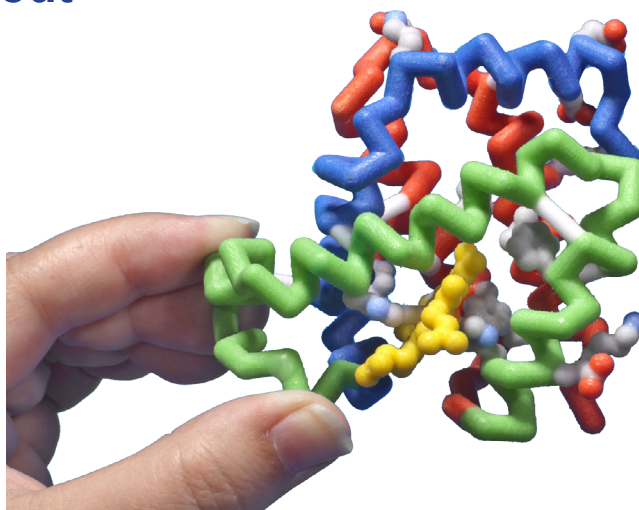


Translation Activity Guide

Student Handout



β-Globin

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. In the process of protein synthesis there are two important types of nucleic acids: DNA and RNA (ribonucleic acid). Messenger RNA (mRNA) has the information for arranging the amino acids in the correct order to make a functional protein.

The key to deciphering DNA is called a **triplet code**, in which the sequence of three adjacent DNA nitrogen bases codes for a specific amino acid. Translation of the mRNA occurs in groups of three nitrogenous bases called codons. The order in which the amino acids are put together depends on the sequence of bases in the mRNA. Proteins can consist of as few as 100 or as many as thousands of amino acids.

1. 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 in transcription. Starting from the 5' end of the mRNA **at the start codon**, every three bases determines a particular amino acid.

Translation Continued

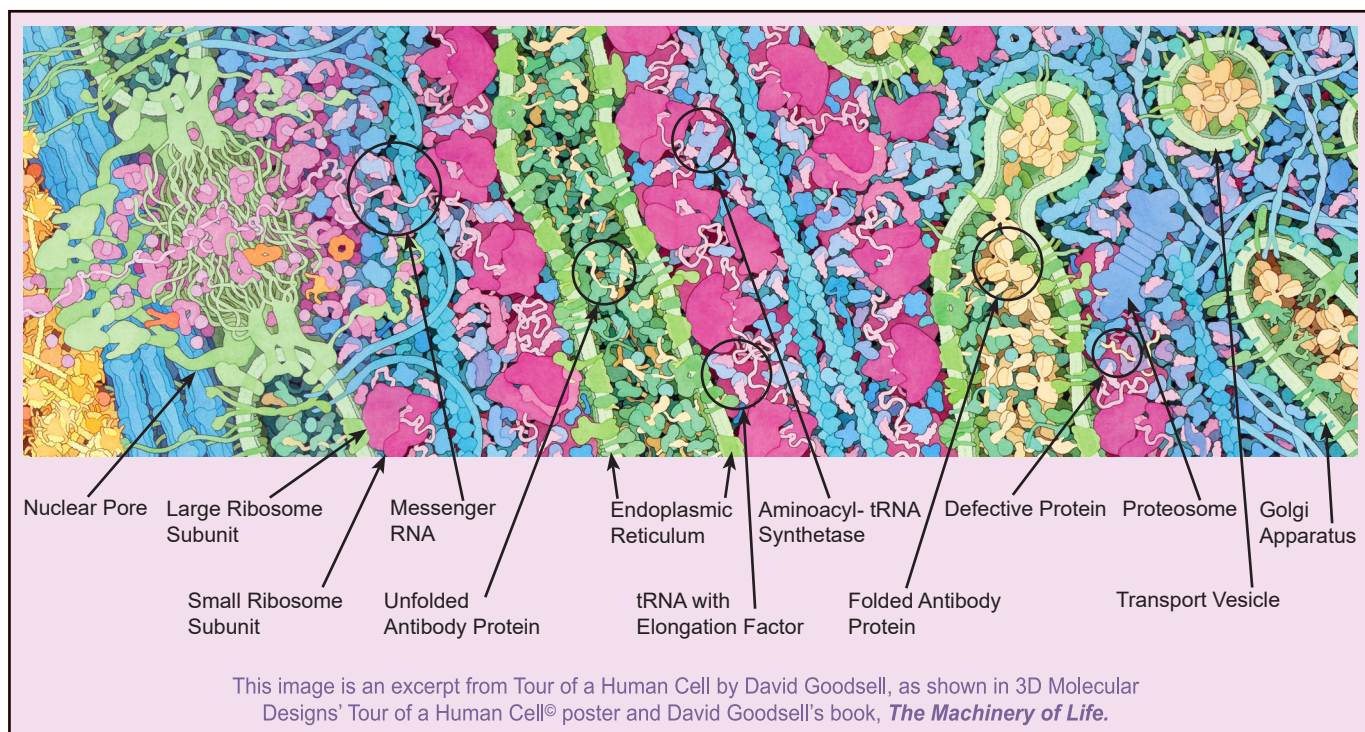
Use The Genetic Codon Chart® included in the kit or the one at the end of this student handout to determine the identity of the correct amino acid for each codon in your mRNA strand.

2. Identify the three-letter and one-letter abbreviation for each amino acid in the table below:

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

The process of deciphering DNA to produce a protein requires two major stages: (1) transcription and (2) translation. Transcription is the process in which DNA is used as a template to produce a single-stranded RNA molecule. Translation is the process in which the DNA code, now contained in the single-stranded RNA, is deciphered into a sequence of linked amino acids that become a protein.

In eukaryotic cells, DNA is found in the nucleus, chloroplasts, and mitochondria, and cannot leave these structures. As a result, transcription occurs inside these organelles in eukaryotic cells. A eukaryote is an organism composed of cells which contain a nucleus and other membrane-bound organelles. An organelle is a differentiated structure within a cell, such as a mitochondrion, vacuole, or chloroplast that performs a specific function (examples include the nucleus, mitochondria or Golgi apparatus). The image below will help you orient the mRNA exiting the nucleus to be translated into protein in the cytoplasm.



Translation Continued

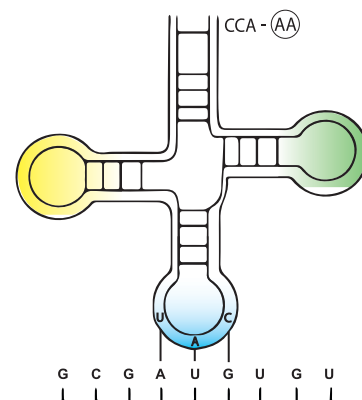
Although the Flow of Genetic Information Kit® model does not illustrate the entire initiation process, the initiation stage of translation brings together mRNA and a second type of RNA called transfer RNA (tRNA), with two subunits of a ribosome.

Proteins are made by ribosomes (workbenches) that are outside of the nucleus in the cytoplasm, in a process called protein synthesis. Synthesis refers to linking together individual monomer subunits (nucleotides or amino acids) into a larger polymer (mRNA or protein).

How does the information carried by DNA get to the ribosomes? The code has been transcribed from the DNA to RNA. RNA must leave the nucleus and carry the code to the ribosome for proteins to be synthesized. The RNA carrying the code is called messenger RNA (mRNA). It is one of three types of RNA (mRNA, tRNA, and rRNA) that play major roles in protein synthesis. Note that the mRNA code is not identical to the DNA code.

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 forms complementary base pairs with the codon of the mRNA. The other functional part of tRNA attaches to a specific amino acid.

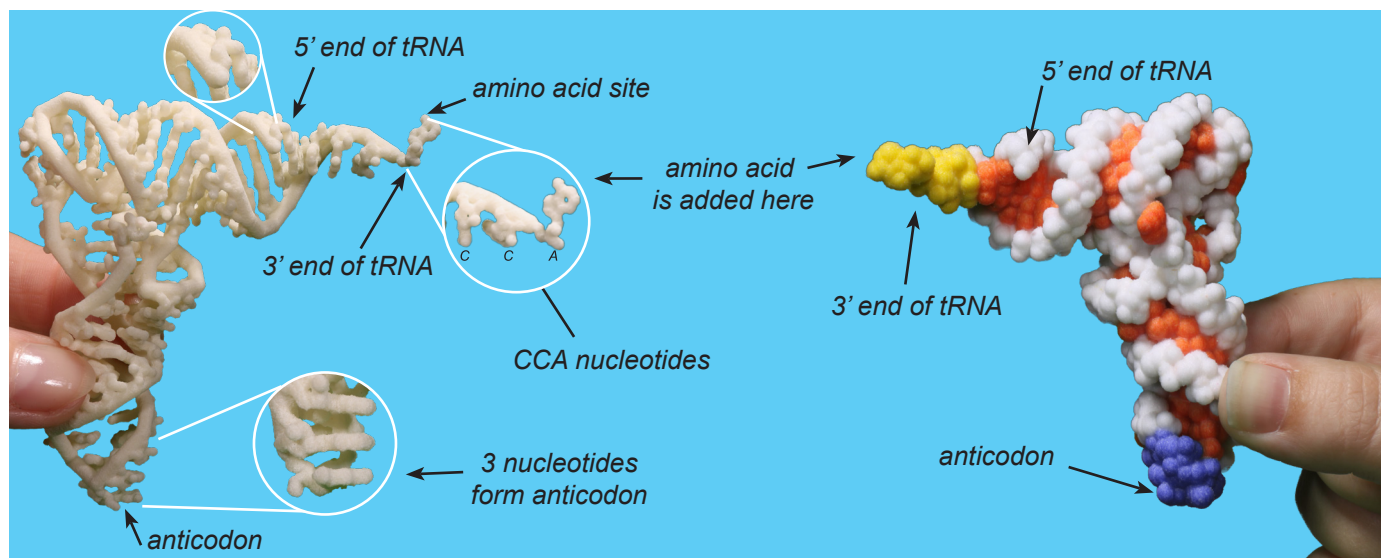
While tRNA is typically shown in textbooks as a cloverleaf design, tRNA actually folds into the triangular 3-D structure that is necessary for it to bind to the small ribosomal subunit. Compare this image to the accurate images of tRNA shown on the next page.



Typical textbook model

Translation Continued

tRNA Models



Left Nylon Backbone Model

- The backbone of the nucleotides is a solid strand.
- Every tRNA has the same CCA sequence (cytosine, cytosine, adenine) at the 3' end of the structure. Specific amino acids – based on the mRNA sequence – are added to the 3' adenine (the terminal end of tRNA) during translation. Note the detail of the CCA nucleotide shapes shown in the top magnified white circle.
- The bottom magnified white circle shows the detail of the nucleotides that form the anticodon. These nucleotides vary in tRNAs, depending on the specific mRNA sequence being translated.
- The shapes of the nucleotides and complimentary base pairing are visible in this model.

Right Plaster Spacefill Model

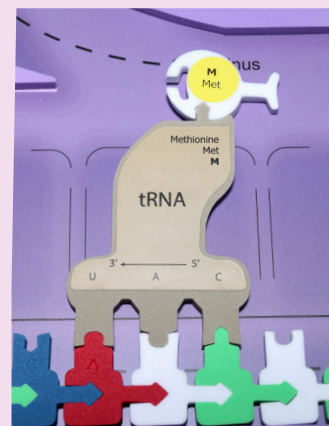
- The atoms of the backbone are shown in white.
- The CCA sequence at the 3' end of the tRNA – where the amino acids attach – is shown in yellow.
- The anticodon is shown in blue.
- The atoms of the nitrogen bases are red.
- Note: When tRNA folds into its triangular shape, the 5' end is near the 3' end. The 5' end is easier to see in this model because of the different colors.

In this handout you will see four different models of tRNA including the two in the photo above, the traditional schematic textbook image on the previous page and the photo of the foam model on the next page that you will use in the activities. None of the models is exactly like a real tRNA. Each representation is useful in various ways. By noting the strengths and weaknesses of the models, you can gain a more complete understanding of the structure and function of tRNA. For more information visit 3dmoleculardesigns.com/Teacher-Resources/Transfer-RNA-Mini-Models.htm.

The models in the above photo are based on crystallographic data from the RCSB Protein Data Bank (4TNA.pdb) and produced by 3D Molecular Designs. The model on the left was built out of nylon using a large SLS laser-based machine. The model on the right was built out of plaster on a large ZCorp 3-D printer.

Translation Continued

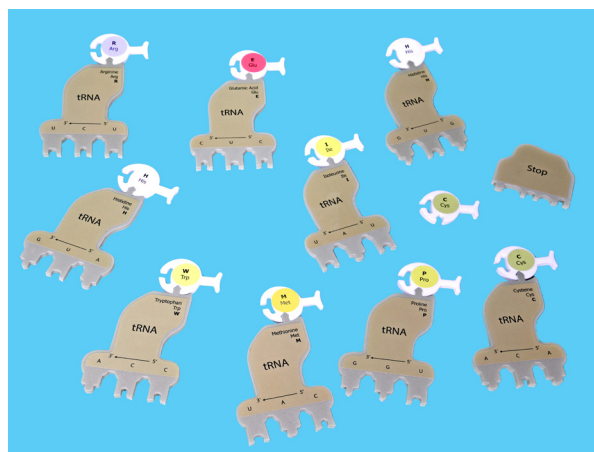
- On the image to the right, label the amino acid that is joined to the 3' end of the tRNA.
- Label the anticodon of the tRNA.
- Label the translation start codon (AUG) of the mRNA.
- What amino acid is associated with the tRNA that will bind to the mRNA start codon AUG?



- Record the tRNA anticodons in the table below:

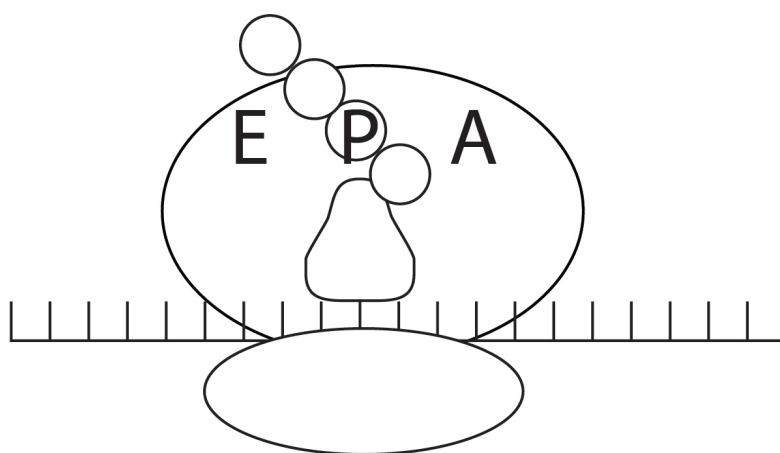
	5'											3'
mRNA Codons	AUG	UGU	GAG	AUA	CAU	UGG	CCA	AGA	CAC	UGU	UAG	
tRNA anticodons	3'←											→5'
Amino Acid												

Add the appropriate amino acids to each of the tRNAs identified in the table above. The amino acids have different colors representing their various chemical properties. Green represents cysteines, which may form disulfide bonds with each other during protein folding. Red indicates acidic amino acids while blue indicates basic amino acids. Yellow amino acids are hydrophobic. White amino acids are hydrophilic. For more information on the properties of amino acids, see page 2 of the Amino Acid Starter Kit® Student Handout 1 found at <http://www.3dmoleculardesigns.com/Teacher-Resources/Amino-Acid-Starter-Kit.htm>.



Translation Continued

8. Draw your own illustration of the model in the space below. Label the **anticodon**, the **ribosomal subunits**, the **tRNA**, the **amino acid**, the **mRNA**, and the **growing polypeptide chain**.



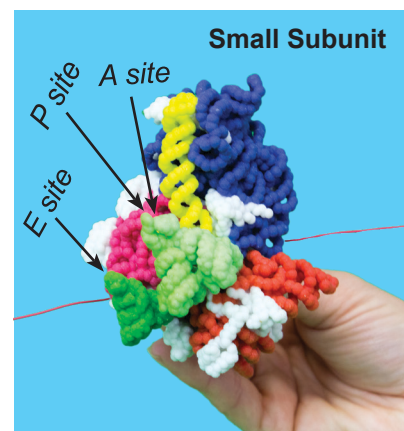
While the tRNA-amino acid complex is being formed in the cytoplasm, mRNA moves towards the ribosome. Ribosomal subunits are made in the nucleolus of eukaryotic cells. The resulting ribosomal subunits are exported via nuclear pores to the cytoplasm. Approximately one-third of the mass of a ribosome is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

The ribosome consists of two parts, the large and the small subunits which are unattached when not in use.

Translation Continued

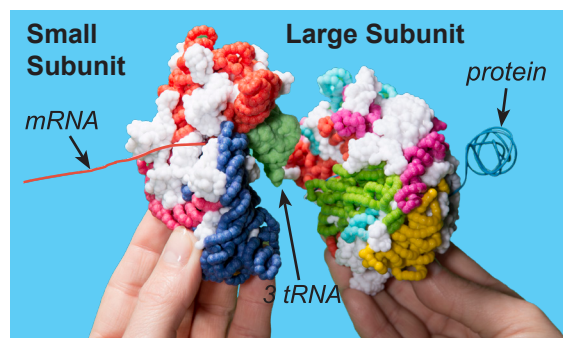
Top photo of small ribosome subunit

- Note the three tRNA on the small ribosome subunit, which are labeled A, P and E sites.
- The mRNA is represented by the red telephone wire in the photo. It passes through a channel in the small subunit to coordinate with the anticodons (nucleotide bases) of the tRNA.



Bottom photo of large and small subunits together

- After the tRNA and mRNA bind to the small subunit, it attaches to the large subunit.
- Once the small and large subunits are fully attached (not shown), the growing polypeptide (amino acids) chain passes through a channel in the large subunit.
- As the polypeptide chain emerges, it spontaneously folds into its 3-dimensional shape following basic principles of chemistry and physics.
- The chemical properties of the amino acids – whether they are hydrophobic, hydrophilic or charged – determine how the polypeptide (protein) will fold into its functional shape.
- The blue telephone wire represents the protein emerging from the large subunit.



These models of the small and large ribosomal subunits are based on crystallography data deposited in the RSCB Protein Data Bank: <http://www.rcsb.org/pdb/home/home.do>. The small subunit is based on 1GIX.pdb and the large subunit is based on 1FFK.pdb. They are produced by 3D Molecular Designs on a large ZCorp 3-D printer. For more information visit <http://www.3dmoleculardesigns.com/Teacher-Resources/Large-and-Small-Ribosome-Mini-Models.htm>.

Color Key

Small Subunit

Protein	○
tRNA A site	●
tRNA P site	●
tRNA E site	●
RNA domains	● ● ● ● ● ● ● ●

Large Subunit

Protein	○
Catalytic residues	●
RNA domains	● ● ● ● ● ● ● ●

9. Which end of the mRNA strand is attached to the small ribosomal subunit?

Translation Continued

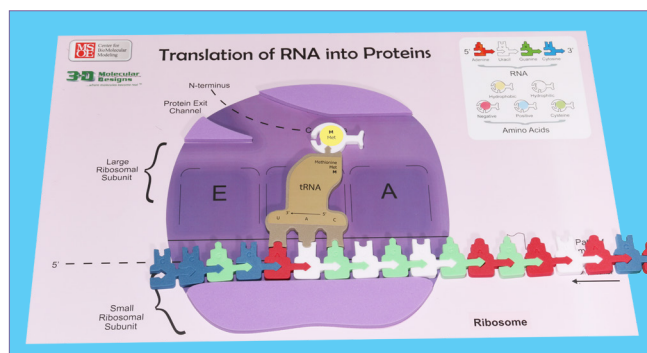
Translation: Initiation

In the next part of this activity you will model the initiation, elongation and termination processes of translation. To initiate protein synthesis, the AUG start codon of the mRNA is bound to the P site of the small ribosomal subunit. The initiation tRNA - charged with Met - base pairs with the AUG codon, and the large ribosomal subunit joins the small subunit to form a functional ribosome. 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).

As you begin, don't forget to look for the start codon. Refer to the photo on the right to ensure the mRNA is in the proper orientation in your ribosome.

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

10. 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.



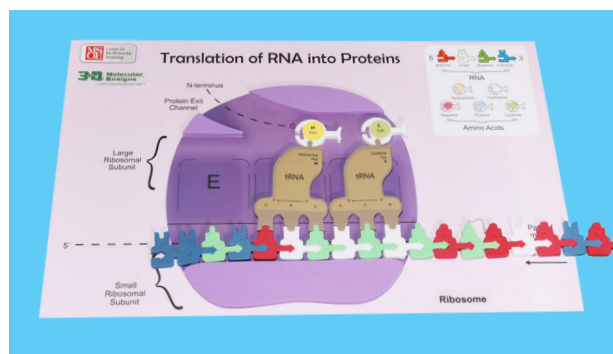
Translation: Elongation

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

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

An rRNA found in the large ribosomal 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.

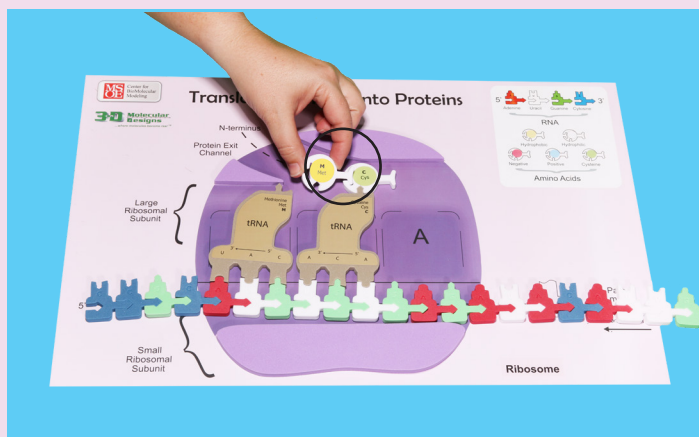


Translation Continued

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

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.

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



13. What characteristic allows tRNA to separate from mRNA at the ribosome?

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

15. Which mRNA codon is now located in the A site? _____

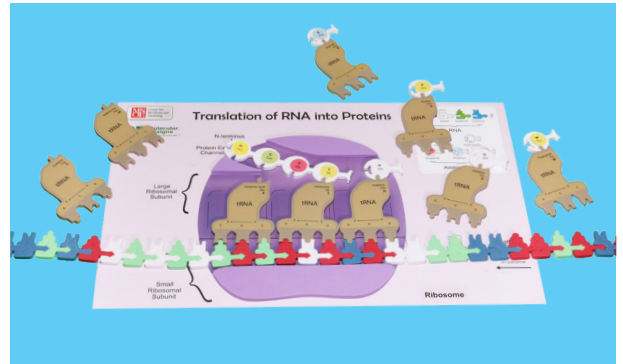
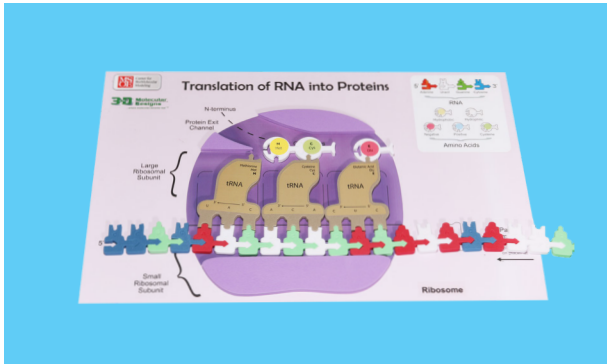
With the A site now available for another tRNA-amino acid complex, these steps can continue. Remember that the growing polypeptide transfers from the P site to the A site. Demonstrate this process using all of your tRNA-amino acid complexes in the appropriate order.

The mRNA is translated in one direction from its 5' → 3' end. New protein is made in the N-terminal to C-terminal direction — meaning that new amino acids are joined to the C-terminal end of the growing protein.

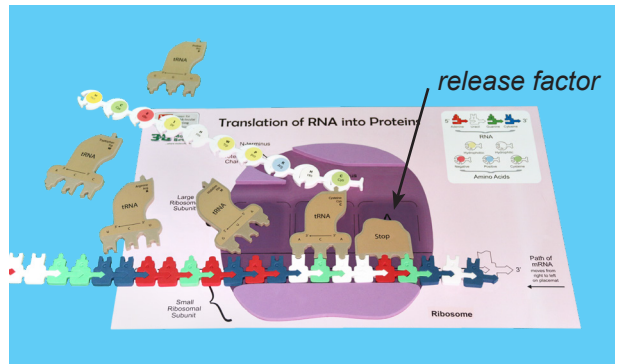
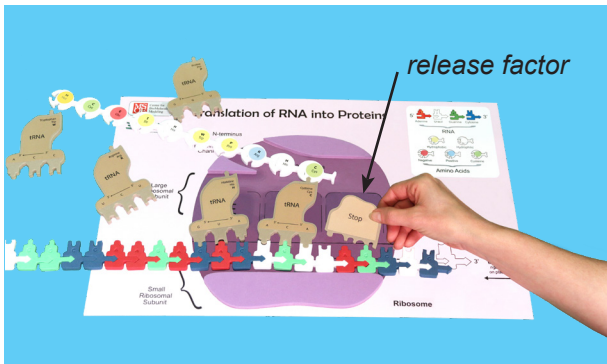
Translation Continued

Translation: Termination

This developing polypeptide will exit the ribosome through the opening in the large ribosomal subunit.



When the ribosome reaches the stop codon on the mRNA, the A site of the ribosome accepts a release factor.



16. Which tRNA need to be "recycled"?

17. Why did the tRNA for the second histidine NOT need to be recycled?

18. What occurs at the mRNA codon UAG?

Translation Continued

19. Using The Genetic Codon Chart® included in the kit or the one at the end of the handout, list the various stop codons.

20. What is the order of amino acids in your polypeptide?

Met									
M									

21. Compare the amino acid sequence of the polypeptide you created to the sequence predicted in question 7. How do your sequences compare?

22. 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

23. What will happen next to the polypeptide?

24. 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?

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

Translation Continued

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° C).

26. At this rate, how long would it take to make a protein such as actin which is 375 amino acids long?

27. 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.

The Genetic Codon Chart®

	U				C				A				G			
	UUU	UUC	UUA	UUG	UCU	UCC	UCA	UCG	UAU	UAC	UAA	UAG	UGU	UGC	UGA	UGG
U	Phe F	Phe F	Leu L	Leu L	Ser S	Ser S	Ser S	Ser S	Tyr Y	Tyr Y	Stop	Stop	Cys C	Cys C	Stop	Trp W
C	Leu L	Leu L	Leu L	Leu L	Pro P	Pro P	Pro P	Pro P	His H	His H	Gln Q	Gln Q	Arg R	Arg R	Arg R	Arg R
A	Ile I	Ile I	Ile I	Met M	Thr T	Thr T	Thr T	Thr T	Asn N	Asn N	Lys K	Lys K	Ser S	Ser S	Arg R	Arg R
G	Val V	Val V	Val V	Val V	Ala A	Ala A	Ala A	Ala A	Asp D	Asp D	Glu E	Glu E	Gly G	Gly G	Gly G	Gly G

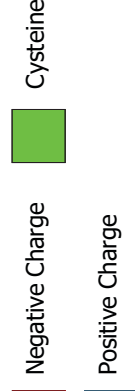
Amino Acid Properties



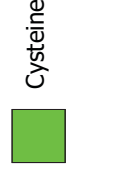
Translation Start Codon
Translation Stop Codon



Hydrophobic / Non-polar
Hydrophilic / Polar



Negative Charge
Positive Charge



Cysteine

