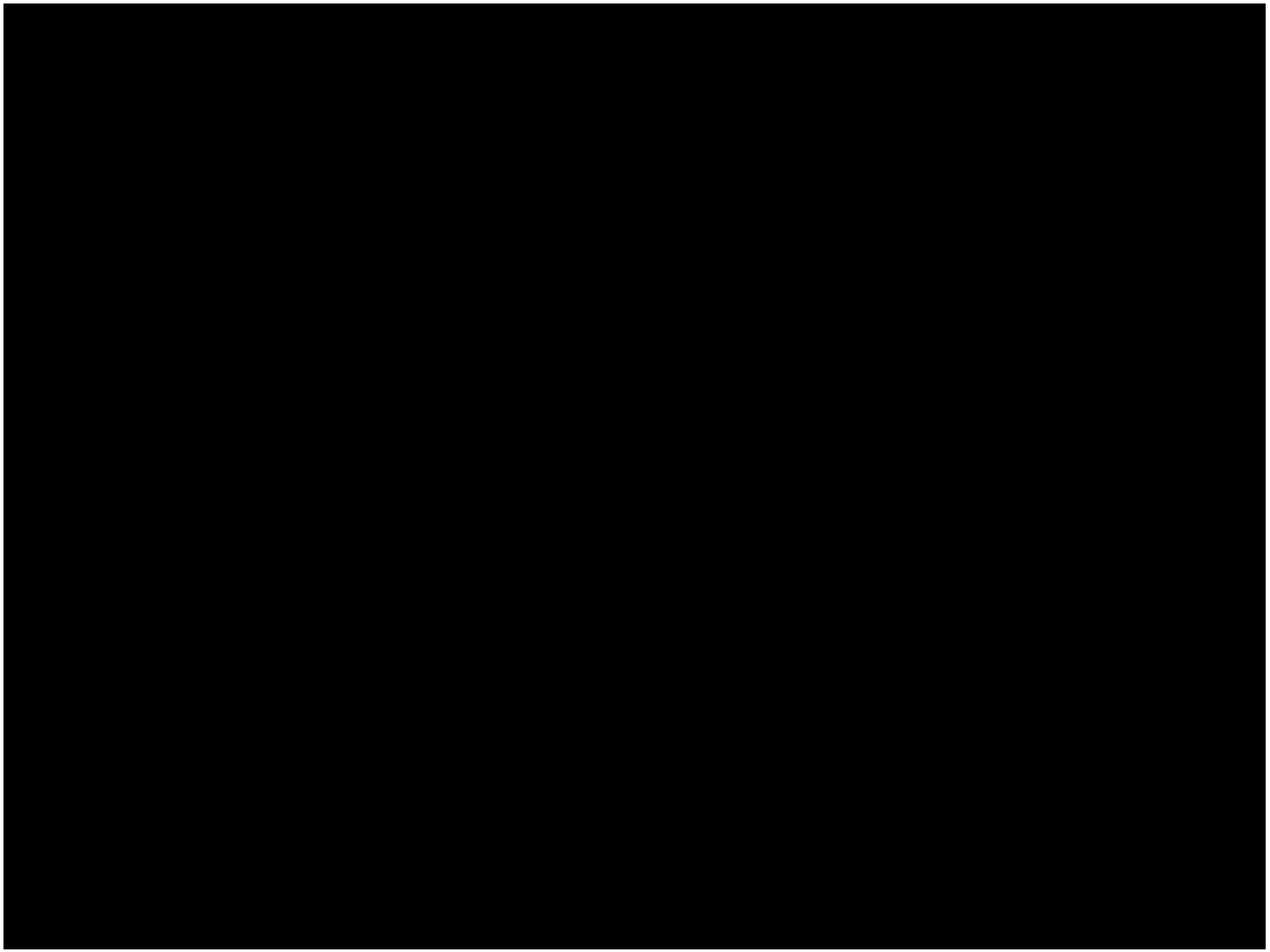
- enzyme-catalyzed polymerization

- deoxyribonucleoside triphosphates

II.B. DNA Replication Mechanisms



II.B. DNA Replication Mechanisms



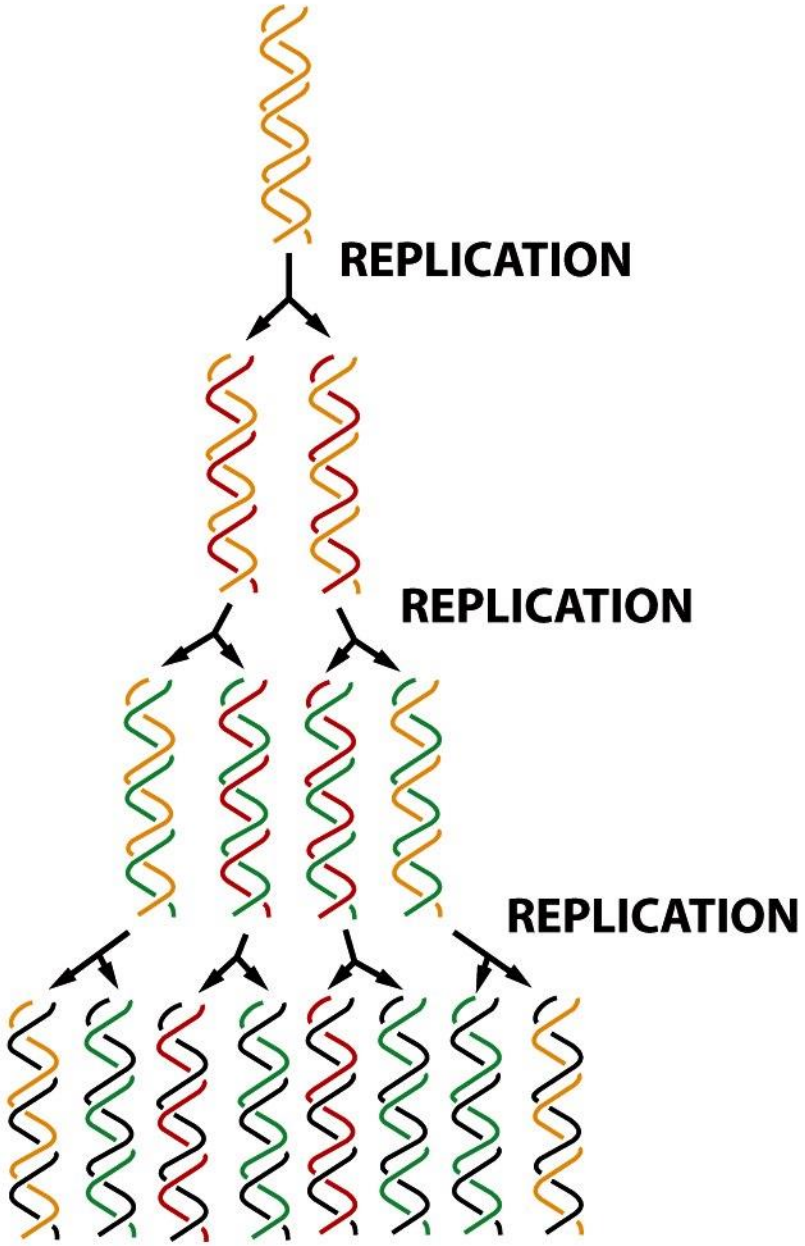
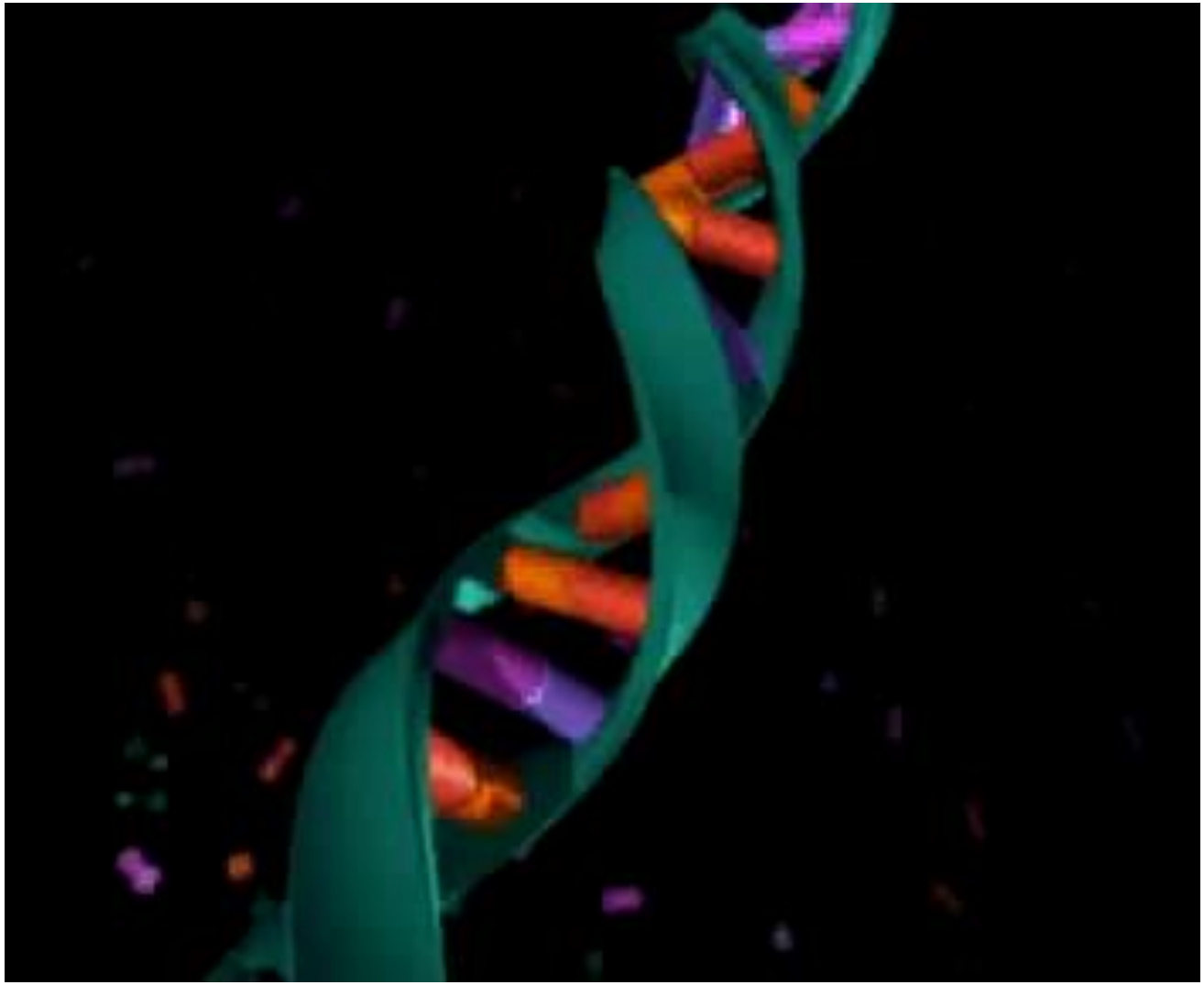


Figure 5-5 *Molecular Biology of the Cell* (© Garland Science 2008)

II.B. DNA Replication Mechanisms



II.B. DNA Replication

- WHEN?

- WHERE (WHICH CELL COMPARTMENT)?

- REPLICATION ORIGIN (ONE/MANY)?

- STEPS?

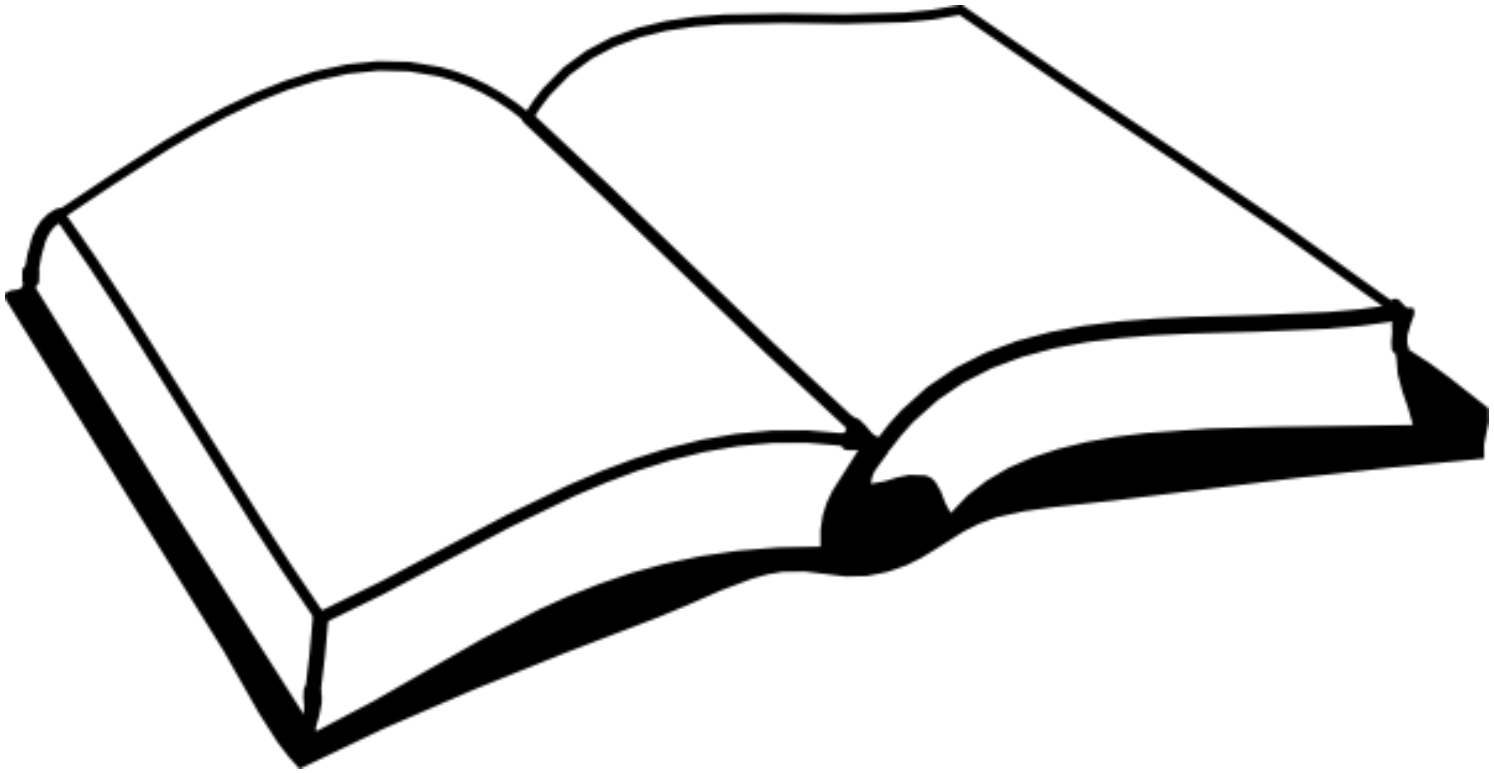
- KEY COMPONENTS?

COPY the BOOK

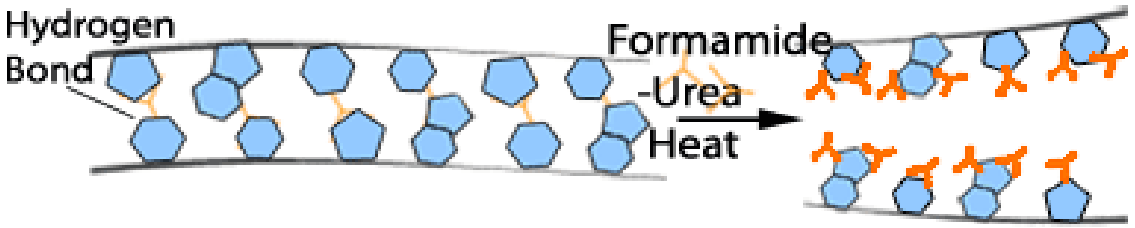
the DNA SEQUENCE

OPEN it!

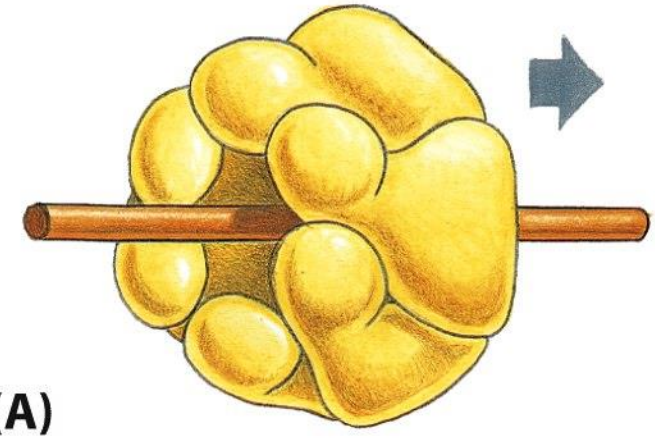
Separate the two DNA strands



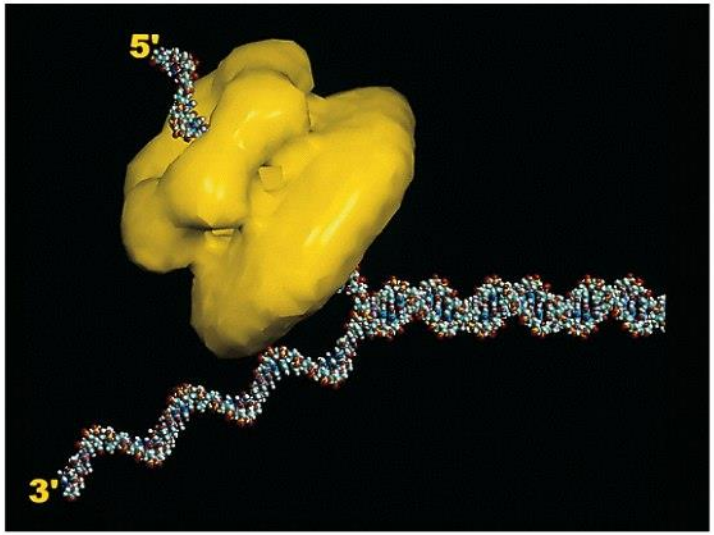
DENATURATION - the hydrogen bonds between the strands are broken;



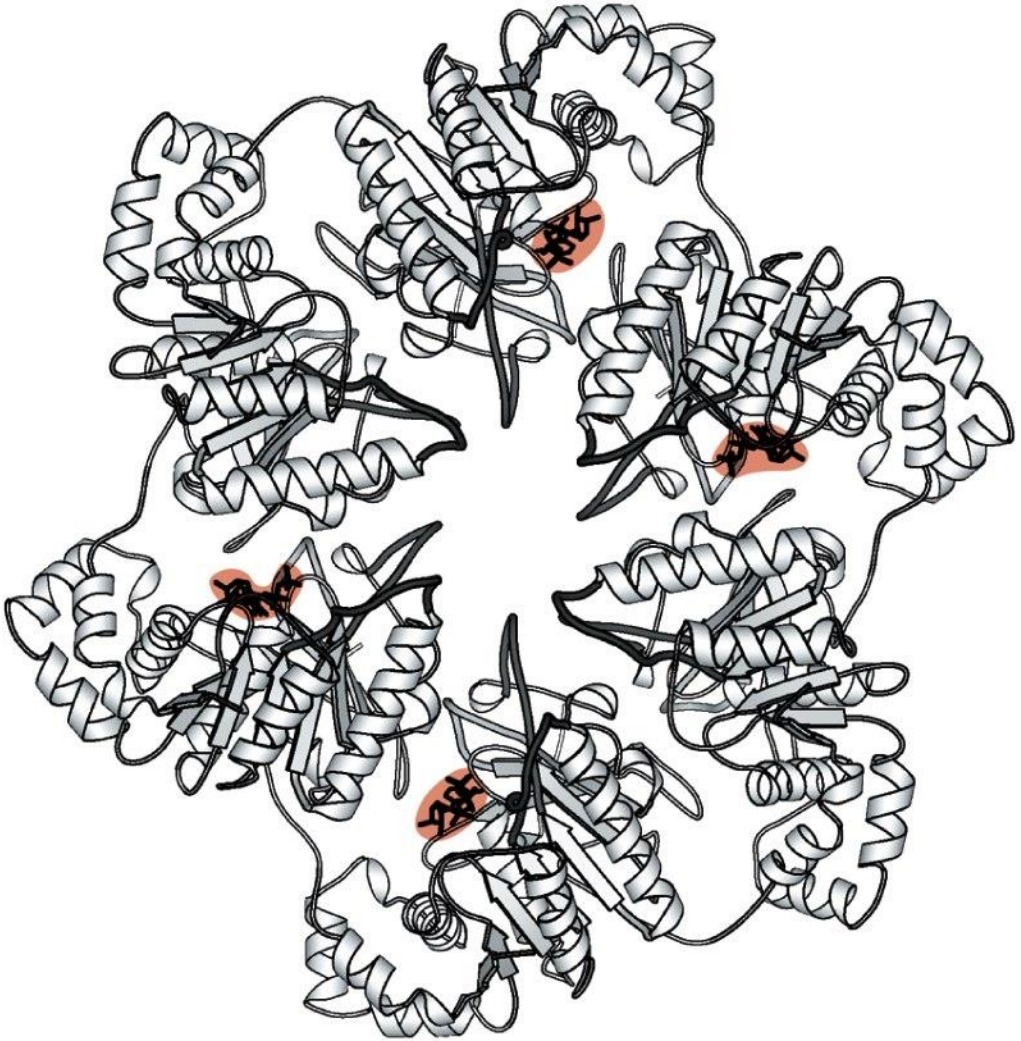
1. DNA helicase.



(A)



(B)



(C)

Figure 5-15 *Molecular Biology of the Cell* (© Garland Science 2008)

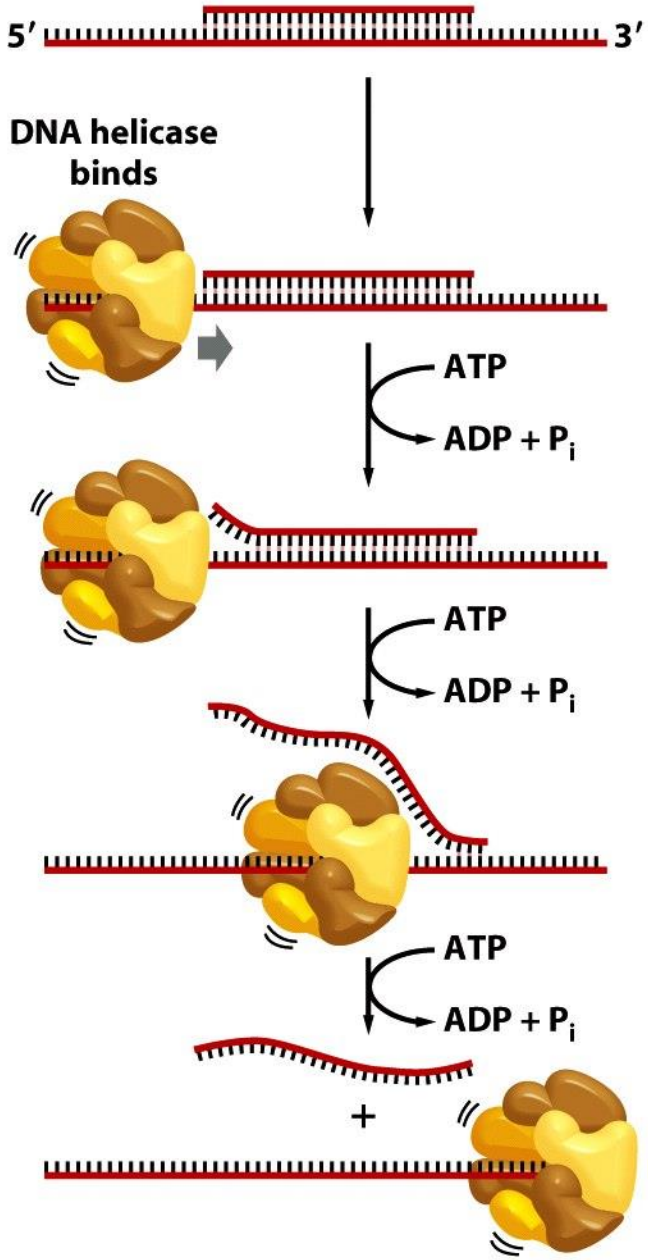


Figure 5-14 *Molecular Biology of the Cell* (© Garland Science 2008)

How to prevent DNA Tangling During Replication?

“The winding problem”

2. topoisomerases: 2 types

Topoisomerase I

- cleaves phosphodiester bond
- transient single-strand break (or nick)
- relieves the tension
- allows free rotation of the DNA around the covalent backbone bonds

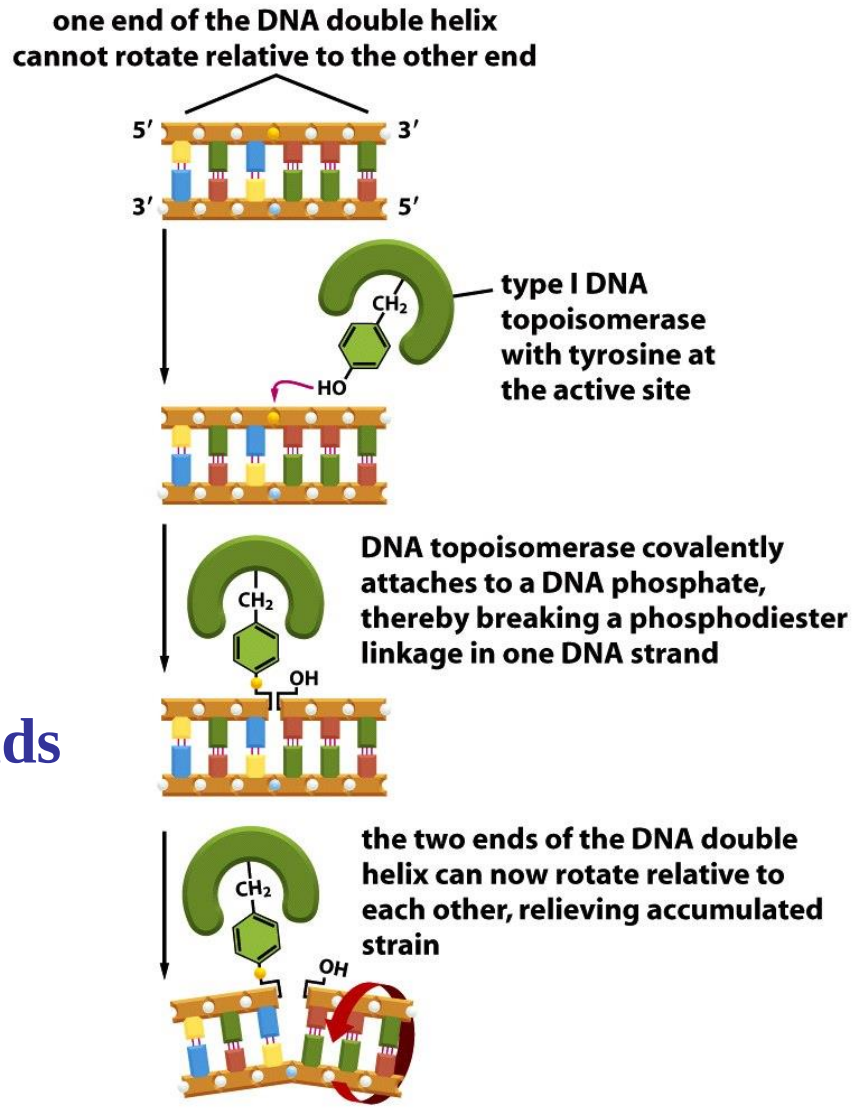


Figure 5-22 (part 1 of 2) *Molecular Biology of the Cell* (© Garland Science 2008)

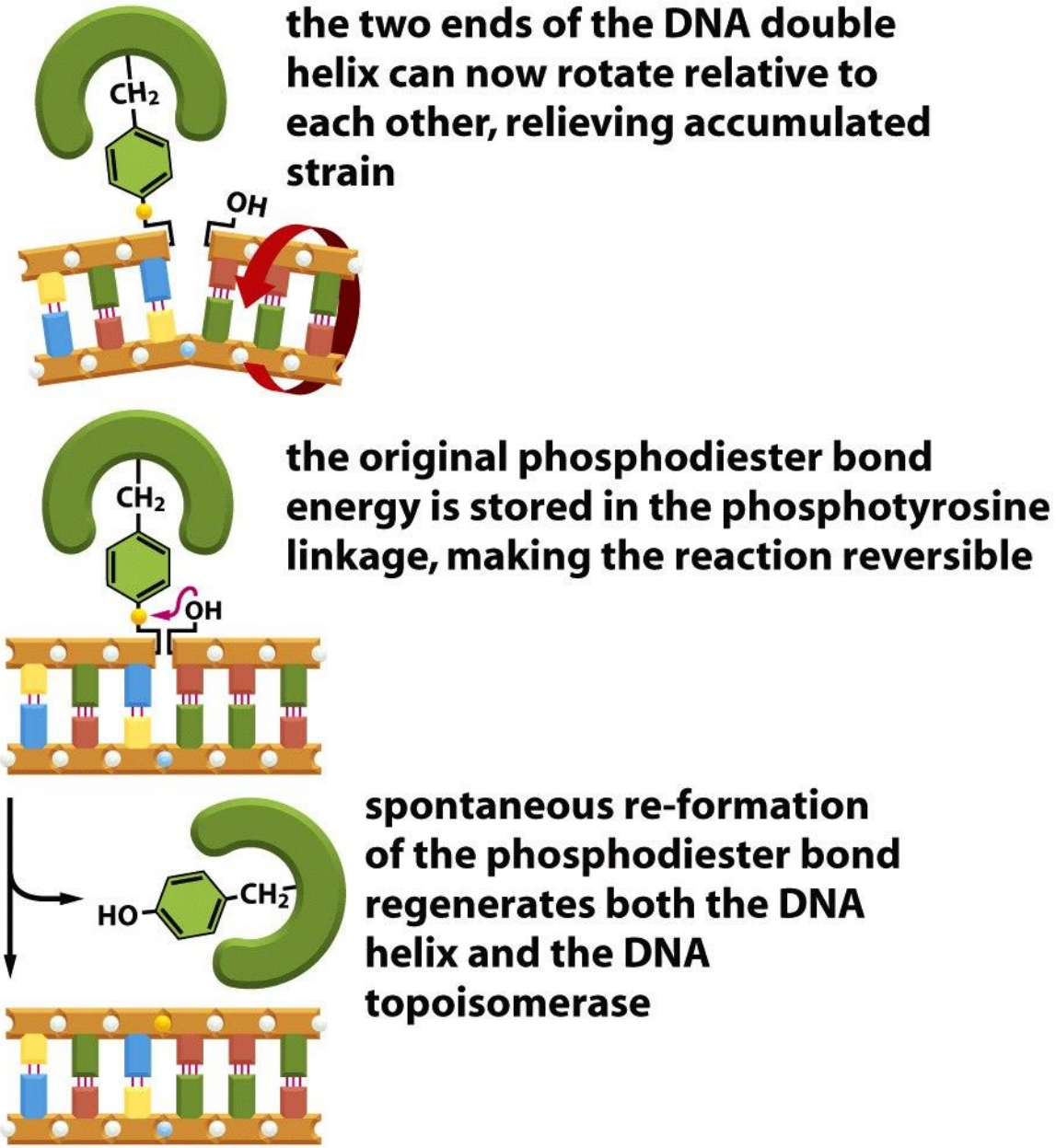


Figure 5-22 (part 2 of 2) *Molecular Biology of the Cell* (© Garland Science 2008)

3. single-strand DNA-binding proteins

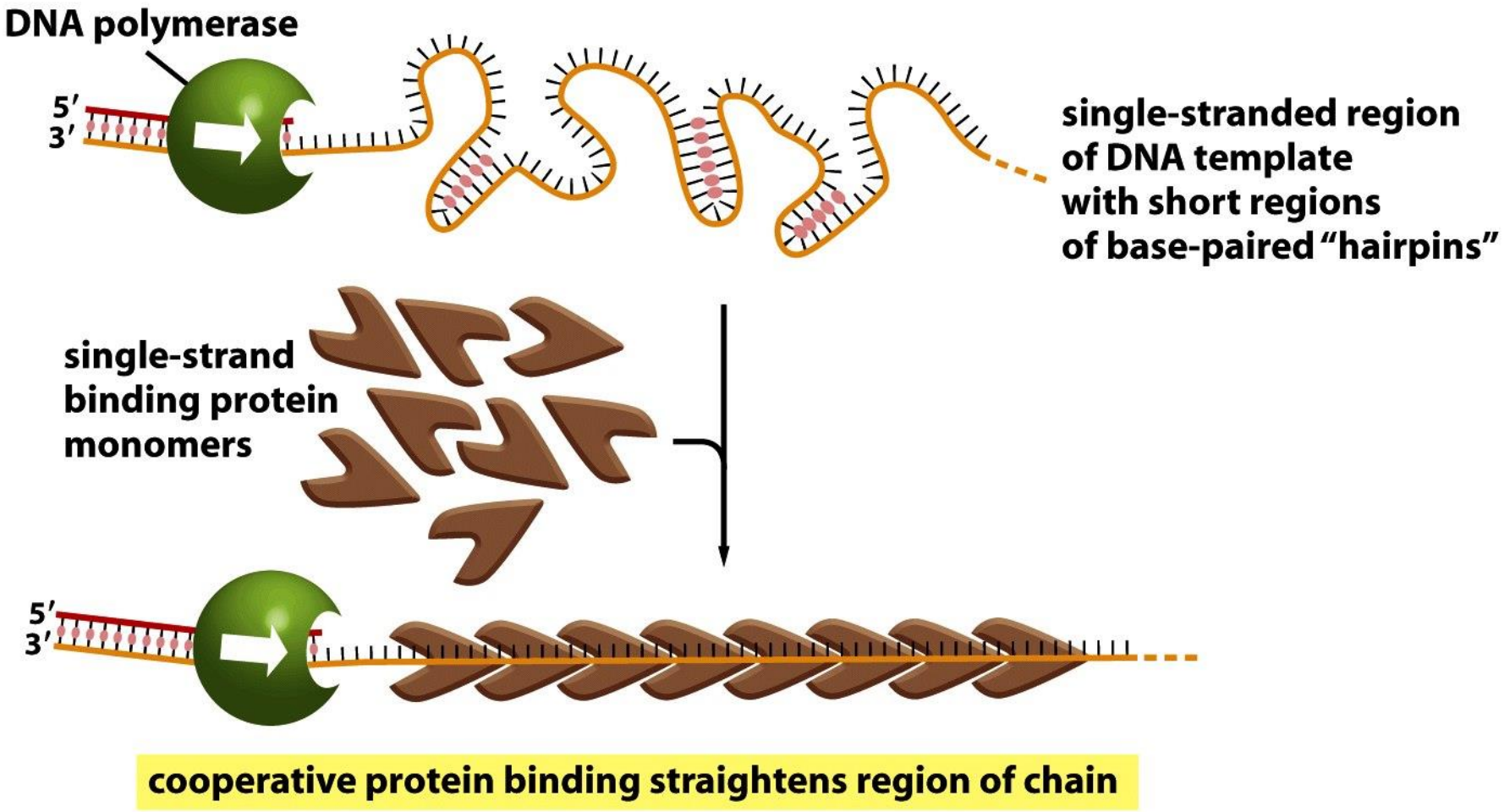


Figure 5-16 *Molecular Biology of the Cell* (© Garland Science 2008)

4. DNA Primase & Primer

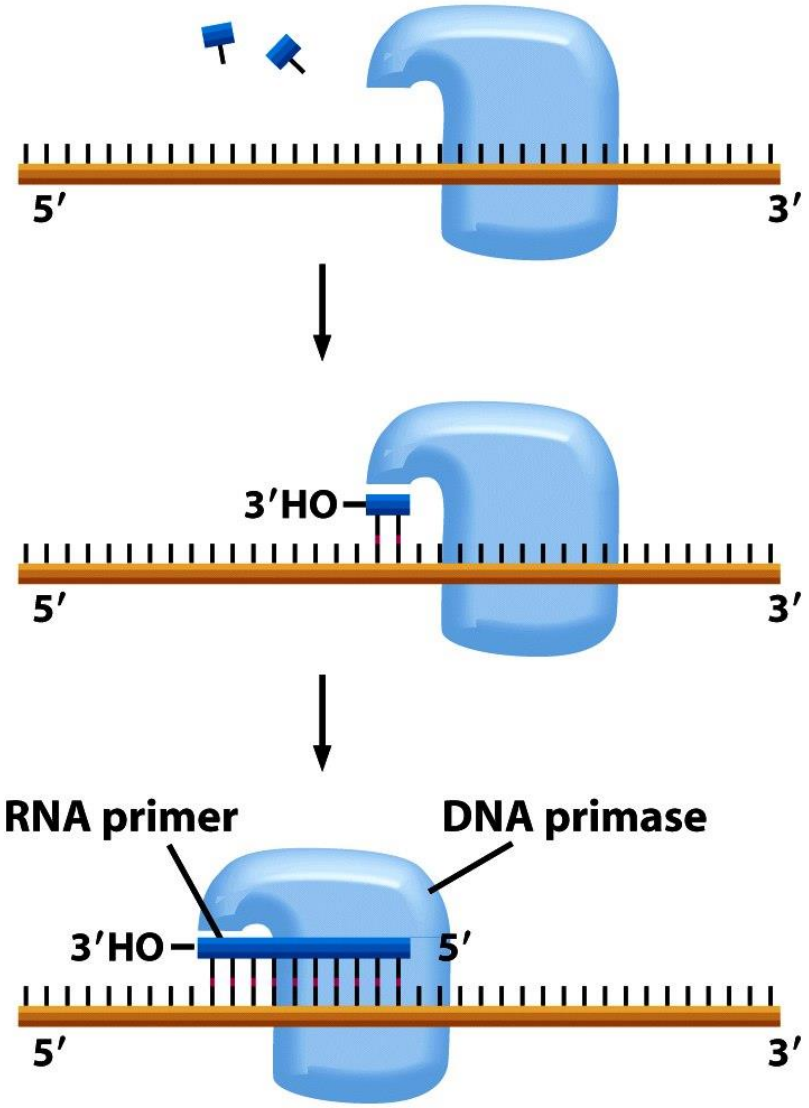


Figure 5-11 *Molecular Biology of the Cell* (© Garland Science 2008)

5. DNA polymerase & dNTs

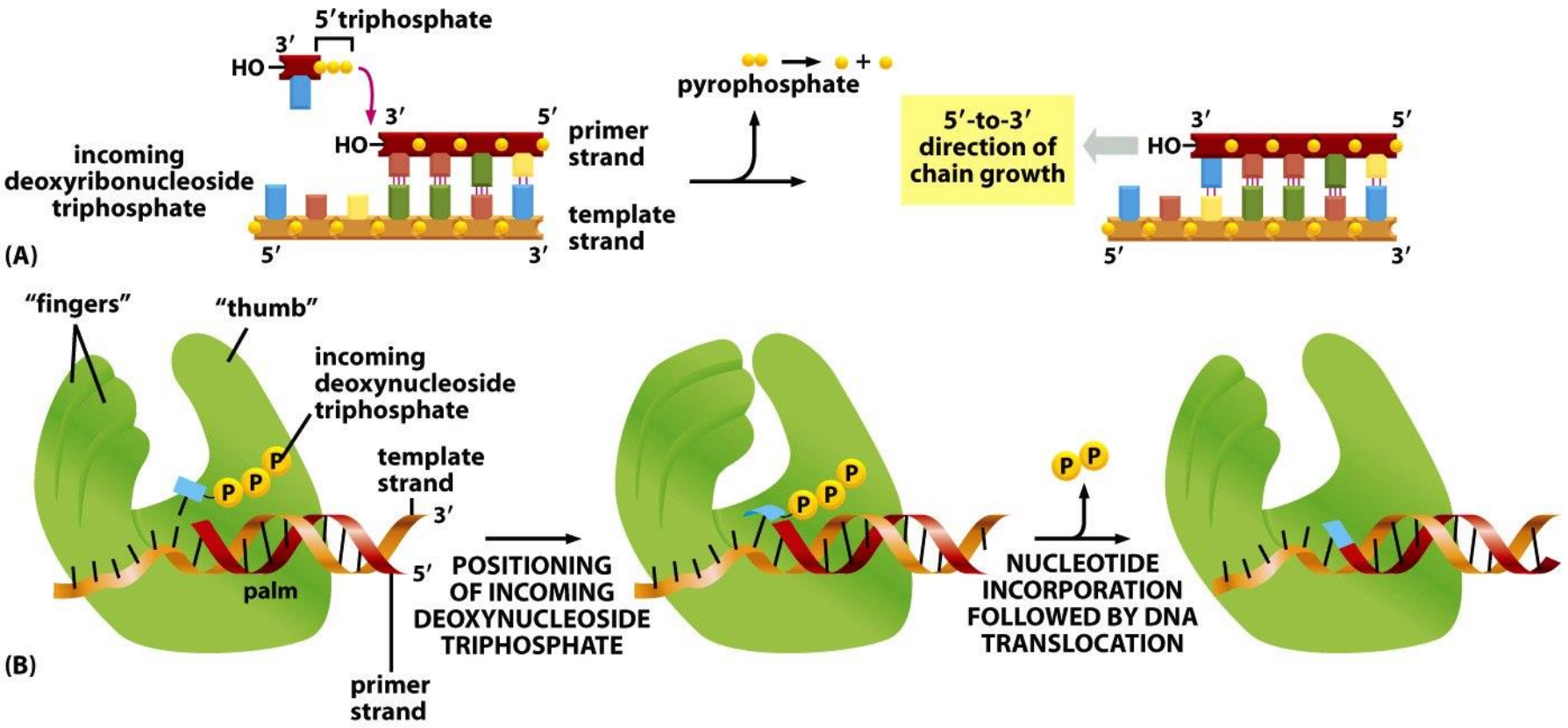


Figure 5-4 *Molecular Biology of the Cell* (© Garland Science 2008)

6. Sliding clamp protein

- keeps DNA polymerase connected to the DNA

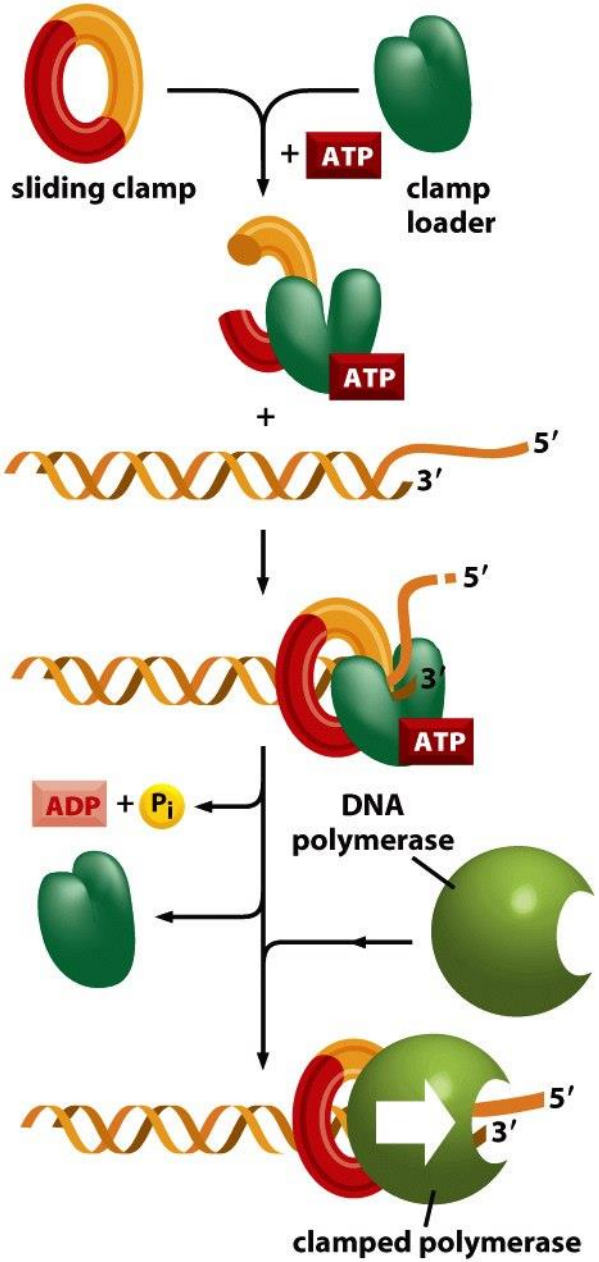


Figure 5-18c *Molecular Biology of the Cell* (© Garland Science 2008)

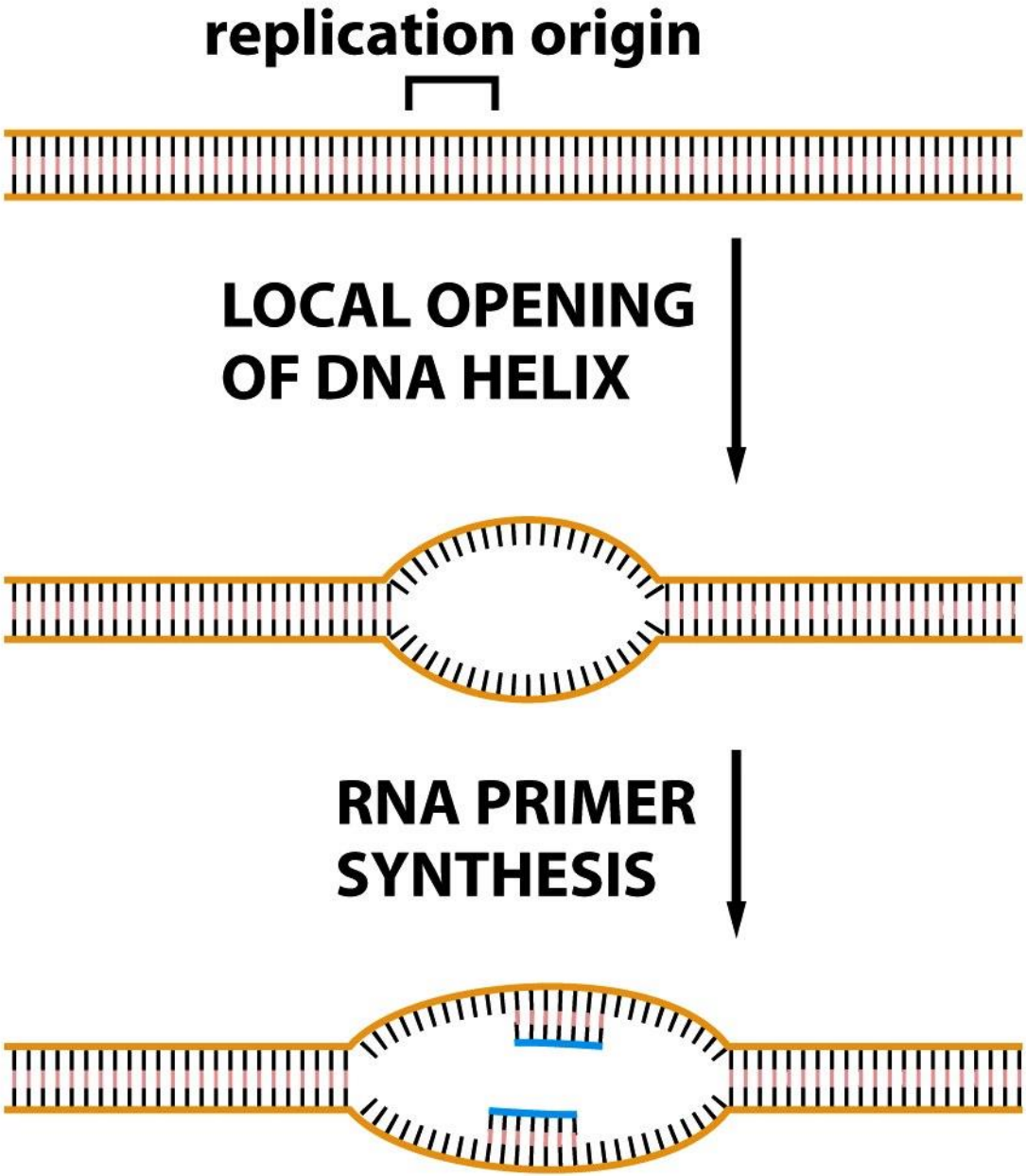


Figure 5-25 (part 1 of 2) *Molecular Biology of the Cell* (© Garland Science 2008)

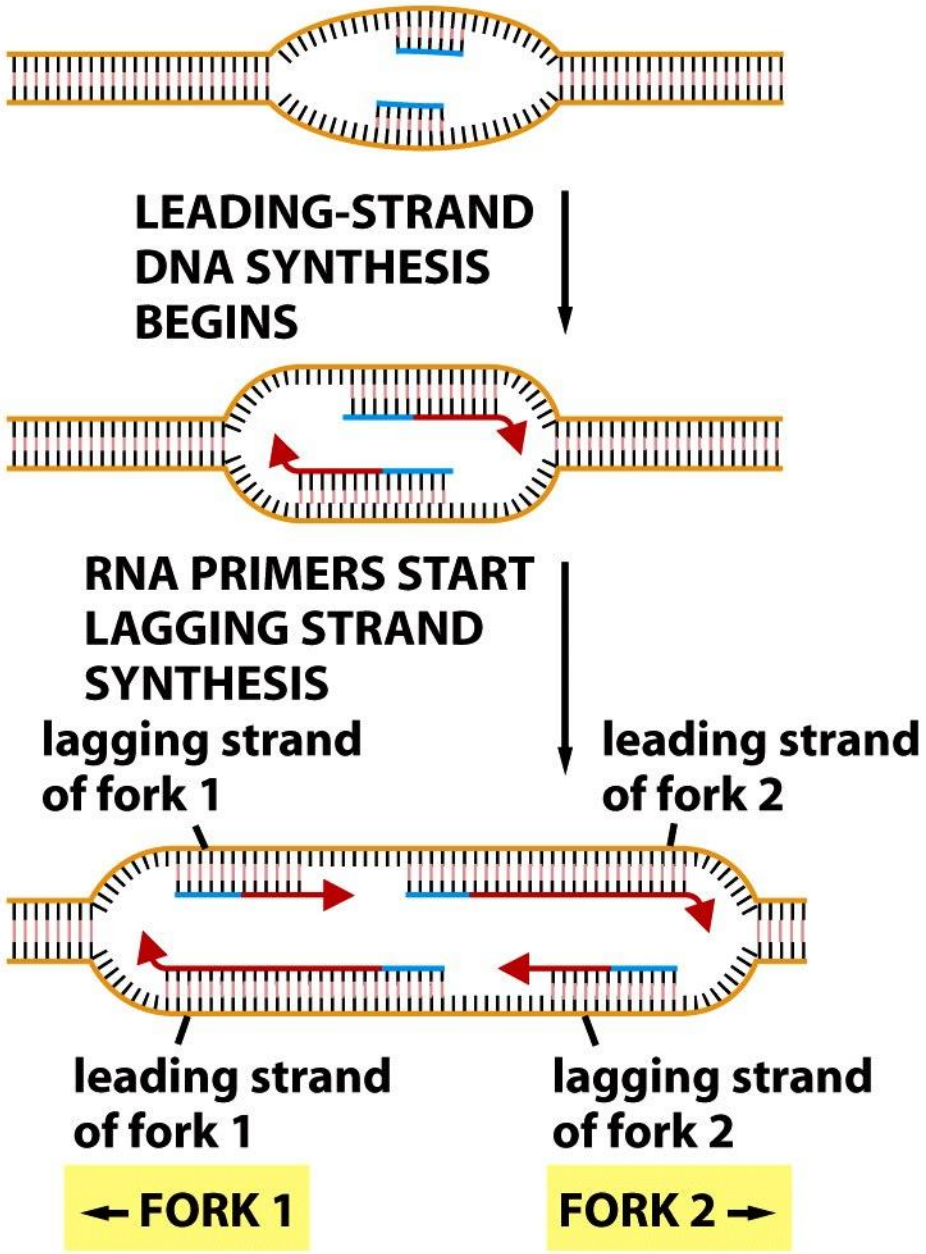


Figure 5-25 (part 2 of 2) *Molecular Biology of the Cell* (© Garland Science 2008)

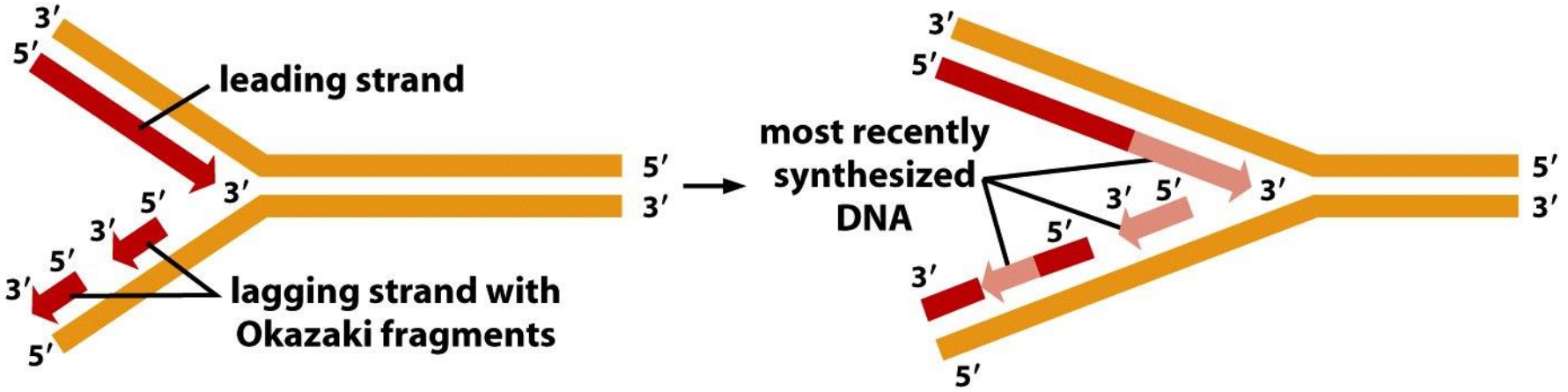
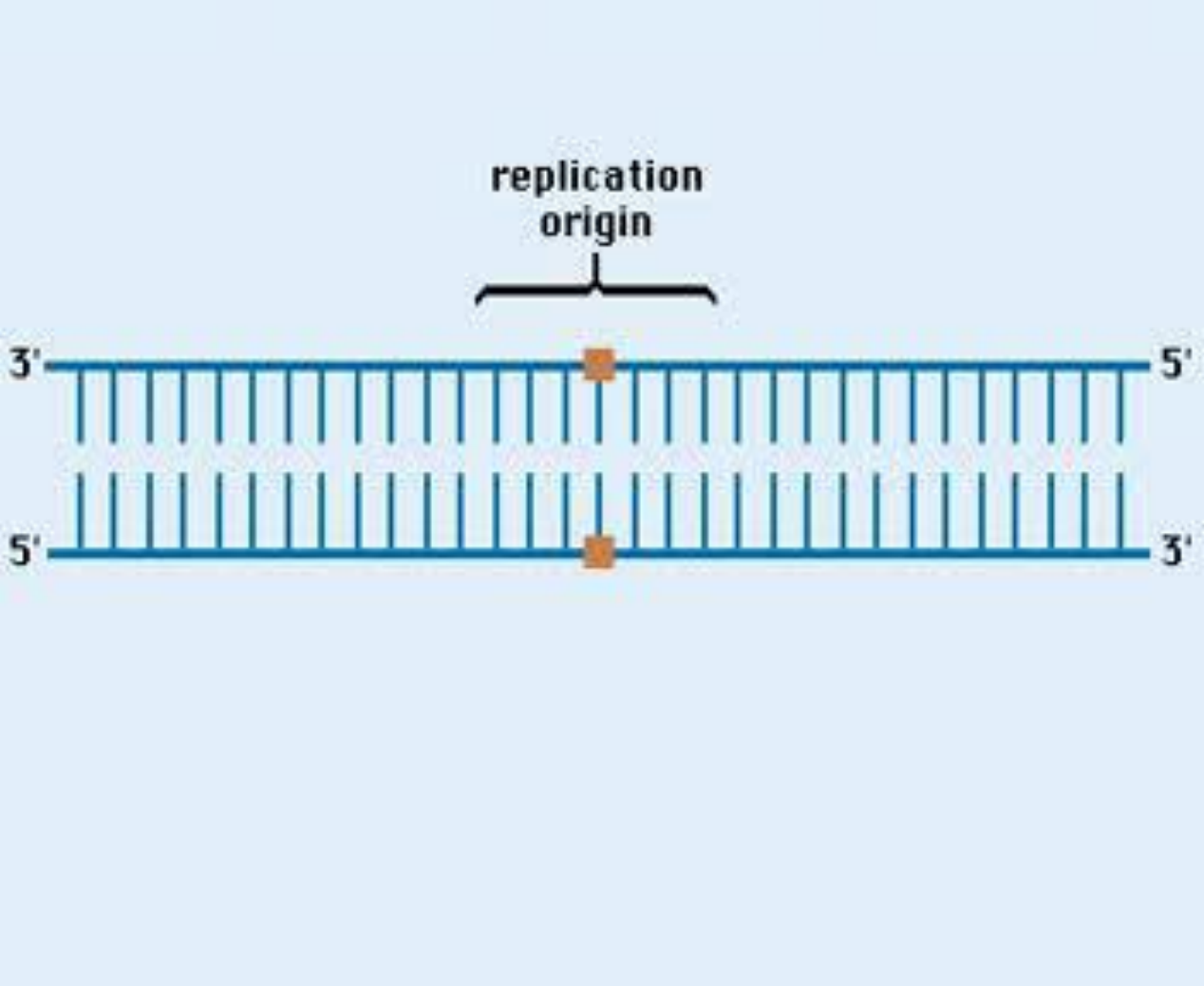


Figure 5-7 *Molecular Biology of the Cell* (© Garland Science 2008)

II.B. DNA Replication Mechanisms



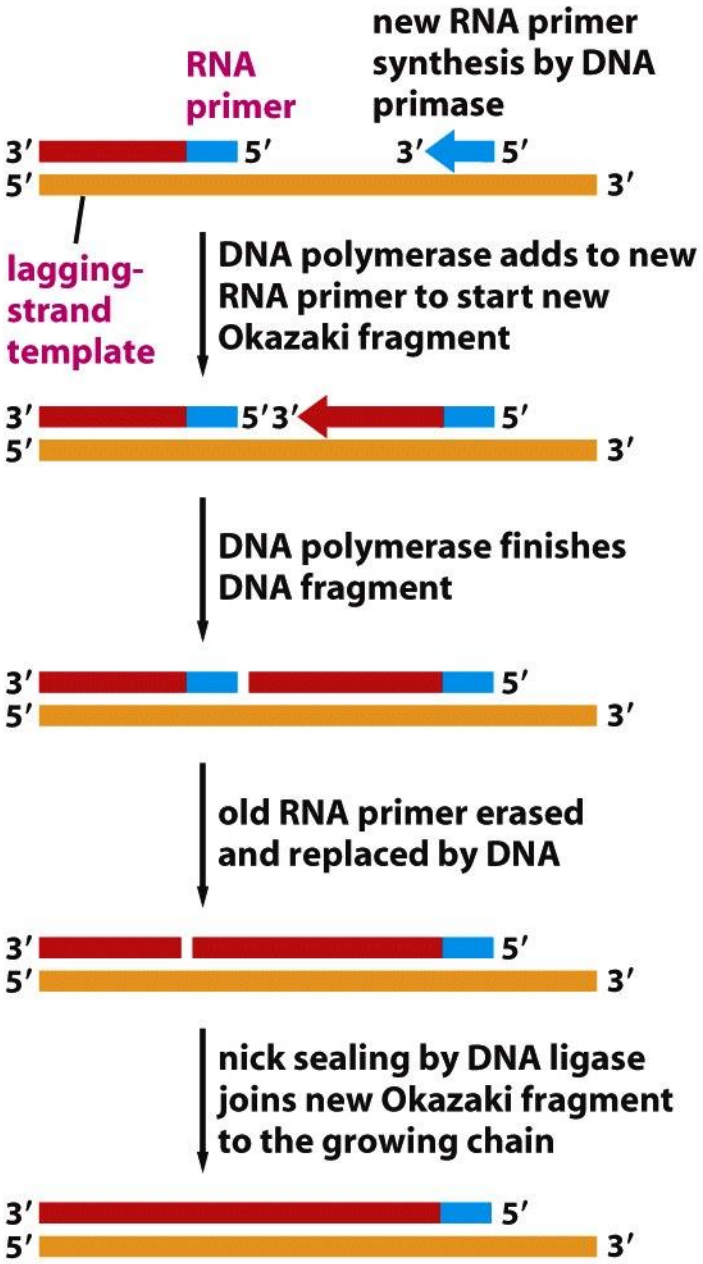


Figure 5-12 *Molecular Biology of the Cell* (© Garland Science 2008)

7. RNase H

- removes the RNA primer

- allows the completion of the newly synthesized DNA.

Figure 5-13 *Molecular Biology of the Cell* (© Garland Science 2008)

8. DNA ligase

- joins the ends of two strands of DNA together with a covalent bond to make a continuous DNA strand.

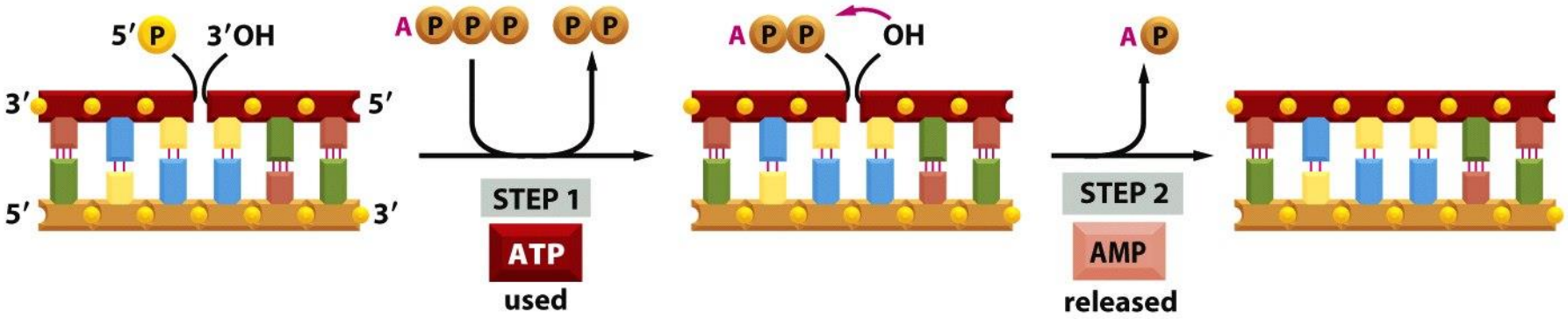


Figure 5-13 *Molecular Biology of the Cell* (© Garland Science 2008)

II.B. DNA Replication Mechanisms

Exonucleolytic proofreading by DNA polymerase during DNA replication

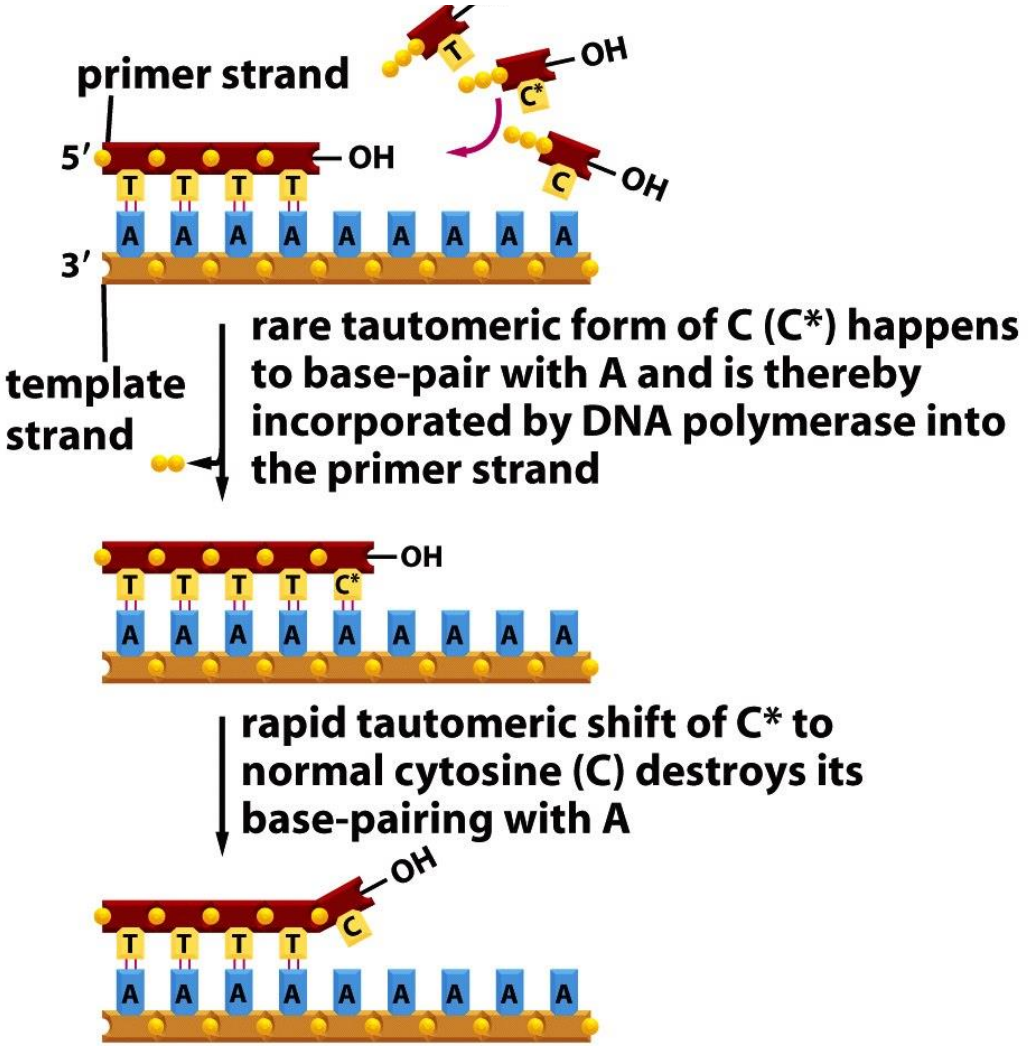


Figure 5-8 (part 1 of 2) *Molecular Biology of the Cell* (© Garland Science 2008)

Exonucleolytic proofreading by DNA polymerase during DNA replication

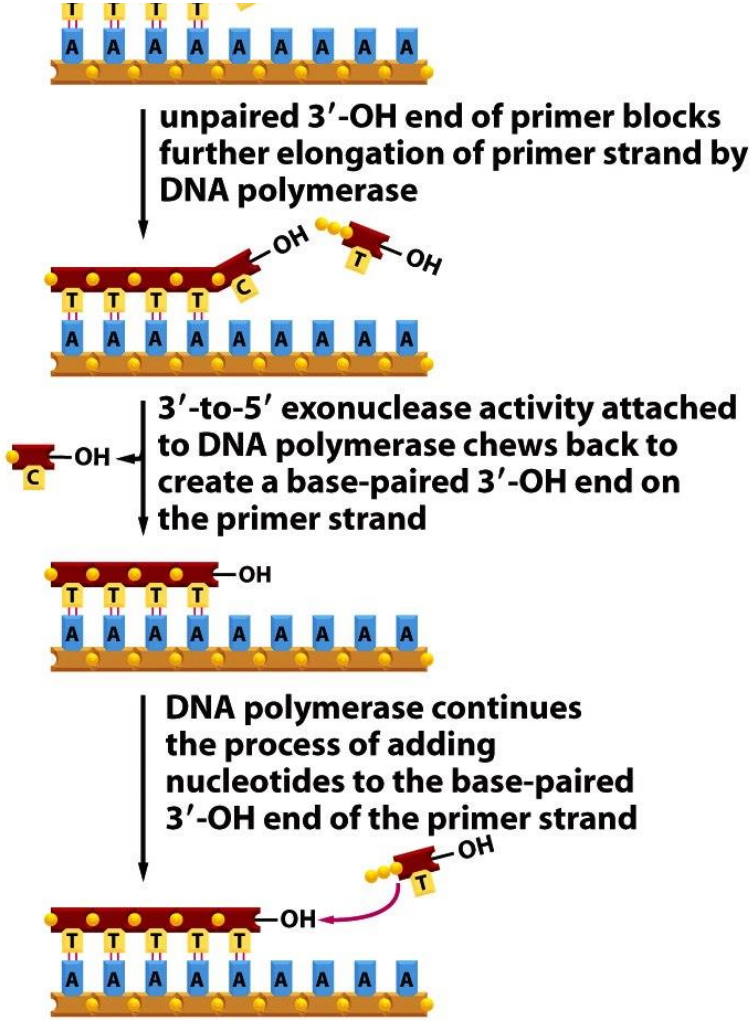


Figure 5-8 (part 2 of 2) *Molecular Biology of the Cell* (© Garland Science 2008)

Table 5–1 The Three Steps That Give Rise to High-Fidelity DNA Synthesis

REPLICATION STEP	ERRORS PER NUCLEOTIDE
5′ → 3′ polymerization	1 in 10^5
3′ → 5′ exonucleolytic proofreading	1 in 10^2
Strand-directed mismatch repair	1 in 10^2
Combined	1 in 10^9

The third step, strand-directed mismatch repair, is described later in this chapter.