

Charles C. Tseng · Xiaoli Yang

Learning Basic Genetics with Interactive Computer Programs

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Preface

Genetics is a basic science, providing the foundation for major biotechnological applications in medicine, agriculture, and other interdisciplinary fields. However, the teaching and learning of genetics have been fraught with difficulty. The abstract nature of genetic concepts, as well as the intricate molecular structures and biochemical processes associated with the concepts, make understanding genetics particularly difficult for beginning students, discouraging them from pursuing degrees in the biological sciences and decreasing educational outcomes in other scientific subjects. There is certainly an urgent need nationwide to improve learning outcomes in basic biology and health science courses with genetics components.

Traditionally, genetics, as are most science courses, is taught with classroom lectures, textbooks, homework assignments, and laboratory exercises. Recently, however, more and more educators are taking advantage of computer technology to enhance the learning experience. For example, nearly all textbooks now are accompanied by a set of CDs/DVDs, which provide students with videos, animations, and other learning tools. To be sure, the quantity of educational materials and tools online for learning genetics is seemingly limitless.

Although all these tools have their merits, not all are equally valuable. Animations with special effects, while entertaining, have been shown to have limited value in cognition. And hands-on programs, although valuable, are ineffective without cognitive involvement.

So what is the best way to enhance learning? To borrow the wise words of the great philosopher and educator Confucius (450 B.C): “Tell me, and I will forget. Show me, and I may remember. Involve me, and I will understand.” To involve students more effectively, we need the three “on’s”: “eyes-on” to read and assimilate information, “minds-on” to think and reason, and “hands-on” to turn knowledge into action.

It is with these three “on’s” in mind that we developed the interactive computer program for learning basic genetics. The program consists of 11 interconnected modules that span the general principles of transmission genetics to the molecular processes in gene expression. It is important to note, however, that this program is not a substitute for a textbook, nor is it a substitute for classroom instruction. Rather,

the program is designed to be a supplementary tool for learning basic concepts, serving as a tutor to guide students.

The learning modules are the result of a long-term collaboration between a geneticist (Charles C. Tseng), with decades of teaching experience plus feedback from thousands of students, and a computer engineer (Xiaoli Yang), with a group of able computer engineering students, who tirelessly wrote the code for the modules. In this collaborative project, it is significant that the geneticist had no knowledge of computer science, and the computer engineers had never taken a biology course at the college level. Not surprisingly, at the beginning, it was virtually impossible for the two groups to communicate. At the end, however, the computer engineers gained and enjoyed their new knowledge of genetics, but the geneticist remained computer illiterate, offering a preview of the effectiveness of the program in conveying basic genetic concepts.

We hope that this program serves as an effective supplementary educational tool for many college biology courses (introductory biology, human biology, plant and animal biology), as well as for a core course in genetics. The modules are suitable not only for biology and health science majors, but also for general students or laymen who are interested in broadening their knowledge of the life sciences.

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Information for Using the Book

This book contains two parts.

- Part I is a hard copy, which includes all the contents for learning basic genetics. There are 11 learning modules. Within each module, several major topics require the users' total involvement with the interactive programs.
- Part II contains interactive programs for all the learning modules. These programs, which constitute an integral part of the learning activities, are available at Extras.Springer.com. To use the programs, .Net Framework 2.0, which exists in all Windows-based computers with Windows XP or newer operating systems, is required. For older computers, “Net Frameworks 2.0” can easily be downloaded for free.

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Module 1

Introduction: What Is Genetics?

What Is Genetics?

- Genetics is the study of genes.
- Generally speaking
 1. Genetics explains why we look like our parents in some ways ... and why we may not look like our parents in other ways.
 2. Genetics explains why certain diseases run in families and why one person has black hair and brown eyes while another person has blonde hair and blue eyes.
- Specifically, genetics answers the following questions:
 1. What is a gene made of (genetic material)?
 2. What does the gene do (gene structure and function)?
 3. How is genetic information transmitted from parent to child generation after generation?
 4. How does the genetic material change (variations through mutations and recombination)?

Applications

- Genetics has applications in
 1. Medicine
 2. Agriculture
 3. Forensics
 4. Environment

Can you think of an example for each of the foregoing applications?

- Examples:
 1. Medicine: diagnosing and treating diseases
 2. Agriculture: making crops grow faster and be more productive
 3. Forensics: DNA fingerprinting
 4. Environment: identifying sources of pollution and contamination

Genetic Material

- If genetics is the study of genes, what are genes?
- To answer this question, it helps to know what makes up a gene.
- In other words, we want to know what the genetic “material” is.

Scientific Method

- To identify the genetic material, we employ the scientific method.
- The scientific method is a system for studying natural phenomena, creating new knowledge, and correcting previous knowledge.
- The scientific process consists of the following stages:
 1. Based on an unexplained situation or event, ask a question or formulate a problem.
 2. Make observations and research similar problems.
 3. Construct a hypothesis to explain the situation or problem.
 4. Design and perform experiments to test the hypothesis.
 5. Analyze the results (data) and draw conclusions about the hypothesis.
- We shall go through these stages one at a time.

Stage 1: Question

- Our question is: What is the genetic material?
- We want to figure out why we look like our parents in some ways.
- We want to know why we have the characteristics that we have.
- To look for the genetic material—the material that is responsible for our traits—it is often easier to study simpler organisms such as bacteria.

Stage 2: Observation and Research (*Interactive Program I*)

- In 1928, Frederick Griffith studied two types of a bacterial species (*Streptococcus pneumoniae*): S (smooth) and R (rough) (Step 1).
- The smooth appearance of S bacteria is caused by the presence of a capsule surrounding the bacteria. R bacteria, which lack such a capsule, are rough in appearance.
- Griffith observed that S bacteria always produced S bacteria while R bacteria always produced R bacteria: the trait is hereditary!
- He also observed that mice died when infected with S bacteria but were unharmed when infected with R bacteria (another genetic trait).

[Use Interactive Program I on the software program available at Extras. Springer.com to familiarize yourself with the following experiment.](#)

[After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “Summary of Observations.”](#)

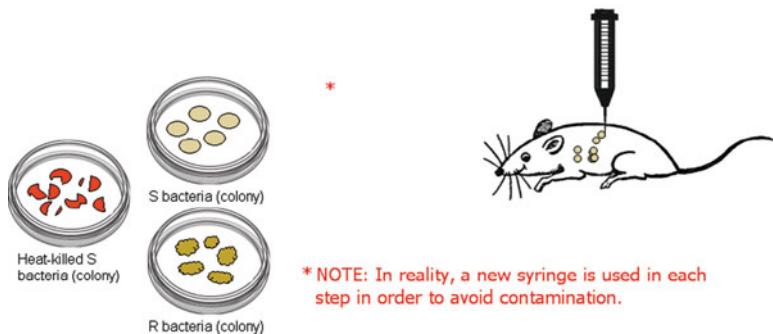
- On the left (Step 1) are plates of three types of bacteria: living S (smooth) bacteria, living R (rough) bacteria, and heat-killed S bacteria.
- In the middle is a syringe. On the right is a mouse.



Step 1 Layout: Griffith's experiment

- Click once on the syringe (do not keep holding the computer “mouse” button down), and then drag the syringe to the S bacteria. Scroll up on the computer “mouse” wheel to fill the syringe. Drag the syringe to the on-screen mouse (the one with whiskers), and scroll down on the computer “mouse” wheel to empty the syringe (Steps 1–3).

- What happens to the mouse?



Step 2 Action: Injection of S bacteria into mouse

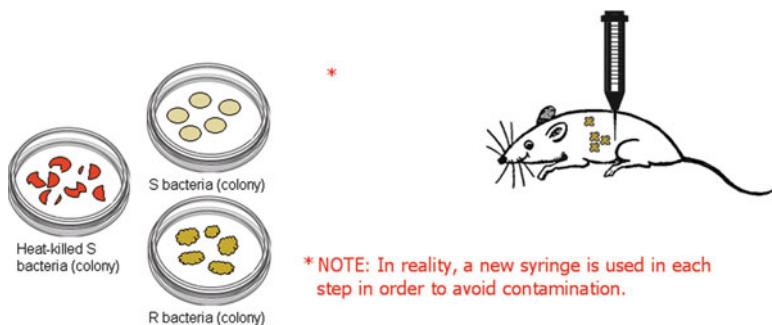


Step 3 Results: Dead mouse from infection of S bacteria

- Similarly, inject the mouse with R bacteria (Steps 4–6).
- What happens to the mouse?

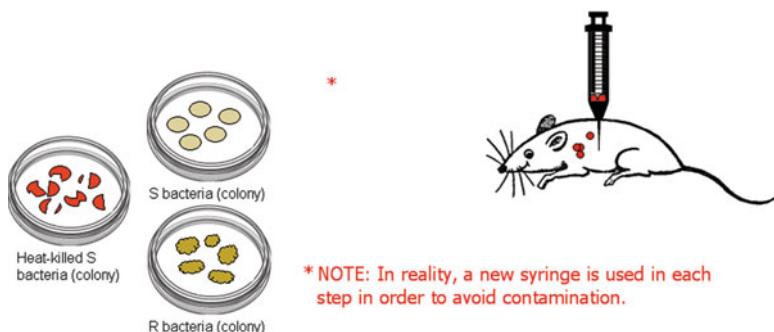


Step 4 Layout: Griffith's experiment (continued)

**Step 5** Action: Injection of R bacteria**Step 6** Results: Live mouse without infection

- Now inject the mouse with heat-killed S bacteria (Steps 7–9).

**Step 7** Layout: Griffith's experiment (continued)



Step 8 Action: Injection of heat-killed S bacteria

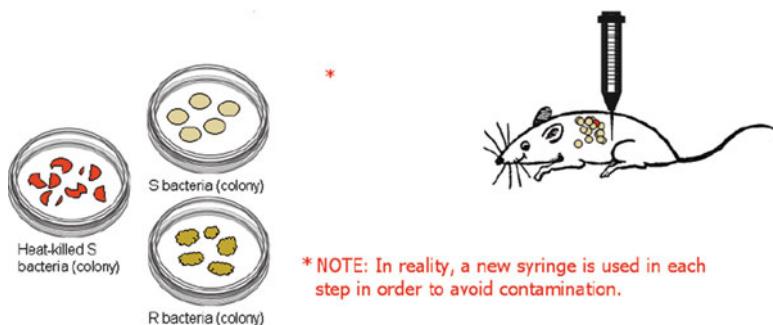


Step 9 Results: Live mouse without infection

- What happens to the mouse?
- Finally, fill the syringe with both the heat-killed S bacteria and the R bacteria before injecting the mixture into the mouse (Steps 10–12).
- What happens to the mouse?



Step 10 Layout: Griffith's experiment (continued)

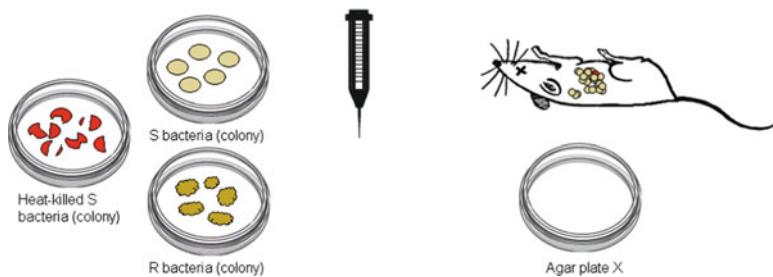


Step 11 Action: Injection with both heat-killed S bacteria and live R bacteria

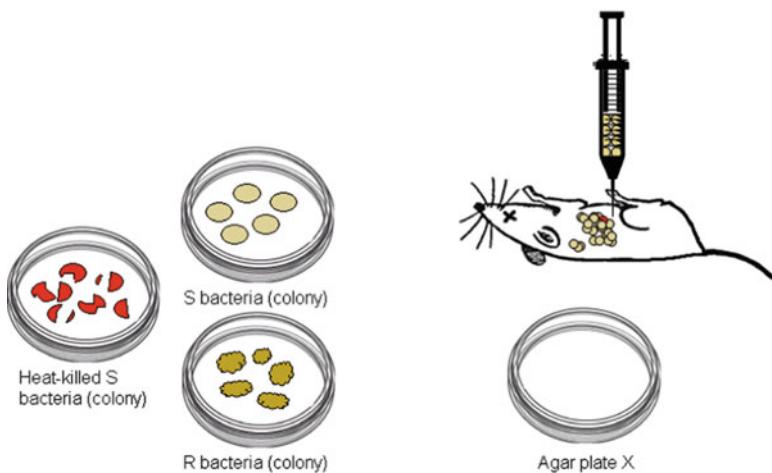


Step 12 Results: Dead mouse from infection

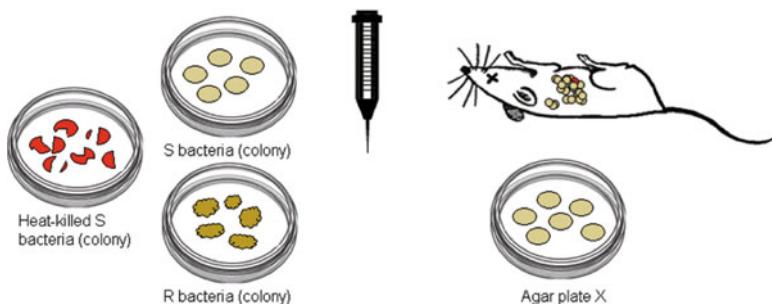
- Let us determine what type of bacteria is in the dead mouse (Steps 13–15).
- Using the syringe, transfer the bacteria from the mouse to the plate (Steps 13–15).*



Step 13 Layout: Griffith's experiment (continued)



Step 14 Action: Transfer of infected bacteria in the mouse to growth medium on agar plate X



Step 15 Results: Growth of S bacteria on agar plate X

- Clearly, there are S bacteria growing on the plate; this means that the live R bacteria have changed into live S bacteria (transformation) after contact with the heat-killed S bacteria.
- Also, the S trait is permanent (hereditary).

Summary of Observations

A summary of the observed results is recorded in Table 1.1.

- What do you think happened?
- Why did the mouse die from a mixture of heat-killed S bacteria and R bacteria, but not from either one of them alone?
- How did R bacteria transform (change) into S bacteria?
- Can you form a hypothesis?

Table 1.1 Observed results from Griffith's experiment

Bacteria	Results
S bacteria	Mouse dies (deadly infection with S bacteria)
R bacteria	Mouse lives (no deadly infection)
Heat-killed S bacteria	Mouse lives (no deadly infection)
Heat-killed S bacteria + R bacteria	Mouse dies (R bacteria change to S bacteria, causing deadly infection)

Stage 3: Hypothesis

- Some material is transferred from heat-killed S bacteria to R bacteria that gives R bacteria the characteristics of S bacteria. The change from R bacteria to S bacteria is called transformation.
- This term means that the material is responsible for making the bacterium smooth and infectious (its traits), and that the material can be transmitted from generation to generation (making it inheritable).
- Anything that is responsible for an organism's traits and can be transmitted to future generations must be the genetic material.

Stage 4: Hypothesis Testing: Avery's Experiment (*Interactive Program 2*)

- Experiments can be used to test a hypothesis.
- An experiment is needed to determine what the genetic material is.
- A scientist named Oswald T. Avery conducted such an experiment in 1944.

[Use Interactive Program 2 on the software program available at Extras. Springer.com to familiarize yourself with the following experiment.](#)

[After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “Stage 5: Results.”](#)

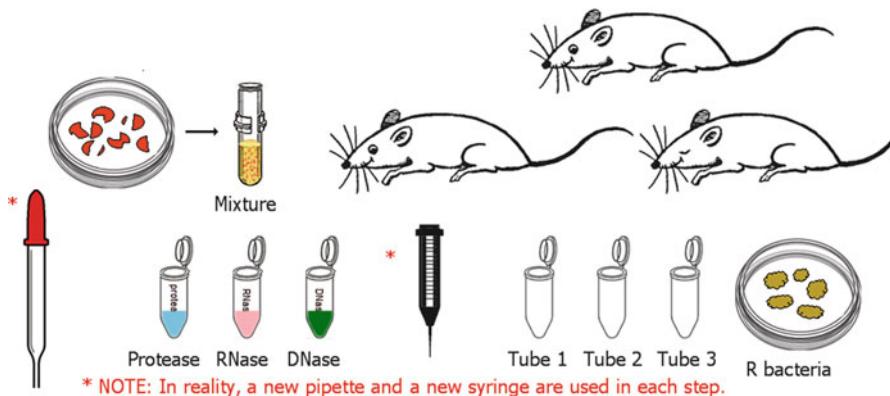
- Oswald T. Avery extracted a mixture of chemicals from S bacteria and found that it contained three types of molecules: (1) proteins, (2) RNA, and (3) DNA. One of the three molecules could be the genetic material. How do we find out?
- Avery then used three different enzymes to destroy the molecules: (1) protease, (2) RNase, and (3) DNase.

Note:

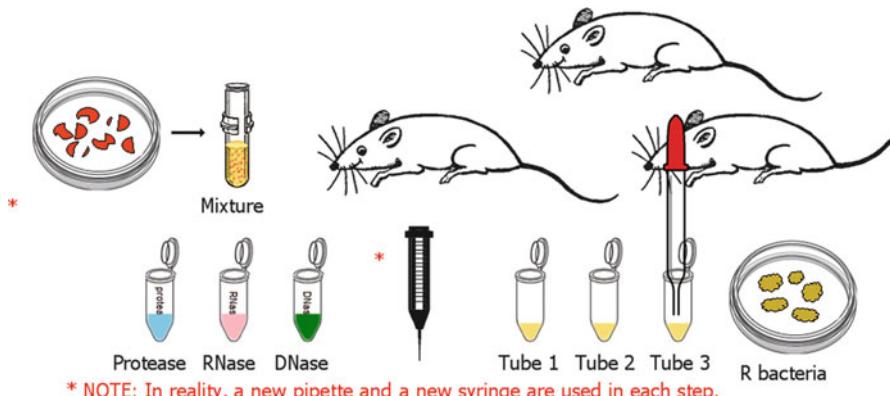
1. Enzymes end with the suffix “-ase.”
2. Enzymes are substances (proteins) that can cause or control chemical reactions.

3. Proteases destroy proteins, RNases destroy RNA, and DNases destroy DNA.

- Examine the following materials from left to right: pipette, plate of heat-killed S bacteria and the extracted chemical mixture, three enzyme tubes, syringe, three mice, three empty tubes, and R bacteria (Step 1).
- *Click once on the pipette and move it to the chemical mixture. Transfer the mixture to the pipette by pressing down on the left mouse button and then releasing it (this squeezes the pipette bulb) (Step 2).*
- *Move the pipette to tube 1 and squeeze the bulb to release the mixture (repeat for tubes 2 and 3) (Step 3).*

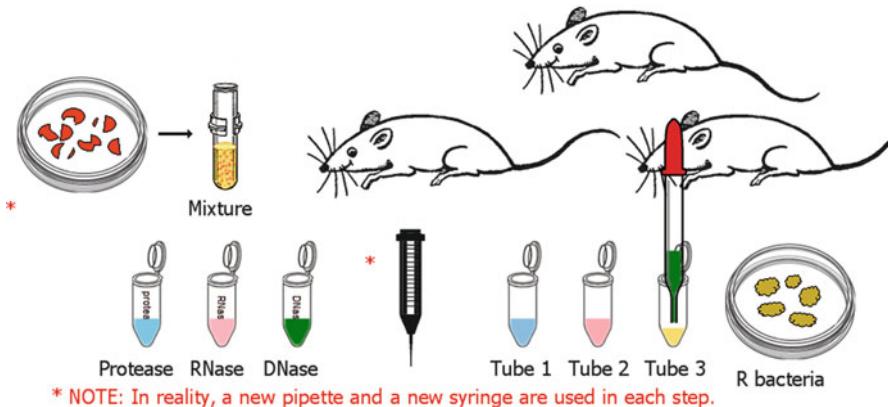


Step 1 Layout: Avery's experiment

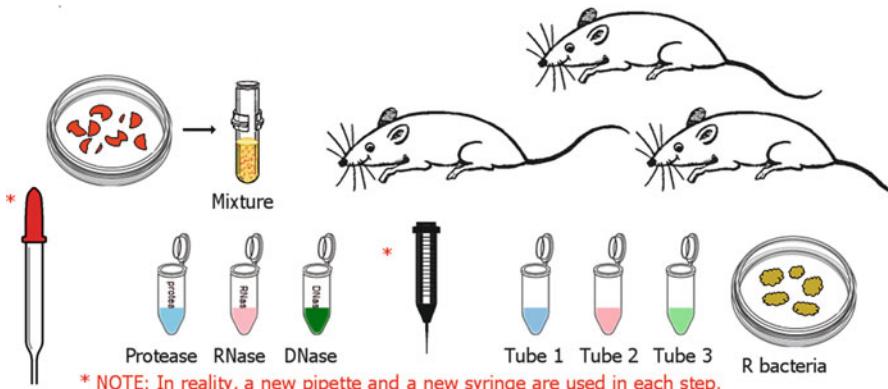


Steps 2–3 Action: Transfer of chemical mixture to tubes 1, 2, and 3

- Use pipette to add protease to tube 1, RNase to tube 2, and DNase to tube 3 (Steps 4–6).

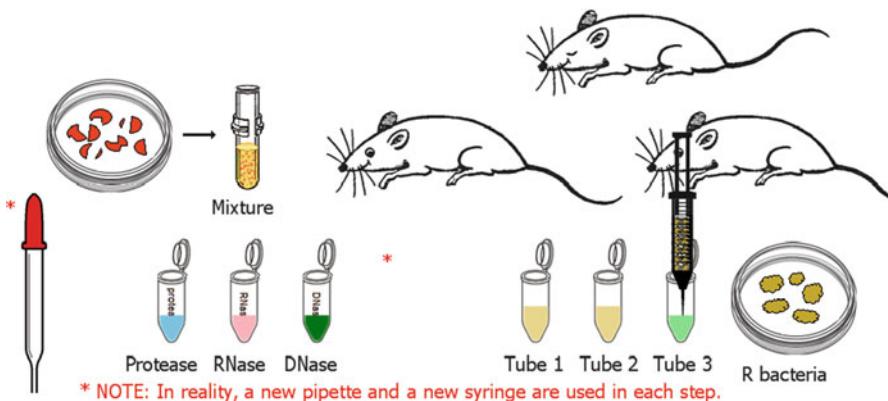


Steps 4–6 Action: Transfer of protease to tube 1, RNase to tube 2, and DNase to tube 3

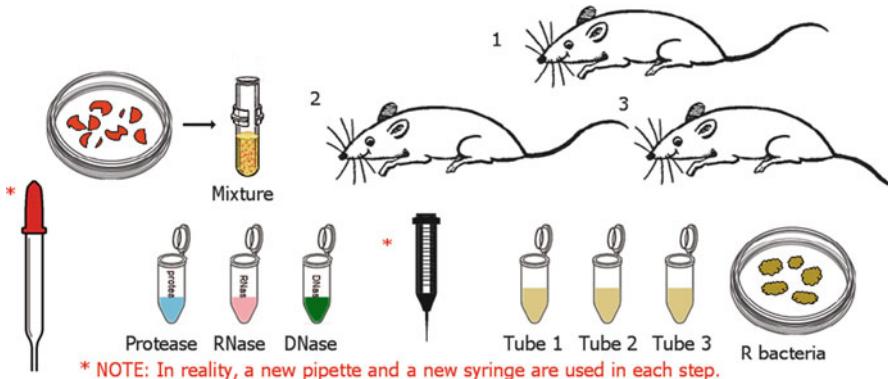


Step 7 Layout for the following steps

- Using the syringe, add R bacteria to each tube (1, 2, and 3) (Steps 7–9).
- Now the proteins in tube 1 are destroyed, RNA in tube 2 is destroyed, and DNA in tube 3 is destroyed (Step 10).

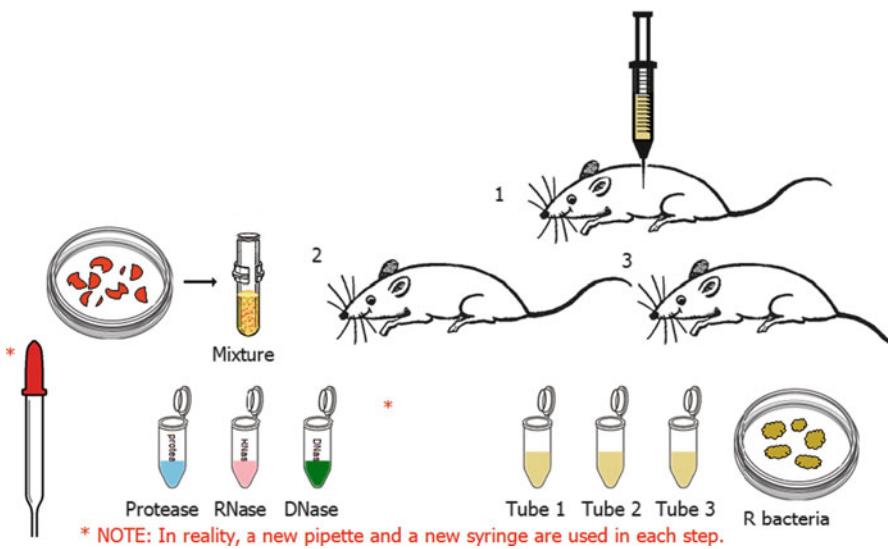


Steps 7–9 Action: Adding R bacteria to tubes 1, 2, and 3

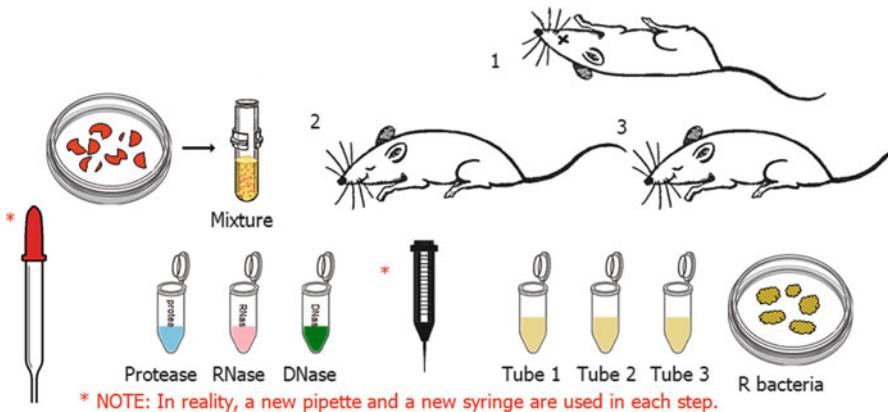


Step 10 Layout for the Steps 11–16

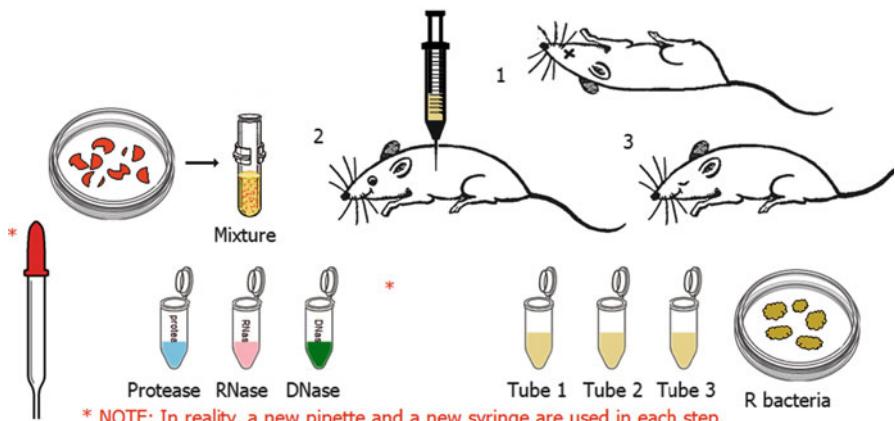
- Inject mouse 1 with the contents of tube 1 (Steps 11 and 12). What happens?
- Inject mouse 2 with the contents of tube 2 (Steps 13 and 14). What happens?
- Inject mouse 3 with the contents of tube 3 (Steps 15 and 16). What happens?



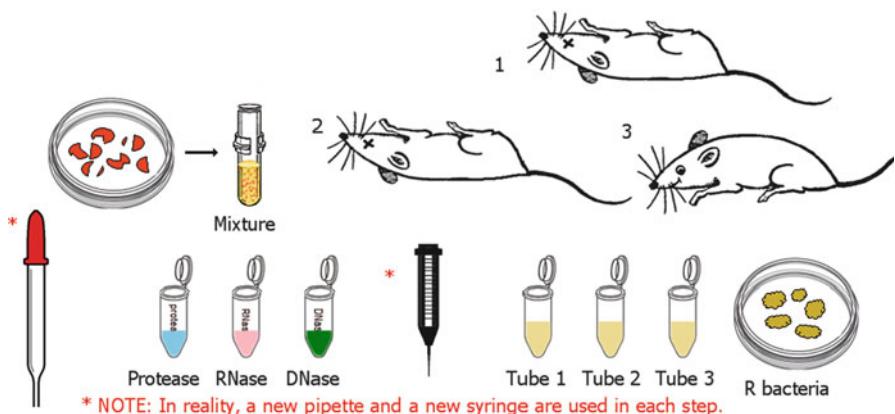
Step 11 Action: Injection of tube 1 content to mouse 1



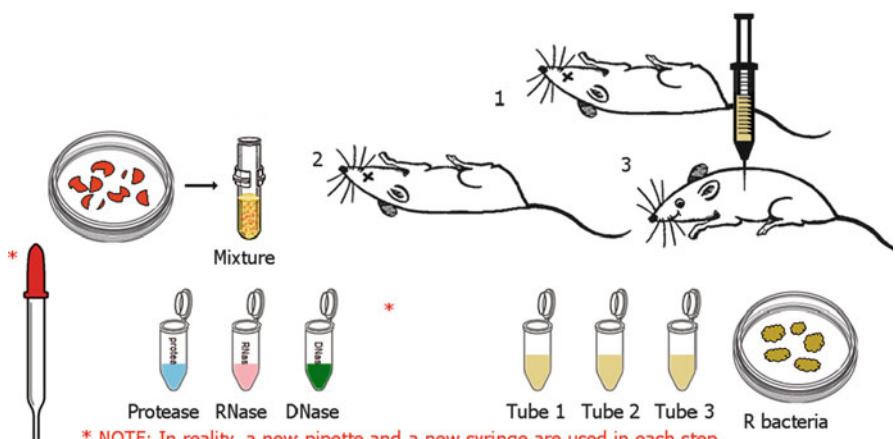
Step 12 Results: Death of mouse 1



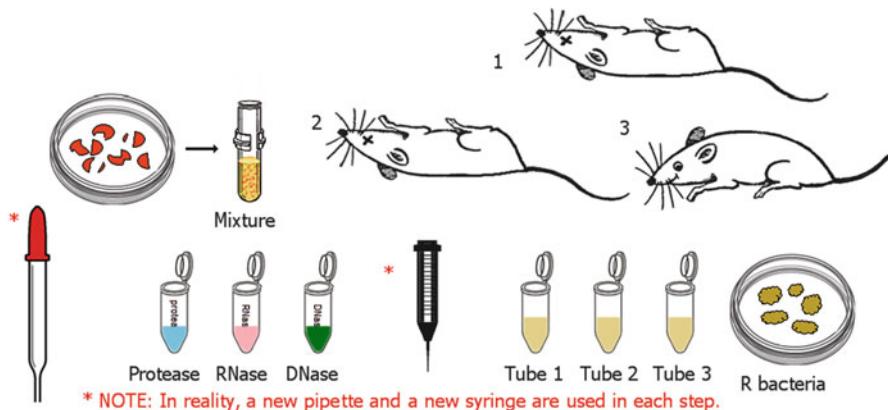
Step 13 Action: Injection of tube 2 content to mouse 2



Step 14 Results: Death of mouse 2



Step 15 Action: Injection of tube 3 content to mouse 3



Step 16 Results: No effect on mouse 3 (no infection)

- Step 16 also shows the final results after three individual injections to these three mice.

Stage 5: Results

A summary of the results is shown below.

Tube	Result
1. Mixture + protease	Mouse 1 dies
2. Mixture + RNase	Mouse 2 dies
3. Mixture + DNase	Mouse 3 lives

- Can you explain these results?
- Why do you think the mouse lived when DNase was added to the mixture?

Data Analysis

- Because the mouse died when the protease mixture was injected, protein cannot be the genetic material (Fig. 1.1). The protease digests all the proteins, but the R bacteria are still able to transform into S bacteria and kill the mouse, so the genetic material must be something other than proteins.
- Because the mouse also died when the RNase mixture was injected, RNA cannot be the genetic material (Fig. 1.2). The RNase digests all the RNA, but the R bacteria are still able to transform into S bacteria and kill the mouse, so the genetic material must be something other than RNA.

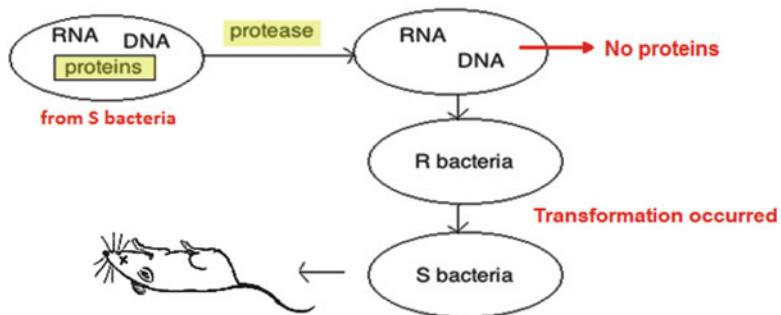


Fig. 1.1 Proteins are not responsible for mouse death (transformation occurs)

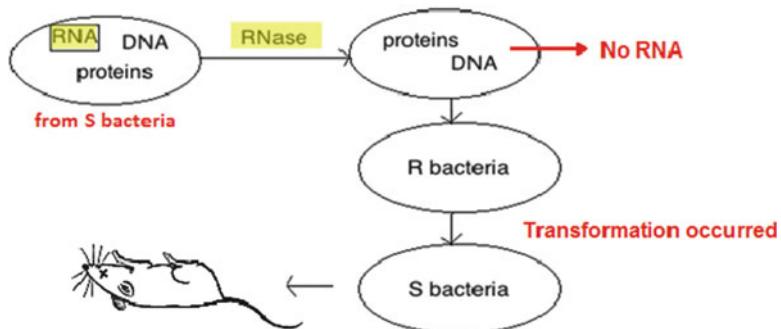


Fig. 1.2 RNA is not responsible for mouse death (transformation occurs)

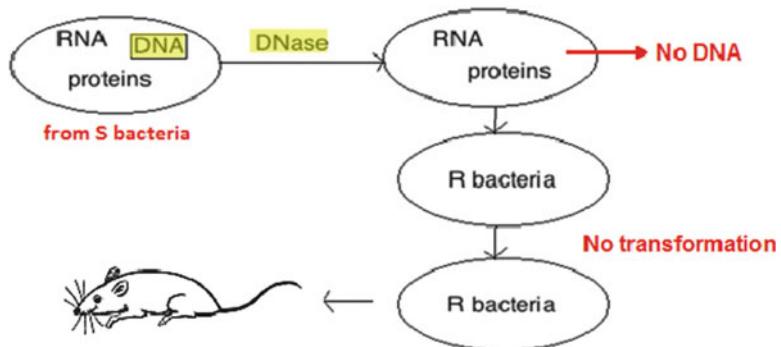


Fig. 1.3 DNA is responsible for mouse death (no transformation occurs when DNA is destroyed)

- Because the mouse lived when the DNase mixture was injected, DNA must be the genetic material (Fig. 1.3). The R bacteria are not able to transform into S bacteria when no specific S bacterial DNA enters the R bacteria. This result confirms that DNA is the genetic material.

Conclusions

- DNA is the genetic material because it is responsible for transforming the R bacteria into S bacteria.
- When the DNA from the S bacteria is destroyed, no transformation occurs.
- Therefore, DNA is the material that determines our characteristics and is heritable (can be passed on to future generations).
- Now we know that DNA is why we look like our parents in some ways.
- To learn more about DNA, let us continue with the following modules.

Quiz

1. The scientific field that focuses on the study of genes is called

- (a) Physiology
- (b) Ecology
- (c) Zoology
- (d) Genetics

2. Genes are made of

- (a) Proteins
- (b) DNA
- (c) RNA
- (d) Enzymes

3. Genetics has applications in

- (a) Medicine
- (b) Agriculture
- (c) Forensics
- (d) Environment
- (e) All the above

4. The steps of the scientific method are ordered as follows:

- (a) Hypothesize, analyze, experiment, question, observe
- (b) Analyze, question, experiment, observe, hypothesize
- (c) Question, observe, hypothesize, experiment, analyze
- (d) Experiment, question, observe, hypothesize, analyze

5. Who performed the first bacterial transformation experiment?

- (a) Frederick Griffith
- (b) Oswald Avery
- (c) James Watson
- (d) Albert Einstein

6. Who did the experiment that proved that DNA is the genetic material?
 - (a) Frederick Griffith
 - (b) Oswald Avery
 - (c) James Watson
 - (d) Albert Einstein
7. The genetic material
 - (a) Is responsible for an organism's traits
 - (b) Can be transmitted to future generations
 - (c) Can be destroyed by proteases
 - (d) Two of the above
 - (e) None of the above
8. Enzymes are proteins that may
 - (a) Catalyze chemical reactions
 - (b) Destroy (digest) proteins
 - (c) Destroy (digest) RNA
 - (d) Destroy (digest) DNA
 - (e) All the above
9. In Avery's experiment, a new pipette and syringe must be used in each step because we want to
 - (a) Control the amount of chemicals used
 - (b) Record the amount of chemicals used
 - (c) Mix the chemicals thoroughly
 - (d) Avoid contamination
10. How did Avery prove that DNA was the genetic material?
 - (a) He showed that protease prevented transformation
 - (b) He showed that DNase prevented transformation
 - (c) He showed that DNase caused transformation
 - (d) He showed that RNase prevented transformation

Answers

1. d 2. b 3. e 4. c 5. a 6. b 7. d 8. e 9. d 10. b

Module 2

DNA Structure: What Is DNA?

The Double Helix of DNA

- DNA stands for deoxyribonucleic acid, a molecule that consists of two long, intertwined polymers forming a structure known as a double helix.
- A polymer is a long molecule made of many identical, or similar, subunits.

Deoxyribonucleotide: Subunit of DNA (*Interactive Program 1*)

- A **nucleotide** is generally composed of a phosphate group, a sugar, and a nitrogenous (nitrogen-containing) base.
- A **deoxyribonucleotide** is a nucleotide that lacks an oxygen atom at the 2' C (number 2 carbon) of the sugar.
- If an oxygen atom is present at the 2' C of the sugar, the nucleotide is called a **ribonucleotide**.
- DNA is composed of deoxyribonucleotides.
- RNA (which is covered in other modules) is composed of **ribonucleotides**.
- Therefore, the DNA nucleotide (Fig. 2.1) is composed of a 5-carbon sugar (called deoxyribose), a phosphate group, and a nitrogenous base (nitrogen-containing structure).
- Note the 5'-carbon (at the top) and the 3'-carbon (at the bottom) of the sugar.
- The phosphate group has the following structure (Fig. 2.2).
- The sugar in a deoxyribonucleotide is called deoxyribose because there is no oxygen (O) atom at the 2' C (Fig. 2.3).

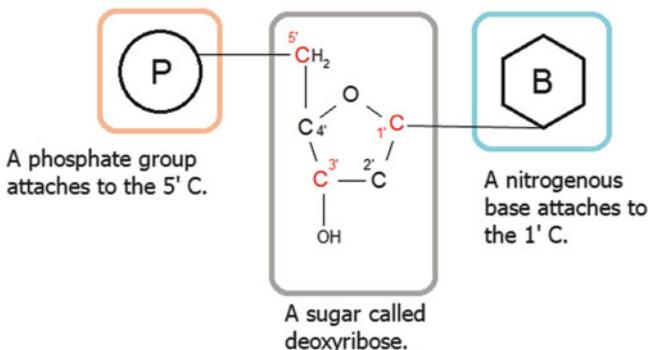


Fig. 2.1 DNA nucleotide (deoxyribonucleotide)

Fig. 2.2 Phosphate

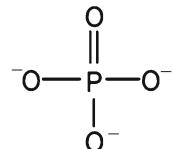
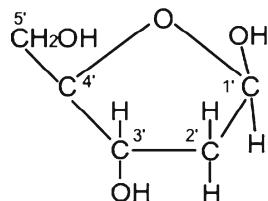


Fig. 2.3 Deoxyribose



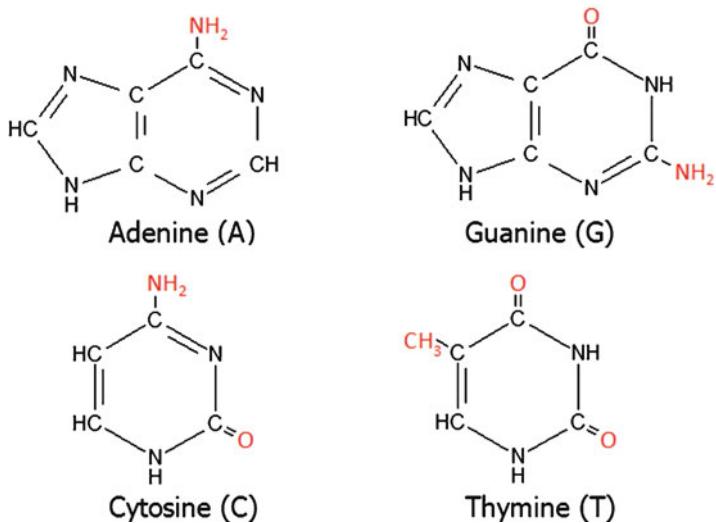
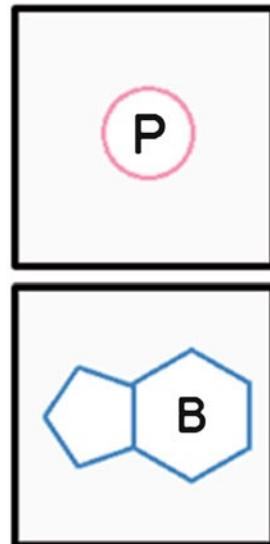
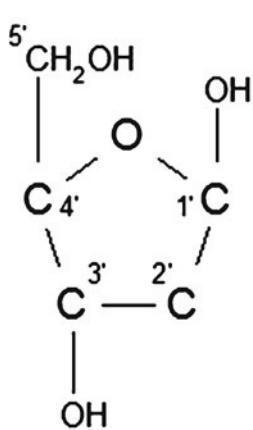
DNA Bases

- There are four types of bases, so there are four types of nucleotides.
- The four bases (Fig. 2.4) are adenine, cytosine, guanine, and thymine.
- Adenine and guanine are called purines because they contain two rings.
- Cytosine and thymine are called pyrimidines because they only contain one ring.

[Use Interactive Program 1 on the software program available at Extras. Springer.com to familiarize yourself with nucleotide structure.](#)

[After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “Polynucleotide: Specific DNA Sequence”](#)

- Drag the phosphate group and the base to the correct carbons on deoxyribose to create a nucleotide (Steps 1 and 2)

**Fig. 2.4** DNA bases

Step 1 Layout: Three separate chemical groups

Step 2 Results: Formation of a deoxyribonucleotide

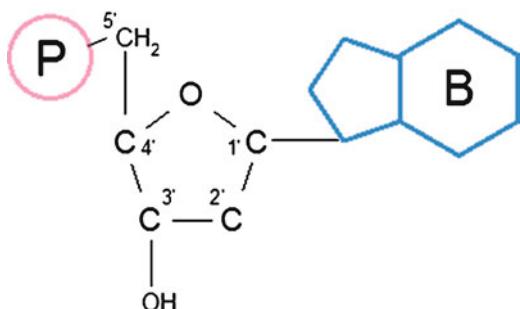
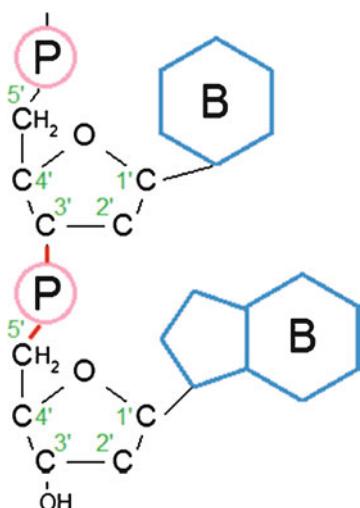


Fig. 2.5 Dinucleotide



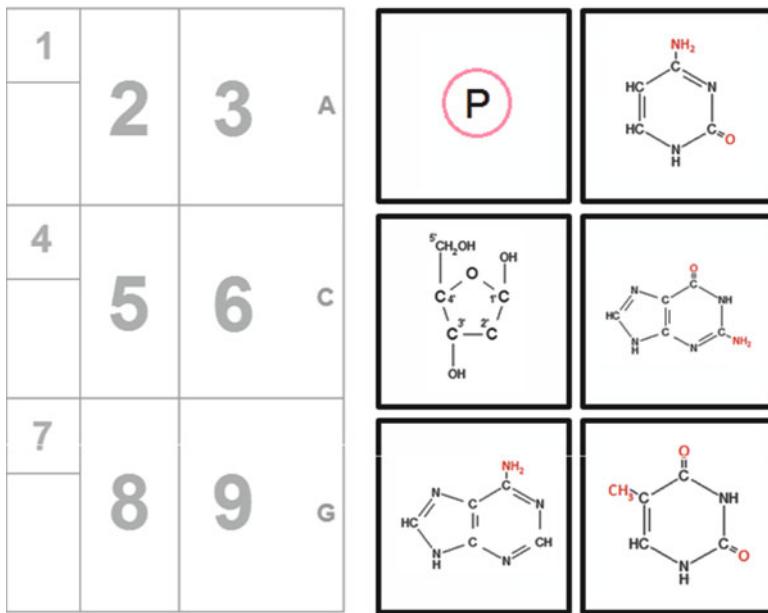
Polynucleotide: Specific DNA Sequence (*Interactive Program 2*)

- Nucleotides are connected by 5'-3' phosphodiester linkages (Fig. 2.5).
- This means that one phosphate group links two sugars by forming two bonds, one to the 3' C of one sugar and the other to the 5' C of another sugar.
- When two nucleotides are joined together, a dinucleotide is formed (Fig. 2.5).
- When more than two nucleotides are joined together, a polynucleotide is formed.
- DNA is a polynucleotide with a sequence of specific bases.

Use Interactive Program 2 on the software program available at Extras. Springer.com to familiarize yourself with DNA base sequence.

After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “DNA structure So Far.”

- Drag the chemical groups on the right to their correct positions on the left to create the nucleotide sequence A-C-G (groups may be used more than once) (Steps 1–4)



Step 1 Layout: Individual chemical groups

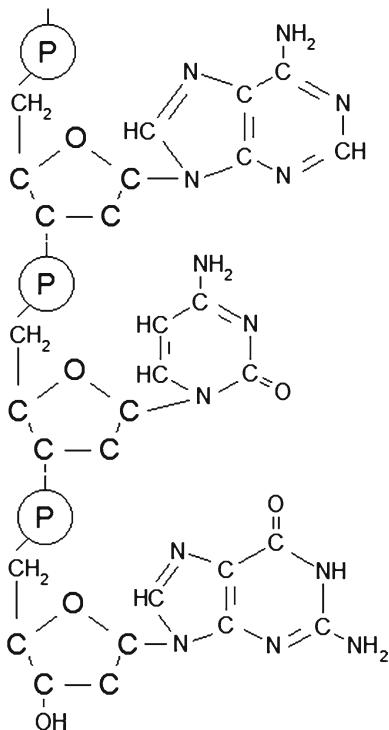


Step 2 Action: Move the phosphate groups to appropriate locations

- Drag the phosphate groups to boxes 1, 4, and 7 (Step 2).
- Drag the sugars to boxes 2, 5, and 8 (Step 3).
- Drag the bases to boxes 3, 6, and 9 (Step 4).
- Hint: Adenine is a two-ringed structure with 1 NH₂. Guanine is also a two-ringed structure with 1 O and 1 NH₂. Cytosine is a one-ringed structure with 1 NH₂ and 1 O. Thymine is also one-ringed structure with 2 O's and 1 CH₃.

P		3	A
P		6	C
P		9	G

Step 3 Action: Move the sugars to appropriate locations



Step 4 Action and Results: Move the bases to appropriate locations to form a trinucleotide (5'-ACG-3")

DNA Structure So Far

- So we now know that
 1. DNA contains four types of nucleotides with four different bases: A, C, G, and T.
 2. The nucleotides are connected linearly (for example, A–A–C–T–G ...).
- What do you think is significant about these two facts?

The Significance of Basic DNA Structure

- These two facts are significant because, taken together, it means that DNA can store information (it forms a **genetic “code”**).
- The nucleotides are similar to the “letters” of the alphabet.
- When the nucleotides are connected together in sequence, they form “words” (information).
- These “words” are further combined to form sentences (detailed information).

Additional DNA Functions

- DNA does more than store information, however.
- The information in DNA is used to create all the traits and functions of a living organism.
- The information in DNA is passed on to future generations.
- We now need to focus on some additional features of the structure of DNA to understand how information in DNA is used and passed on.

Additional Observations of DNA Structure

- The DNA found in living organisms is twice as heavy as a single chain (or length) would indicate.
- X-ray diffraction (which investigates chemical structure) shows that DNA is a helix with a constant diameter of 2 nm (nanometer).
- Measurements show that in DNA the proportion of G is always the same as the proportion of C and the proportion of A is always the same as the proportion of T.

What Kind of Structure Has These Three Properties?

- Think first and check your answer in the following pages.

DNA Structure: The Double Helix (*Interactive Program 3*)

- Because the density (weight in a specified volume) of DNA is twice as heavy as one chain would indicate, DNA must contain two polynucleotide chains (Fig. 2.6).

Pyrimidine–Purine Pairing

- Because diffraction data show that DNA is a double helix with a constant diameter, the two polynucleotide chains must wind around each other such that a purine is always opposite a pyrimidine. **Why must a purine always pair with a pyrimidine?** See Fig. 2.7 for the explanation.

A–T and C–G Pairing

- Chemical analysis shows that in DNA the proportion of G is always the same as the proportion of C and the proportion of A is always the same as the proportion of T. Therefore, G must always be paired with C, and A must always be paired with T: This is called complementary base pairing (Fig. 2.8).

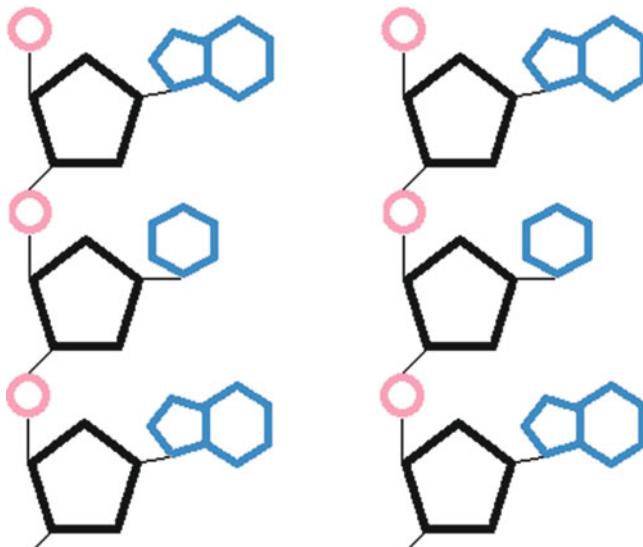


Fig. 2.6 Two polynucleotide chains

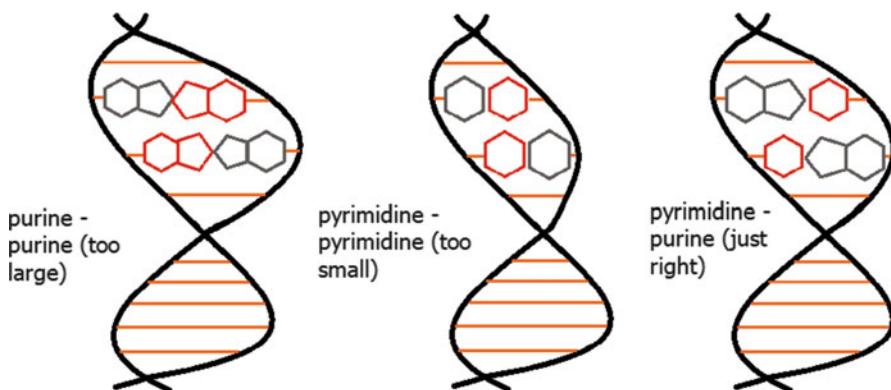
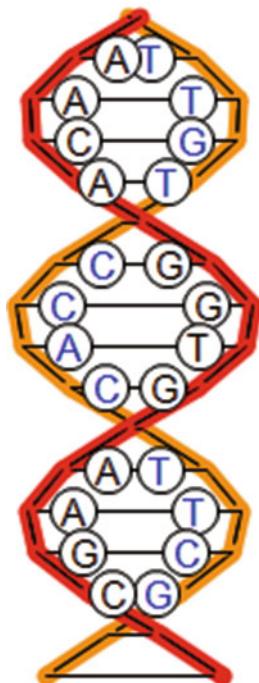


Fig. 2.7 Of the three possible ways to base pair, only the last one (pyrimidine–purine pairing) leads to the right width

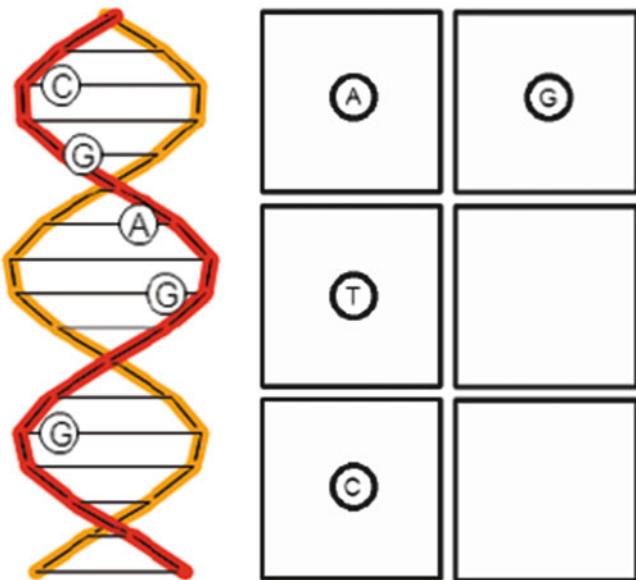
Fig. 2.8 Complementary base paring



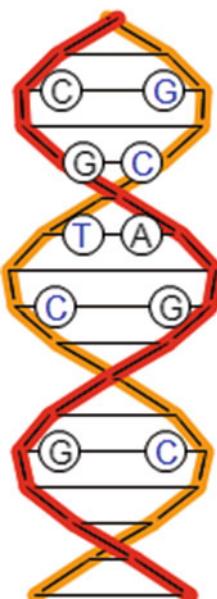
Use Interactive Program 3 on the software program available at Extras. Springer.com to familiarize yourself with DNA base pairing.

After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “Hydrogen Bonds.”

- For practice, drag the appropriate bases to the helix so that there is complementary base pairing (Steps 1 and 2).



Step 1 Layout



Step 2 Results: Pairing of complementary bases

Hydrogen Bonds: The Attractive Force Between Two DNA Strands (*Interactive Program 4*)

- The next question is
- How do the two polynucleotide chains of the DNA double helix stay together?
- Answer:** The two polynucleotide chains of DNA stay together because of hydrogen bonds between the complementary bases.
- A hydrogen bond is an attractive force between a slightly positive hydrogen (H) atom and a slightly negative atom (such as N or O).

Adenine (A) and Thymine (T) Are Connected by Two Hydrogen Bonds (Fig. 2.9)

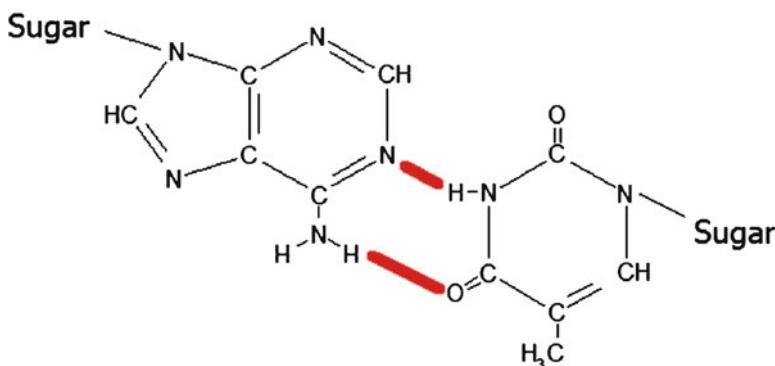


Fig. 2.9 Two hydrogen bonds between adenine and thymine

Cytosine (C) and Guanine (G) Are Connected by Three Hydrogen Bonds (Fig. 2.10)

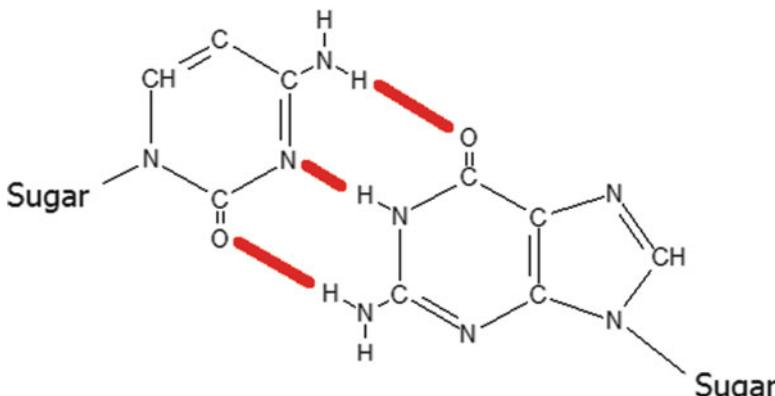


Fig. 2.10 Three hydrogen bonds between cytosine and guanine

Antiparallel Arrangement (Organization)

- For the bases on the two strands to be in the correct positions to form hydrogen bonds, however, the two strands have to run in opposite directions (antiparallel). That is, one strand runs in the 5' C to 3' C direction, while the other strand runs in the 3' C to 5' C direction (Fig. 2.11).

Use Interactive Program 4 on the software program available at Extras. Springer.com to familiarize yourself with hydrogen bonding between bases.

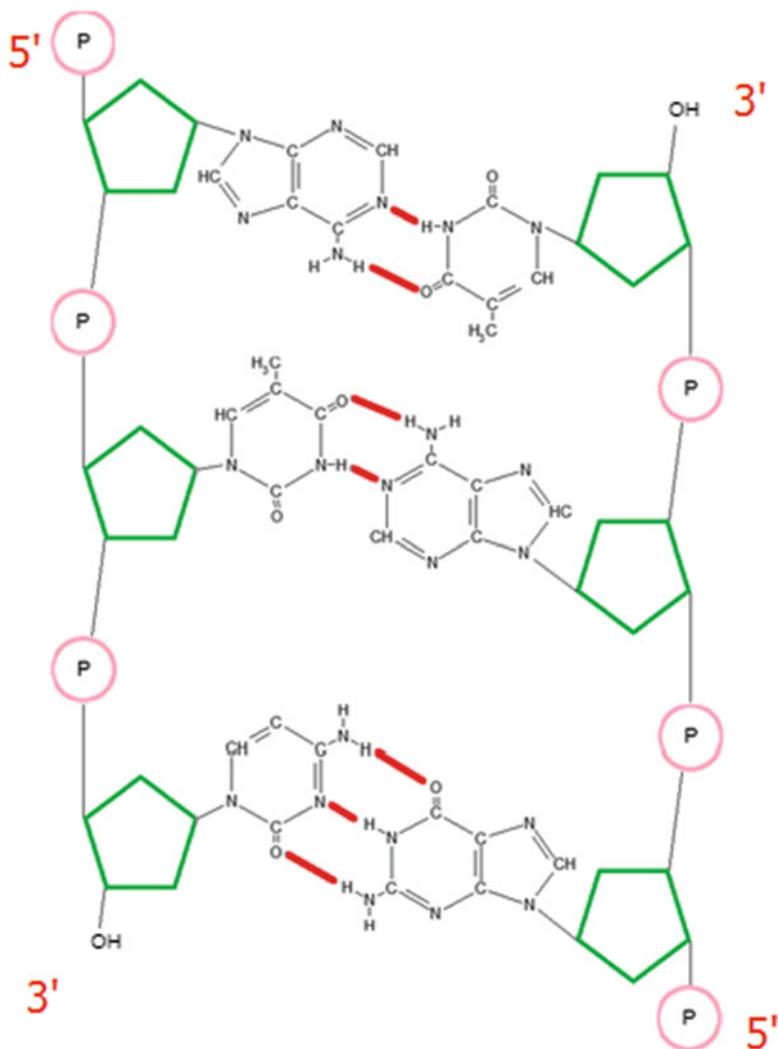
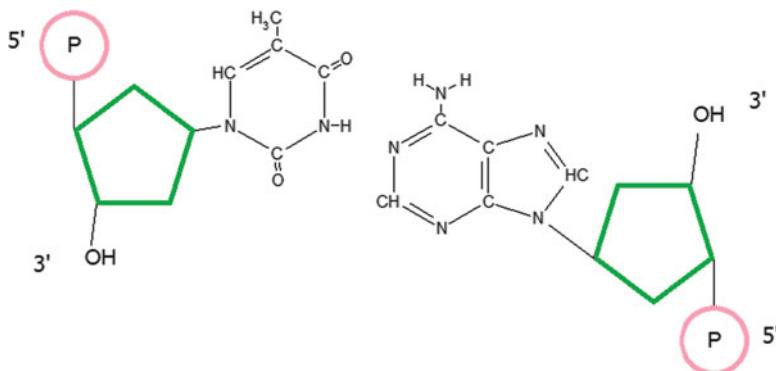


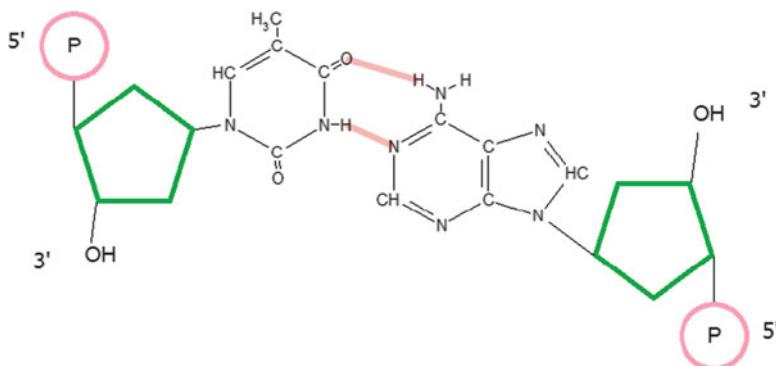
Fig. 2.11 Antiparallel organization of the DNA double helix

After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “What We Have Learned.”

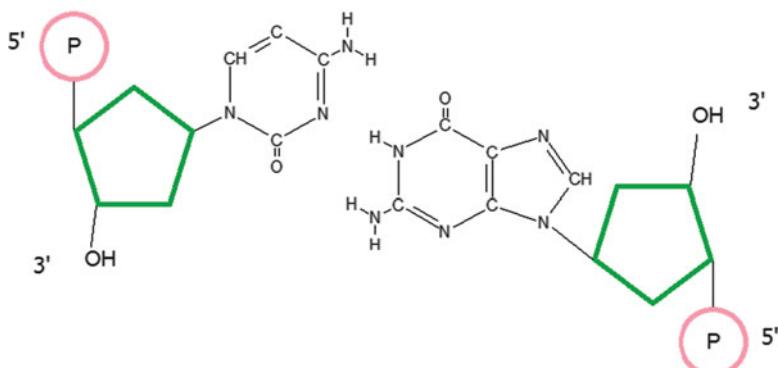
- Click on the two atoms (one at a time) that are hydrogen bonded (Steps 1–4).



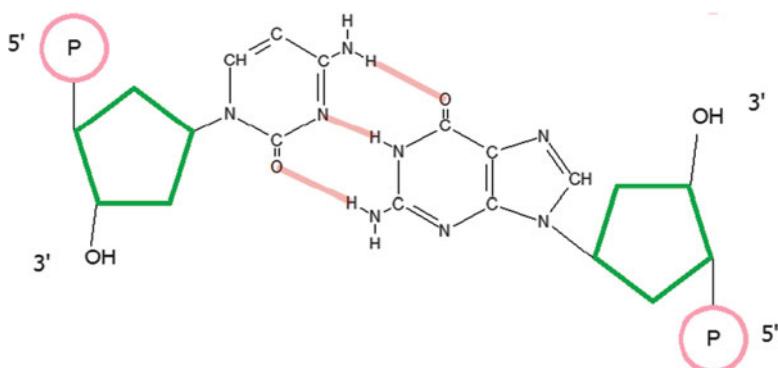
Step 1 Layout



Step 2 Results: Formation of hydrogen bonds



Step 3 Layout



Step 4 Results: Formation of three hydrogen bonds

- How many hydrogen bonds are between these two bases (Step 2)?
- What are these two bases?
- How many hydrogen bonds are between these two bases (Step 4)?
- What are these two bases?

What We Have Learned

- Now we know the following:
 1. DNA is the genetic material.
 2. DNA consists of a sequence of four types of nucleotides, each with a different base (A, C, T, G). These DNA sequences exist as pairs of strands that run in opposite directions in a double helix.
 3. A and T form a base pair through two hydrogen bonds. C and G form another base pair through three hydrogen bonds.

- We still need to know exactly
 1. How DNA stores information (to be learned in this module).
 2. How the information is used (to be learned in a future module).
 3. How the information is passed on to future generations (to be learned in a future module).

How DNA Stores Information

- As we said before, the bases of DNA resemble the letters of the alphabet.

5' AACTAGCGCTCTGGGCCGGATAGGTGCCCGGA 3'

- In DNA, three consecutive bases form a “word,” called a codon.

5' AAC TAG CGC TCT GGG CCG GAT AGG TGC CCC GGA 3'

- A series of codons forms a “sentence,” called a gene.
- Genes are the basic units of inheritance. They are responsible for the synthesis of proteins of all living organisms. These genes are called protein coding genes. Certain genes, however, only code for RNA without ensuing protein synthesis, and those are called non-protein coding genes.
- How do genes provide the information for making all proteins? We shall learn more about the process in Modules 8, 9, and 10.
- In the next several modules, we shall see what happens to DNA when cells divide.

Quiz

1. The subunits of DNA are called

- (a) Amino acids
- (b) Sugars
- (c) Nucleosides
- (d) Nucleotides

2. A DNA nucleotide contains

- (a) A ribose, a phosphate group, and a nitrogenous base
- (b) A deoxyribose, a phosphate group, and an amino acid
- (c) A deoxyribose, a sugar, and a nitrogenous base
- (d) A deoxyribose, a phosphate group, and a nitrogenous base

3. Which of the following are purines?

- (a) Adenine and thymine
- (b) Cytosine and guanine
- (c) Adenine and guanine
- (d) Guanine and thymine

4. If the proportion of guanine in a particular DNA molecule is 30 %, what is the proportion of adenine?
 - (a) 20 %
 - (b) 30 %
 - (c) 40 %
 - (d) 50 %
5. In a double-stranded DNA molecule, if one strand runs in the 5' C to 3' C direction, the other strand must run from
 - (a) 4' C to 2' C
 - (b) 3' C to 6' C
 - (c) 3' C to 5' C
 - (d) 1' C to 5' C
6. The phenomenon in Question 5 is referred to as
 - (a) Parallel organization
 - (b) Unidirectional organization
 - (c) Bidirectional organization
 - (d) Antiparallel organization
7. Which one of the following base pairs is possible in DNA?
 - (a) A and G
 - (b) C and T
 - (c) G and T
 - (d) C and G
8. In DNA, how many hydrogen bonds are between A and T?
 - (a) 1
 - (b) 2
 - (c) 3
 - (d) 4
9. DNA contains genetic information, because DNA nucleotides can be grouped into coding units, each unit coding for an amino acid. These coding units are called
 - (a) Codons
 - (b) Anticodons
 - (c) Nucleotides
 - (d) Polynucleotides

10. Phosphodiester bonds connect

- (a) Two phosphates
- (b) Two nitrogenous bases
- (c) Two sugars
- (d) Two strands of DNA

Answers

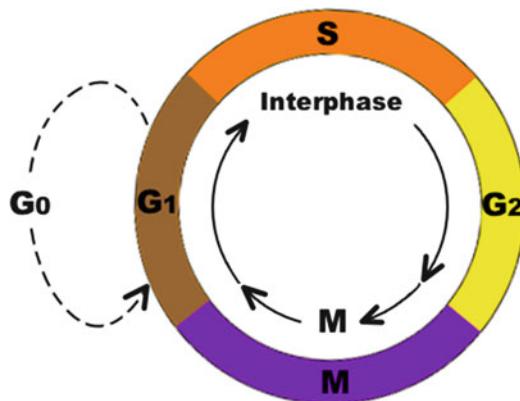
1. b 2. d 3. c 4. a 5. c 6. d 7. d 8. b 9. a 10. c

Module 3

Cell Cycle and DNA Replication: How Does DNA Replicate in Preparation for Cell Division?

What Is the Cell Cycle?

- Cells are structural and functional units of organisms.
- All cells contain DNA, which encodes all the genetic information of an organism.
- The DNA in each cell can be transmitted to future generations, that is, the genetic material is heritable.
- There are different ways to describe and classify organisms. For example:
- **Unicellular organisms versus multicellular organisms:** Unicellular organisms are composed of just one cell, whereas multicellular organisms are composed of many cells.
- **Prokaryotes versus eukaryotes:** Prokaryotes (such as bacteria) lack nuclear membranes and are unicellular. Eukaryotes contain nuclear membranes and are often multicellular organisms, although most protists and certain fungi are unicellular.
- Some unicellular organisms live together, forming colonies, but each cell in the colony is independent, carrying out its own life processes.
- Multicellular organisms contain specialized (differentiated) cells that perform specific functions.
- Cells are mortal: no cell lives forever. After passing through certain growth phases, a cell either dies or divides to form daughter cells, which undergo their own growth and death or division. Each generation of cell growth and division is known as a **cell cycle**.
- **Why is DNA replication necessary?**
- Before cell division, the DNA molecules must be replicated (through a process called DNA synthesis), so that the resulting daughter cells will each have the same DNA content as the original cell.
- In prokaryotes, cell division is called **binary fission**, a process in which the single, circular DNA molecule of most prokaryotic cells is replicated and distributed to the daughter cells.

Fig. 3.1 Cell cycle

- In most eukaryotes, such as animal and plants, the somatic (body) cells are diploid, containing **two sets of chromosomes (two genomes)**, one from each parent.
- The cell cycle of eukaryotes can be divided into four phases: G₁ (Gap 1), S (DNA synthesis), G₂ (Gap 2), and M (mitosis) (Fig. 3.1). The G₁, S, and G₂ phases comprise interphase, during which the chromosomes are stretched out and engaged in various life processes. During the M phase (mitosis), the chromosomes are highly condensed (and thus visible under the light microscope) to allow for their segregation into daughter nuclei.

The Constancy and Dynamic Nature of Genomic DNA During the Cell Cycle

- A diploid cell in the G₁ phase contains two genomes. In humans, each genome is a set of 23 chromosomes from one of the parents. Thus, in diploid organisms, chromosomes come in pairs. The members of each pair are called **homologous chromosomes**; chromosomes from different pairs are called **nonhomologous chromosomes**. In G₁, each human chromosome contains a single DNA molecule. Each human genome, therefore, contains 23 different DNA molecules.
- In the G₁ phase, the chromosomes are in the form of fibers (chromatin) in which the DNA molecules wrap around negative proteins called histones.
- Some sections of the chromatin fiber are about 10 nm in diameter (euchromatin), whereas other sections of the chromatin fiber are 30 nm in diameter (heterochromatin) (Fig. 3.2).
- DNA replication takes place in the S phase. After DNA replication, each chromosome contains two identical copies of DNA: each DNA copy is contained in one of the two structural subunits of a chromosome called the chromatid. Therefore, the number of genomes in the cell is doubled from two genomes in

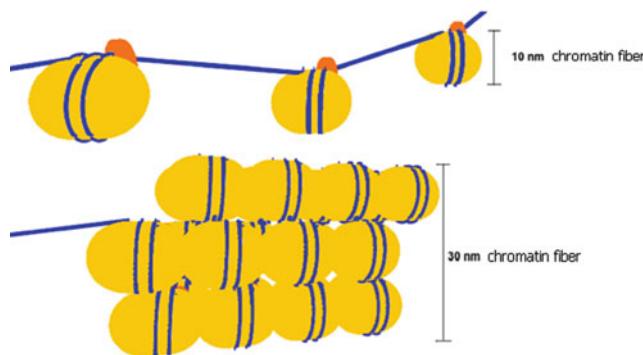


Fig. 3.2 Chromatin: 10- and 30-nm fibers

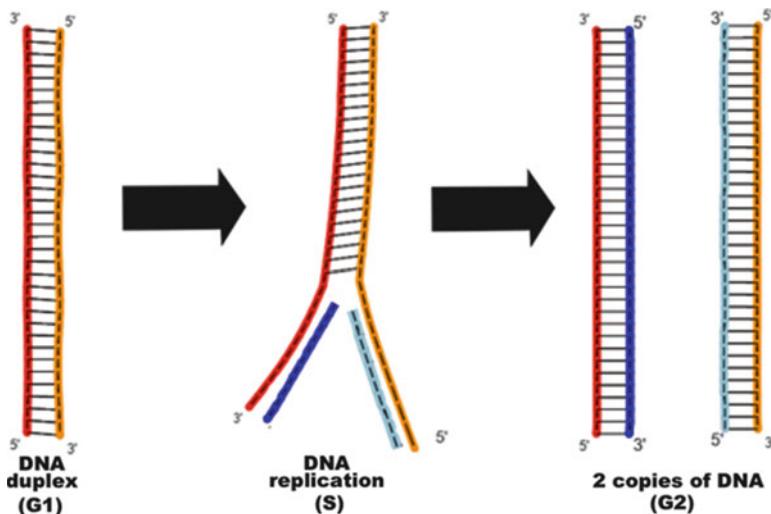


Fig. 3.3 Replication of a DNA duplex to form two sister chromatids, which are connected by a common region called centromere

the G₁ phase to four genomes after the S phase. In other words, after the S phase, each chromosome consists of two sister chromatids (Fig. 3.3).

- The G₂ phase follows the S phase, and the cell continues to contain four genomes. Because DNA replication is semiconservative (reviewed later), after replication, each DNA duplex contains an old strand and a new strand. The two DNA duplexes (Fig. 3.3) eventually become the two sister chromatids visible during mitosis.
- Although human chromosomes in prophase and metaphase often appear to be solitary structures, careful examination of the metaphase chromosomes often reveals the existence of two sister chromatids. The sister chromatids are separated during anaphase by spindle fibers attached to the centromeres, the

region that connects two sister chromatids. Once separated, the chromatids become chromosomes. Decondensation of the chromosomes during telophase, followed by cytokinesis, results in two daughter cells, each with two genomes. (For details, see the module on mitosis).

- After many cell cycles, a fertilized egg (zygote) develops into a mature organism. The cell cycle is controlled at checkpoints, where there are mechanisms in place to ensure that the DNA is faithfully replicated and properly distributed to the daughter cells. A complex array of proteins (e.g., cyclins and cyclin-dependent kinases) maintains the integrity of the DNA during the cell cycle and ushers the cell from one phase to the next.
- During the RP (restriction checkpoint) of late G₁, cells that are destined for differentiation (most human cells) enter the G₀ phase. The cells in the G₀ phase may later return to the G₁ phase (e.g., liver cells) for limited divisions, or they may become permanently differentiated (e.g., blood or nerve cells).

Regulation of the Cell Cycle

- Because DNA replication involves the synthesis of polynucleotides with millions and billions of nucleotides, errors are inevitable. Three mechanisms are employed at the checkpoints to deal with damaged DNA: (1) a sensor/repair mechanism that detects and repairs the damaged DNA, (2) a signaling mechanism that prolongs the cell-cycle phase until the repair process is completed, and (3) an effector mechanism that initiates apoptosis (programmed cell death) in the event that the damaged DNA is beyond repair. Failure of any of these mechanisms may lead to uncontrolled cell growth (cancer).

The General Process of DNA Replication

- DNA replication is a complex process involving many steps, each step controlled by numerous proteins/enzymes. The following model of DNA replication is based on the model developed for the *Escherichia (E.) coli* bacterium.
- **Essential enzymes/proteins for DNA replication:** Let us first familiarize ourselves with some important enzymes and proteins in the process.
 1. **Initiation protein:** The initiation protein initiates the separation of the two strands of the double helix.
 2. **Topoisomerase I and II:** These two enzymes are used to cut one DNA strand and two DNA strands, respectively, to loosen the tension in the double helix caused by the formation and enlargement of the DNA bubble during strand separation.
 3. **Helicase:** Helicase is an enzyme that separates the dsDNA (double-stranded DNA), creating ssDNA (single-stranded DNA), after the initial DNA bubble

is formed. Helicase is a hexamer (composed of six units) forming a donut-shaped structure that often encloses the lagging strand (discussed later) template.

4. **Single-strand binding proteins (SSBs):** SSBs are proteins that bind to the newly separated strands of DNA to prevent renaturation (reformation of double-stranded DNA).
5. **RNA primase:** RNA primase is an enzyme that catalyzes the synthesis of a short RNA strand called the RNA primer. You will soon learn that DNA polymerase (the enzyme that catalyzes DNA synthesis) cannot synthesize a new DNA strand without a preexisting strand serving as a primer.
6. **DNA polymerase III:** DNA polymerase III is the principal enzyme used for DNA synthesis in *E. coli*.
7. **RNase H:** RNase H is an enzyme that removes the RNA primer, creating a gap that is subsequently filled through DNA synthesis.
8. **DNA exonuclease:** DNA exonuclease is an enzyme that removes the last RNA nucleotide (the nucleotide RNase H cannot remove).
9. **DNA polymerase I:** DNA polymerase I is an enzyme that synthesizes a new DNA fragment to fill the gap created by the removal of the RNA primer.
10. **DNA ligase:** DNA ligase is an enzyme that seals the nick between the DNA fragment synthesized by DNA polymerase III and the fragment synthesized by DNA polymerase I. DNA ligase seals the break in the DNA backbone by forming a phosphodiester bond between the 3'-hydroxyl end of one nucleotide with the 5'-phosphate end of another nucleotide.
11. **Sliding (β) clamp loader and sliding (β) clamp:** In DNA synthesis, two DNA polymerase III core enzymes (one for leading strand synthesis, one for lagging strand synthesis) are linked together to form a DNA polymerase III holoenzyme at the replication fork. The two core enzymes are held together by a tau (τ) linker dimer, which is linked to the sliding clamp loader. The sliding clamp loader is able to pick up a protein called “clamp” (β -clamp), a donut-shaped structure that clamps DNA polymerase III to the DNA template for prolonged DNA synthesis.

Origin of DNA Replication (*Interactive Program 1*)

- Origin of replication: DNA replication starts at a specific A–T (adenine and thymine)-rich region that is recognized by the DNA initiator protein (e.g., dnaA). This region is called the origin of replication (Ori).

Use Interactive Program 1 on the software program available at Extras. Springer.com to familiarize yourself with the origin of replication.

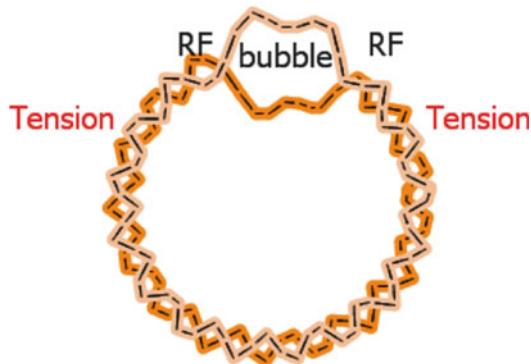
After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “Tension Release.”

- Click on the *Ori* inside the circular DNA (Steps 1 and 2).



Step 1 Layout: Circular DNA to be replicated

- The initiator protein creates a small bubble with two replication forks (RF) (Step 2)



Step 2 Action and Results: Separation of DNA strands causes tension in the remaining parts of the double helix

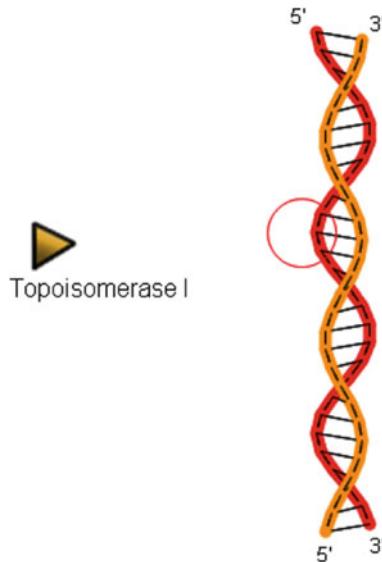
Tension Release (*Interactive Program 2*)

- Because the bacterial DNA is a closed circular structure made up of two intertwined strands (double helix), if the bubble continues to grow, the adjacent double-stranded regions will eventually become so tight (from overtwisting) that further separation of the remaining dsDNA becomes impossible.
- To loosen the tightly coiled structures near the replication forks, the cell uses the enzymes DNA topoisomerase I and II (or gyrase in bacteria).

[Use Interactive Program 2 on the software program available at Extras. Springer.com to familiarize yourself with the origin of replication.](#)

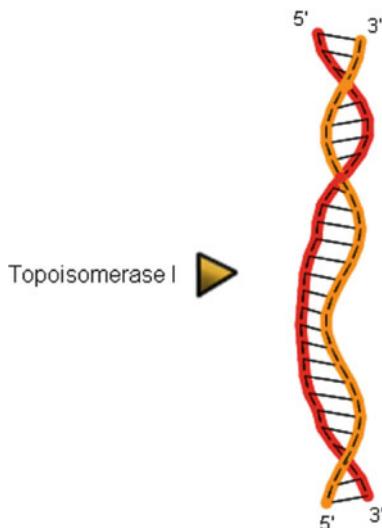
After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “Density Gradient Centrifugation.”

- How does topoisomerase I loosen the overtwisted double helix?
- Drag the enzyme to the region of the DNA indicated by the red circle (Step 3).



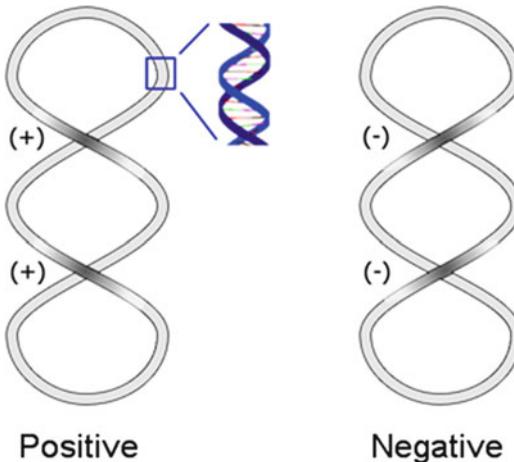
Step 3 Layout: Topoisomerase I cuts a single DNA strand

- *The enzyme cuts one strand, allows the ends to rotate and untwist, and then rejoins the free ends (Step 4).*



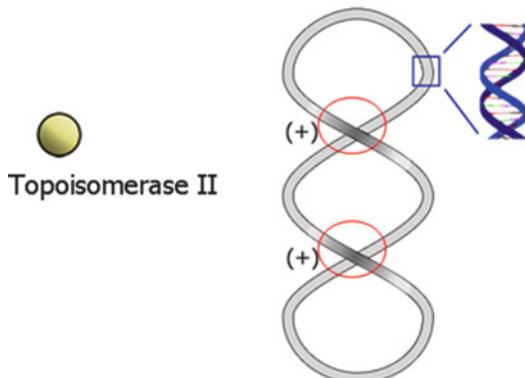
Step 4 Results: Partially untwisted DNA

- Because bacterial DNA is circular, it is frequently supercoiled in nature (either positive (+) or negative (-)) (Step 5).
- Positive supercoiling creates tension in the double helix (tighter), whereas negative supercoiling loosens the double helix.



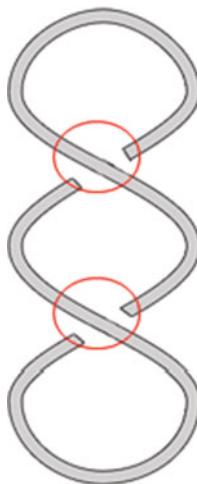
Step 5 Layout: Positively and negatively supercoiled DNA

- How does topoisomerase II (gyrase) release tension? When the double helix gets tighter, the circular DNA becomes positively supercoiled, which prevents DNA strand separation. On the other hand, negatively supercoiled DNA facilitates strand separation. Therefore, to facilitate DNA strand separation, the positive supercoil must be changed to a negative supercoil. To achieve this change, the enzyme topoisomerase II makes a double-stranded cut. The free ends then pass to the other side of the coil and rejoin to form a negative supercoil, facilitating DNA strand separation.
- Drag the enzyme topoisomerase II to the two positively supercoiled regions (red circles) (Steps 6 and 7).*



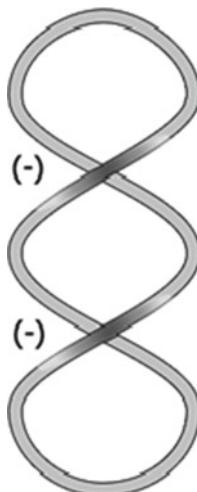
Step 6 Layout: Preparation for the double-stranded cut by topoisomerase II

- Can you see the double-stranded cut (Step 6)?
- *Click inside the red circle to rejoin the ends on the other side of the double helix* (Steps 7 and 8).



Step 7 Action: Broken ends after double-stranded cuts

- Negative supercoiling facilitates the unwinding of the double helix (Step 8).



Step 8 Results: Rejoining of the ends to form a negative supercoil

Density Gradient Centrifugation (*Interactive Program 3*)

- DNA replication is semiconservative because each of the two original DNA strands serves as a template for synthesizing a new strand. Thus, newly replicated DNA contains one old strand and one new strand (Fig. 3.3).
- How do we know that DNA replication is semiconservative?
- In 1958, Matthew Meselson and Franklin Stahl performed the following experiment.
- The experiment takes advantage of the fact that atoms of the same element may have different numbers of neutrons (isotopes).
- The most common isotope of nitrogen is ^{14}N (light nitrogen), but ^{15}N (heavy nitrogen) is also present in the environment in small amounts.
- Because DNA contains nitrogenous bases, all DNA molecules normally contain ^{14}N .
- Meselson and Stahl maintained a culture of *E. coli* in a medium containing only ^{15}N until all the bacterial DNA molecules contained only ^{15}N . We call this **generation 0**.
- They then isolated the DNA and spun it at a high speed in a heavy salt solution of cesium chloride (CsCl) (density gradient ultracentrifugation).
- After centrifugation, the salt solution formed a density gradient (top = low density, bottom = high density), and the DNA settled to form a band in the gradient according to its density. Let us call it band 0, since the DNA came from generation 0. The DNA in band 0 (Fig. 3.4, left) contains two strands with ^{15}N bases ($^{15}\text{N}^{15}\text{N}$).
- The *E. coli* from generation 0 was then transferred to a medium containing ^{14}N . After 25 min (one cell cycle), the bacterial DNA (**generation 1**) was subjected to density gradient ultracentrifugation again. The band (band 1) that was observed this time was slightly higher (less dense) than band 0 (Fig. 3.4, right).
- When the experiment was repeated (growing *E. coli* in ^{14}N medium for 50 min, yielding **generation 2** DNA), two bands were observed: one band had the same density as band 1, whereas the other band (band 2) was less dense (higher in the gradient) (Fig. 3.5, right).

Fig. 3.4 DNA band 0 from *E. coli* generation 0 (left) and band 1 from *E. coli* generation 1 (right)



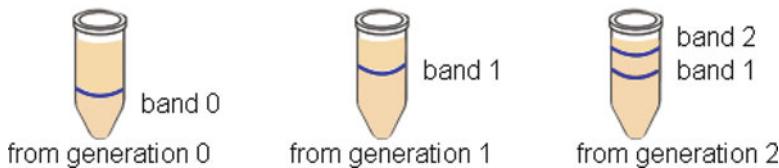


Fig. 3.5 DNA bands 1 and 2 from *E. coli* generation 2 (right) in comparison with band 1 from generation 1 (middle) and band 0 from generation 0 (left)



Fig. 3.6 DNA bands 1 and 2 from *E. coli* generation 3 (right) in comparison with bands from generations 0, 1, and 2 (left three tubes)

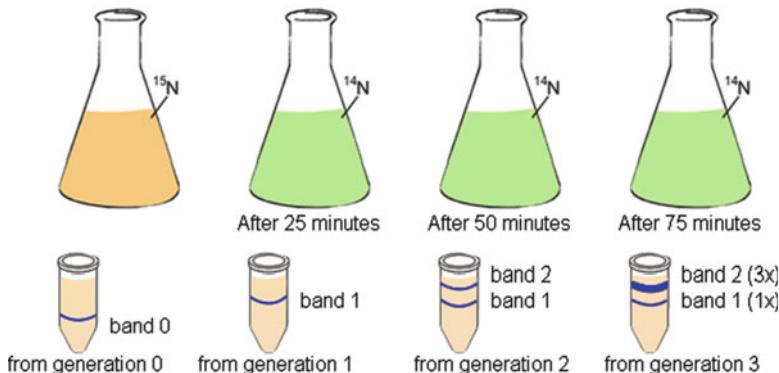


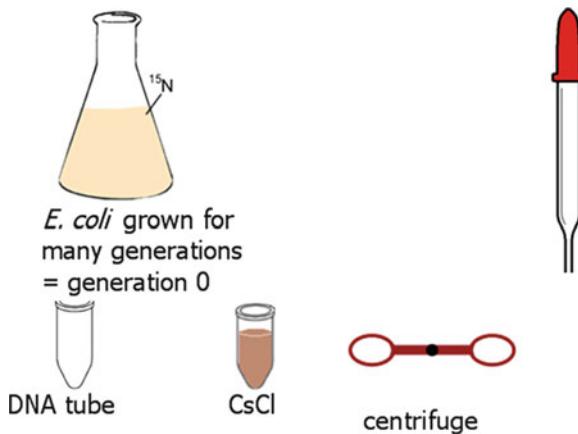
Fig. 3.7 Comparison of DNA bands (position and thickness) from various *E. coli* generations after density gradient centrifugation

- Repeated again (yielding generation 3 DNA by growing *E. coli* in ^{14}N medium for 75 min), the experiment produced the same two bands: band 1 and band 2. However, band 2 was threefold thicker than band 1 (Fig. 3.6, right).

Use Interactive Program 3 on the software program available at Extras.Springer.com to familiarize yourself with the experiment.

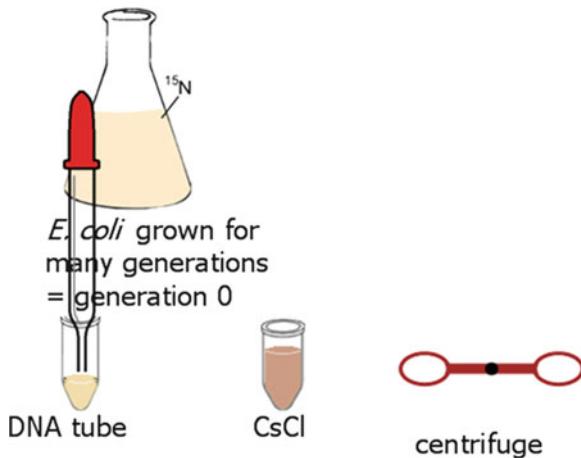
After completing the interactive program, continue reading the following text for further review “Comparison of DNA bands (position and thickness) from various *E. coli* generations after density gradient centrifugation.”

- Let us perform Meselson and Stahl's experiment (Step 1).

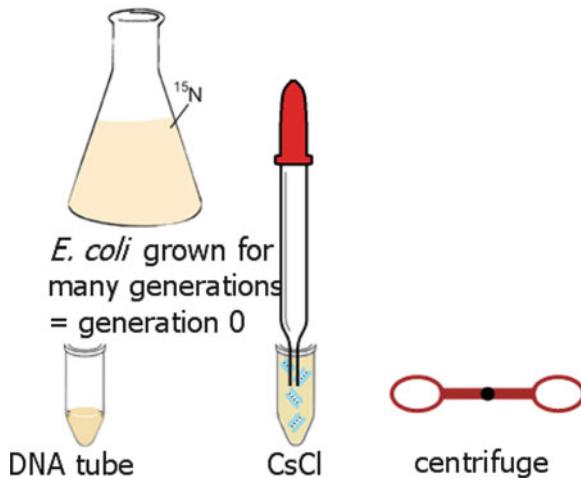


Step 1 Layout: Meselson and Stahl's experiment

- Use the pipette to transfer *E. coli* in generation 0 to the DNA tube (for DNA isolation) (Step 2), and then transfer the DNA to the CsCl tube (Step 3).

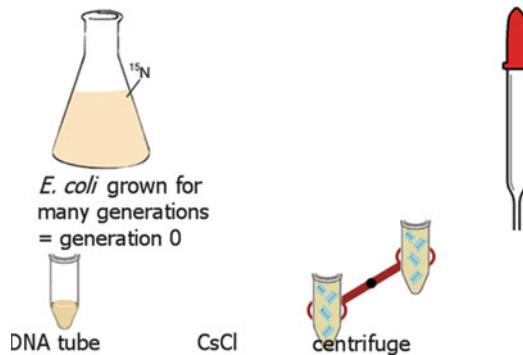


Step 2 Action: Generation 0 DNA isolation

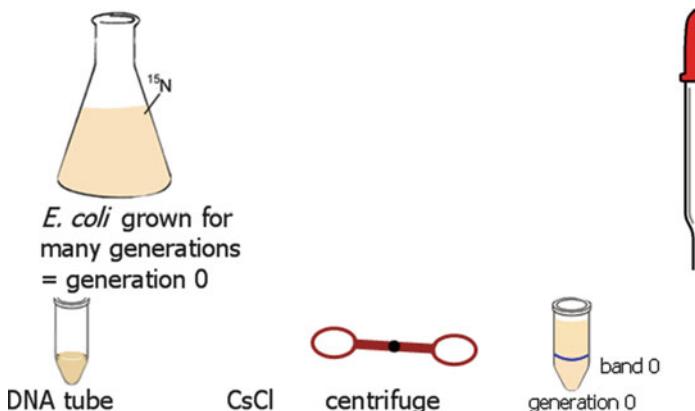


Step 3 Action: Adding generation 0 DNA to CsCl

- Click and drag the CsCl tube to the centrifuge (Step 4). Band 0 can be observed after centrifugation (Step 5).

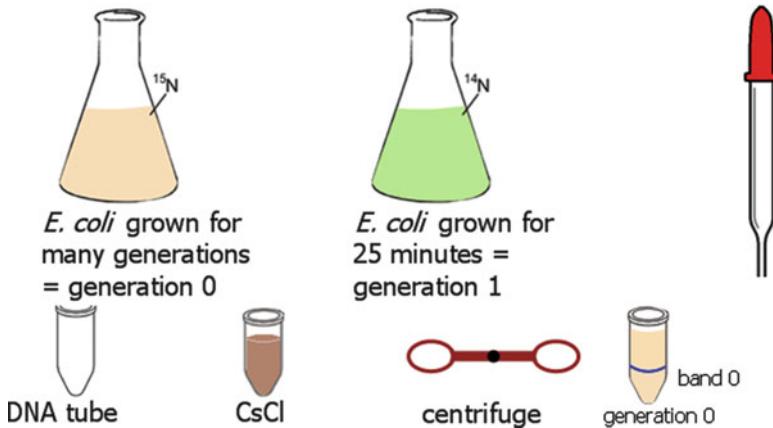


Step 4 Action: Centrifugation

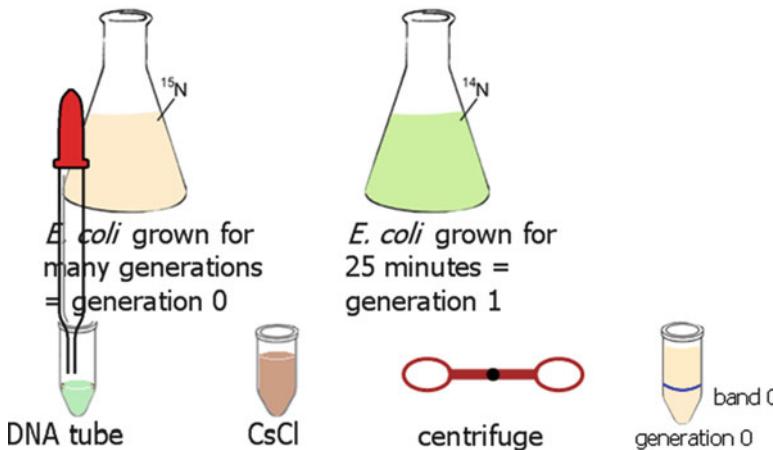


Step 5 Results: Band 0

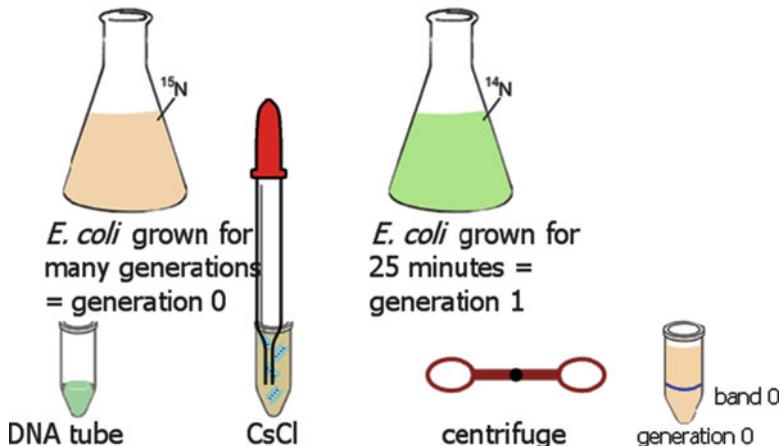
- Repeat the same procedure for generations 1, 2, and 3.
- Use the pipette to transfer *E. coli* at generation 1 to the DNA tube (for DNA isolation), and then transfer the isolated DNA to the CsCl tube for centrifugation to see DNA band 1 (Steps 6–10).



Step 6 Layout

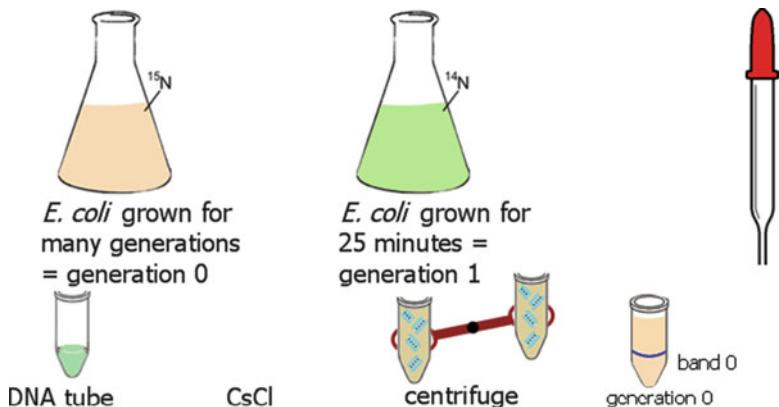


Step 7 Action: Generation 1 DNA isolation

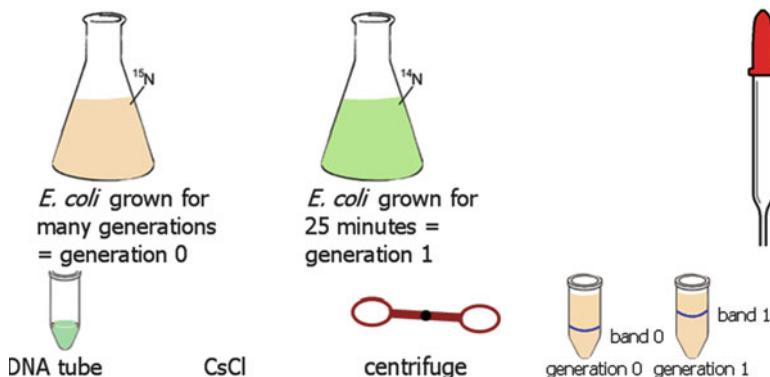


Step 8 Action: Add generation 1 DNA to CsCl

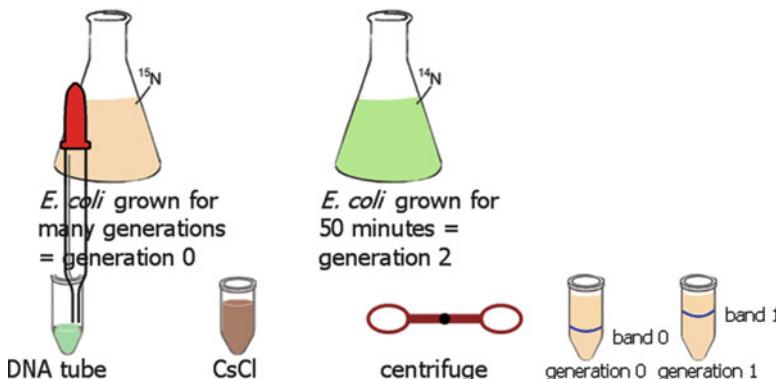
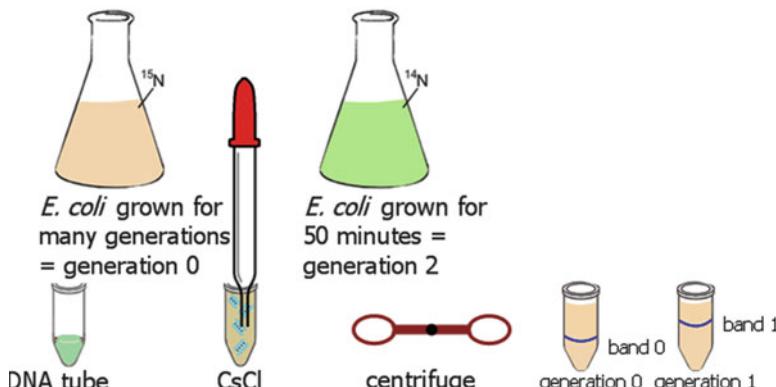
- Click and drag the CsCl tube to the centrifuge. Band 1 can be observed after centrifugation.

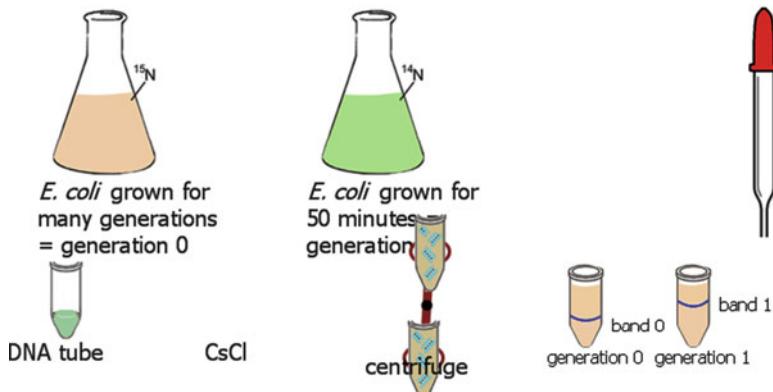
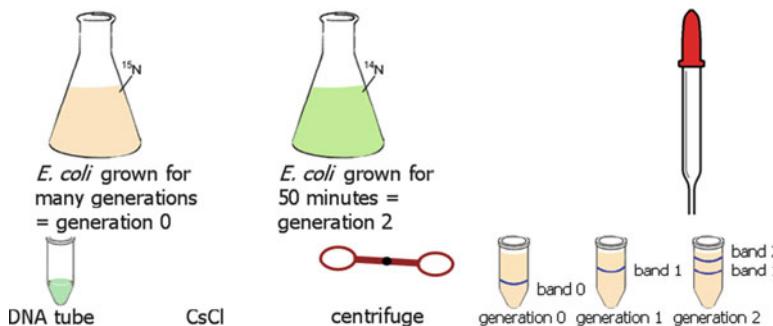


Step 9 Action: Centrifugation

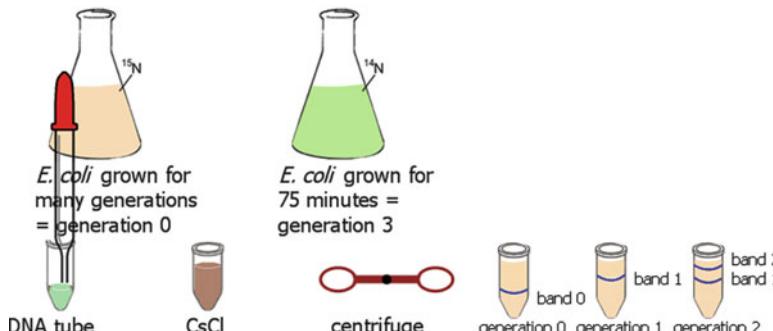
**Step 10** Results: DNA band 1

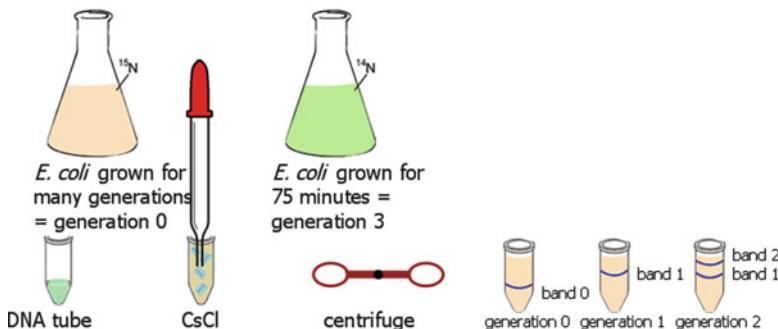
- Repeat the same procedure for generations 1, 2, and 3 (continued).
- Use the pipette to transfer *E. coli* at generation 2 to the DNA tube (for DNA isolation), and then transfer the isolated DNA to the CsCl tube for centrifugation to see DNA bands 1 and 2 (Steps 11–14).

**Step 11** Action: Generation 2 DNA isolation**Step 12** Action: Add generation 2 DNA to CsCl

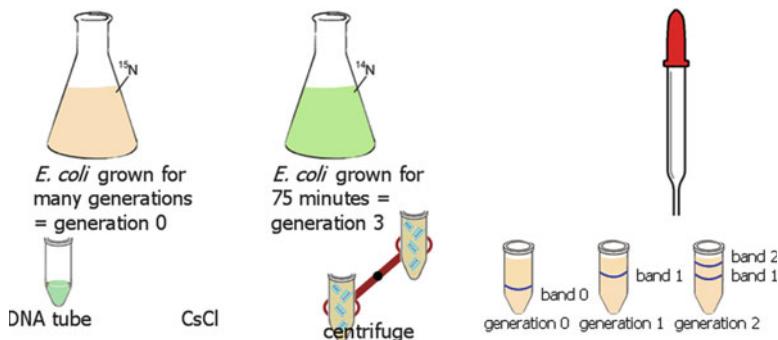
**Step 13** Action: Centrifugation**Step 14** Results: DNA bands 1 and 2 from *E. coli* generation 2

- Repeat the same procedure for generations 1, 2, and 3 (continued).
- Use the pipette to transfer *E. coli* at generation 3 to the DNA tube (for DNA isolation), and then transfer the isolated DNA to the CsCl tube for centrifugation to see DNA bands 1 and 2 (Steps 15–18).

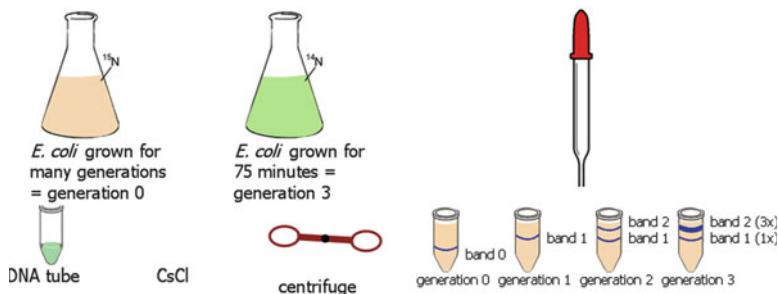
**Step 15** Action: Generation 3 DNA isolation



Step 16 Action: Add generation 3 DNA to CsCl



Step 17 Action: Centrifugation



Step 18 Results: DNA bands 1 and 2 from generation 3 in comparison with bands from previous generations

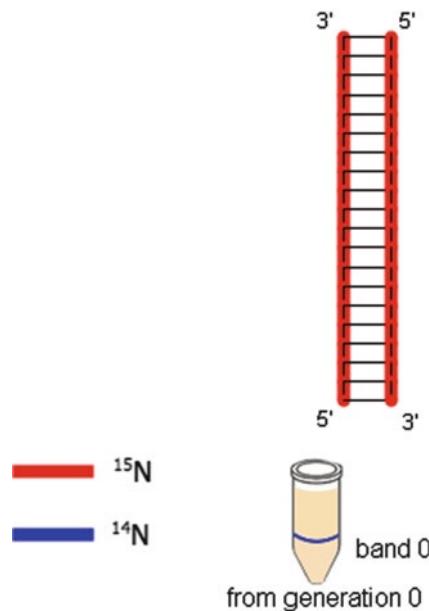
- The density gradient centrifugation experiment (Steps 1–18) is also summarized in (Fig. 3.7).
- On the basis of the foregoing results, what conclusions can you reach?
- These results provide convincing evidence that DNA replication is **semiconservative**. Can you see why?

Semiconservative DNA Replication (*Interactive Program 4*)

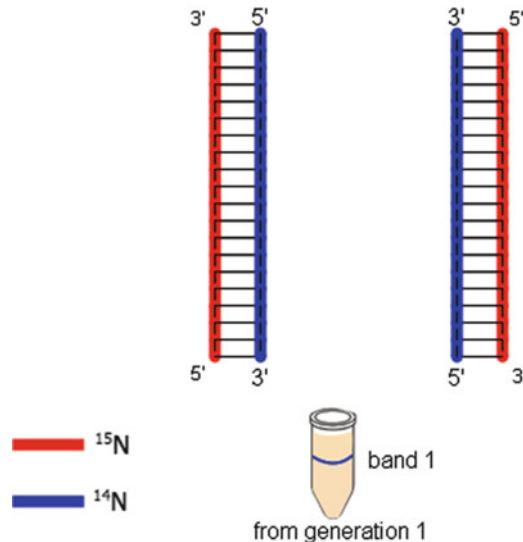
Use *Interactive Program 4* on the software program available at Extras.Springer.com to familiarize yourself with the experiment.

After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “Detailed Process of DNA Replication.”

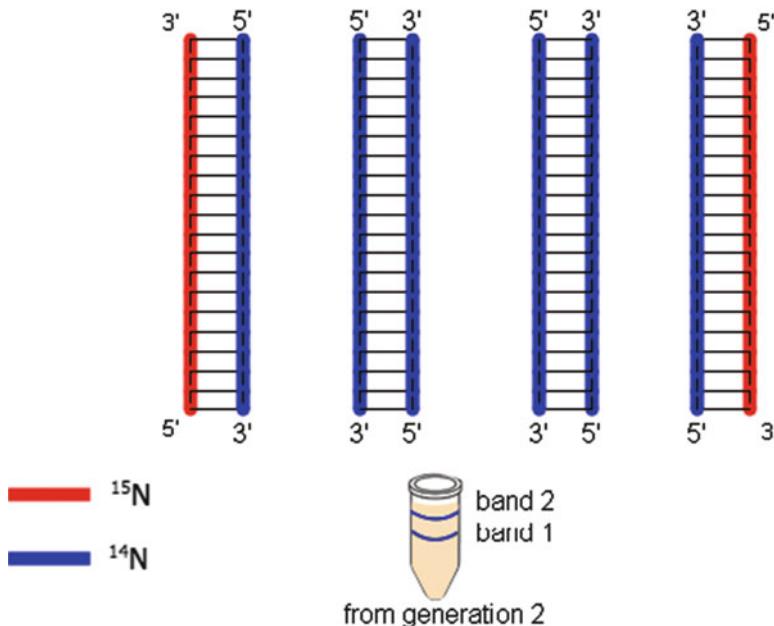
- The following semiconservative model of DNA replication (Steps 1–4) will explain the *E. coli* DNA band patterns after density gradient ultracentrifugation.
- Click to see an original DNA duplex ($^{15}\text{N}^{15}\text{N}$) isolated from generation 0, which forms band 0 after density gradient centrifugation (Step 1).
- Repeat clicking to see the formation of band 1 from generation 1 (Step 2), bands 1 and 2 from generation 2 (Step 3), and bands 1 and 2 from generation 3 (Step 4).



Step 1 Action: Formation of DNA band 0 ($^{15}\text{N}^{15}\text{N}$)

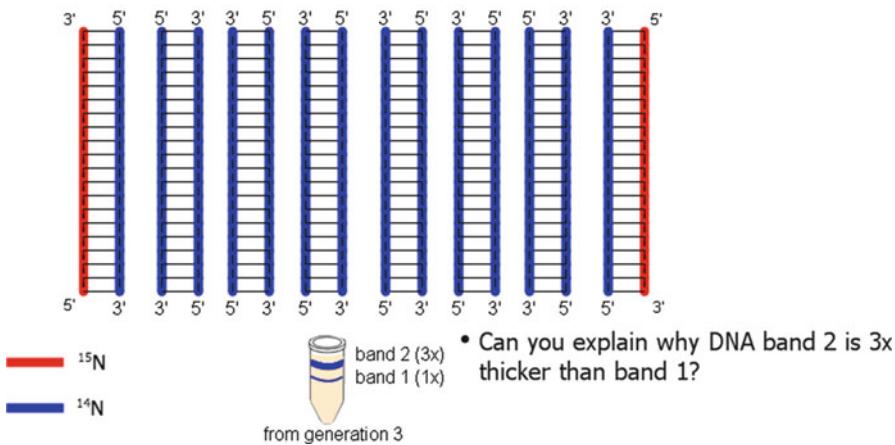


Step 2 Action: DNA band 1 from generation 1 is $^{14}\text{N}^{15}\text{N}$, with the ^{15}N strand being the original template and the ^{14}N strand the newly synthesized strand (semiconservative replication)



Step 3 Action: DNA band 1 is $^{14}\text{N}^{15}\text{N}$ and band 2 is $^{14}\text{N}^{14}\text{N}$ (again, the results of semiconservative replication)

- Can you explain why DNA band 2 is threefold thicker than band 1? See Step 4.



Step 4 Action and results: After one more round of DNA replication, the ratio of $^{14}\text{N}^{14}\text{N}$ DNA to $^{14}\text{N}^{15}\text{N}$ DNA is 6:2 (or 3:1)

Detailed Process of DNA Replication (*Interactive Program 5*)

The Overall View

- We are now convinced that DNA replication is semiconservative.
- Let us look at the overall view followed by the detailed process of DNA replication.

[Use Interactive Program 5 on the software program available at Extras.Springer.com to familiarize yourself with the detailed process of DNA replication.](#)

[After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “Questions That Still Remain.”](#)

- The interactive program will show you the overall process of semiconservative DNA replication.
- A DNA molecule is shown below (Step 1). Note that the two strands of the double helix run in opposite directions (5' to 3' and 3' to 5').
- Thus, the two strands are antiparallel.



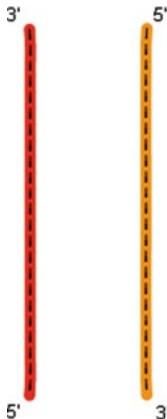
Step 1 Layout: A DNA double helix

- For simplicity, two straight lines are used to represent the double helix (Step 2). Note again that the two strands are antiparallel to each other.



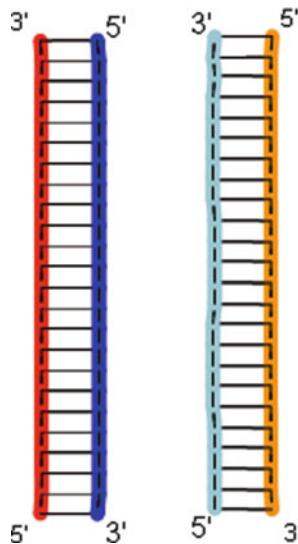
Step 2 Layout: A simplified DNA duplex

- For DNA replication to occur, the double-stranded DNA (dsDNA) must be separated into two single strands (ssDNA), a process called DNA denaturation (Step 3).



Step 3 Action: DNA denaturation

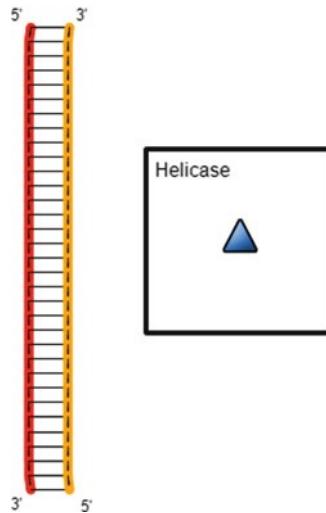
- Each original DNA strand serves as a template for synthesizing a new complementary strand (dark blue and light blue) (Step 4).



Step 4 Action and results: New DNA strands are complementary to old DNA templates

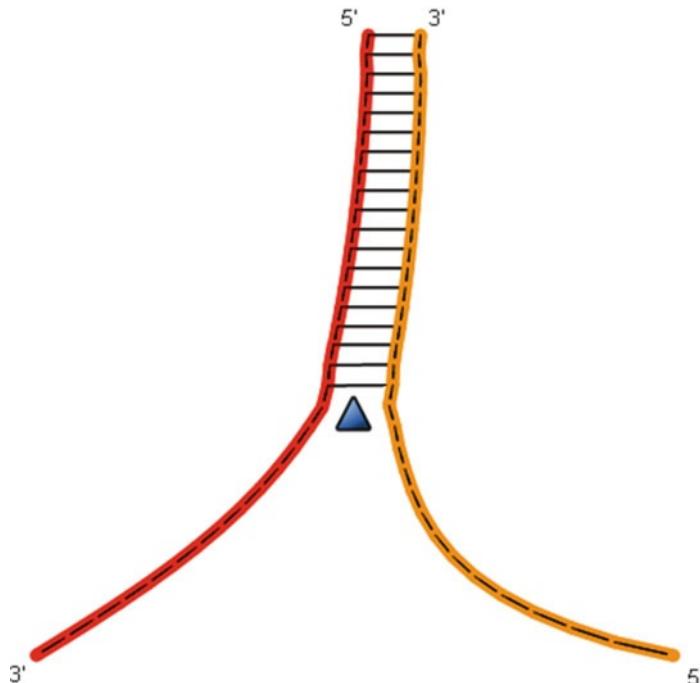
- Next, we examine DNA replication in detail with the enzymes involved.
- In the first step of DNA replication, the enzyme helicase breaks the hydrogen bonds between the complementary bases of the double helix, turning double-stranded DNA into single-stranded DNA.

- Drag the helicase to the bottom of the double helix between the two strands (Steps 1 and 2).



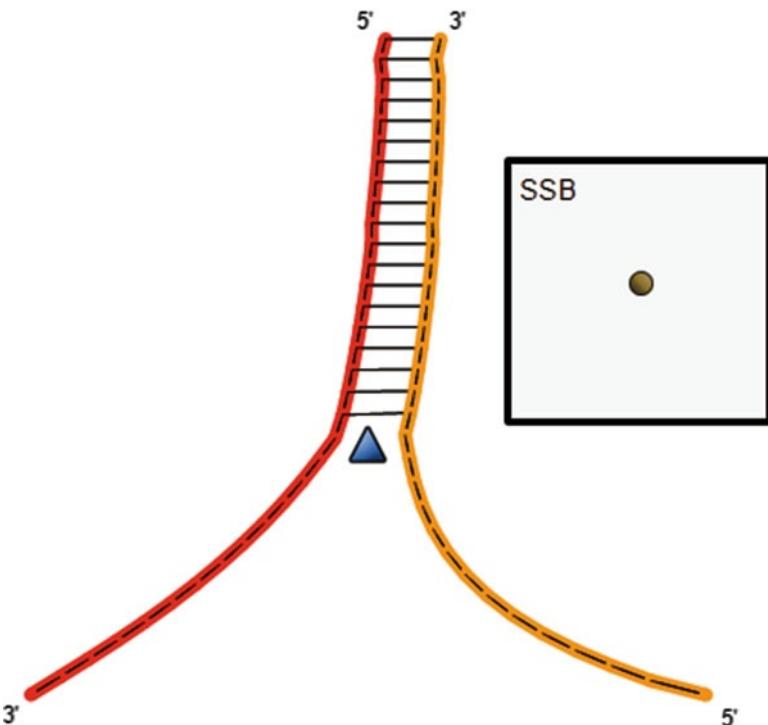
Step 1 Layout

- The junction between the double-stranded and the single-stranded DNA is called the **replication fork**.



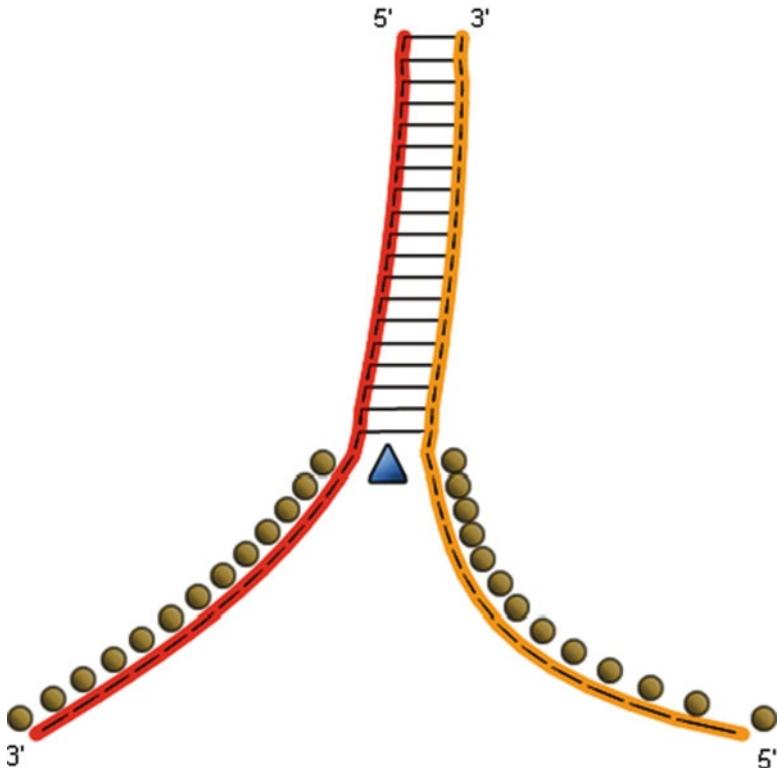
Step 2 Action: Breaking of hydrogen bonds by helicase

- Drag one SSB (single-strand binding protein) to the 3'-end of one single strand, and then drag another SSB to the 5'-end of the other single strand (Steps 3 and 4).



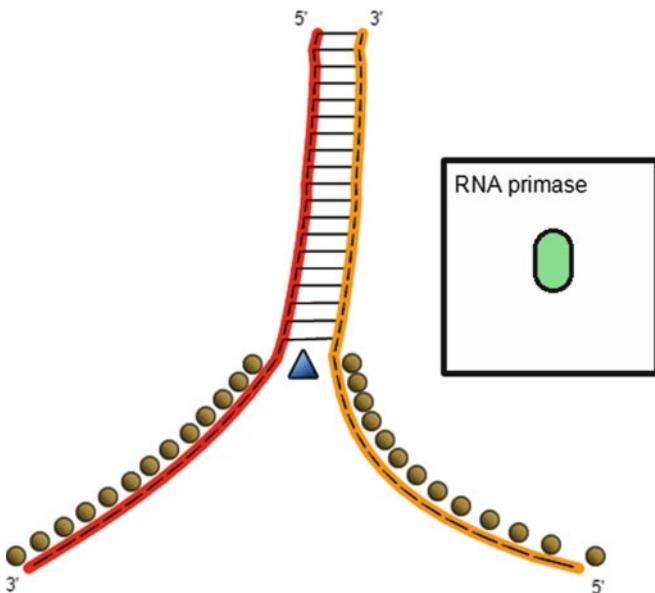
Step 3 Layout

- SSBs continue to bind to the newly separated DNA strands to prevent the strands from reforming the double helix (Step 4).



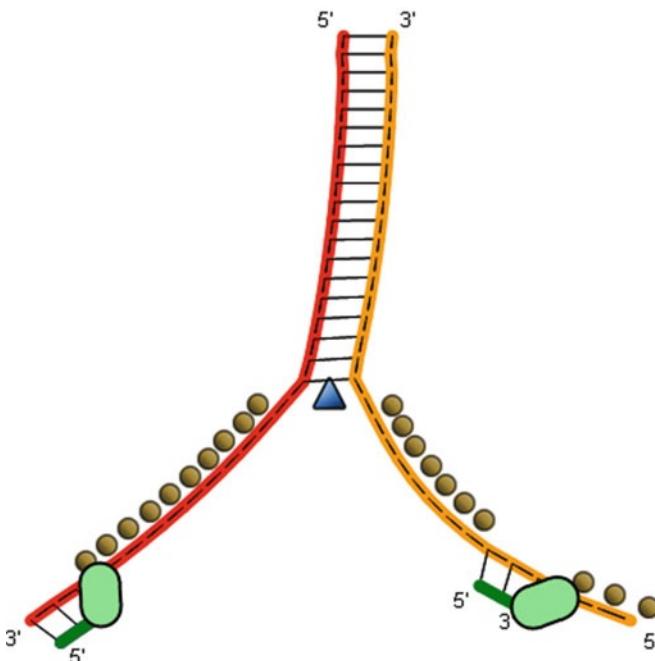
Step 4 Action: Binding of SSBs to single-stranded DNA

- Next, RNA primase attaches to each unwound strand.
- RNA primase is an enzyme that generates a short RNA primer to initiate DNA synthesis.
- The RNA primer is complementary to the DNA segment it pairs.
- *Drag one RNA primase molecule to the 3'-end of the single-stranded DNA. Then drag another RNA primase molecule to the middle of the other single strand (Steps 5 and 6).*



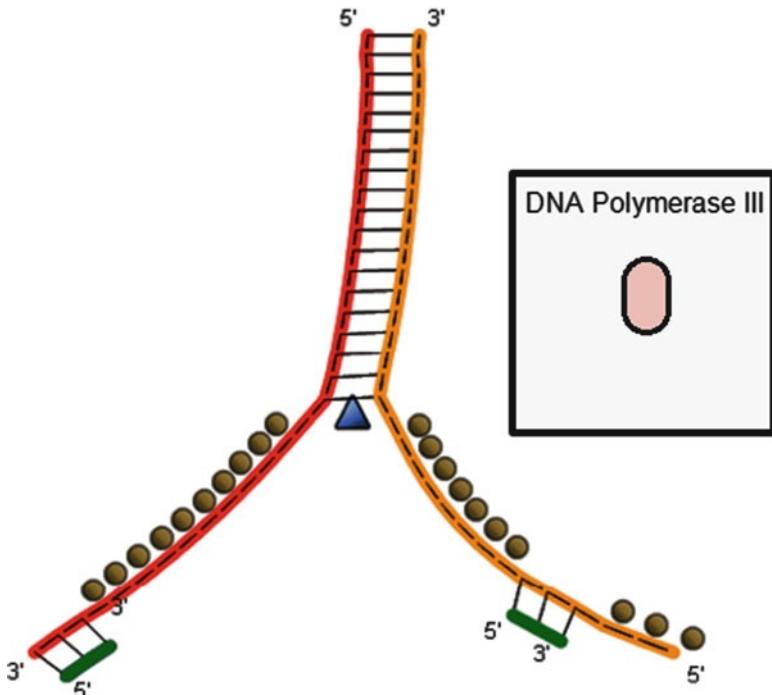
Step 5 Layout

- As you can see, on both strands a short segment of RNA, called a RNA primer, is synthesized in the 5'- to 3'-direction (Step 6).



Step 6 Action: Binding of RNA primase and synthesis of RNA primers

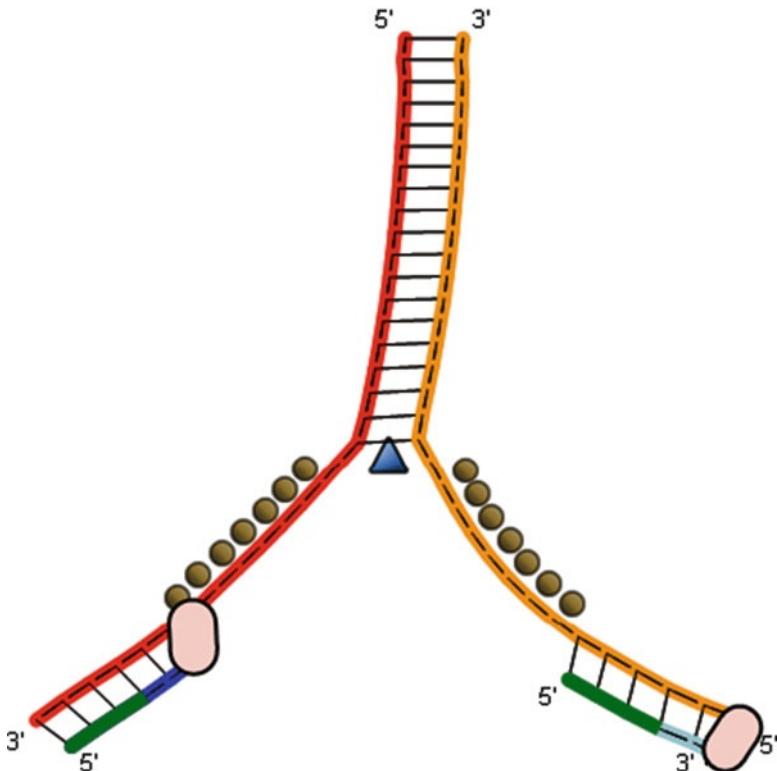
- The RNA primers serve as the starting points for DNA synthesis.
- After the RNA primers are generated (Step 6), the RNA primases leave the DNA strands (Step 7). Then, DNA polymerase III attaches to the RNA primers, synthesizing a new complementary DNA strand in the 5'- to 3'-direction (Step 7).
- Drag a DNA polymerase III molecule to each primer (Steps 7 and 8).



Step 7 Layout: Replacement of RNA primase by incoming DNA polymerase III

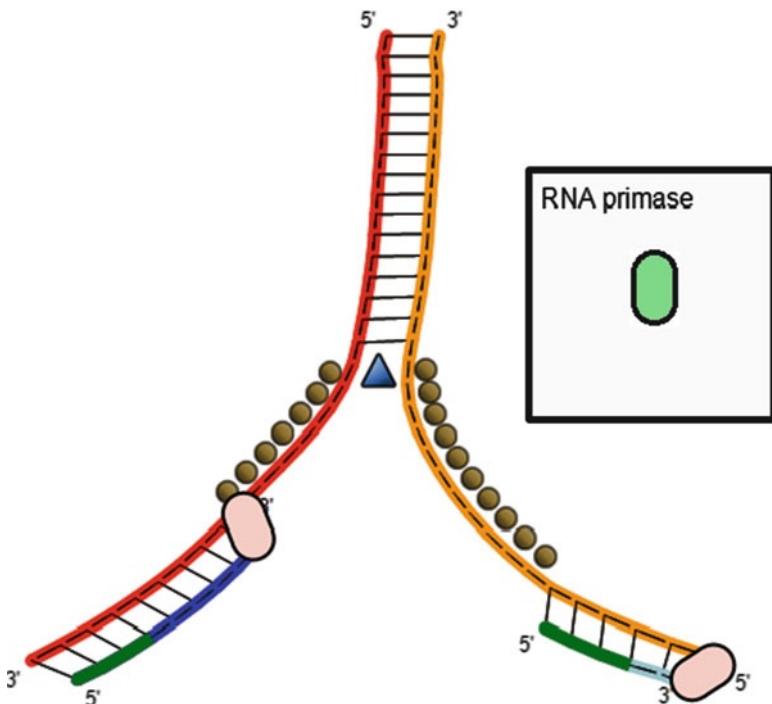
- On the left strand, DNA polymerase III moves up the template strand in the 3' - to 5'-direction. Thus, the new DNA strand is synthesized in the 5'-to 3'-direction.
- On the right strand, DNA polymerase III moves down the template strand in the 3' - to 5'-direction. Again, this means that the new DNA strand is synthesized in the 5'-to 3'-direction.

- On the left strand, DNA polymerase III continues to synthesize DNA. The new strand that is continuously synthesized is called the leading strand.
- On the right strand, however, DNA synthesis is away from the replication fork. As new sections of the template become available, additional DNA fragments must be synthesized.
- Of course, an RNA primase must first synthesize a new RNA primer before DNA synthesis.



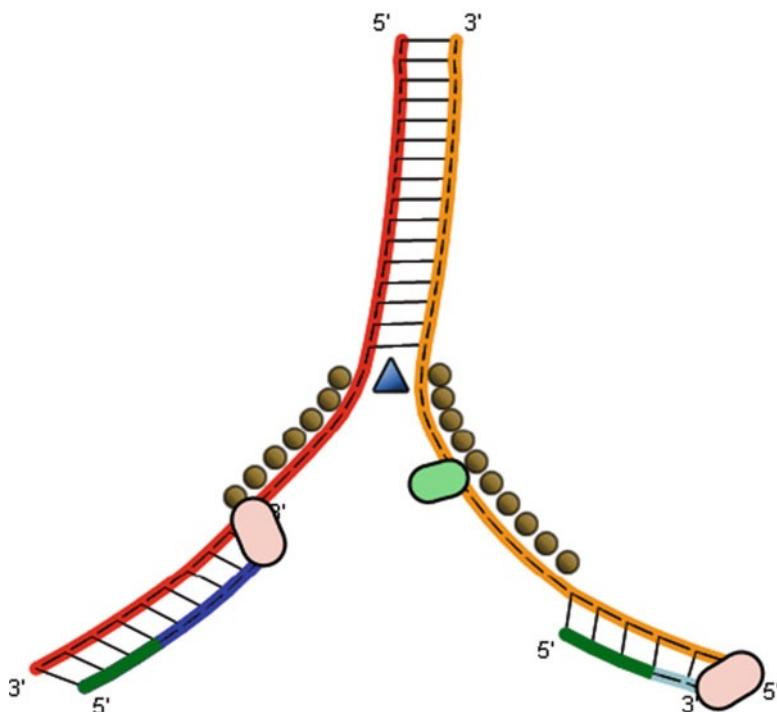
Step 8 Action: DNA synthesis by DNA polymerase III (primer extension)

- Drag RNA primase to the lagging strand template just below the helicase (Steps 9 and 10).



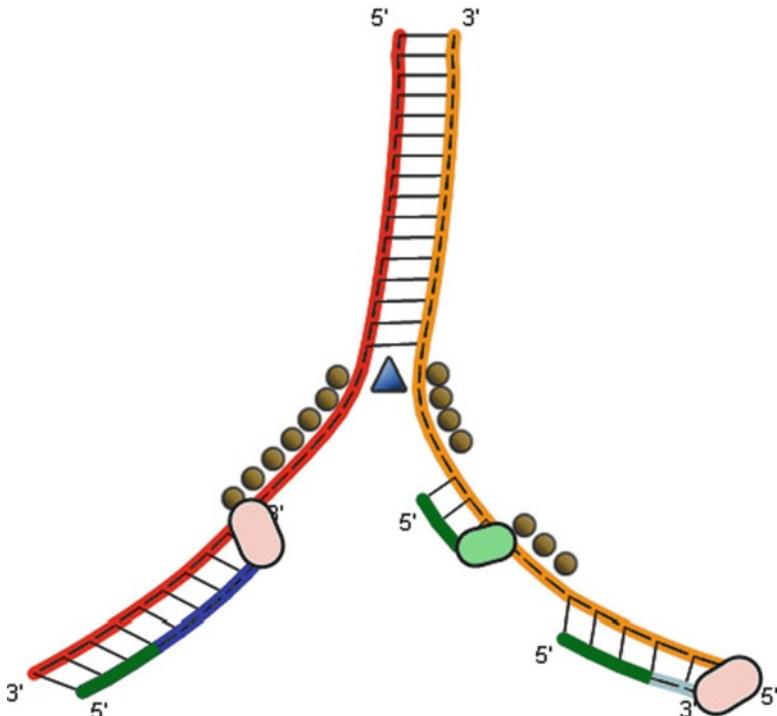
Step 9 Layout: New segment of lagging strand template for incoming RNA primase and another round of RNA primer synthesis

- The RNA primase synthesizes another RNA primer (Step 10).



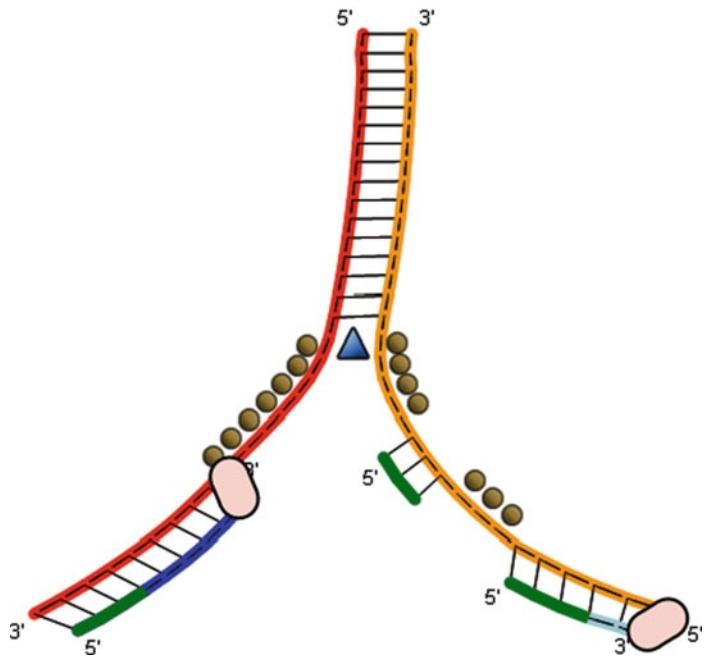
Step 10 Action: Binding of RNA primase to the lagging strand template

- Click to generate the RNA primer (Step 11).

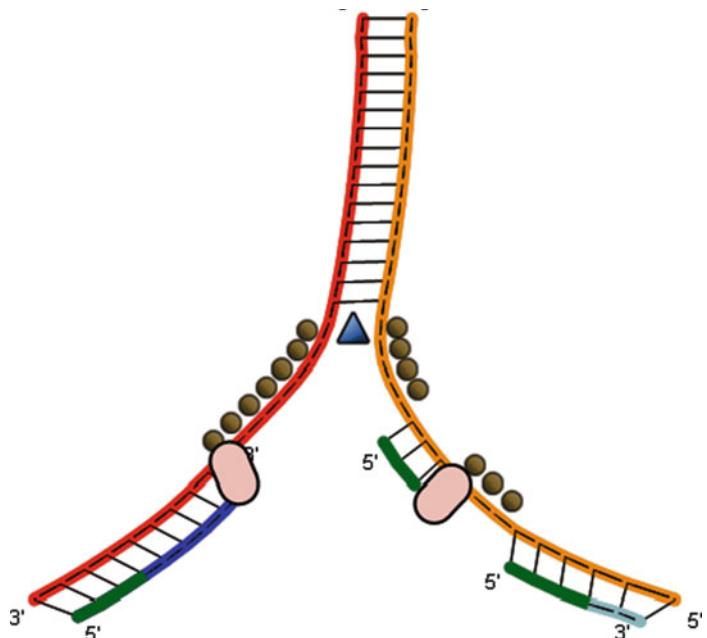


Step 11 Action: New RNA primer synthesis

- After the RNA primer is generated, the RNA primase leaves and another DNA polymerase molecule attaches to the RNA primer (Steps 12 and 13).

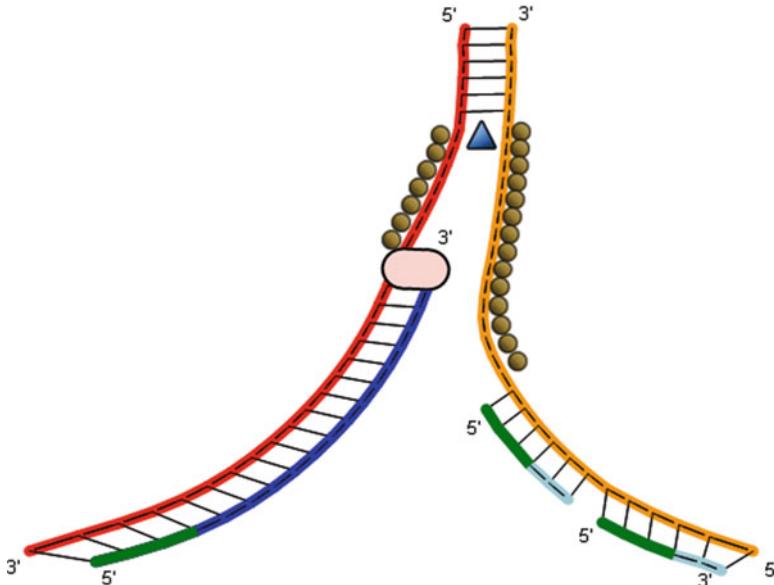


Step 12 Action: RNA primase is replaced by DNA polymerase III again



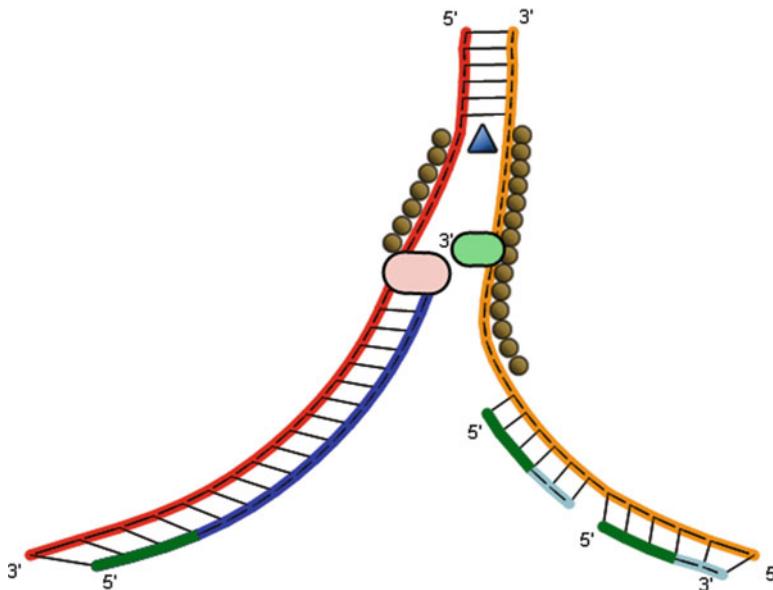
Step 13 Action: Attachment of new DNA polymerase III for synthesizing another fragment of the lagging strand

- Note that the leading strand on the left is continuously being synthesized (Step 14).

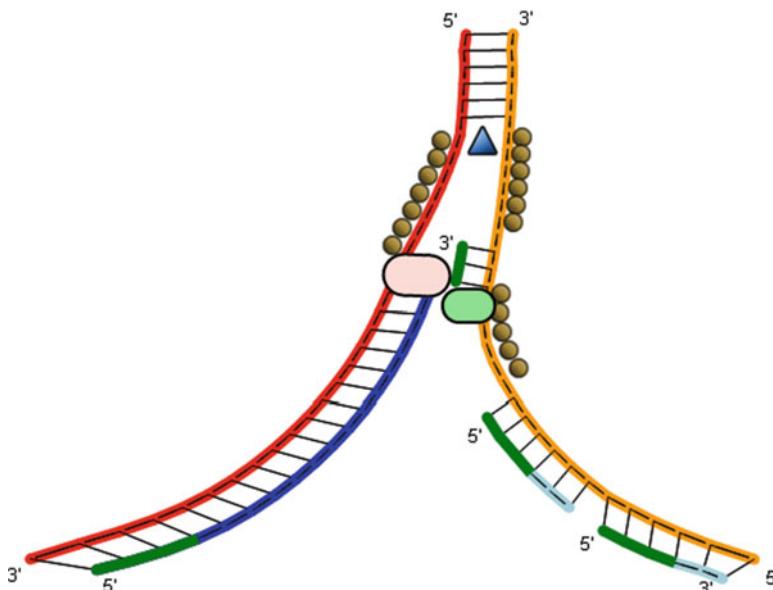


Step 14 Action: Continuous synthesis of leading strand DNA

- While DNA replication continues on the leading strand, RNA primase continues to bind to the newly separated sections of the lagging strand template (Steps 15 and 16) to initiate the synthesis of more DNA fragments (Okazaki fragments) (Step 17).

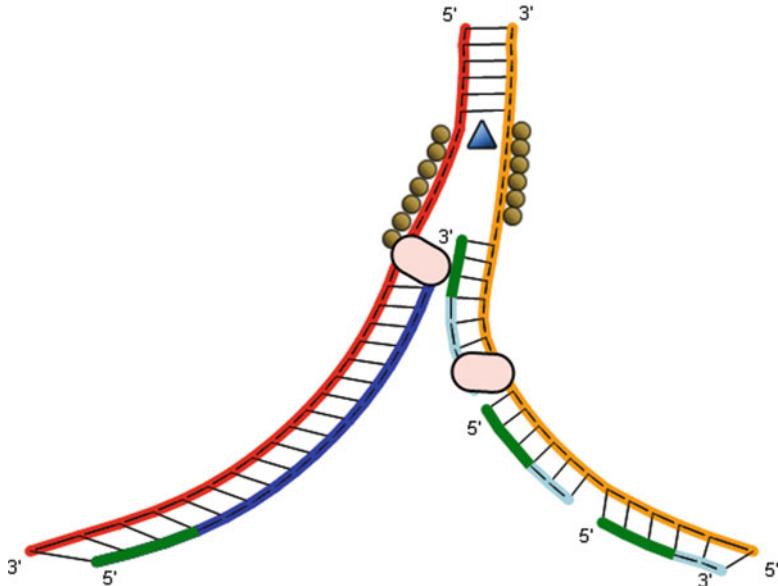


Step 15 Action: Binding of RNA primase



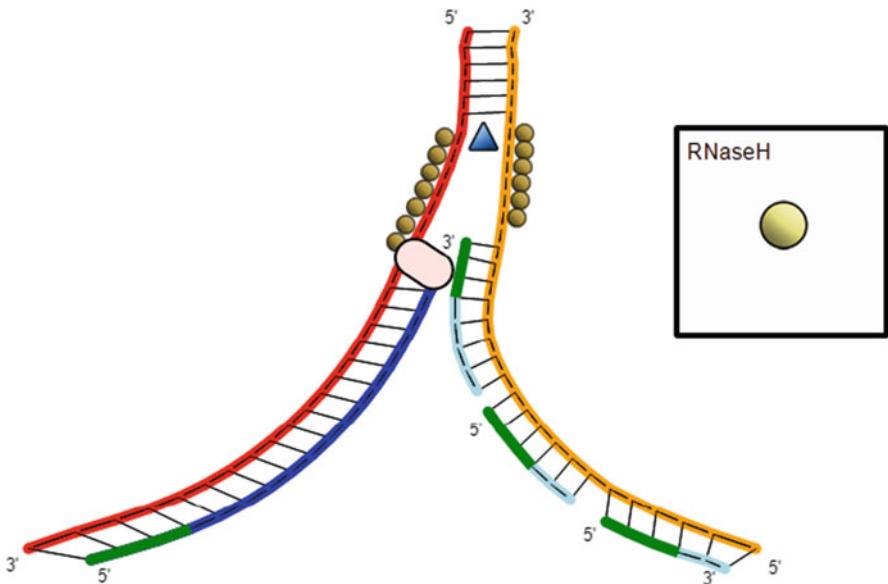
Step 16 Action: Synthesis of new RNA primer on the lagging strand template

- Because the primer is composed of RNA nucleotides, not DNA nucleotides, it eventually must be removed. The primers are removed by the enzymes RNase H (Steps 18–20) and 5'-exonuclease (Step 21).

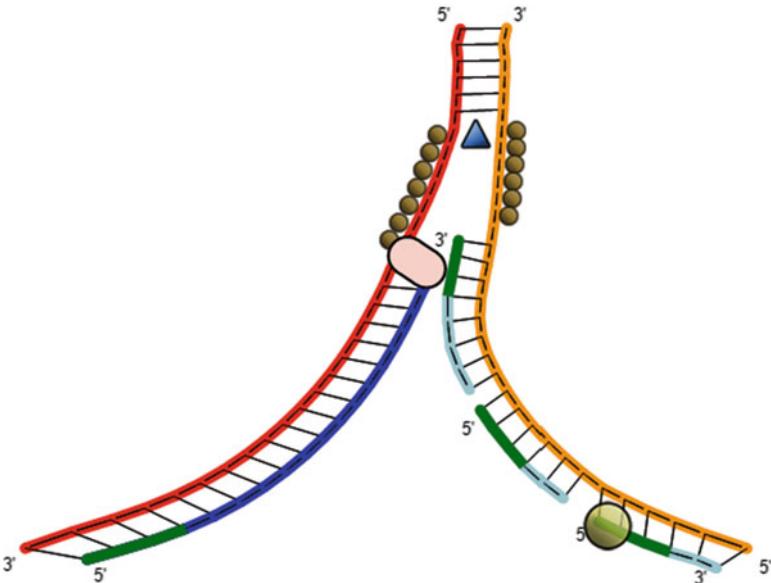


Step 17 Action: Synthesis of the third Okazaki fragment

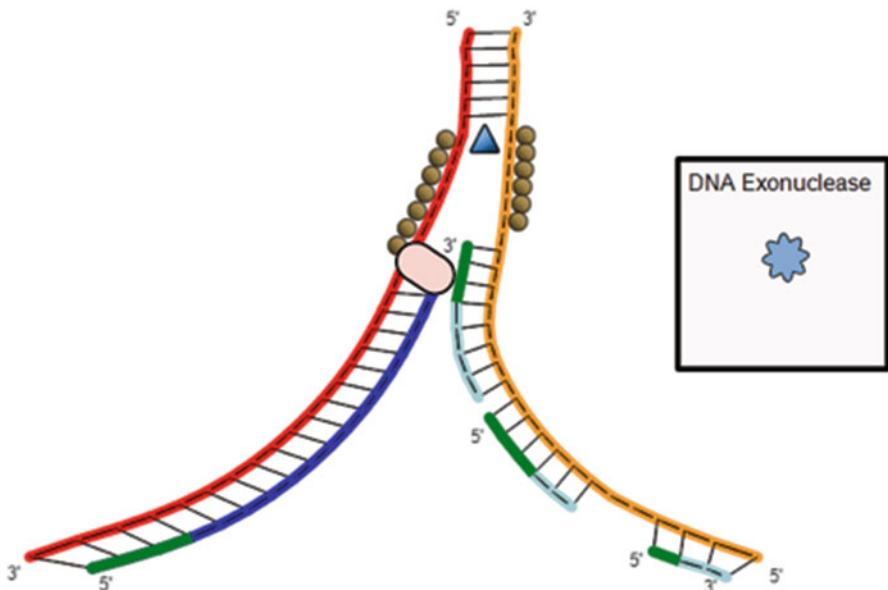
- Drag RNase H to the primer (Steps 18–20).



Step 18 Layout: For RNA primer removal

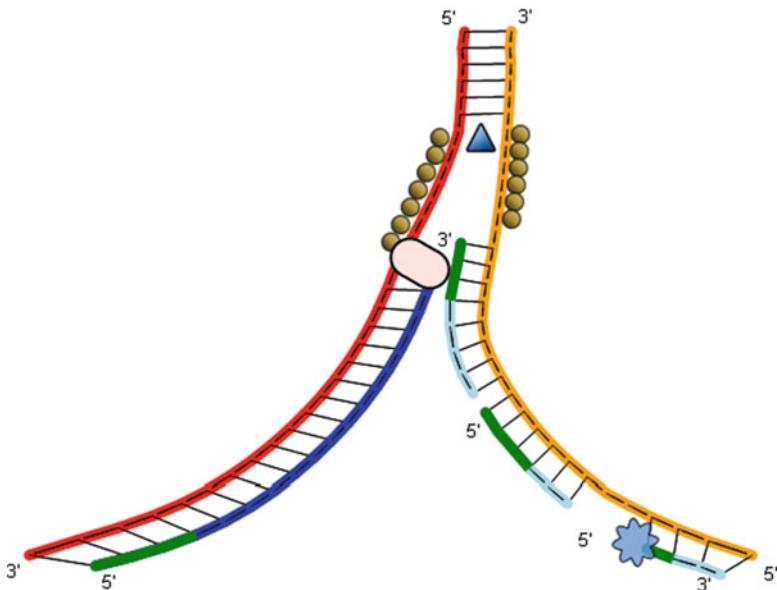


Step 19 Action: Attachment of RNase H at 5'-end of RNA primer



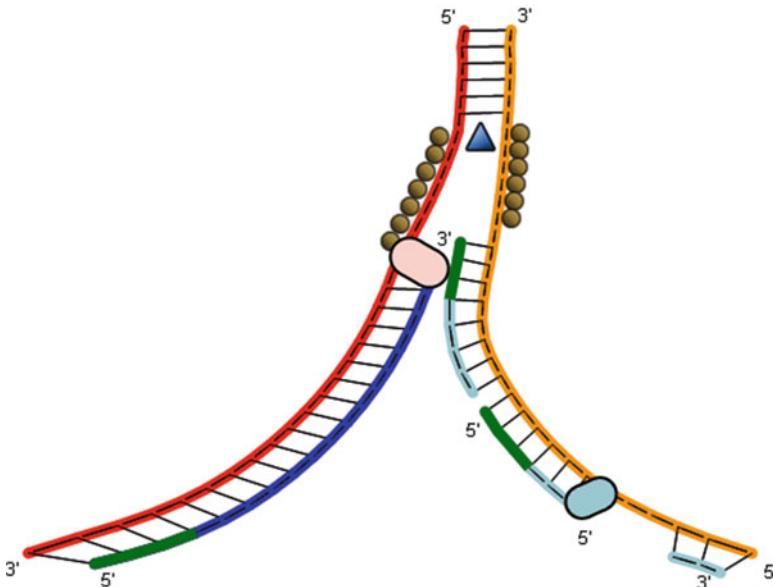
Step 20 Action: Removal of all the nucleotides of the RNA primer by RNase H except for the last nucleotide, which will be removed by exonuclease

- Drag the exonuclease to the last RNA nucleotide at the end of the lagging strand (Steps 20 and 21).



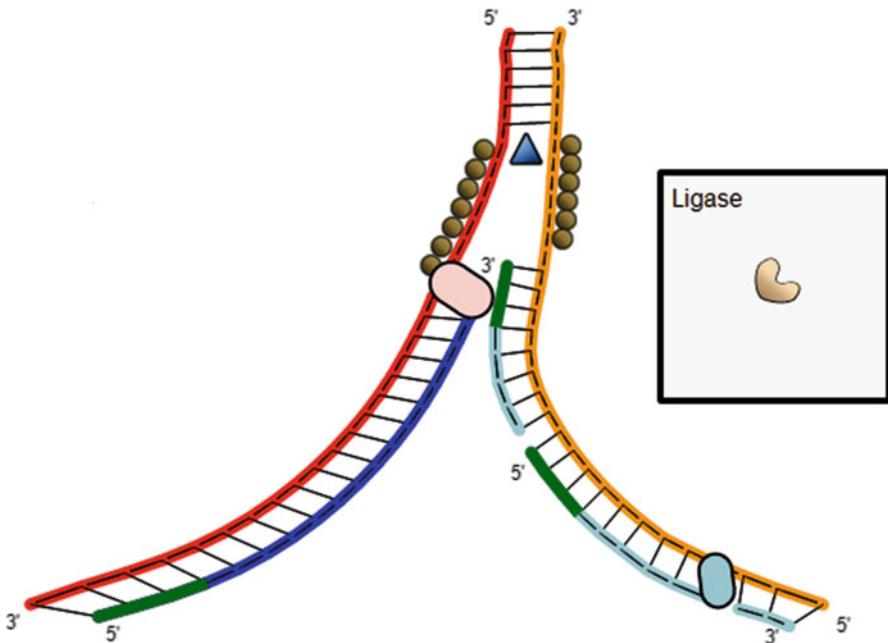
Step 21 Action: Binding of exonuclease for the removal of the last RNA primer nucleotide

- The gap is filled by DNA polymerase I (a proofreading and repairing enzyme) through synthesis of a new DNA strand (Step 22).



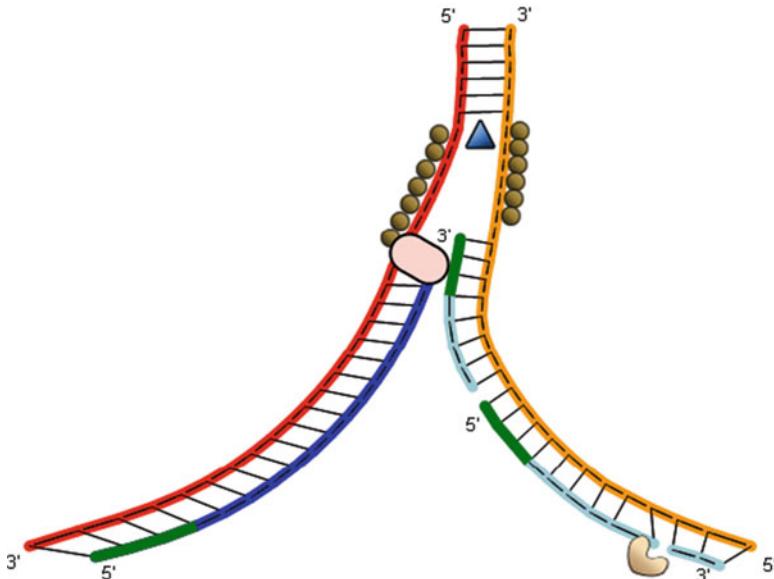
Step 22 Action: Binding of DNA polymerase I, filling the gap created by the removal of the RNA primer

- The newly added stretch of DNA (added by the DNA polymerase I repairing enzyme) is not connected to the previously synthesized stretch of DNA.
- Therefore, the nick between the two stretches of DNA (the two Okazaki fragments) must be sealed by the enzyme ligase through phosphodiester bond formation (Steps 23–25).
- *Drag DNA ligase to the nick* (Step 23).

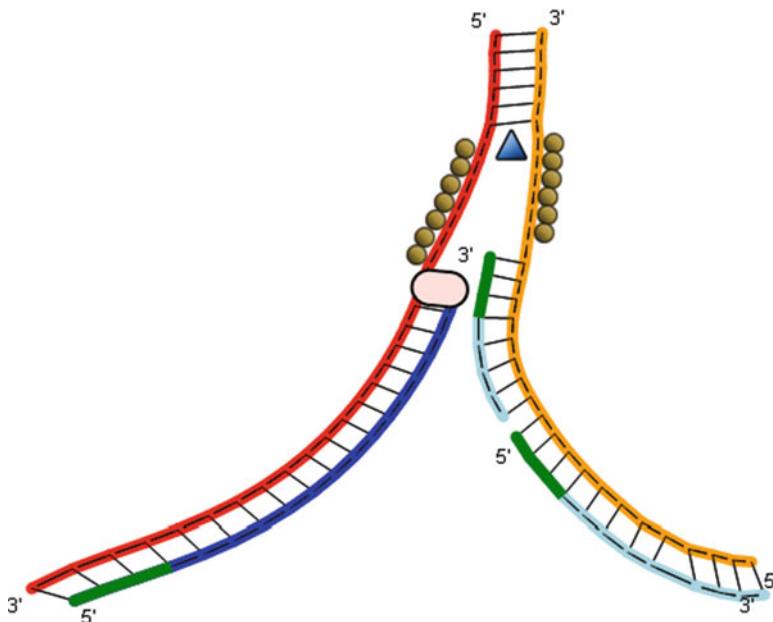


Step 23 Action: A nick between two Okazaki fragments to be sealed by DNA ligase

- The process continues. When all the nicks between the Okazaki fragments are sealed by DNA ligase, two intact double helixes are formed (Steps 24 and 25).



Step 24 Action: Sealing of the nick between two Okazaki fragments by DNA ligase

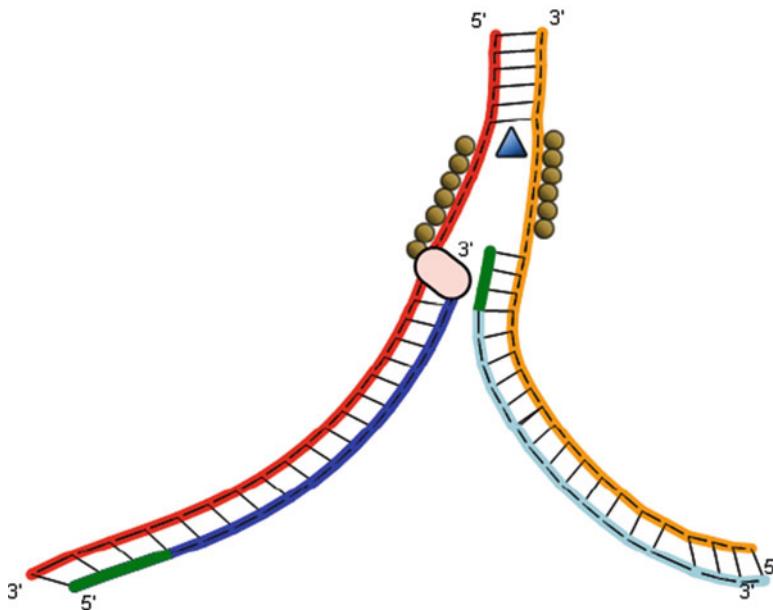


Step 25 Results: Joining of two Okazaki fragments

Conclusions

- DNA replication occurs before cell division so that there is no loss of DNA; the resulting daughter cells end up with the same amount of DNA as the original cell.
- DNA replication in all known cells occurs semiconservatively.
- Each of the original strands of DNA serves as a template for synthesizing a new DNA strand.
- The leading strand is synthesized continuously, whereas the lagging strand is synthesized discontinuously.
- Before the discontinuous stretches of newly synthesized DNA are joined together, they are known as Okazaki fragments.
- DNA synthesis requires a preexisting primer, which is synthesized by the enzyme RNA primase.
- The key enzymes in DNA replication are (1) topoisomerase I and II, which unwind the double helix; (2) helicase, which separates the double-stranded DNA; (3) RNA primase, which synthesizes RNA primers; (4) DNA polymerase, which synthesizes DNA; (5) RNase H, which removes all the nucleotides of the RNA primer except the last one; (6) DNA exonuclease, which removes the last RNA nucleotide; (7) DNA-repairing enzyme, which replaces the removed RNA primer

by synthesizing a new DNA segment; and (8) DNA ligase, which seals the nick between the Okazaki fragments through the formation of phosphodiester bonds (Step 26).



Step 26 Results: Continuous synthesis of leading strand and joining of discontinuously synthesized DNA lagging strand

Questions That Still Remain

- Note: Up to now, the two core enzymes of DNA polymerase III seem to move in opposite directions for leading and lagging strand synthesis. In reality, however, the two core enzymes stay together (forming a holoenzyme) joined by the tau subunit dimer, which are connected to a sliding clamp loader.
- In fact, all enzymes form a factory, through which the DNA double helix moves during DNA synthesis.
- How do the two core enzymes of DNA polymerase III stay together at the replication fork?
- We shall see the animated process in the next section.

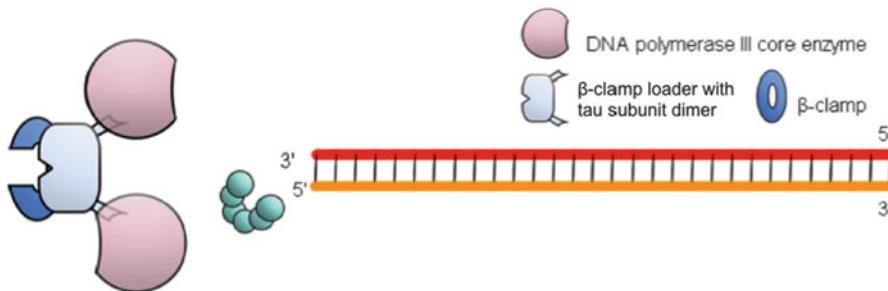
Replication Machinery at the Replication Fork (*Interactive Program 6*)

- Previously, we visualized leading and lagging strand synthesis occurring in opposite directions; the two DNA polymerase III core enzymes moved in different directions. However, the two core enzymes are joined together by a clamp loader with a tau (τ) subunit dimer to form a holoenzyme. Therefore, the two core enzymes are connected and do not actually move in different directions. To accomplish leading and lagging strand synthesis, the lagging strand template must first move to the right, then to the left (follow the motion), and then to the right again during DNA synthesis. In this way, the two core enzymes stay together, and the elongation of the leading and lagging strands appears to be in the same direction.

Use Interactive Program 6 on the software program available at Extras. Springer.com to familiarize yourself with the holoenzyme.

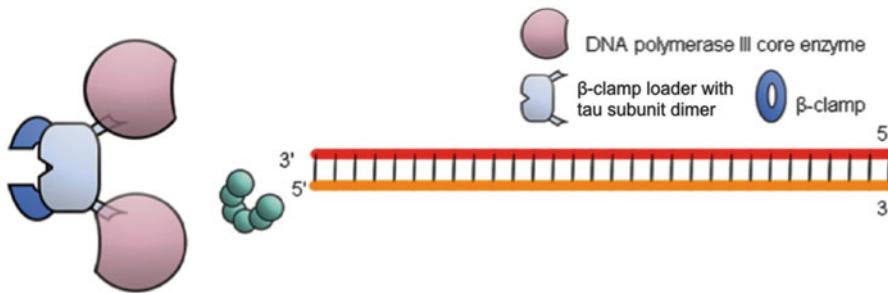
After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “Bidirectional DNA Replication.”

- Study the following animated process of DNA synthesis at the replication fork (Steps 1–8).



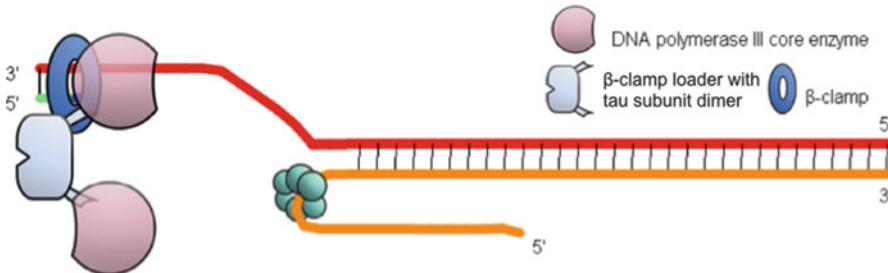
Step 1 Layout: From *left to right*: two core enzymes of polymerase III are joined by the β -clamp loader with tau subunit dimer, a helicase, and a DNA duplex

- You may click “ll” to stop the animation at any time to review and think about the process before moving to the next step.



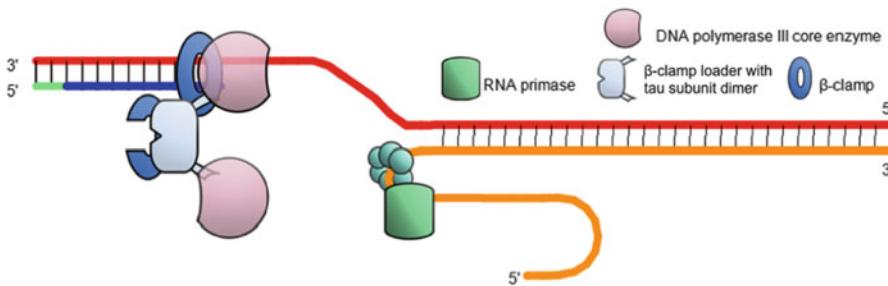
Step 2 Action: Binding of helicase to the lagging strand template, movement of DNA toward DNA polymerase III holoenzyme, and binding of RNA primase (green (not shown)) to the leading strand template for RNA primer synthesis

- As the partially dissociated dsDNA approaches the holoenzyme, RNA primase (green) binds to the leading strand template (upper strand) for RNA primer synthesis (shown in the interactive program). Pause here to view the binding of RNA primase and then click the play button to view the synthesis of a new RNA primer. The newly formed DNA–RNA duplex is recognized by the clamp loader, which then loads the β -clamp on to the new duplex and localizes the leading strand template for leading strand synthesis. The lagging strand template, on the other hand, is bent to the right in preparation for lagging strand synthesis (Step 3).



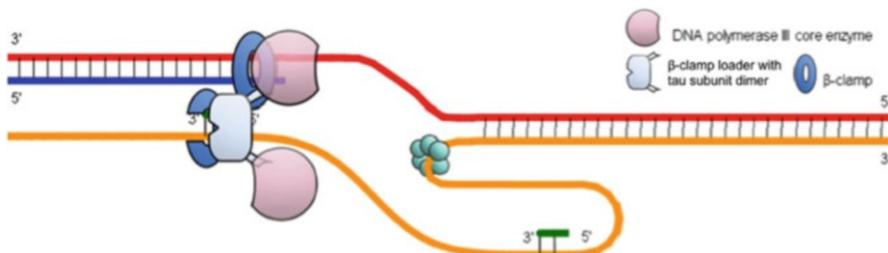
Step 3 Action: Entry of DNA leading strand template with its RNA primer into the core enzyme before being locked by the β -clamp for leading strand synthesis.

- Primer extension or DNA synthesis of the leading strand occurs at the core enzyme (upper). Look how the core enzyme stays with the leading strand template. Pause at this point to study how the leading strand interacts with DNA polymerase III (Step 3).
- The lagging strand template continues to be unwound and moves to the left (Step 4).



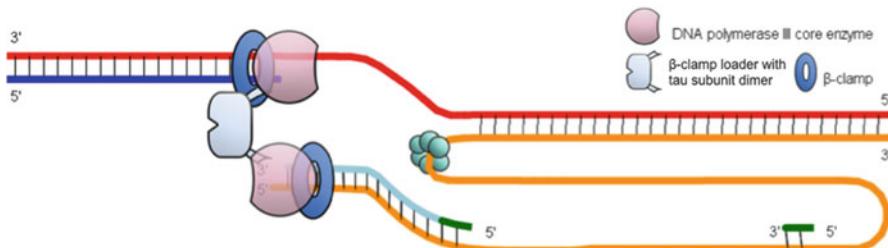
Step 4 Action: Bending of the lagging strand template toward the holoenzyme; the addition of a new β -clamp to its loader; and the attachment of RNA primase to the lagging strand template

- While the leading strand is continuously synthesized in the 5'- to 3'-direction, a new β -clamp joins the β -clamp loader in preparation for lagging strand synthesis (Step 4)
- When enough of the lagging strand is separated, the enzyme RNA primase binds to it (Step 4).
- As leading strand synthesis continues, the RNA primase generates a short RNA primer (5' to 3', left to right) to start lagging strand synthesis (Step 5). The 5' end of the lagging strand template is now bent to the left and has entered the holoenzyme.



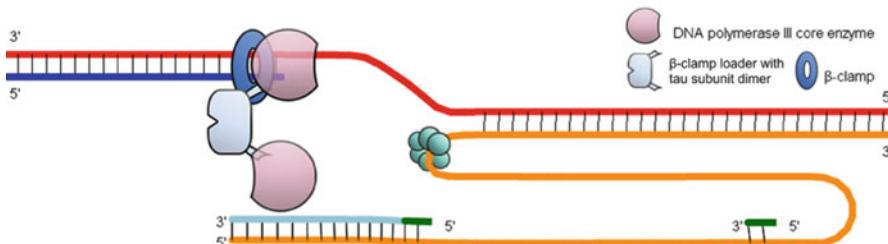
Step 5 Action: RNA primer synthesis on the lagging strand template: movement of the lagging strand template through the clamp loader

- Note that the sliding clamp is loaded on the right side of the lower core enzyme (Steps 5–6). Thus, lagging strand synthesis is from right to left (still 5' to 3') as the lagging strand template retracts from the left to the right (Step 6). In other words, the lagging strand template moves from right to left and then from left to right during lagging strand synthesis. In this way, both core enzymes stay together during the synthesis of both leading and lagging strands (Step 6).
- You should also notice that the second β -clamp has enclosed the lagging strand template along with the RNA primer. At this point, a second RNA primer is synthesized by RNA primase.



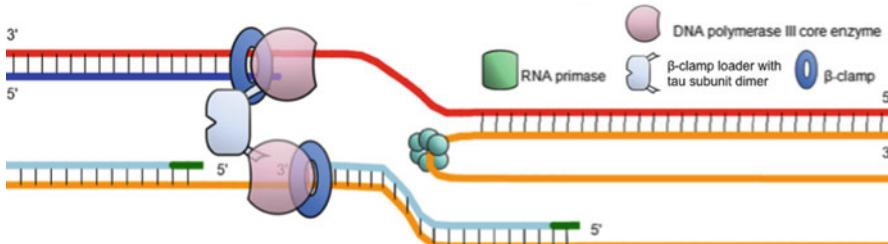
Step 6 Action: While the leading strand template moves from left to right for DNA synthesis, the lagging strand template retracts from right to left for DNA synthesis

- Here, a complete Okazaki fragment (of the lagging strand) is synthesized (Step 6). In the following steps, you will see more Okazaki fragments being synthesized (Step 7).
- After the Okazaki fragments are synthesized, all the nucleotides of the RNA primer are removed by RNase H except for the last RNA nucleotide, which is removed by an exonuclease (see earlier).



Step 7 Action: Completion of the first Okazaki fragment and the synthesis of a new RNA primer for the future Okazaki fragment synthesis

- Complete removal of the RNA primer is followed by DNA synthesis by DNA polymerase I. (Not shown here. See previous section for details.)
- As the process continues, more Okazaki fragments are made and joined (Step 8).



Step 8 Results: More Okazaki fragments are made and joined, while the leading strand is being synthesized continuously

- The foregoing animated process explains how the two core enzymes of DNA polymerase III stay together at the replication fork during DNA replication.
- We now shall take a look at both replication forks during the bidirectional DNA replication process.

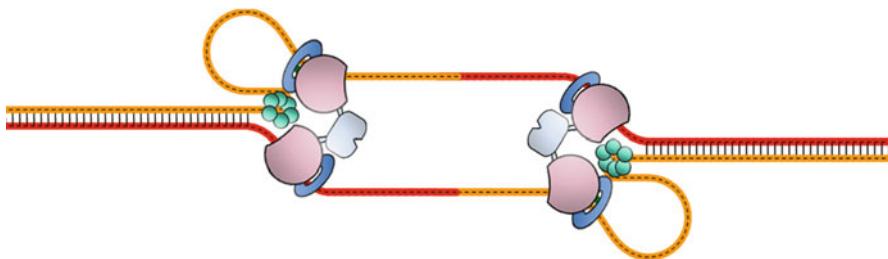
Bidirectional DNA Replication (*Interactive Program 7*)

- In the previous section, we saw that DNA synthesis takes place at a replication fork.
- However, the opening of dsDNA at the origin of replication results in the generation of two replication forks (RFs), one on each side. Thus, DNA replication is generally bidirectional.

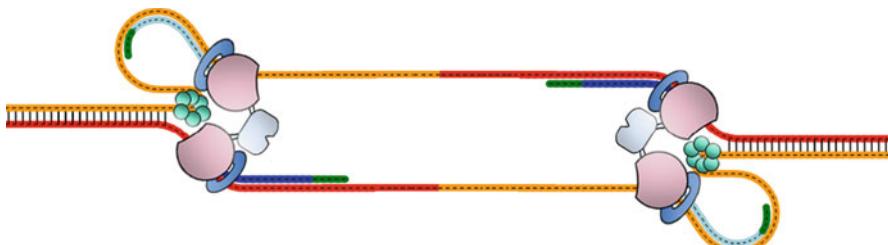
[Use Interactive Program 7 on the software program available at Extras. Springer.com to familiarize yourself with bidirectional DNA replication.](#)

[After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “Circular DNA Replication.”](#)

- Click to see the two opposite replication forks during DNA replication (Step 1).
- It should be noted that the directions of DNA replication at the two replication forks are opposite to each other. In other words, if the lower DNA strand in the left RF serves as a leading strand template, the same strand in the right RF serves as the lagging strand template. Similarly, if the upper strand in the left RF acts as a lagging strand template, then it acts as the leading strand template in the right RF.
- Click to see the movement of the replication forks during DNA replication (Step 2).



Step 1 Layout: Two opposite and inverted replication forks



Step 2 Action: Movement of two replication forks

- As the DNA replication process continues, the replication bubble continues to enlarge as a result of the movement of the replication forks away from each other until the entire genome is replicated.
- Are you ready to see the replication of the entire circular DNA bacteria? Go to the next section.

Circular DNA Replication (*Interactive Program 8*)

- Bacterial chromosomes are circular: each bacterial cell contains one circular dsDNA molecule.
- The model indicates that the DNA synthesis is bidirectional with two replication forks.

[Use Interactive Program 8 on the software program available at Extras. Springer.com to familiarize yourself with the replication of circular DNA.](#)

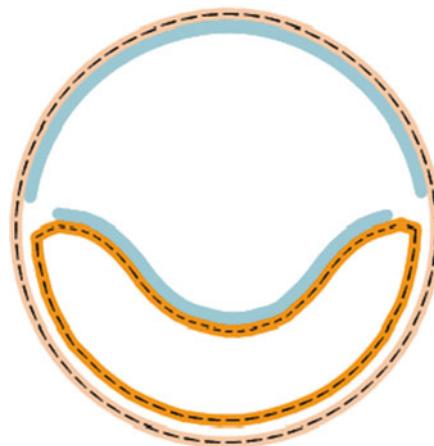
[After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “Linear DNA Replication.”](#)

- Click to see an early stage of DNA replication (Step 1).

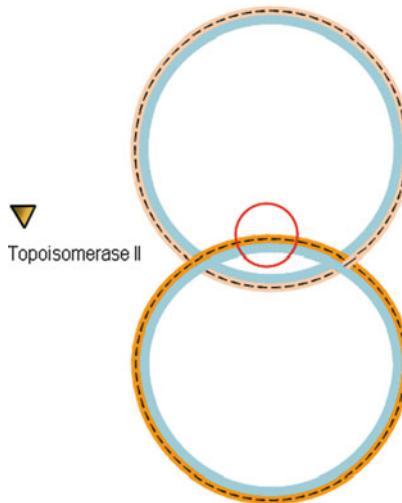


Step 1 Layout: Early stage of DNA replication

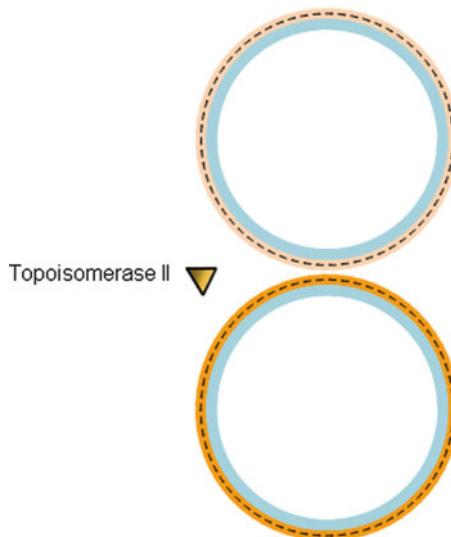
- Click to see a late stage of DNA replication (Step 2).

**Step 2** Action: Continuation of DNA replication

- Two circular DNA duplexes are formed but still interlocked (Step 3).

**Step 3** Action: Double-stranded cut by topoisomerase II to resolve the interlocked DNA molecules

- Drag the enzyme topoisomerase II into the red circle (Step 4).
- Breakage and reunion of the two interlocked DNA molecules form two separate DNA molecules ready for cell division (Step 4).
- In eukaryotic cells, chromosomes are linear. See the next section for eukaryotic DNA replication.



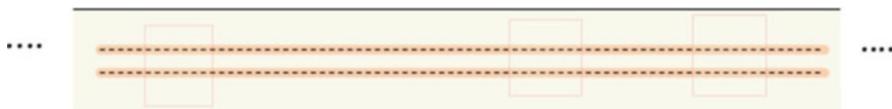
Step 4 Results: Separation of two DNA molecules

Linear DNA Replication (*Interactive Program 9*)

Use Interactive Program 9 on the software program available at Extras.Springer.com to familiarize yourself with the replication of linear DNA.

After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “Shortening of Telomeric DNA: Implications for Mortality and Cancer”

- The model shows a dsDNA molecule inside the chromosome (Step 1).
- The linear DNA contains many *Ori*; each *Ori* is a point of bilateral DNA replication, such that the replicated fragments meet and join to form a continuous molecule (Step 2).



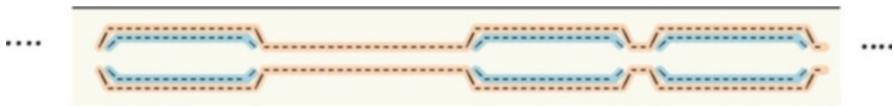
Step 1 Layout: A DNA duplex in a chromosome

- Click on the flashing area (left) to initiate DNA replication (Step 2).



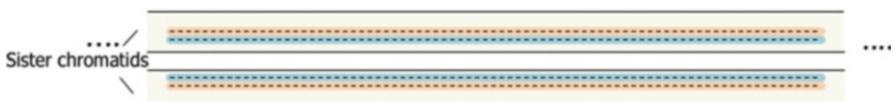
Step 2 Action: DNA replication at three origins of replication

- Click on the flashing area (left) to continue DNA replication (Step 3).



Step 3 Action: Continuation of DNA replication at three origins

- Replication of chromosomal DNA is complete: two DNA duplexes are formed, with each in a chromatid (Step 3).



Step 4 Results: Completion of DNA replication and formation of sister chromatids

- In the next section, we see what happens to the DNA at both ends of the chromosome (telomeres).

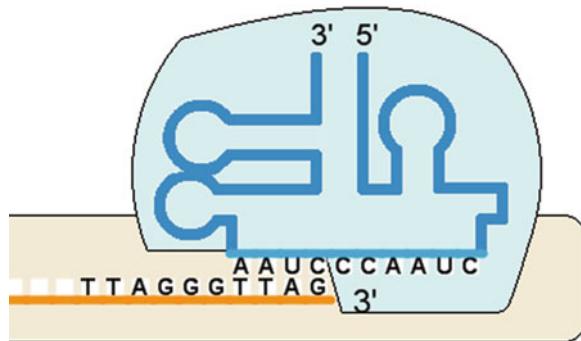
Shortening of Telomeric DNA: Implications for Mortality and Cancer (*Interactive Program 10*)

- The telomere is located at the tip of the chromosome. A telomere contains repetitive DNA (e.g., TTAGGG in humans) sequences. Because DNA in the eukaryotic chromosome is linear (rather than circular), the RNA primer at the 5'-end of the lagging strand cannot be converted to DNA because there is no additional DNA in front of it. Thus, DNA at the end of the chromosome is not replicated. As a result, the telomeres become shorter and shorter after each cell division, leading to the eventual death of the cell.
- The enzyme telomerase, however, is able to lengthen telomeric DNA. In most cells, the activity of telomerase is low; thus, cells have a finite lifespan. After a limited number of cell division, the cells eventually die as a result of the shortening of the telomeres. Cancer cells divide uncontrollably and indefinitely because the telomerase enzyme in cancer cells is activated and the shortened telomeric DNA is lengthened (Step 1).

Use Interactive Program 10 on the software program available at Extras. Springer.com to familiarize yourself with telomeric DNA and telomerase.

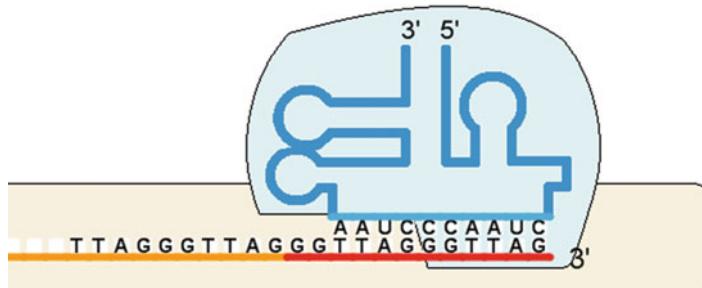
After completing the interactive program, you may continue reading the following text for further review or directly jump to the section “Final Thoughts.”

- Telomerase is a RNA–protein complex, which is able to elongate telomeric DNA by sliding along the end of the chromosome to provide a single-stranded RNA template for extending the 3'-end of the telomeric DNA (Step 1).



Step 1 Layout: Matching the RNA from the telomerase to the 3'-end of the telomere

- *The sliding of the telomerase along the chromosome provides a new template for telomere synthesis (Steps 2 and 3).*



Steps 2-3 Action and results: Sliding of telomerase–telomere DNA synthesis because of the availability of new templates from telomerase

- As a result of the sliding motion, new templates become available for the synthesis of telomeric DNA. In fact, increased telomerase activity is commonly observed in cancer cells.
- Can you think of a way to prolong to prolong human life?
- Can you also think of a way to treat cancer cells?

Final Thoughts

- By now you should be familiar with the process of DNA replication and understand the roles of the numerous enzymes involved.
- Because DNA molecules are extremely long, consisting of millions or hundreds of millions of nucleotides, it is inevitable that errors (mutations) occur during replication. Some mutations are neutral, but many others are detrimental. Thanks to repairing enzymes that work in concert with the DNA replication enzymes, the majority of new mutations are repaired.
- In eukaryotes, DNA replication in the S phase is a key step in controlling cell growth and development. No cell division is possible without the completion of the S phase.
- Armed with the knowledge in this module, you are now prepared to study the consequences of DNA replication: cell division (mitosis and meiosis in the eukaryotes).
- In the next module, you will learn a powerful technology called PCR (polymerase chain reaction) with which we can amplify DNA into millions or even billions of copies in the laboratory.

Quiz

1. In diploid species, how many genomes per nucleus are expected during G₁ of the cell cycle?
 - 1
 - 2
 - 3
 - 4
2. In diploid species, how many genomes per nucleus are expected during prophase of mitosis?
 - 1
 - 2
 - 3
 - 4
3. Human cells contain 46 chromosomes (or 23 homologous chromosome pairs). How many DNA molecules are present in the nucleus during metaphase of mitosis?
 - 23
 - 46
 - 92
 - 184

4. Which enzyme listed below separates the two strands of the DNA double helix by breaking the hydrogen bonds between base pairs?
 - (a) DNA polymerase
 - (b) DNA ligase
 - (c) Topoisomerase II
 - (d) Helicase
5. In addition to a DNA template, the synthesis of a new DNA strand requires a short piece of nucleic acid (RNA or DNA), which serves as a primer for the elongation of the new strand. In the cell, what enzyme is used for synthesizing such a primer?
 - (a) RNA primase
 - (b) Protease
 - (c) DNA polymerase
 - (d) DNA exonuclease
6. In DNA replication, the new strand grows
 - (a) In the 3'- to 5'-direction
 - (b) In the 5'- to 3'- direction
 - (c) From either the 3'- or 5'-end
 - (d) From neither the 3'- nor 5'-end
7. Which of the following experiments did Meselson and Stahl perform to prove that DNA replication is semiconservative?
 - (a) Bacterial transformation
 - (b) Bacteriophage T4 labeled with ^{32}P and ^{35}S
 - (c) Replacement of ^{15}N DNA in *E. coli* with ^{14}N followed by CsCl centrifugation
 - (d) Comparison of the old and new DNA sequences
8. During DNA replication, the lagging strand is synthesized
 - (a) Continuously
 - (b) Bidirectionally
 - (c) Discontinuously
 - (d) In the 3'- to 5'-direction
9. At the *E. coli* DNA replication fork, the two core enzymes of DNA polymerase III are linked together by a
 - (a) Tau subunit dimer
 - (b) Sigma protein
 - (c) Alpha protein
 - (d) Beta protein

10. Eukaryotic chromosomes may become shorter after each cycle of replication because there is no replacement of the
- (a) RNA primer at the 5'-end of the new DNA strand
 - (b) RNA primer at the 3'-end of the new DNA strand
 - (c) DNA primer at the 5'-end of the new DNA strand
 - (d) DNA primer at the 3'-end of the new DNA strand

Answers

1. b 2. d 3. c 4. d 5. a 6. b 7. c 8. c 9. a 10. a

Module 4

PCR: How Do We Amplify DNA in the Laboratory?

- We have just learned, in Module 3, how living systems replicate DNA. Let us examine a very powerful technology that utilizes this knowledge: PCR.
- PCR, or polymerase chain reaction, is a process that uses the enzyme DNA polymerase to amplify (replicate) DNA in the laboratory.
- Using PCR, we are able to make millions, and even billions, of copies of DNA in which we are interested. Why?
- Most of the time, the DNA we want to study is present in very small amounts (for example, in small pieces of hair or skin, or drops of blood at the crime scene).
- If we could make many copies of the DNA, then we would have enough material to test and study for a variety of purposes in medicine, agriculture, forensics, and the environment.

How PCR Works

- Suppose that we want to make many copies of a specific gene or segment of DNA.
- To do PCR, we must prepare the following:

1. Reagents

- (a) DNA template: a segment of the DNA duplex to be amplified
- (b) DNA primers: A primer is a short, single-stranded DNA sequence that pairs with the DNA template to initiate DNA synthesis. As you have learned, during DNA replication, RNA primers must first be synthesized before DNA synthesis can take place. It is no different in the laboratory. However, instead of RNA primers, PCR employs DNA primers. Two primers (primer 1 or forward primer and primer 2 or reverse primer) are needed.

- (c) A high temperature resistant DNA polymerase (e.g., Taq polymerase) for DNA elongation (synthesis).
- (d) dNTPs (dATP or deoxyadenosine triphosphate, dCTP or deoxycytidine triphosphate, dGTP or deoxyguanosine triphosphate, dTTP or deoxythymidine triphosphate): These triphosphonucleosides serve as building blocks for new copies of the DNA segment.

2. Thermocycler

The thermocycler is a machine that can be programmed to create the conditions necessary for PCR, including the number of the desired cycles and the temperatures in each cycle. A thermocycler may accommodate many microtubes (samples).

- For PCR, the following must also be considered:
 1. Length of DNA to be amplified: generally, the lengths range from 100 bases to thousands of bases.
 2. Number of cycles: because of the limitations of DNA polymerase, the number of PCR cycles is usually limited to 30 to 40.
 3. Size of primers: the specificity of amplification depends on the size of the primers. To avoid random amplification, primers must be a certain size. To be sure, the larger the primer, the more specific the amplification. Specificity is also related to the size of the template. For the human genome (3×10^9 bases), a primer with 20 or more bases is large enough to avoid random amplification.
 4. Temperature: the three temperatures in each cycle are
 - (a) 94 °C for denaturation (separation) of double-stranded DNA (template) into single-stranded DNA
 - (b) +/– 55 °C for annealing the primers to the single-stranded DNA (Annealing temperature chosen for a PCR depends on length and composition of the primer.)
 - (c) 72 °C for DNA elongation (primer extension), so that the denatured, single-stranded DNA template becomes double stranded again

The Process of PCR (*Interactive Program 1*)

[Use Interactive Program 1 on the software program available at Extras.Springer.com to familiarize yourself with the process of PCR.](https://extras.springer.com/familiarize-yourself-with-the-process-of-pcr/)

[After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “Conclusions.”](#)

- Cycle 1: denaturation: Set temperature to 94 °C for DNA denaturation (Steps 1 and 2).

• Cycle 1

- 1) Move the slide bar on the right to set the denatureing temperature at 94°C.



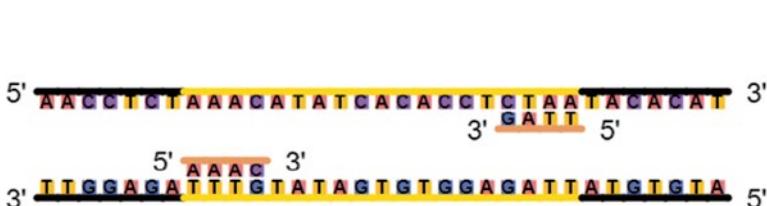
Step 1 Layout: Segment of DNA (yellow) to be amplified



- 2) Move the slide bar to set the annealing temperature at 55°C, and drag primers 1 and 2 to the appropriate location (red circle).

Step 2 Action: Set temperature to 94 °C

- Cycle 1: Annealing: Set temperature to 55 °C so that the primer can anneal (Step 3).

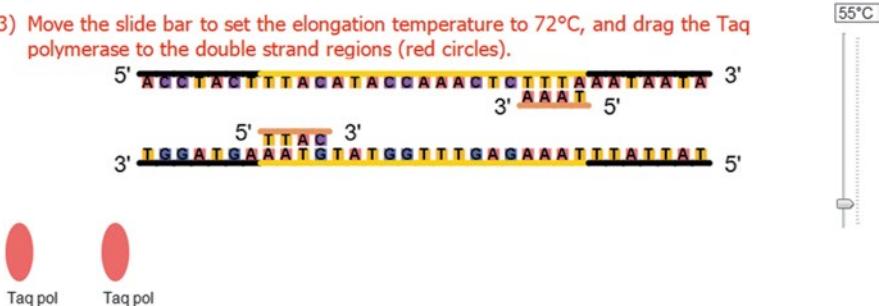


Step 3 Action: Primer annealing at 55 °C

- Cycle 1: Elongation: Set temperature at 72 °C and drag the Taq polymerase to the double-stranded (primer temperate) region (Steps 4 and 5).

• **Cycle 1**

- 3) Move the slide bar to set the elongation temperature to 72°C, and drag the Taq polymerase to the double strand regions (red circles).



Step 4 Action: Set temperature to 72 °C

- This is the end of cycle 1.
- Please note that the desired fragment to be amplified is the middle section (yellow). Because Taq polymerase continues to elongate DNA beyond the desired region, the cycle 1 products are not desirable (too long).

Step 5 Results: Products of cycle 1: no desirable product

- Cycle 2: Denaturation–Annealing–Elongation: At the end of cycle 2, four products are formed, but none of them is useful because the products are still too long (amplified beyond the desired borders of the fragment) (Step 6).

- Cycle 2.



- Continue the process for elongation.



- Again, no desired fragment has been amplified.



Step 6 Results: End products of cycle 2: no desirable product

- Cycle 3: Denaturation–Annealing–Elongation: Eight products are formed at the end of cycle 3 (Steps 7 and 8). Among the eight, two amplified DNA fragments are desirable (they are the right size).

- Cycle 3.

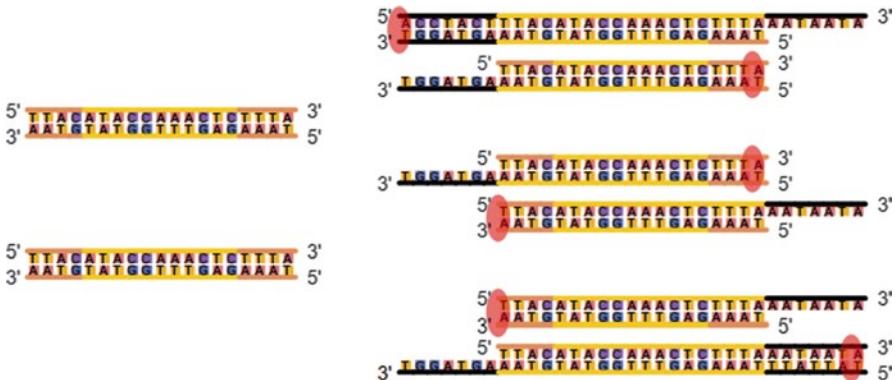


- The last step: elongation

- See, we get 2 desired fragments with the correct size!



Step 7 Results: End products of cycle 3: Two of the eight products are the right size



Step 8 Results: The products of cycle 3 are rearranged. The two products with the right sizes are shown on the left

- Now we can clearly see that of the eight DNA duplexes (products), two are desired fragments. For ease of visualization, the desired fragments are shown on the left (Step 8).

Conclusions

- More cycles of PCR will yield more desired fragments (of correct size). We can use the following formula to calculate the theoretical number of copies at the end of each cycle N:

$$X(2^N - 2)$$

where X is the initial number of desired segments, and N is the number of cycles. For simplicity, assume X=1. The number of copies after each cycle is calculated below:

At the end of cycle 1: $2^1 - 2 \times 1 = 0$ (no desired fragment)

At the end of cycle 2: $2^2 - 2 \times 2 = 0$

At the end of cycle 3: $2^3 - 2 \times 3 = 2$ (desired copies)

At the end of cycle 4: $2^4 - 2 \times 4 = 8$

At the end of cycle 25: $2^{25} - 2 \times 25 = 33,554,432$ (desired copies)

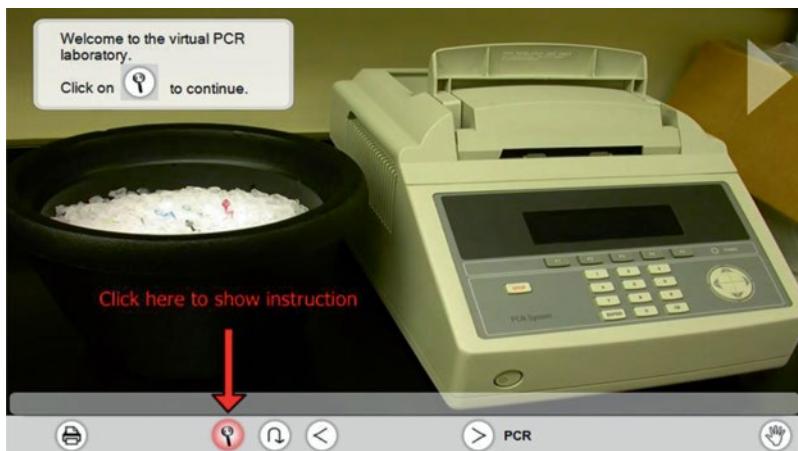
- In reality, the Taq polymerase enzyme does not work at 100 % efficiency, so the actual number of copies is smaller.
- In the next section, we enter a **virtual PCR laboratory**.

Virtual PCR Laboratory (*Interactive Program 2*)

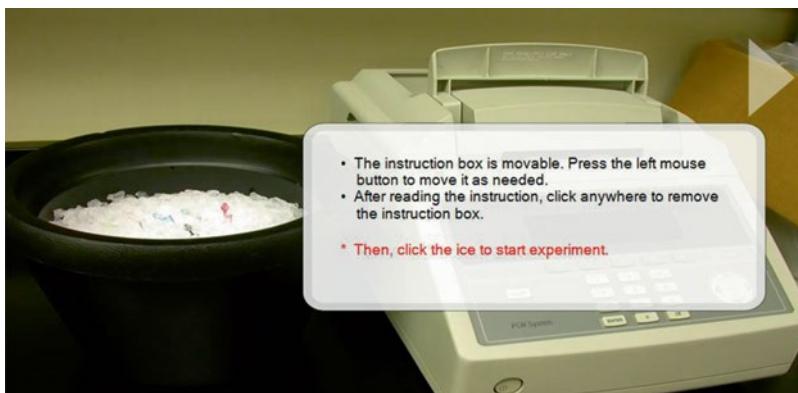
Use Interactive Program 2 on the software program available at Extras.Springer.com to familiarize yourself with the PCR laboratory.

After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “Agarose gel electrophoresis.”

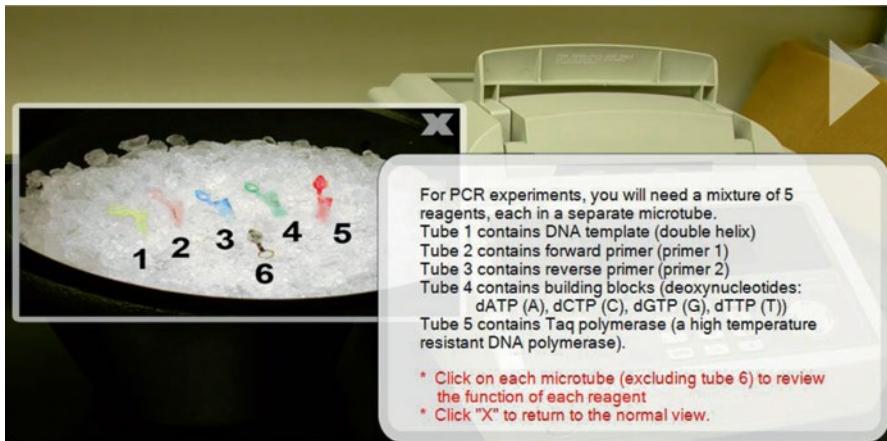
- The following screenshots represent the PCR laboratory (Steps 1–21).
- The interactive program is ideal for performing the virtual laboratory exercise on PCR.
- The following are representative screen snapshots for the PCR laboratory (Steps 1–21).



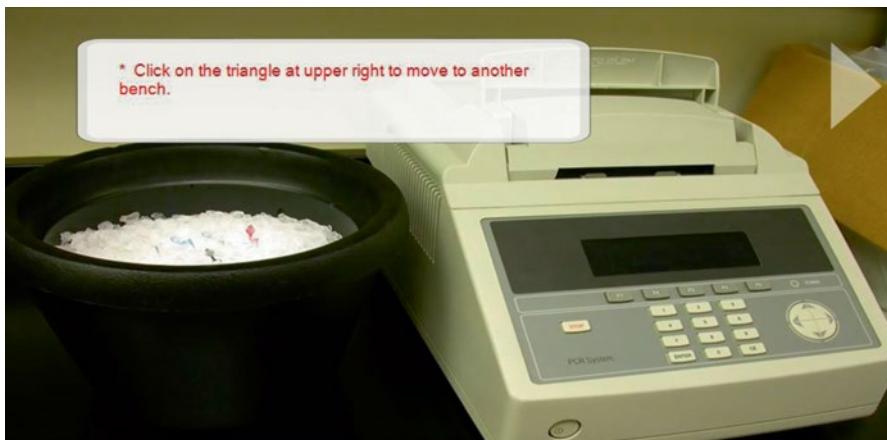
Step 1 Layout: Bench 1 (Left: ice bucket with six microtubes containing PCR reagents; right: a thermocycler)



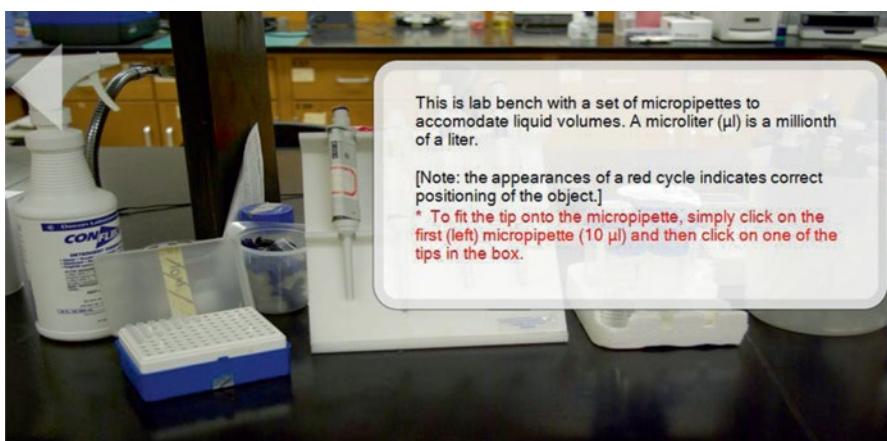
Step 2 Action: Enlarge the ice bucket to view contents



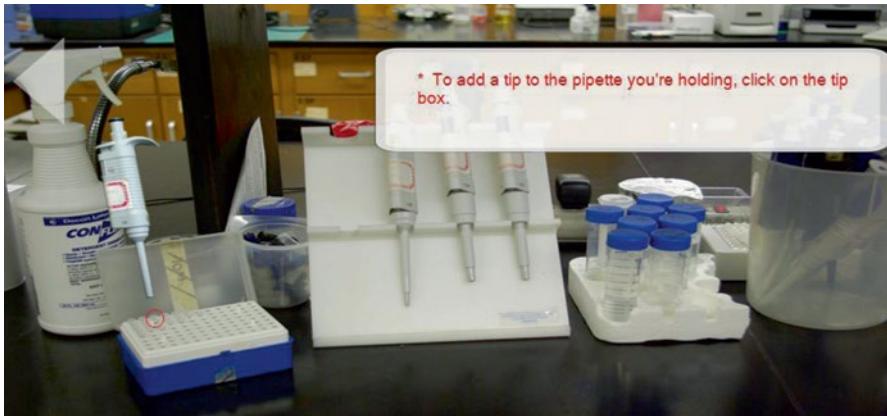
Step 3 Action: Add reagents to tube 6



Step 4 Action: Go to laboratory bench 2



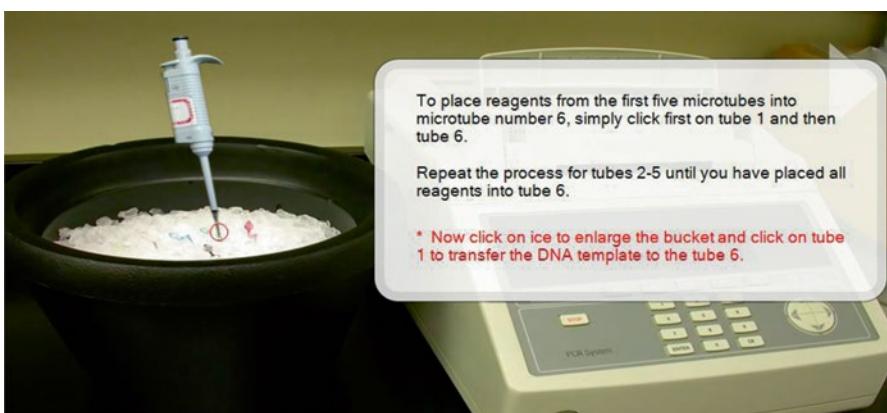
Step 5 Action: Pick up a micropipette



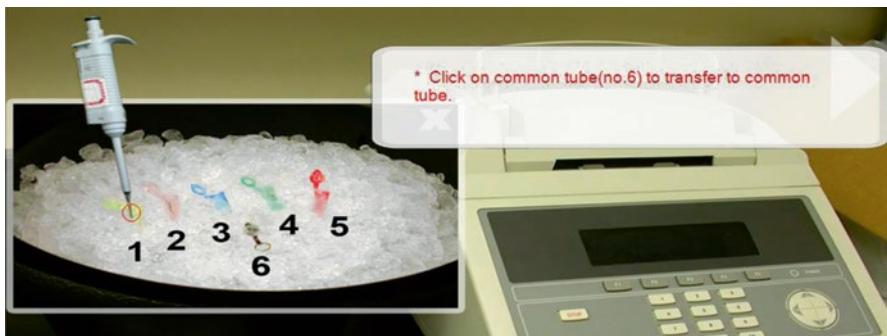
Step 6 Action: Attach a pipette tip to the micropipette



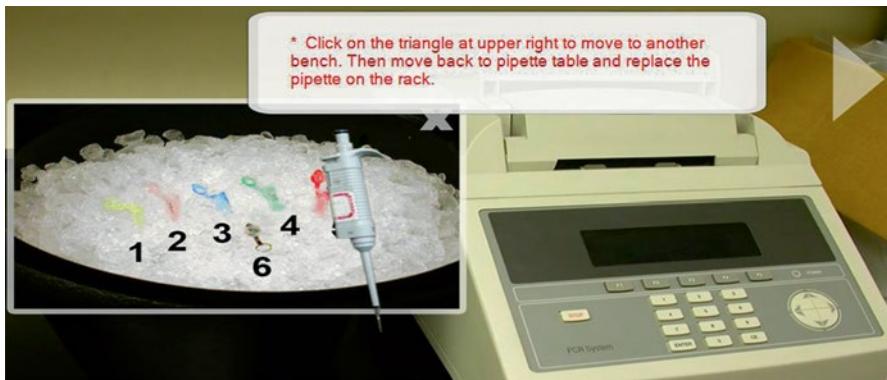
Step 7 Action: Return to the original lab bench



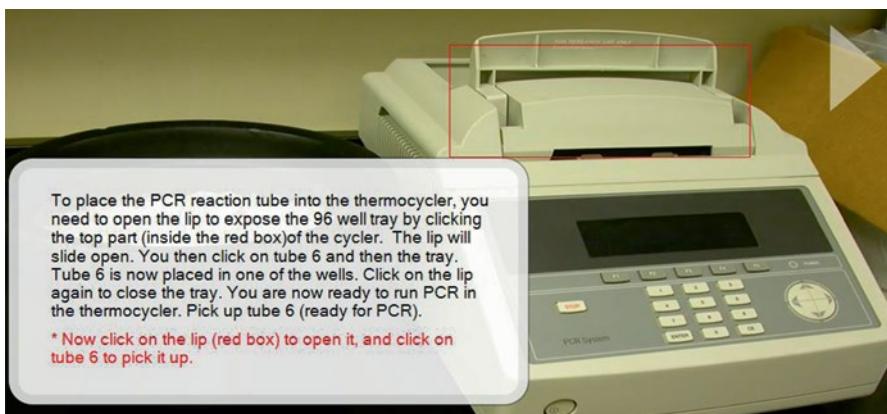
Step 8 Action: Enlarge the ice bucket



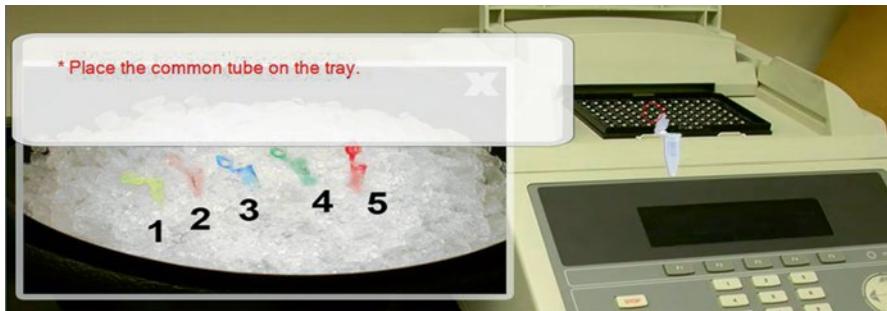
Step 9 Action: Add reagents to tube 6



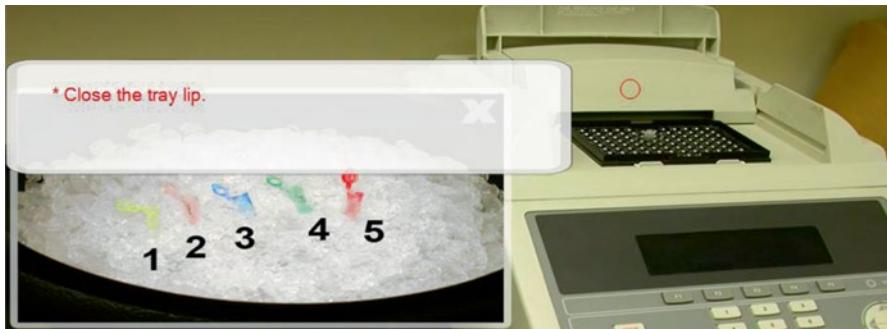
Step 10 Action: Return the micropipette to bench 2



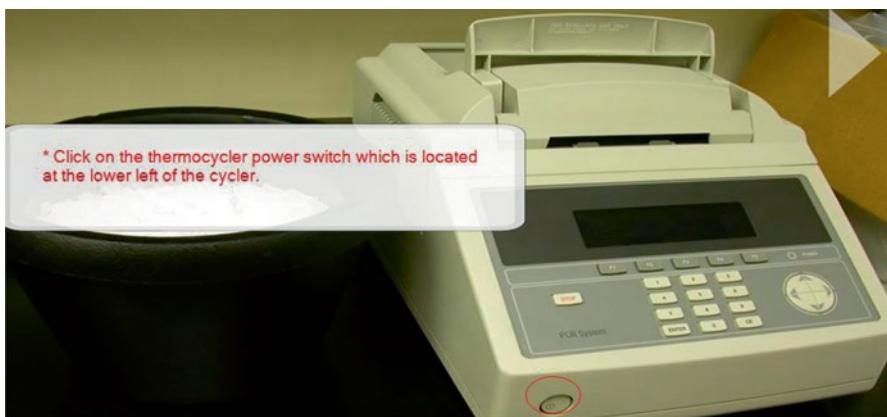
Step 11 Action: Open the thermocycler



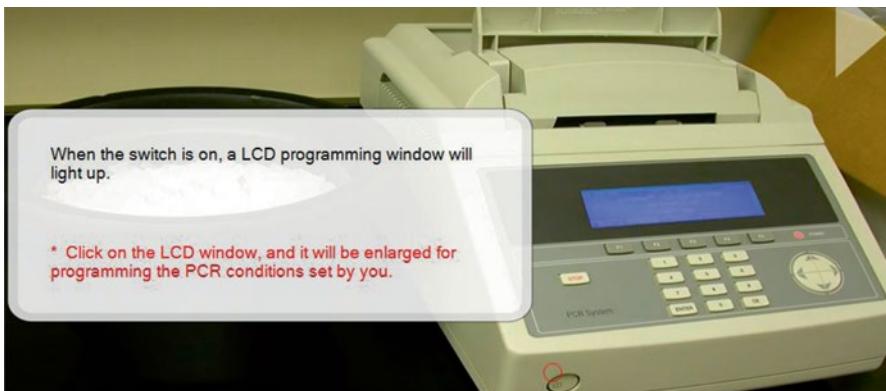
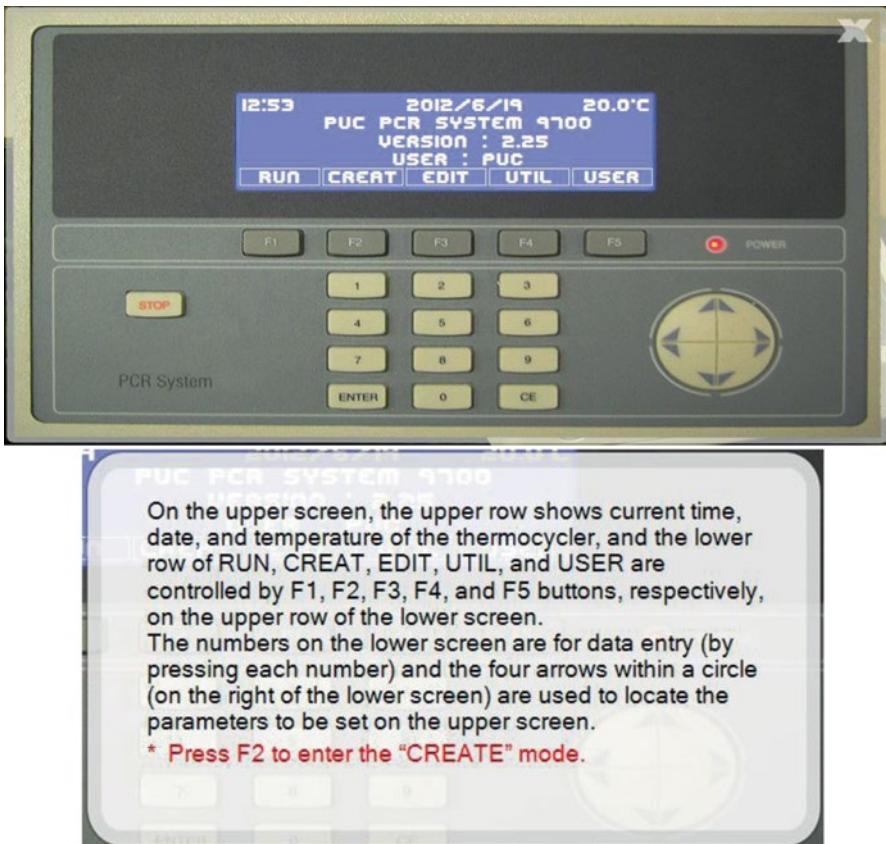
Step 12 Action: Place tube 6 into a well of the PCR plate



Step 13 Action: Close the thermocycler



Step 14 Action: Turn on the thermocycler

**Step 15** Action: Enlarge the LCD window**Step 16** Action: Set the PCR parameters according to the instructions

Setting conditions for running PCR. Suppose that you are ready to run your PCR with the following conditions set by you.

- 1) Number of cycles: 25.
 - 2) Initial holding temperature: 95°C for 5 minutes.
 - 3) The three temperatures for each cycle are 94°C, 55°C, 72°C, each for 30 seconds.
 - 4) Holding 72°C for 7 minutes at the end of the run followed by 4 cycles indefinitely until that you are ready to pick up the reaction tube for electrophoresis.
- * Use the "down" arrow to locate the initial holding temperature first...

Step 17 Action: Continue following the instructions

Press the "down" arrow to locate the initial holding temperature, a highlighted box will show up (on the left), press 9, 5, 0, and 0 at the lower screen to enter 95 in the box. To hold this initial temperature for 5 minutes, press the down arrow again to highlight the box below the 95°C box. The remaining settings are the same as yours; therefore, there is no need to readjust the conditions.

* Press F1 to run.

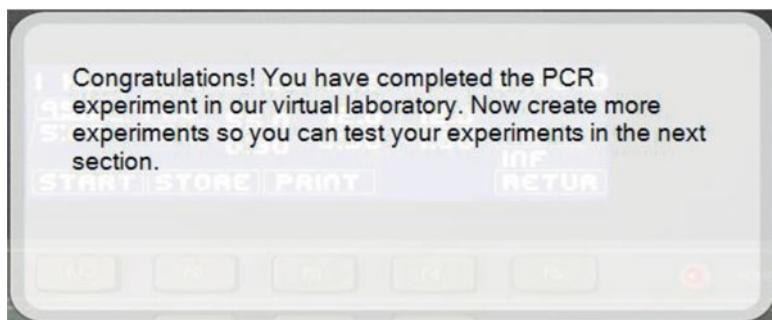
Step 18 Action: Continue following the instructions



Step 19 Results: Initial holding temperature is changed to 95.0°



Step 20 Results: Completion of PCR (stop)



Step 21 Results: Final message

- We have now seen the molecular process of PCR, and we now know how to calculate the theoretical number of copies generated after each cycle of PCR.
- We have also experienced the virtual PCR laboratory.
- The next question is: How do you visualize the products of PCR?
- The easiest way to visualize the PCR product is with a technique called agarose gel electrophoresis.

Agarose Gel Electrophoresis (*Interactive Program 3*)

- As was just mentioned, to visualize the DNA fragment amplified by PCR, we need to use another technique called agarose gel electrophoresis.

Use Interactive Program 3 on the software program available at Extras. Springer.com to familiarize yourself with agarose gel electrophoresis.

After completing the interactive program, you may continue reading the following text for further review or take the quiz.

- In agarose gel electrophoresis, a DNA sample is placed in a well at one end of a gel matrix (Steps 1–5)

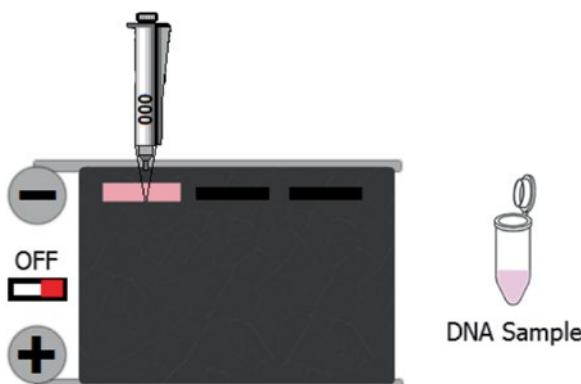


Step 1 Layout: *Left:* An electrophoresis chamber with an agarose plate containing three wells. *Right:* A DNA sample in a tube and a micropipette

- Use the pipette to pick up a predetermined amount of the DNA sample (Step 2).



Step 2 Action: Pick up DNA



Step 3 Action: Load DNA into a well

- The power source is then turned on to establish an electric field (negatively charged at the sample wells and positively charged at the bottom of the plate) (Step 4).
- *Click on the switch to turn it on* (Step 4).



Step 4 Action: Turn on power

- Since DNA is negatively charged, the DNA fragments in the sample move through the gel matrix toward the “+” pole.



Step 5 Results: Separation of DNA bands according to size

- Smaller fragments move faster and larger fragments move slower through the gel matrix.
- In this example, three DNA bands appear in the gel (Step 5).
- **If PCR is performed correctly, only one DNA band should appear: the PCR product.**
- The PCR product (with the expected fragment size) can be verified by comparing the position of the band with the position of bands of known molecular size (the DNA ladder) in the next lane.

Quiz

1. PCR, or polymerase chain reaction, is a process that uses the enzyme DNA polymerase to
 - (a) Repair damaged DNA in the laboratory
 - (b) Sequence DNA in the laboratory
 - (c) Amplify DNA in the laboratory
 - (d) Create a gene in the laboratory
2. The chemical components of PCR include
 - (a) DNA template, RNA primers, Taq polymerase, dNTPs
 - (b) DNA template, DNA primers, Taq polymerase, dNTPs
 - (c) DNA template, DNA primers, polymerase III, dNTPs
 - (d) DNA template, Taq polymerase, ligase, dNTPs
3. Each cycle of PCR requires three temperature settings. What temperature is most appropriate for DNA denaturation?
 - (a) 50 °C
 - (b) 65 °C
 - (c) 72 °C
 - (d) 94 °C
4. The appropriate temperature for primer annealing is normally a few (5) degrees lower than the primer's T_m (melting temperature), the temperature at which one half of the DNA is single stranded and the other half remains double stranded. If the T_m of a primer is 60 °C, what is the most appropriate annealing temperature for the primer?
 - (a) 45 °C
 - (b) 50 °C
 - (c) 55 °C
 - (d) 60 °C
5. To avoid random amplification of nonspecific DNA segments from a large genome (e.g., human genome, 3×10^9 bases long), the size of the PCR primers should be
 - (a) Very small (e.g., ≤ 5 bases long)
 - (b) Small (e.g., between 5 and 10 bases long)
 - (c) Large (e.g., ≥ 18 bases long)
 - (d) Very large (e.g., ≥ 30 bases long)
6. Suppose one DNA molecule undergoes amplification by PCR. After four cycles, how many desired (correctly sized) DNA fragments are produced?
 - (a) 2
 - (b) 4
 - (c) 8
 - (d) 16

7. To obtain good results from PCR, which of these steps must be followed?
 - (a) Pipette accurately
 - (b) Avoid contamination
 - (c) Increase the number of cycles to more than 40
 - (d) Two of the above
 - (e) Three of the above
8. The negative charge of DNA molecules is caused by their
 - (a) Phosphate groups
 - (b) Sugars
 - (c) Adenine bases
 - (d) Guanine bases
9. Agarose gel electrophoresis is commonly used to separate DNA fragments of
 - (a) The same size
 - (b) Different sizes
 - (c) More than 1 million base pairs
 - (d) Less than 10 base pairs
10. Because DNA fragments are (uniformly) negatively charged, they will migrate through a agarose gel matrix under an electric field according to the following rule:
 - (a) The smaller the DNA fragment, the faster it moves toward the positive electrode
 - (b) The smaller the DNA fragment, the faster it moves toward the negative electrode
 - (c) The larger the DNA fragment, the faster it moves toward the positive electrode
 - (d) The larger the DNA fragment, the faster it moves toward the negative electrode

Answers

1. c 2. b 3. d 4. c 5. c 6. c 7. d 8. a 9. b 10. a

Module 5

Packaging DNA into Chromosomes: How Do the Long Threads of DNA Fit into the Small Interphase Nucleus?

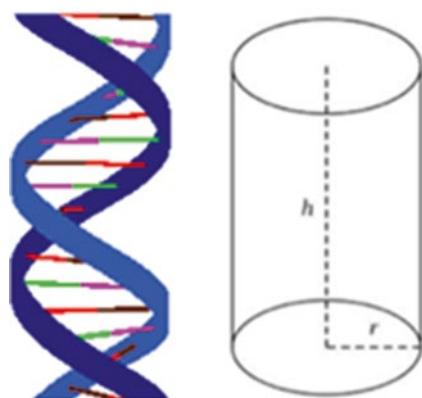
Let's Consider the Following Scenarios

- Human cells (and cells from most other species) are so small that they can only be seen with a microscope.
- Yet, if you connect all the DNA molecules in a single human cell at G₁ phase linearly, the total DNA length is approximately 2 m (see calculation later).
- And, if you connect all the DNA molecules in a single human cell at the end of the S phase linearly, the total DNA length is doubled (to about 4 m).
- How can the DNA molecules fit into a cell, let alone a small nucleus?
- To answer this question, we first need to do some calculations.
- Let us examine the size of the human genome in the cell.
- In the nucleus of each human cell, there are 46 chromosomes (in the stretched state called chromatin), of which 23 chromosomes constitute a genome (one whole set of DNA).
- There are two genomes per nucleus, one from each parent.
- Each genome contains approximately 3×10^9 base pairs, so there are 6×10^9 base pairs per nucleus in the G₁ phase.
- Each nucleotide of the base pair measures approximately 0.34 nm (3.4×10^{-10} m) in length (Fig. 5.1).
- Therefore, the total length of DNA in each nucleus (G₁ phase) is

$$2 \times (3 \times 10^9 \text{ nucleotide}) \times (3.4 \times 10^{-10} \text{ m / nucleotide}) \approx 2 \text{ m}$$

- However, the diameter of a typical nucleus is approximately 10×10^{-6} m, making the total length of DNA in the nucleus 200,000 times longer than the diameter of the nucleus:

$$2 \text{ m} \div (10 \times 10^{-6} \text{ m}) = 200,000$$

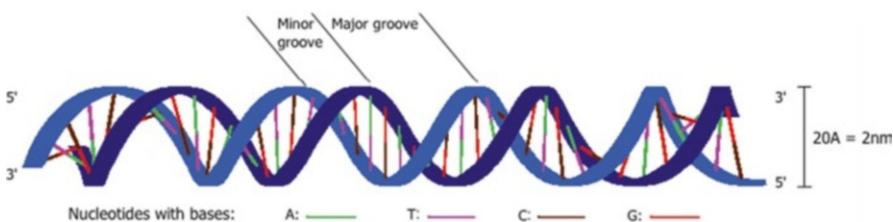
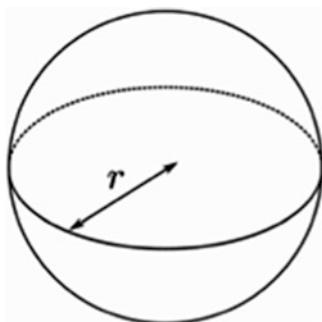
Fig. 5.1 DNA double helix**Fig. 5.2** DNA volume

- How can such long strings of DNA fit into the small nucleus?
- Clearly, the DNA must be folded.
- Let us examine how much space the DNA occupies in the nucleus if it is somehow folded so that it will fit.
- DNA exists in a double helix, which can be approximated by a cylinder with diameter 20 Å (2 nm or 20×10^{-10} m) (Fig. 5.2).

$$\pi r^2 h = 3.14159 \times (10 \times 10^{-10} \text{ m})^2 \cdot 2 \text{ m} = 6.4 \times 10^{-18} \text{ m}^3 \quad (\text{total volume of DNA})$$

- Assume that the diameter of a nucleus is approximately 10 μm (10^{-6} m), making the volume of the spherical nucleus (Fig. 5.3):

$$\frac{4}{3} \pi r^3 = \frac{4}{3} \times 3.14159 \times (5 \times 10^{-6} \text{ m})^3 = 5.24 \times 10^{-16} \text{ m}^3 \quad (\text{Volume of a nucleus})$$

Fig. 5.3 Nucleus volume**Fig. 5.4** DNA model (double helix with alternating major and minor grooves)

- Consequently, the fraction of the nucleus occupied by DNA is

$$(6.4 \times 10^{-18} \text{ m}^3) \div (5.24 \times 10^{-16} \text{ m}^3) \times 100 = 1.22 \%$$

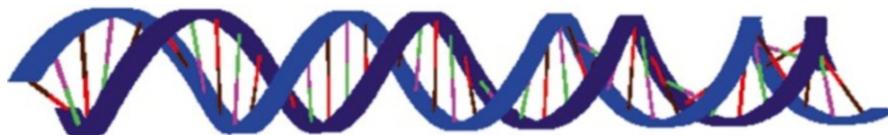
- There is clearly enough room in the nucleus for housing DNA and its activities, including packaging and unpackaging (e.g., nucleosome dissociation and association) and gene expression (e.g., dissociation of nucleosomes and protein-DNA complex formation for synthesis of RNA as well as RNA processing).
- Having reviewed the foregoing scenarios, we are now ready to focus on our main topic: **How is the DNA folded (organized) in the cell nucleus?**
- Let us look at the DNA double helix next (Fig. 5.4).
- Note that the major and minor grooves alternate along the molecule (Fig. 5.4).
- In the major grooves, the DNA sugar-phosphate backbone is spread out (more DNA bases are exposed), which allows the DNA to interact with proteins.

Three-Dimensional (3D) View of DNA (*Interactive Program 1*)

Use **Interactive Program 1** on the software program available at Extras.Springer.com to familiarize yourself with the 3D DNA structure.

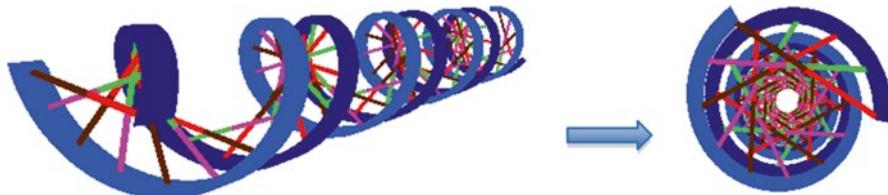
After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “Nucleosomes and Linker DNA.”

- To view the dynamic nature of DNA, press the  button to start the 3D animation (Step 1).



Step 1 Layout: 3D view of DNA

- Use the mouse to move the DNA; move it clockwise to see a cross section of the molecule (Steps 2 and 3).



Steps 2 and 3 Action: Move DNA clockwise and back

- Move the DNA counterclockwise to return it to the original position (Step 1).
- To better visualize the DNA packaging process, the molecule is now reduced to a single line (Step 4).

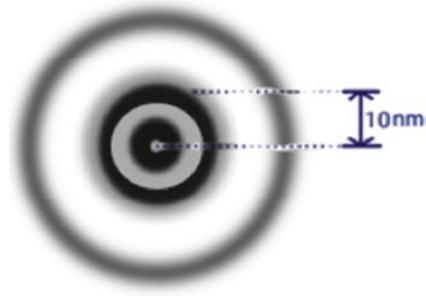


Step 4 Action: Reduction of DNA size

Nucleosomes and Linker DNA (*Interactive Program 2*)

- DNA associates with proteins called histones to form chromatin. Histones are basic proteins (positively charged). There are five different kinds of histones: H1, H2A, H2B, H3, and H4.
- The X-ray diffraction image of chromatin (Fig. 5.5) has a repeating pattern of 10-nm (100-Å) intervals, which is different from the repeating pattern of the DNA double helix.

Fig. 5.5 X-ray diffraction pattern of chromatin



- What does the 10-nm chromatin look like?
- Figure 5.6 shows a chromatin fiber with the beads on a string structure (beads are nucleosomes and the string is DNA).



Fig. 5.6 A 10-nm chromatin fiber (DNA + plus nucleosomes)

- Nucleosomes (beads) are histones complexes wrapped by DNA, and the DNA between nucleosomes is called linker DNA (string).
- How do we know that?

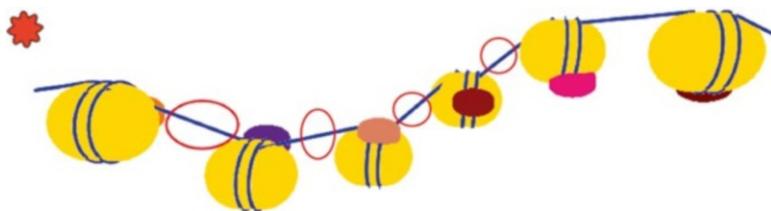
Length of DNA Between Nucleosomes

- The enzyme micrococcal nuclease (MNase) is used to digest the chromatin.
- Represented below (Step 1) is a chromatin fiber.

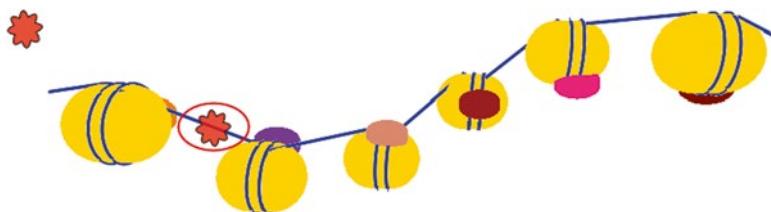
[Use Interactive Program 2 on the software program available at Extras. Springer.com to familiarize yourself with the enzyme digestion experiment.](#)

[After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section Fig. 5.8 “DNA bands separated by electrophoresis.”](#)

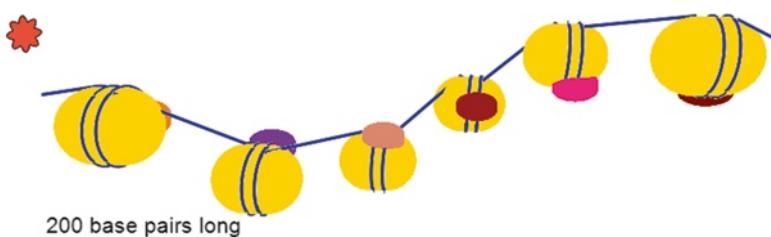
- “” represents the enzyme MNase, and the red circles along the DNA represent the linker (unprotected by histones) to be digested by MNase. Let us practice the experiment using an enzyme called micrococcal nuclease (MNase) to digest the chromatin.



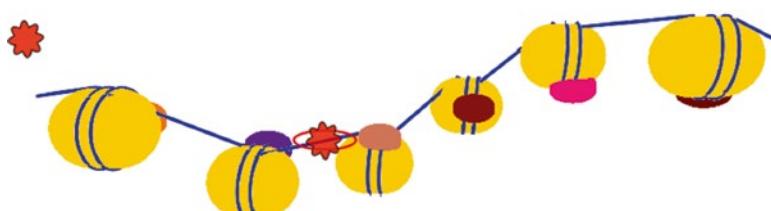
Step 1 Layout: A 10-nm chromatin fiber



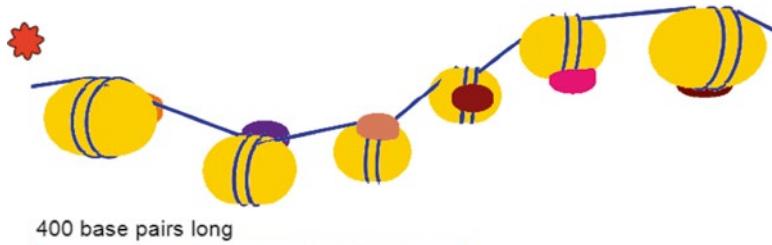
Step 2 Action: Cleavage at the first DNA linker



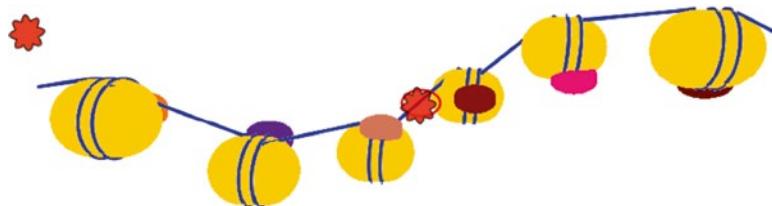
Step 3 Results: Generation of a 200-nm DNA fragment



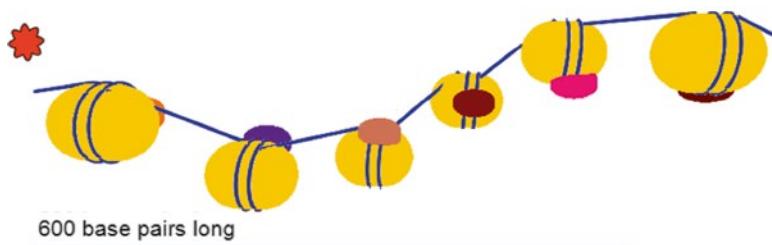
Step 4 Action: Cleavage at the second linker DNA



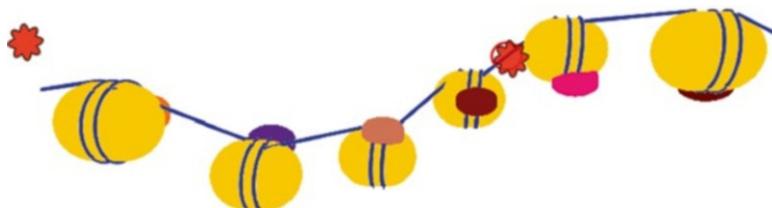
Step 5 Results: Generation of a 400-nm DNA fragment



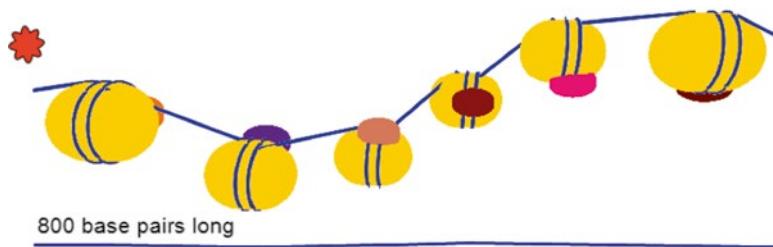
Step 6 Action: Cleavage at the third linker DNA



Step 7 Results: Generation of a 600-nm DNA fragment



Step 8 Action: Cleavage at the fourth linker DNA



Step 9 Results: Generation of a 800-nm DNA fragment

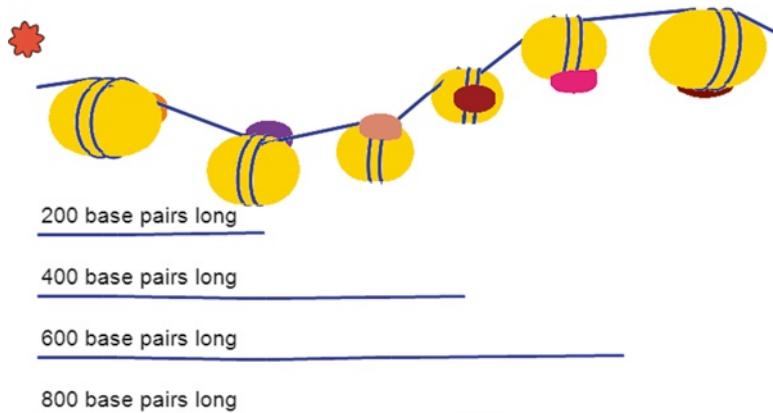
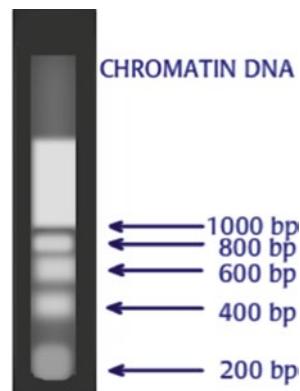


Fig. 5.7 Results of the MNase digestion experiment

- Drag the enzyme to the designated locations (red circles), one by one, to digest the fiber (Steps 2–9).
- The MNase digestion results are summarized below (Fig. 5.7).
- The DNA fragments can be separated in an electrophoresis gel (Fig. 5.8).
- Clearly, a segment of DNA 200 bases long is needed to wrap the histone complex and link two nucleosomes.
- These results suggest that histones somehow protect the DNA from being completely digested. Because the fragments from the nuclease-treated chromatin are multiples of 200 bp (200, 400, 600, 800 bp ...), it stands to reason that the histones are evenly distributed on the DNA, with only the unprotected linker DNA digested by the enzyme.

Fig. 5.8 DNA bands separated by electrophoresis

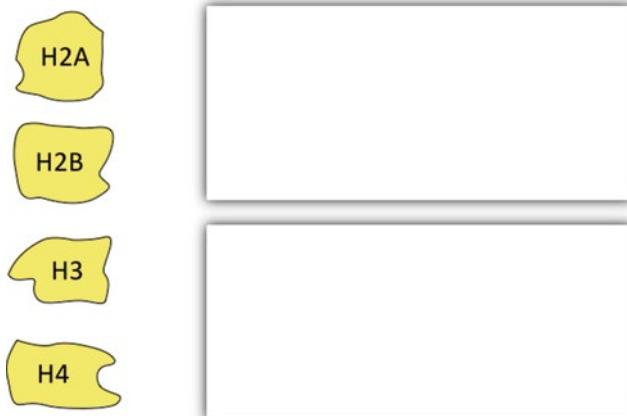


Histones and Higher Levels of DNA Packaging (*Interactive Program 3*)

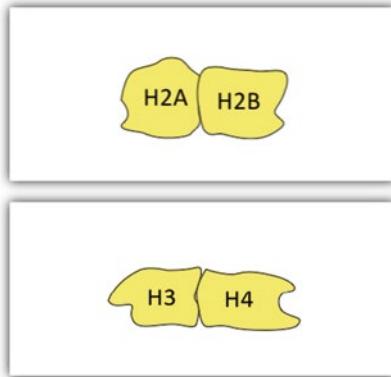
Use Interactive Program 3 on the software program available at Extras.Springer.com to familiarize yourself with the histones and DNA packaging.

After completing the interactive program, you may continue reading the following text for further review or take the quiz.

- How are the histone molecules arranged?
- When the histones were purified, it was found that H2A and H2B tended to stick together, as did H3 and H4.
- *Drag H2A and H2B to the upper box, and drag H3 and H4 to the lower box (Step 1).*

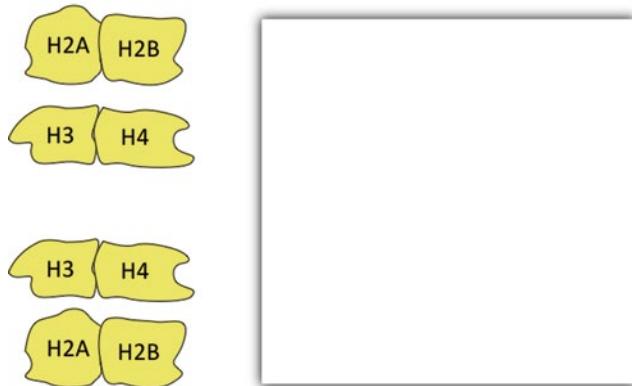


Step 1 Layout

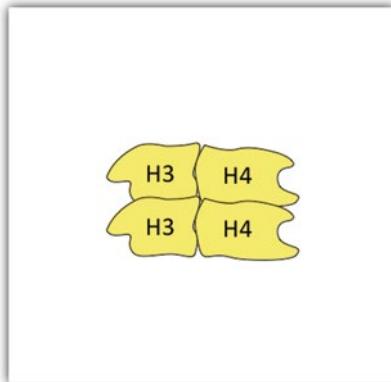


Step 2 Action: Association of histones: H2A–H2B and H3–H4

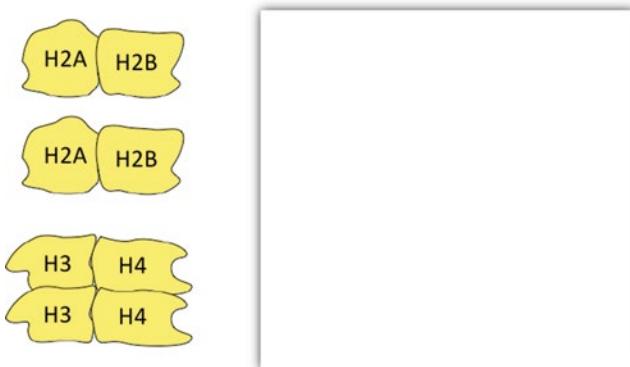
- In fact, cross-linking studies showed that H2A and H2B form a heterodimer, whereas H3 and H4 form a heterotetramer, resulting in two H2A–H2B heterodimers and one H3–H4 tetramer per core nucleosome.
- *Drag the two H3–H4 dimers to the window (Steps 3 and 4).*

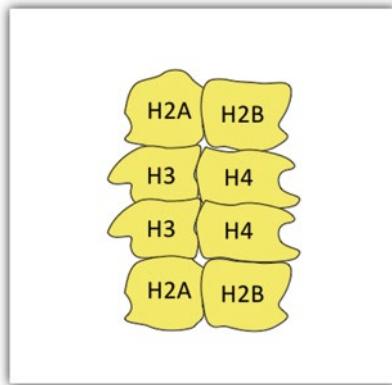


Step 3 Action: Move two H3–H4 heterodimers to form a heterotetramer

**Step 4** Results: A heterotetramer

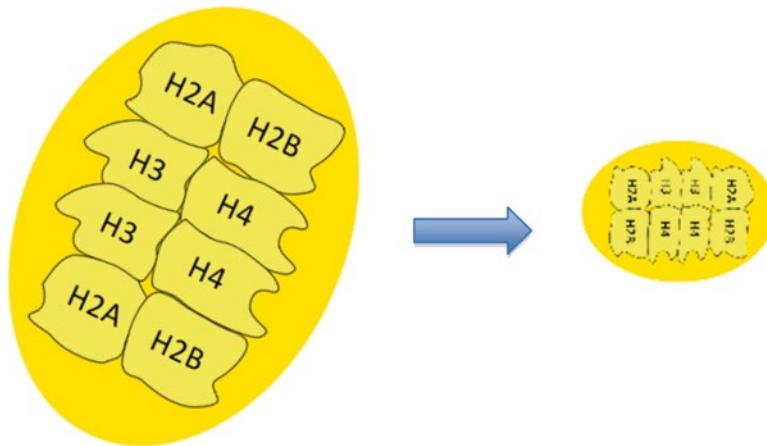
- At high salt concentrations, a mixture of one H3–H4 tetramer and two H2A–H2B dimers forms an octamer.
- The H2A–H2B dimer binds to the H3–H4 tetramer as the result of interactions between H4 and H2B, so that the H3–H4 tetramer is sandwiched between the two H2A–H2B dimers.
- *Drag the two dimers and the one tetramer to the window* (Steps 5 and 6).

**Step 5** Layout: Two dimers and one tetramer



Step 6 Results: Formation of the histone octamer (the H3–H4 tetramer is sandwiched between two H2A–H2B dimers)

- The size of the histone octamer is now reduced to better visualize DNA packaging (step 7).



Step 7 Action: Reduction of histone octamer size for viewing higher levels of packaging

- The histone octamers are now to the right of the DNA duplex, which is represented by a single line (Step 8).



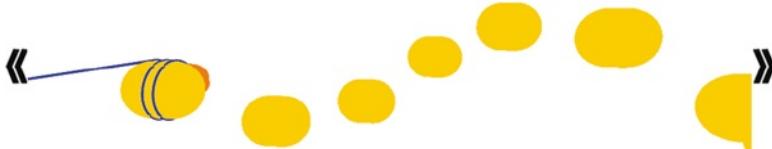
Step 8 Layout: DNA plus histone octamers

- Click the » sign four times to see more histone octamers (Steps 8 and 9).



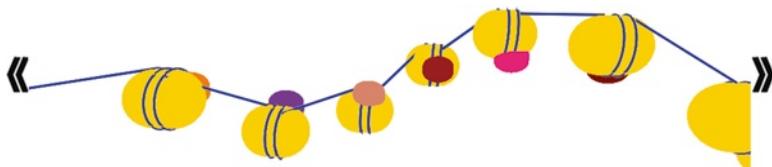
Step 9 Results: DNA plus six histone octamers

- Click to see the DNA wrap around a histone octamer: H1 (in various colors) binds to the resulting nucleosome to stabilize it (Steps 10 and 11).



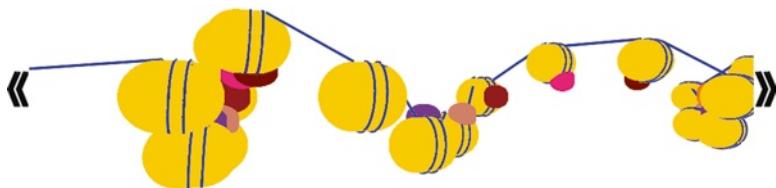
Step 10 Action: DNA wraps around the octamer

- Click again until all the histone octamers are wrapped by DNA (Steps 10 and 11).



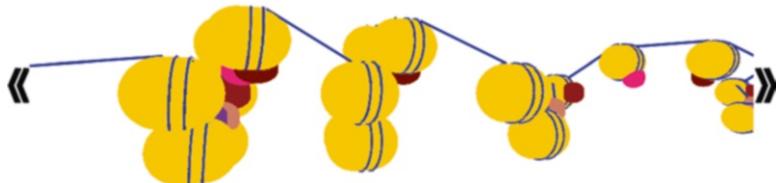
Step 11 Action: Continuation of Step 10

- Click to see the first six nucleosomes form a solenoid through H1 interactions (Step 12).



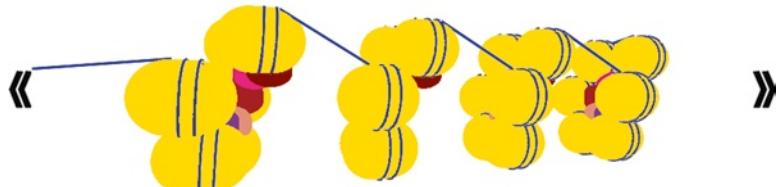
Step 12 Action: Linking of six histone octamers through H1 interactions

- Click again to see the next six H1 histones form a solenoid (Step 13).



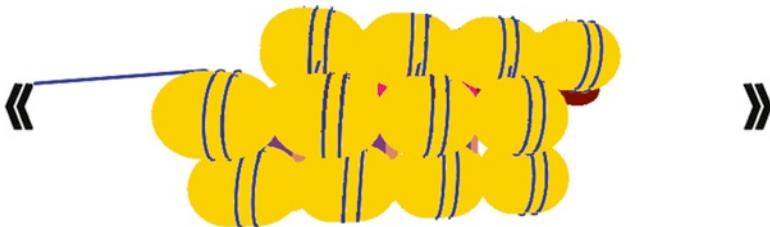
Step 13 Action: Formation of spiral turns

- Continue to click to see the formation of other spiral turns (Step 14).



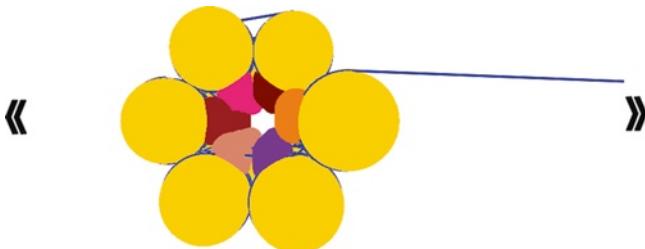
Step 14 Action: Continued formation of spiral turns

- Clearly, six nucleosomes form a solenoid (Step 14).
- Click  to see four solenoids come together (Step 15).



Step 15 Results: Joining of four solenoids to form a 30-nm chromatin fiber

- In cross section, you can see that six nucleosomes (one spiral turn) are brought together by the H1 histones (Step 16).



Step 16 Results: Cross section of a 30-nm chromatin fiber

- The 30-nm chromatin fiber is now reduced to a single line to visualize higher levels of packaging: looping (Step 17).



Step 17 Results: Reduction of 30-nm chromatin fiber

- In metaphase, the DNA has been replicated, so that each chromosome consists of two sister chromatids, each containing a copy of the DNA molecule: this action is possible because of looping of the 3-nm chromatin fibers (Step 18).



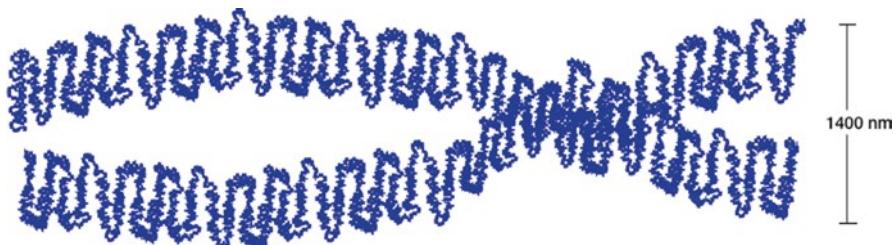
Step 18 Action: Looping of chromatin

- The looped chromatin is now reduced to a line (Step 19) to show another level of packaging, representing the highly condensed state of a metaphase chromosome.



Step 19 Action: Another level of looping

- Finally, a metaphase chromosome (with two chromatids), which represents the highest level of DNA packaging, is formed (Step 20).



Step 20 Results: Metaphase chromosome: the highest level of DNA packaging

Summary: Different Levels of DNA Packaging Are Shown in Fig. 5.9

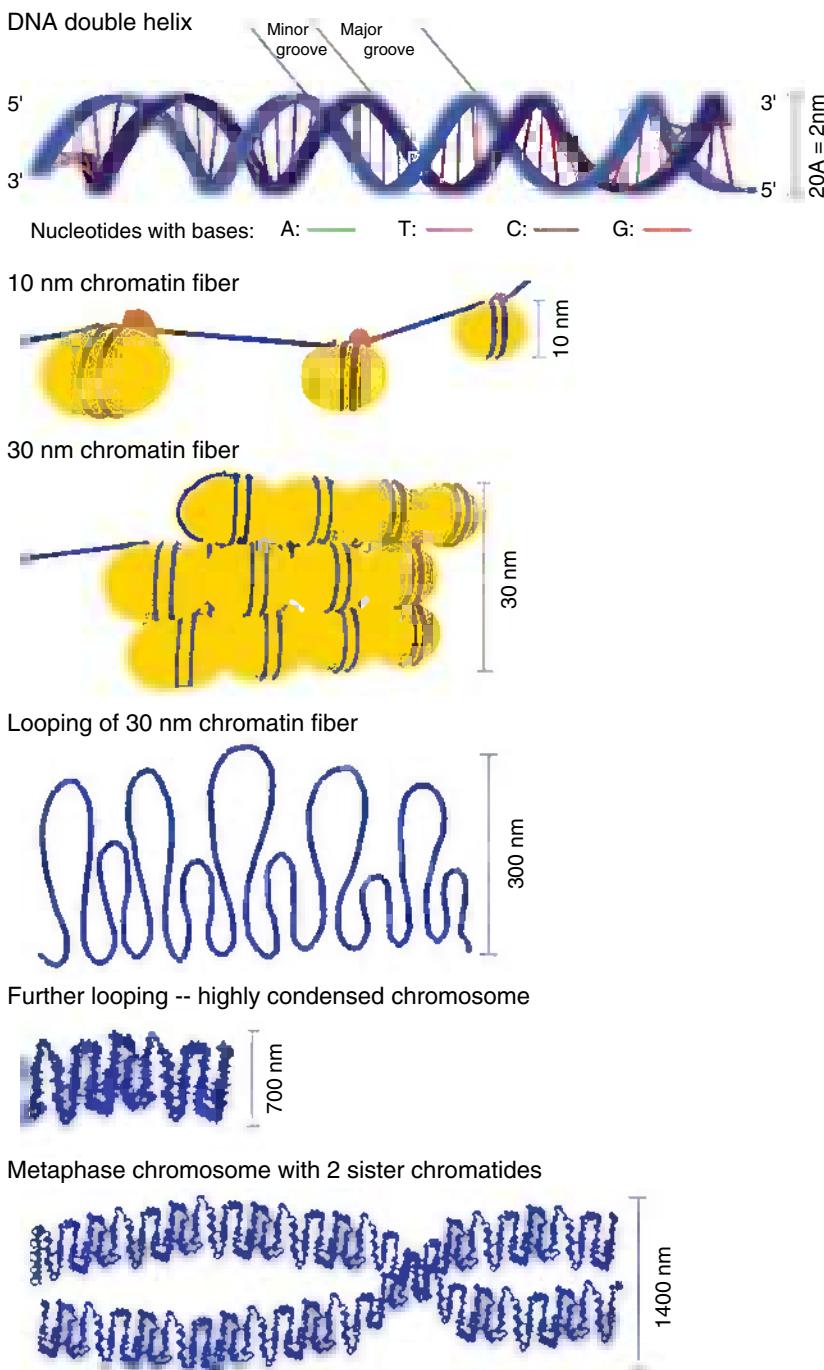


Fig. 5.9 Summary: Different levels of DNA packaging

Quiz

1. In the first level of packaging, the DNA double helix wraps around a group of basic proteins called
 - (a) Histones
 - (b) Enzymes
 - (c) Cytoskeletons
 - (d) Transcription factors
2. A histone octamer comprises two of each of the following molecules:
 - (a) H1, H2, H3, and H4
 - (b) H2, H3, H4, and H5
 - (c) H2A, H2B, H3, and H4
 - (d) H2, H3A, H3B, and H4
3. Which of the following histones binds to both the nucleosome and linker DNA, stabilizing both the 10- and 30-nm chromatin fibers?
 - (a) H1
 - (b) H2A
 - (c) H3
 - (d) H4
4. When 10-nm chromatin is subjected to micrococcal nuclease digestion, the DNA fragments that are generated are multiples of how many base pairs?
 - (a) 30
 - (b) 80
 - (c) 150
 - (d) 200
5. Euchromatin, which contains potentially active genes, is approximately
 - (a) 5 nm thick
 - (b) 10 nm thick
 - (c) 20 nm thick
 - (d) 30 nm thick
6. Heterochromatin, which contains inactive genes, is approximately
 - (a) 5 nm thick
 - (b) 10 nm thick
 - (c) 20 nm thick
 - (d) 30 nm thick
7. During the formation of the 30-nm chromatin fiber, how many nucleosomes join together to form one spiral turn?
 - (a) 5
 - (b) 6
 - (c) 7
 - (d) 8

8. The formation of 30-nm chromatin is facilitated by
 - (a) H2A and H2B interactions
 - (b) H3 and H4 interactions
 - (c) H1 interactions
 - (d) H3 interactions
9. Through different levels of looping, the 30-nm chromatin fibers are packed together such that genomic DNA reaches its most condensed state during
 - (a) Prophase
 - (b) Metaphase
 - (c) Anaphase
 - (d) Telophase
10. During prophase of mitosis, a chromosome consists of how many chromatids?
 - (a) 1
 - (b) 2
 - (c) 3
 - (d) 4

Answers

1. a 2. c 3. a 4. d 5. b 6. d 7. b 8. c 9. b 10. b

Module 6

Mitosis: How Are the Chromosomes of the Parent Cell Evenly Parceled Out to the Daughter Cells?

What Is Mitosis?

- As we have learned in [Module 3](#), a eukaryotic cell cycle consists of four phases: G₁, S, G₂, and M.
- The first three phases comprise what is known as interphase, a state in which chromosomes are stretched in the form of chromatin fibers, which cannot be seen under a light microscope.
- In the M phase, mitosis, a process in which the sister chromatids separate and the nucleus divides, and cytokinesis, a process in which the cytoplasm divides, occur.
- Because a diploid cell contains two sets of chromosomes (two genomes, one from each parent), mitosis ensures that the DNA content of the original cell is properly separated and equally distributed to the daughter cells.
- You may recall that during the S phase each chromosome is duplicated, forming a new chromosome with two sister chromatids, which become visible microscopically early in the M phase.
- The key function of mitosis is to separate the sister chromatids and deliver them to the two daughter cells, reducing the DNA content from four genomes to two genomes.

Terminology

- At this point we need to define some important terms, which we use frequently to describe mitosis, as well as meiosis (in a later module).
- Ploidy:** Ploidy refers to the number of sets of chromosomes that a cell contains. A cell with two sets of chromosomes (one set from the father and the other set from the mother) is called a diploid cell. We use 2N to designate a diploid cell, such as a somatic (body) cell. Haploid cells are gametes (sperm or egg cells), which contain only one set of chromosomes (1N). Polyploid cells have three (triploid) or more sets of chromosomes.

2. **Homologous chromosomes (homologs or homologues):** Homologous chromosomes are members of the same chromosome pair: they are similar in size, centromere position, and staining pattern. The genes for the same traits are located at corresponding loci on the chromosomes. One homologous chromosome is inherited from the organism's mother; the other is from the organism's father. In humans, there are 22 pairs of homologous chromosomes (known as autosomes because they are not directly involved in sex determination), plus a pair of sex chromosomes: XX for female and XY for male. X chromosomes are larger than Y chromosomes, and only small regions at the tip of the X and Y chromosomes are homologous.

Also, homologous chromosomes physically pair (synapse) during prophase I and metaphase I of meiosis, the type of cell division that leads to the formation of gametes.

3. **Chromatids:** after the S phase, DNA content is doubled. As a result of DNA replication, each chromosome now contains two sister chromatids, each containing a DNA duplex. A homologous chromosome pair, therefore, consists of four chromatids or four DNA duplexes (see below). Chromatids belonging to the same chromosome (joined by a centromere) are called sister chromatids, whereas chromatids from homologous chromosomes are called non-sister chromatids. Thus,

$$\begin{aligned} \text{1 homologous chromosome pair} &= 2 \text{ chromosomes} \\ &= 4 \text{ chromatids} = 4 \text{ DNA molecules} \end{aligned}$$

4. **Genome:** The total DNA content of a gamete is a genome. For example, a set of 23 human chromosomes constitutes a genome. In the G₁ phase, a cell contains two genomes, but after the S phase, the cell contains four genomes (i.e., there are four copies of each gene per cell). After mitosis and cytokinesis, each daughter cell ends up with two genomes in the G₁ phase.

Sister Chromatids versus Non-Sister Chromatids (Interactive Program 1)

[Use Interactive Program 1 on the software program available at Extras.Springer.com to familiarize yourself with chromatids within the same chromosome and chromatids between homologous chromosomes.](https://extras.springer.com)

[After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “Additional Terminology.”](#)

- Below is a chromosome pair. Can you tell which are sister chromatids and which are non-sister chromatids?

Click on any two chromatid numbers, one at a time, to check your answer (Steps 1–3).



Step 1 Layout: A pair of homologous chromosomes, each with two chromatids



Step 2 Action and Results: 2 and 4 are non-sister chromatids



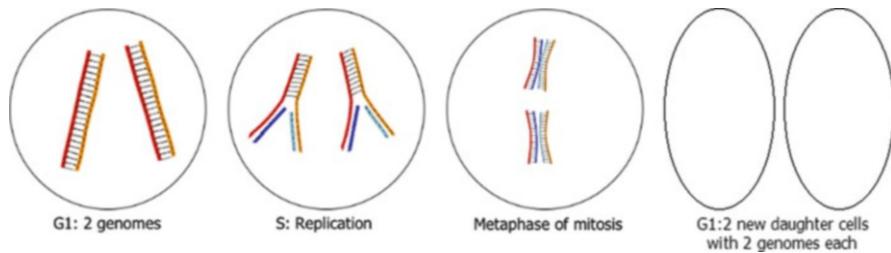
Step 3 Action and Results: 1 and 2 are sister chromatids

Separation of Chromatids and Equal Distribution of Chromosomes (*Interactive Program 2*)

Use Interactive Program 2 on the software program available at Extras.Springer.com to familiarize yourself with changes in genome number.

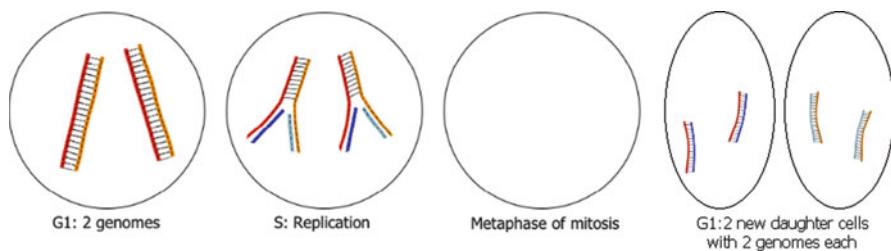
After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “Additional Terminology.”

- For simplicity, we use a pair of homologous chromosomes to represent two genomes (i.e., one genome = one chromosome). Also, we use a DNA duplex to simulate a chromosome (in G₁) or a chromatid (in metaphase).
- DNA replication in the S phase changes the number of genomes from two to four (Step 1).



Step 1 Layout: Change of genomic number during S phase

- Drag each chromatid in metaphase to a daughter cell (Step 2), so that each daughter cell ends up with two genomes.



Step 2 Results: Returning of genome number to two (2) after mitosis

Additional Terminology

- Allele:** In a diploid cell, there are two copies of each gene (in G₁ phase), one from each parent. The two copies of the gene may be identical (have identical DNA sequences) or be slightly different (have somewhat different DNA sequences). The different forms of a gene are called alleles. If the two alleles in a diploid cell in the G₁ phase are the same, then the alleles are homozygous. If the two alleles are different, then they are heterozygous.
- Wild-type alleles versus mutant alleles:** Because a gene in a population of many individuals may have many different alleles (caused by mutations), each allele may be represented by a symbol: a_1 , a_2 , a_3 , etc. We pick the allele with the greatest frequency in the population to be the wild type (a^+); the rest of the alleles are known as mutant alleles.
- Genotype versus phenotype:** The genotype of an organism refers to its particular set of genes: it represents the organism's exact genetic makeup. For example, in a diploid organism, a particular trait might be represented by A/A , each letter representing a gene, and two gene pairs might be represented by A/A ; B/B . The phenotype of an organism refers to its actual, observed properties, ranging from morphology to physiology to behavior. The phenotype results from the expression of the organism's genes in a particular environment.
- Dominance versus recessiveness:** In a diploid cell, if the two alleles of a gene pair are different (heterozygous), then the allele that determines the phenotype (the allele which is expressed) is considered dominant over the unexpressed allele. The unexpressed allele is considered recessive. We use capital letters to indicate dominant alleles and lowercase letters to indicate recessive alleles. If both alleles are expressed (such as in someone with blood type AB), the alleles are codominant. If the expression of both alleles results in an intermediate phenotype, then there is incomplete dominance (e.g., pink flowers resulting from a cross between red flowers and white flowers).

Chromosome Behavior and the Genetic Consequences of Mitosis (*Interactive Program 3*)

- The different stages of mitosis in white fish cells (Fig. 6.1) are pictured below. As each stage is described in the following pages, match the description with one of the micrographs.

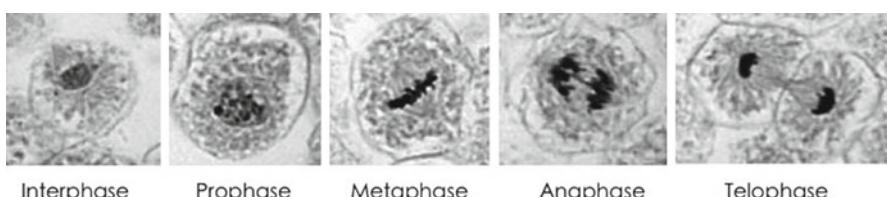


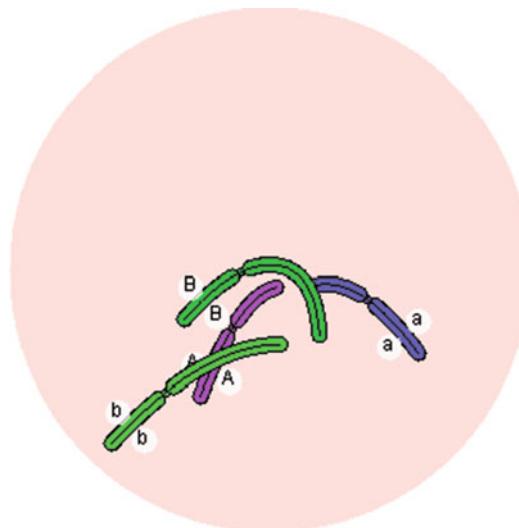
Fig. 6.1 Interphase and mitotic phases

1. Interphase (G_1 , S, or G_2): Chromosomes are stretched out and not visible microscopically.
 2. Mitosis–prophase: Chromosomes (each consisting of two sister chromatids) thicken and become recognizable; the nuclear envelope begins to break down.
 3. Mitosis–metaphase: Chromosomes move to the equatorial plane of the cell; nuclear envelope completely breaks down.
 4. Mitosis–anaphase: Sister chromatids of each chromosome separate and move to opposite poles of the cell; each chromatid is now considered a chromosome with its own centromere.
 5. Mitosis–telophase: Chromosomes begin to stretch and a nuclear envelope is reformed around each daughter nucleus. Cytokinesis follows, creating two daughter cells in the G_1 phase, ready to start a new cell cycle.
- We use two examples to illustrate chromosome behavior during mitosis:

Use Interactive Program 3 on the software program available at Extras. Springer.com to familiarize yourself with chromosome behavior and genetic consequences of mitosis.

After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “Conclusions.”

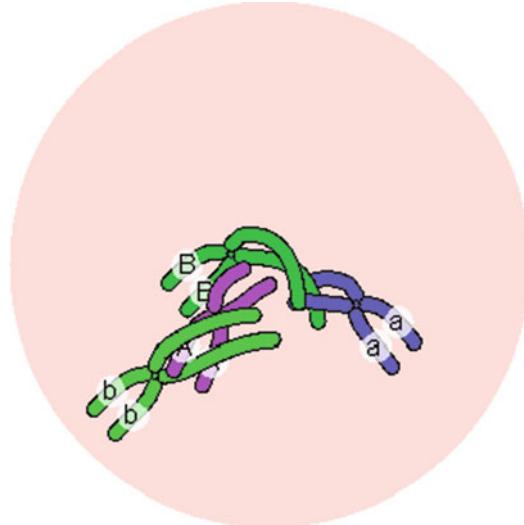
- **Example 1:** A cell with chromosome number $2N=4$. We will follow two pairs of heterozygous genes (A/a ; B/b) through mitosis. Note that these gene pairs are located on different chromosome pairs, so we call them unlinked genes.
- **Early prophase:** *Use the mouse to untangle each chromosome, if needed.* Can you see the two homologous chromosome pairs (Step 1)? The dark and light purple chromosomes are homologous to each other, carrying the allelic genes A and a , respectively. Similarly, the dark and light green chromosomes are homologous,



Step 1 Layout: Early prophase: two homologs, $2N=4$

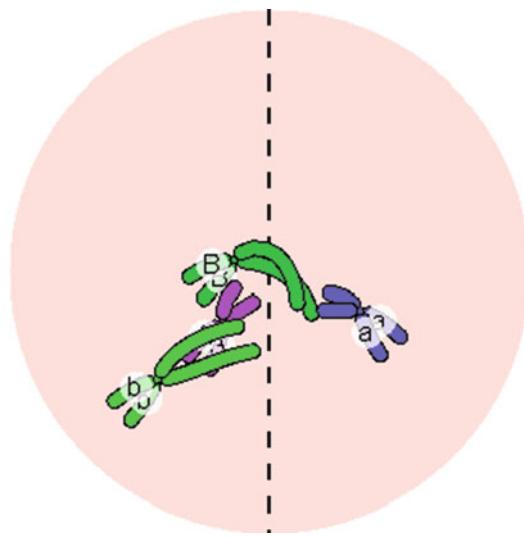
carrying allele *B* and allele *b*, respectively. Because each chromosome consists of two sister chromatids, there are really two copies of each gene (*AA*)/(*aa*); (*BB*)/(*bb*).

- **Late prophase:** Chromosomes continue to shorten and the sister chromatids become more distinguishable (Step 2). Note that each chromosome behaves independently (there is **no physical pairing between homologous chromosomes**). Use the mouse to untangle chromosomes, if needed.

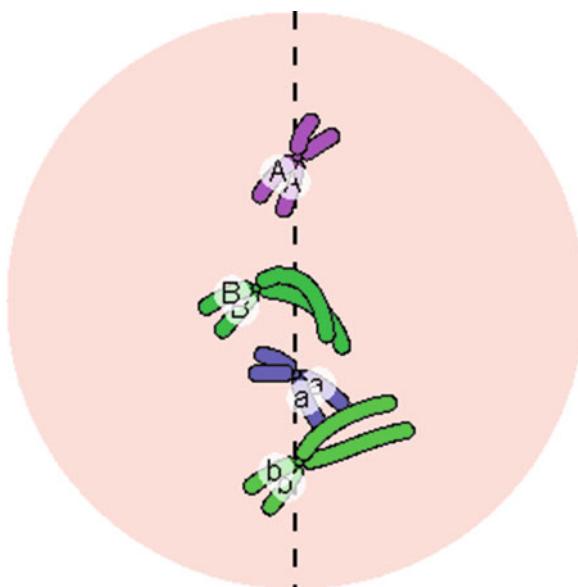


Step 2 Layout: Late prophase

- **Metaphase:** Drag the chromosomes by the centromeres to the equatorial plane (Steps 3 and 4). Note that the distribution of chromosomes at the equator is random; just the centromeres need to be accurately lined up.

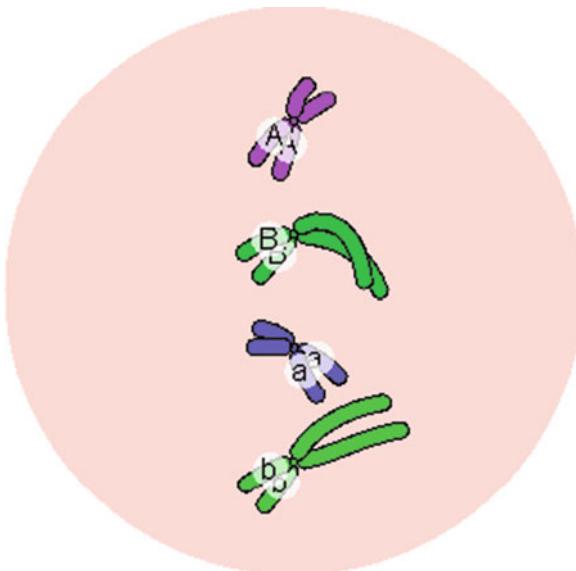


Step 3 Action: Move chromosomes to the equatorial plane

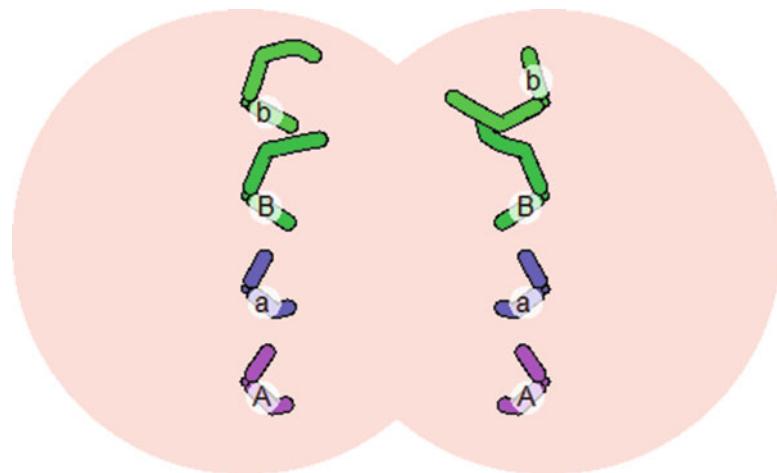


Step 4 Results: Centromeres aligned at the equatorial plane

- **Anaphase:** Before anaphase, the centromere of each chromosome resolves into two functional centromeres. *Click on each centromere to separate the sister chromatids* (Step 5). Note that each chromatid (once separated) is called a chromosome (Step 6).

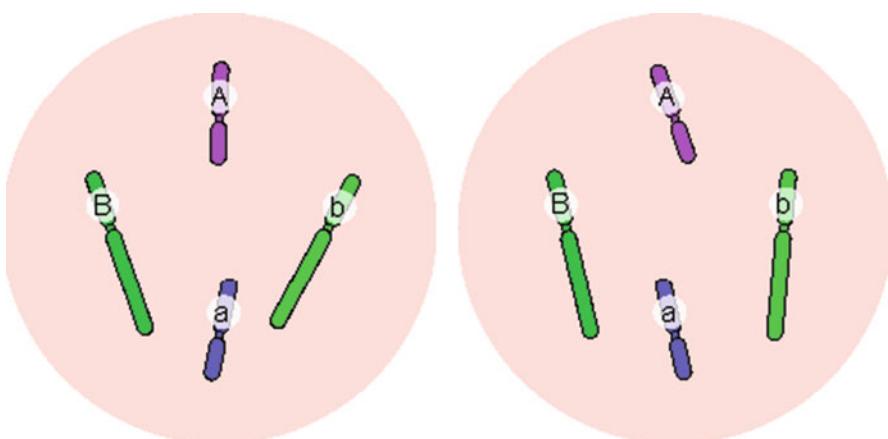


Step 5 Action: Click on each centromere to start chromatid separation



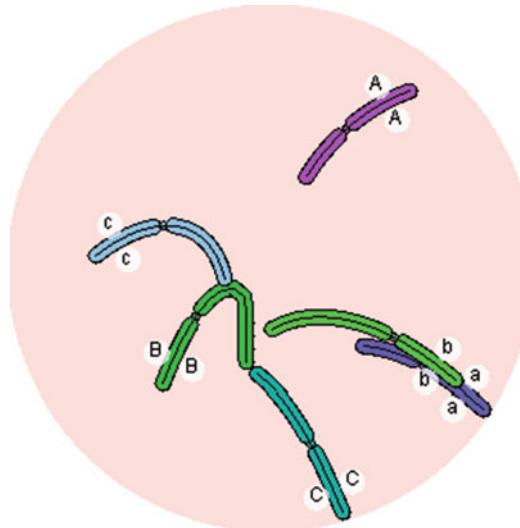
Step 6 Results: Anaphase: each chromatid is now a chromosome

- **Telophase:** In telophase, the chromosomes stretch and unpack while a new nuclear envelope (not shown) forms around each daughter nucleus. After cytokinesis (separation of cytoplasm), two daughter cells are formed, each entering the G₁ phase with genotype A/a; B/b (Step 7). Therefore, the daughter cells contain the same number of chromosomes (2N=4), and have the same genotype (A/a; B/b) as the parent cell.



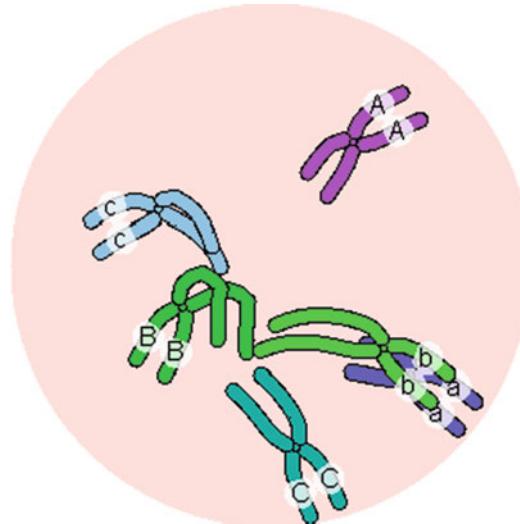
Step 7 Results: Telophase and formation of two daughter cells

- Example 2:** A cell with chromosome number $2N=6$. We will follow three pairs of heterozygous genes (A/a ; B/b ; C/c) through mitosis. Note again that these gene pairs are located on different chromosome pairs, so we call them unlinked genes (Step 8).



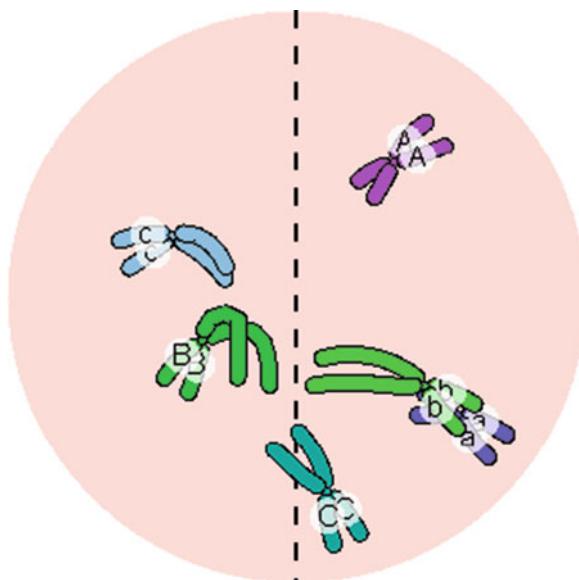
Step 8 Layout: Early prophase of a cell with $2N=6$

- Move the chromosomes as you did in the previous example (Steps 9–13). The resulting daughter cells should both have the genotype A/a ; B/b ; C/c .

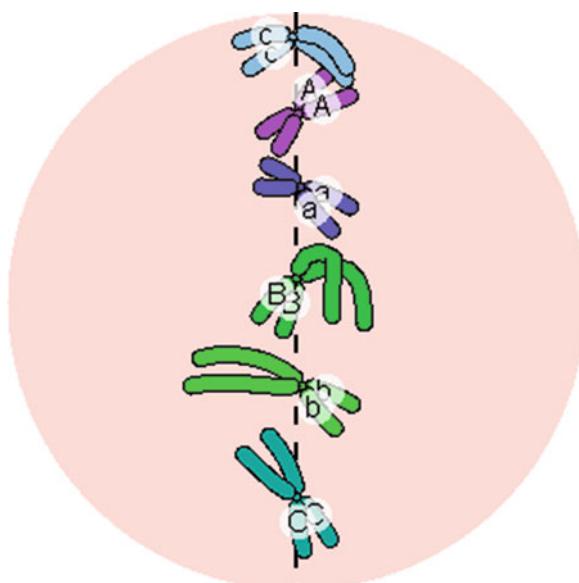


Step 9 Action: Late prophase

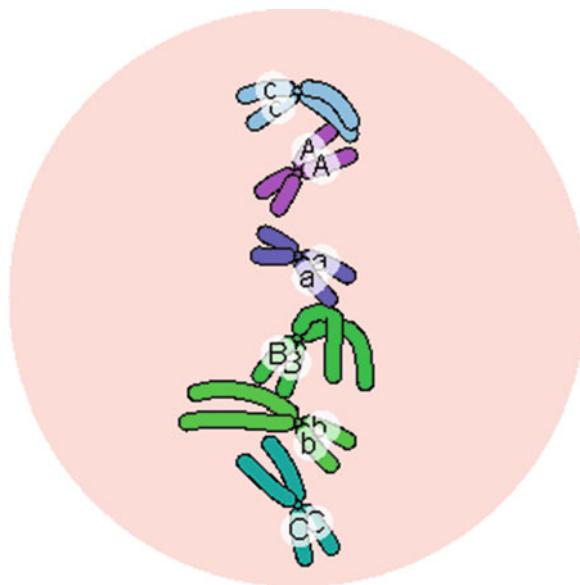
- Drag the centromere of each chromosome to the equator (line) (Steps 10 and 11).



Step 10 Action: Move chromosomes to the equator

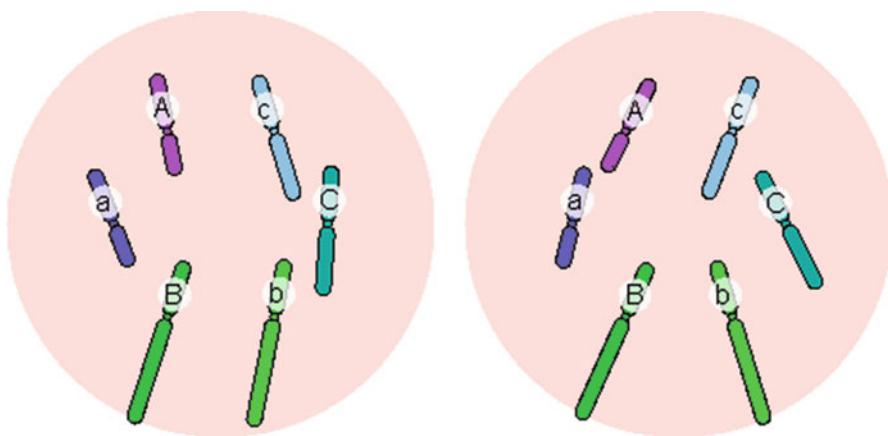


Step 11 Results: Metaphase: Chromosomes at the equator



Step 12 Action: Separation of sister chromatids to enter anaphase

- The daughter cells contain the same number of chromosomes ($2N=6$), and have the same genotype (A/a ; B/b ; C/c), as the parent cell (Step 12).



Step 13 Results: Telophase: formation of daughter cells

Conclusions

- Mitosis ensures the equal separation of sister chromatids, so that the number of genomes remains constant.
- Mitosis ensures that the same genotype is maintained from generation to generation, so that there is continuity in DNA content.
- Mitosis ensures the equal separation and distribution of genes.
- In contrast to meiosis (during gamete formation), homologous chromosomes do not physically pair at prophase or metaphase in mitosis.

Quiz

1. Which of the following cells divide by mitosis?
 - (a) Bacterial cells
 - (b) Plant leaf cells
 - (c) Animal liver cells
 - (d) Two of the above
2. Which of the following is true about mitosis?
 - (a) Homologous chromosomes physically pair during prophase.
 - (b) Crossing over between non-sister chromatids occurs during prophase.
 - (c) Chromosomes are individually aligned at the equatorial plane of the cell during metaphase.
 - (d) Each chromosome contains a single DNA molecule during prophase and metaphase.
3. Each chromosome is duplicated to form two sister chromatids during
 - (a) G1
 - (b) S
 - (c) G2
 - (d) M
4. When does a human cell contain four genomes?
 - (a) During G1
 - (b) During G2
 - (c) After M
 - (d) b and c
5. There are 46 chromosomes in a human somatic cell. How many chromosomes constitute a human genome?
 - (a) 12
 - (b) 23
 - (c) 46
 - (d) 92

6. Sister chromatids

- (a) Are identical
- (b) Lie on homologous chromosomes
- (c) Lie on the same chromosome
- (d) a and c

7. Sister chromatids separate during

- (a) Prophase
- (b) Metaphase
- (c) Anaphase
- (d) Telophase

8. During metaphase of mitosis, how many physically paired homologs in a human cell do you expect to see at the equatorial plane of the cell?

- (a) 0
- (b) 23
- (c) 46
- (d) 92

9. Chromatids that share the same centromere are called

- (a) Non-sister chromatids
- (b) Sister chromatids
- (c) Homologous chromatids
- (d) Nonhomologous chromatids

10. A cell that contains two genomes during the G₁ or G₀ phase is called a

- (a) Haploid cell
- (b) Diploid cell
- (c) Triploid cell
- (d) Tetraploid cell

Answers

1. d 2. c 3. b 4. b 5. b 6. d 7. c 8. a 9. b 10. b

Module 7

Meiosis: How Is DNA Content Reduced and Recombined During the Formation of Gametes?

- **Review of mitosis** (Fig. 7.1): A diploid cell in the G₁ phase contains two genomes, but in preparation for cell division, the DNA content is doubled to four genomes during the S phase. Mitosis, then, is the process by which the replicated DNA is precisely separated and distributed to the daughter cells, so that each ends up with a complete set of genes (two genomes). Specifically, during mitosis, each chromosome lines up at the cell's equatorial plane, where the two sister chromatids of each chromosome are pulled apart before being distributed to the daughter nuclei.
- **Meiosis, a unique way to produce gametes:** Meiosis, a special type of cell division, is essential for sexual reproduction. The cells produced by meiosis are called gametes (in higher animals) or spores (in flowering plants). Male gametes are also known as sperm; female gametes are known as eggs, or ova. In this learning module, we study meiosis in higher animals.
- Only specific cells undergo meiosis to produce gametes. **Primary spermatocytes** undergo spermatogenesis to produce sperm, and **primary oocytes** undergo **oogenesis** to produce eggs.

Overall process of meiosis (Figs. 7.2 and 7.3)

- Let us first briefly outline the overall process of meiosis.
 1. Cells (with two genomes initially) undergoing mitosis or meiosis must first complete the S phase, during which DNA is replicated. Therefore, the cell contains **four genomes at the start of mitosis or meiosis**.
 2. In contrast to mitosis, in which one cell produces two identical daughter cells, meiosis involves two cell divisions (**meiosis I and II**), producing four daughter cells, each with only half the DNA content (one genome) of the original cell. The major steps of meiosis are summarized below:
- **Summary of meiosis:** Meiosis consists of two divisions. The first division results in two daughter cells, each with two genomes. The second division results in four daughter cells, each with one genome. In human males, the daughter cells

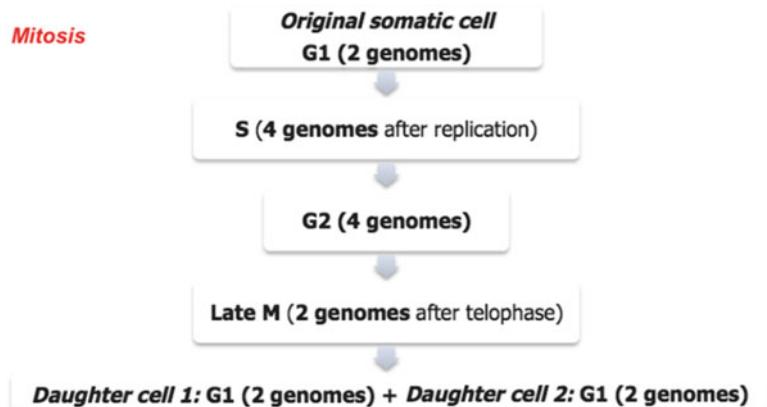


Fig. 7.1 Overall process of mitosis

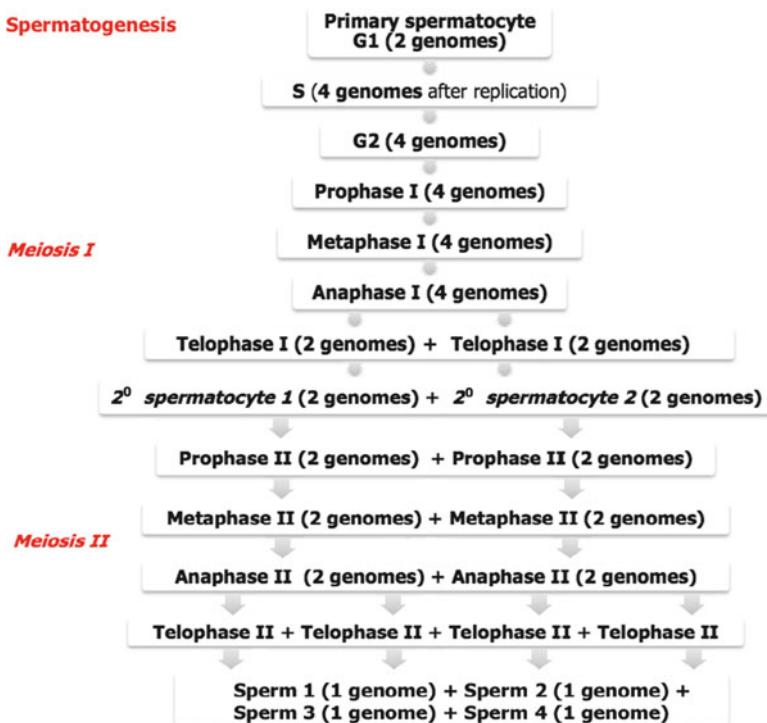


Fig. 7.2 Meiosis: spermatogenesis

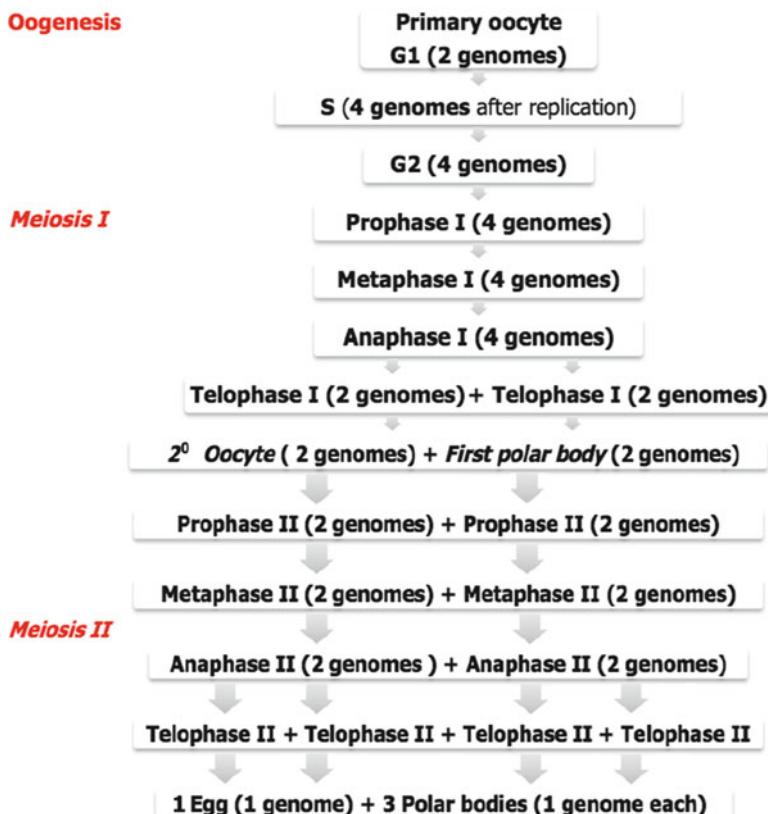


Fig. 7.3 Meiosis: oogenesis

after the first division are called secondary spermatocytes; the daughter cells after the second division develop into sperm. In human females, cell division is unequal. The larger daughter cell after the first division is called the secondary oocyte; the smaller cell is called the first polar body. The secondary oocyte and the polar body then both divide, yielding an ovum (egg) and a polar body, and two polar bodies, respectively. Thus, oogenesis ultimately yields one egg and three polar bodies.

Genetic Consequences

- One of the consequences of meiosis is that the number of genomes is reduced from two (in the G₁ phase of the meiotic cell) to one (in each of the four daughter cells)
- In addition to reducing the genome number from two to one, meiosis provides mechanisms for the recombination of paternal and maternal DNA. Consequently,

no two gametes are genetically identical. The random fusion of these gametes during fertilization further increases genetic variation.

- How do paternal and maternal DNA recombine during meiosis?
- Let us examine two significant aspects of meiosis:
 1. **Principle of segregation:** Segregation of homologous chromosomes and allelic genes.
 2. **Recombination of parental DNA:** There are two ways by which genes are recombined.
 - (a) **Principle of independent assortment:** genetic recombination of unlinked genes (genes on different chromosomes).
 - (b) **Homologous recombination:** genetic recombination of linked genes (genes on the same chromosome).

Principle of Segregation

- As you may recall, a gene may exist in different forms called alleles. In a diploid cell, each gene has two alleles, which lie at corresponding loci on a homologous chromosome pair. Even though there are technically four alleles of each gene after the S phase, the alleles on sister chromatids are identical, so the genotype is denoted with only two letters. For example, we write Aa for the heterozygous genotype (in G_1 phase) instead of $AAaa$ (after S phase). During the formation of gametes, allelic genes are segregated. Thus, each gamete contains either A or a , but not both (Fig. 7.4):

Chromosome-Based Segregation of Allelic Genes (Interactive Program 1)

Use Interactive Program 1 on the software program available at Extras.Springer.com to familiarize yourself with the principle of segregation.

After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “Independent Assortment of Unlinked Genes.”

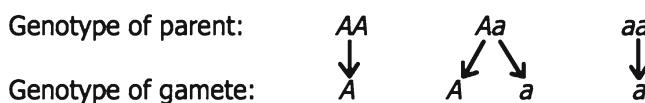
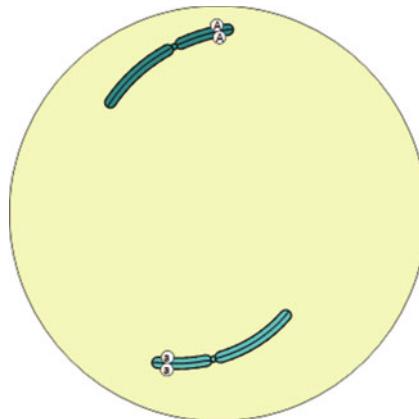


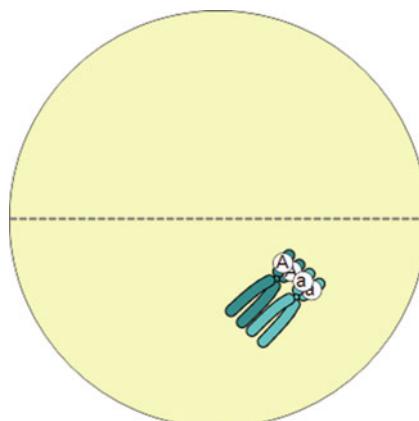
Fig. 7.4 Segregation of allelic genes (homozygous parents generate only one type of gamete; a heterozygous parent generates two types of gametes)

- To demonstrate the segregation of allelic genes during meiosis, we use a homologous chromosome pair, one chromosome carrying the duplicated A allele, the other carrying the duplicated a allele (Step 1).



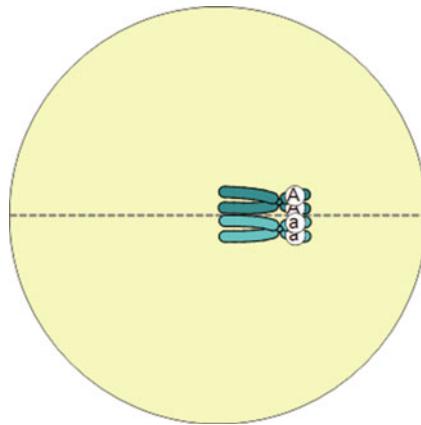
Step 1 Layout: Cell with a pair of chromosomes to demonstrate the segregation of allelic genes

- Note again that each chromosome (Step 1) contains two sister chromatids, so there are really two A genes and two a genes (AAaa).
- Click on each chromosome* (Step 1) so homologous pairing (Step 2) can take place in prophase I.



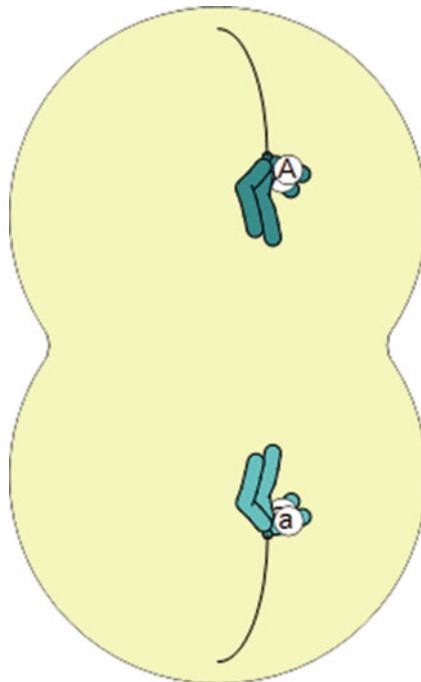
Step 2 Action: Pairing of homologous chromosomes

- Drag a centromere and move the homologous pair to the equator of the cell (Step 3).



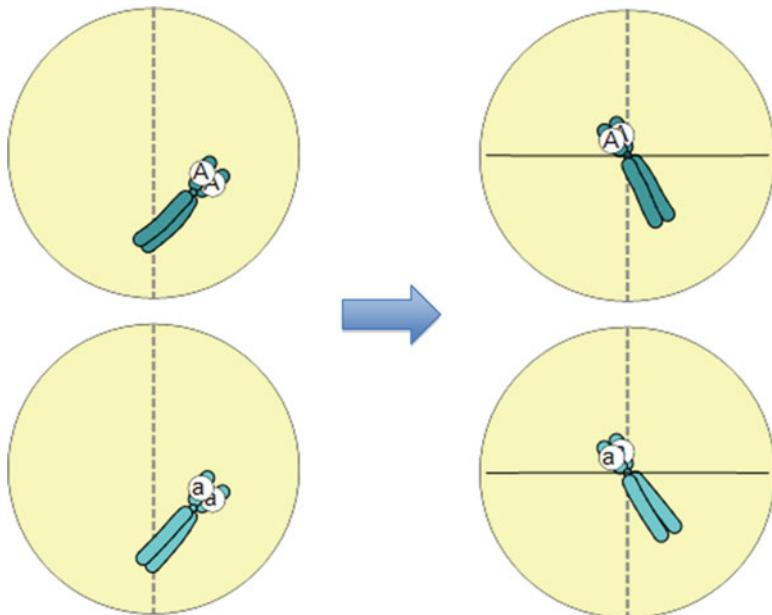
Step 3 Action: Move homologous pair to the equator

- Click on the centromeres to move the chromosomes to opposite poles (anaphase I) (Step 4).



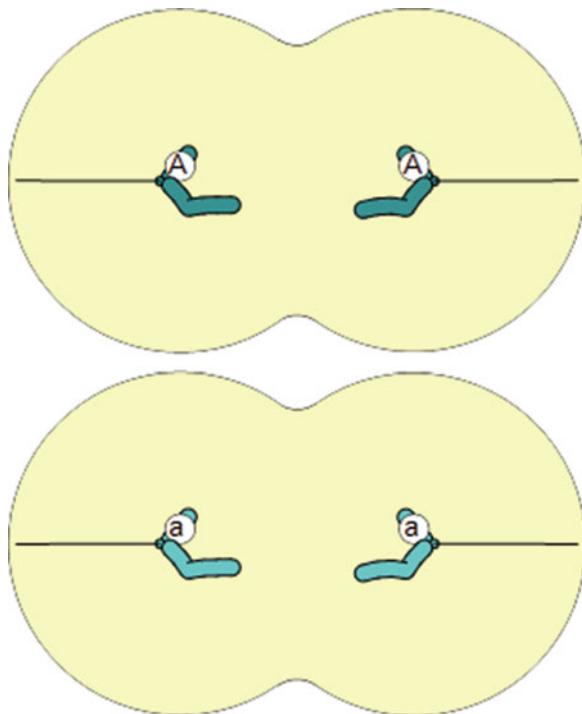
Step 4 Results: Anaphase: separation of each homolog

- Although the homologous chromosome pair is separated into two single chromosomes in **telophase I**, each chromosome still contains two sister chromatids. Thus, each daughter cell after the first meiotic division contains either AA or aa.
- In each prophase II cell, drag the centromere of the chromosome to the cell's equator to enter metaphase II (Step 5).*



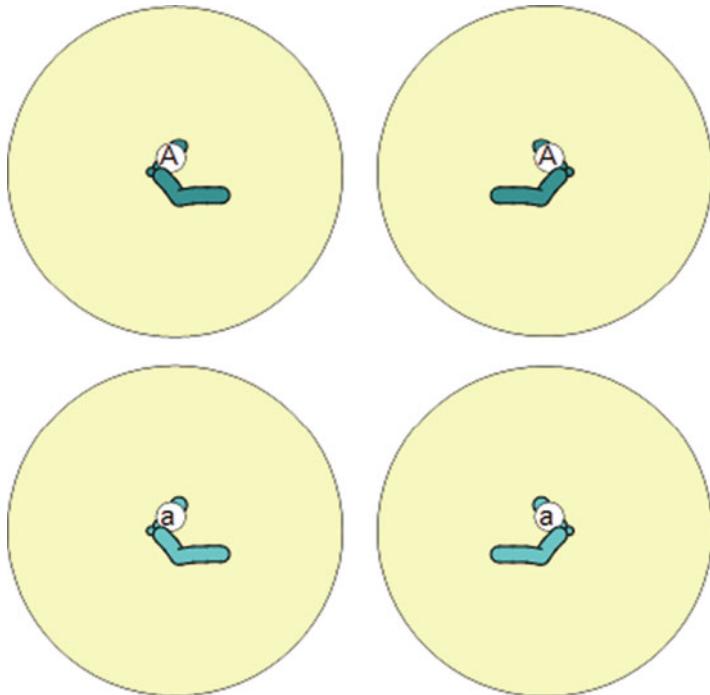
Step 5 Action: Prophase II becomes metaphase II

- Click on the centromere in each daughter cell to separate the sister chromatids to enter anaphase II (Step 6).



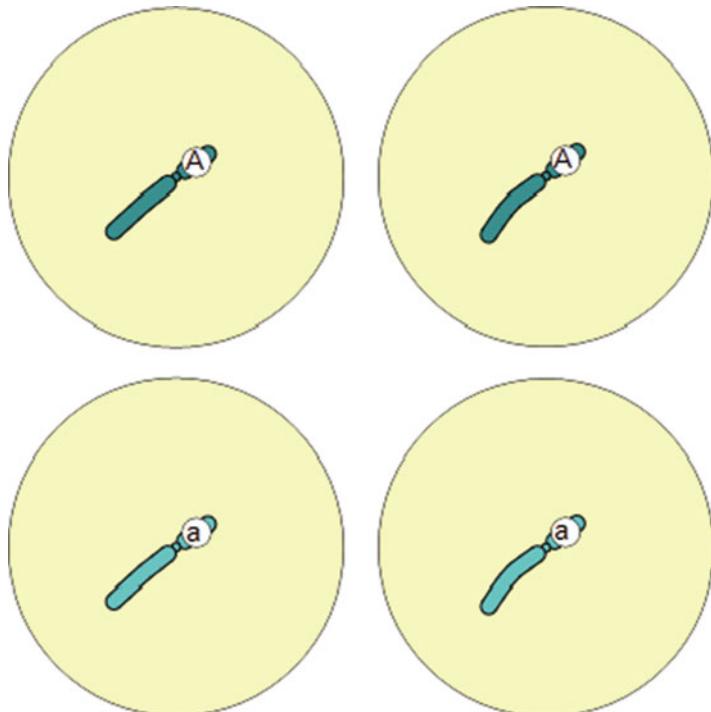
Step 6 Action: Separation of sister chromatids (anaphase II)

- *Chromosomes continue to stretch during telophase II, after which four gametes are formed (Step 7).*



Step 7 Action: From telophase to gamete

- Each gamete contains one chromosome (with a single chromatid). Two gametes contain gene *A* and two gametes contain gene *a*.
- Therefore, the two alleles (*A/a*) are now segregated into two types of gametes: one with *A* and the other with *a* (Step 8).



Step 8 Results: A heterozygous parent generates two types of gametes: *A* and *a*

Independent Assortment of Unlinked Genes *(Interactive Program 2)*

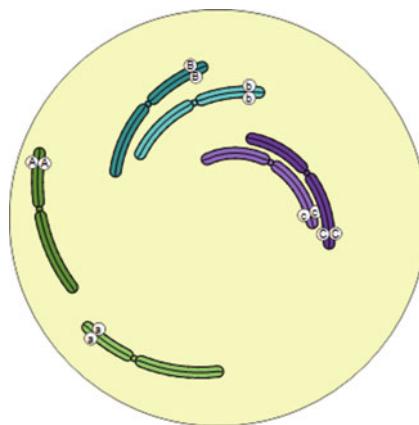
- During the formation of gametes, the segregation of allelic genes from nonhomologous chromosomes is independent (random). That is, the segregation of alleles from one homologous chromosome pair does not affect the segregation of alleles from another homologous chromosome pair. Take, for example, the two heterozygous gene pairs *A/a* and *B/b*. If *A* and *a* are segregated into gametes 1 and 2, respectively, then *B* and *b* are equally as likely to be segregated into gametes 1 and 2 as to be segregated into gametes 2 and 1, resulting in four kinds of gametes: *AB*, *Ab*, *aB*, or *ab*.

- Thus, a cell with three heterozygous gene pairs A/a , B/b , and C/c on three different chromosomes will produce eight kinds of gametes: ABC , ABc , AbC , Abc , aBC , aBc , abC , and abc (independent assortment). How do we know that?
- Let us follow these three chromosome pairs through each step of meiosis. Let alleles A , B , and C be from the mother and alleles a , b , and c be from the father.
- In early prophase I, the three homologous chromosome pairs are depicted by three different colors. Note the genes on each chromosome.
- As you can see, each chromosome contains two sister chromatids, which are barely distinguishable at this stage (Step 1).
- One of the most significant events in meiosis—that which distinguishes meiosis from mitosis—is the physical pairing of homologous chromosomes in early prophase I.

[Use Interactive Program 2 on the software program available at Extras. Springer.com to familiarize yourself with the principle of independent assortment.](#)

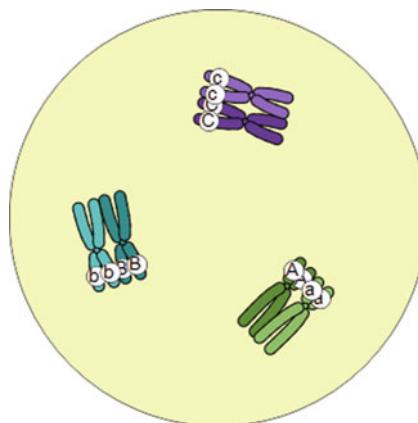
[After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “Genetic recombination of linked genes.”](#)

- For simplicity, no nuclear envelope is shown.



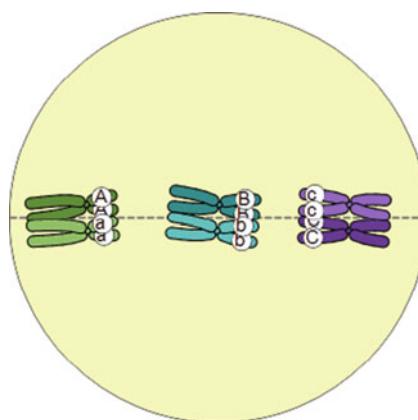
Step 1 Layout: Early prophase I

- Identify the three homologs by clicking on each member of a homologous chromosome pair (starting with pair 1) to initiate physical pairing (based on DNA homology) (Steps 1 and 2).



Step 2 Action: Physically paired homologs in prophase I

- After the homologous chromosomes are physically paired, each resulting structure is called a bivalent, consisting of two homologs and four chromatids (Step 2).
- During the physical pairing of homologous chromosomes, another significant event may occur: crossing over. Crossing over is a process that leads to the exchange of DNA between chromatids. Because sister chromatids are identical, crossing over is only important between non-sister chromatids, where DNA exchanges may create new sequences. For convenience, we will examine the process of crossing over in detail in the next section.
- *Drag any centromere of each chromosome pair to the equator* (Step 3).



Step 3 Action: Alignment of three homologs at the equator (the metaphase chromosome

configuration above, $\frac{ABC}{abC}$, is only one of the four possible configurations)

- When all the homologs are lined up on the equator, the cell is in metaphase I. During this time, the chromosomes are in their most condensed state.
- The configuration of homologous chromosome pairs at the equator during metaphase I is the key to understanding the principle of independent assortment of unlinked genes.
- For a cell with three homologous chromosome pairs ($2N=6$), four different configurations are possible:

$\begin{matrix} A & B & C \\ a & b & c \end{matrix}$

Config. 1

$\begin{matrix} a & B & C \\ A & b & c \end{matrix}$

Config. 2

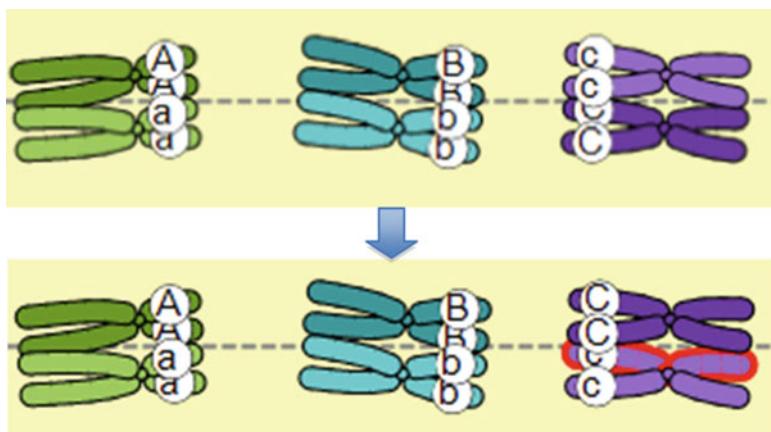
$\begin{matrix} A & b & C \\ a & B & c \end{matrix}$

Config. 3

$\begin{matrix} A & B & c \\ a & b & C \end{matrix}$

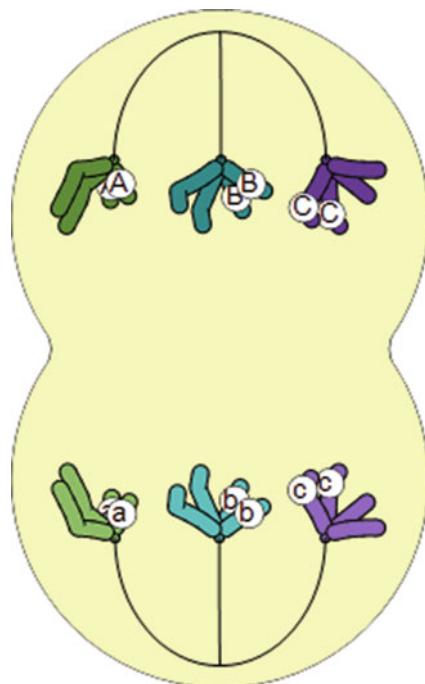
Config. 4

- Click on the genes (Step 4) to generate the configuration in which the maternal genes A , B , and C are at the top and the paternal genes a , b , and c are at the bottom. In other words, we want $\frac{ABC}{abc}$ as configuration 1.



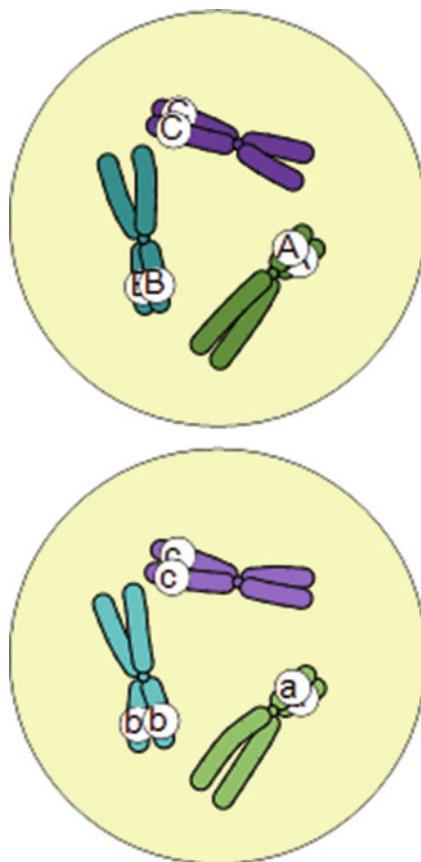
Step 4 Action: Change the metaphase chromosome configuration by clicking the appropriate genes

- Click on each of the centromeres to separate the homologous chromosomes, moving the chromosomes to opposite poles (Step 5).



Step 5 Action: Separation of homologous chromosomes: anaphase I

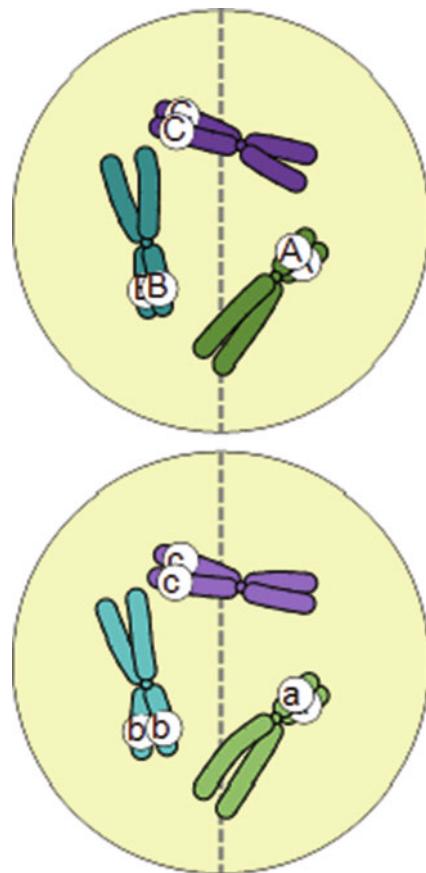
- The cell is now in anaphase I. Note that the chromosomes in anaphase I of meiosis, unlike the chromosomes in anaphase of mitosis, still consist of two chromatids.
- In telophase I (Step 6), the chromosomes stretch out.
- Telophase I marks the conclusion of the first meiotic division. At this point, we pause to review the consequences of the first meiotic division.

**Step 6** Results: Telophase I or prophase II

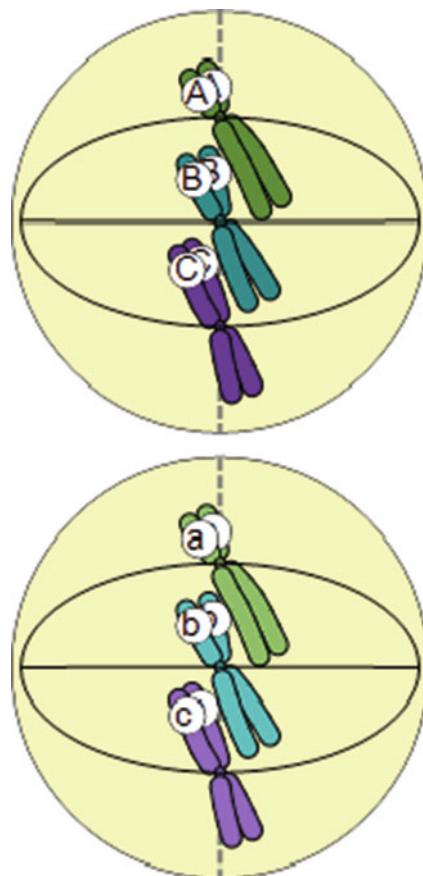
1. How many daughter cells are formed after the first meiotic division?
 2. How many chromosomes does each daughter cell contain (compare with the chromosome number in prophase I)?
 3. **In prophase I**, there are six chromosomes (or three homolog pairs), and each chromosome has two sister chromatids, making a total of twelve chromatids. Therefore, there are four copies of each gene ($AAaa$, $BBbb$, $CCcc$). Strictly speaking, the cell contains four genomes.
 4. **In telophase I**, each daughter cell contains only three chromosomes and six chromatids. The four alleles of each gene are reduced to two alleles. As a result of the chromosome configuration at metaphase I, one daughter cell has the genotype ABC (all maternal in origin), while the other daughter cell has the genotype abc (all paternal in origin). We will examine the consequences of the other three configurations later.
- Continue clicking to view the second meiotic division. Prophase II looks like telophase I, although chromosome behavior during these two phases are

opposite in nature. During telophase I, the chromosomes start to stretch out; the chromosomes start condensing during **prophase II**. There may or may not be a short interphase period between the two meiotic divisions. For simplicity, we omit it.

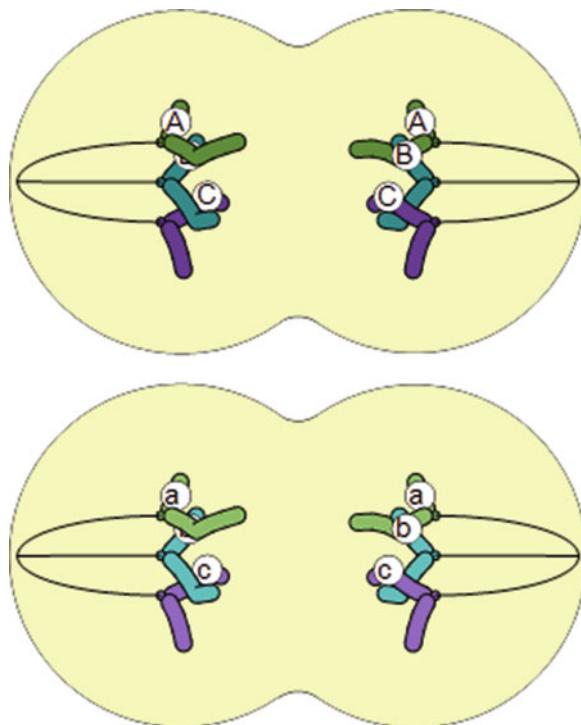
- *Drag the centromere of each chromosome to the cell's equator (do it for both daughter cells) (Step 7). The cells are now in **metaphase II** (Step 8).*



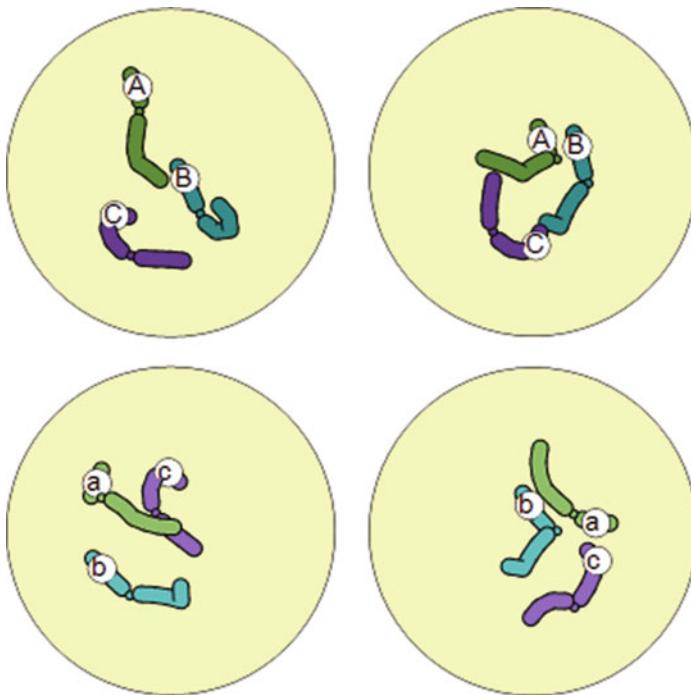
Step 7 Action: Move chromosomes to equator

**Step 8** Action: Entering metaphase II

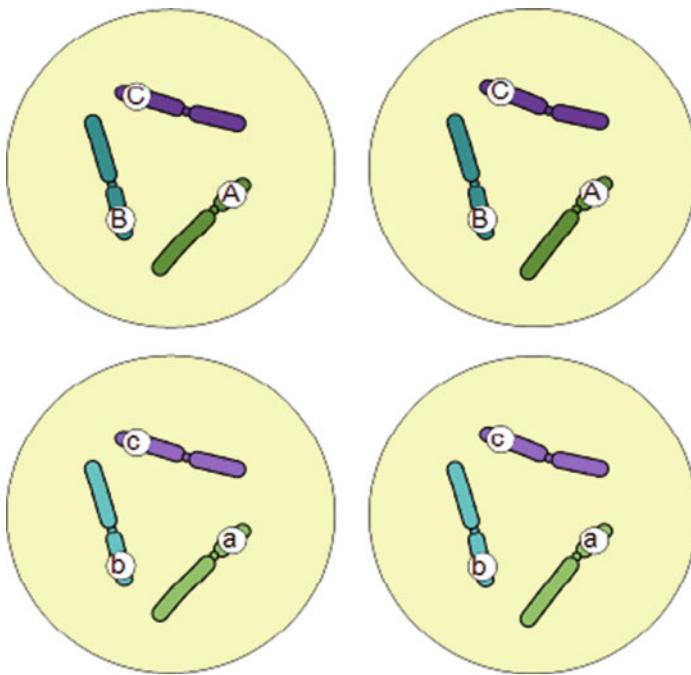
- Attention: Here each cell contains three unique chromosomes; there are no homologs.
- *Click on each centromere to separate the sister chromatids* (Step 9). We are now in anaphase II.

**Step 9 Action:** Anaphase II: separation of sister chromatids

- As the chromosomes move to opposite poles and start stretching out, the cell enters **telophase II** (steps 10 and 11). Each previous daughter cell produces two new daughter cells (granddaughter cells), resulting in a total of four daughter cells (gametes) after two meiotic divisions.
- If you closely examine the four gametes, you should see that the top two gametes have the same genotype (ABC , from the three maternal chromosomes), and the bottom two gametes have the same genotype (abc , from the three paternal chromosomes). Each chromosome consists of just one chromatid.



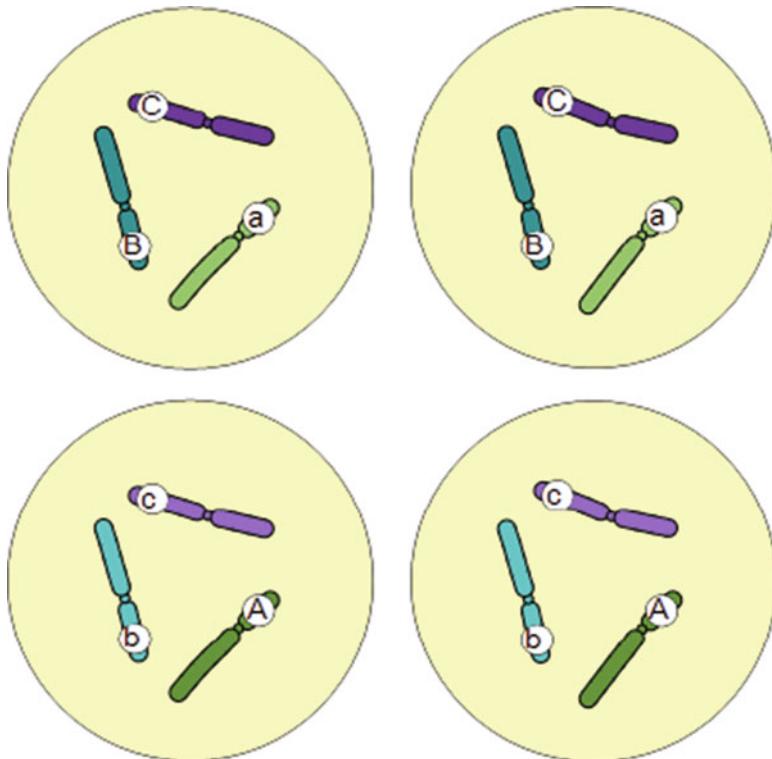
Step 10 Action: Telophase II



Step 11 Results: Based on configuration 1, four gametes but only two genotypes, ABC and abc , are produced

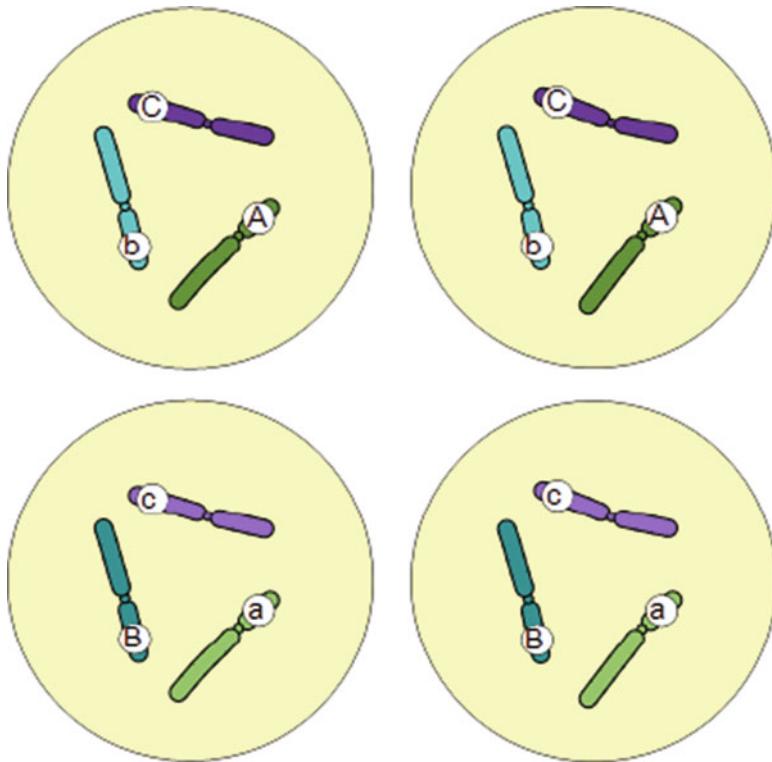
- Let us go back to the beginning. A cell (primary spermatocyte or oocyte) with chromosome number $2N=6$, or three chromosomes with three pairs of unlinked genes (A/a , B/c , and C/c), may have any one of four types of chromosome configurations in metaphase I. Configuration 1 (described above) yields two types of gametes: ABC and abc .
- To view the gametes produced from the other three chromosome configurations in metaphase I, simply click ' $>$ ' and then the centromeres to achieve the desired configurations. See below.
- Click genes to produce configuration 2 $\frac{aBC}{Abc}$*

Then continue clicking ' $>$ ' until you see all four gametes with two genotypes of aBC and Abc (Step 12).



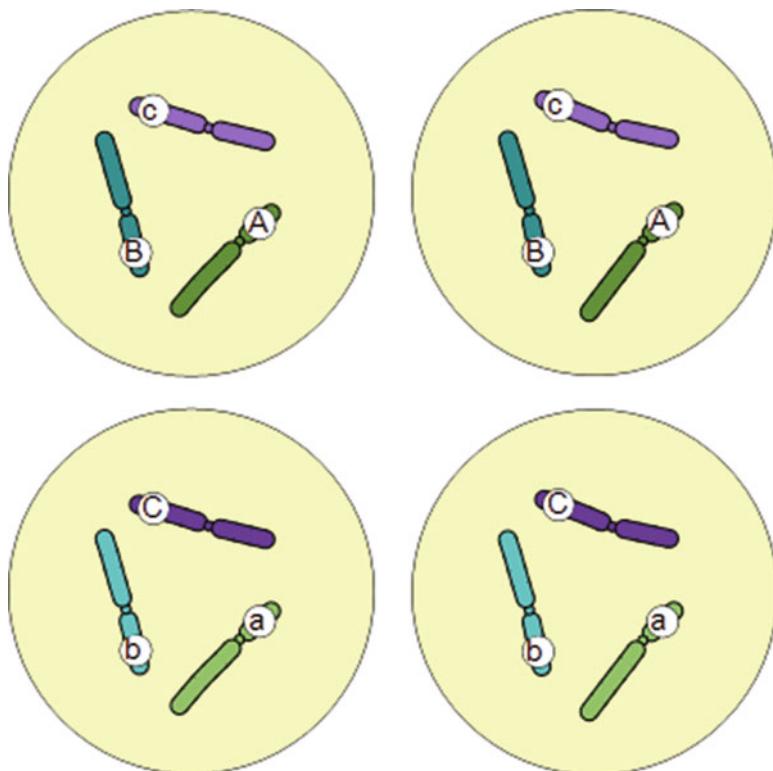
Step 12 Results: Based on configuration 2, four gametes but only two genotypes, aBC and Abc , are produced

- Click on the gene symbols, as needed, to produce configuration 3 $\frac{AbC}{aBc}$
Then continue clicking '>' until you see all four gametes with two genotypes of AbC and aBc (Step 13).



Step 13 Results: Based on configuration 3, four gametes but only two genotypes, AbC and aBc , are produced

- Click on the gene symbols, as needed, to produce configuration 4 $\frac{ABc}{abC}$
Then continue clicking '>' until you see all four gametes with the genotypes ABc and abC (Step 14).



Step 14 Results: Based on configuration 4, four gametes but only two genotypes, ABc and abC , are produced

| Summary of gamete genotypes | ABC |
|-----------------------------|-------|-------|-------|-------|-------|-------|-------|-------|
| | ABC |

Fig. 7.5 The gametic genotypes from three independently assorted gene pairs (A/a ; B/b ; C/c)

- Conclusion: If a parent has three pairs of independently assorted gene pairs, eight different gametes, each with 1 in 8 chances of occurring, are possible (Fig. 7.5).
- Note that the four configurations generate eight types of gametes: ABC and abc (from configuration 1), aBC and Abc (from configuration 2), AbC and aBc (from configuration 3), and ABc and abC (from configuration 4).

- Because the chromosome configurations at metaphase I are the result of random processes, each of the eight different gametes has an equal chance of being generated: 1 in 8 for each genotype.
- For a cell with $2N=4$ and two pairs of independently assorted genes (A/a , B/b), there are two possible chromosome configurations at metaphase I, leading to four different types of gametes with genotypes AB , Ab , aB , and ab .
- Similarly, a cell with four independently assorted heterozygous gene pairs can produce eight possible chromosome configurations at metaphase I, leading to 16 different types of gametes.
- The number of possible gametic genotypes = 2^N , where N is the number of independently assorted heterozygous gene pairs.
- Note that (1) homozygous gene pairs do not increase the number of gametic genotypes, and (2) the principle of independent assortment only applies to genes on different chromosome pairs (**unlinked genes**).

Genetic Recombination of Linked Genes

- We have just learned that nonallelic genes on different chromosomes (unlinked genes) assort independently during meiosis, providing a major mechanism for the recombination of parental genes.
- Can parental genes on the same chromosome (linked genes) be recombined during meiosis to generate new genotypes?
- The answer is “yes.” We now examine the **genetic recombination of linked genes**.
- The recombination of homozygous alleles leads to no new genotypes because the alleles are the same. Therefore, we will only be concerned with the recombination of heterozygous allelic genes.
- If a maternal chromosome carries allele A and allele B and its homologous paternal chromosome carries allele a and allele b , then we say the linkage is *cis*.
The *cis* linkage genotype is written $\frac{AB}{ab}$.
- If a chromosome carries allele A and allele b and its homologous chromosome carries allele a and allele B, then the linkage is *trans*. The *trans* linkage genotype is written $\frac{Ab}{aB}$.
- Prophase I is a critical moment for the recombination of linked genes. At this time the homologs are physically paired.
- After the homologs are paired, breakage and reunion (crossing over) at any point along the paired maternal and paternal DNA may occur, leading to the genetic recombination of parental genes. The result of crossing over is a recombinant (crossover) chromosome, which contains a mixture of genes from the two parental chromosomes.

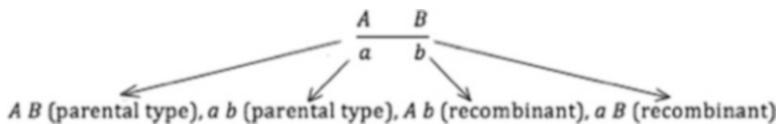


Fig. 7.6 Crossing over between a pair of linked heterozygous genes results in four genotypes of gametes (two parental types and two recombinants)

- A homologous chromosome pair contains four chromatids (two pairs of sister chromatids). As we alluded to earlier, crossing over between sister chromatids leaves the chromosomes unchanged. Therefore, crossing over only generates new genotypes when it is between two non-sister chromatids. The chromosome that carries the original genes is a **parental type** or **nonrecombinant** chromosome.
- Every crossover between two genes during prophase I generates four types of gametes (Fig. 7.6):
- In the example above, 50 % of the gametes have parental-type chromosomes and 50 % of the gametes have recombinant chromosomes (provided that there is crossing over between the two genes in question for every meiotic cell). In reality, however, crossing over between two specific genes generally does not occur in every meiotic division. Consequently, parental type chromosomes are always predominant over recombinant chromosomes.
- For example, if a single crossover between two linked genes occurs in 20 % of the meiotic cells and there is no crossing over in the rest (80 %) of the meiotic cells, 90 % of the gametes will be parental type and 10 % of the gametes will be recombinant. Why? See the explanation below:
- Let us assume that the original parent has the linkage genotype AB/ab (Fig. 7.7).
- The calculation in Fig. 7.7 is based on a *cis* linkage (*A* and *B* genes on one chromosome and *a* and *b* genes on the other chromosome). If the linkage is *trans*, the percentages are reversed.
- If the number of recombinant gametes is divided by the total number of gametes (recombinants plus parental types), the recombinant frequency can be obtained.
- Furthermore, recombinant frequencies are proportional to the distance between the two genes in question: the longer the distance (more chances of crossing over within the region), the higher the frequency of recombination.
- Genes on the same chromosome are said to be linked, so a chromosome is also known as a linkage group. We can use the frequency of genetic recombination between two genes to estimate the distance between them (measured in map units). This is known as **linkage analysis**.

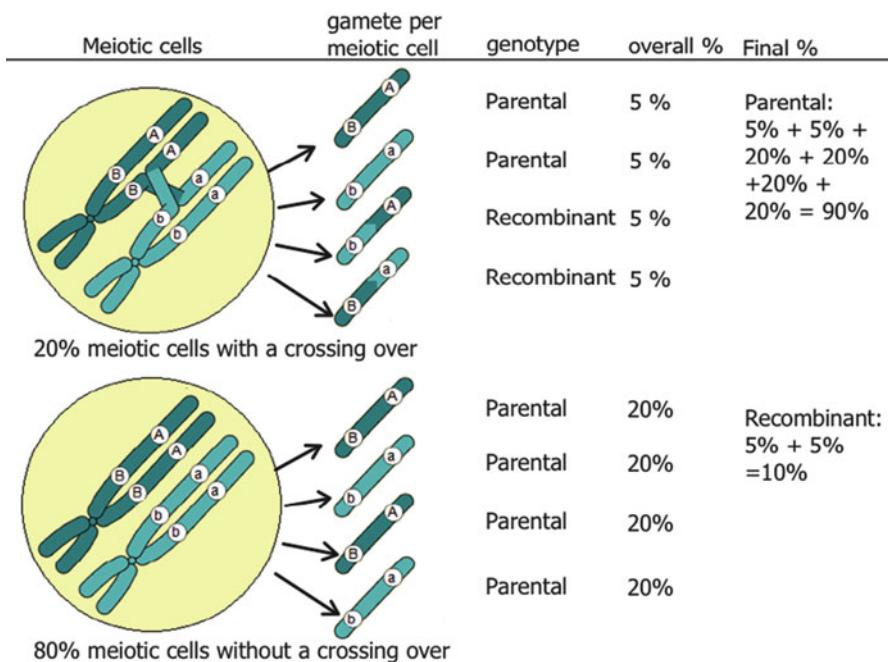


Fig. 7.7 Calculating the percentage of parental and recombinant gametes

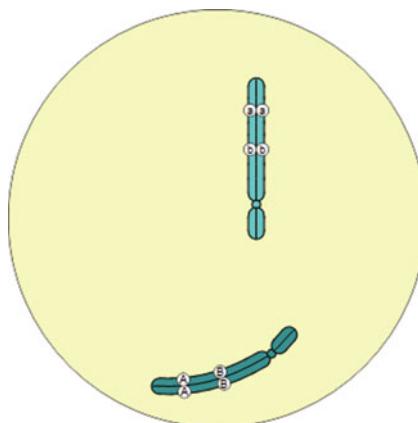
Genetic Recombination of Linked Genes: Linkage Analysis (*Interactive Program 3*)

- Using linkage analysis, we will determine the distance between two heterozygous gene pairs (AB/ab).

Use *Interactive Program 3* on the software program available at Extras. Springer.com to familiarize yourself with linkage analysis.

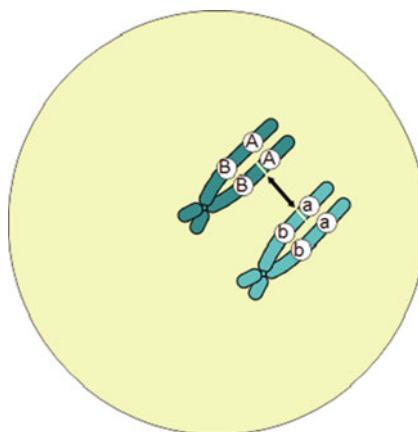
After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “Homologous Recombination: Molecular Mechanism.”

- The following cell is in early prophase I (Step 1).
- Click > to see a homologous chromosome pair with four chromatids (Step 2).



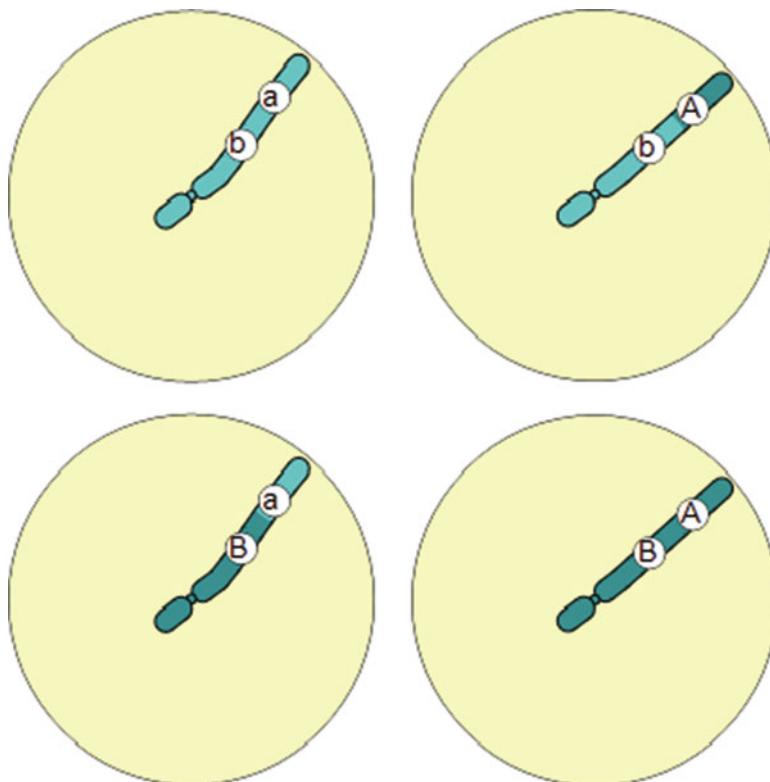
Step 1 Layout: Early prophase I with homologous chromosomes

- Click > again to initiate crossing over. Crossing over occurs during early prophase I of meiosis, but only becomes evident in late prophase I when the homologs begin to separate, revealing the point of chromosome breakage and reunion. This point is called the **chiasma** (Step 2).



Step 2 Action: Pairing of homologous chromosomes and crossing over between two *cis* genes

- To simplify matters, we skip the intermediate steps of meiosis and go directly to the gametes that are formed (Step 3) (also see Fig. 7.7).

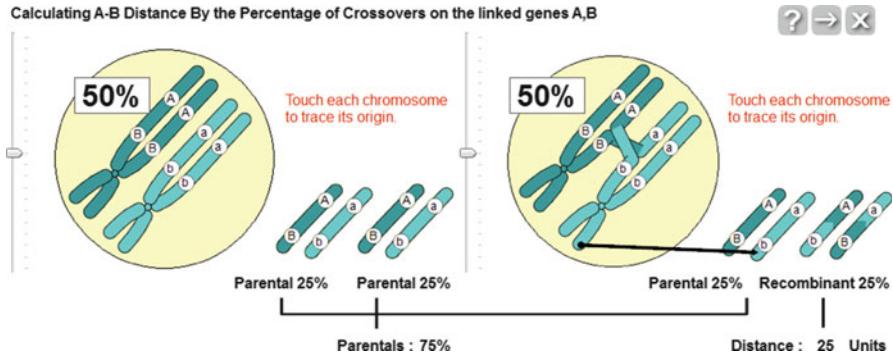


Step 3 Results: Formation of four gametes from a single meiotic cell with a single crossing cover

- In the cell (Step 2), there is a pair of homologous chromosomes with four chromatids, each containing two genes. A and B are maternal in origin, while a and b are paternal in origin. As was stated earlier, when both dominant alleles lie on one chromosome and both recessive alleles lie on the other chromosome, the linkage is *cis*. If one dominant allele and one recessive allele are on the same chromosome, the linkage is *trans*.
- The molecular process of crossing over is discussed in the next section. At this time, we want to know what happens to the linked genes after the non-sister chromatids exchange their DNA content (crossing over).
- To simplify matters, we assume that there is a single crossover between two non-sister chromatids (chromatids 2 and 3) in **some** of the meiotic cells.
- Click to proceed* (Step 2). The four gametes that are produced by the meiotic cells with a crossover have the following genotypes: AB , ab , Ab , and aB .

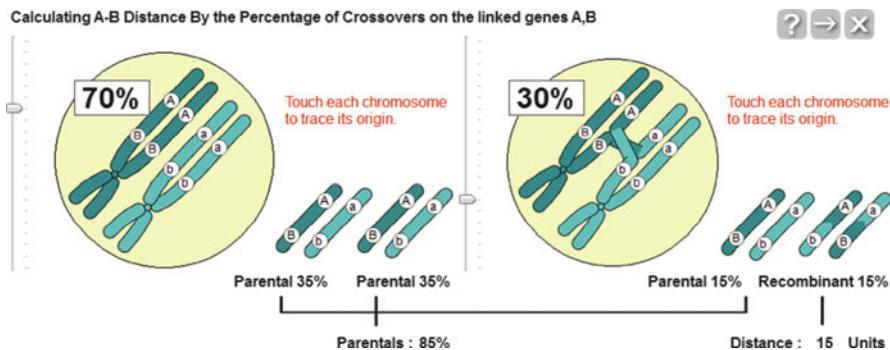
- The gametes with genotypes AB and ab are parental types, because they reflect the original linkages of the parent cell. The gametes with genotypes Ab and aB are recombinant, because they result from crossing over between non-sister chromatids.
- If crossing over occurs in a cell (e.g., primary spermatocyte), then the ratio of parental types to recombinants is 1:1.
- However, as we mentioned previously, crossing over between two gene loci does not always occur.
- In most cases, crossing over only occurs sporadically, the frequency depending on the distance between the two gene loci. For example, if two genes are very close together on the chromosome, then the probability of a crossover between the genes is very low.
- On the other hand, if two linked genes are far apart, then the probability of a crossover between the genes is very high.
- Therefore, the frequency of crossing over between two linked genes is proportional to the distance between the genes. Because crossing over results in two recombinant chromosomes out of a total of four chromosomes, we can use the frequency of recombinant gametes to estimate the distance between two specific genes.
- Although the genotype of a gamete cannot be observed, we can infer its genotype by analyzing its progeny, which are produced by means of a test cross. In a test cross, a heterozygous organism (AB/ab or Ab/aB) is crossed with a homozygous recessive organism (ab/ab). In this way, the phenotypes of the progeny (F_1 generation) only reflect the genotypes of the heterozygous parent (because the homozygous recessive parent only contributes recessive genes to the progeny).
- Mapping is based on the notion that 1 % recombinant progeny = 1 map unit.
- We are now interested in mapping the distance between the A/a gene locus and the B/b gene locus.
- First, we must cross an organism with heterozygous genotype $A/a; B/b$ with an organism with homozygous genotype $a/a; b/b$. As we mentioned earlier, this type of genetic cross is called a test cross.
- We assume nothing about the linkage condition of these two gene pairs. If they happen to be on different chromosome pairs (unlinked), then there is nothing to map.
- If the two genes are linked, then the linkage is either *cis* (both dominant alleles on one parental chromosome and both recessive alleles on the other parental chromosome) or *trans* (each parental chromosome carries one dominant allele and one recessive allele).
- Some points to consider:
 1. Not every pair of linked genes in a cell undergoing meiotic crosses over. Generally, crossing over between a specific pair of linked genes occurs in only a fraction of the meiotic cells.
 2. Even if crossing over occurs, only two out of the four chromatids are affected. Therefore, crossing over results in two parental type gametes and two recombinant gametes.

3. If the original linkage is *cis*, then the parental types in the gametes are *AB* and *ab* and the recombinants are *Ab* and *aB*. If the original linkage is *trans*, then the parental types are *Ab* and *aB* and the recombinants are *AB* and *ab*. Of course, the genotypes can only be determined after a test cross.
- Let us examine the meiotic cells below (Step 4):
Note that the linked genes are *cis*. Assume that there is no crossing over in the cell at the left and that there is one cross over in the right cell.



Step 4 Layout: *Left:* 50 % of meiotic cells do not contain a cross over, resulting in all the gametes being parental types (25 % *AB* and 25 % *ab*); *Right:* 50 % of the meiotic cells contain a cross over, resulting in recombinant and parental type gametes (25 % parental types (12.5 % *AB* and 12.5 % *ab*) and 25 % recombinants (12.5 % *Ab* and 12.5 % *aB*))

- In this case, therefore, there is crossing over in 50 % of the cells undergoing meiosis.
- When there is no crossing over (left), all the gametes are parental types. When there is crossing over (right), half the gametes are parental types and half are recombinants. Therefore, taken together, 25 % of the gametes are recombinant (1/4). Because 1 % recombination = 1 map unit, the distance between *A/a* and *B/b* loci is 25 map units.
- The foregoing example represents only one of many situations, because the frequency of crossing over between the linked genes varies, depending on the distance between them (See also Fig. 7.7). Follow the procedure below to change the recombination frequency.
- Slide the center bar up and down to change the relative frequencies of the two types of cells (Step 5).



Step 5 Action: Change the recombination frequency

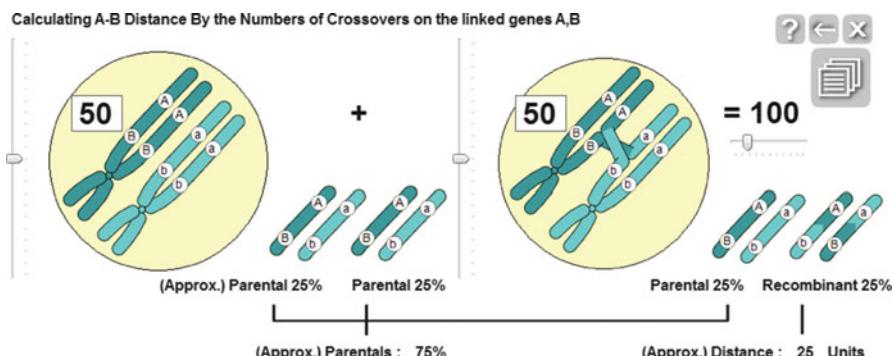
- Note that the **recombinant frequency is always 1/2 the frequency of meiotic cells with one crossover** (the cells on the right).
- If crossing over occurs in 100 % of the meiotic cells (no cells on the left, 100 % cells on the right), then the recombinant frequency is 50 % ($1/2 \times 100\%$). Therefore, the maximum map distance between two genes is 50 map units.
- In practice, the recombinant frequency is calculated as follows:

$$\frac{\text{Number of gametes with recombinants}}{\text{Total number of gametes} (\text{recombinants} + \text{parental types})} \times 100\%$$

- Because the phenotypes of the progeny from a test cross reflect the genotypes of the gametes, the recombinant frequency (map distance) is also calculated as follows:

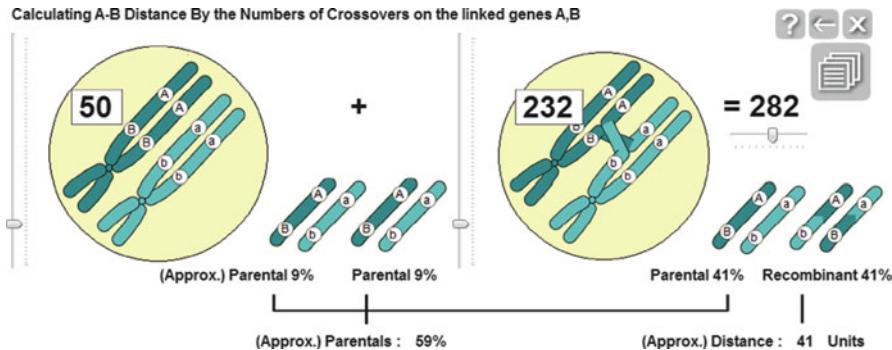
$$\frac{\text{Number of recombinants in the progeny}}{\text{Total number of progeny} (\text{recombinants} + \text{parental types})} \times 100\%$$

- To perform some actual calculations (by counting the number of meiotic cells), *click on the arrow in the upper right-hand corner*. The number of gametes is initially set to 100, half of which are recombinant (Step 6).



Step 6 Action: The actual number of meiotic cells with a crossover

- Slide the bar on the horizontal scale, which appears at the right, left and right to change the total number of cells (Step 7). Then slide the bars on one of the vertical scales up and down to change the number of parental or recombinant cells (Step 7). The map distance is displayed in the lower right-hand corner.



Step 7 Results: Of the 282 meiotic cells, if 232 cells contain a crossover between the two loci, then the map distance between the two loci is 41 map units

- Let's try a problem. A female animal with the genotype $A/a; B/b$ is crossed with a male animal with genotype $a/a; b/b$. A =long body, a =short body, B =black hair, and b =pale hair. The progeny (F_1) include 402 long and black, 388 short and pale, 11 long and pale, and 9 short and black.

 1. Are the two genes linked?
 2. If they are linked, are they *cis* or *trans*?
 3. Which progeny are parental types and which progeny are recombinants?
 4. Calculate the distance between the two gene loci.

- Because chromosomes contain DNA (organized into functional units or genes), chromosome behavior during meiosis explains the reduction of genomes, the segregation of allelic genes, the independent assortment of unlinked genes, and the homologous recombination of linked genes.
- Chromosome behavior during meiosis, however, does not fully explain the recombination of linked genes (e.g., chiasma formation).
- We visit the recombination of linked genes at the molecular level below.

Homologous Recombination: Molecular Mechanism (*Interactive Program 4*)

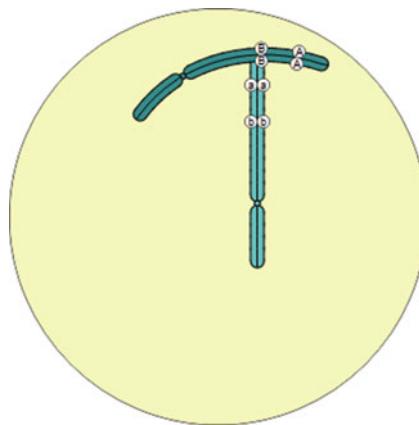
- At the DNA level, homologous recombination involves the exchange of nucleotide sequences between two similar or identical DNA molecules.

- The mechanisms involved in homologous recombination are ubiquitous in nature. For example, they are often used to repair double-strand breaks in DNA, which are harmful to the cell. They are also used to transfer genes between different species, especially in prokaryotes.
- In eukaryotes, the mechanisms involved in homologous recombination are used to produce new nucleotide sequences during meiosis. We examine this process in the following pages.
- Remember that homologous recombination (crossing over) occurs in prophase I when a pair of the homologous chromosomes or four chromatids are physically paired?

Use Interactive Program 4 on the software program available at Extras. Springer.com to familiarize yourself with homologous recombination.

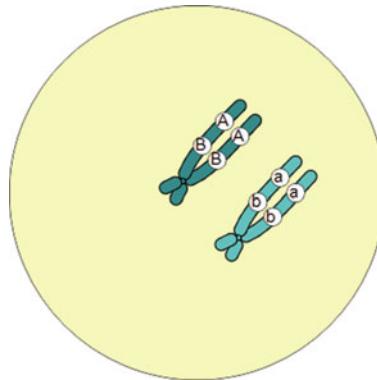
After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “Conclusions.”

- The following two homologous chromosomes are used to demonstrate the mechanisms involved in eukaryotic homologous recombination (Step 1).



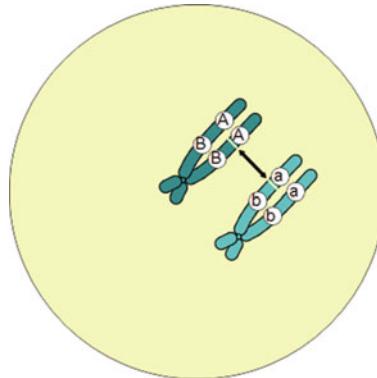
Step 1 Layout: Two homologs with two *cis* linked genes

- Although different models of recombination have been proposed, we examine in detail the double-strand break recombination (DSBR) model (also called the double Holliday junction model).
- Look at the screen with two homologous chromosomes (Step 2). The linkage is *cis*.
- First, the two homologs physically pair up (Step 2).



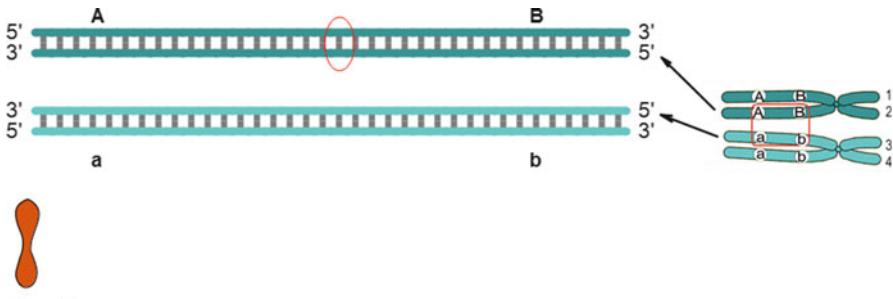
Step 2 Action: Physical pairing of two homologs

- Second, in the pachytene stage of prophase I, which consists of five stages (leptotene, zygotene, pachytene, diplotene, and diakinesis), a crossover between the non-sister chromatids appears (Step 3).
- Click the two-headed arrow to explore the molecular process in detail (Step 3).



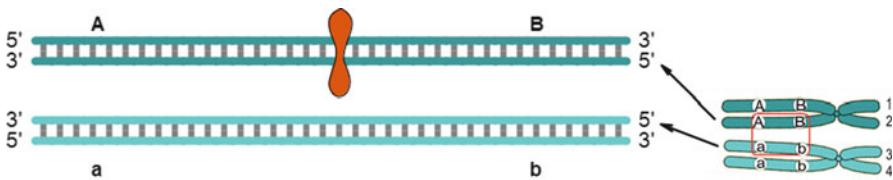
Step 3 Action: Crossing over between two loci

- The two DNA duplexes of the non-sister chromatids (2 and 3) are enlarged to show more detail (Step 4).

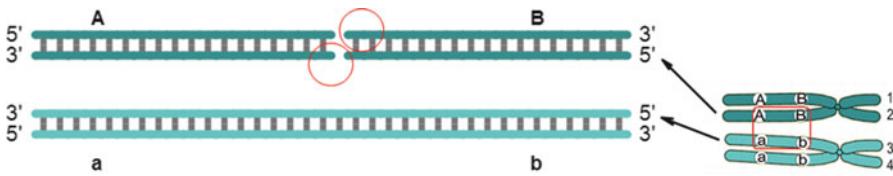


Step 4 Layout: Two DNA duplexes from the non-sister chromatids 2 and 3; Spo 11 is an enzyme

- Note the direction of each strand (the 5'- and 3'-ends).
- Crossing over does not occur randomly along the chromosome. Rather, crossing over is generally limited to 1,000- to 2,000-base, CG-rich regions between genes known as recombination hotspots.
- Homologous recombination involves several major steps as shown below.
- **Double-strand break:** Crossing over is initiated by the endonuclease Spo 11, which generates a double-strand break in one of the two DNA duplexes (Step 5).
- *Drag Spo 11 to the area circled in red* (Steps 4 and 5).

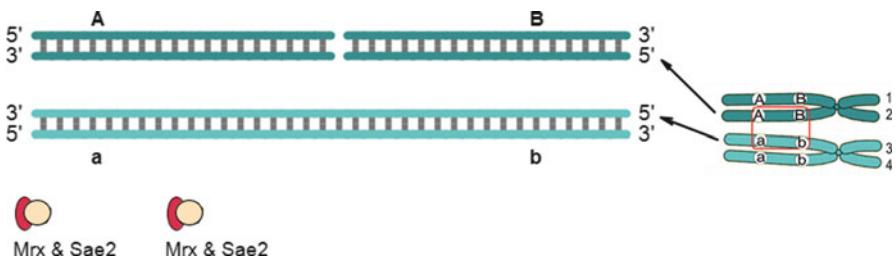


Step 5 Action: Binding of Spo 11 to one of the DNA duplexes

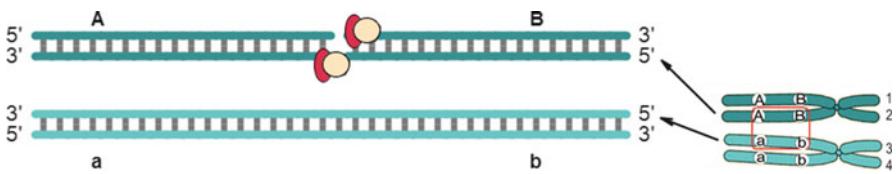


Step 6 Results: Double-strand break by Spo 11

- **Recession:** At this time, the 5'-ends of the break are processed (nucleotides removed).
 1. Two proteins, MRX and Sae2, first process the 5'-ends (removing nucleotides), creating short, 3'-overhangs of single-stranded DNA on the complementary strands.
 2. Then, processing continues with Sgs 1 (a helicase) separating the double-stranded DNA, allowing Exo1 and Dna2 (nucleases) to remove more nucleotides from the 5' ends.
- *Drag MRX and Sae2 to the target area (red circles in Step 6) for initial processing* (Steps 7 and 8).

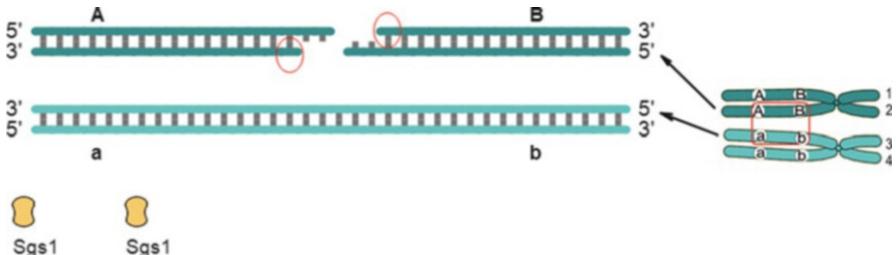


Step 7 Action: New 5'-ends ready for binding by Mrx and Sae2

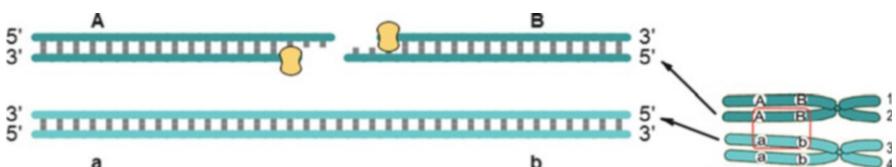


Step 8 Action: Binding of Mrx and Sae2 to the new 5'-ends

- Drag Sgs1 to the target area (red circle) to separate the double helix (Steps 9–11).

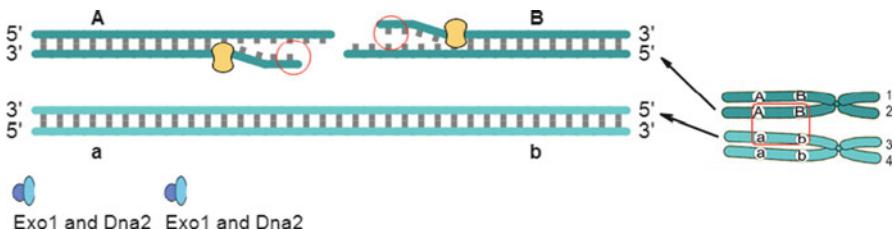


Step 9 Results: Recessed areas ready for Sgs 1 binding

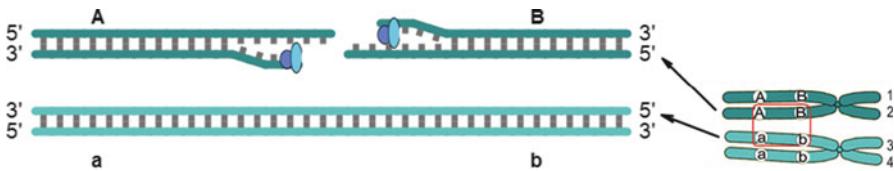


Step 10 Action: Binding of Sgs 1

- Drag Exo1 and Dna2 to the new target areas (red circles) for further processing (Steps 11 and 12).

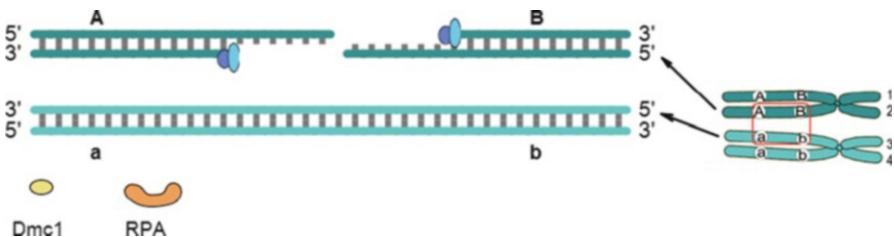


Step 11 Action: Separation of the double helix by Sgs 1, providing new target areas for Exo1 and Dna2

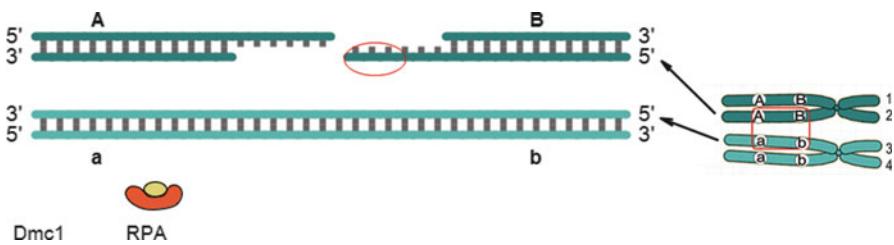


Step 12 Action: Binding of Exo1 and Dna2

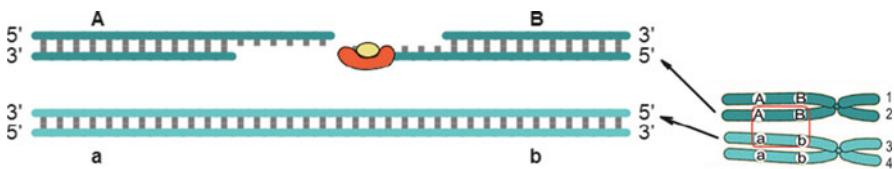
- **First strand invasion/D-loop formation:** At this time, in concert with the proteins Dmc1 and RPA, one of the hanging 3'-ends invades one of the DNA duplexes of the homologous chromosome (non-sister chromatid).
- *Drag Dmc1 to RPA (Steps 13 and 14), and then drag the activated Dmc1–RPA complex to one of the free 3'-ends (red circle) (Step 15).*



Step 13 Action: Layout: Showing Dmc1 and RPA

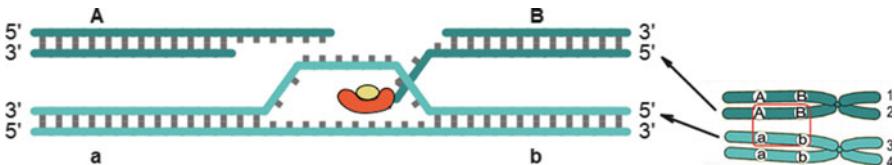


Step 14 Action: Formation of Dmc1–RPA complex



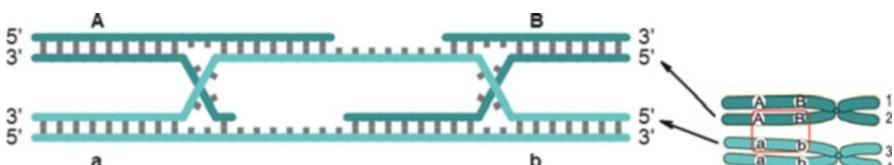
Step 15 Action: Binding of Dmc1–RPA complex to one of the 3'-ends

- The Dmc1–RPA-coated 3'-end invades one of the duplexes of the homologous chromosome, displacing one of its strands to create a cross-shaped structure known as a Holliday junction (Step 16).



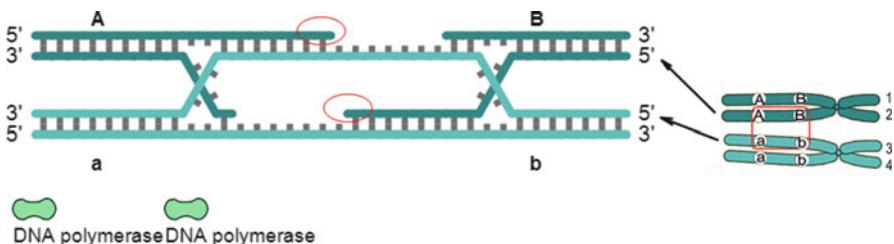
Step 16 Action: Holliday junction formation

- Second strand invasion:** The second strand invasion is similar to the first one, resulting in a second Holliday junction (Step 17).

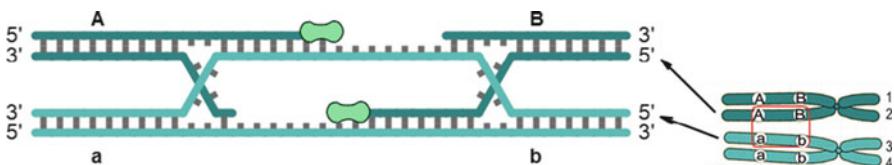


Step 17 Action: Second Holliday junction formation

- **DNA synthesis:** Drag DNA polymerase to the 3'-ends (red circles) to initiate DNA synthesis (Steps 18–20).

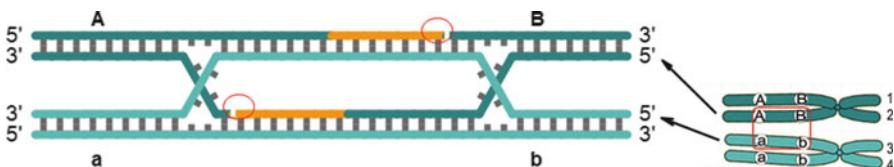


Step 18 Layout: 3'-ends as target areas for DNA polymerase binding



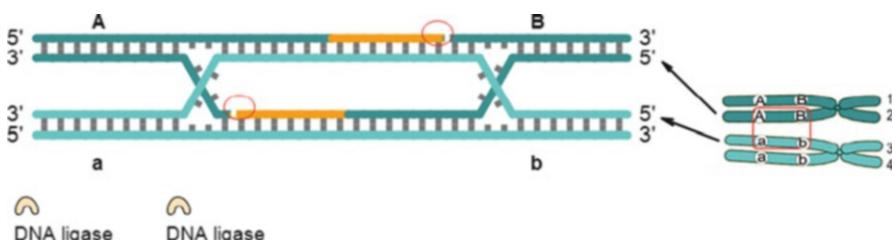
Step 19 Action: Binding of DNA polymerase

- The nicks between the new and old strands are sealed with DNA ligase, and a second Holliday junction is formed at each original junction.

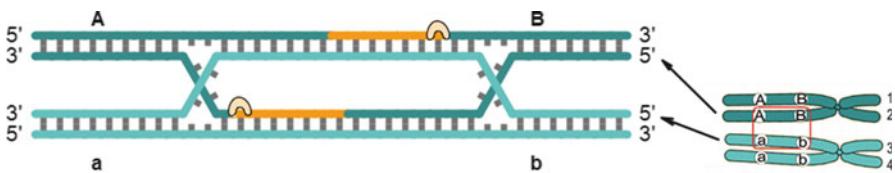
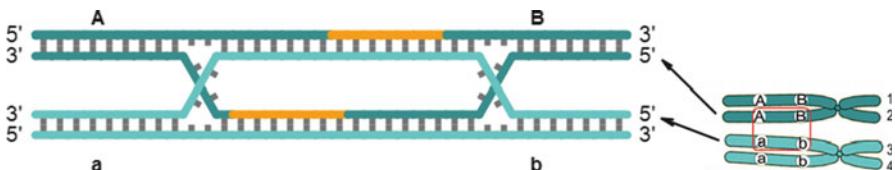


Step 20 Results: New DNA fragments fill in the gaps.

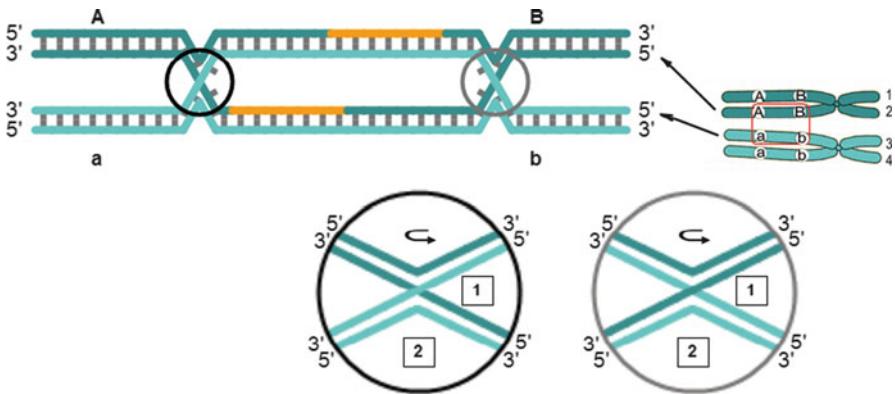
- *Drag DNA ligase to the nicks to seal them* (Steps 21–23).



Step 21 Layout: Nicks to be sealed by DNA ligase

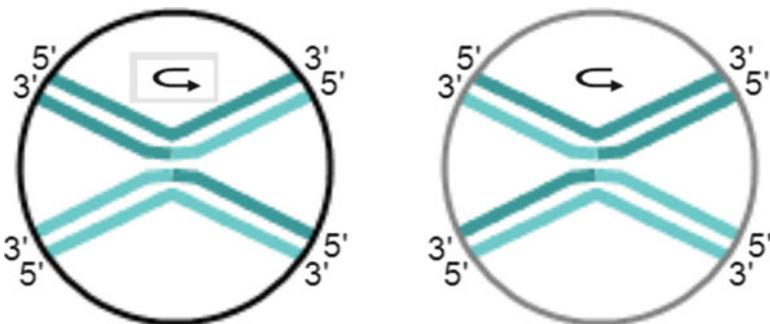
**Step 22 Action: Binding of DNA ligase****Step 23 Results: Sealing of nicks and completion of DNA synthesis**

- Branch migration and resolution:** The Holliday junctions may migrate along the linked DNA before being resolved, that is, split up by nicking endonucleases so that the two linear DNA duplexes are restored.
- The regions around the Holliday junctions are enlarged to show more detail (Step 24).

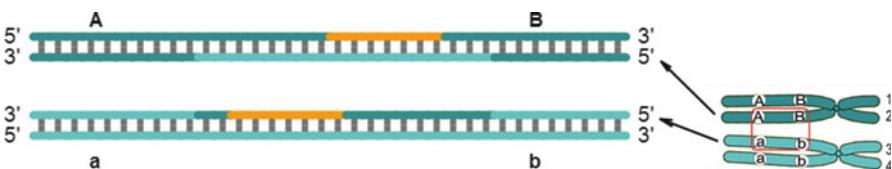
**Step 24 Layout: Enlargement of Holliday junctions**

- At each junction, there are two sites (1 and 2) that the nicking endonuclease may cut, making four cut combinations possible.
 - Both junctions are cut at site 1.
 - Both junctions are cut at site 2.
 - Junction 1 is cut at site 1 and junction 2 is cut at site 2.
 - Junction 1 is cut at site 2 and junction 2 is cut at site 1.

- Combinations 1 and 2 result in non-crossover products, that is, there is no genetic recombination except for a small patch of heteroduplex (two strands with different origins) DNA.
- Combinations 3 and 4 result in crossover products, that is, the genes on both sides of the break are recombined.
- *At both junctions, click site 1 twice (to cut and recombine)* (Steps 24 and 25).

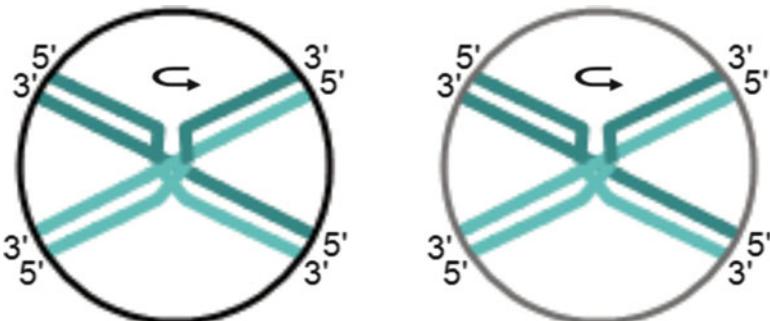


Step 25 Action: Cutting and resolving at site 1 for both junctions

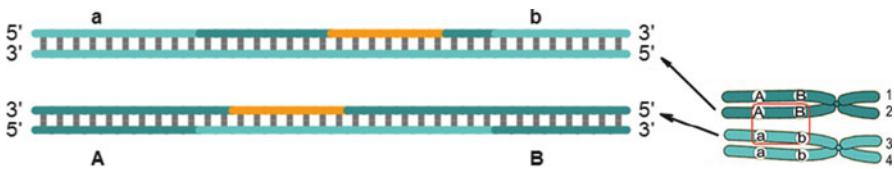


Step 26 Results: No recombination except at the heteroduplex regions

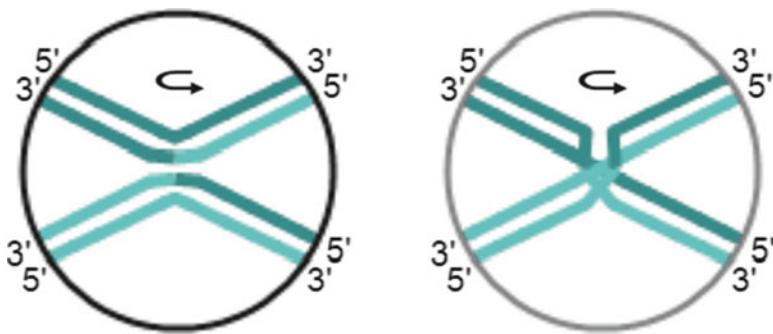
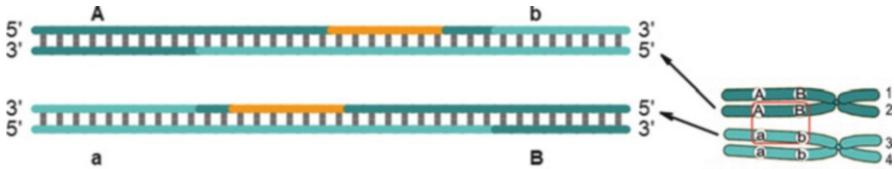
- *This results in a non-crossover (Step 26).*
- *At both junctions, click site 2 twice (Step 27).*



Step 27 Action: Cutting and resolving at site 2 for both junctions

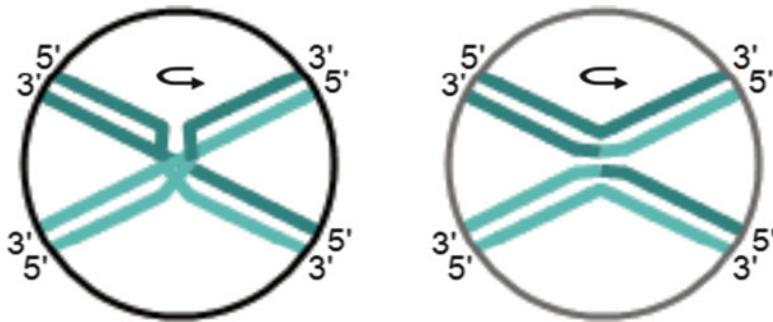
**Step 28** Results: A non-crossover

- This also results in a non-crossover (Step 28).
- At junction 1, click site 1 twice, and at junction 2, click site 2 twice (Steps 29).

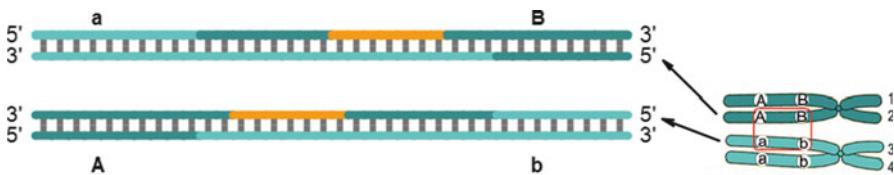
**Step 29** Action: Cutting and resolving at site 1 for junction 1 and cutting and resolving at site 2 for junction 2**Step 30** Results: Formation of a crossover: new recombinants of A-b and a-B genes

- This results in a crossover (Step 30).

- Finally, at junction 1, click site 2 twice, and at junction 2, click site 1 twice (Step 31).



Step 31 Action: Cutting and resolving at site 1 for junction 1 and cutting and resolving at 1 for junction 2)



Step 32 Results: Formation of a crossover: new recombinants of *a-B* and *A-b* genes

- This also results in a crossover (Step 32).

Conclusions

- The foregoing process demonstrates how homologous recombination may take place at the molecular level.
- The results are either a crossover or a non-crossover for the two genes in question.
- The heteroduplex (brown and green strands) region, which contains two patches of different DNA strands, will become a homoduplex again after one run of DNA replication.
- The molecular mechanism of recombination of linked genes is a complex process. Further research will lead to a better understanding of the mechanism.

Summary

- Meiosis is a key cellular and molecular process in sexual reproduction.
- Through chromosome behavior during meiosis (segregation of homologous chromosomes and sister chromatids), two genomes (two sets of chromosomes) in the original diploid cell are reduced to one genome (one set of chromosomes) in the gamete.
- The diploid chromosome number (two genomes) is regained after fusion of the male and female gametes.
- Another major function of meiosis is to provide genetic variation for enriching the gene pool, which is vital for the survival of a species.
- There are two ways to increase genetic variation during meiosis: one is independent assortment of genes on paternal and maternal chromosomes, and the other is homologous recombination of paternal and maternal genes on the homologs.
- Mutations, of course, can also increase genetic variation, but most mutations harm the organism. In the short run, therefore, most genetic variations among individual family members are the result of genetic recombination rather than mutations.

Quiz

1. Meiosis is a special type of cell division for producing
 - (a) Gametes
 - (b) Spores
 - (c) Two of the above
 - (d) None of the above
2. How many genomes comprise the primary spermatocyte or primary oocyte?
 - (a) 1
 - (b) 2
 - (c) 3
 - (d) 4
3. How many genomes are present in sperm?
 - (a) 1
 - (b) 2
 - (c) 3
 - (d) 4
4. Based on 46 human chromosomes per cell, fill in the following table

Stage of meiosis	Number of genomes	Number of chromosomes	Number of chromatids	Number of DNA molecules
Prophase I				
Metaphase I				
Telophase I or prophase II				
Metaphase II				
Telophase II				
Sperm/egg				

5. Which of the following is not true during meiosis?
- (a) Homologous chromosomes and allelic genes are segregated in gametes.
 - (b) Homologous chromosomes are never physically paired during prophase.
 - (c) Unlinked genes (genes on different chromosomes) are assorted independently during the formation of gametes.
 - (d) Linked genes (genes on the same chromosome) may be recombined through crossing over.
6. Cells with two heterozygous gene pairs (A/a and B/b on two different chromosome pairs) undergoing meiosis will produce how many types of gametes?
- (a) 2
 - (b) 4
 - (c) 6
 - (d) 8
7. The types of gametes produced in question 6 are the result of how many kinds of metaphase I chromosome configurations?
- (a) 1
 - (b) 2
 - (c) 3
 - (d) 4
8. In a genetic cross for gene mapping, assume that the original heterozygous parent has the *cis* linkage genotype AB/ab and that a single crossover between the two linked genes occurs in 10 % of the meiotic cells (there is no crossing over in the rest, i.e., 90 %, of the meiotic cells). What proportion of the gametes will have the recombinant genotype aB ?
- (a) 5 %
 - (b) 10 %
 - (c) 15 %
 - (d) 20 %
9. A plant with the genotype Ab/aB is crossed with another with the genotype ab/ab : A =tall, a =short, B =red petal, and b =white petal. The progeny (F_1) include 450 tall and white, 448 short and red, 50 tall and red, and 52 short and white plants. What is the map distance between these two genes?
- (a) 10.2 mu
 - (b) 11.0 mu
 - (c) 12.7 mu
 - (d) 15.4 mu
10. According to the double-strand cut model, two Holliday junctions may be cut by an endonuclease, making four possible cut combinations possible: (1) both junctions are cut at site 1, (2) both junctions are cut at site 2, (3) junction 1 is

cut at site 1 and junction 2 is cut at site 2, and (4) junction 1 is cut at site 2 and junction 2 is cut at site 1. Which two of these combinations will lead to crossover products?

- (a) 1 and 2
- (b) 2 and 3
- (c) 3 and 4
- (d) None of the above

Answers

1. c 2. d 3. a

4.

Stage of meiosis	Number of genomes	Number of chromosomes	Number of chromatids	Number of DNA molecules
Prophase I	4	46	92	92
Metaphase I	4	46	92	92
Telophase I or prophase II	2	23	46	46
Metaphase II	2	23	46	46
Telophase II	1	23	23	23
Sperm/egg	1	23	23	23

5. b 6. b 7. b 8. a 9. a 10. c

Module 8

Genes and Proteins: How Do Genes Work?

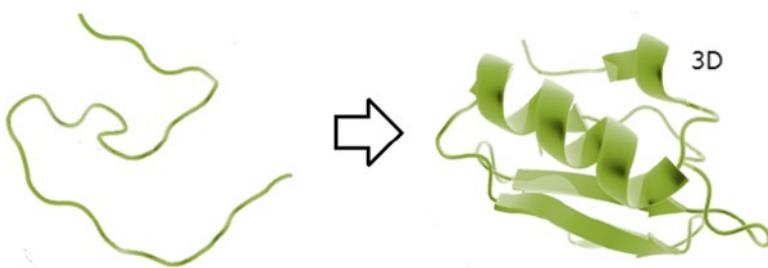
- We now know how DNA stores information in the form of genes, but how do genes control what we look like?
- To answer this question, we must understand the genetic code, which explains how information is transferred from DNA to RNA to a protein.
- We therefore need to study both proteins and RNA molecules to understand how genes work.

Proteins (*Interactive Program 1*)

- Similar to DNA, a protein is a macromolecule (large molecule) composed of connected subunits.
- The subunits of proteins are called amino acids (AA).
- There are 20 kinds of amino acids in living organisms.
- The sequence of amino acids determines the property of a protein.
- See Table 8.1 for the names of these amino acids and their abbreviations.
- Each kind of protein has a unique three-dimensional structure, which is determined by its amino acid sequence.
- The three-dimensional (3D) structure of a protein determines its function (Fig. 8.1).
- Some proteins are structural (Fig. 8.2), forming muscles, hair, and cytoskeletal structure (filamentous or tubular proteins in the cell), among many other entities.

Table 8.1 List of amino acids and their abbreviations

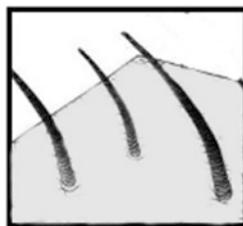
Alanine	(Ala)	Arginine	(Arg)	Asparagine	(Asn)
Aspartic acid	(Asp)	Cysteine	(Cys)	Glutamic acid	(Glu)
Glutamine	(Gln)	Glycine	(Gly)	Histidine	(His)
Isoleucine	(Ile)	Leucine	(Leu)	Lysine	(Lys)
Methionine	(Met)	Phenylalanine	(Phe)	Proline	(Pro)
Serine	(Ser)	Threonine	(Thr)	Tryptophan	(Trp)
Tyrosine	(Tyr)	Valine	(Val)		

**Fig. 8.1** Folding of a protein to form a 3D structure

• muscles



• hair



• cytoskeletal structure

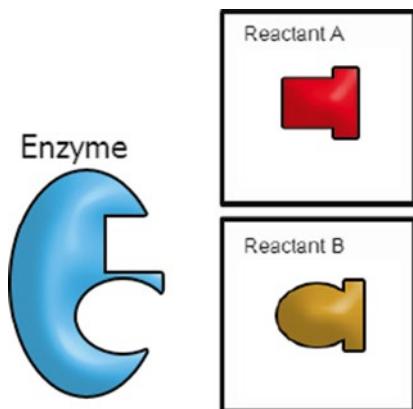
**Fig. 8.2** Examples of structural proteins

Enzymes: Proteins that Catalyze Chemical Reactions

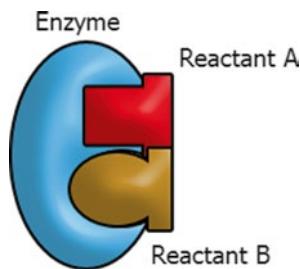
Use Interactive Program 1 on the software program available at Extras.Springer.com to familiarize yourself with proteins.

After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “Phenotype.”

- Other proteins are catalytic, meaning that they cause, or control the speed of, chemical reactions. These proteins are called enzymes.
- For example, reactants A and B may interact, with help of an enzyme, to form product C.
- *Drag reactants A and B from the pop-up window to the enzyme (shape is important) (Steps 1–4).*

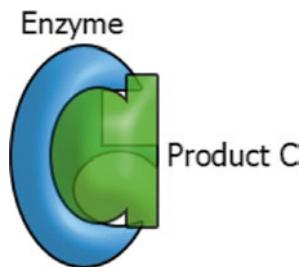


Step 1 Layout: Reactants A and B to be catalyzed by the enzyme



Step 2 Action: Fitting reactants A and B into active sites of the enzyme: bringing the reactants closer

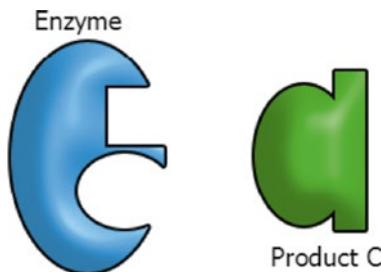
- As you can see, the surface of an enzyme contains specific sites (called active sites) into which reactants (chemicals) can fit. The enzyme, therefore, brings reactants (A and B) closer together so that they can interact to form a product (C).



Step 3 Action: Formation of product C

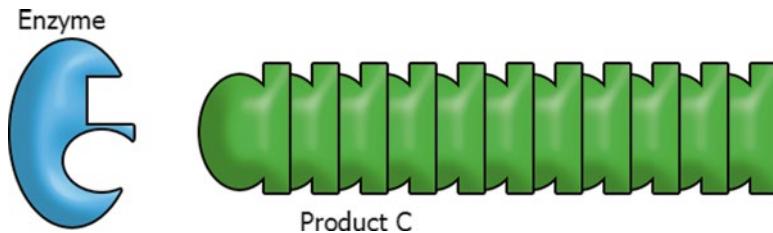
- Because product C is shaped differently than the reactants, it no longer fits the enzyme site, and it soon leaves.
- The reaction is $A + B \xrightarrow{\text{Enzyme}} C$

- When the product (C) leaves the enzyme, the enzyme is free to bind more reactants (A and B), generating more products (Steps 4 and 5).



Step 4 Results: Release of product C

- As the process continues, large amounts of reactants A and B are combined to form product C. Thus, small amounts of an enzyme can do large amounts of work (chemical reaction) (Step 5).



Step 5 Results: High efficiency of enzymes: only a small amount of enzyme is needed to catalyze a reaction

Phenotype

- The physical characteristics of an organism (its phenotype) are determined by (1) its structural components (formed by structural proteins) and (2) the results of chemical reactions (catalyzed by enzymes).
- Because proteins form the structural components and catalyze the chemical reactions in living systems, proteins are responsible for what we look like and what we are (our phenotype).
- However, we said previously that DNA (in the form of genes) is the genetic material. We also said that DNA is responsible for what we look like and what we are.
- Therefore, there must be a relationship between genes and proteins. Indeed, genes are responsible for protein structures.
- We will look into the relationship between genes and proteins in the following section.
- Protein function is determined by its three-dimensional structure, which depends on its linear sequence of amino acids.

- From this information, can you guess how proteins are related to genes?
- If you can, then you know how the information stored in genes controls how we look.
- See the following pages for detailed information on genes and proteins.

The Genetic Code (*Interactive Program 2*)

- Polypeptides are chains of amino acids. They represent the basic form of proteins.
- In other words, proteins are made up of one or more polypeptides.
- Genes are related to proteins (polypeptides) because the linear sequence of nucleotides in a gene codes for the linear sequence of amino acids in a protein (polypeptide).
- In fact, each three DNA nucleotides in a DNA sequence form a codon (the coding unit), which codes for one amino acid (Fig. 8.3).
- However, DNA codons do not directly code for the amino acids in a polypeptide or protein. Another molecule, which transfers the information from DNA to proteins, is needed: **RNA**.

RNA

- Although RNA is composed of a linear series of nucleotides resembling DNA, it differs from DNA in the following ways:
 1. RNA is usually single stranded, not a double helix.
 2. RNA nucleotides contain the sugar ribose (with an extra O) (Fig. 8.4, top) instead of deoxyribose.



Fig. 8.3 Relationship between DNA codons and amino acids of polypeptide: one DNA codon, one amino acid

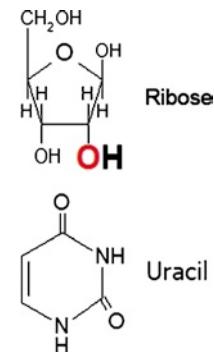


Fig. 8.4 Sugar of RNA (ribose) and base of RNA (uracil to replace DNA base thymine)

3. The pyrimidine thymine in DNA is replaced by uracil (U) in RNA (Fig. 8.4, bottom).
4. There are different kinds of RNA. We start with messenger RNA (mRNA).

Messenger RNA (mRNA): The Intermediate Molecule Between DNA and Protein

- In living systems, DNA does not directly code for proteins. Rather, an intermediate molecule called messenger RNA (mRNA) is needed.
- Therefore, there are two major steps in the transfer of genetic information.

• DNA (gene) → RNA → protein (polypeptide)

- Each step is explained in detail later.
- Right now, you only need to know that DNA codons are converted into mRNA codons, which then directly code for amino acids. The coding steps (Fig. 8.5) are as follows:
- The genetic code (Table 8.2) below shows the amino acid coded by each mRNA codon.

DNA codons (in DNA coding strand):	5' AGA	GCG	AAC	CTG	GTC	AGC	GTC	GTA 3'
mRNA codons (in mRNA):	5' AGA	GCG	AAC	CUG	GUC	AGC	GUC	GUU 3'
Amino acids (in protein):	Arg	Ala	Asn	Leu	Val	Ser	Val	Val

Fig. 8.5 Coding steps: from DNA codons to mRNA codons to amino acids

Table 8.2 The genetic code table

First	Second				Third
	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Stop	Stop	A
	Leu	Ser	Stop	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

- Three letters (Phe, Leu, etc.) are used to represent an amino acid. For example, Phe stands for phenylalanine and Leu for leucine.

Use Interactive Program 2 on the software program available at Extras. Springer.com to familiarize yourself with the genetic code.

After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “Coding Strand Versus Template Strand.”

- From the genetic code table, you can see that if
- The first mRNA base is U (highlighted),
- The second base is U (highlighted), and
- The third base is U (highlighted),
- Then the corresponding amino acid is Phe (upper red circle) (Step 1).

1 st	2 nd	U	C	A	G	3 rd
		Phe	Ser	Tyr	Cys	U
		Phe	Ser	Tyr	Cys	C
U		Leu	Ser	Stop	Stop	A
		Leu	Ser	Stop	Trp	G

Step 1 Results: mRNA codon UUU coding for Phe

- Thus, the mRNA codon UUU codes for Phe. Similarly, the codon UUC also codes for Phe (lower red circle in Step 1).

Thus, different codons may code for the same amino acid.

Some codons (UAA, UAG, and UGA) that code for no amino acids are called **stop codons** (Step 2, highlighted codons).

- Click ‘>’ to see the stop codons (Step 2).

1 st	2 nd	U	C	A	G	3 rd
		Phe	Ser	Tyr	Cys	U
		Phe	Ser	Tyr	Cys	C
U		Leu	Ser	Stop	Stop	A
		Leu	Ser	Stop	Trp	G

Step 2 Results: Three stop codons: highlighted

- There are 64 possible codons (Step 3), but only 20 amino acids.
- Of the 64 codons, 3 are stop codons (they do not code for any amino acids).
- Because more than one codon may code for a particular amino acid, the genetic code is called “degenerate.”
- For example, six codons (UUA, UUG, CUU, CUC, CUA, CUG) code for the amino acid leucine (Leu), whereas only one codon (AUG) codes for methionine (Step 3).

1 st	2 nd	U	C	A	G	3 rd
U	Phe	Ser	Tyr	Cys	U	
	Phe	Ser	Tyr	Cys	C	
	Leu	Ser	Stop	Stop	A	
	Leu	Ser	Stop	Trp	G	
C	Leu	Pro	His	Arg	U	
	Leu	Pro	His	Arg	C	
	Leu	Pro	Gln	Arg	A	
	Leu	Pro	Gln	Arg	G	
A	Ile	Thr	Asn	Ser	U	
	Ile	Thr	Asn	Ser	C	
	Ile	Thr	Lys	Arg	A	
	Met	Thr	Lys	Arg	G	
G	Val	Ala	Asp	Gly	U	
	Val	Ala	Asp	Gly	C	
	Val	Ala	Glu	Gly	A	
	Val	Ala	Glu	Gly	G	

Step 3 Results: Six codons for Leu and AUG codes for Met

- Drag an appropriate amino acid on the right (some are not shown here) to the amino acid box specified by the codon (Steps 4 and 5).

	2 nd	U	C	A	G	3 rd
1 st						
U	Phe	Ser	Tyr	Cys	U	
	Phe	Ser	Tyr	Cys	C	
	Leu	Ser	Stop	Stop	A	
	Leu	Ser	Stop	Trp	G	
C	Leu	Pro	His	Arg	U	
	Leu	Pro	His	Arg	C	
	Leu	Pro	Gln	Arg	A	
	Leu	Pro	Gln	Arg	G	
A	Ile	Thr	Asn	Ser	U	
	Ile	Thr	Asn	Ser	C	
	Ile	Thr	Lys	Arg	A	
	Met	Thr	Lys	Arg	G	
	G	Val	Ala	Asp	Gly	U
Val		Ala	Asp	Gly	C	
Val		Ala	Glu	Gly	A	
Val		Ala	Glu	Gly	G	

* Drag the appropriate amino acid to the box specified by the codon.

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Genes and Proteins

Step 4 Action: Move appropriate amino acids to the boxes next to the codons

	2 nd	U	C	A	G	3 rd
1 st						
U	Phe	Ser	Tyr	Cys	U	
	Phe	Ser	Tyr	Cys	C	
	Leu	Ser	Stop	Stop	A	
	Leu	Ser	Stop	Trp	G	
C	Leu	Pro	His	Arg	U	
	Leu	Pro	His	Arg	C	
	Leu	Pro	Gln	Arg	A	
	Leu	Pro	Gln	Arg	G	
A	Ile	Thr	Asn	Ser	U	
	Ile	Thr	Asn	Ser	C	
	Ile	Thr	Lys	Arg	A	
	Met	Thr	Lys	Arg	G	
	G	Val	Ala	Asp	Gly	U
Val		Ala	Asp	Gly	C	
Val		Ala	Glu	Gly	A	
Val		Ala	Glu	Gly	G	

amino acid	codon
Tyr	UAU
Gly	GGU
Ser	AGU
Asn	AAU
Glu	GAG
Ala	GCU
Thr	ACU
His	CAU

Step 5 Results: Assignment of amino acids to the appropriate boxes

- In the following figure are the results (Step 6) of the exercise above (Step 5).
- We now want (Step 7) to determine the original DNA codons coding for the mRNA codons.



Amino acid (protein)	Tyr	Gly	Ser	Asn	Glu	Ala	Thr	His
mRNA codon (mRNA)	UAU	GGU	AGU	AAU	GAG	GCU	ACU	CAU
DNA codon (DNA coding strand)								

Step 6 Action: Assign DNA codons to the mRNA codons

- As you already know, DNA is double stranded. The strand containing the codons is called the coding strand, and its complementary strand is called the template, or noncoding, strand. **In other words, the noncoding strand is a template for mRNA and the coding strand is the non-template strand.**
- Drag the DNA codons on the right to the table above (Step 6) to create the DNA coding strand (Step 7).

Amino acid (protein)	Tyr	Gly	Ser	Asn	Glu	Ala	Thr	His
mRNA codon (mRNA)	UAU	GGU	AGU	AAU	GAG	GCU	ACU	CAU
DNA codon (DNA coding strand)	TAT	GGT	AGT	AAT	GAG	GCT	ACT	CAT

Step 7 Results: The original DNA codons, which code for mRNA codons, which, in turn, code for amino acids

Coding Strand Versus Template Strand

- Use the following double-stranded DNA to understand some important terms.

5' TAT GGT AGT ... 3'
3' ATA CCA TCA ... 5'

- Traditionally, DNA codons are written from the left to the right and from the 5'-end to the 3'-end.
- In this case, the upper strand is the coding strand (or sense strand), and the lower strand is the template (also called noncoding strand or antisense strand).
- What is the nucleotide base sequence of the mRNA based on the foregoing information?
- Hint: The mRNA base sequence is the same as the sequence on the coding strand but complementary to the sequence on the template strand.
- Therefore, the mRNA sequence is

5' UAU GGU AGU 3'

- Even today, these terms confuse many biologists (and textbooks). If you can understand all these terms, you should be proud of yourself.

Conclusions

- So far, you have learned the following:
 - Genes contain DNA codons, which generate mRNA codons.
 - mRNA codons are used to code for amino acids in a protein.
 - Proteins are responsible for the formation of the phenotype (genetic traits).
- In the next two modules, we will learn how information in DNA is transferred to mRNA (transcription) and how the information in mRNA is used to produce proteins (translation).

Quiz

1. The subunits of a protein are called
 - (a) DNA nucleotides
 - (b) RNA nucleotides
 - (c) Amino acids
 - (d) Sugars
2. Amino acids are directly coded for by
 - (a) DNA codons
 - (b) mRNA codons
 - (c) Individual DNA nucleotides
 - (d) Individual RNA nucleotides
3. mRNA codons are coded for by
 - (a) DNA codons
 - (b) Amino acids
 - (c) Deoxyriboses
 - (d) Riboses
4. In a double-stranded DNA molecule, the strand that codes for mRNA is called the
 - (a) Coding strand
 - (b) Noncoding strand
 - (c) Template strand
 - (d) None of the above
5. The DNA coding strand contains
 - (a) Amino acids
 - (b) mRNA codons
 - (c) DNA codons
 - (d) None of the above
6. The noncoding DNA strand serves as a template for synthesizing
 - (a) RNA
 - (b) A polypeptide
 - (c) A dipeptide
 - (d) None of the above
7. In living organisms, how many kinds of amino acids are used to make proteins?
 - (a) 10
 - (b) 15
 - (c) 18
 - (d) 20

8. There are four RNA bases. Since three bases form an RNA codon, how many different RNA codons can be formed? (Hint: Use the genetic code table as a reference.)
- (a) 60
 - (b) 64
 - (c) 70
 - (d) 74
9. Which of the following statements is correct?
- (a) Often more than one codon may code for the same amino acid
 - (b) One codon can only code for one amino acid
 - (c) As many as 10 codons may code for one amino acid
 - (d) One codon often codes for more than one amino acid
10. Of the possible 64 codons, how many are stop codons?
- (a) 1
 - (b) 2
 - (c) 3
 - (d) 4

Answers

1. c 2. b 3. a 4. a 5. c 6. a 7. d 8. b 9. a 10. c

Module 9

Gene Expression I (Transcription and RNA Processing): How Is Information Transferred from DNA to RNA?

- As we have learned, genes are sections of DNA that contain information, information which is ultimately expressed through the processes of transcription and translation.
- Although genes are sections of DNA, not all sections of DNA are genes: this is particularly true in higher eukaryotes. In humans, for example, only a small percentage of the genome is genic DNA. Because most of the intergenic DNA has no known functions, these DNAs are known as junk or selfish DNA.
- Furthermore, not all genes code for proteins: only genes that are transcribed into mRNA code for proteins. Some genes are transcribed into other types of RNA, which directly participate in many important cellular functions. For example, tRNA and rRNA serve as part of the protein synthesis machinery, snRNA is needed for RNA splicing, and microRNA and small interfering (si)RNA are essential for silencing target genes.
- In short, all genes are transcribed into RNAs, but only mRNA is translated into proteins.

Prokaryotic Transcription: Overall Process

(*Interactive Program 1*)

- Prokaryotes are cells (such as bacteria) without a nuclear envelope.
- Through the process of transcription, the information in DNA is transferred to RNA.
- Specifically, the enzyme RNA polymerase (RNAP) synthesizes RNA from a DNA template.
- Logically, a gene lies next to a structure (*cis* control element) that is recognized by RNA polymerase. This important control element is called the **promoter**. In prokaryotes (bacteria), certain genes cluster together to form an operational unit (operon). In other words, these genes (also called structural genes) are controlled



Fig. 9.1 The overall structure of a gene

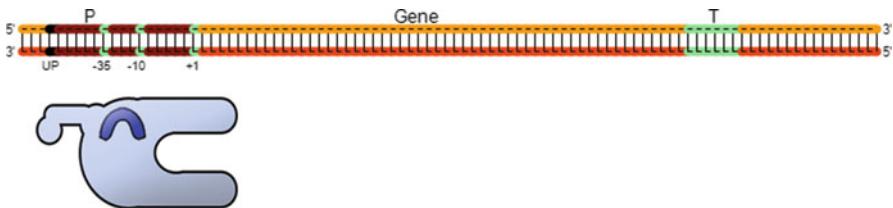
by the same promoter and transcribed into a single mRNA molecule, which is then translated into different proteins.

- For simplicity, we use a single gene to illustrate the transcription process.
- The last part of a gene is called the terminator, which is where transcription ends.
- The entire RNAP enzyme (holoenzyme) consists of a core enzyme (with several protein units) and a sigma (σ) factor.
- Within the promoter are a few conserved nucleotide sequences known as consensus sequences, which RNA polymerase recognizes and binds.
- Let us examine the basic structure of a gene (Fig. 9.1).
- Note that the two DNA strands run in opposite directions. Only one strand is used as the template for RNA synthesis. The non-template strand is called the coding (sense) strand, because its codons are the same as the RNA codons that are synthesized from the template strand (except that the T's in DNA are replaced by U's in RNA). The noncoding strand is, therefore, the template strand.
- Which strand in Fig. 9.1 is the coding strand?
- Because all RNAs are synthesized in the 5'- to 3'-direction, the DNA template strand must run in the 3'- to 5'-direction. Therefore, the template strand, or noncoding strand, is the bottom strand, and the top strand is the coding strand. Note that the promoter is upstream (toward the 5'-end) of the gene (coding strand) and that the terminator is downstream (toward the 3'-end) of the gene (coding strand).
- Within the promoter (P) are the following regions: -35, -10, and UP. These are the so-called consensus sequences (conserved sequences) to which the RNA polymerase attaches.
- The nucleotides of the promoter are designated by negative numbers, which become progressively larger the further upstream the nucleotides are.
- Transcription starts at +1.
- RNA synthesis stops at the terminator (T).
- The overall process of transcription is composed of (1) **initiation**: binding and aligning of RNA polymerase at the promoter; (2) **elongation**: RNA synthesis; and (3) **termination**: completion and release of the newly synthesized RNA from the template strand. We examine these steps in detail below.

Use Interactive Program 1 on the software program available at Extras. Springer.com to familiarize yourself with prokaryotic transcription (overall process).

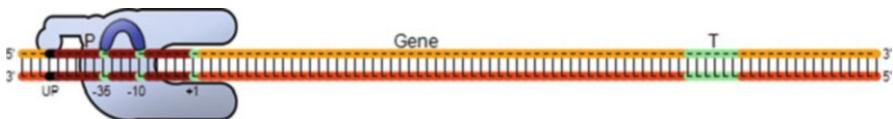
After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “Prokaryotic Transcription.”

- Initiation:** Drag RNAP (with the σ factor) to the promoter (Steps 1 and 2). *Escherichia (E.) coli* RNAP consists of two parts: the core enzyme (gray) and the σ factor (dark purple), which functions to lead the core RNAP to the -35 and -10 regions of the promoter. Before transcription, the two combine to form a holoenzyme.



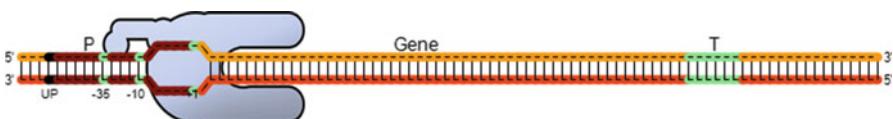
Step 1 Layout: A gene with its promoter and the RNA polymerase holoenzyme

- Click on the RNAP to initiate RNA synthesis (Step 2). The dsDNA (double-stranded DNA) is separated, forming an open complex as the σ factor leaves.



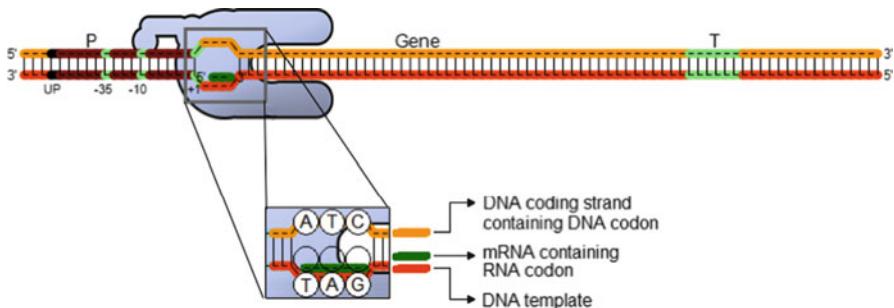
Step 2 Action: Binding of RNA polymerase: initiation

- Elongation:** Click on the RNAP again to continue RNA synthesis (Steps 3–6).



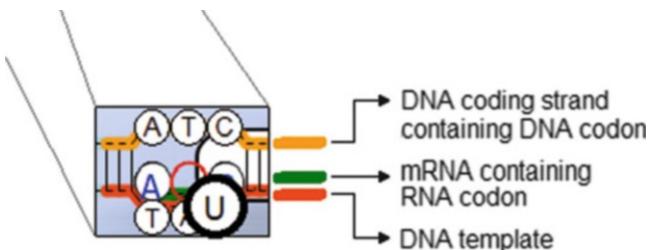
Step 3 Action: Formation of an open complex for RNA synthesis

- Because RNA is synthesized in the 5'- to 3'-direction, the RNA nucleotides base pair with the nucleotides on the 3'- to 5'-DNA strand (template) (Step 4).



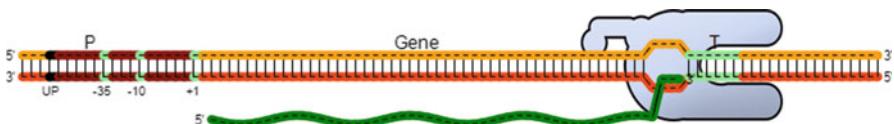
Step 4 Action: Pairing of appropriate RNA nucleotides with the complementary DNA nucleotides of the noncoding strand (template)

- Drag the appropriate RNA nucleotides to pair with the nucleotides of the noncoding DNA strand (Step 5).



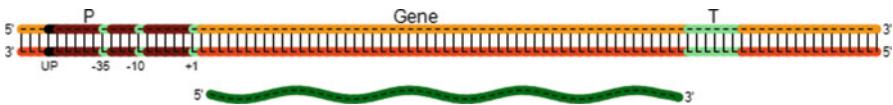
Step 5 Action: Drag the complementary RNA nucleotides to the correct positions

- Elongation continues (the RNA strand is separated from the DNA template and the denatured DNA strands are renatured) until the ribosome reaches the terminator (Step 6).



Step 6 Action: Continuation of the elongation process

- **Termination:** When the RNAP reaches the terminator, it leaves the DNA template, and RNA synthesis is complete (Step 7).



Step 7 Results: Completion of transcription: termination

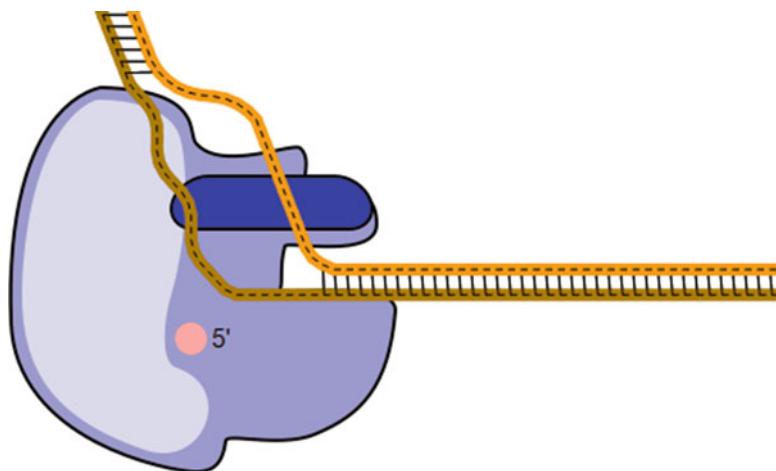
- There are two ways for transcription to terminate in prokaryotes:
 1. Rho-independent termination (hairpin formation)
 2. Rho-dependent termination
- See the following sections for additional information on the error-prone initial RNA fragments and the termination processes.

Prokaryotic Transcription: Error-Prone RNA Fragments and Rho-Independent Termination (Hairpin Formation) (Interactive Program 2)

[Use Interactive Program 2 on the software program available at Extras.Springer.com to learn more about initiation and Rho-independent termination \(hairpin formation\).](#)

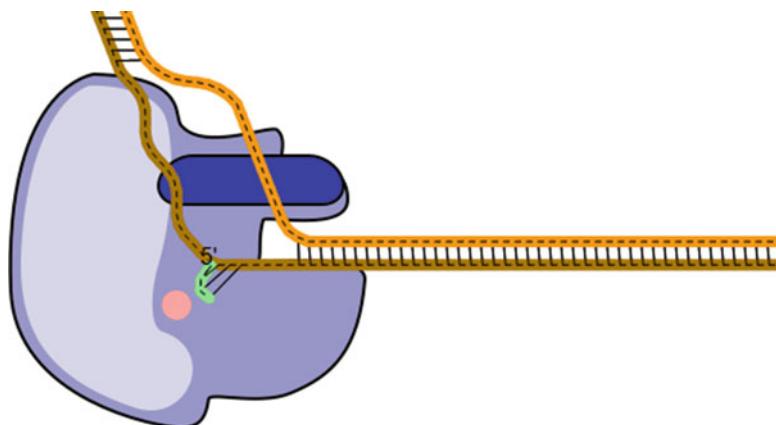
[After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “Prokaryotic Transcription and Rho-Dependent Termination.”](#)

- As you have seen, at the start of transcription, the RNAP holoenzyme attaches to the double helix, aligning itself on the promoter (Step 1). The core enzyme is light purple in color; the σ factor is dark blue.
- Magnesium, which RNAP needs to initiate transcription, is shown as a pink dot.
- The upper strand (yellow) is the coding strand, making the lower strand (brown) the template or noncoding strand.
- At this point, the RNAP holoenzyme has separated the dsDNA to create an open complex.

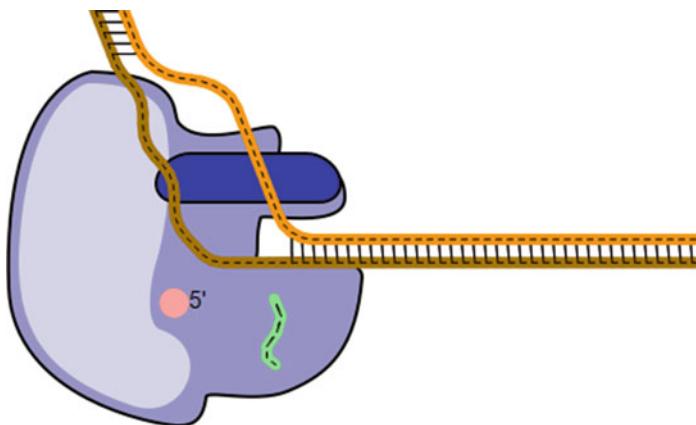


Step 1 Layout: Initiation: attachment of RNAP to form an open complex for RNA synthesis

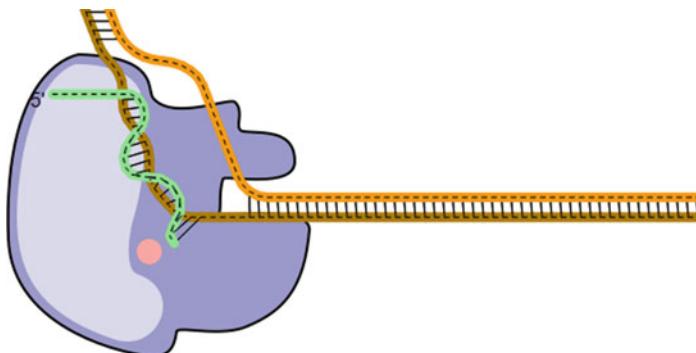
- Click on the enzyme to start RNA synthesis (Steps 2 and 3).
- Transcription is initially inaccurate (within the first 10 bases), and the short, error-prone RNA fragments (green) are aborted repeatedly (Step 3).
- Once the correct RNA sequence is synthesized, the σ factor is released and elongation continues (Step 4).



Step 2 Action: The first RNA nucleotide (green) enters to start abortive RNA synthesis

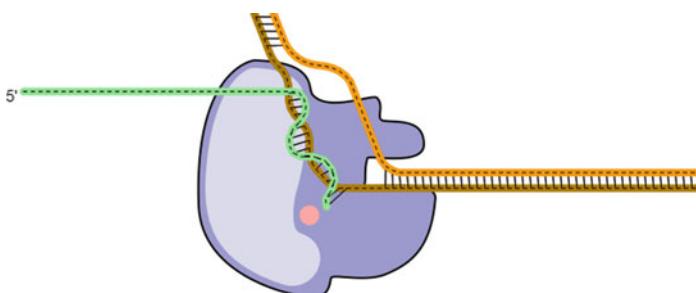


Step 3 Action: The aborted RNA fragment falls off

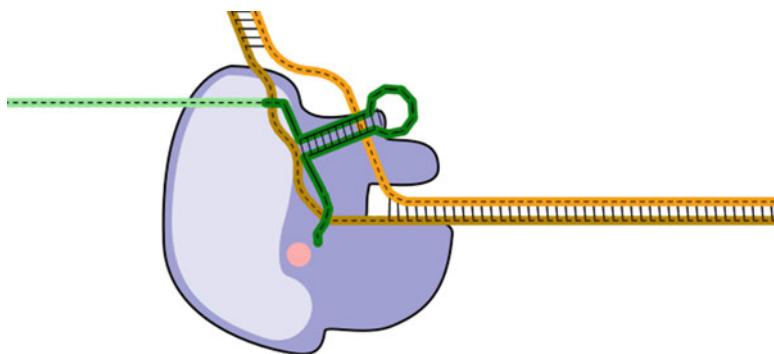


Step 4 Action: Release of the σ factor and continuation of the elongation process

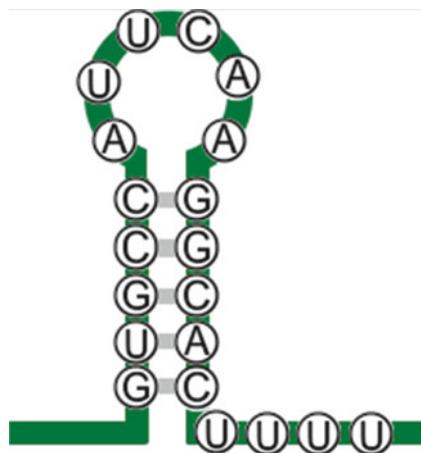
- Click to prepare for termination (Step 5).
- At the terminator, there is a sequence of GC repeats (Steps 5–8) that, once transcribed, causes sections of the newly synthesized RNA to pair with each other, creating a hairpin structure.



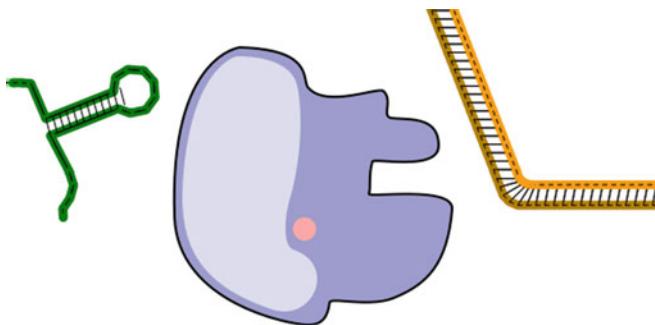
Step 5 Action: Beginning of GC repeats (dark green) in preparation for termination

**Step 6** Action: Formation of hairpin structure

- Click the magnifying glass (highlighted) to see the detailed stem-and-loop structure of the hairpin (Step 7).

**Step 7** Layout: Detailed view of the hairpin structure, which is followed by a series of U's

- The hairpin is followed by a series of uracil nucleotides (Step 7).
- The configuration of the hairpin disrupts base pairing between the newly synthesized RNA strand and the DNA template. This disruption, coupled with weak base pairing between the uracil nucleotides of the RNA strand and the adenine nucleotides of the DNA template (only two hydrogen bonds between each base pair), leads to the termination of transcription (Step 8).



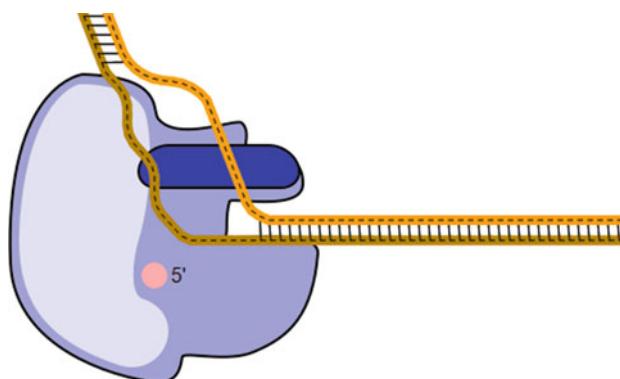
Step 8 Results: Termination: separation of newly synthesized RNA, RNAP, and DNA

Prokaryotic Transcription: Error-Prone RNA Fragments and Rho-Dependent Termination (*Interactive Program 3*)

[Use Interactive Program 3 on the software program available at Extras.Springer.com to learn more about initiation and Rho dependent termination.](http://www.springer.com/extra)

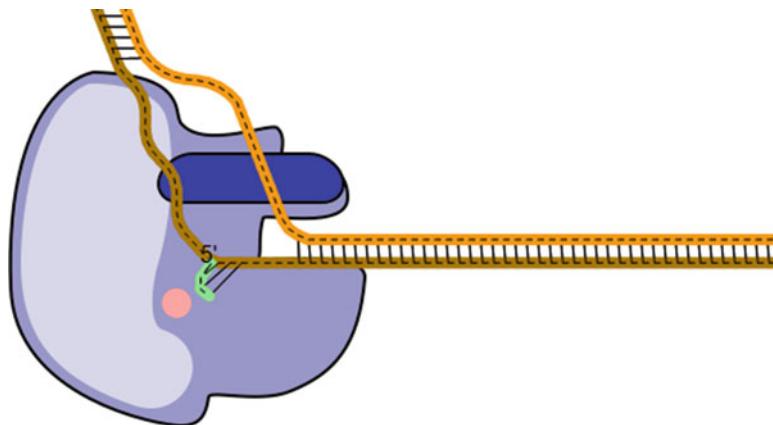
[After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “Eukaryotic Transcription and RNA Processing.”](#)

- To fully understand the transcription process, initiation and elongation of transcription are repeated below.
- As you have seen, at the start of transcription, the RNAP holoenzyme attaches to the double helix, aligning itself on the promoter (Step 1). The core enzyme is light purple in color and the σ factor is dark blue.
- Magnesium, which RNAP needs to initiate transcription, is shown as a pink dot.
- The upper strand (yellow) is the coding strand, making the lower strand (brown) the template or noncoding strand.

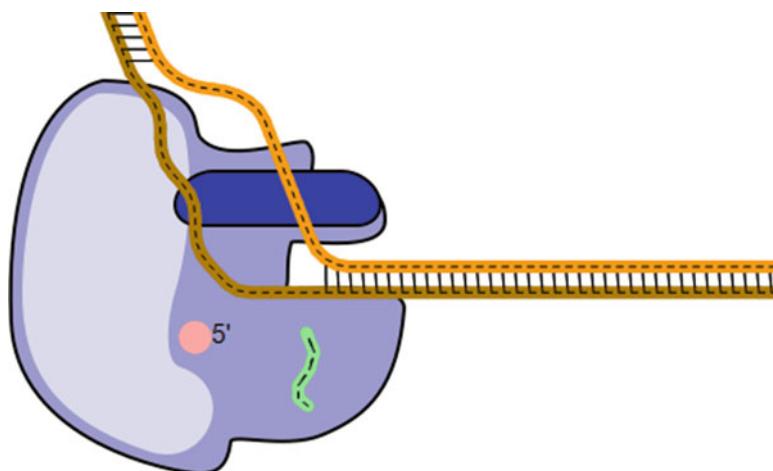


Step 1 Layout: Initiation: attachment of RNAP to form an open complex for RNA synthesis

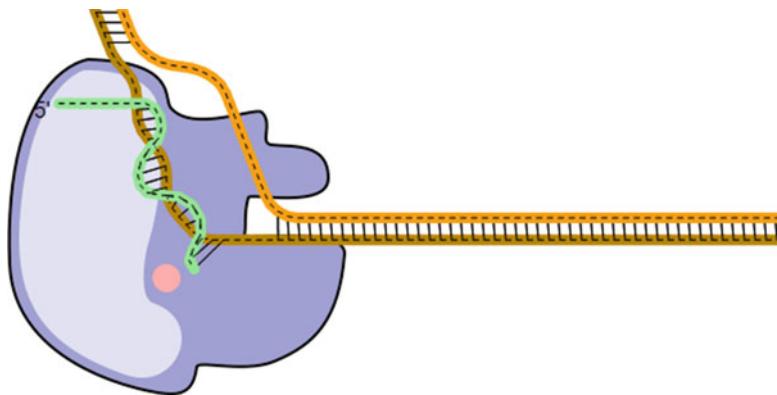
- At this point, the RNAP holoenzyme has separated the dsDNA to create an open complex.
- *Click on the enzyme to start RNA synthesis* (Steps 2 and 3).
- Transcription is initially inaccurate (within the first 10 bases), and the short, error-prone RNA fragments (green) are aborted repetitively (Step 3).
- Once the correct RNA sequence is synthesized, the σ factor is released and elongation continues (Step 4).



Step 2 Action: The first RNA nucleotide (green) enters to start abortive RNA synthesis

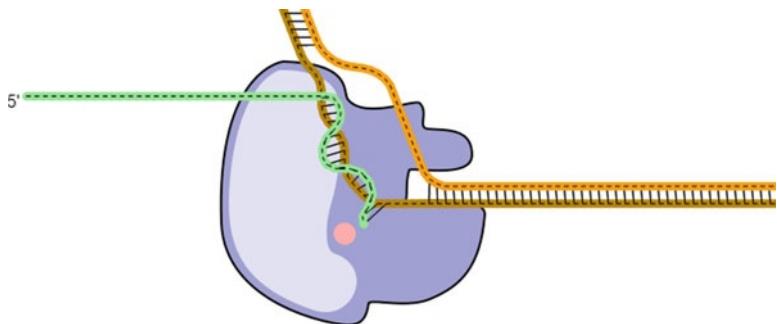


Step 3 Action: The aborted RNA fragment falls off



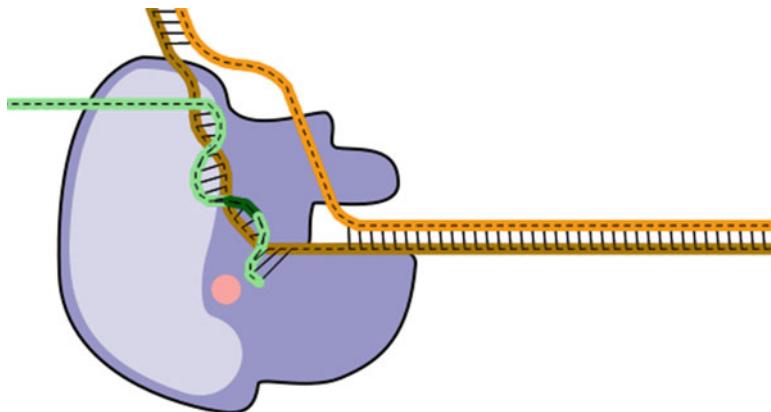
Step 4 Action: Release of the σ factor and continuation of the elongation process

- *Click to prepare for termination (Step 5).*
- In Rho-dependent termination, no hairpin structure is formed. Instead, a protein known as the Rho factor (gray and pink) recognizes a special nucleotide sequence (dark green) near the end of the newly synthesized RNA strand and binds there (Steps 5–7).

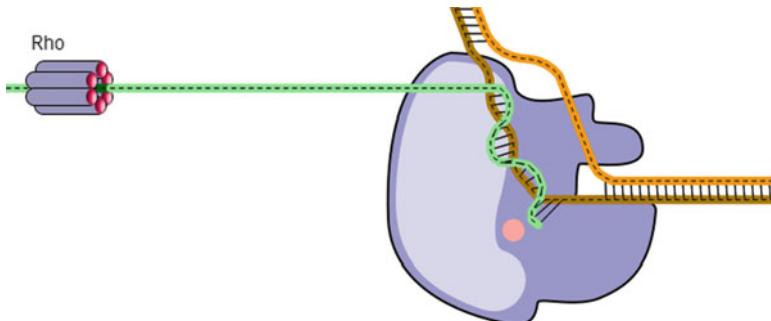


Step 5 Action: A special nucleotide sequence (dark green) is being synthesized

- When the special RNA sequence is fully synthesized (Step 6), it is available to bind with the Rho factor (Step 7).

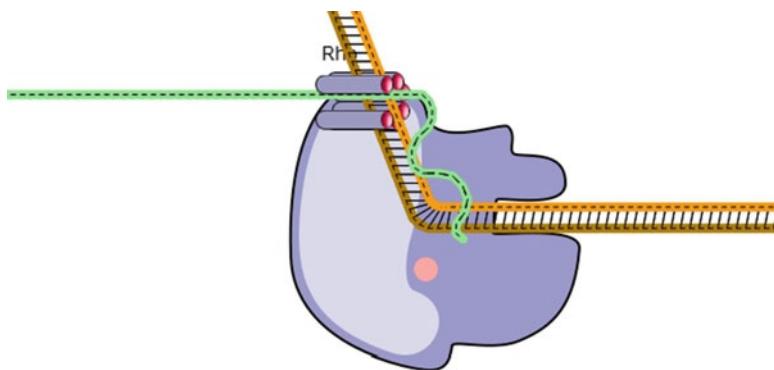


Step 6 Action: Availability of Rho factor-binding sequence

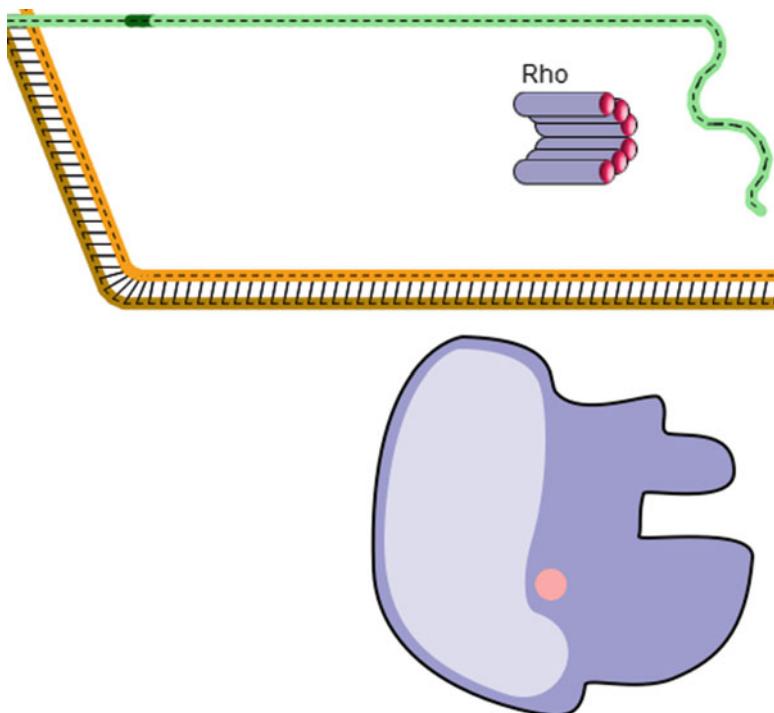


Step 7 Action: Binding of Rho factor

- Once the Rho factor attaches itself to the special sequence (Step 7), it starts to move along the RNA strand at a rate much faster than the rate at which RNA polymerase moves along the RNA strand.
- When the Rho factor collides with the RNA polymerase, the transcription machinery falls apart and transcription terminates (Steps 8 and 9).



Step 8 Action: Rho factor collides with RNAP



Step 9 Results: The transcription machinery breaks apart: termination

Eukaryotic Transcription and RNA Processing (Interactive Program 4)

- In addition to initiation, elongation, and termination, mRNA transcription is followed by a series of RNA processing steps: capping, polyadenylation, and splicing.
- Furthermore, in eukaryotes, there are three classes of RNA polymerase:
 1. RNA polymerase I transcribes rRNA genes (except 5S rRNA).
 2. RNA polymerase II transcribes mRNA genes.
 3. RNA polymerase III transcribes small, functional RNA genes (tRNA, snRNA, 5S RNA, etc.).
- Here, we focus on RNA polymerase II, which transcribes the protein-coding genes.
- Eukaryotic genes, unlike prokaryotic genes, have more than one *cis* control element:
 1. Promoter: Many RNA pol II promoters contain a TATA box around the -30 region (consensus sequence).
 2. Enhancer: The enhancer is another *cis* control element that augments gene transcription. Differing from promoters, an enhancer may lie far from the gene it controls (upstream, downstream, or even on another chromosome).
 3. Silencer: The silencer is a DNA region that suppresses transcription. We will not discuss this control element here.
- Gene structure: Eukaryotic genes are often interrupted by intervening sequences called introns, which are transcribed but not translated. The sections of the gene that are expressed (translated) are called exons (expressed sequences). Clearly, a series of introns and exons must alternate along the length of the gene.
- The primary transcript, or pre-mRNA, contains both introns and exons.
- In a process of RNA splicing, the introns of the primary transcript are excised and the exons are joined together.
- RNA splicing is just one way in which RNA is processed. RNA processing also includes capping (adding a methylated guanosine triphosphate to the 5'-end of the mature mRNA) and polyadenylation (adding a poly-A tail to the 3'-end of the mature RNA).
- In other words, a primary transcript (pre-mRNA) must undergo capping, splicing, and polyadenylation to become a functional (mature) mRNA.
- Because RNA pol II does not have a high affinity for the promoter, a number of protein factors (transcription factors, activators, mediators, etc.) are needed to facilitate binding.
- The general structure of a eukaryotic gene is shown in Fig. 9.2.

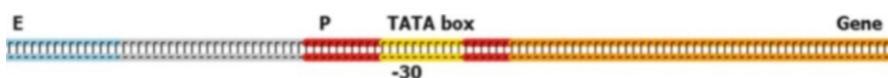


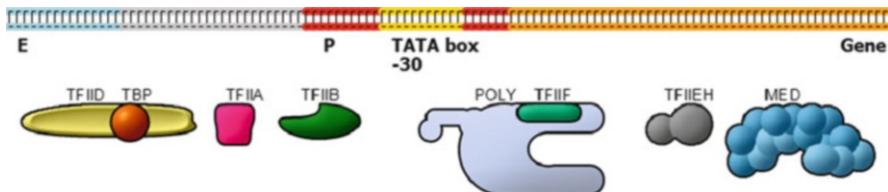
Fig. 9.2 General structure of a eukaryotic gene

- The two control elements are E (enhancer) and P (promoter).
- Many RNAP II promoters contain a consensus sequence known as the TATA box at the -30 region, where the preinitiation complex forms.
- The TATA box is recognized by one of the subunits of the **general transcription factor TFIID**: TBP (TATA box-binding protein).
- After TFIID is bound, other transcription factors bind in the following order: TFIIA, TFIIB, TFIIF with RNAP II, TFIIE, and TFIIH.
- The formation of the preinitiation complex leads to the melting of the promoter.
- Other proteins needed for transcription include activators and mediators. Activators act on enhancers to facilitate the binding of RNA pol II, whereas mediators join transcription factors and activators together, securing the preinitiation complex.
- As in prokaryotic transcription, there is a series of abortive attempts at RNA synthesis before RNAP II is able to leave the promoter and initiate elongation.

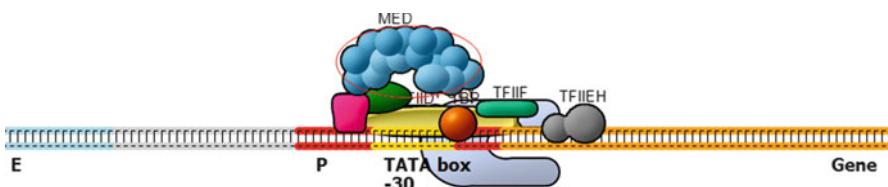
[Use Interactive Program 4 on the software program available at Extras. Springer.com to familiarize yourself with eukaryotic transcription and RNA processing.](#)

[After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “Conclusions at the end of Module 9.”](#)

- Drag the proteins below (from left to right) (Steps 1 and 2) to the proper locations on the DNA (promoter) to form the preinitiation complex.

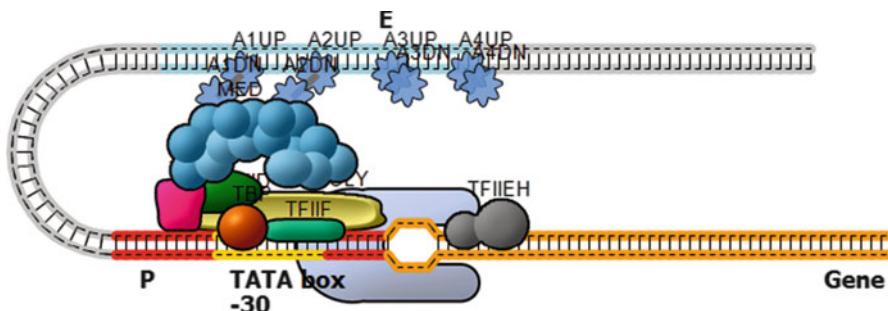


Step 1 Layout: Series of key proteins helping RNAP II bind to the promoter



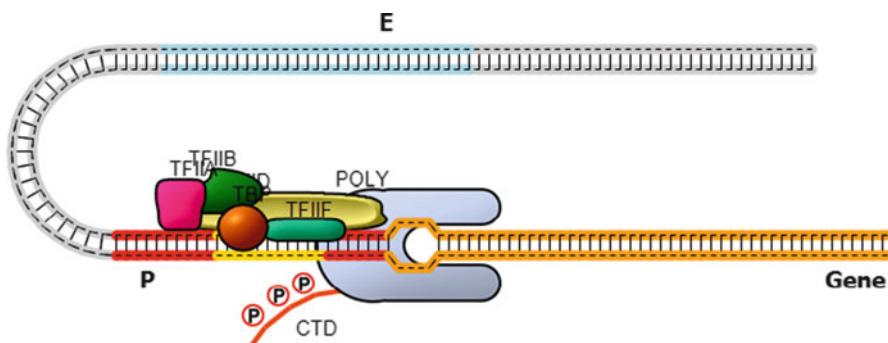
Step 2 Action: Binding of proteins to secure RNAP II to the promoter

- Additional proteins (activators), which bind to both the enhancer and the mediators, further strengthen RNAP II binding (Step 3).



Step 3 Action: RNAP II binding is secured when activators (with spikes) join the transcription factors and mediators together to form a promoter–enhancer complex

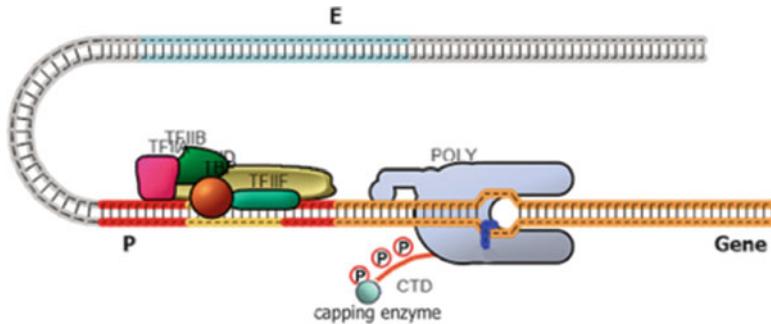
- Elongation begins when most of the proteins dissociate (with the exception of TFIIF, TFIID, TPB, TFIIA, and TFIIB) (Step 4).



Step 4 Action: Dissociation of most proteins and the formation of an open complex for RNA synthesis

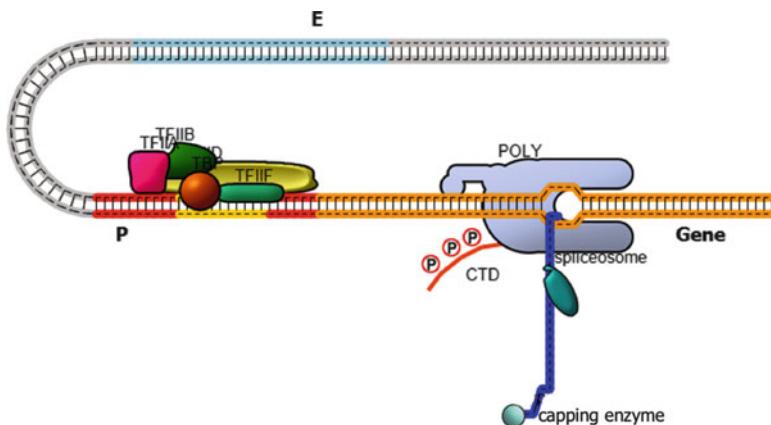
- Note the tail-like structure at the end of RNAP II (Step 4). Called the CTD (carboxyl tail domain), it serves, when phosphorylated, as an entry route for enzymes needed in capping, splicing, and termination.
- Short segments of error-prone RNA (similar to prokaryotic transcription) are synthesized early on.

- Click on the capping enzyme so that it enters the transcription machinery through the CTD (Step 5). Capping is part of the RNA processing, which is explained shortly.



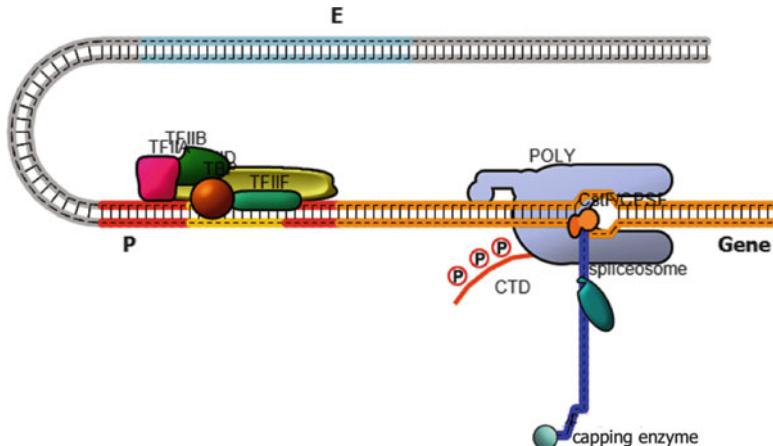
Step 5 Action: The capping enzyme enters the CTD

- At this point, all the TFs have dissociated from the transcription machinery and only RNAP II is involved in elongation (Step 6). Note that the capping enzyme is now attached to the 5'-end of the pre-mRNA.
- Click on the spliceosome (an RNA–protein complex for RNA splicing) so that it enters the transcription machinery through the CTD and then attaches to pre-mRNA (Step 6).



Step 6 Action: Attachment of the spliceosome to the pre-mRNA

- Near the final stages of transcription, the cleavage-stimulating factor (CstF) and the cleavage and polyadenylation factor (CPSF) enter and bind to the poly-A signal sequence in preparation for termination (Step 7).
- Click on CstF and CPSF so that they enter the transcription machinery through the CTD and bind to the 3'-end of the pre-mRNA (Step 7).*

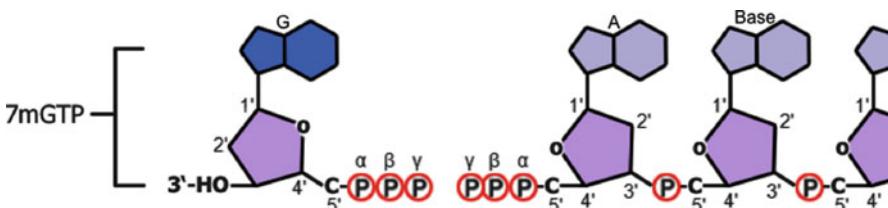


Step 7 Action: CstF-CPSF binds to the 3'-end of the pre-mRNA

- After transcription, the pre-mRNA needs to be processed to produce the mature mRNA.
- In the following sections, we examine RNA processing in detail.

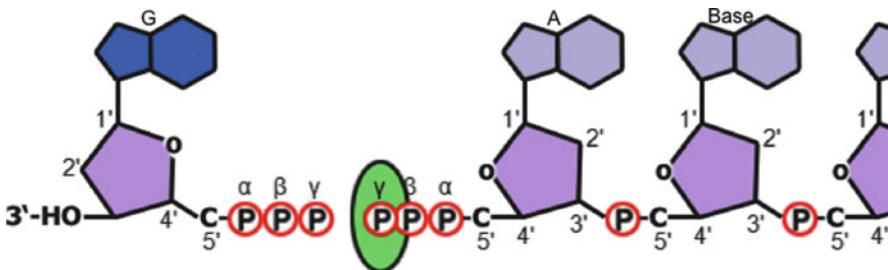
RNA Processing: Capping

- Below is the layout for RNA capping (Step 8).



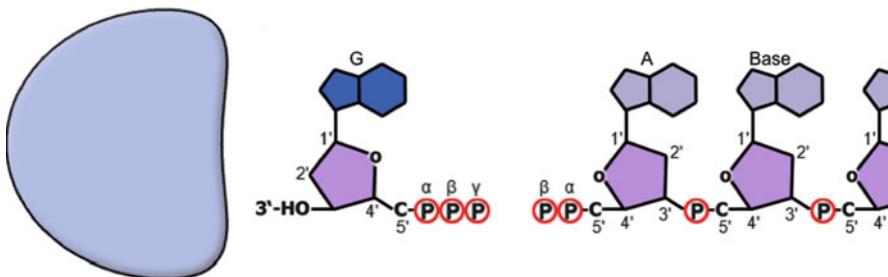
Step 8 Layout: for RNA capping

- The structure on the left is GPPP (guanosine triphosphate) and structure on the right is the 5'-end of the pre-mRNA.
- The 5'-end of the pre-mRNA is adenosine triphosphate (ATP). Both triphosphate groups (one from the cap and the other from the pre-mRNA) are attached to the 5' C of a sugar (ribose).
- The capping process begins when the enzyme RNA triphosphatase (green) binds to the γ phosphate (Step 9) of the first pre-mRNA nucleotide.
- Click on the enzyme RNA triphosphatase (green) to remove the γ phosphate at the 5'-end of the RNA (Steps 9 and 10).*

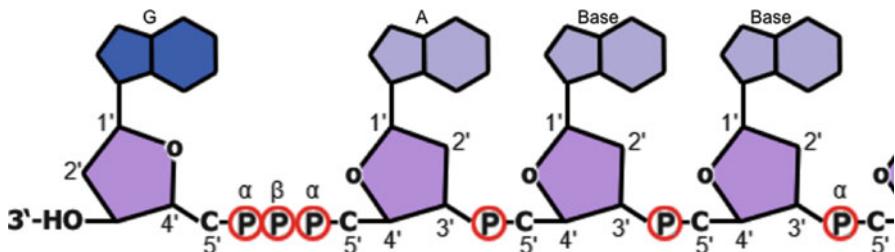


Step 9 Action: Binding of RNA triphosphatase

- Click on the enzyme guanylyltransferase (light blue) to (1) remove two phosphate groups from guanosine triphosphate (GTP) and (2) add the resulting guanosine monophosphate (GMP) to the terminal phosphate of the first RNA nucleotide, creating a 5'- to 5'-triphosphate linkage (Step 11).*

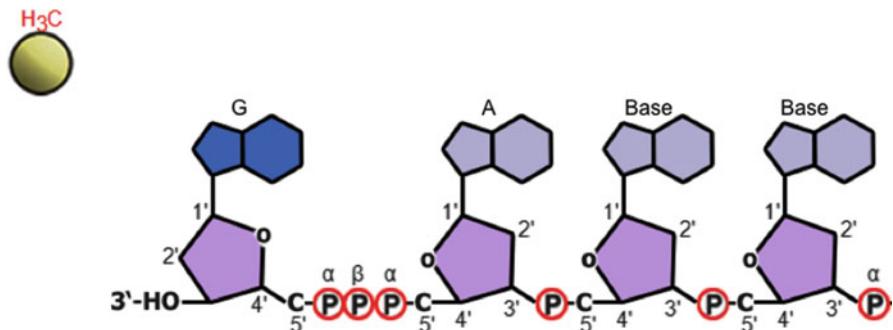


Step 10 Action: Removal of the γ phosphate at the 5'-end of the pre-mRNA along with the appearance of guanylyltransferase (left)



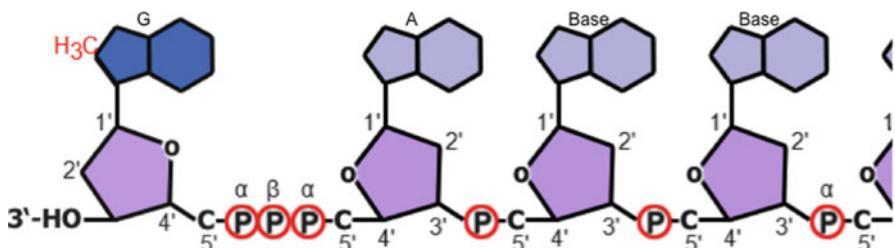
Step 11 Action: Removal of two phosphate groups from GTP followed by the addition of the resulting GMP to the terminal phosphate of the first pre-mRNA nucleotide, creating a 5'- to 5'-triphosphate linkage

- Click on the enzyme methyl transferase (gold) to add a methyl group to the 7' N of guanine to form 7mGTP (Steps 12 and 13). Sometimes, the enzyme also adds a methyl group to the purine at the original 5'-end of the mRNA.



Step 12 Action: Methyl transferase adds a methyl group to the 7' N of guanine to form m7GTP

- 7mGTP is the cap.

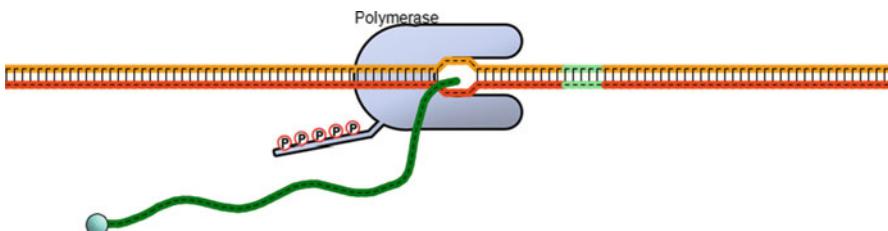


Step 13 Results: Formation of the cap: 7mGTP

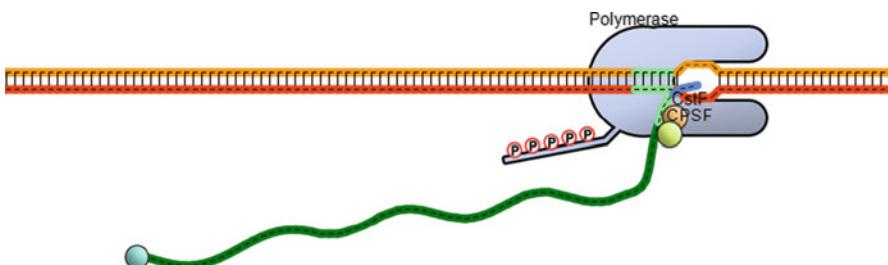
- What are the functions of the cap?
 1. Protection: preventing mRNA degradation from exonucleases.
 2. Transport: facilitating the transport of mRNA from the nucleus to the cytoplasm.
 3. Translation: enabling translation by increasing the affinity of the mRNA for ribosomes.
 4. Intron excision: allowing 5'-proximal intron excision.

RNA Processing: Polyadenylation

- Polyadenylation is linked to termination (of transcription). When the poly-A signal sequence (light green) is transcribed (Steps 14 and 15), a complex of two proteins, CPSF (cleavage and polyadenylation specificity factor) and CstF (cleavage stimulation factor), enters the CTD of RNAP II and attaches to the poly-A signal sequence (Steps 15 and 16).

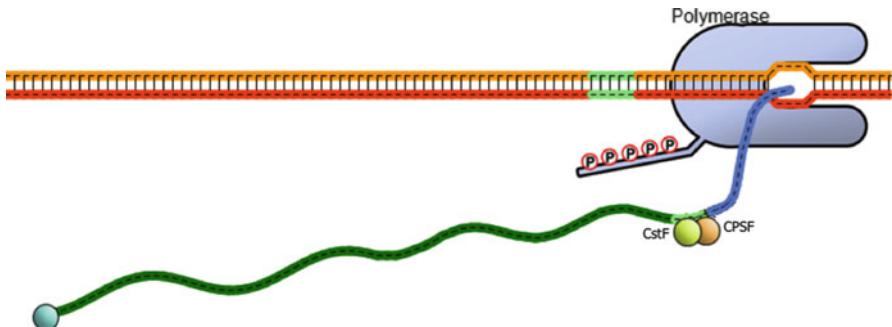


Step 14 Action: Elongation of pre-mRNA before the appearance of the poly-A signal sequence



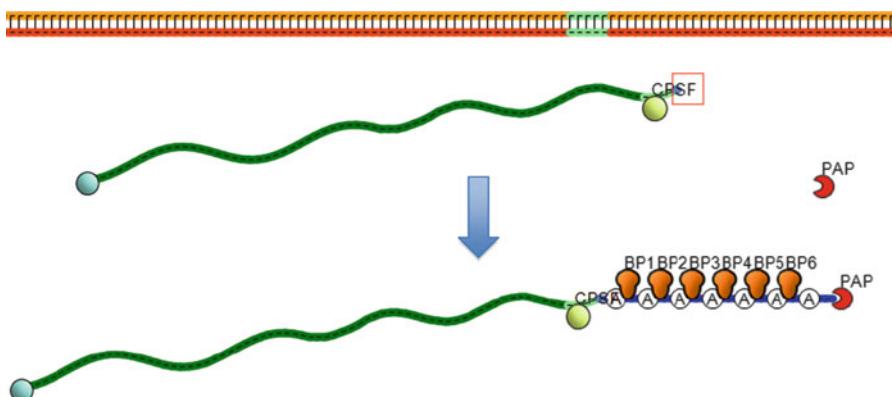
Step 15 Action: The CstF-CPSF complex enters the RNA synthesis machinery

- The complex then moves and binds to the poly-A signal sequence (Step 16).
- Click on the complex to cleave the RNA at the 3'-end and release CstF (Step 17).*



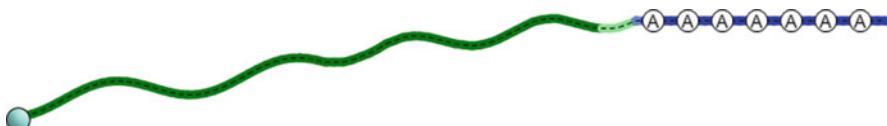
Step 16 Action: CstF-CPSF binds to the poly-A signal sequence

- To add adenines to the 3'-end, drag the enzyme poly-A polymerase (PAP) to the 3'-end of the pre-mRNA. A series of adenines (approximately 200) is added to the 3'-tail with the help of additional poly-A-binding proteins (orange) (Steps 17 and 18).



Step 17 Action: Polyadenylation of the 3'-end

- Polyadenylation is complete when the proteins and enzymes are released.



Step 18 Results: Completion of polyadenylation

Conclusions

- Polyadenylation and termination (of transcription) go hand in hand. Once the poly-A signal sequence is transcribed, a group of proteins immediately starts cleaving the RNA and adding a poly-A tail to the 3'-end.
- In certain genes, poly-A tails may be added to more than one site on the mRNA transcript (although not at the same time), a phenomenon called alternative polyadenylation. Thus, the same gene may generate different mRNA transcripts, transcripts that differ at the 3'-end. Alternative polyadenylation is similar to alternative mRNA splicing, which is discussed below.
- What is the function of polyadenylation? Similar to the cap, the poly-A tail increases the stability of mRNA, facilitates nuclear export to the cytoplasm, and enhances translation.
- Over time, the poly-A tail may become shorter, decreasing its protective power.
- We have now seen two of the three ways in which RNA is processed.

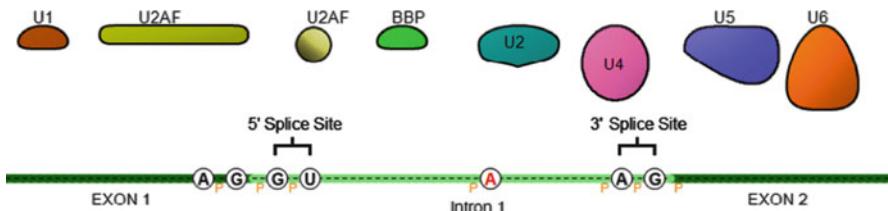
RNA Processing: Splicing

- As we stated previously, most eukaryotic genes are discontinuous, consisting of exons and introns.
- Both introns and exons are transcribed, but only exons are translated: this means that the primary transcript (pre-mRNA) is much longer than the mature mRNA.
- The process of removing introns and joining exons—transforming pre-mRNA into mature mRNA—is called **RNA splicing**.
- In eukaryotes, the major splicing enzyme, comprising multiple RNA–protein subunits, is called the spliceosome.
- In the RNA transcript below, two exons flank an intron (Step 19).



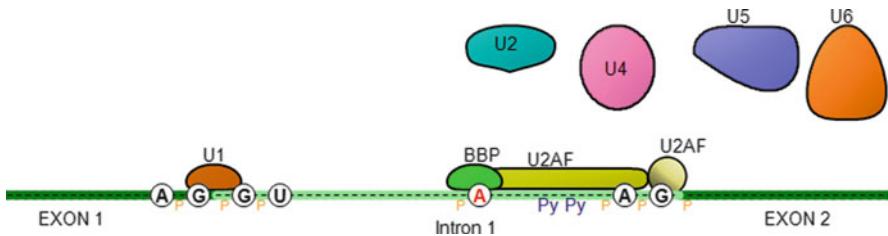
Step 19 Layout: One intron sandwiched between two exons

- RNA splicing is a complicated process. Nucleotide sequences at the junctions between the exons and introns signal the spliceosome to bind. In most cases, the 5'-splice site of the intron contains the nucleotides GU, whereas the 3'-splice site contains the nucleotides AG, prompting the formulation of the GU-AG rule.
- Another important splice site is the specific adenine nucleotide in the intron called the branching point, where the first 5'-intron base (G) attaches after cleavage.
- The spliceosome consists of approximately 150 proteins, along with five small nuclear RNAs (snRNA): U1, U2, U4, U5, and U6. Each of these snRNAs is associated with several proteins, forming a small nuclear ribonucleoprotein complex (snRNP, which is pronounced “snurp”) (Step 20).



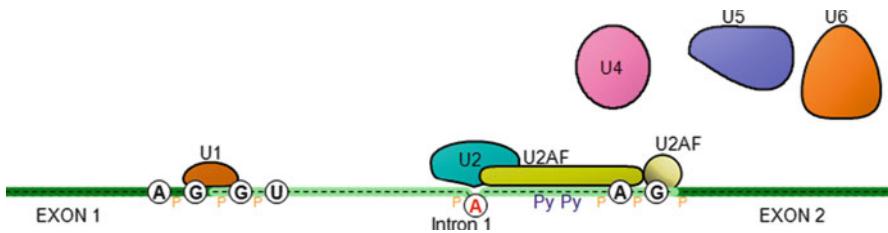
Step 20 Layout: Snurps and the pre-mRNA

- Splicing proceeds as follows:
 1. Binding of several complexes (Step 21):
 - (a) Drag *U1snRNP* (brown) to the 5'-splice site.
 - (b) Drag one unit of *U2AF* (dark yellow) to the polypyrimidine (*Py*) sequence between the branching point and the 3'-splice site and the second unit of *U2AF* (light yellow) to the 3'-splice site.
 - (c) The binding of *U2AF* induces *BBP* (branch-binding protein) (green) to bind to the branching point.



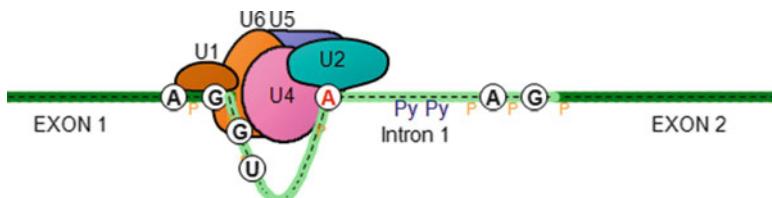
Step 21 Action: Binding of several complexes

2. Replacement of BBP with U2: *Drag U2 (aqua) to the branching point* (Step 22).



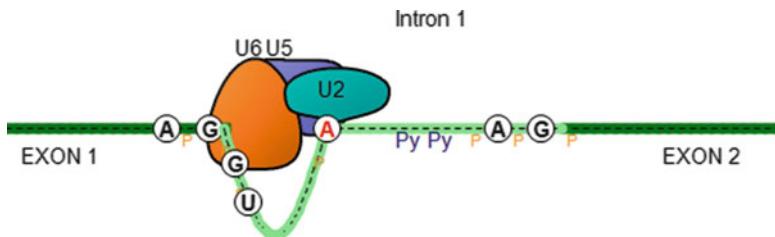
Step 22 Action: Replacement of BBP with U2

3. Removal of U2AF and the binding of U4, U5, and U6 (Step 23): *Drag U4 (pink), U5 (purple), and U6 (orange) to the proper sites to bring the 5' site and the branching point closer together.*



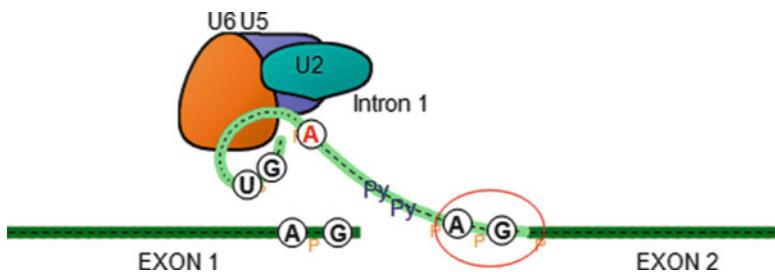
Step 23 Action: Removal of U2AF and the binding of U4, U5, and U6

4. Removal of U1 and U4 (Step 24): *Click on U1 (brown) and U4 (pink) to remove them from the binding sites.*



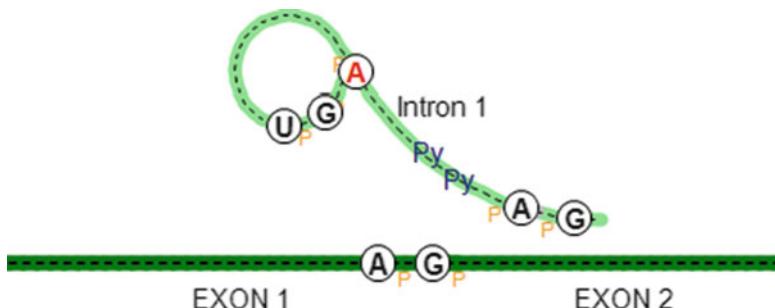
Step 24 Action: Removal of U1 and U4

5. Cleavage of G at the 5'-splice site and the joining of the 5' G to A at the branching point (Step 25): *Click on the U2–U5–U6 complex to accomplish this task (Step 25).*



Step 25 Action: Cleavage of G at the 5'-splice site and the joining of the 5' G to A at the branching point

6. Cleavage at the 3'-splice site, removal of the intron, and the joining of the exons (Step 26): *Click on the last intron nucleotide at the 3'-splice site to accomplish this task.*



Step 26 Action and results: Cleavage at the 3'-splice site, removal of the intron, and the joining of the exons

- After the intron (lariat structure) is removed, it is further degraded.
- Splicing is complete when all the introns are removed and all the exons are joined together.
- Splicing transforms pre-mRNA into a smaller, mature mRNA, ready for translation.

Conclusions

- Transcription is the first step of gene expression.
- The key product of transcription is mRNA, which reflects the DNA code structure: DNA codon to mRNA codon.
- Transcription also results in many other types of RNAs, but they are used for the transcription machinery or for regulatory functions.
- Eukaryotic genes are more complicated than prokaryotic genes. In eukaryotes the pre-mRNA must be processed to form the mature mRNA before translation can take place in the cytoplasm.
- Often, the same gene may be spliced in more than one way, that is, the splice sites may change so that the same pre-mRNA is spliced into different mature mRNAs. As a result, one gene may code for more than one type of proteins. This phenomenon is called alternative splicing.
- Through alternative splicing, two proteins may be produced in two different tissues from one gene, each serving a specific function locally.
- In the next module, you will learn the second step of gene expression: translation.

Quiz

1. The enzyme that catalyzes the transcription of DNA into RNA is called
 - (a) DNA polymerase
 - (b) DNA ligase
 - (c) RNA polymerase
 - (d) Protease
2. During the initiation of transcription, RNA polymerase attaches to a region of DNA called the
 - (a) Promoter
 - (b) Origin of replication
 - (c) Initiator
 - (d) Starter
3. If the mRNA sequence is 5'-AUG CAG UUU-3', the sense strand sequence is
 - (a) 5'-TAC GTC AAA-3'
 - (b) 3'-AAA GTC CAT-5'
 - (c) 5'-ATG CAG TTT-3'
 - (d) 3'-UUU CUG CAU-3'

4. A bacterial protein that complexes with the RNA polymerase core enzyme to form a holoenzyme during the initiation of transcription is called the
 - (a) α factor
 - (b) β factor
 - (c) γ factor
 - (d) σ factor
5. In *E. coli*, transcription termination may be caused by
 - (a) A stop codon
 - (b) A Rho factor
 - (c) Formation of a hairpin structure
 - (d) Two of the above
 - (e) None of the above
6. In eukaryotes, there are three major types of RNA polymerase. Which of the following is responsible for mRNA synthesis?
 - (a) RNA polymerase I
 - (b) RNA polymerase II
 - (c) RNA polymerase III
 - (d) DNA ligase
7. In addition to promoters, eukaryotic genes may have other *cis* control elements, which include
 - (a) Enhancers
 - (b) Silencers
 - (c) Two of the above
 - (d) None of the above
8. In eukaryotes, many RNAP II promoters contain a consensus sequence known as the TATA box at the -30 region, where the preinitiation complex forms. The TATA box is recognized by which of the following general transcription factors?
 - (a) TFIIA
 - (b) TF2B
 - (c) TFIIE
 - (d) TBP of TFIID
9. A primary transcript (pre-mRNA) is 5,000 bases long, whereas the mature mRNA is only 600 bases long. The removed RNA portions are
 - (a) Exons
 - (b) RNA segments with repetitive sequences
 - (c) Introns
 - (d) RNA segments with high G-C content

10. Which of the following is used for RNA splicing?

- (a) Spliceosome
- (b) RNase H
- (c) RNA primase
- (d) snRNA

Answers

1. c 2. a 3. c 4. d 5. d 6. b 7. c 8. d 9. c 10. a

Module 10

Gene Expression II (Translation): How Is Information Transferred from RNA to Proteins?

- In the previous module, we learned how DNA codons are transcribed into RNA codons in a process known as transcription or RNA synthesis.
- In this module, we examine how mRNA transcripts from protein-coding genes are translated.
- Translation in prokaryotes and translation in eukaryotes are similar. However, in prokaryotes, translation occurs in concert with transcription, that is, translation takes place as the mRNA is still being synthesized. This is possible because prokaryotes have no nuclear membrane separating the two processes. In eukaryotes, transcription occurs in the nucleus, whereas translation occurs in the cytoplasm.

General Requirements for Translation

- Three types of RNA are needed for translation:
 1. mRNA, which serves as a template.
 2. tRNA, which serves as an adaptor.
 3. rRNA, which serves as a component of the ribosome.
- The mRNA contains the codons that code for amino acids.
- All mRNAs contain a 5'-UTR (5'-untranslated region) and a 3'-UTR (3'-untranslated region). The 5'-UTR comprises the sequence before the transcription start site, and the 3'-UTR comprises the sequence after the stop codon.
- See the mRNA structure in Fig. 10.1 (below).
- The 5'-UTR is a translation control element, because it serves as a binding site for the ribosome. The 5'-UTR or 3'-UTR also provides the mRNA with varying degrees of protection against degradation by RNase.



Fig. 10.1 Basic mRNA structure

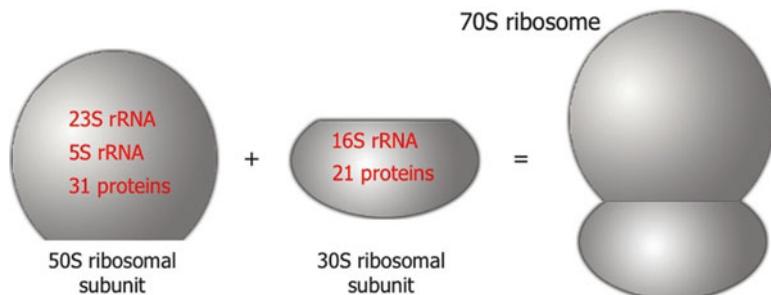


Fig. 10.2 The prokaryotic ribosome and its subunits

- Between the UTR regions is the ORF (open reading frame), which contains the codons to be translated. The ORF usually starts with the codon AUG (start codon) and ends just before the stop codon.
- Translation occurs on a RNA–protein complex known as the ribosome.
- Ribosomes are characterized by their sedimentation rate (S). Larger particles generally have larger S values.
- In prokaryotes, the 70S ribosome is composed of two subunits: the 50S ribosomal subunit with 23S rRNA and 5S rRNA plus 31 proteins, and the 30S ribosomal subunit with 16S rRNA plus 21 proteins (Fig. 10.2).
- In eukaryotes, the 80S ribosome is composed of two subunits: the 60S ribosomal subunit with 28S rRNA, 5.8S rRNA, and 5S rRNA plus 49 proteins, and the 40S ribosomal subunit with 18S rRNA plus 33 proteins (Fig. 10.3).
- Because there is no natural affinity between mRNA codons and amino acids, tRNA (transfer RNA) serves as an adaptor, connecting the codons of the mRNA to amino acids.

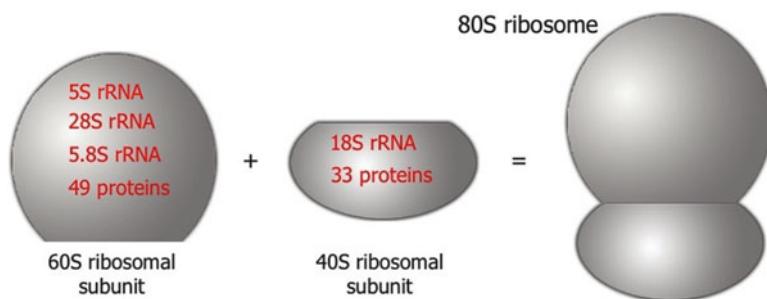


Fig. 10.3 The eukaryotic ribosome and its subunits

- The ribosomal subunits exist as separate entities: they only come together in the presence of mRNA during translation.
- In addition to mRNA, tRNA, and rRNA, many enzymes and protein factors are involved in protein synthesis. For example, aminoacyl tRNA synthetases link amino acids to tRNA, and peptidyl transferases catalyze the formation of peptide bonds between amino acids. After an amino acid is linked to a tRNA, the tRNA is said to be activated.

The Genetic Code: The Flow of Information from DNA to RNA to Protein

- Information is transferred from DNA to RNA to a protein through a series of reactions. To understand the genetic code, we need to examine the relationships between DNA codons and mRNA codons, mRNA codons and tRNA anticodons, and tRNA anticodons and amino acids.
- If you have forgotten all or part of [Module 8](#), please review the entire program before proceeding to the following program.

Key Points

- The mRNA contains all the genetic information in the gene because mRNA codons are synthesized through complementary base pairing with the noncoding strand of the DNA double helix.
- An mRNA codon is connected to an amino acid through an adaptor molecule known as tRNA.

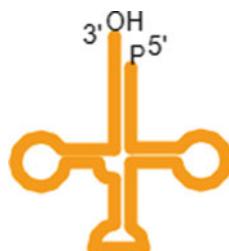
tRNA: The Adaptor (*Interactive Program 1*)

- tRNA has the following structure:
- From one perspective, it is a linear strand with a phosphate group (P) at the 5'-end and a hydroxyl group (OH) at the 3'-end (Step 1). The last three bases at the 3'-end are always CCA.



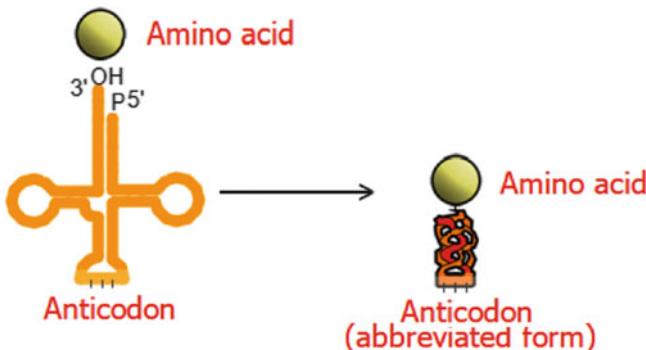
Step 1 Layout: Linear structure of tRNA

- Through complementary base pairing, the molecule is folded into a “cloverleaf”-like structure (Step 2).



Step 2 Action: Folding of tRNA: two-dimensional (2D) view

- The 3'-OH end is linked to an amino acid by the enzyme aminoacyl tRNA synthetase (Step 3).
- The “bottom” part of the tRNA contains three free (unpaired) bases called the anticodon (Step 3). The anticodon is complementary to the mRNA codon that codes for the attached amino acid.



Step 3 Action: Attachment of an amino acid to the 3'-end (adenine) of the tRNA with the anticodon positioned away from the amino acid

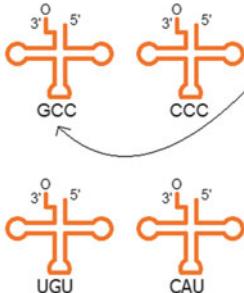
- Based on your knowledge of the codon–anticodon relationship and the codon–amino acid relationship (see the genetic code table below), determine the appropriate amino acid that can be linked to each of the four tRNAs below (Steps 4–6)

[Use Interactive Program 1 on the software program available at Extras. Springer.com to familiarize yourself with tRNA.](#)

[After completing the interactive program, you may either continue reading the following text for further review or directly jump to the Step 6.](#)

* Drag the correct amino acid to each of the 4 tRNA molecules below:

	2 nd	U	C	A	G	3 rd
1 st	Phe	Ser	Tyr	Cys	U	
U	Phe	Ser	Tyr	Cys	C	
	Leu	Ser	Stop	Stop	A	
	Leu	Ser	Stop	Trp	G	
	Leu	Pro	His	Arg	U	
C	Leu	Pro	His	Arg	C	
	Leu	Pro	Gln	Arg	A	
	Leu	Pro	Gln	Arg	G	
	Ile	Thr	Asn	Ser	U	
A	Ile	Thr	Asn	Ser	C	
	Ile	Thr	Lys	Arg	A	
	Met	Thr	Lys	Arg	G	
	Val	Ala	Asp	Gly	U	
G	Val	Ala	Asp	Gly	C	
	Val	Ala	Glu	Gly	A	
	Val	Ala	Glu	Gly	G	

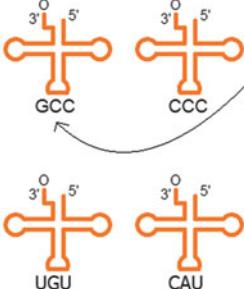


For example, if the anticodon of a tRNA is GCC, then the codon that is complementary to it is CGG. What amino acid is coded for by CGG?

Step 4 Layout: A genetic code table and four tRNA molecules, each with an anticodon for determining the appropriate amino acid that can be linked to the tRNA

* Drag the correct amino acid to each of the 4 tRNA molecules below:

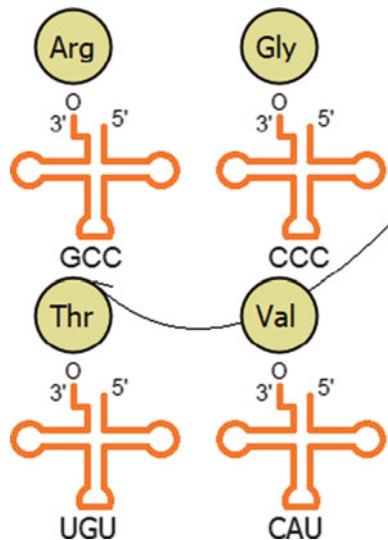
	2 nd	U	C	A	G	3 rd
1 st	Phe	Ser	Tyr	Cys	U	
U	Phe	Ser	Tyr	Cys	C	
	Leu	Ser	Stop	Stop	A	
	Leu	Ser	Stop	Trp	G	
	Leu	Pro	His	Arg	U	
C	Leu	Pro	His	Arg	C	
	Leu	Pro	Gln	Arg	A	
	Leu	Pro	Gln	Arg	G	
	Ile	Thr	Asn	Ser	U	
A	Ile	Thr	Asn	Ser	C	
	Ile	Thr	Lys	Arg	A	
	Met	Thr	Lys	Arg	G	
	Val	Ala	Asp	Gly	U	
G	Val	Ala	Asp	Gly	C	
	Val	Ala	Glu	Gly	A	
	Val	Ala	Glu	Gly	G	



<input type="radio"/> Ala
<input type="radio"/> Arg
<input type="radio"/> Asn

< 1 >

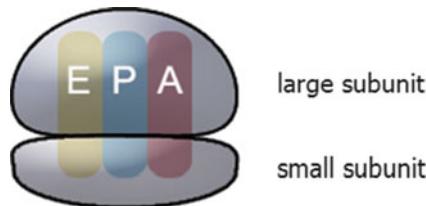
Step 5 Action: Drag the correct amino acid to each of the four tRNA molecules

**Step 6 Results:** The tRNA molecules with their corresponding amino acids

- Through tRNA, we now see the physical relationship between mRNA codons and their corresponding amino acids.
- The actual synthesis of proteins from mRNA occurs on the ribosome, which is a two-subunit particle composed of another type of RNA, ribosomal RNA (rRNA), and proteins.

Ribosome: The Translation Machinery (*Interactive Program 2*)

- The ribosome consists of a small subunit and a large subunit with three sites for binding (Step 1).

**Step 1 Layout:** Ribosome with three sites

- The A (aminoacyl) site is where activated tRNA, also called aminoacyl tRNA (with an attached amino acid), enters the ribosome (Step 2). However, the first aminoacyl tRNA molecule enters the ribosome at the P (peptidyl) site, not the A site.

**Step 2** Layout: A site

- The P site, where the first aminoacyl tRNA enters, holds the growing polypeptide chain (Step 3).

**Step 3** Layout: P site

- The E (exit) site is where the tRNA (without its amino acid) leaves the ribosome (Step 4).

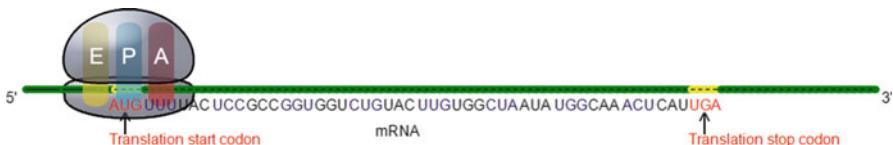
**Step 4** Layout: E site

- Protein synthesis starts at the start codon of the mRNA (AUG) and ends at the stop codon (UGA, UAA or UAG).
- Protein synthesis is initiated when the ribosome binds to the mRNA and the charged tRNA with its anticodon complementary to AUG enters the P site.

Use Interactive Program 2 on the software program available at Extras. Springer.com to familiarize yourself with the translation process.

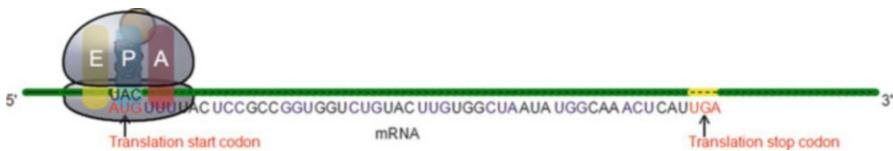
After completing the interactive program, you may either continue reading the following text for further review or directly jump to the section “Conclusions.”

- Drag the ribosome to the mRNA so that the P site is over the start codon AUG (Step 5).



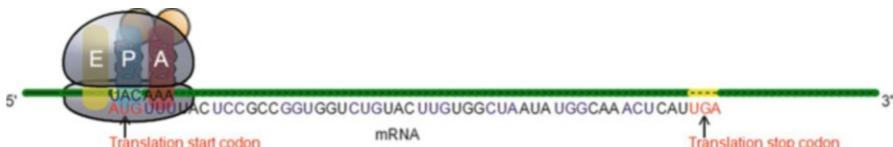
Step 5 Action: Alignment of mRNA on the ribosome so that the start codon AUG is at the P site

- Drag the charged (activated) tRNA into the P site so that its anticodon is complementary to the start codon (Step 6).



Step 6 Action: Codon–anticodon pairing at the P site: initiation

- After initiation, another charged tRNA with an anticodon complementary to the codon in the A site enters the A site.
- Drag the tRNA-AA complex to the A site (Step 7).



Step 7 Action: Second codon-anticodon pairing in the A site

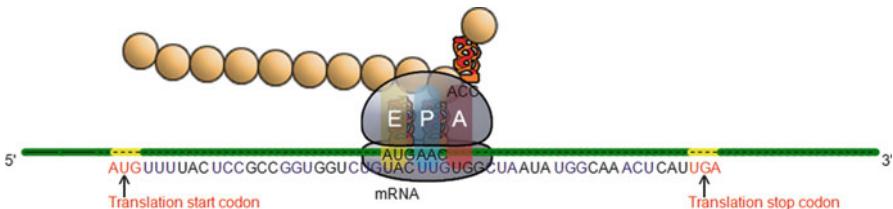
- After a tRNA-AA complex enters the A site, a bond is formed between the amino acid in the P site and the amino acid in the A site by the enzyme peptidyl transferase, and the resulting dipeptide (two linked amino acids) is transferred to the A site (Step 8).

- Click on the amino acid in the P site so that it forms a peptide bond with the amino acid in the A site (Step 8).



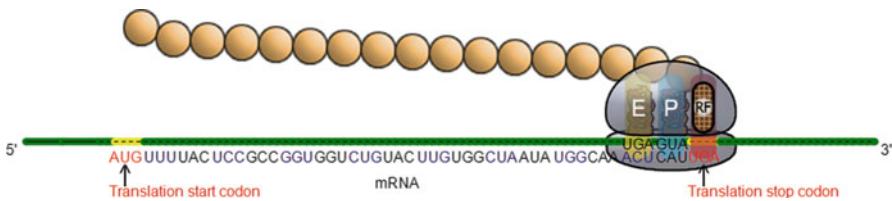
Step 8 Action: Formation of peptide bond by peptidyl transferase: elongation

- The enzyme that catalyzes the formation of the peptide bond is peptidyl transferase.
- In the next step of protein synthesis, the ribosome moves, and the following events occur:
 - The uncharged tRNA in the P site (now without an amino acid) enters the E site.
 - The tRNA with the dipeptide (the two amino acids) enters the P site.
 - The A site moves over a new codon and is ready for another charged tRNA molecule to enter.
- Click on the ribosome to continue polypeptide synthesis (Step 9).



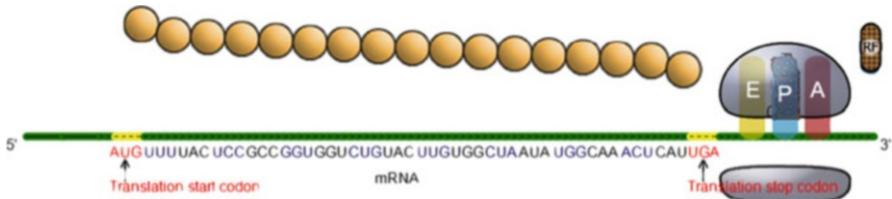
Step 9 Action: Continuation of protein synthesis

- The polypeptide continues to grow as the ribosome moves to the right.
- When the ribosome reaches the stop codon, no tRNA enters because no tRNA molecule has an anticodon that is complementary to a stop codon. Rather, a releasing factor (RF) binds to the A site to terminate translation (Steps 10 and 11).



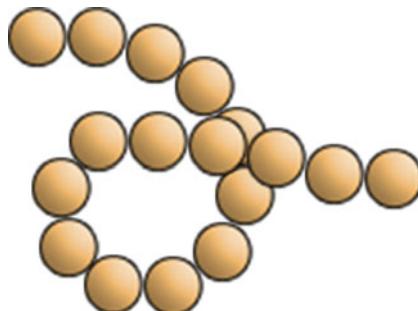
Step 10 Action: Binding of RF in the A site

- Click on the ribosome to terminate translation (Step 11).



Step 11 Results: Translation termination

- The polypeptide then folds into a functional protein with a specific shape (Step 12).

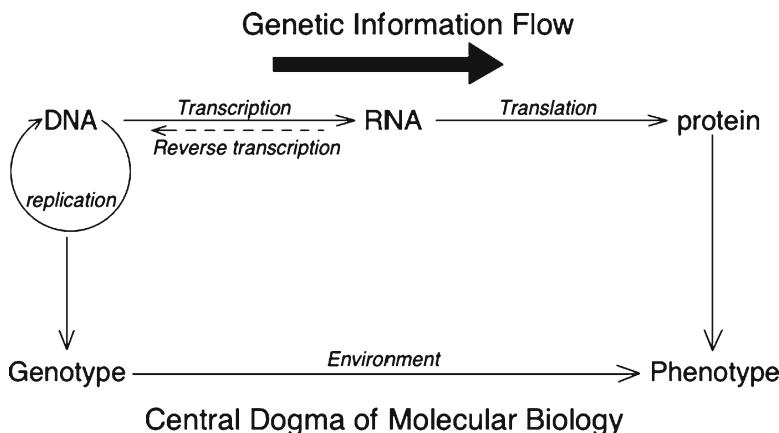


Step 12 Results: Folding of a polypeptide

- Sometimes more than one polypeptide may interact with another to form a protein complex.
- A protein molecule may be modified with additional molecules: lipids or carbohydrates.
- The protein may be either a structural protein or an enzyme.

Conclusions

- Through the processes of transcription and translation, proteins are produced from genetic material.
- Because proteins are responsible for all our physical characteristics, we now know why we look the way we do.
- It is our proteins (including enzymes) that determine our phenotype.
- There are many differences between prokaryotic and eukaryotic translation.
- In prokaryotes, there is no nuclear membrane, so translation takes place as mRNA is still being synthesized.
- In eukaryotes, transcription and RNA processing occur in the nucleus, and translation takes place in the cytoplasm.
- In eukaryotes, ribosomes are either free in the cytosol or attached to the endoplasmic reticulum (ER).
- Proteins produced on ribosomes attached to the ER enter the lumen of the ER, where they are processed or modified. The protein may be further modified in the Golgi apparatus before being secreted out of the cell.
- Proteins are key molecules that participate in cellular structure or control various metabolic activities for the organism, leading to what we call the phenotype.
- Thus, the central dogma of molecule biology states:



Quiz

1. Which of the following is not needed for translation?
 - (a) mRNA
 - (b) rRNA
 - (c) tRNA
 - (d) microRNA
2. mRNA contains the codons that code for
 - (a) Sugars
 - (b) Amino acids
 - (c) DNA
 - (d) Lipids
3. Which sequence occurs after the stop codon?
 - (a) 3'-UTR
 - (b) 5'-UTR
 - (c) 3'-ATR
 - (d) 5'-ATR
4. Translation occurs on a RNA–protein complex known as the
 - (a) Holoenzyme
 - (b) Spliceosome
 - (c) Ribosome
 - (d) Endoplasmic reticulum
5. In both prokaryotes and eukaryotes, the ribosome is composed of how many subunits?
 - (a) 1
 - (b) 2
 - (c) 3
 - (d) 4
6. Which of the following serves as an adaptor molecule during translation, positioning amino acids according to mRNA codons?
 - (a) mRNA
 - (b) rRNA
 - (c) snRNA
 - (d) tRNA
7. At which site on the ribosome does the first charged tRNA enter?
 - (a) E site
 - (b) P site
 - (c) A site
 - (d) Any one of the above

8. After the first site is occupied, which site do all subsequently charged tRNAs enter?
 - (a) E site
 - (b) P site
 - (c) A site
 - (d) Any one of the above
9. In what part of the eukaryotic cell does translation take place?
 - (a) Nucleus
 - (b) Mitochondrion
 - (c) Cytoplasm
 - (d) Either nucleus or cytoplasm
10. The end product of translation is
 - (a) A polypeptide
 - (b) A carbohydrate
 - (c) DNA
 - (d) RNA

Answers

1. d 2. b 3. a 4. c 5. b 6. d 7. b 8. c 9. c 10. a

Module 11

Human Cytogenetics: Are You Interested in Becoming a Clinical Geneticist or Just Curious About Human Chromosomes?

- You have worked hard to complete the previous ten modules on learning basic genetics. Here is something extra as a reward, especially for those who wish to gain insight into their own chromosomes, or who want to become clinical cytogeneticists.
- The study of chromosomes is called cytogenetics. Because human chromosome morphology is so dynamic and beautiful under the microscope, the study of human chromosomes is not only a basic science with clinical significance, but it is also an art form.
- Instead of chromosome models, here we present virtual images of real human chromosomes.
- Human cytogenetics is clinically significant because chromosome abnormalities are the leading causes of many genetic diseases. Chromosome abnormalities have been linked to mental retardation, autism, and inborn errors of metabolism.
- Chromosome analysis is performed for diagnosing congenital disorders prenatally, detecting the causes of miscarriage, and determining certain forms of cancers.
- Cytogenetics is also used for human population monitoring, in biological dosimetry after radiation accidents, and for monitoring astronauts in space. After all, DNA double-strand breaks leading to chromosome aberrations can be induced by ionizing radiation. Furthermore, high frequencies of sister chromatid exchanges are characteristic of certain cancers and have been used to measure the effects of high-energy radiation and other mutagens.

Changes in Chromosome Number

- There are two ways to describe changes in chromosome number:
 1. **Euploidy:** Euploidy refers to the number of chromosome sets: 1N (1 set) is the haploid number, 2N (2 sets) is the diploid number, and 3N (3 sets) is the

triploid number, etc. In humans, the number of chromosomes in a normal somatic cell is 46 (2N). In rare cases, 69 (triploid) or 92 (tetraploid) chromosomes are found in aborted tissues.

2. **Aneuploidy:** Aneuploidy refers to the addition or deletion of individual chromosomes, rather than sets of chromosomes: $2N+1$ (trisomy) or $2N-1$ (monosomy).

A person with Down syndrome has an extra copy of chromosome 21 (trisomy 21), and a person with Turner syndrome has only one X chromosome (monosomy).

Changes in Chromosome Structure

- Structural changes in human chromosomes include
 1. **Deletions:** A chromosome segment is missing
 2. **Duplications:** A chromosome segment is repeated
 3. **Inversions:** A chromosome segment is reversed
 4. **Translocations:** Parts of nonhomologous chromosomes are rearranged
 5. **Ring formations:** Formation of a ring-shaped chromosome through breakage and reunion of the chromosome tips (telomeres)
 6. **Isochromosome formation:** Formation of a chromosome with two identical arms (e.g., two long arms)

Chromosome Techniques

- Various banding and staining techniques are employed for human chromosome analysis. The standard technique is G-banding, which is used in clinical genetics laboratories throughout the United States.
- G-banded chromosomes are prepared as follows:
 1. Cell culture: Cells from peripheral blood, bone marrow, amniotic fluid, chorionic villous samples (CVS), and the products of conception (POC) are commonly used for cell culture. In this example, we use peripheral blood cells for chromosome analysis. Peripheral blood cells are cultured in the medium RPMI1640 plus 10 % fetal bovine serum (FBS). PHA (phytohemagglutinin) is added as a mitogen for T cells. The blood cells are incubated at 37 °C and 5 % CO₂ for 3–4 days.
 2. Chromosome harvest: Before harvesting, colcemid is added to the medium to destroy the spindle so that the dividing cells are arrested at metaphase.
 3. Hypotonic treatment: Cells are treated with a hypotonic solution (KCl) to expand cell volume and spread the chromosomes.

4. Fixation: Chromosomes are fixed with a solution containing three parts methanol and one part acetic acid.
5. Slide preparation: The fixed cells are dropped with a Pasteur pipette onto a microscope slide and dried at 90 °C for 40 min.
6. G-banding: Slides containing human chromosomes are treated with trypsin or pancreatin (protease), followed by Giemsa staining. The G-banded chromosomes contain the dark (A-T rich) and light (G-C rich) regions characteristic of each chromosome pair.
7. Criteria for human chromosome analysis: (a) relative chromosome size (ranging from large to small), (b) centromere position (from metacentric to submetacentric to acrocentric), and (c) G-banding.
8. Chromosome groupings: 23 chromosome pairs are placed into the following groups: group A (1–3), group B (4–5), group C (6–12), group D (13–15), group E (16–18), group F (19–20), group G (21–22), and sex chromosomes (XX or XY).

Chromosome Descriptions

- A chromosome has two arms (P for short arm and Q for long arm). Each arm is divided into regions, starting at the centromere. Each region is then subdivided into bands, and each band is subdivided into subbands.
- Idiograms are diagrams of G-banded chromosomes.
- Each chromosome has its G-banded landmarks. Therefore, normal and abnormal chromosomes can be recognized on the basis of these landmarks.

Karyotyping

- Karyotyping refers to the organization of chromosomes into groups and individual numbers on a sheet of paper for identification purposes.
- Karyotyping exercises are traditionally performed with the “cut-and-paste” method.
- However, the traditional method is time consuming and ineffective.
- In response to this problem, we developed an interactive method for karyotyping that is efficient and ideal for training human cytogenetics technologists.

Karyotyping Exercises (*Interactive Program I*)

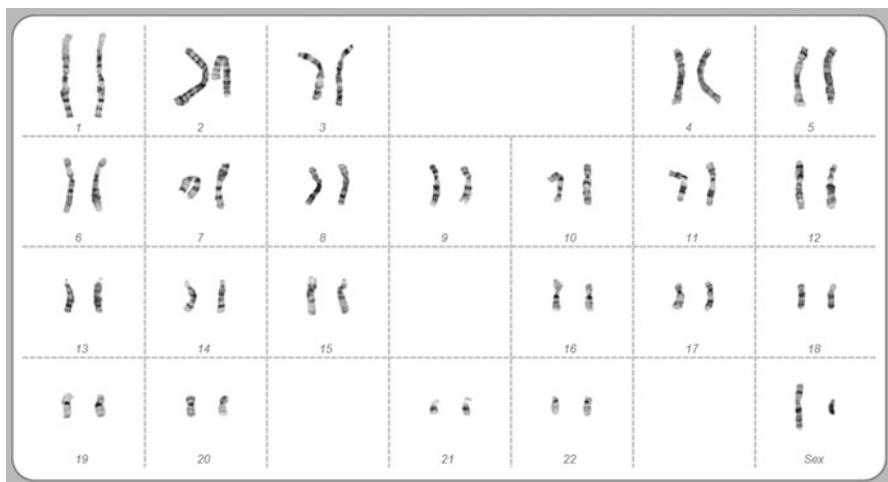
The software package contains comprehensive karyotyping exercises for human chromosome identification. It is a innovative tool for learning human cytogenetics.

Use Interactive Program 1 on the software program available at Extras.Springer.com to familiarize yourself with chromosome identification and karyotyping.

Below are screen shots showing 1 of the 21 different metaphases (Step 1) and a completed karyotype from that metaphase (Step 2).



Step 1 Layout: Metaphase of G-banded chromosomes



Step 2 Results: Completed karyotype

Back Page

The 11 modules of this program form an integrated system for learning the basic concepts of genetics. The program innovation lies in its unique content design that incorporates cognitive feedback and experiential learning through the novel use of interactive applications. Each of the modules is designed to enhance learning by simultaneously employing “eyes-on,” “minds-on,” and “hands-on” activities. Topics are motivated by questions, information is presented in simple terms, and concepts are clarified by interactive prompts. Used as a supplement to traditional lectures and textbook assignments, the program not only serves as a foundation upon which to build more complex and detailed topics, but as a tutor to clarify and guide when there is confusion.

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