Flow of Genetic Information

DNA Replication

Pre-Lab:

1? State at least three reasons why a cell must undergo division.

Scenario: Imagine you are cutting a bagel (one of the most common household injuries) and you get a cut. The cut heals.

2? How do the new cells compare to the original (pre-cut) cells?

3? How does your body ensure that the new cells are the same?

4? How does DNA get into the new cells?

Lab

Student Introduction:

No molecular structure has gained world-wide notoriety more than the double helical structure of DNA. The famous *Nature* paper written by James Watson and Francis Crick in 1953 entitled, "Molecular Structure of Nucleic Acids" ends with the statement, "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material." Since the release of the paper, the focus of the research of a number of scientists has been on elucidating the mechanism of DNA replication.

Modeling DNA Replication

In this lesson you will learn how a copy of DNA is replicated for each cell.

STEP 1 You will model a 2D representation of DNA replication using the foam pieces provided. Assemble the non-template strand of the abbreviated sequence of the beta-globin gene using the pattern shown below.

STEP 2 Base pair the nucleotides of the template strand in order to the non-template strand of DNA you have previously constructed to create a double stranded DNA model.

2a Record the template strand bases in the blank spaces provided above.

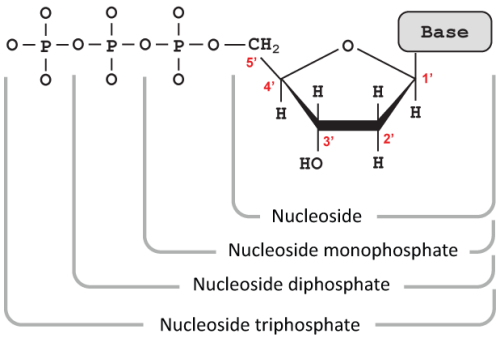
2b Examine the strands of DNA. What can you observe about the "arrow" ends of the model?

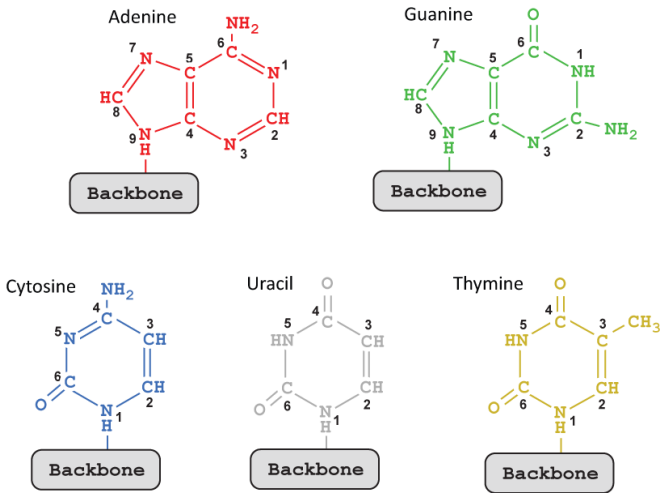
_____

_____

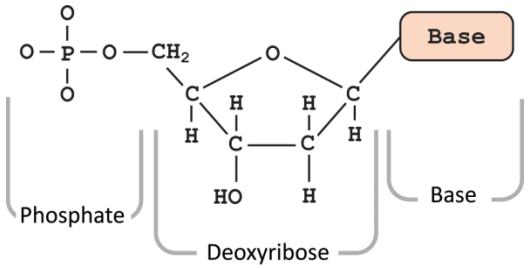
The arrow indicates the 3' end of the DNA molecule. Examine the diagrams below.

STEP 3: Examine the diagrams below.

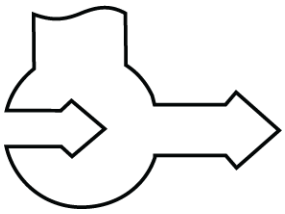




3a? Circle and label the 3' carbon and the 5' carbon in the DNA nucleotide shown in the diagram to the right. Primes are used in the numbering of the carbons on the sugar portion of the nucleotide to distinguish them from the nitrogen base carbons.



3b? Identify and label the nitrogen base, phosphate group, hydroxyl group and sugar in the representation pictured to the right. Label the locations of the 3' and 5' carbons.



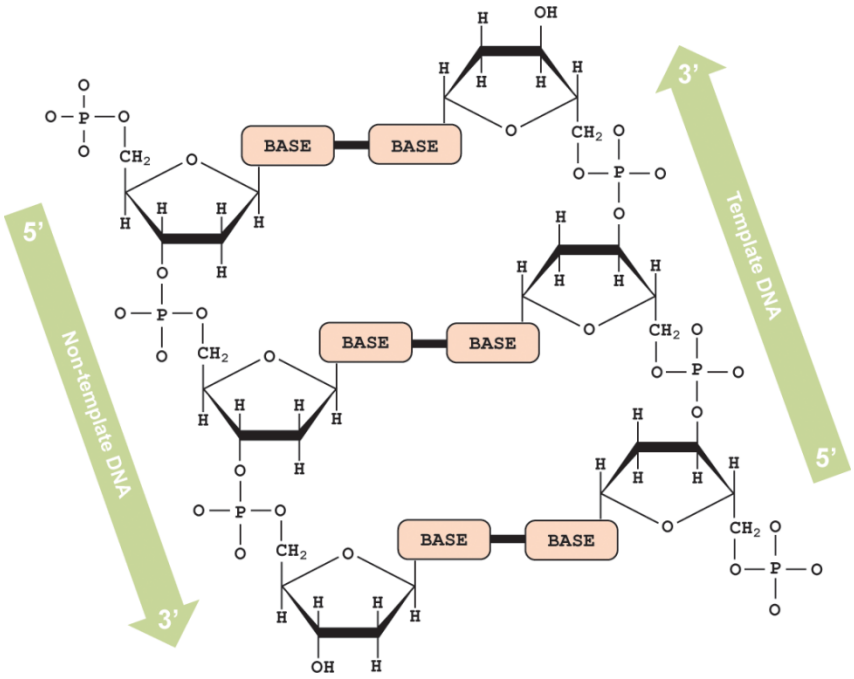
3c? How are the 3' and 5' carbons oriented in the strands of the DNA molecule you assembled?

_____

_____

STEP 4: Examine the detailed diagram of the DNA model below.

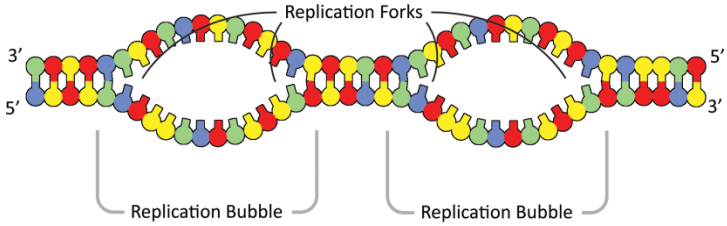
Double stranded DNA is composed of **<u>two anti-parallel strands</u>**! Each DNA strand has "**<u>directionality</u>**". The two sugar-phosphate backbones run in opposite 5' → 3' directions from each other. It is important to keep this directionality in mind as you model the process of DNA replication.

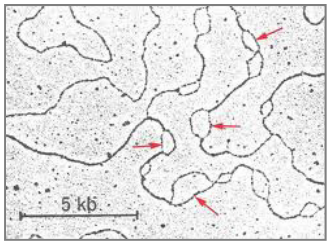


4a? Circle and label the 3' carbons and the 5' carbons in the DNA molecule above.

4b? What group is attached to the 3' carbon? What group is attached to the 5' carbon?

Replication of DNA begins at specific sites referred to as origins of replication. A eukaryotic chromosome may have hundreds or even a few thousand replication origins. Proteins that start DNA replication attach to the DNA and separate the two strands creating a replication "bubble". At each end of the **<u>replication bubble</u>** is a Y-shaped region where the parental strands of DNA are being unwound. This region is referred to as the **<u>replication fork</u>**.





http://kootation.com/origins-of-replication.html

STEP 5: Observe your teacher create a model of a DNA replication bubble using two toobers.

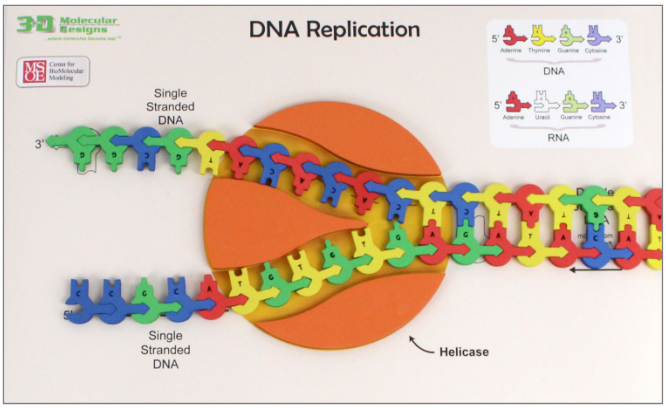
5a? Identify and label the replication bubble and replication forks in the model below.

5b? Looking at the toober model, what do you think might be the first step of replication?

5c? Nucleotides are added at an approximate rate of 50 nucleotides per second in eukaryotic cells. The human genome contains 6.4 billion nucleotides (3.2 billion base pairs) which must be copied. Calculate the length of time in days that it would take to copy the human genome. Show all calculations including units.

5d? Why do you think multiple replication bubbles form during the process of DNA replication?

STEP 6: Begin the process of DNA replication by feeding the strands of the constructed DNA into the top of the **helicase** enzyme on the replication mat. Be sure to position the 5' and 3' ends of the DNA appropriately as you place the DNA on the mat. Continue feeding the DNA through the enzyme until you have 11 bases emerging from the bottom of the helicase. Notice that helicase moves into the replication fork NOT away from it.



6a? What does the helicase appear to be doing?

_____

_____

6b? Identify which type of bond is broken.

_____

_____

6c? Why is the helicase able to break these bonds?

_____

_____

Note: Replication occurs on both sides of the replication fork simultaneously. For simplicity and clarification you will simulate replication on one side of the fork at a time.

STEP 7: **DNA polymerase** catalyzes the synthesis of new DNA by adding nucleotides to a preexisting chain. **New DNA can elongate only in the 5' → 3' direction.** The DNA strand that is made continuously is referred to as the **leading strand**.

Simulate replication in the **leading strand** by placing one DNA polymerase at the point of origin (refer to Diagram 2 on the Replication Placemat) and adding nucleotides in the active site to the parent strand. Continue adding nucleotides as you move the DNA polymerase until you reach the fork.

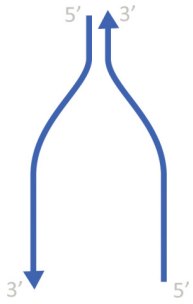
7a? As a new nucleotide is added to the growing DNA strand, which part of the new nucleotide forms a bond with the 3' OH group?

_____

_____

Additional Note: The 3' OH group is essential for adding a new nucleotide to the growing DNA strand. If this group is not present, for example, if there is a 3' H instead of a 3' OH, then DNA synthesis cannot continue. This is the basis for the Sanger Sequencing method used in determining the sequence of nucleotides.

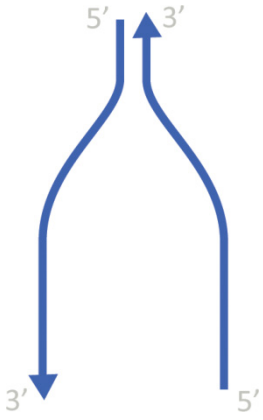
7b? Insert a sketch of the helicase on the diagram below and indicate the directionality of the newly replicated **leading strand** of DNA:



7c? Will you be able to synthesize the other strand of DNA in a continuous manner when using the model? Explain why or why not.

STEP 8: Place the second DNA polymerase at the fork on the other strand of DNA. Notice that the DNA polymerase must move away from the fork instead of toward the fork as it did in the leading strand. In order to accommodate the 5′ → 3′ synthesis of DNA, short fragments are made on the second strand referred to as the **lagging strand**. Continue adding nucleotides in the active site as you move the DNA polymerase away from the fork until you reach the end.

8a? Sketch and indicate the directionality of the fragments composing the **lagging strand** of DNA below:



STEP 9: Feed the next eleven nucleotides through the helicase. Continue sliding the DNA polymerase along the **leading strand**, adding more nucleotides as you progress.

STEP 10: The **lagging strand** requires that you move the DNA polymerase! Place the DNA polymerase back at the fork junction to create the next fragment. Move the DNA polymerase so that the bases may be added from the 5' → 3' direction. (Refer to the third diagram on the DNA Replication Placemat.) You have now created a second fragment of DNA on the lagging strand. These fragments are referred to as **Okazaki fragments** and are usually 100-200 nucleotides long in eukaryotic cells.

When you "bump" into the first fragment, you will need to remove the DNA polymerase and join the two fragments together with the appropriate nucleotide. The actual process of joining the Okazaki fragments together is a bit more complicated and involves several other molecules.

STEP 11: Complete the process of DNA replication with the remaining 11 nucleotides on both the leading and the lagging strands. DNA replication is considered to be a **semi-discontinuous** process.

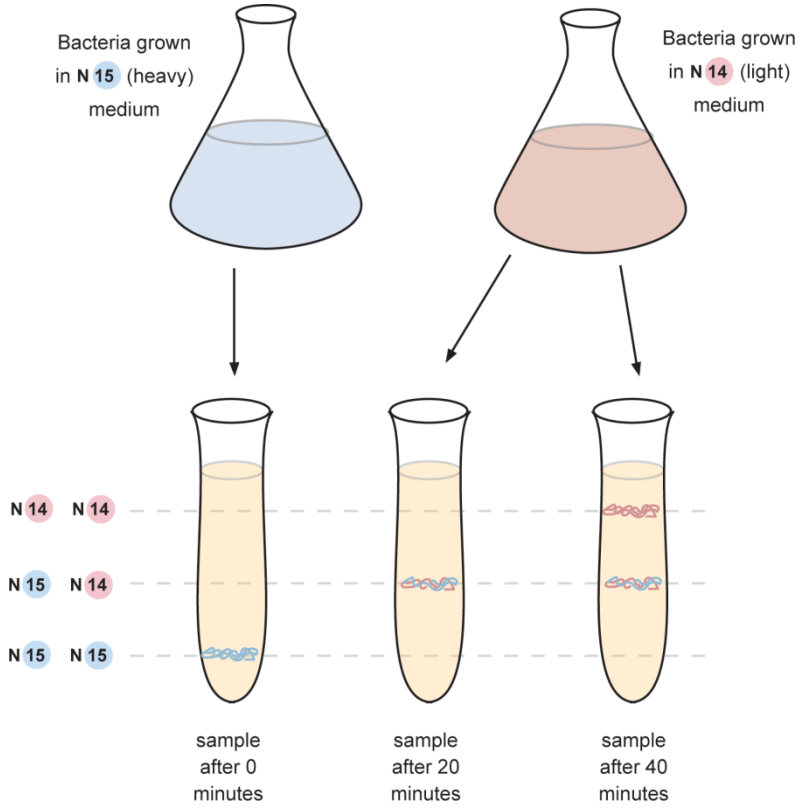
11a? Why is DNA replication considered to be a **semi-discontinuous** process?

11b? Create a sketch which models the semi-discontinuous process of DNA replication. Be sure to label the following aspects of your representation: leading and lagging strands, helicase, Okazaki fragments, parental strands, 3' ends and 5' ends.

11c? How do these two new strands compare to the original (parental) strand?

Three Models for the Process of DNA Replication:

In 1958 at the California Institute of Technology Matthew Meselson and Franklin Stahl devised an elegant series of experiments to discern which one of three models explained the mechanism of DNA replication. Meselson and Stahl cultured *E. coli* in a medium containing nucleotides labeled with a heavy isotope of nitrogen, ^{15}N. They transferred the bacteria to a medium with only ^{14}N, a lighter isotope. A sample was taken after the DNA had replicated once. Another sample was taken after the DNA replicated again. The DNA was extracted from the bacteria in the samples and then centrifuged to separate the DNA of different densities. Their results are shown below:



STEP 1: Obtain and assemble 11 nucleotide basepairs of the colored DNA foam pieces. Find the matching gray basepair pieces but DO NOT assemble them. These colored DNA strands represent the parental strands from *E. coli* grown in a medium tagged with ^{15}N nucleotides. The gray foam pieces represent the nucleotides used to synthesize new DNA.

You will create a physical representation of the three mechanisms of DNA replication; (1) conservative, (2) semiconservative, and (3) dispersive. Begin with modeling the first round of replication of the DNA after the bacteria were transferred to a medium with only ^{14}N.

You will use the foam DNA models to discern which mechanisms of replication would most likely explain Meselson and Stahl's results

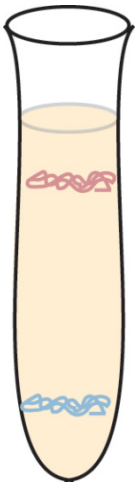
Conservative model:

In the conservative model of DNA replication the parental strands are used as templates for the new DNA molecule and somehow come back together to "conserve" the parental molecule.

STEP 2: Using the colored DNA parental strands you have just created and the gray nucleotides, model the end result of the conservative method of DNA replication. You should have 1 parental model made entirely of colored pieces and 1 daughter molecule with the same sequence of base pairs but made entirely of gray foam nucleotides.

2a? Sketch the new and old strands after one round of replication. It will be helpful if you have two different colored pens or pencils to create your sketches.

STEP 3: A sketch of a test tube showing the density gradient of ^{15}N tagged DNA after one round of conservative replication is shown below.



Semiconservative model:

In the semiconservative model of DNA replication, each of the two daughter molecules will have one old strand from the parental molecule and one newly made strand.

STEP 4: Now using the colored DNA parental strands you have created and the gray nucleotides, model the semiconservative method of DNA replication.

4a? Sketch the results of one round of DNA synthesis after the semiconservative method of replication.

4b? Sketch a test tube showing the density gradient of ^{15}N tagged DNA after one round of semi-conservative replication. Refer to the Meselson and Stahl experiment to help you create your sketch.

Dispersive model:

In the dispersive model of DNA replication, each strand of both daughter molecules contains a mixture of old and newly synthesized DNA.

STEP 5: Finally, using the colored DNA parental strands you have just created and the gray nucleotides, model the dispersive method of DNA replication.

5a? Sketch the results of one round of DNA synthesis after the dispersive method of replication.

5b? Sketch a test tube showing the density gradient of ^{15}N tagged DNA after one round of dispersive replication.

5c? Which of the methods can now be eliminated based on the results that Meselson and Stahl got after one round of replication? Why?

_____

_____

_____

STEP 6: Use the foam pieces to visualize what the newly synthesized strands of DNA would look like after a second round of replication in each of the methods. Sketch your results in the first column in the table below. In the second column, sketch what the DNA density gradient would look like in the test tube.

DNA Synthesized After A Second Round of Replication	DNA Density gradient
Conservative Model	
Semi-conservative Model	
Dispersive Model	

6a? Which method of DNA replication may now be eliminated after the second round of DNA replication based on the results of the Meselson and Stahl experiments? Why?

_____

_____

_____

6b? Based on the results of Meselson and Stahl's experiments, DNA is shown to replicate in a

_____ manner.

Post-Lab Questions:

1. What is the relationship of DNA replication to cell division?

2. Of the representations of DNA models (foam pieces, paper diagram, toobers), identify the strengths and weaknesses of each.

3. Based on what you have learned from this activity, explain why semi-conservative replication is the preferred process of DNA replication as opposed to dispersive or conservative.

For a detailed description suitable for IB or AP Biology:

http://www.youtube.com/watch?v=teV62zrm2P0
http://www.youtube.com/watch?v=-mtLXpgjHL0
(these descriptions include RNA primer)

For a general overview animation of continuous and discontinuous replication:

http://www.wehi.edu.au/education/wehitv/molecular_visualisations_of_dna/
http://www.dnalc.org/resources/3d/04-mechanism-of-replication-advanced.html

A group of videos on DNA replication:

http://www.youtube.com/watch?v=AGUuX4PGlCc&list=PL38E7B903667B4498

Links to the Next Generation Standards

Scientific and Engineering Practices:

- Asking Questions (for science) and Defining Problems (for engineering)
- Developing and Using Models
- Using Mathematics and Computational Thinking
- Constructing Explanations (for science) and Designing Solutions (for engineering)

Crosscutting Concepts:

- Patterns
- Cause and Effect: Mechanism and Explanation
- Scale, Proportion, and Quantity
- Structure and Function
- Systems and System Models
- Stability and Change

Disciplinary Core Ideas:

- LS 1: From Molecules to Organisms: Structures and Processes
- HS-LS1-1: Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.

- LS 2: Heredity: Inheritance and Variation of Traits
- HS-LS3-1: Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.
- HS-LS3-2: Make and defend a claim based on evidence that inheritable genetic variations may result from (1) new genetic combinations through meiosis, (2) viable errors occurring during replication, and/or (3) mutations caused by environmental factors.

- HS-ETS1: Engineering Design
- HS-ETS1-4: Use a computer simulation to model the impact of proposed solutions to a complex real-world problem with numerous criteria and constraints on interactions within and between systems relevant to the problem.

Students will:

- **Identify** the directionality of a DNA strand.
- **Explain** the implications of the anti-parallel structure of DNA on replication.
- **Model** the replication process of the leading and lagging strands of DNA.
- **Describe** the semi-conservative nature of DNA replication.
- **Describe** the semi-discontinuous process of DNA replication.
- **Explain** how a change in the DNA code may occur.

Prerequisite Knowledge and Skills:

- Hydrogen bonding and covalent bonding
- Cell structure
- DNA structure
- Cell cycle basics
- Prokaryotic and eukaryotic cell structure

Materials:

- DNA toober model
- Student Lab Packet
- DNA Replication Placemat, recommended one kit per group of three students

Flow of Genetic Information

DNA Replication

Pre-Lab:

1. State at least three reasons why a cell must undergo division.

(possible answers include: growth, repair, reproduction, the cell gets too big (surface area to volume ratio))

Scenario: Imagine you are cutting a bagel (one of the most common household injuries) and you get a cut. The cut heals.

2. How do the new cells compare to the original (pre-cut) cells?

(*answers may include: exactly the same, scar forms, cells are different ages)*

3. How does your body ensure that the new cells are the same?

(possible answers: DNA contains the information in the old cells as well as the new cells. The DNA is the same in each cell)

4. How does DNA get into the new cells?

(*answers will vary. Answers may not be accurate, but lead to discussions regarding DNA replication)*

Lab

Student Introduction:

No molecular structure has gained world-wide notoriety more than the double helical structure of DNA. The famous *Nature* paper written by James Watson and Francis Crick in 1953 entitled, "Molecular Structure of Nucleic Acids" ends with the statement, "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material." Since the release of the paper, the focus of the research of a number of scientists has been on elucidating the mechanism of DNA replication.

Modeling DNA Replication

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STEP 2 Base pair the nucleotides of the template strand in order to the non-template strand of DNA you have previously constructed to create a double stranded DNA model.

2a Record the template strand bases in the blank spaces provided above.

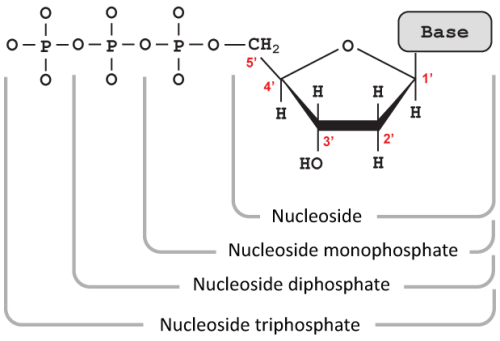
2b Examine the strands of DNA. What can you observe about the "arrow" ends of the model?
(The arrows are on opposite ends of the strands.)

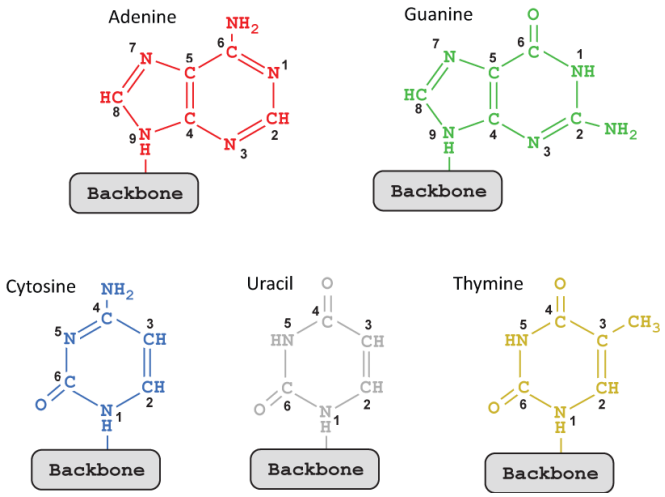
_____

_____

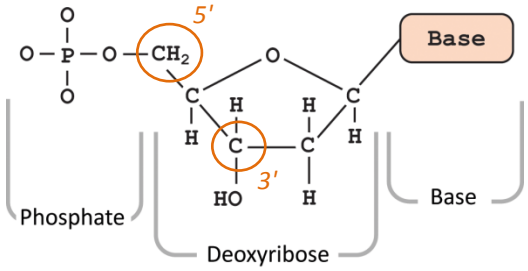
The arrow indicates the 3' end of the DNA molecule. Examine the diagrams below.

STEP 3: Examine the diagrams below.

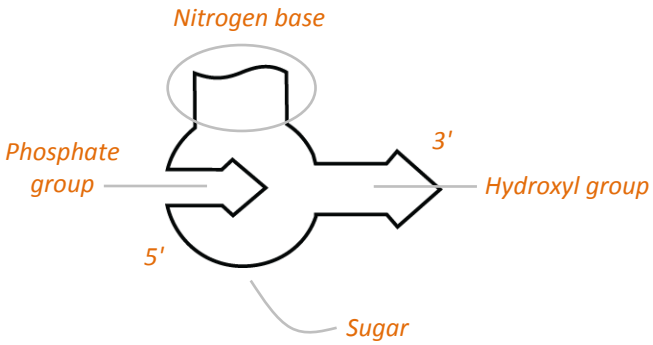




3a? Circle and label the 3' carbon and the 5' carbon in the DNA nucleotide shown in the diagram to the right. Primes are used in the numbering of the carbons on the sugar portion of the nucleotide to distinguish them from the nitrogen base carbons.



3b? Identify and label the nitrogen base, phosphate group, hydroxyl group and sugar in the representation pictured to the right. Label the locations of the 3' and 5' carbons.

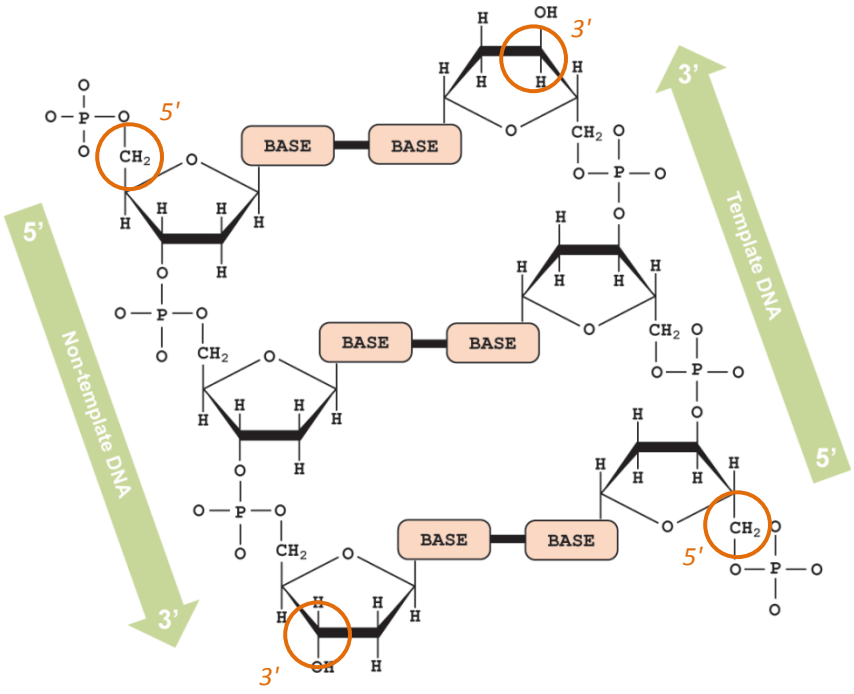


3c? How are the 3' and 5' carbons oriented in the strands of the DNA molecule you assembled?

(The 3' and 5' carbons are on opposite ends of each strand and the strands are "anitparallel" to each other)

STEP 4: Examine the detailed diagram of the DNA model below.

Double stranded DNA is composed of **<u>two anti-parallel strands</u>**! Each DNA strand has "**<u>directionality</u>**". The two sugar-phosphate backbones run in opposite 5' → 3' directions from each other. It is important to keep this directionality in mind as you model the process of DNA replication.

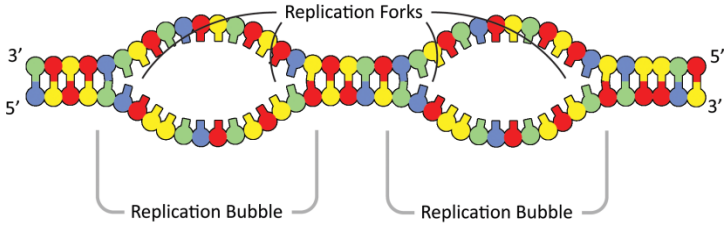


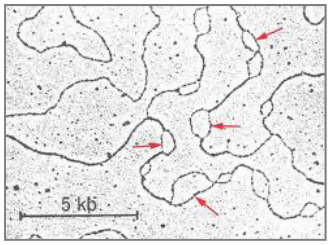
4a? Circle and label the 3' carbons and the 5' carbons in the DNA molecule above.

4b? What group is attached to the 3' carbon? What group is attached to the 5' carbon?

(The hydroxyl group is attached to the 3' carbon while the phosphate group is attached to the 5' carbon.)

Replication of DNA begins at specific sites referred to as origins of replication. A eukaryotic chromosome may have hundreds or even a few thousand replication origins. Proteins that start DNA replication attach to the DNA and separate the two strands creating a replication "bubble". At each end of the **<u>replication bubble</u>** is a Y-shaped region where the parental strands of DNA are being unwound. This region is referred to as the **<u>replication fork</u>**.

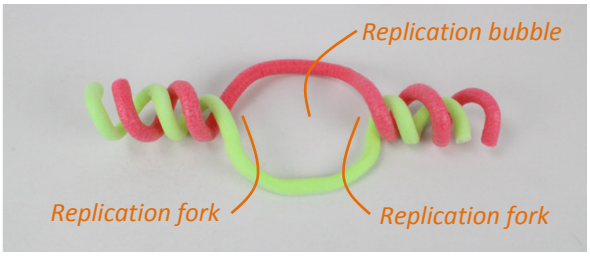




http://kootation.com/origins-of-replication.html

STEP 5: Observe your teacher create a model of a DNA replication bubble using two toobers.

5a? Identify and label the <u>replication bubble</u> and <u>replication forks</u> in the model below.



5b? Looking at the toober model, what do you think might be the first step of replication?

(The unwinding of DNA)

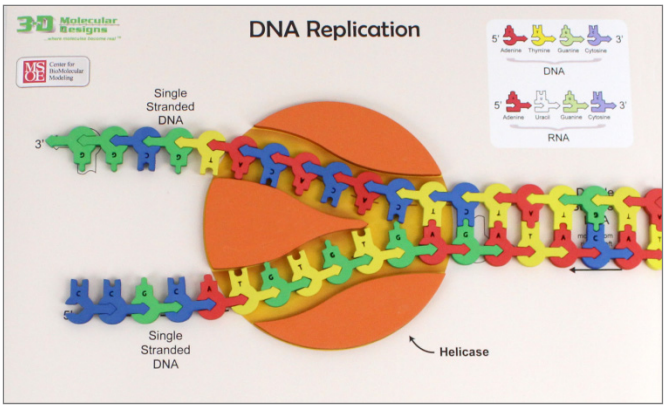
5c? Nucleotides are added at an approximate rate of 50 nucleotides per second in eukaryotic cells. The human genome contains 6.4 billion nucleotides (3.2 billion base pairs) which must be copied. Calculate the length of time in days that it would take to copy the human genome. Show all calculations including units.

(1.5 X 10^3 days) (6.4 X10^9 nucleotides X 1 second/50 nucleotides X 1hour/60 seconds X 1day/24 hours)

5d? Why do you think multiple replication bubbles form during the process of DNA replication?

(The replication process would be too slow if DNA replication occurred at a single bubble)

STEP 6: Begin the process of DNA replication by feeding the strands of the constructed DNA into the top of the **<u>helicase</u>** enzyme on the replication mat. Be sure to position the 5' and 3' ends of the DNA appropriately as you place the DNA on the mat. Continue feeding the DNA through the enzyme until you have 11 bases emerging from the bottom of the helicase. Notice that helicase moves into the replication fork NOT away from it.



6a? What does the helicase appear to be doing?

(Helicase appears to be separating the two DNA strands.)

6b? Identify which type of bond is broken.

(Hydrogen bond.)

6c? Why is the helicase able to break these bonds?

(Helicase is an enzyme that facilitates breaking the hydrogen bonds as shown by the "active site", depicted by the pointy orange wedge in the model).

Note: Replication occurs on both sides of the replication fork simultaneously. For simplicity and clarification you will simulate replication on one side of the fork at a time.

STEP 7: **DNA polymerase** catalyzes the synthesis of new DNA by adding nucleotides to a preexisting chain. **New DNA can elongate only in the 5' → 3' direction.** The DNA strand that is made continuously is referred to as the **leading strand**.

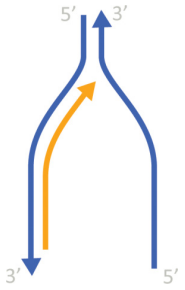
Simulate replication in the **leading strand** by placing one DNA polymerase at the point of origin (refer to Diagram 2 on the Replication Placemat) and adding nucleotides in the active site to the parent strand. Continue adding nucleotides as you move the DNA polymerase until you reach the fork.

7a? As a new nucleotide is added to the growing DNA strand, which part of the new nucleotide forms a bond with the 3' OH group?

(the phosphate group)

Additional Note: The 3' OH group is essential for adding a new nucleotide to the growing DNA strand. If this group is not present, for example, if there is a 3' H instead of a 3' OH, then DNA synthesis cannot continue. This is the basis for the Sanger Sequencing method used in determining the sequence of nucleotides.

7b? Insert a sketch of the helicase on the diagram below and indicate the directionality of the newly replicated **leading strand** of DNA:

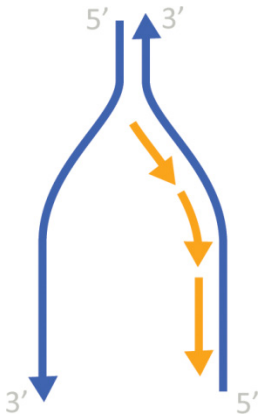


7c? Will you be able to synthesize the other strand of DNA in a continuous manner when using the model? Explain why or why not.

(DNA may be synthesized only in the 5′ → 3′ direction. Because DNA is anti-parallel, the other strand would be synthesized in the 3′ → 5′ direction if it were continuous synthesis.)

STEP 8: Place the second DNA polymerase at the fork on the other strand of DNA. Notice that the DNA polymerase must move away from the fork instead of toward the fork as it did in the leading strand. In order to accommodate the 5′ → 3′ synthesis of DNA, short fragments are made on the second strand referred to as the **lagging strand**. Continue adding nucleotides in the active site as you move the DNA polymerase away from the fork until you reach the end.

8a? Sketch and indicate the directionality of the fragments composing the **lagging strand** of DNA below:



STEP 9: Feed the next eleven nucleotides through the helicase. Continue sliding the DNA polymerase along the **leading strand**, adding more nucleotides as you progress.

STEP 10: The **lagging strand** requires that you move the DNA polymerase! Place the DNA polymerase back at the fork junction to create the next fragment. Move the DNA polymerase so that the bases may be added from the 5' → 3' direction. (Refer to the third diagram on the DNA Replication Placemat.) You have now created a second fragment of DNA on the lagging strand. These fragments are referred to as **Okazaki fragments** and are usually 100-200 nucleotides long in eukaryotic cells.

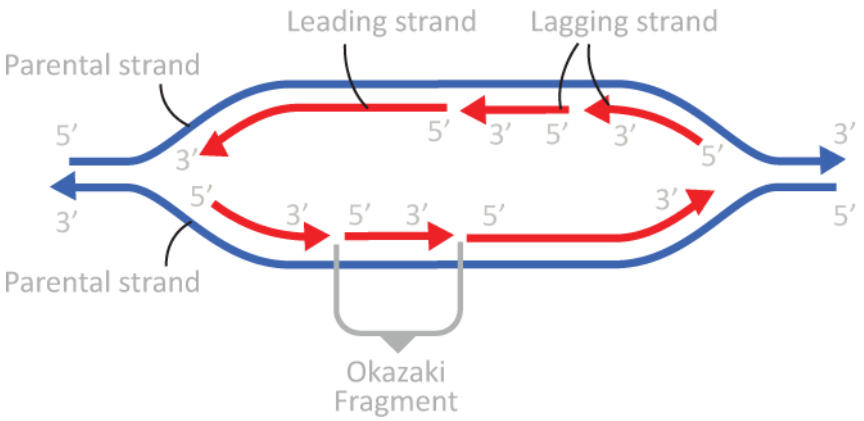
When you "bump" into the first fragment, you will need to remove the DNA polymerase and join the two fragments together with the appropriate nucleotide. The actual process of joining the Okazaki fragments together is a bit more complicated and involves several other molecules.

STEP 11: Complete the process of DNA replication with the remaining 11 nucleotides on both the leading and the lagging strands. DNA replication is considered to be a **semi-discontinuous** process.

11a? Why is DNA replication considered to be a **semi-discontinuous** process?

(DNA may be synthesized only in the 5' → 3' direction. Because DNA is anti-parallel, the other strand would be synthesized in the 3' → 5' direction if it were continuous synthesis.)

11b? Create a sketch which models the semi-discontinuous process of DNA replication. Be sure to label the following aspects of your representation: leading and lagging strands, helicase, Okazaki fragments, parental strands, 3' ends and 5' ends.

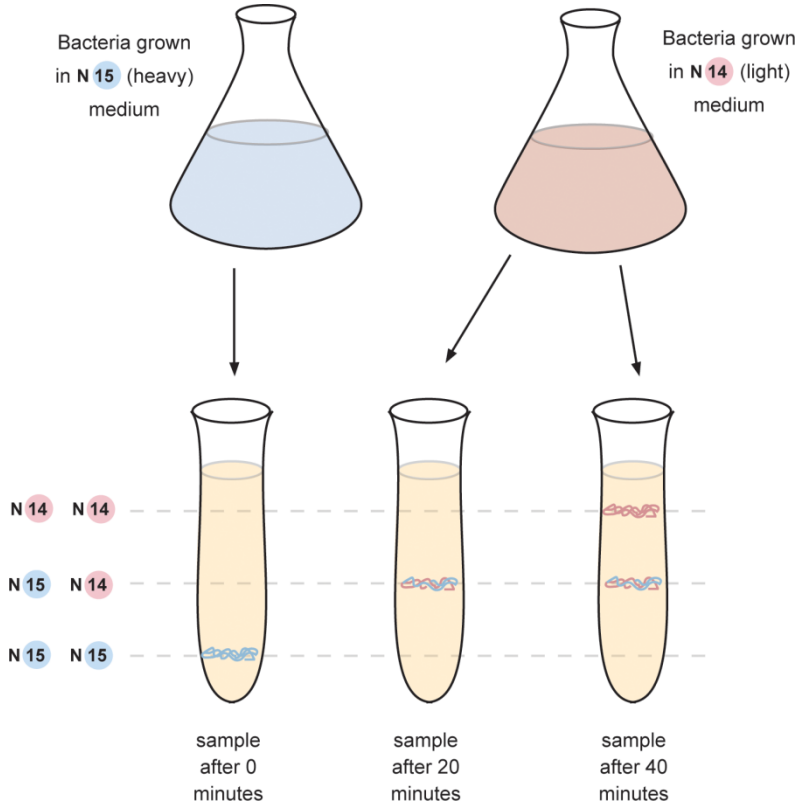


11c? How do these two new strands compare to the original (parental) strand?

(Answers may include the fact that the two daughter molecules are identical to the parent molecule, that each daughter molecule is composed of ½ parental (template) DNA and ½ new DNA)

Three Models for the Process of DNA Replication:

In 1958 at the California Institute of Technology Matthew Meselson and Franklin Stahl devised an elegant series of experiments to discern which one of three models explained the mechanism of DNA replication. Meselson and Stahl cultured *E. coli* in a medium containing nucleotides labeled with a heavy isotope of nitrogen, ^{15}N. They transferred the bacteria to a medium with only ^{14}N, a lighter isotope. A sample was taken after the DNA had replicated once. Another sample was taken after the DNA replicated again. The DNA was extracted from the bacteria in the samples and then centrifuged to separate the DNA of different densities. Their results are shown below:



STEP 1: Obtain and assemble 11 nucleotide basepairs of the colored DNA foam pieces. Find the matching gray basepair pieces but DO NOT assemble them. These colored DNA strands represent the parental strands from *E. coli* grown in a medium tagged with ^{15}N nucleotides. The gray foam pieces represent the nucleotides used to synthesize new DNA.

You will create a physical representation of the three mechanisms of DNA replication; (1) conservative, (2) semiconservative, and (3) dispersive. Begin with modeling the first round of replication of the DNA after the bacteria were transferred to a medium with only ^{14}N.

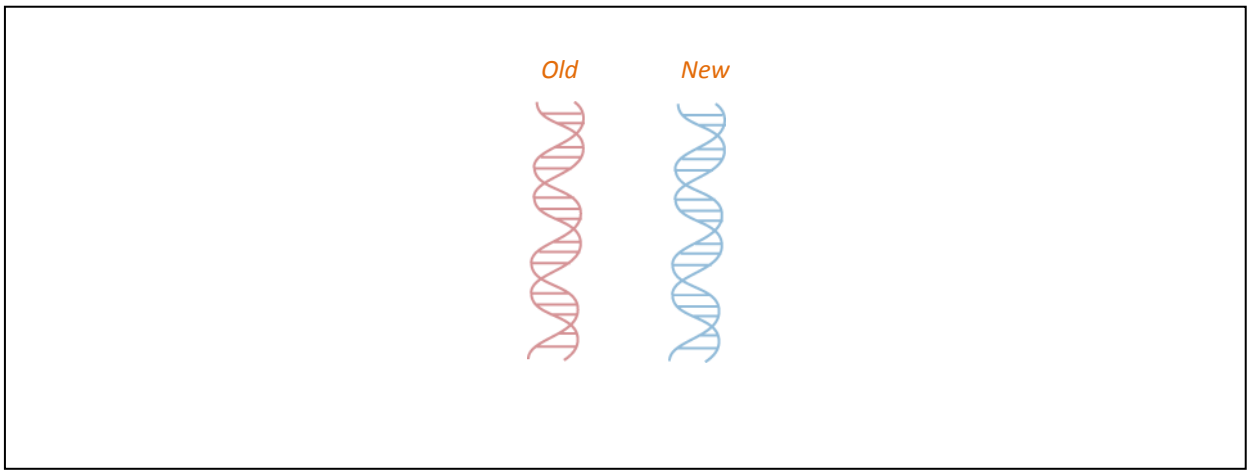
You will use the foam DNA models to discern which mechanisms of replication would most likely explain Meselson and Stahl's results

Conservative model:

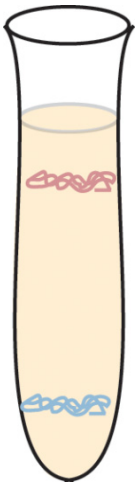
In the conservative model of DNA replication the parental strands are used as templates for the new DNA molecule and somehow come back together to "conserve" the parental molecule.

STEP 2: Using the colored DNA parental strands you have just created and the gray nucleotides, model the end result of the conservative method of DNA replication. You should have 1 parental model made entirely of colored pieces and 1 daughter molecule with the same sequence of base pairs but made entirely of gray foam nucleotides.

2a? Sketch the new and old strands after one round of replication. It will be helpful if you have two different colored pens or pencils to create your sketches.



STEP 3: A sketch of a test tube showing the density gradient of ^{15}N tagged DNA after one round of conservative replication is shown below.

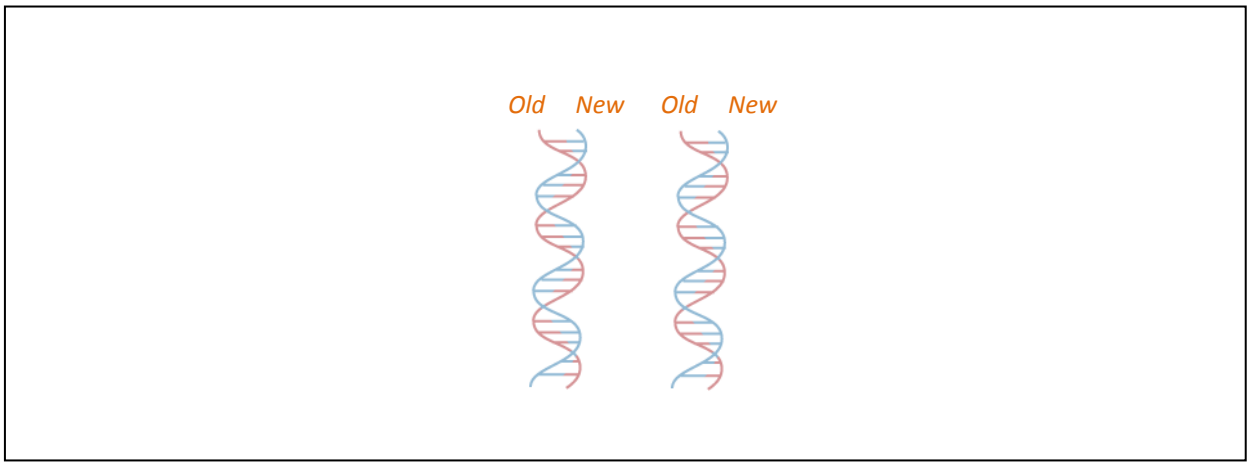


Semiconservative model:

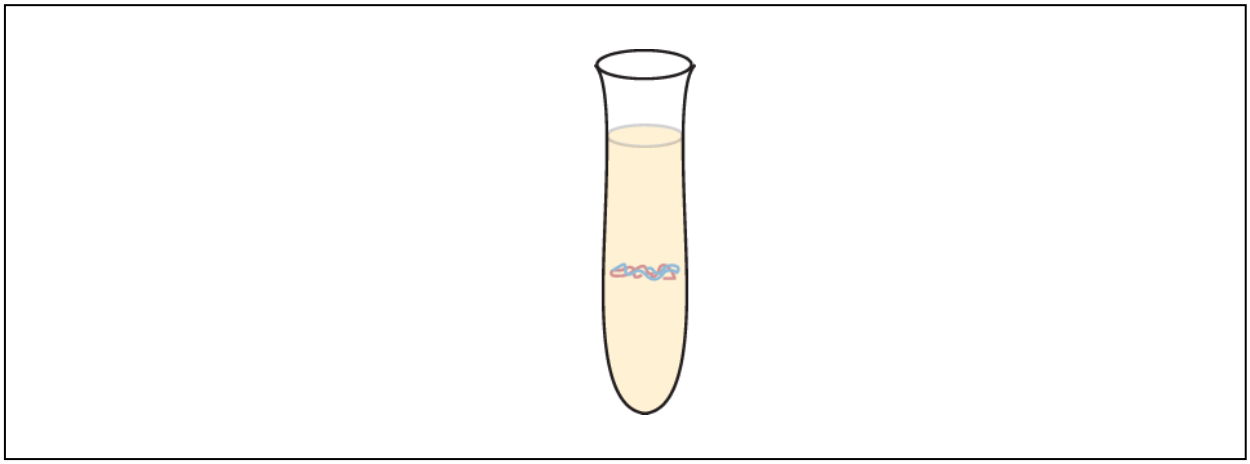
In the semiconservative model of DNA replication, each of the two daughter molecules will have one old strand from the parental molecule and one newly made strand.

STEP 4: Now using the colored DNA parental strands you have created and the gray nucleotides, model the semiconservative method of DNA replication.

4a? Sketch the results of one round of DNA synthesis after the semiconservative method of replication.



4b? Sketch a test tube showing the density gradient of ^{15}N tagged DNA after one round of semi-conservative replication. Refer to the Meselson and Stahl experiment to help you create your sketch.

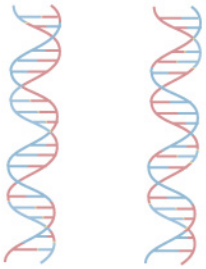


Dispersive model:

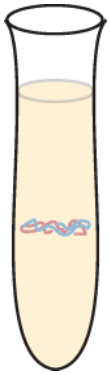
In the dispersive model of DNA replication, each strand of both daughter molecules contains a mixture of old and newly synthesized DNA.

STEP 5: Finally, using the colored DNA parental strands you have just created and the gray nucleotides, model the dispersive method of DNA replication.

5a? Sketch the results of one round of DNA synthesis after the dispersive method of replication.



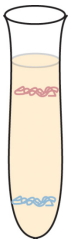
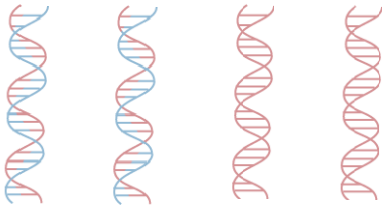
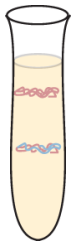
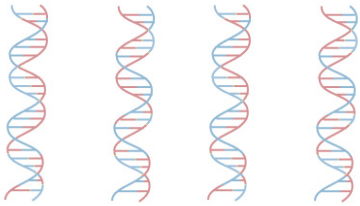
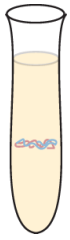
5b? Sketch a test tube showing the density gradient of ^{15}N tagged DNA after one round of dispersive replication.



5c? Which of the methods can now be eliminated based on the results that Meselson and Stahl got after one round of replication? Why?

(The conservative mechanism of DNA replication may be eliminated because it produces two bands in the density gradient test tube. Meselson and Stahl's experiment showed only one band after one round of replication.)

STEP 6: Use the foam pieces to visualize what the newly synthesized strands of DNA would look like after a second round of replication in each of the methods. Sketch your results in the first column in the table below. In the second column, sketch what the DNA density gradient would look like in the test tube.

DNA Synthesized After A Second Round of Replication	DNA Density gradient
Conservative Model 	
Semi-conservative Model 	
Dispersive Model 	

6a? Which method of DNA replication may now be eliminated after the second round of DNA replication based on the results of the Meselson and Stahl experiments? Why?

(The dispersive method may be eliminated after the second round of DNA replication because 1 band is shown in the density gradient while Meselson and Stahl's experiment showed two bands in the density gradient.)

6b? Based on the results of Meselson and Stahl's experiments, DNA is shown to replicate in a

(Semi-conservative) manner.

Post-Lab Questions:

1. What is the relationship of DNA replication to cell division?

(DNA replication is the process by which cells make a copy of DNA for the daughter cells.)

2. Of the representations of DNA models (foam pieces, paper diagram, toobers), identify the strengths and weaknesses of each.

(Various.)

3. Based on what you have learned from this activity, explain why semi-conservative replication is the preferred process of DNA replication as opposed to dispersive or conservative.

(Semi-conservative replication is an efficient, controlled process with directionality. The other two methods lack these properties. The other two methods would introduce far more error (mutation) into the process than does the semi-conservative method.)

For a detailed description suitable for IB or AP Biology:

http://www.youtube.com/watch?v=teV62zrm2P0
http://www.youtube.com/watch?v=-mtLXpgjHL0
(these descriptions include RNA primer)

For a general overview animation of continuous and discontinuous replication:

http://www.wehi.edu.au/education/wehitv/molecular_visualisations_of_dna/
http://www.dnalc.org/resources/3d/04-mechanism-of-replication-advanced.html

A group of videos on DNA replication:

http://www.youtube.com/watch?v=AGUuX4PGlCc&list=PL38E7B903667B4498

Links to the Next Generation Standards

Scientific and Engineering Practices:

- Asking Questions (for science) and Defining Problems (for engineering)
- Developing and Using Models
- Using Mathematics and Computational Thinking
- Constructing Explanations (for science) and Designing Solutions (for engineering)

Crosscutting Concepts:

- Patterns
- Cause and Effect: Mechanism and Explanation
- Scale, Proportion, and Quantity
- Structure and Function
- Systems and System Models
- Stability and Change

Disciplinary Core Ideas:

- LS 1: From Molecules to Organisms: Structures and Processes
- HS-LS1-1: Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.

- LS 2: Heredity: Inheritance and Variation of Traits
- HS-LS3-1: Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.
- HS-LS3-2: Make and defend a claim based on evidence that inheritable genetic variations may result from (1) new genetic combinations through meiosis, (2) viable errors occurring during replication, and/or (3) mutations caused by environmental factors.

- HS-ETS1: Engineering Design
- HS-ETS1-4: Use a computer simulation to model the impact of proposed solutions to a complex real-world problem with numerous criteria and constraints on interactions within and between systems relevant to the problem.

Students will:

- **Identify** the directionality of a DNA strand.
- **Explain** the implications of the anti-parallel structure of DNA on replication.
- **Model** the replication process of the leading and lagging strands of DNA.
- **Describe** the semi-conservative nature of DNA replication.
- **Describe** the semi-discontinuous process of DNA replication.
- **Explain** how a change in the DNA code may occur.

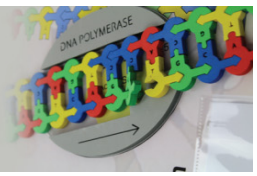
Prerequisite Knowledge and Skills:

- Hydrogen bonding and covalent bonding
- Cell structure
- DNA structure
- Cell cycle basics
- Prokaryotic and eukaryotic cell structure

Materials:

- DNA toober model
- Student Lab Packet
- DNA Replication Placemat, recommended one kit per group of three students

Transcription of DNA into RNA



DNA carries the instructions for making the proteins that are found in our bodies.

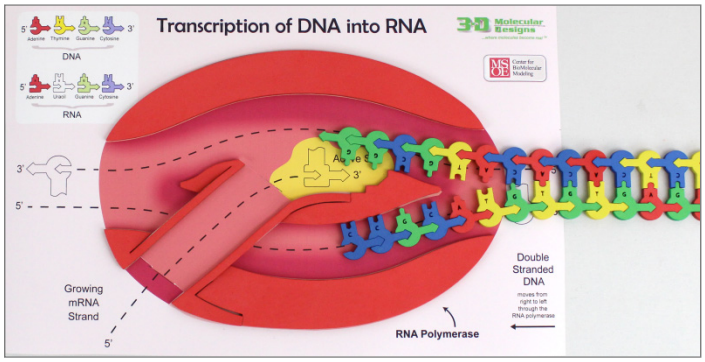
Template

Non-template

Transcription: Initiation

RNA polymerase binds to DNA.
DNA is transcribed by an enzyme called RNA polymerase. The enzyme pries apart the two DNA strands and starts transcribing the template strand (diagram 1). Specific nucleotide sequences tell RNA polymerase where to begin and where to end on the DNA. These sequences are not included in this model.

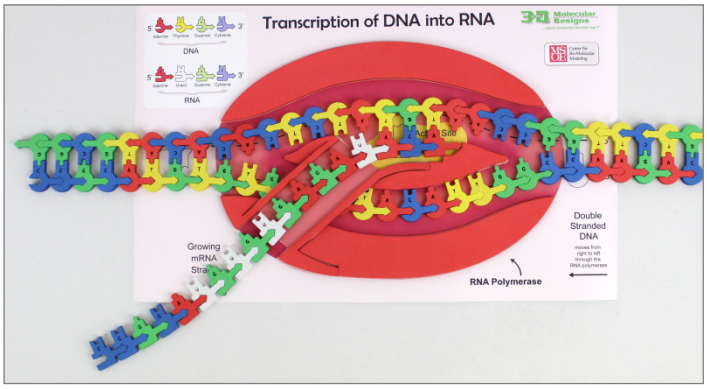
Diagram 1



Transcription: Elongation

The mRNA is synthesized.
RNA polymerase moves downstream breaking the hydrogen bonds between the DNA base pairs exposing 10-20 DNA nucleotides at a time. Messenger RNA is assembled in the 5′ → 3′ direction using the template DNA strand (diagram 2). When RNA polymerase transcribes DNA, guanine pairs with cytosine and adenine pairs with uracil.

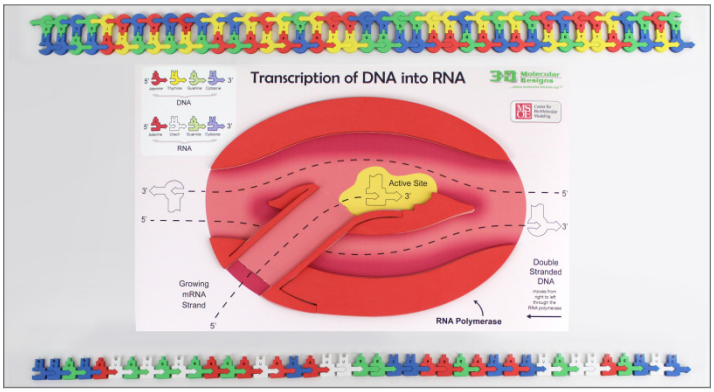
Diagram 2



Transcription: Termination

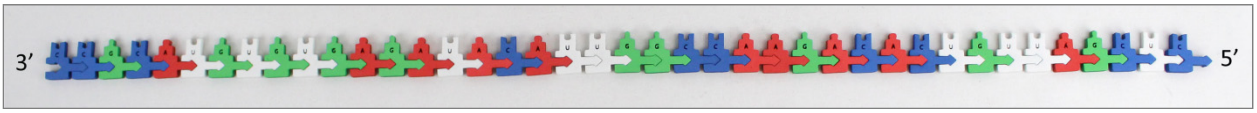
The mRNA transcript is released.
Eventually the single stranded mRNA transcript is released, and the polymerase detaches from the double stranded DNA (diagram 3).

Diagram 3



Translation of RNA into Proteins

mRNA carries the nucleotide sequence for synthesizing proteins.



Translation: Initiation

Ribosomal subunits bind to the mRNA.

The small ribosomal subunit binds to the mRNA. Note the orientation of the mRNA in this subunit shown in the diagram 1 to the right. An initiator tRNA, with the anticodoon UAC, base-pairs with the start codon, AUG. This tRNA carries the amino acid methionine. The large ribosomal subunit completes the intiation complex. In addition to a binding site for mRNA, each ribosome has three binding sites for tRNA. The **P site** (**p**eptidyl-tRNA binding site) holds the tRNA carrying the growing polypeptide chain. The **A site** (**a**minoacyl-tRNA binding site) holds the tRNA carrying the next amino acid to be added to the chain. Discharged tRNAs leave the ribosome from the **E site** (**e**xit site)

Diagram 1

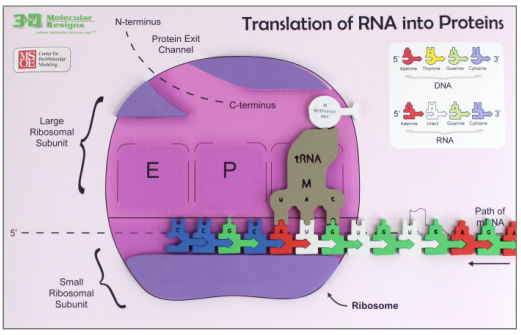
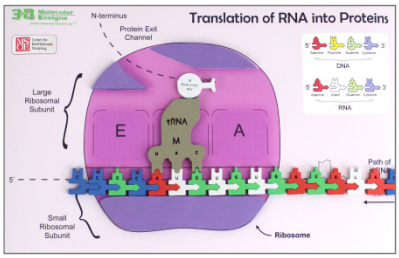


Diagram 2



Translation: Elongation

Amino acids are added to the growing protein.

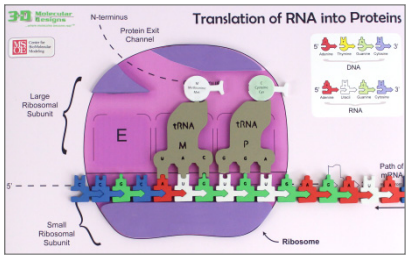
1. The initiator tRNA is in the P site; the A site is available to the tRNA carrying the next amino acid (diagram 2)

2. An rRNA molecule of the large ribosomal subunit catalyzes the formation of a peptide bond between the amino group of the new amino acid in the A site and the growing protein chain in the P site (diagram 3).

3. The ribosome translocates the tRNA in the A site to the P site. The empty tRNA in the P site is moved to the E site where it is released (diagram 4).

4. Elongation continues as each amino acid is added to the chain until the polypeptide is completed (diagram 5).

Diagram 3



Translation: Termination

The mRNA transcript is released.

Elongation continues until a stop codon in the mRNA reaches the A site of the ribosome. The mRNA codons UAG, UAA and UGA do not code for amino acids but act as signals to stop translation.

Diagram 4

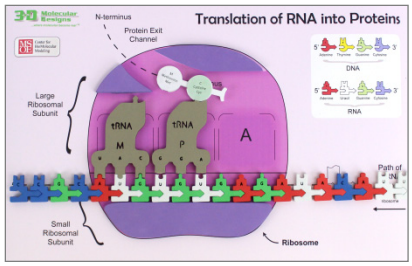
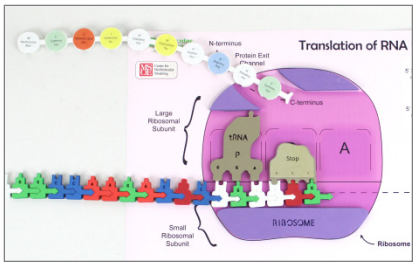


Diagram 5



Flow of Genetic Information

Transcription and Translation

PreLab

Student Introduction:

Almost all dynamic functions in a living organism depend on proteins. A wide variety of essential functions carried out by proteins have been identified including support, movement, transport, buffering, metabolic regulation, coordination and control and defense. More than 50% of the dry mass of an average cell is composed of protein. The current accepted number of proteins in the human body is approximately 25,000. Given the important role that these molecules play in an organism's survival, it is understandable that scientists focus a considerable amount of attention studying them. Central to their study is the question of how these biologically crucial molecules are produced in a cell. The molecular chain of command that dictates the directional flow of genetic information from DNA to RNA to protein was dubbed the "central dogma" by Francis Crick in 1956.

DNA carries all of the instructions for making the proteins that are found in our bodies. In fact, DNA is the universal code for the characteristics of simple organisms such as bacteria as well as for complex organisms such as plants or animals. DNA codes for the characteristics of all LIVING THINGS!! In this lesson you will learn how to interpret the DNA code to make proteins which determine these characteristics.

DNA has only four nitrogen bases; A, T, G, and C. But there are 20 amino acids that serve as the building blocks (monomers) for all proteins. How can only four letters code for all of these proteins? In order to accomplish this task DNA combines these four nitrogen bases into a three letter code called a **triplet code**.

1? How many possible combinations of these four base letters can be formed in total?

_____

_____

2? Given that there are more possible combinations for amino acids than amino acids themselves what does this imply about the number of codes for each amino acid?

_____

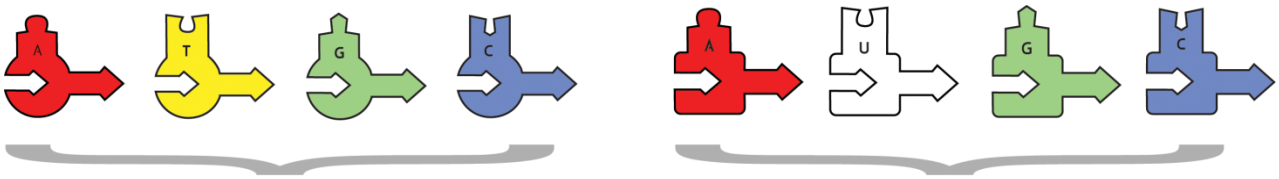
_____

Getting DNA to protein requires two major stages: (i) **transcription** and (ii) **translation**. The process by which a DNA template is used to produce a single-stranded RNA molecule is referred to as **transcription**.

In eukaryotic cells, DNA can found in the nucleus, chloroplasts, and mitochondria and cannot leave these structures. As a result, transcription occurs inside these organelles of eukaryotic cells.

3? Why can't DNA leave the nucleus?

Proteins are made on ribosomes (workbenches) that are outside of the nucleus in the cytoplasm. How does the information carried by DNA get to the ribosomes? Another molecule must carry this code from the DNA to the ribosome for the manufacture of proteins. In the process of protein synthesis there are two important types of nucleic acids; DNA and RNA (ribonucleic acid). Three different types of RNA (mRNA, tRNA, and rRNA) are major contributors to this process. The molecule that receives a copy of the DNA code in the nucleus and carries it to the ribosomes is called **messenger RNA** (mRNA).
The mRNA code is not identical to the DNA code.

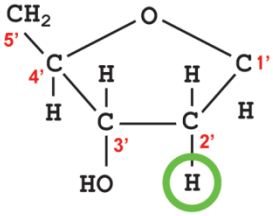


DNA RNA

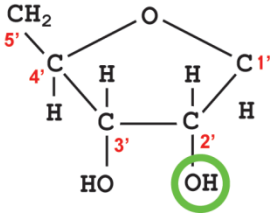
4? Examine the foam DNA pieces and compare them to the foam mRNA pieces. Identify any similarities and differences in the bases that comprise each nucleic acid.

Other differences between RNA and DNA are not readily visible in the model. The RNA backbone contains the sugar ribose which has an extra oxygen atom not found in the deoxyribose sugar of DNA. The model depicts this difference in the rounded shape of the DNA nucleotides as compared to the squared shape of the RNA nucleotides.

Deoxyribose
found in DNA



Ribose
found in RNA

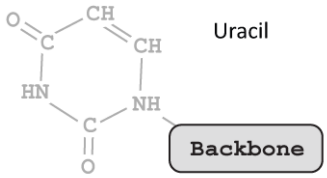


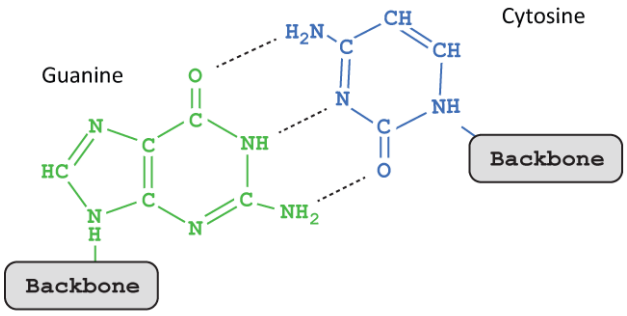
Transcription may be thought of in three stages: (1) initiation, (2) elongation and (3) termination. DNA acts as a blueprint for making mRNA. In eukaryotes, initiation begins with a collection of proteins called transcription factors mediating the binding of an enzyme **RNA polymerase** to the DNA. RNA polymerase breaks the hydrogen bonds between the two strands of DNA apart and joins the RNA nucleotides as they base-pair along the DNA template. When this happens only one side of the DNA will be used as a template for mRNA nucleotides to complementary base pair to the DNA.

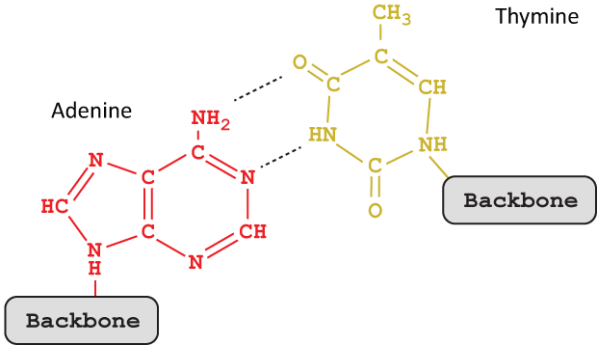
Base paring rules still apply with one exception. Guanine still pairs with cytosine while adenine pairs with uracil (recall that RNA contains the base uracil instead of the base thymine).

5? Complete the following chart by matching the correct RNA complementary base to the DNA base:

DNA Base	RNA Base
T	
G	
C	
A	
C	
A	







Lab

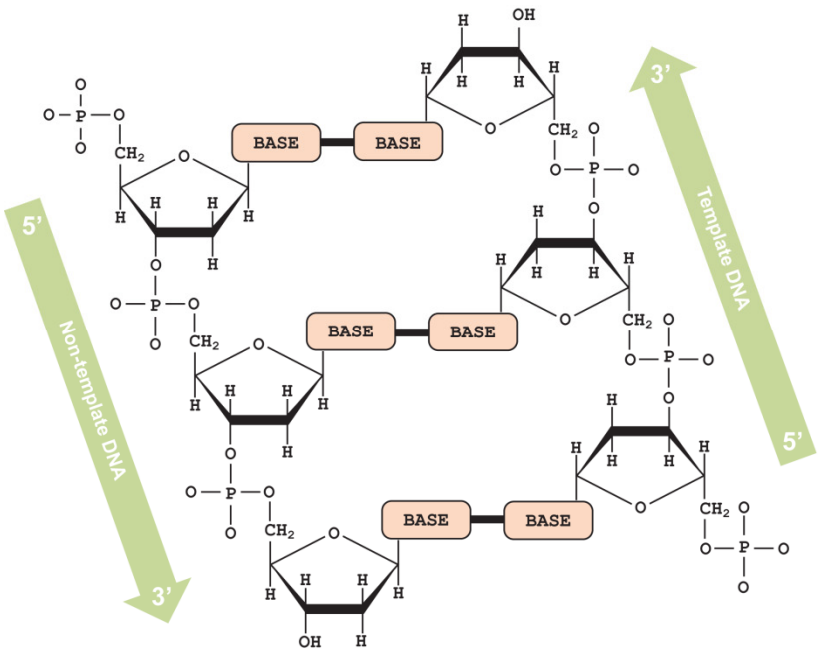
Modeling the Flow of Genetic Information

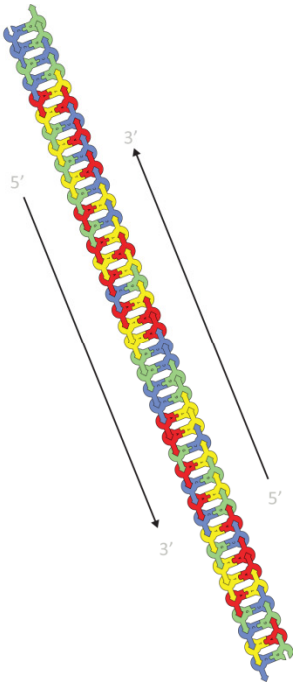
Note to Teacher: If you choose to pursue a more rigorous lesson, you may elect to introduce pre-mRNA, introns, exons, splicing and post transcriptional modification. The details of these processes are shown on the 3DMD Map of the Human β-Globin Gene.

Part I: Transcription

STEP 1: Using the rounded DNA foam pieces and following the code listed in question 1a or on the placemat, create a **non-template strand** of DNA. On the DNA backbone the sugar end is the 3' end (arrow end of the foam piece) and the phosphate end is the 5' end. In order for DNA to be interpreted correctly the 3' → 5' direction must be maintained.

Important Note! Refer to the diagram to ensure correct initiation of the protein synthesis process. Recall the antiparallel nature of the DNA molecule.





1a? Fill in the correct base pairs in the template strand below and build the DNA template strand.

STEP 2: Build the template strand of DNA to create a double stranded DNA model. Attach the two strands together following the rules of complementary DNA base pairing.

2a? Recalling from the lesson on DNA structure identify the type of bond that holds the two strands of DNA together.

_____

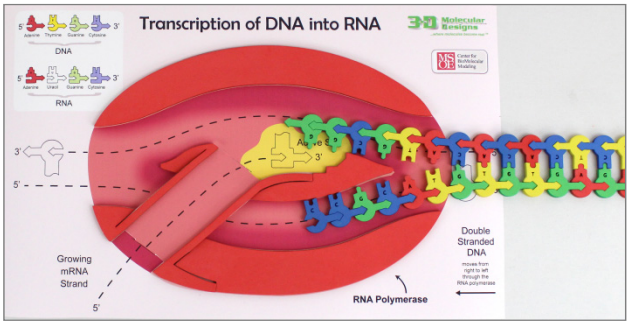
_____

STEP 3: Compare and contrast the foam model to the DNA Discovery Kit model or DNA Starter kit model on display.

3a? Identify and label the 3′ and 5′ ends in each of the models above.

3b? Identify two similarities and two differences between these models.

Transcription: Initiation



STEP 4: RNA polymerase assembles the mRNA only in its 5' → 3' direction. In order for this to properly occur, the template strand of DNA must be oriented in the top slot with the 3' end (arrow end) entering the polymerase first. (Please refer to the photo to ensure proper setup.)

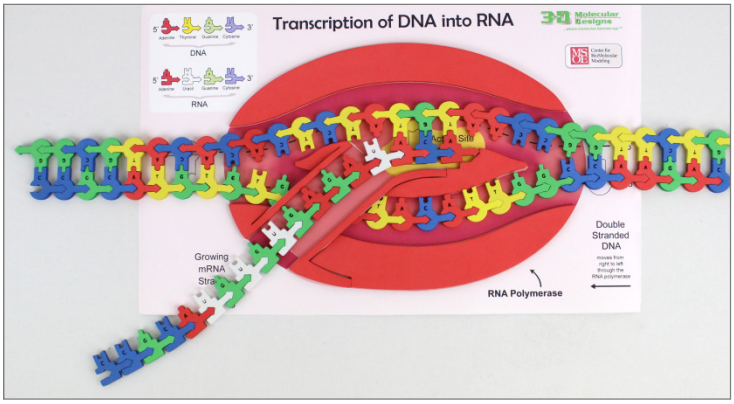
4a? Label the DNA template strand and non-template strand in the photo above.

Transcription: Elongation

STEP 5: Feed the DNA into the RNA polymerase (refer to diagram 1 on the Transcription Placemat).

5a? What will happen when RNA polymerase acts on DNA?

STEP 6: Sprinkle free RNA nucleotides around the enzyme. RNA polymerase uses the template strand of DNA to synthesize the mRNA. You will use the template strand of DNA to complementary base pair the correct sequence of mRNA nucleotides. Complete the base pairing process on your placemat.



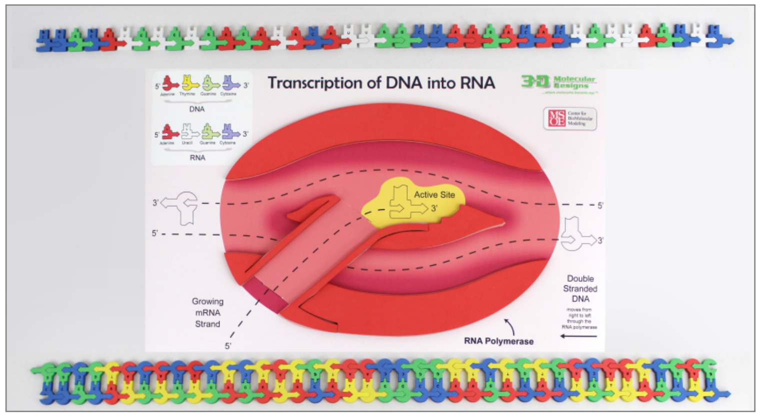
6a? Using your mRNA model record the correct sequence of mRNA base pairs:

5′ _ 3′

☆ **Note to Teacher:** Reinforce differences between RNA structure and DNA structure.

Transcription: Termination

STEP 7: At this point the mRNA will separate from the DNA and may be processed into its final form. The template strand of DNA will rejoin with the nontemplate strand. Complete this step with your model. Refer to diagram 3 on the Transcription Placemat.

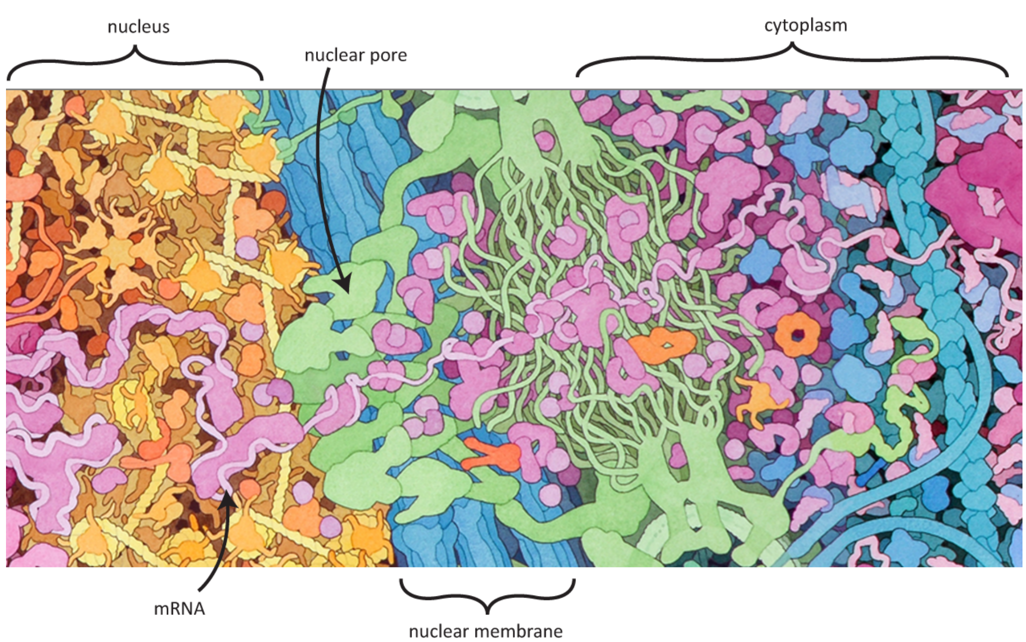


7a? What type of bond is broken when mRNA separates from DNA and what characteristic of this bond allows for this separation?

____

____

In eukaryotic cells the mRNA leaves the nucleus through nuclear pores after being processed into its final form.



Part II: Translation

Translation occurs in the cytoplasm of the cell and is defined as the synthesis of a protein (polypeptide) using information encoded in an mRNA molecule. Messenger RNA (mRNA) has the information for arranging the amino acids in the correct order to make a functional protein.

Translation of the mRNA occurs in groups of three nitrogenous bases called **codons**. The three nitrogen bases in one codon will indicate a specific amino acid. The order in which the amino acids are put together depends on the sequence of bases in the mRNA. Typically one mRNA strand will result in a protein (polypeptide strand) that can be 100 – 1000's of amino acids long.

7b? What part of the mRNA nucleotide contains the information to make a protein?

_____

_____

The identity of the amino acids in the protein sequence can be determined using the mRNA strand you created above. Starting from the 5' end of the mRNA every three bases determines a particular amino acid.

STEP 8: Use the table to the right to determine the identity of the correct amino acid for each codon in your mRNA strand.

8a? Identify the three letter and one letter abbreviation for each amino acid in the table below.

	U	C	A	G	
U	UUU → Phe F	UCU → Ser S	UAU → Tyr T	UGU → Cys C	U
	UUC → Phe F	UCC → Ser S	UAC → Tyr T	UGC → Cys C	C
	UUA → Leu L	UCA → Ser S	UAA → Stop	UGA → Stop	A
	UUG → Leu L	UCG → Ser S	UAG → Stop	UGG → Trp W	G
C	CUU → Leu L	CCU → Pro P	CAU → His H	CGU → Arg R	U
	CUC → Leu L	CCC → Pro P	CAC → His H	CGC → Arg R	C
	CUA → Leu L	CCA → Pro P	CAA → Gln Q	CGA → Arg R	A
	CUG → Leu L	CCG → Pro P	CAG → Gln Q	CGG → Arg R	G
A	AUU → Ile I	ACU → Thr T	AAU → Asn N	AGU → Ser S	U
	AUC → Ile I	ACC → Thr T	AAC → Asn N	AGC → Ser S	C
	AUA → Ile I	ACA → Thr T	AAA → Lys K	AGA → Arg R	A
	AUG → Met M	ACG → Thr T	AAG → Lys K	AGG → Arg R	G
G	GUU → Val V	GCU → Ala A	GAU → Asp D	GGU → Gly G	U
	GUC → Val V	GCC → Ala A	GAC → Asp D	GGC → Gly G	C
	GUA → Val V	GCA → Ala A	GAA → Glu E	GGA → Gly G	A
	GUG → Val V	GCG → Ala A	GAG → Glu E	GGG → Gly G	G

5' → 3'

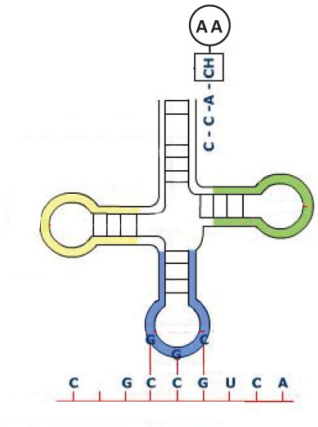
Codon	*AUG*	*UGU*	*GAG*	*AUA*	*CAU*	*UGG*	*CCA*	*AGA*	*CAC*	*UGU*	*UAG*
Amino Acid Abbreviations											

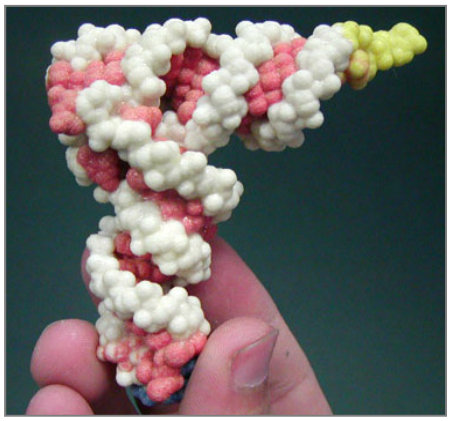
Note: Translation may also be thought of in three stages: (1) initiation, (2) elongation and (3) termination.

Translation: Initiation

Although this particular model does not illustrate the entire initiation process, the initiation stage of translation brings together mRNA, a second type of RNA called transfer RNA (tRNA) and the two subunits of a ribosome.

Two functional portions of the tRNA are necessary for protein synthesis to continue. One functional part of tRNA is a series of three nitrogen bases referred to as an **anticodon**. This anticodon complementary base pairs with the codon of the mRNA. The other functional part of tRNA attaches to a specific amino acid.





8b? On the preceding diagrams, label the 5' and 3' ends, anticodon, amino acid binding site of each tRNA model.

Note to Teacher: You may elect to include the following interesting note:

If one tRNA anticodon variety existed for each mRNA codon specifying an amino acid, there would be 61 tRNAs. In fact, there are only about 45, implying that some tRNAs must be able to bind to more than one codon. Such flexibility is possible because the rules for base pairing between the third nucleotide base of the mRNA codon and the corresponding tRNA anticodon are relaxed. Flexible base pairing at this codon position is referred to as wobble. For example, a tRNA with the anticodon 3'-CGU-5' can base pair with either the mRNA codon 5'-GCA-3' or 5'-GCG-3' both of which code for alanine.

8c? What amino acid is associated with the tRNA that will bind to the mRNA start codon AUG?

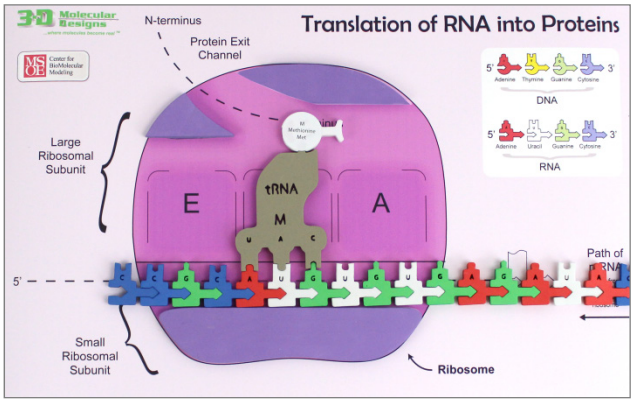
_____

8d? In the table below insert the mRNA codons from ?13 above and record the tRNA anticodons:

5′ ——————————————————————→ 3′

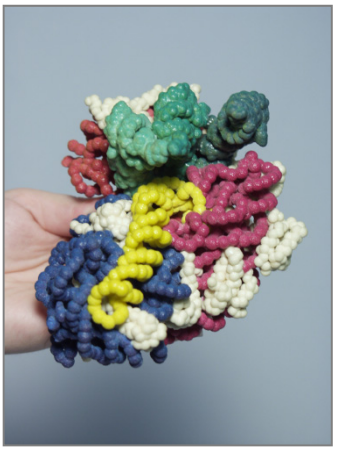
mRNA codons	*AUG*	*UGU*	*GAG*	*AUA*	*CAU*	*UGG*	*CCA*	*AGA*	*CAC*	*UGU*	*UAG*
tRNA anticodons											
Amino acids											

STEP 9: Bond the appropriate amino acids to each of the tRNAs identified in the table above. The amino acids have different colors which represent their various chemical properties such as acidic, basic, hydrophobic, and hydrophilic. Refer to Diagram 1 on the Translation Placemat.



9a? Draw your own illustration of the model and label the, anticodon and the amino acid on the mRNA or tRNA in the space below.

While the tRNA-amino acid complex is being assembled in the cytoplasm, mRNA moves towards the ribosome. Ribosomal subunits are made in the nucleolus of eukaryotic cells. The resulting ribosomal subunits are exported via nuclear pores to the cytoplasm. Approximately one third of the mass of a ribosome is made up of protein while the rest is composed of a third type of RNA, ribosomal ribonucleic acid (rRNA).



The ribosome consists of two separate parts; the large and small subunits which are unattached when not in use. First, the small ribosome subunit binds to both mRNA and a specific initiator tRNA bearing the amino acid methionine. The attachment of the large ribosomal subunit completes the translation initiation complex.

The large and small subunits join to form a functional ribosome only when they attach to an mRNA. Each ribosome has three binding sites for tRNA. The **P site** (peptidyl-tRNA binding site) holds the tRNA carrying the growing polypeptide chain). The **A site** (aminoacyl-tRNA binding site) holds the tRNA carrying the next amino acid to be added to the chain. Discharged tRNAs leave the ribosome from the **E site** (exit site).

In the next part of this activity you will model the elongation and termination processes of translation.

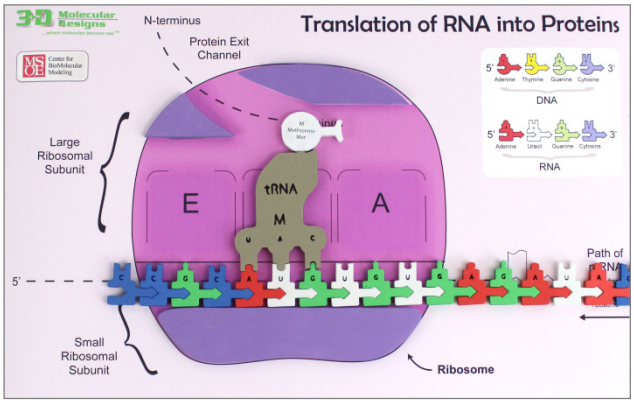
9b? Which end of the mRNA strand attaches to the small ribosomal subunit?

_____

_____

Refer to your place mat to ensure the mRNA is in the proper orientation in your ribosome.

STEP 10: Slide your mRNA into the small ribosomal subunit. Now attach the first tRNA-amino acid complex to the mRNA in the P site.



10a? Referring to the previous amino acid codon table you completed, record which tRNA anticodon and accompanying amino acid will attach first in this P site.

_____

_____

Translation: Elongation

STEP 11: The anticodon of another tRNA base pairs with the mRNA in the A site. Complete this process using your model.

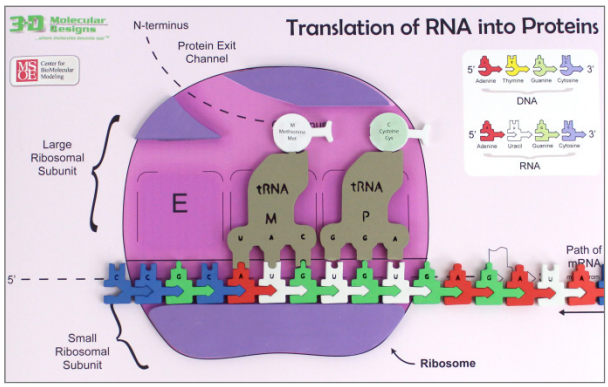
11a? Which tRNA-amino acid complex will attach into the A site at this time?

_____

_____

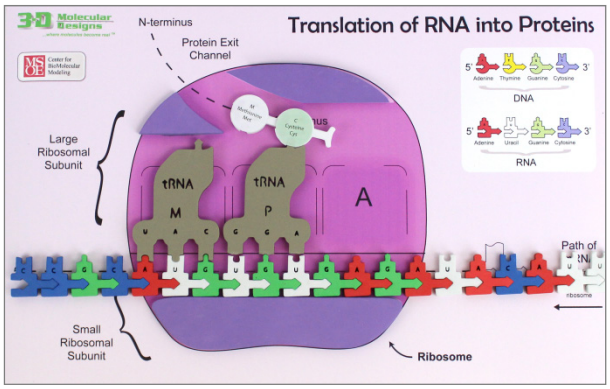
STEP 12: An rRNA found in the large ribosmal subunit catalyzes the formation of a peptide bond between the amino group of the amino acid in the A site and the carboxyl end of the amino acid in the P site.

Simulate the peptide bond formation with your model.



12a? Label the peptide bond in the photo to the right.

STEP 13: The ribosome translocates the tRNA in the A site to the P site. The tRNA in the P site is simultaneously moved to the E site where it is released.



STEP 14: Separate your tRNA in the E site from mRNA and return the tRNA to the cytoplasm.

14a? What characteristic allows tRNA to separate from mRNA at the ribosome?

_____

_____

14b? Why would tRNA get recycled for use in future translation?

_____

_____

14c? Which mRNA codon is now located in the A site? _____

STEP 15: With the A site now available for another tRNA-amino acid complex these steps can continue. Remember that the growing polypeptide transfers from the P site to the A site. Demonstrate this process using all of your tRNA-amino acid complexes in the appropriate order.

The mRNA is translated in one direction from its 5' → 3' end.

Translation: Termination

This developing polypeptide will exit the ribosome through the opening in the large ribosomal subunit. A stop codon is also present to indicate the end of the protein.

15a? Using the reference mRNA Codon/Amino Acid Chart list the various stop codons.

_____

15b? What is the order of amino acids in your polypeptide?

Met M									

15c? Compare the amino acid sequence of the poly peptide you created to the sequence predicted in question 13. How do your sequences compare?

15d? When you reach the end of the mRNA strand in your modeling of the translation process, describe what has happened to the polypeptide.

For Further Exploration

15e? What will happen next to the polypeptide?

15f? As you have followed this process of translation what steps are now left to be completed? What will happen to the mRNA, tRNA, and the ribosome at the end of this process?

15g? How long did this process of translation take for you and your lab group? Do you think the cell could operate at this rate?

mRNA, tRNA, and ribosomes can be reused over and over. The same protein can be made again if needed, or a new piece of mRNA can be translated. Ribosomes add new amino acids to the polypeptide at a rate of 20 amino acids per second (at 37^{o} C).

15h ? At this rate, how long would it take to make a protein such as actin 375 amino acids long?

15i ? Develop a new model summarizing the entire process of transcription and translation with your lab group. You will be asked to communicate and share your model with the class.

Links to the Next Generation Standards

Scientific and Engineering Practices:

- Asking Questions (for science) and Defining Problems (for engineering)
- Developing and Using Models
- Analyzing and Interpreting Data
- Using Mathematics and Computational Thinking
- Constructing Explanations (for science) and Designing Solutions (for engineering)

Crosscutting Concepts:

- Patterns
- Cause and effect: Mechanism and Explanation
- Scale, Proportion, and Quantity
- Structure and Function
- Systems and System Models
- Stability and Change

Disciplinary Core Ideas:

- LS 1: From Molecules to Organisms: Structures and Processes
- HS-LS1-1: Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.

- LS 2: Heredity: Inheritance and Variation of Traits
- HS-LS3-1: Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.
- HS-LS3-2: Make and defend a claim based on evidence that inheritable genetic variations may result from (1) new genetic combinations through meiosis, (2) viable errors occurring during replication, and/or (3) mutations caused by environmental factors.
- HS-LS3-3: Apply concepts of statistics and probability to explain the variation and distribution of expressed traits in a population.

- HS-ETS1: Engineering Design
- HS-ETS1-4: Use a computer simulation to model the impact of proposed solutions to a complex real-world problem with numerous criteria and constraints on interactions within and between systems relevant to the problem.

Students will:

- Identify different types of RNA.
- **Demonstrate** how a molecule of messenger RNA is created from the template of DNA using the model.
- Compare and **contrast** the structures of RNA and DNA.
- Explain the structure and function of codons and anticodons in the formation of proteins.
- Model the flow of genetic information from DNA → RNA → protein (also known as the Central Dogma).
- Explain how changing the DNA code, a mutation, may ultimately change the sequence of amino acids in the protein.

Prerequisite Knowledge and Skills:

- Hydrogen bonding and covalent bonding
- Cell structure
- DNA structure
- Structure of amino acids and proteins
- Prokaryotic and eukaryotic cell structure

Materials:

- One DNA Discovery Kit, assembled for display
- Student Lab Packet
- Protein Synthesis Kit, recommended one kit per group of four students

Flow of Genetic Information

Transcription and Translation

PreLab

Student Introduction:

Almost all dynamic functions in a living organism depend on proteins. A wide variety of essential functions carried out by proteins have been identified including support, movement, transport, buffering, metabolic regulation, coordination and control and defense. More than 50% of the dry mass of an average cell is composed of protein. The current accepted number of proteins in the human body is approximately 25,000. Given the important role that these molecules play in an organism's survival, it is understandable that scientists focus a considerable amount of attention studying them. Central to their study is the question of how these biologically crucial molecules are produced in a cell. The molecular chain of command that dictates the directional flow of genetic information from DNA to RNA to protein was dubbed the "central dogma" by Francis Crick in 1956.

DNA carries all of the instructions for making the proteins that are found in our bodies. In fact, DNA is the universal code for the characteristics of simple organisms such as bacteria as well as for complex organisms such as plants or animals. DNA codes for the characteristics of all LIVING THINGS!! In this lesson you will learn how to interpret the DNA code to make proteins which determine these characteristics.

DNA has only four nitrogen bases; A, T, G, and C. But there are 20 amino acids that serve as the building blocks (monomers) for all proteins. How can only four letters code for all of these proteins? In order to accomplish this task DNA combines these four nitrogen bases into a three letter code called a **triplet code**.

1? How many possible combinations of these four base letters can be formed in total?

(64, calculation: 4 different bases, in groups of three, $4^3 = 64$)

2? Given that there are more possible combinations for amino acids than amino acids themselves what does this imply about the number of codes for each amino acid?

(*Some but not all amino acids may be coded for in more than one way. Therefore there is redundancy in the code.)*

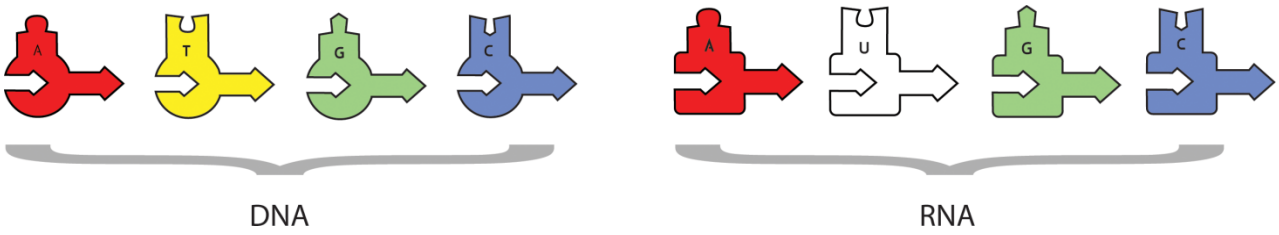
Getting DNA to protein requires two major stages: (i) **transcription** and (ii) **translation**. The process by which a DNA template is used to produce a single-stranded RNA molecule is referred to as **transcription**.

In eukaryotic cells, DNA can found in the nucleus, chloroplasts, and mitochondria and cannot leave these structures. As a result, transcription occurs inside these organelles of eukaryotic cells.

3? Why can't DNA leave the nucleus?

(DNA is too large; its information is too valuable to risk exposure to harmful chemicals in the cytoplasm.)

Proteins are made on ribosomes (workbenches) that are outside of the nucleus in the cytoplasm. How does the information carried by DNA get to the ribosomes? Another molecule must carry this code from the DNA to the ribosome for the manufacture of proteins. In the process of protein synthesis there are two important types of nucleic acids; DNA and RNA (ribonucleic acid). Three different types of RNA (mRNA, tRNA, and rRNA) are major contributors to this process. The molecule that receives a copy of the DNA code in the nucleus and carries it to the ribosomes is called **messenger RNA** (mRNA).
The mRNA code is not identical to the DNA code.

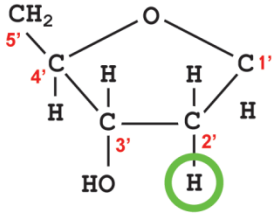


4? Examine the foam DNA pieces and compare them to the foam mRNA pieces. Identify any similarities and differences in the bases that comprise each nucleic acid.

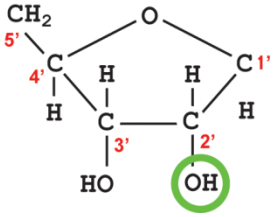
(DNA and RNA both contain adenine, guanine and cytosine. RNA contains the base uracil while DNA contains the base thymine.)

Other differences between RNA and DNA are not readily visible in the model. The RNA backbone contains the sugar ribose which has an extra oxygen atom not found in the deoxyribose sugar of DNA. The model depicts this difference in the rounded shape of the DNA nucleotides as compared to the squared shape of the RNA nucleotides.

Deoxyribose
found in DNA



Ribose
found in RNA

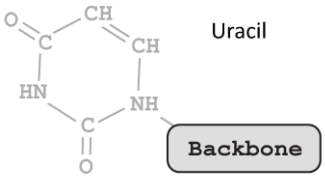


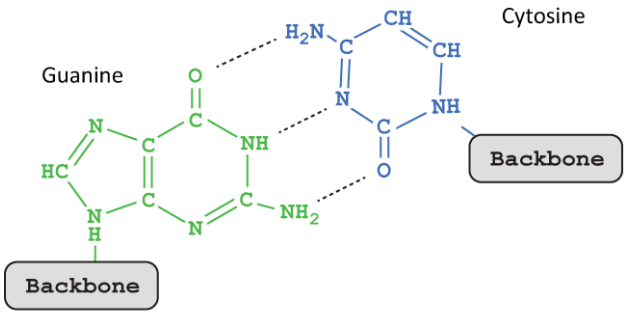
Transcription may be thought of in three stages: (1) initiation, (2) elongation and (3) termination. DNA acts as a blueprint for making mRNA. In eukaryotes, initiation begins with a collection of proteins called transcription factors mediating the binding of an enzyme **RNA polymerase** to the DNA. RNA polymerase breaks the hydrogen bonds between the two strands of DNA apart and joins the RNA nucleotides as they base-pair along the DNA template. When this happens only one side of the DNA will be used as a template for mRNA nucleotides to complementary base pair to the DNA.

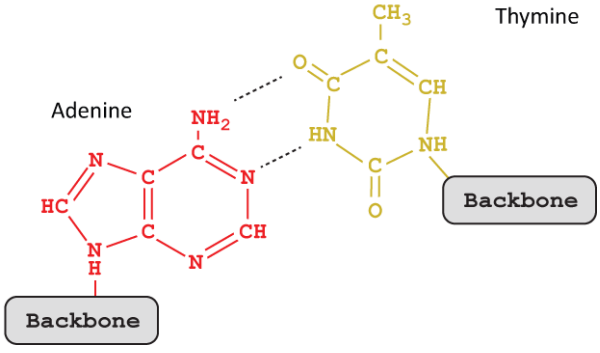
Base paring rules still apply with one exception. Guanine still pairs with cytosine while adenine pairs with uracil (recall that RNA contains the base uracil instead of the base thymine).

5? Complete the following chart by matching the correct RNA complementary base to the DNA base:

DNA Base	RNA Base
T	*(A)*
G	*(C)*
C	*(G)*
A	*(U)*
C	*(G)*
A	*(U)*







Lab

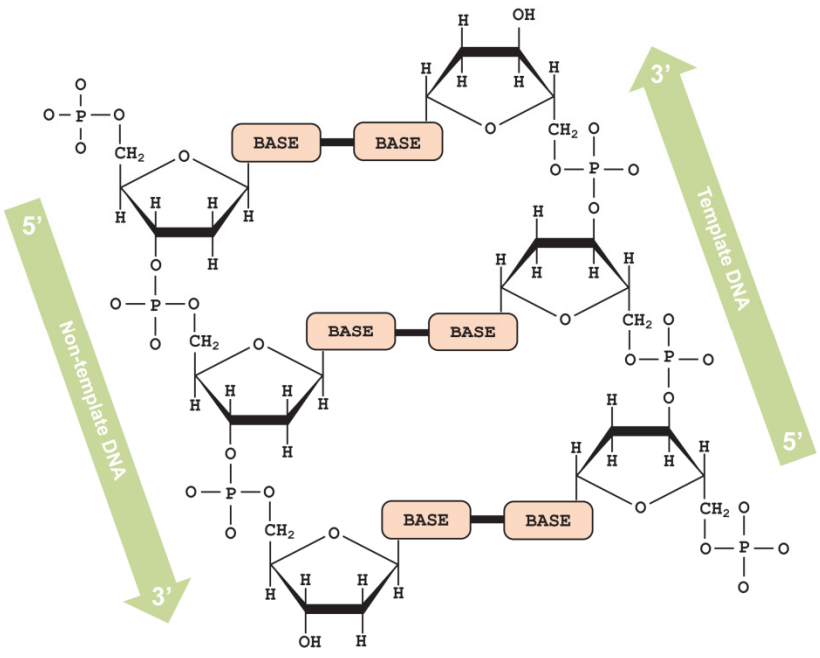
Modeling the Flow of Genetic Information

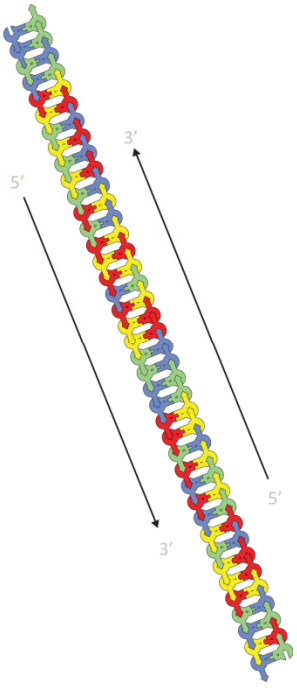
Note to Teacher: If you choose to pursue a more rigorous lesson, you may elect to introduce pre-mRNA, introns, exons, splicing and post transcriptional modification. The details of these processes are shown on the 3DMD Map of the Human β-Globin Gene.

Part I: Transcription

STEP 1: Using the rounded DNA foam pieces and following the code listed in question 1a or on the placemat, create a **non-template strand** of DNA. On the DNA backbone the sugar end is the 3' end (arrow end of the foam piece) and the phosphate end is the 5' end. In order for DNA to be interpreted correctly the 3' → 5' direction must be maintained.

Important Note! Refer to the diagram to ensure correct initiation of the protein synthesis process. Recall the antiparallel nature of the DNA molecule.





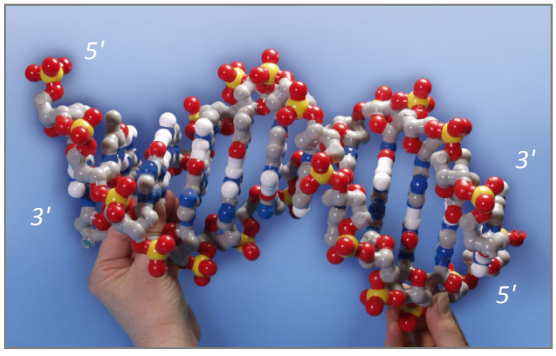
1a Fill in the correct base pairs in the template strand below and build the DNA template strand.

STEP 2: Build the template strand of DNA to create a double stranded DNA model. Attach the two strands together following the rules of complementary DNA base pairing.

2a Recalling from the lesson on DNA structure identify the type of bond that holds the two strands of DNA together.

(Hydrogen bonds)

STEP 3: Compare and contrast the foam model to the DNA Discovery Kit model or DNA Starter kit model on display.



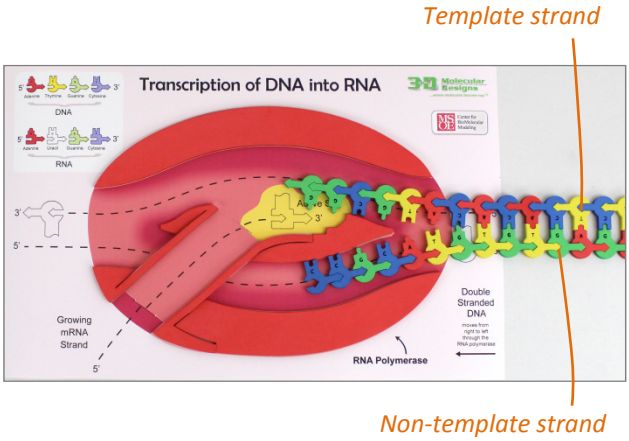
3a Identify and label the 3’ and 5’ ends in each of the models above.

3b? Identify two similarities and two differences between these models.

(Similarities: Base pairing rules are consistent, DNA is antiparallel; Differences: foam model is two dimensional and does not show the detail of the three dimensional model, major and minor grooves are missing from the foam model, cannot see the sugar phosphate backbone, foam model does not show the twisted ladder structure.)

Transcription: Initiation

STEP 4: RNA polymerase assembles the mRNA only in its 5' → 3' direction. In order for this to properly occur, the template strand of DNA must be oriented in the top slot with the 3' end (arrow end) entering the polymerase first. (Please refer to the photo to ensure proper setup.)



4a? Label the DNA template strand and non-template strand in the photo above.

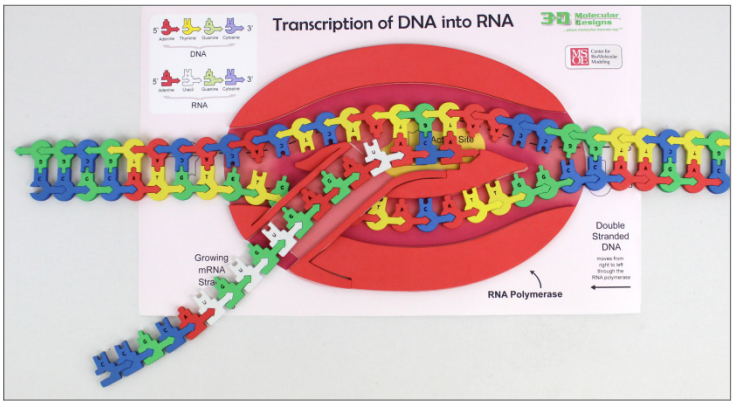
Transcription: Elongation

STEP 5: Feed the DNA into the RNA polymerase (refer to diagram 1 on the Transcription Placemat).

5a? What will happen when RNA polymerase acts on DNA?

(RNA polymerase breaks the hydrogen bonds between the DNA base pairs to open up the DNA)

STEP 6: Sprinkle free RNA nucleotides around the enzyme. RNA polymerase uses the template strand of DNA to synthesize the mRNA. You will use the template strand of DNA to complementary base pair the correct sequence of mRNA nucleotides. Complete the base pairing process on your placemat.



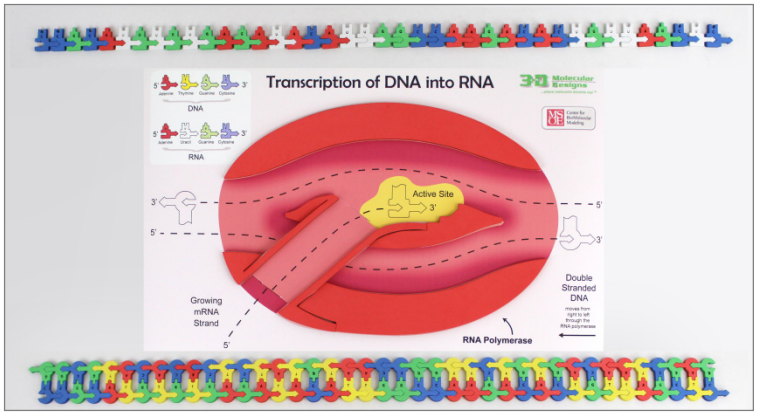
6a? Using your mRNA model record the correct sequence of mRNA base pairs:

5′ C C G C A U G U G U G A G A U A C A U U G G C C A A G A C A C U G U U A G C U C 3′

Note to Teacher: Reinforce differences between RNA structure and DNA structure.

Transcription: Termination

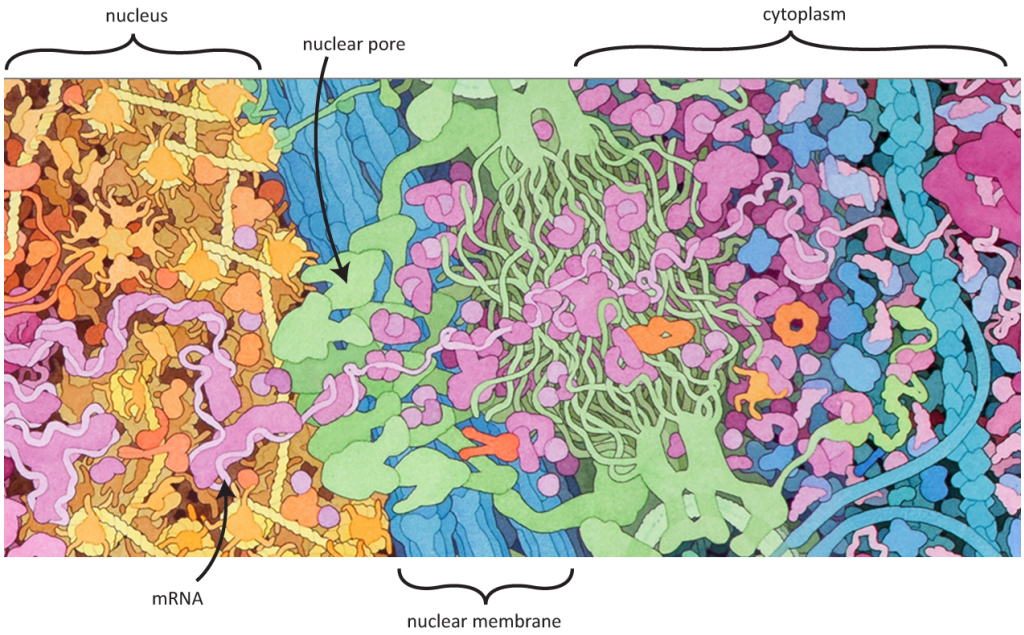
STEP 7: At this point the mRNA will separate from the DNA and may be processed into its final form. The template strand of DNA will rejoin with the nontemplate strand. Complete this step with your model. Refer to diagram 3 on the Transcription Placemat.



7a? What type of bond is broken when mRNA separates from DNA and what characteristic of this bond allows for this separation?

(hydrogen bond, weak bond)

In eukaryotic cells the mRNA leaves the nucleus through nuclear pores after being processed into its final form.



Part II: Translation

Translation occurs in the cytoplasm of the cell and is defined as the synthesis of a protein (polypeptide) using information encoded in an mRNA molecule. Messenger RNA (mRNA) has the information for arranging the amino acids in the correct order to make a functional protein.

Translation of the mRNA occurs in groups of three nitrogenous bases called **codons**. The three nitrogen bases in one codon will indicate a specific amino acid. The order in which the amino acids are put together depends on the sequence of bases in the mRNA. Typically one mRNA strand will result in a protein (polypeptide strand) that can be 100 – 1000's of amino acids long.

7b? What part of the mRNA nucleotide contains the information to make a protein?

(the order of the various nitrogen bases, the codon)

STEP 8: Use the table to the right to determine the identity of the correct amino acid for each codon in your mRNA strand.

8a? Identify the three letter and one letter abbreviation for each amino acid in the table below.

	U	C	A	G	
U	UUU → Phe F	UCU → Ser S	UAU → Tyr T	UGU → Cys C	U
	UUC → Phe F	UCC → Ser S	UAC → Tyr T	UGC → Cys C	C
	UUA → Leu L	UCA → Ser S	UAA → Stop	UGA → Stop	A
	UUG → Leu L	UCG → Ser S	UAG → Stop	UGG → Trp W	G
C	CUU → Leu L	CCU → Pro P	CAU → His H	CGU → Arg R	U
	CUC → Leu L	CCC → Pro P	CAC → His H	CGC → Arg R	C
	CUA → Leu L	CCA → Pro P	CAA → Gln Q	CGA → Arg R	A
	CUG → Leu L	CCG → Pro P	CAG → Gln Q	CGG → Arg R	G
A	AUU → Ile I	ACU → Thr T	AAU → Asn N	AGU → Ser S	U
	AUC → Ile I	ACC → Thr T	AAC → Asn N	AGC → Ser S	C
	AUA → Ile I	ACA → Thr T	AAA → Lys K	AGA → Arg R	A
	AUG → Met M	ACG → Thr T	AAG → Lys K	AGG → Arg R	G
G	GUU → Val V	GCU → Ala A	GAU → Asp D	GGU → Gly G	U
	GUC → Val V	GCC → Ala A	GAC → Asp D	GGC → Gly G	C
	GUA → Val V	GCA → Ala A	GAA → Glu E	GGA → Gly G	A
	GUG → Val V	GCG → Ala A	GAG → Glu E	GGG → Gly G	G

5′ → 3′

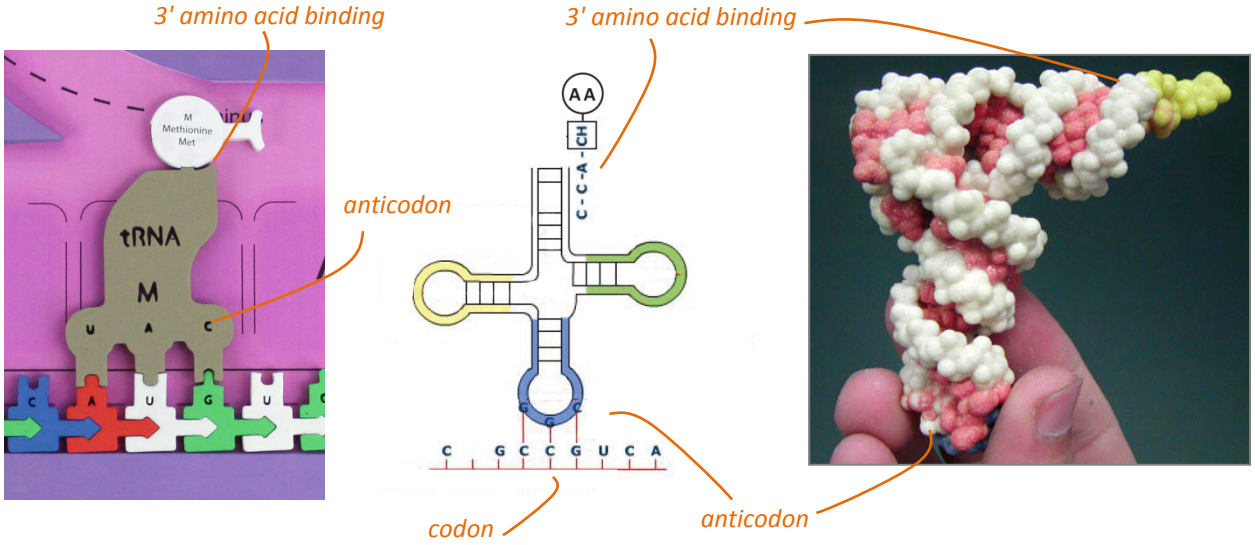
Codon	*AUG*	*UGU*	*GAG*	*AUA*	*CAU*	*UGG*	*CCA*	*AGA*	*CAC*	*UGU*	*UAG*
Amino Acid Abbreviations	*Met M (Start)*	*Cys C*	*Glu E*	*Ile I*	*His H*	*Trp W*	*Pro P*	*Arg R*	*His H*	*Cys C*	*STOP*

Note: Translation may also be thought of in three stages: (1) initiation, (2) elongation and (3) termination.

Translation: Initiation

Although this particular model does not illustrate the entire initiation process, the initiation stage of translation brings together mRNA, a second type of RNA called transfer RNA (tRNA) and the two subunits of a ribosome.

Two functional portions of the tRNA are necessary for protein synthesis to continue. One functional part of tRNA is a series of three nitrogen bases referred to as an **anticodon**. This anticodon complementary base pairs with the codon of the mRNA. The other functional part of tRNA attaches to a specific amino acid.



8b On the preceding diagrams, label the 5' and 3' ends, anticodon, amino acid binding site of each tRNA model.

Note to Teacher: You may elect to include the following interesting note:

If one tRNA anticodon variety existed for each mRNA codon specifying an amino acid, there would be 61 tRNAs. In fact, there are only about 45, implying that some tRNAs must be able to bind to more than one codon. Such flexibility is possible because the rules for base pairing between the third nucleotide base of the mRNA codon and the corresponding tRNA anticodon are relaxed. Flexible base pairing at this codon position is referred to as wobble. For example, a tRNA with the anticodon 3'-CGU-5' can base pair with either the mRNA codon 5'-GCA-3' or 5'-GCG-3' both of which code for alanine.

8c What amino acid is associated with the tRNA that will bind to the mRNA start codon AUG?

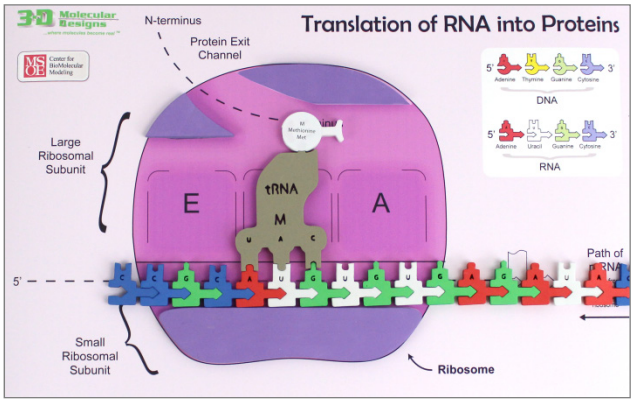
(methionine)

8d? In the table below insert the mRNA codons from ?13 above and record the tRNA anticodons:

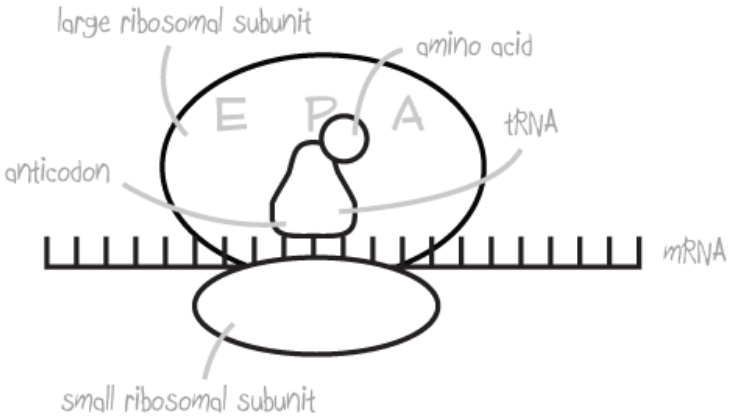
5′ ⟶ 3′

mRNA codons	AUG	UGU	GAG	AUA	CAU	UGG	CCA	AGA	CAC	UGU	UAG
tRNA anticodons	UAC	ACA	CUC	UAU	GUA	ACC	GGU	UCU	GUG	ACA	
Amino acids	M	C	E	I	H	W	P	R	H	C	STOP

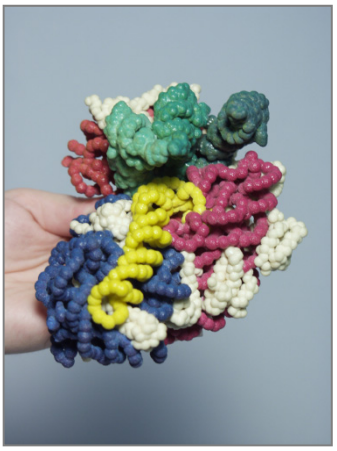
STEP 9: Bond the appropriate amino acids to each of the tRNAs identified in the table above. The amino acids have different colors which represent their various chemical properties such as acidic, basic, hydrophobic, and hydrophilic. Refer to Diagram 1 on the Translation Placemat.



9a? Draw your own illustration of the model and label the, anticodon and the amino acid on the mRNA or tRNA in the space below.



While the tRNA-amino acid complex is being assembled in the cytoplasm, mRNA moves towards the ribosome. Ribosomal subunits are made in the nucleolus of eukaryotic cells. The resulting ribosomal subunits are exported via nuclear pores to the cytoplasm. Approximately one third of the mass of a ribosome is made up of protein while the rest is composed of a third type of RNA, ribosomal ribonucleic acid (rRNA).



The ribosome consists of two separate parts; the large and small subunits which are unattached when not in use. First, the small ribosome subunit binds to both mRNA and a specific initiator tRNA bearing the amino acid methionine. The attachment of the large ribosomal subunit completes the translation initiation complex.

The large and small subunits join to form a functional ribosome only when they attach to an mRNA. Each ribosome has three binding sites for tRNA. The **P site** (peptidyl-tRNA binding site) holds the tRNA carrying the growing polypeptide chain). The **A site** (aminoacyl-tRNA binding site) holds the tRNA carrying the next amino acid to be added to the chain. Discharged tRNAs leave the ribosome from the **E site** (exit site).

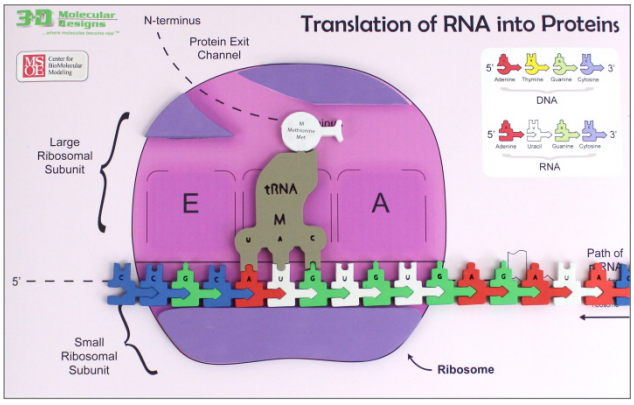
In the next part of this activity you will model the elongation and termination processes of translation.

9b? Which end of the mRNA strand attaches to the small ribosomal subunit?

(the part with the start codon, 5' end)

Refer to your place mat to ensure the mRNA is in the proper orientation in your ribosome.

STEP 10: Slide your mRNA into the small ribosomal subunit. Now attach the first tRNA-amino acid complex to the mRNA in the P site.



10a? Referring to the previous amino acid codon table you completed, record which tRNA anticodon and accompanying amino acid will attach first in this P site.

(UAC, or methionine)

Translation: Elongation

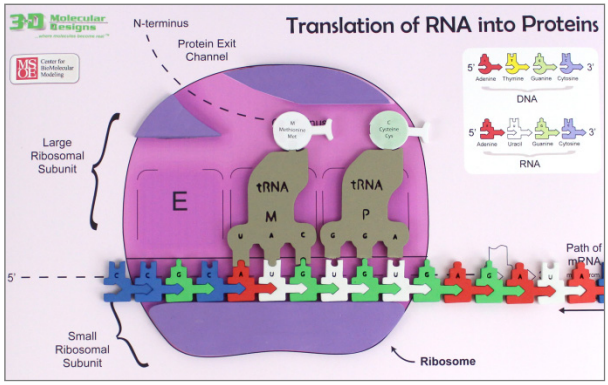
STEP 11: The anticodon of another tRNA base pairs with the mRNA in the A site. Complete this process using your model.

11a? Which tRNA-amino acid complex will attach into the A site at this time?

(UGU or cysteine)

STEP 12: An rRNA found in the large ribosmal subunit catalyzes the formation of a peptide bond between the amino group of the amino acid in the A site and the carboxyl end of the amino acid in the P site.

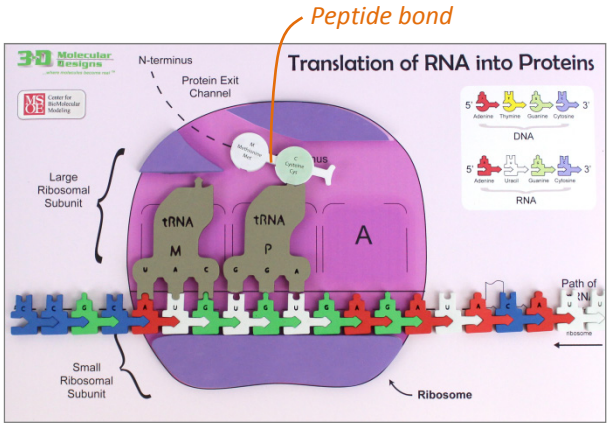
Simulate the peptide bond formation with your model.



12a? Label the peptide bond in the photo to the right.

STEP 13: The ribosome translocates the tRNA in the A site to the P site. The tRNA in the P site is simultaneously moved to the E site where it is released.

STEP 14: Separate your tRNA in the E site from mRNA and return the tRNA to the cytoplasm.



14a? What characteristic allows tRNA to separate from mRNA at the ribosome?

(the hydrogen bond formation between codon and anticodon allows tRNA to separate from mRNA as the hydrogen bond is a weak bond)

14b? Why would tRNA get recycled for use in future translation?

(tRNA picks up the correct amino acid in the cytoplasm. These amino acids are products of protein digestion.)

14c? Which mRNA codon is now located in the A site? *(UGU)*

STEP 15: With the A site now available for another tRNA-amino acid complex these steps can continue. Remember that the growing polypeptide transfers from the P site to the A site. Demonstrate this process using all of your tRNA-amino acid complexes in the appropriate order.

The mRNA is translated in one direction from its 5' → 3' end.

Translation: Termination

This developing polypeptide will exit the ribosome through the opening in the large ribosomal subunit. A stop codon is also present to indicate the end of the protein.

15a? Using the reference mRNA Codon/Amino Acid Chart list the various stop codons.

(UGA, UAA, UAG)

15b? What is the order of amino acids in your polypeptide?

Met M	*Cys* *C*	*Glu* *E*	*Ile* *I*	*His* *H*	*Trp* *W*	*Pro* *P*	*Arg* *R*	*His* *H*	*Cys* *C*

15c? Compare the amino acid sequence of the poly peptide you created to the sequence predicted in question 13. How do your sequences compare?

(the sequence of amino acids match)

15d? When you reach the end of the mRNA strand in your modeling of the translation process, describe what has happened to the polypeptide.

(polypeptide is emerging from the opening in the large ribosomal subunit, polypeptide is longer, eleven amino acids long)

For Further Exploration

15e? What will happen next to the polypeptide?

(polypeptide will separate from the mRNA strand and leave the ribosome through the opening in the large subunit; it may be a functional protein at this time or may require further modification in which case it will be transported to the rough ER.)

15f? As you have followed this process of translation what steps are now left to be completed? What will happen to the mRNA, tRNA, and the ribosome at the end of this process?

(the last tRNA will separate, the mRNA will leave the ribosome, and the large and small ribosomal subunits will separate and could be reused later)

15g? How long did this process of translation take for you and your lab group? Do you think the cell could operate at this rate?

(varies; no, too slow for cellular processes)

mRNA, tRNA, and ribosomes can be reused over and over. The same protein can be made again if needed, or a new piece of mRNA can be translated. Ribosomes add new amino acids to the polypeptide at a rate of 20 amino acids per second (at 37^{o} C).

15h ? At this rate, how long would it take to make a protein such as actin 375 amino acids long?

(approximately 20 seconds, 375 amino acids x 1 sec/20 amino acids = 18.75 sec., actin is used in muscle contractions and found in the cytoskeleton)

15i ? Develop a new model summarizing the entire process of transcription and translation with your lab group. You will be asked to communicate and share your model with the class.

(various answers)

Links to the Next Generation Standards

Scientific and Engineering Practices:

- Asking Questions (for science) and Defining Problems (for engineering)
- Developing and Using Models
- Analyzing and Interpreting Data
- Using Mathematics and Computational Thinking
- Constructing Explanations (for science) and Designing Solutions (for engineering)

Crosscutting Concepts:

- Patterns
- Cause and effect: Mechanism and Explanation
- Scale, Proportion, and Quantity
- Structure and Function
- Systems and System Models
- Stability and Change

Disciplinary Core Ideas:

- LS 1: From Molecules to Organisms: Structures and Processes
- HS-LS1-1: Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.

- LS 2: Heredity: Inheritance and Variation of Traits
- HS-LS3-1: Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.
- HS-LS3-2: Make and defend a claim based on evidence that inheritable genetic variations may result from (1) new genetic combinations through meiosis, (2) viable errors occurring during replication, and/or (3) mutations caused by environmental factors.
- HS-LS3-3: Apply concepts of statistics and probability to explain the variation and distribution of expressed traits in a population.

- HS-ETS1: Engineering Design
- HS-ETS1-4: Use a computer simulation to model the impact of proposed solutions to a complex real-world problem with numerous criteria and constraints on interactions within and between systems relevant to the problem.

Students will:

- Identify different types of RNA.
- **Demonstrate** how a molecule of messenger RNA is created from the template of DNA using the model.
- Compare and **contrast** the structures of RNA and DNA.
- Explain the structure and function of codons and anticodons in the formation of proteins.
- Model the flow of genetic information from DNA → RNA → protein (also known as the Central Dogma).
- Explain how changing the DNA code, a mutation, may ultimately change the sequence of amino acids in the protein.

Prerequisite Knowledge and Skills:

- Hydrogen bonding and covalent bonding
- Cell structure
- DNA structure
- Structure of amino acids and proteins
- Prokaryotic and eukaryotic cell structure

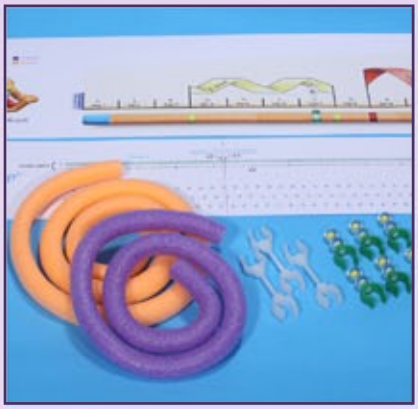
Materials:

- One DNA Discovery Kit, assembled for display
- Student Lab Packet
- Protein Synthesis Kit, recommended one kit per group of four students

Insulin mRNA to Protein Kit©

A 3DMD Paper BioInformatics and Mini-Toober Folding Activity©

Student Handout



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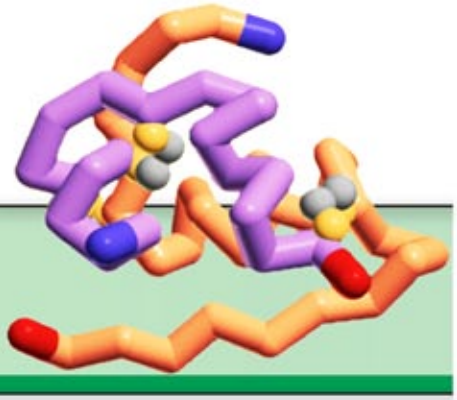
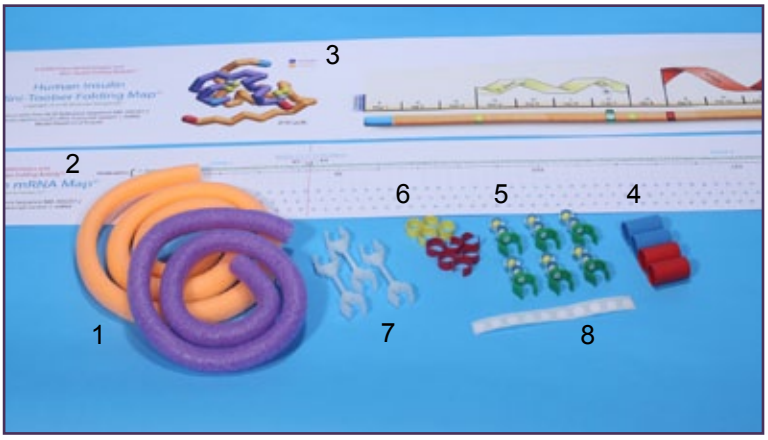


Insulin mRNA to Protein Kit©

Contents

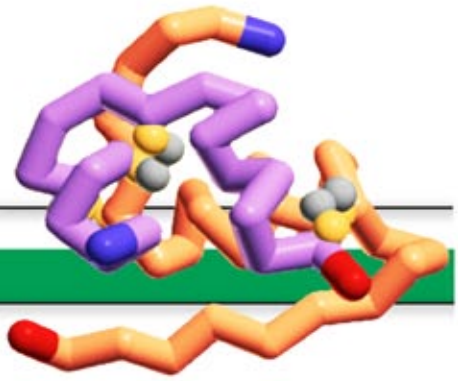
Parts

1. Mini-Toobers (orange and purple)
2. Insulin mRNA Map
3. Insulin Mini-Toober Folding Map
4. Endcaps
5. Cysteine with Plastic Clips
6. Plastic Markers
7. Support Posts
8. White Dots



Why Is Insulin Important?

Insulin is a protein (peptide hormone) that plays a major role in glucose homeostasis – the regulation of your blood sugar levels. After you eat insulin is normally released into your blood, triggering your liver, muscle, and fat cells to take up glucose from your bloodstream. Once inside these cells, the glucose can be used to fuel the production of ATP (adenosine triphosphate). ATP is frequently called the universal molecular currency because it transfers energy in our cells. *See the animation at* 3dmoleculardesigns/Teacher-Resources.htm *for more information on the role insulin plays in regulating blood sugar and the uptake of insulin.*



Insulin Paper BioInformatics Activity

In this activity, you will explore the steps involved in the synthesis of the insulin, starting with insulin mRNA. Specifically, you will consider how this mRNA is translated by the ribosome into a precursor form of insulin, and how the precursor is *processed* to create the final, functional protein. As the final step in this activity, you will create a physical model of insulin by folding two mini toobers (foam-covered wires) into the precise 3-D shape of the A-chain and the B-chain of this protein.

Becoming Familiar with the Data

A gene encoded within the DNA of a chromosome is transcribed into mRNA in the nucleus of a cell. The mRNA is then transported into the cytoplasm*, where a ribosome reads the code and builds a protein (translation). This activity focuses on how the insulin mRNA is translated into the insulin protein.

1. Unroll your Insulin mRNA Map and look at the green-colored sequence of letters at the top of the map.

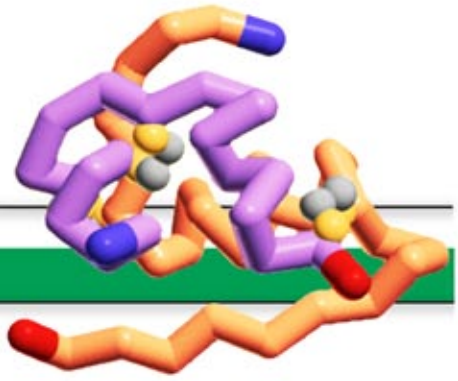
a. What different letters appear in this sequence?

_____

b. What do these letters represent?

_____

_____



The Standard Genetic Code

When RNA polymerase initially transcribes the insulin gene into messenger RNA, two introns – totaling 966 additional nucleotides – are included in the precursor form of the insulin mRNA. These intron sequences are removed from the mRNA in a splicing reaction as the mRNA is being transported out of the nucleus of the cell. You might want to discuss why almost all eukaryotic genes contain introns.

Second Letter

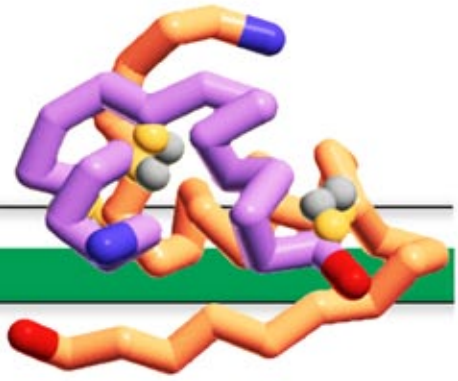
First Letter	U	C	A	G	Third Letter
U	UUU → Phe	UCU → Ser	UAU → Tyr	UGU → Cys	U
	UUC → Phe	UCC → Ser	UAC → Tyr	UGC → Cys	C
	UUA → Leu	UCA → Ser	UAA → Stop	UGA → Stop	A
	UUG → Leu	UCG → Ser	UAG → Stop	UGG → Trp	G
C	CUU → Leu	CCU → Pro	CAU → His	CGU → Arg	U
	CUC → Leu	CCC → Pro	CAC → His	CGC → Arg	C
	CUA → Leu	CCA → Pro	CAA → Gln	CGA → Arg	A
	CUG → Leu	CCG → Pro	CAG → Gln	CGG → Arg	G
A	AUU → Ile	ACU → Thr	AAU → Asn	AGU → Ser	U
	AUC → Ile	ACC → Thr	AAC → Asn	AGC → Ser	C
	AUA → Ile	ACA → Thr	AAA → Lys	AGA → Arg	A
	AUG → Met	ACG → Thr	AAG → Lys	AGG → Arg	G
G	GUU → Val	GCU → Ala	GAU → Asp	GGU → Gly	U
	GUC → Val	GCC → Ala	GAC → Asp	GGC → Gly	C
	GUA → Val	GCA → Ala	GAA → Glu	GGA → Gly	A
	GUG → Val	GCG → Ala	GAG → Glu	GGG → Gly	G

- translation start codon
- translation stop codon
- hydrophobic amino acids
- hydrophilic non-charged amino acids
- - charged amino acids
- + charged amino acids
- cysteine

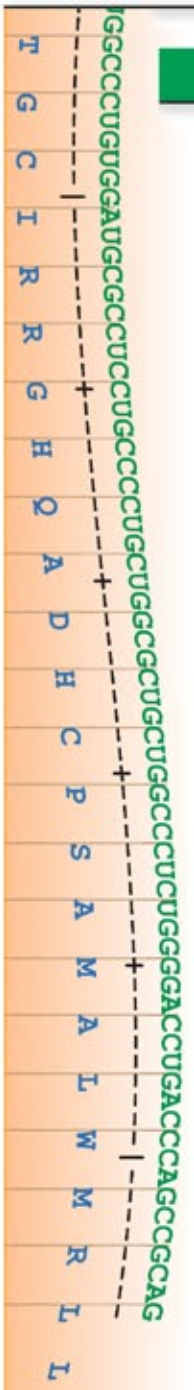
Translation Reading Frames

2. Look at the three **blue sequences** at the bottom of the Insulin mRNA map.

a. What different letters appear in these blue sequences? How many different letters appear in these sequences?



Translation Reading Frames (continued)



b. What do these letters represent?

_____

_____

c. What is the relationship between the green letters at the top of the strip to the blue letters at the bottom?

_____

_____

d. Why are there three blue sequences?

_____

_____

e. What do you think the asterisks (*) represent in the blue sequences?

_____

_____

Identifying the A-Chain and the B-Chain

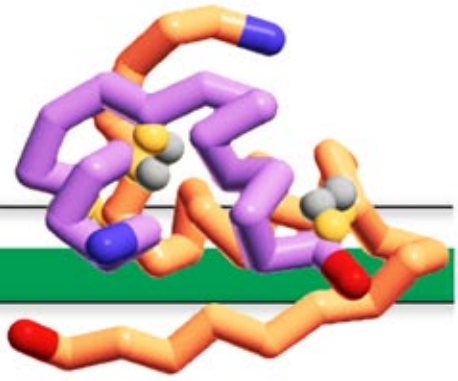
The insulin protein actually consists of two separate chains, known as the **A-chain** and the **B-chain**. The amino acid sequences of the two chains are shown below:

A-Chain

G I V E Q C C T S I C S L Y Q L E N Y C N

B-Chain

F V N Q H L C G S H L V E A L Y L V C G E R G F F Y T P K T



Identifying the A-Chain and the B-Chain (continued)

3. Locate, highlight and label the A-chain and the B-chain amino acid sequences on your Insulin mRNA Map.

   a. What do you notice about the location of the A-chain and B-chain amino acid sequences within the bioInformatics map?

_____

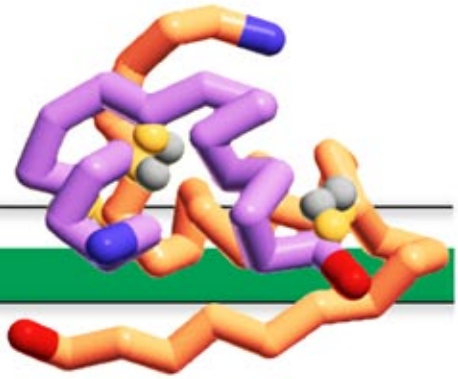
_____

Note: The subunit composition of insulin (two chains) was known before the sequence of the gene was determined. Unfortunately, when the gene was sequenced and the two chains were named, it was discovered that the B-chain was encoded before the A-chain – which has been confusing biology students ever since!

Translating mRNA into Protein

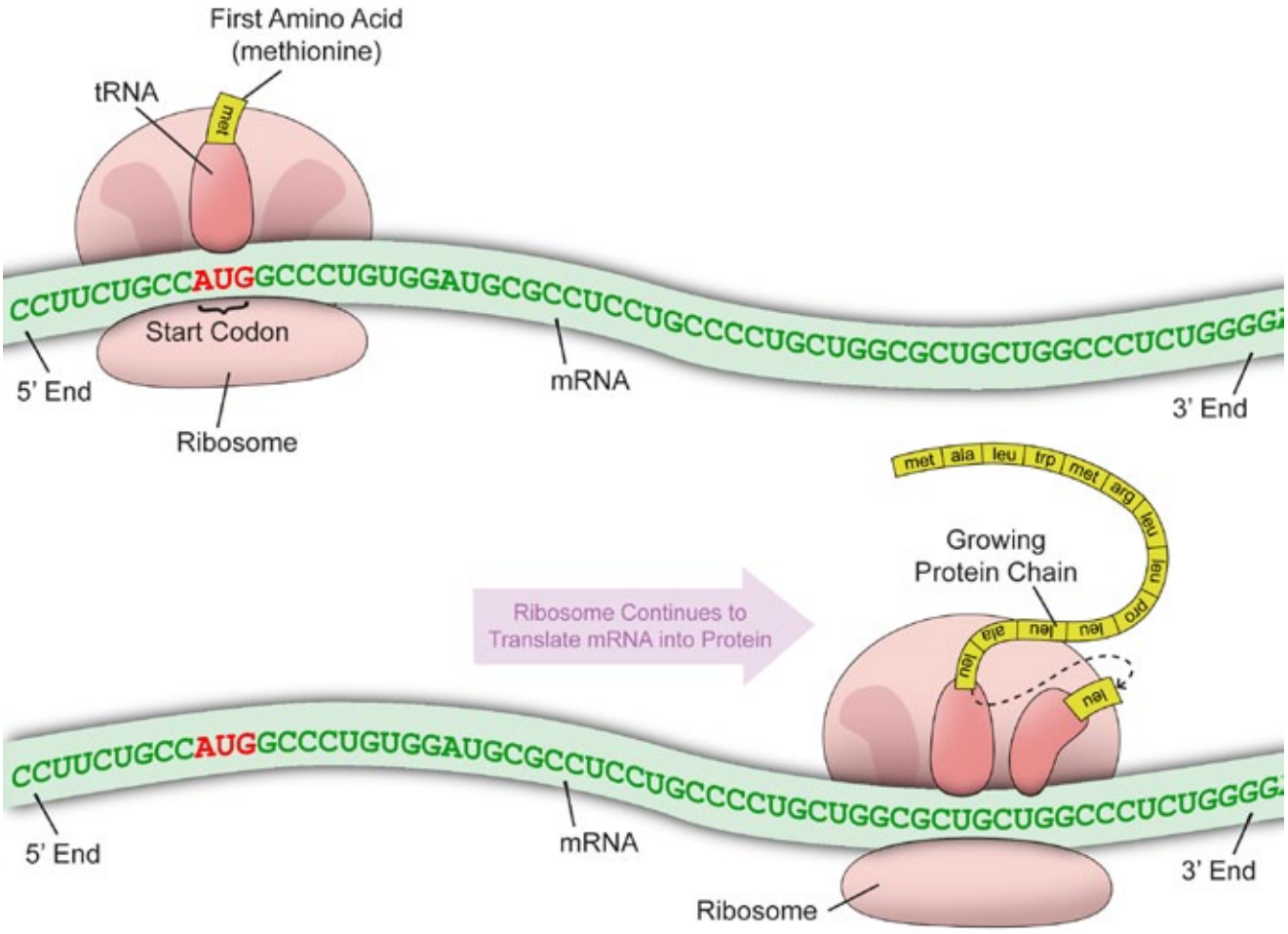
To translate mRNA into protein, the ribosome recognizes an AUG codon – and begins decoding the mRNA as it moves from left to right (5' to 3') down the mRNA sequence. As a result, all proteins begin with the amino acid methionine (Met, M) at their N-terminal end.

In humans and other eukaryotes the ribosome begins synthesizing proteins at the first AUG codon from the 5' end of the mRNA.



Translating mRNA Into Protein

Protein Synthesis of Insulin Protein



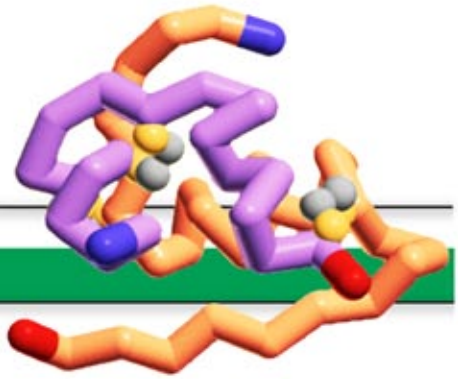
4. Highlight the protein that is synthesized by a ribosome. The ribosome binds to the first AUG located downstream (to the right) of the 5' end of the mRNA to begin synthesis.

a. Where does the protein stop?

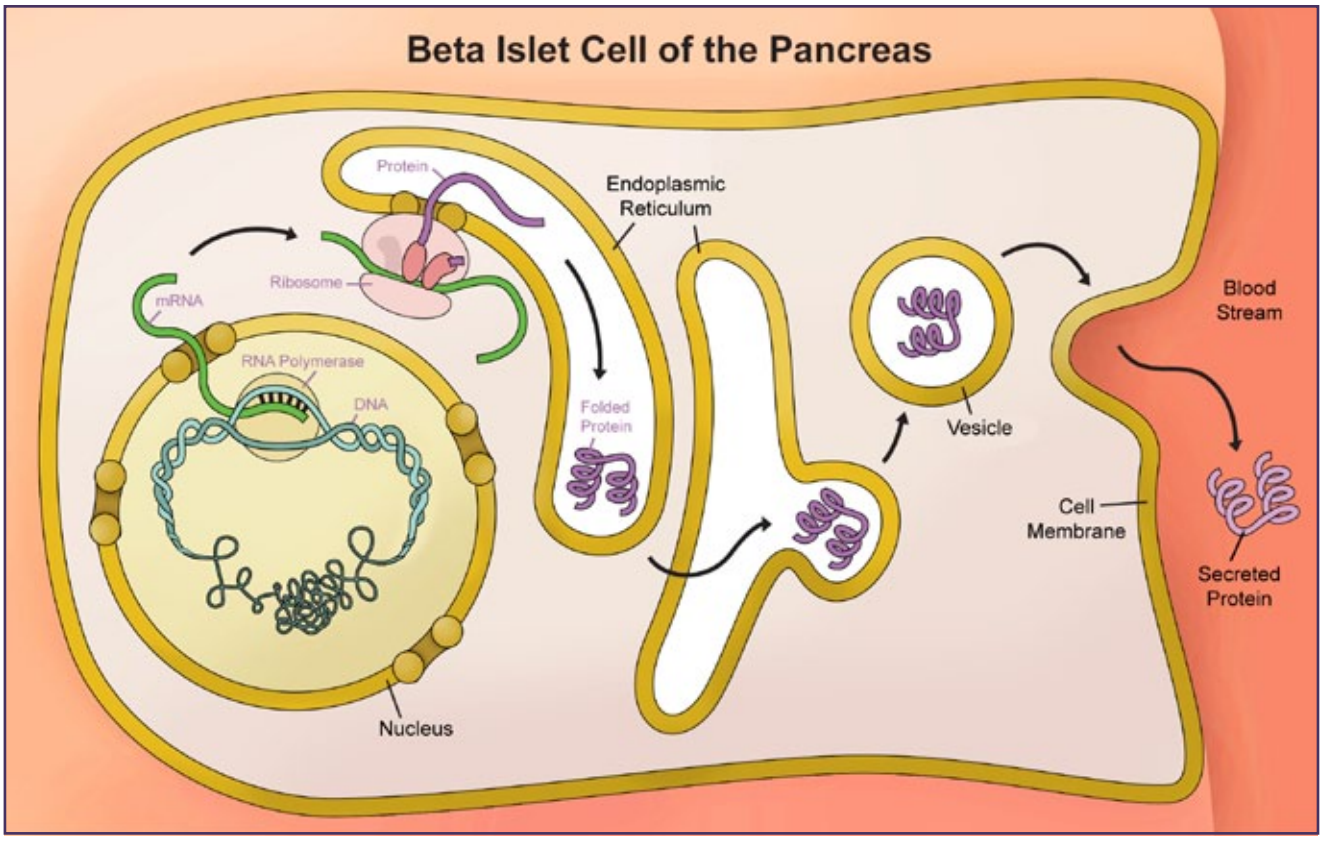
_____

b. How many amino acids are in the insulin protein?

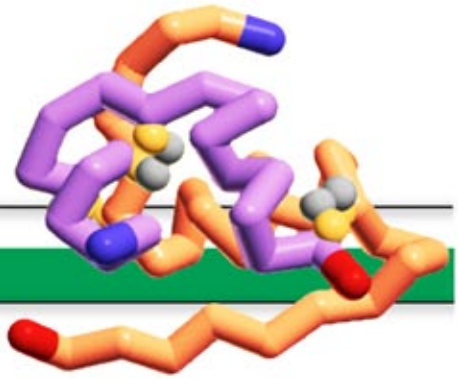
_____



Preproinsulin - the Precursor Form of Insulin



Insulin is synthesized in beta islet cells of the pancreas. Following a meal, it is secreted from these cells into the bloodstream. Proteins that are destined to be released from the cell travel through the endoplasmic reticulum and Golgi apparatus of pancreatic cells to the cell surface where they can be secreted.



Preproinsulin - the Precursor Form of Insulin

Precursor Insulin

The precursor (inactive) form of insulin is known as *preproinsulin*. The first 24 amino acids of preproinsulin make up the **endoplasmic reticulum*** (ER) **signal sequence**. As the protein is being synthesized, this signal sequence begins to emerge from the ribosome. Other proteins in the cell recognize this peptide and dock the ribosome onto the ER. As the rest of the protein is synthesized, it is directed through this membrane, into the lumen of the ER. From there, the preproinsulin is further processed (cleaved into four pieces) as it moves through the ER to the Golgi, and to the cell surface.

5. Locate, highlight and label the ER Signal Sequence on your Insulin Bioinformatics map.

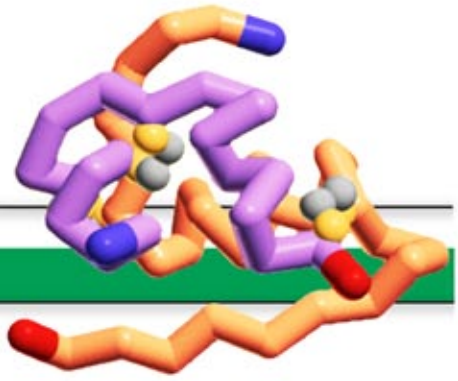
Signal Peptide

M A L W M R L L P L L A L L A L W G P D P A A A

a. Referring to the Standard Genetic Code table, *categorize* the chemical properties of each of the 24 amino acids that make up the ER Signal Peptide (hydrophobic, hydrophilic, positive charge, or negative charge). What is notable about the chemical properties of the amino acids that make up the ER Signal Peptide?

_____

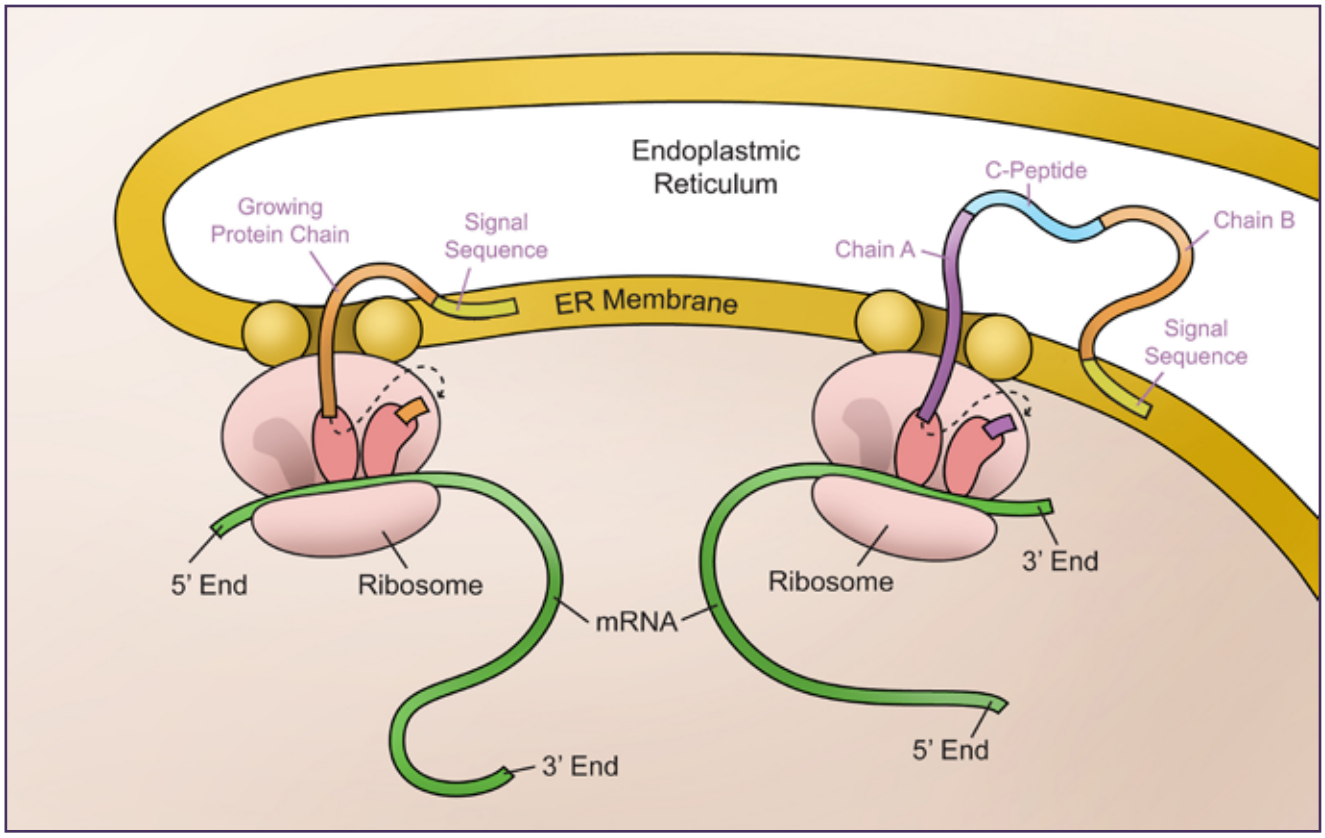
_____



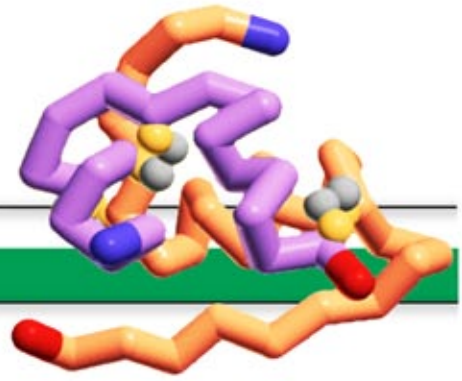
Preproinsulin to Proinsulin

Soon after the ribosome that is synthesizing preproinsulin is docked onto the ER, a protease in the ER cuts the precursor protein between amino acids 24 and 25 (Alanine, Ala, A and Phenylalanine, Phe, F). The 24 amino acid signal peptide is rapidly degraded, while the remaining 86 amino acid proinsulin begins its journey toward the Golgi and cell surface.

Proinsulin consists of the B-chain (30 amino acids) and the A-chain (21 amino acids), separated by the 35 amino-acid C-peptide.



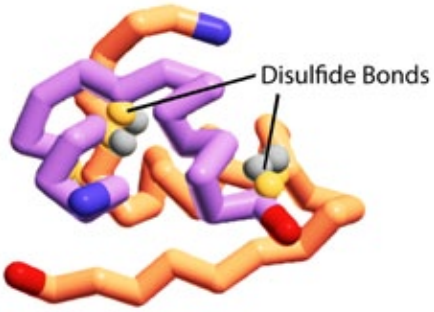
As proinsulin spontaneously folds into its final 3-D shape in the ER, another protease cuts the protein at two sites: between amino acids 54 and 55 (Threonine, Thr, T and Arginine, Arg, R) and between amino acids 89 and 90 (Agrinine, Arg, R and Glycine, Gly, G). As the C-peptide is released from the folded B-chain and A-chain complex, it floats away and is degraded.



Preproinsulin to Proinsulin (continued)

6. Locate, highlight, and label the C-peptide on your Insulin BioInformatics Map.

   a. Since the C-peptide is cut out of proinsulin to create the final mature insulin (B-chain and A-chain) what role do you think the C-peptide might play in the biosynthesis of the mature insulin protein?



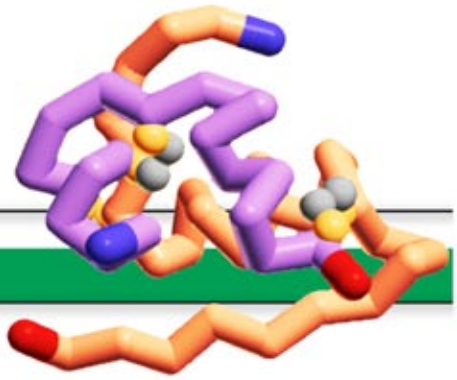
As with many secreted proteins that must function in the harsh environment outside the cell, insulin is stabilized by two covalent disulfide bonds that join the B-chain to the A-chain. Each chain contributes one cysteine amino acid (Cys, C) to each disulfide bond.

Cys7 of the B-chain forms a disulfide bond with Cys7 of the A-chain.

Cys19 of the B-chain forms a disulfide bond with Cys20 of the A-chain.

A third disulfide bond forms between Cys6 and Cys11, both from the A-chain.

7. Circle each Cys on your Insulin mRNA to Protein© map that participates in disulfide bond formation, and connect (with a line) the pairs that interact to form each disulfide bond.



Folding the Physical Model of Insulin

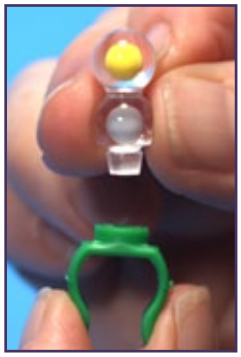
Like all proteins, insulin folds into a specific 3-D shape, following basic principles of chemistry. It is this 3-D shape that allows it to bind to the insulin receptor protein on the surface of liver, muscle, and fat cells to trigger the uptake of glucose from the bloodstream.

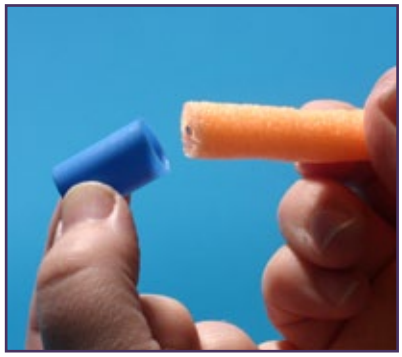
In this final activity, you will shape two mini-toobers into the 3-D shape of the insulin protein.

1. Gather all of the parts you need (see contents photo on page 2).
   - Insulin mini-toober folding map
   - Orange and purple mini toobers
   - Bag with parts for mini toobers
     - Cysteine sidechains and plastic clips
     - Support posts
     - White dots
     - Plastic markers
     - Endcaps

As you proceed with the directions (2) through (6) below you can work with the two chains at the same time or you can complete the B-chain (orange mini toober) and then repeat with the A-chain (purple mini toober).

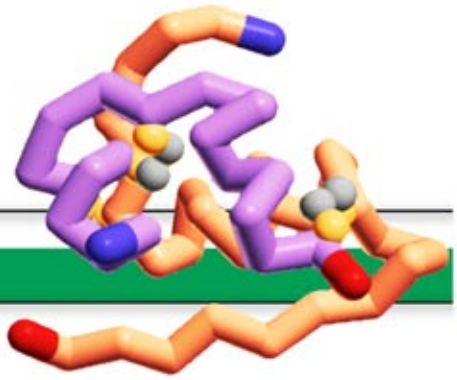
2. Insert each cysteine into a green plastic clip





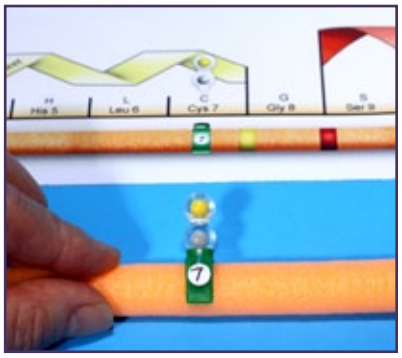
3. Unroll your Insulin Mini Toober Folding Map and identify the **N-terminus (blue)** and the **C-terminus (red)** of each protein chain by putting one red and one blue end cap onto the ends of each mini toober.

4. Using the map, locate the cysteine amino acids on each protein chain. Write the number of each of the six cysteines on the white dots and add these numbered dots to six plastic clips.

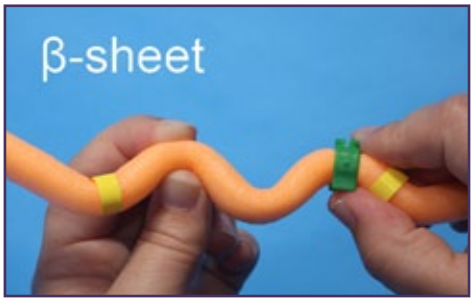


Folding the Physical Model Of Insulin (continued)

5. Carefully align each mini toober with the corresponding chain on the Insulin Mini Toober Folding Map matching the end caps to the images of the end caps on the map. Add the appropriately numbered plastic clips to the mini toober. The plastic clips represent the alpha-carbon of each cysteine amino acid.

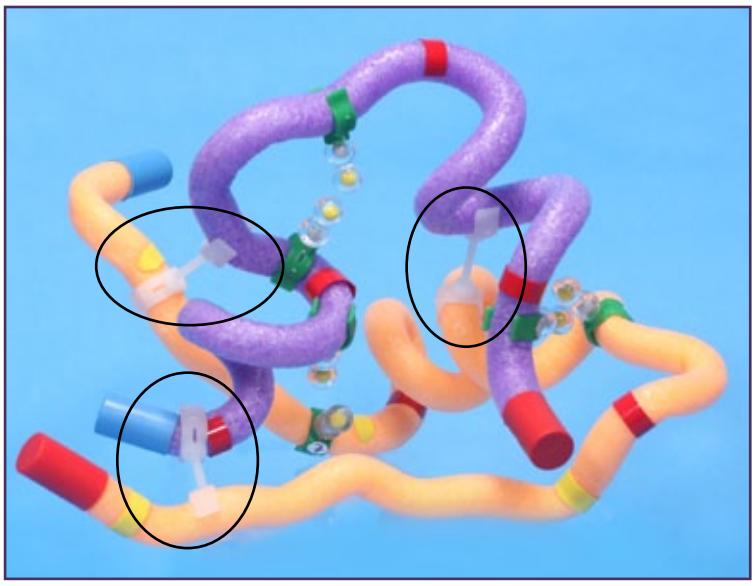


6. Indicate where the α-helicies are on each protein chain by placing the red plastic markers at the beginning and the end of each α-helix. Indicate where the β-sheets are on each protein chain – by placing the yellow plastic markers on the mini-toober at the beginning and the end of each β-sheet shown on the map.



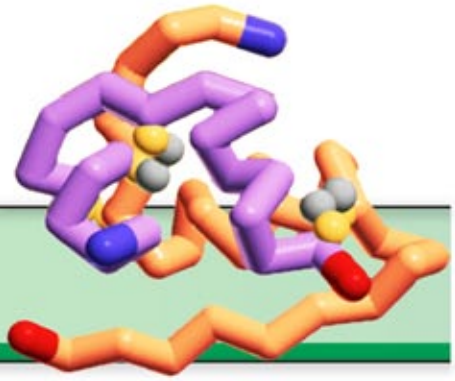
7. Fold the mini toobers to create the α-helicies (right-handed) and the β-sheet strands (extended zig-zag) in each protein chain. See photos above.

   To fold the overall 3-D shape of each protein chain, use the online Jmol visualization tool at 3dmoleculardesigns/Teacher-Resources.htm and/or the images at the end of the map to fold your insulin.



8. Assemble the two chains into the final insulin model by positioning the chains as shown in the photo using the images on the map and/or the Jmol visualization tool.

Hint: The three pairs of cysteine amino acids that form covalent disulfide bonds should be close to each other in the final model. Use the three plastic support posts to stabilize the protein, as shown in the photo.



Insulin In Review

- The insulin gene is located on the short arm of chromosome 11 in humans.
- The insulin gene is transcribed into an insulin mRNA molecule in the nucleus of the beta islet cells of the pancreas.
- *Insulin* mRNA is transported to the cytoplasm of the cell where a ribosome recognizes the first AUG near the 5'-end of the mRNA and begins translating the protein, starting with methionine.
- The ribosome synthesizes a precursor form of insulin, known as preproinsulin.
- Preproinsulin is processed to become mature, functional insulin as it proceeds through the endoplasmic reticulum and Golgi apparatus, moving toward the cell membrane where it can be secreted from the cell.
- When there are high levels of sugar in the blood, insulin is released from the beta cells. It binds to receptors on the surface of liver, muscle, and fat cells. This binding results in a series of reactions within the cell, (called a signal cascade), leading to the fusion of vesicles containing glucose transporter proteins (GLUTS) with the membrane. The GLUTS transport glucose into the cells, where it is stored.