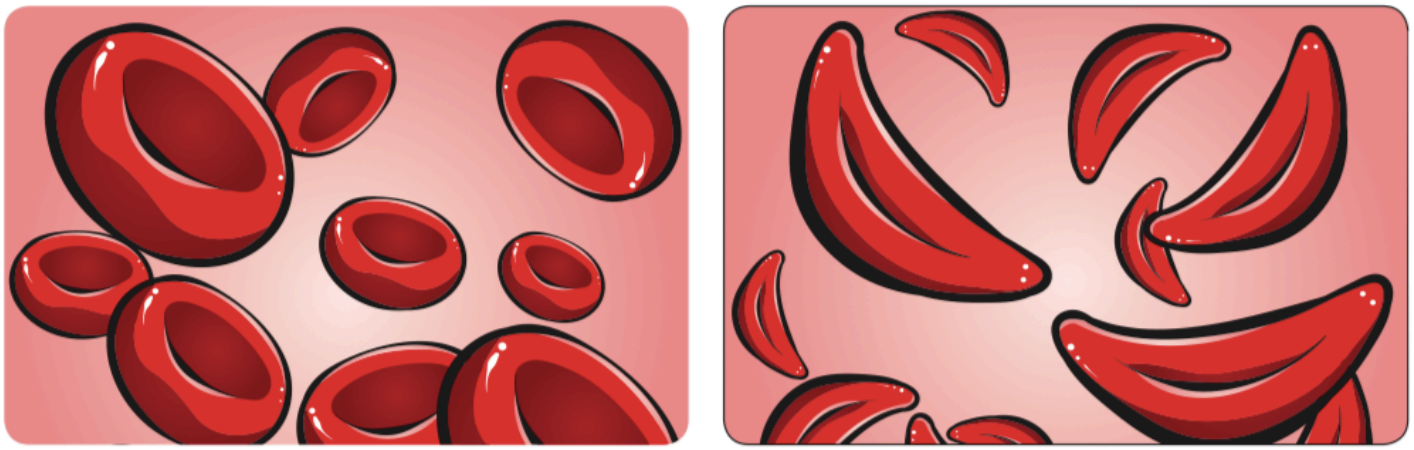
LT: I can follow a multistep procedure to model protein translation and inheritable genetic variations that may result from mutations occurring during replication.

Phenomenon

Weighing a healthy 7 lb., 9 oz at birth, Terra is a beautiful baby girl. As part of their mandatory medical screening procedures, nurses at the hospital collected a small sample of Terra's blood from her heel. Terra's hemoglobin, a component of her blood, was analyzed. The results of the test indicated that Terra was likely to develop a red blood cell disease known as sickle-cell anemia. Her parents were shocked and wondered how this could happen, because neither of them has the disease.

Activate Prior Knowledge

Examine the illustrations of normal and sickled red blood cells, and then answer the following questions.



Normal and sickled red blood cells

Engage:

a. What differences do you observe between normal and sickled red blood cells?

Making Connections:

b. To function properly, our cells need a constant supply of oxygen. Hemoglobin is a protein that carries oxygen in red bloods cells throughout the body. Use your prior knowledge and information from the illustrations to predict how the sickling of red blood cells could influence their functioning in Terra's body.

Making Sense:

c. Neither Terra's older brother nor her parents suffer from sickle-cell disease, yet her medical team knows that Terra has inherited the disease from her parents. Knowing this, record some questions you now have about sickle-cell anemia, the transmission of genetic diseases such as sickle-cell disease, Terra's family history, or the methods used to diagnose Terra.

Explore:

Additional tests confirm that baby Terra does indeed have sickle-cell disease. The medical team informs Terra's parents that sickle-cell disease is caused by abnormal hemoglobin, called hemoglobin S. Your group will model the process of transcription in the same region of the normal and the sickle-cell beta hemoglobin gene in humans to determine any differences in the resulting mRNA sequences.

You will use the set of nucleotide magnets representing the components of the base pairs that make up DNA. The four DNA nucleotides are adenine, thymine, cytosine, and guanine and are represented by the letters A, T, C, and G. Using the magnetic whiteboard provided, your group will share the tasks of reading the sequence, arranging the nucleotides on the whiteboard, and checking the accuracy of the arrangements.

Transcription Procedure

1. Hemoglobin carries oxygen in the blood. Make the following DNA sequence—a portion of the normal human beta hemoglobin gene—with your nucleotide magnets on the magnetic whiteboard. For the sake of this exercise, we will call this the "original" strand. Orient this strand of magnetic nucleotides with the bonding regions facing downward, as shown below.

TAC CAC GTA GAC TGA GGA CTC

2. DNA is double stranded; each nucleotide is paired with its complementary base. Adenine binds to thymine, and cytosine binds to guanine. Link the complementary nucleotide magnets to your original strand to make the complementary DNA strand. The first three nucleotides of the complementary strand have been shown:

d. Record the complementary sequence

3. To simulate the beginning of transcription, spread apart the two DNA strands.

4. Match and link complementary RNA nucleotide magnets to the DNA of the original strand. Remember that in RNA, thymine is replaced by uracil (U). The first three nucleotides of the growing mRNA strand are shown:

e. Record the mRNA sequence

5. Make the following DNA sequence, a portion of the sickle-cell anemia human beta hemoglobin gene, with your nucleotide magnets on the magnetic whiteboard. Remember to orient this strand of magnetic nucleotides with the bonding regions facing downward, as shown below.

TAC CAC GTA GAC TGA GGA CAC

6. DNA is double stranded; each nucleotide is paired with its complementary base. Adenine is bound to thymine, and cytosine is bound to guanine. Link the complementary nucleotide magnets to your original strand to make the complementary DNA strand.

f. Record the complementary sequence

7. To simulate the beginning of transcription, spread apart the two DNA strands.

8. Match and link complementary RNA nucleotide magnets to the DNA of the original strand. Remember that in RNA, thymine is replaced by uracil (U).

g. Record the mRNA sequence.

Questions

h. Compare the DNA sequences in the normal and sickle-cell anemia hemoglobin genes, and then make a claim about the structure of Terra's red blood cells.

i. After simulating transcription, identify any differences between the mRNA sequences in normal and sickle-cell hemoglobin. How did these differences arise?

Stop and Think

Examine the 3-D structure of normal the hemoglobin protein in Figure 2. The iron is the site of oxygen binding in the molecule. People suffering from sickle-cell disease sometimes become easily fatigued and may experience dizziness, especially at high altitudes. In your lab notebook, make a claim about the structure of sickle-shaped red blood cells that may explain these symptoms.

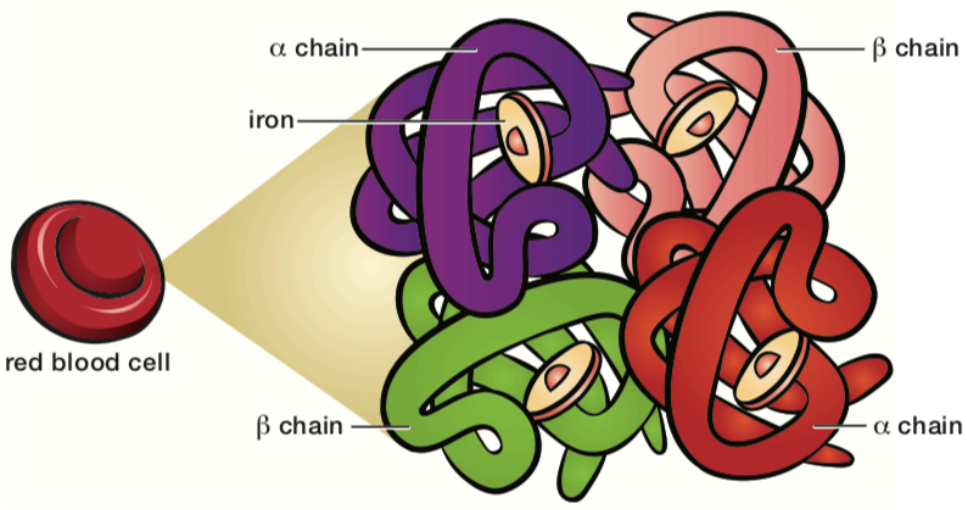


Figure 2. Normal hemoglobin protein

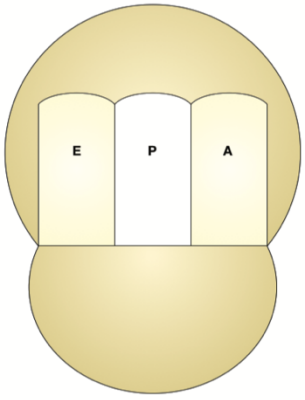
Translating Normal Hemoglobin Protein Procedure

Codons are groups of three mRNA bases. Each codon signifies a specific amino acid during protein synthesis.

1. During the Prelab investigation, your group used magnetic nucleotides to transcribe a portion of the human hemoglobin gene. Review your answer to Prelab Step 4 and use your nucleotide magnets to make the mRNA sequence for normal hemoglobin in the middle section of your magnetic whiteboard. The sequence represents the codons of the normal hemoglobin mRNA sequence.

Stop and Think

Composed of a large and a small subunit, the ribosome is the site of protein synthesis within the cytoplasm. Examine the three sites of the ribosome transparency (A site, P site, and E site), and then answer questions j through l.



j. Which ribosomal domain likely functions as the **Acceptor** for a growing protein?

k. Which ribosomal domain likely functions to link amino acids in the growing protein chain via **Peptide** bonds?

l. Which ribosomal domain likely functions as the **Exit** site?

The first step of translating a mRNA sequence into a protein is **initiation**. Follow these steps to model the process of initiation.

2. The codons do not start at a random site; the first mRNA codon is always AUG. Locate the AUG codon, and then place the P site of the ribosome transparency over this "Start" codon. Refer to the example in Figure 3.

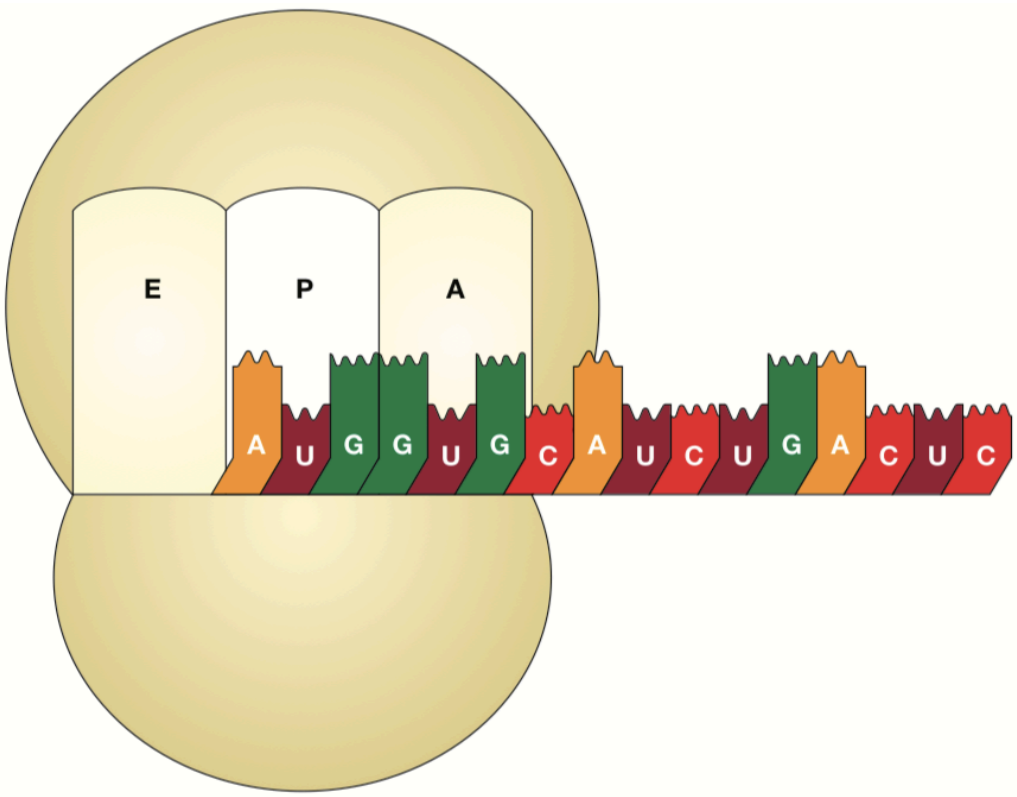


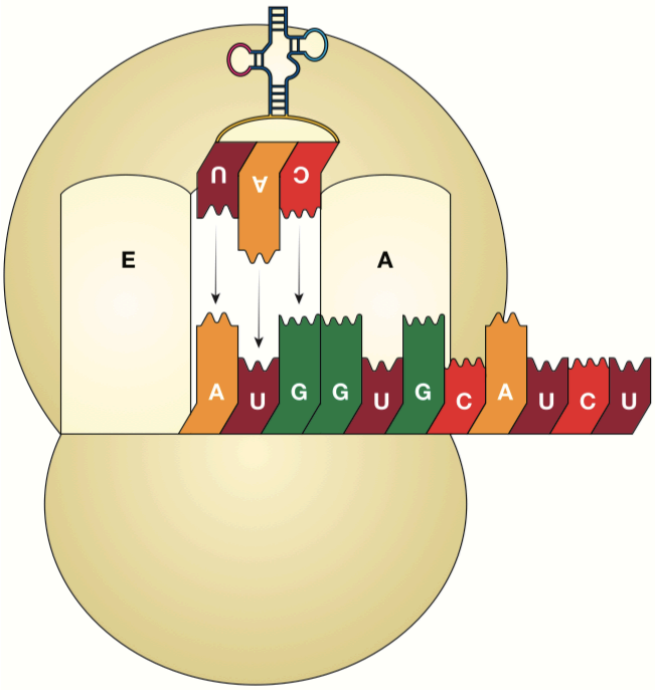
Figure 3. Initiation of transcription

3. The order of the nucleotides in mRNA determines the specific order of amino acids incorporated into the growing protein chain. Use the "mRNA Codons for Amino Acids" chart found on page 9 to determine the amino acid that matches the AUG codon.

Note: *The base-pair combinations for each of the 20 essential amino acids are followed by the name of the amino acid and (in parentheses) its three-letter and one-letter abbreviations*.

m. Record the one letter abbreviation of the amino acid that matches the AUG start codon here.

4. Using RNA base-pairing rules (A pairs with U, and C pairs with G) locate the tRNA magnet containing the complementary RNA sequence to the AUG start codon. Refer to the example that follows.



Stop and Think

The three nucleotides located at the bottom of the tRNA molecule are known as an "anticodon." Examine the relationship between the AUG start codon found on your group's nucleotide magnet sequence and the tRNA associated with this start codon.

n. Why do you suppose they call this region of the tRNA an "anticodon?"

o. Examine the structure of the magnetic mRNA codon and its complementary anticodon. What are the base- pairing rules between mRNA codons and tRNA anticodons?

5. During protein synthesis, tRNA molecules carry specific amino acids from the cytoplasm to their matching mRNA codon. These amino acids will then be "transferred" to the growing protein chain (represented by a colored pipe cleaner) as it elongates.

p. Record the color of the normal hemoglobin pipe cleaner.

6. The plastic bag with beads serves as the cytoplasmic reservoir of unbound amino acids. Carry one pipe cleaner and the tRNA magnet corresponding to the AUG start codon to this amino acid reservoir.

7. Remember, the purpose of the transfer RNA (tRNA) is to move the correct amino acid to the growing protein chain. Use the codon chart to locate the bead inscribed with the one-letter abbreviation of the amino acid that corresponds to the AUG start codon, and then thread the amino acid onto the pipe cleaner to simulate the placement of the first amino acid in the growing hemoglobin protein chain.

8. Place this tRNA/amino acid complex on the P site of the large ribosomal subunit. It may help to bend the bottom 4 cm of the pipe cleaner to form an "L" to achieve a better fit when using the model (Figure 4).

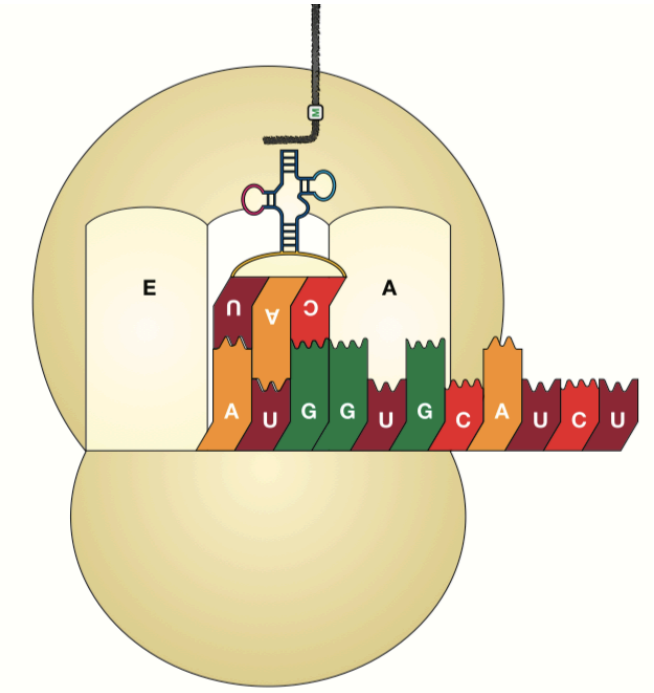


Figure 4. Initiation

The next step of translating a mRNA sequence into a protein is **elongation**. Follow these steps to model the process of **elongation**.

9. The mRNA codon located at the current A site (attachment site) of the ribosome specifies the next amino acid to be added to the growing chain. Use the codon chart to determine the amino acid that corresponds to the codon at the A site.

10. Locate the tRNA magnet that corresponds to the mRNA codon at the current A site of the ribosome, and then carry this tRNA to the amino acid reservoir.

11. Retrieve the correct amino acid. Remember, the mRNA codon occupying the A site of the ribosome, *not the tRNA anti-codon*, specifies the correct amino acid.

12. Place the appropriate tRNA magnet and its corresponding amino acid on the current A site of the ribosome (Figure 5).

13. Move the pipe cleaner representing the growing amino acid chain from the P site to the A site.

14. Transfer the amino acid corresponding to the tRNA found at the A site to the growing amino acid chain to simulate a peptide bond linking the amino acids together (Figure 5).

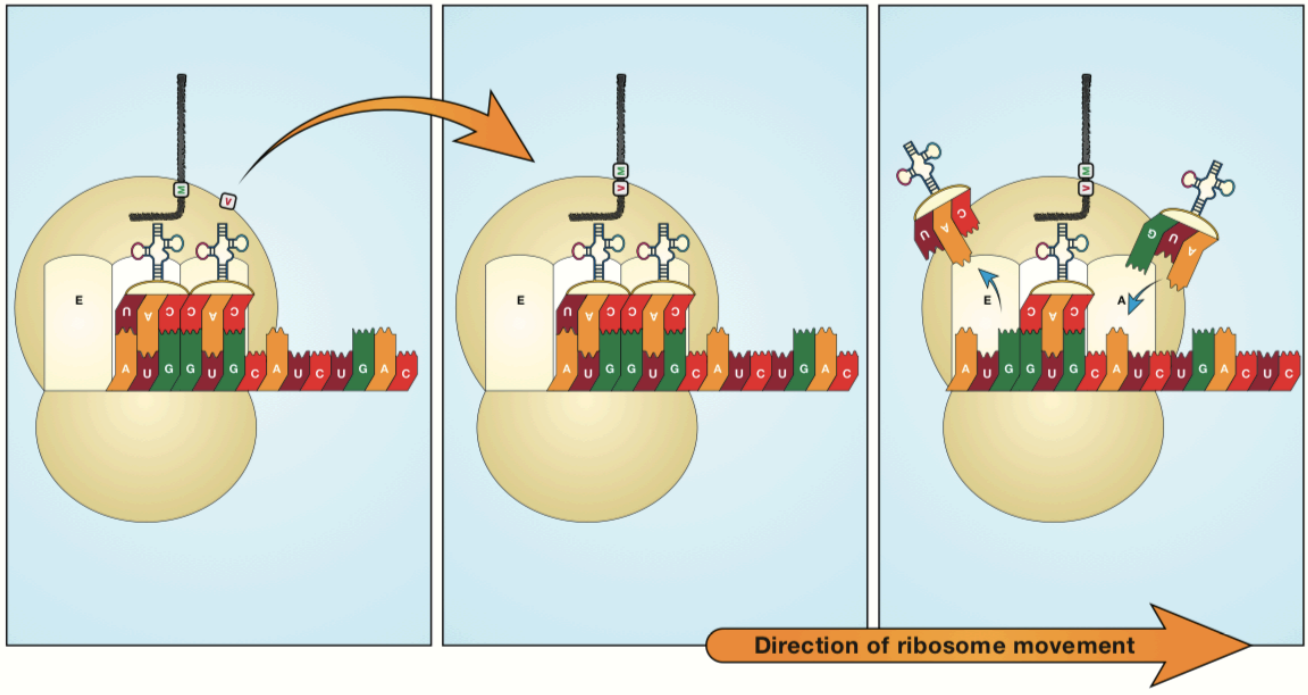


Figure 5. Elongation of translation

15. Use one hand to hold the tRNA magnets in place, and then advance the ribosome transparency a distance of one codon to the right (moving in a 5' to 3' direction on the mRNA sequence). The current amino acid chain should reside on the tRNA found in the P site.

16. The transfer RNA that carried the first amino acid in the sequence (methionine, or M) is now released from the E site and may be reused (Figure 5). Simulate this event by removing the magnetic tRNA from the E site.

17. The next tRNA may now move into the now-vacant A site position, carrying with it the appropriate amino acid that corresponds with the A site codon (Figure 5). Repeat steps of protein elongation using the appropriate tRNA magnets and amino acid beads. **Important:** *Remember to advance the ribosome transparency a distance of one codon after transferring each amino acid to the growing protein chain*.

The final step of translating a mRNA sequence into a protein is **termination**. Follow these steps to model the process of **termination**.

18. Elongation of the protein chain is terminated when a stop codon moves into the A site of the ribosome complex. A stop codon *does* have a corresponding tRNA, it *does not* specify an amino acid.

19. A protein called a release factor now binds to the stop codon located at the A site. To simulate this event, place the release factor magnet over the A site.

20. When the release factor occupies the A site, the completed protein (amino acid chain) may be released. Separate the newly translated portion of the hemoglobin protein chain (pipe cleaner containing a sequence of beads that represent a chain of amino acids) from the tRNA located at the P site and remove the ribosome transparency from the mRNA strand.

q. Record the one-letter abbreviations for the amino acid sequence of the normal hemoglobin.

21. Place this translated protein chain aside for analysis in Investigation 2.

<u>Translating Sickle-Cell Hemoglobin Protein Procedure</u>

22. Reference your answer for Step 8 of the Transcription investigation. Make this mRNA sequence for sickle-cell hemoglobin with your nucleotide magnets on the middle section of the magnetic whiteboard.

23. Using a different colored pipe cleaner, repeat procedure Step 3 through Step 20 to translate the mRNA sequence for sickle-cell hemoglobin.

r. Record the color of the sickle-cell pipe cleaner

24. Separate the newly translated sickle-cell hemoglobin protein chain from the tRNA located at the P-site.

s. Record the one-letter abbreviations for the amino acid sequence of the sickle-cell hemoglobin

25. Place this translated sickle-cell hemoglobin protein next to the normal hemoglobin protein for analysis in Investigation 2.

26. Clean up according to your instructor's directions.

Modeling Sickle-cell

1. Examine the models you created with pipe cleaners and beads representing a portion of the amino acid sequence for normal and sickle- shaped hemoglobin.

t. Use the one-letter amino acid abbreviations found on each pipe cleaner hemoglobin model to complete the following chart.

u. In the chart, circle any differences in amino acid sequence between both types of hemoglobin.

Type of Hemoglobin	1st amino acid	2nd amino acid	3rd amino acid	4th amino acid	5th amino acid	6th amino acid	7th amino acid
Normal							
Sickle-cell							

2. Divide your portion of clay into two equal pieces.

3. Further divide each piece of clay into 10 spheres of equal size, creating 20 identical spheres.

4. Hemoglobin S (sickle-cell hemoglobin) molecules tend to clump together, leading to the formation of sickle-shaped red blood cells that are rigid and sticky. This physiological deformation explains the tendency of sickle-shaped red blood cells to clog small blood vessels such as capillaries. Roll the 10 of the clay spheres representing normal red blood cells in flour or cornstarch to limit "stickiness." The other 10 clay portions should not be rolled in flour or cornstarch.

5. Hemoglobin, the oxygen-transport protein in red blood cells, can make up about one-third of a red blood cell's total content. Humans who inherit two normal beta hemoglobin genes (one from each parent) will produce red blood cells containing normal hemoglobin. Model normal red blood cells flowing through blood vessels into narrow capillaries, as follows:

- The spherical clay pieces (rolled in flour) represent red blood cells containing normal hemoglobin.
- The funnel represents a small blood vessel of the human body.
- The movement of the clay pieces through the funnel represents red blood cells flowing through a small blood vessel.

Place the 10 normal, spherical red blood cells into the funnel representing the blood vessel.
v. Record your observations

6. Humans who inherit two sickle-cell hemoglobin genes (one from each parent) will produce red blood cells that, under some conditions, will become sickle shaped. To simulate sickle-shaped red blood cells, roll each of 10 clay spheres into a crescent shape that is roughly twice as long, and half as wide, as the "normal" hemoglobin spheres modeled in Step 4. Model sickle-shaped red blood cells flowing through blood vessels into narrow capillaries, as follows:

- The crescent-shaped clay pieces represent red blood cells containing sickled hemoglobin.
- The funnel represents a small blood vessel of the human body.
- The movement of the clay pieces through the funnel represents red blood cells flowing through a small blood vessel.

Place the 10 sickle-shaped red blood cells into the funnel representing the blood vessel.
w. Record your observations

7. A person with sickle-cell trait inherits one normal beta hemoglobin gene and one sickle-cell gene. Assume that each gene is translated, *producing both normal and sickle-cell hemoglobin*.
x. Model the flow of red blood cells in this individual, and then record your observations.

8. Clean up according to your instructor's directions.

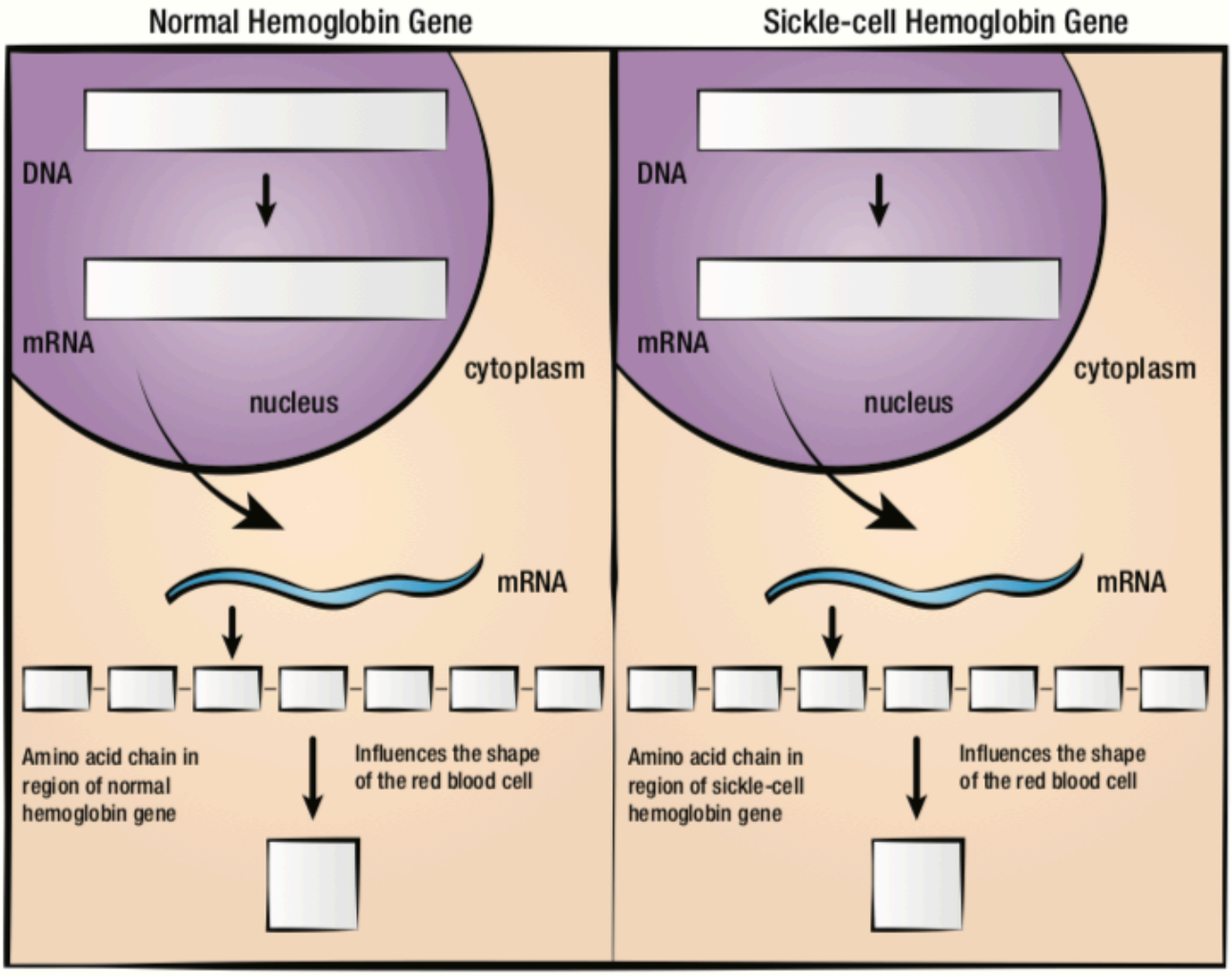
mRNA Codons for Amino Acids

First Base	Second Base: U	Second Base: C	Second Base: A	Second Base: G	Third Base
U	**UUU** Phenylalanine (Phe, F)	**UCU** Serine (Ser, S)	**UAU** Tyrosine (Tyr, Y)	**UGU** Cysteine (Cys, C)	U
	UUC Phenylalanine (Phe, F)	**UCC** Serine (Ser, S)	**UAC** Tyrosine (Tyr, Y)	**UGC** Cysteine (Cys, C)	C
	UUA Leucine (Leu, L)	**UCA** Serine (Ser, S)	**UAA** Stop	**UGA** Stop	A
	UUG Leucine (Leu, L)	**UCG** Serine (Ser, S)	**UAG** Stop	**UGG** Tryptophan (Trp, W)	G
C	**CUU** Leucine (Leu, L)	**CCU** Proline (Pro, P)	**CAU** Histidine (His, H)	**CGU** Arginine (Arg, R)	U
	CUC Leucine (Leu, L)	**CCC** Proline (Pro, P)	**CAC** Histidine (His, H)	**CGC** Arginine (Arg, R)	C
	CUA Leucine (Leu, L)	**CCA** Proline (Pro, P)	**CAA** Glutamine (Gln, Q)	**CGA** Arginine (Arg, R)	A
	CUG Leucine (Leu, L)	**CCG** Proline (Pro, P)	**CAG** Glutamine (Gln, Q)	**CGG** Arginine (Arg, R)	G
A	**AUU** Isoleucine (Ile, I)	**ACU** Threonine (Thr, T)	**AAU** Asparagine (Asn, N)	**AGU** Serine (Ser, S)	U
	AUC Isoleucine (Ile, I)	**ACC** Threonine (Thr, T)	**AAC** Asparagine (Asn, N)	**AGC** Serine (Ser, S)	C
	AUA Isoleucine (Ile, I)	**ACA** Threonine (Thr, T)	**AAA** Lysine (Lys, K)	**AGA** Arginine (Arg, R)	A
	AUG Start Methionine (Met, M)	**ACG** Threonine (Thr, T)	**AAG** Lysine (Lys, K)	**AGG** Arginine (Arg, R)	G
G	**GUU** Valine (Val, V)	**GCU** Alanine (Ala, A)	**GAU** Aspartic Acid (Asp, D)	**GGU** Glycine (Gly, G)	U
	GUC Valine (Val, V)	**GCC** Alanine (Ala, A)	**GAC** Aspartic Acid (Asp, D)	**GGC** Glycine (Gly, G)	C
	GUA Valine (Val, V)	**GCA** Alanine (Ala, A)	**GAA** Glutamic Acid (Glu, E)	**GGA** Glycine (Gly, G)	A
	GUG Valine (Val, V)	**GCG** Alanine (Ala, A)	**GAG** Glutamic Acid (Glu, E)	**GGG** Glycine (Gly, G)	G

Conclusion/Analysis

1. The following model represents the process of transcription and translation in a portion of the normal and the sickle-cell hemoglobin genes.

a. Copy the following model neatly, using color. Be sure to include the Title of each image. (6 points)
b. Fill in the blanks using information such as specific DNA and mRNA nucleotide sequences collected during the Prelab. (4 points)
c. The arrows on the model represent processes (transcription and translation). Label each process. (4 points)
d. Revise the model by drawing and labeling tRNAs, amino acids, and ribosomes. (6 points)
e. Draw the shape of the resulting red blood cell represented by your clay model after each gene has been translated into normal or sickle-cell hemoglobin. (2 points)



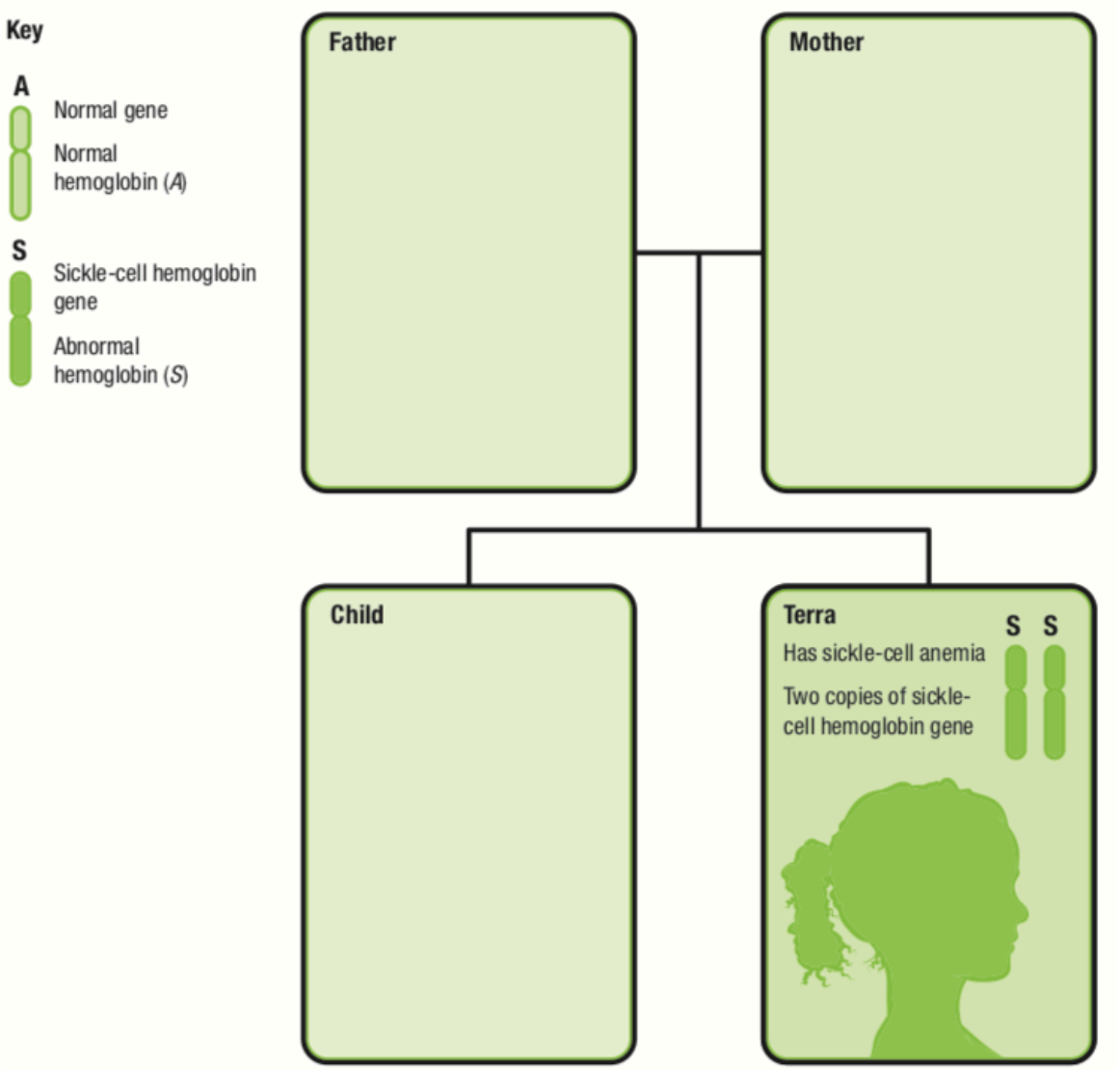
2. The results of medical tests indicated that baby Terra was likely to develop a red blood cell disease known as sickle-cell anemia. Using the model created in Conclusion/Analysis Question #1, explain the genetic cause of sickle-cell disease to Terra's parents. Write a concise description of the components and processes depicted in the model. You will be asked to communicate your model and explanation to the class. Be sure to explain the following words in context: mutation; hemoglobin gene; mRNA codon; transcribed; amino acid; polypeptide; structural, sickle-shape. (7 points)

3. Combine your observations of the clay models and prior knowledge of the structure and function of blood cells in humans to describe how the sickle-cell mutation may cause symptoms of the sickle-cell disease. (2 points)

Mendelian Genetics and Sickle-Cell Disease

Offspring inherit one copy of a gene from each parent. Individuals with two copies of the normal hemoglobin gene (genotype *AA*) do not have sickle-cell anemia. Individuals with two copies of the sickle-cell hemoglobin gene (genotype *SS*) will develop sickle-cell anemia. Heterozygous individuals (genotype *AS*) have one copy of the normal gene, and one copy of the sickle-cell hemoglobin gene. Genotype *AS* individuals rarely suffer from complications associated with sickle-cell disease (breakdown of red blood cells, buildup of sickled cells in the spleen, etc.).

4. Use the family tree template below to construct a model that explains how Terra inherited sickle-cell disease, even though her parents do not have the disease. Label each family member in your model, their likely genotype (*AA*, *AS*, or *SS*), their disease status (has sickle-cell anemia, is a carrier, or is normal), and the number of each gene (normal or sickle) possessed. List all likely possibilities for individuals when multiple genotypes are possible. Baby Terra's data has been completed for you as an example.



a. Copy the model neatly. Be sure to include a Title and Terra's COMPLETE information. (5 points)
b. For each family member, draw a silhouette as displayed in Terra's box. (3 points)
c. For each family member, identify the likely genotype. (6 points)
d. For each family member, identify the number of each gene (normal or sickle) possessed. (6 points)
e. For each family member, identify the disease status (has sickle-cell anemia, is a carrier, or is normal). (3 points)