

## 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.

T G C I R R G H Q A D H C P S A M A T W M R L I  
 G G C C C U G U G G A U G C G C C C U C C U G C C C C U G C C C U G C C C U G G G A C C U G A C C C A G C C C G C A G

### Second Letter

		Second Letter					
		U	C	A	G		
First Letter	U	UUU → Phe UUC → Phe UUA → Leu UUG → Leu	UCU → Ser UCC → Ser UCA → Ser UCG → Ser	UAU → Tyr UAC → Tyr UAA → Stop UAG → Stop	UGU → Cys UGC → Cys UGA → Stop UGG → Trp	U C A G	<div style="border: 1px solid green; width: 10px; height: 10px; display: inline-block;"></div> translation start codon <div style="border: 1px solid red; width: 10px; height: 10px; display: inline-block;"></div> translation stop codon <div style="background-color: yellow; width: 10px; height: 10px; display: inline-block;"></div> hydrophobic amino acids <div style="background-color: white; width: 10px; height: 10px; display: inline-block;"></div> hydrophilic non-charged amino acids <div style="background-color: red; width: 10px; height: 10px; display: inline-block;"></div> - charged amino acids <div style="background-color: blue; width: 10px; height: 10px; display: inline-block;"></div> + charged amino acids <div style="background-color: lightgreen; width: 10px; height: 10px; display: inline-block;"></div> cysteine
	C	CUU → Leu CUC → Leu CUA → Leu CUG → Leu	CCU → Pro CCC → Pro CCA → Pro CCG → Pro	CAU → His CAC → His CAA → Gln CAG → Gln	CGU → Arg CGC → Arg CGA → Arg CGG → Arg	U C A G	
	A	AUU → Ile AUC → Ile AUA → Ile AUG → Met	ACU → Thr ACC → Thr ACA → Thr ACG → Thr	AAU → Asn AAC → Asn AAA → Lys AAG → Lys	AGU → Ser AGC → Ser AGA → Arg AGG → Arg	U C A G	
	G	GUU → Val GUC → Val GUA → Val GUG → Val	GCU → Ala GCC → Ala GCA → Ala GCG → Ala	GAU → Asp GAC → Asp GAA → Glu GAG → Glu	GGU → Gly GGC → Gly GGA → Gly GGG → Gly	U C A G	

## 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?

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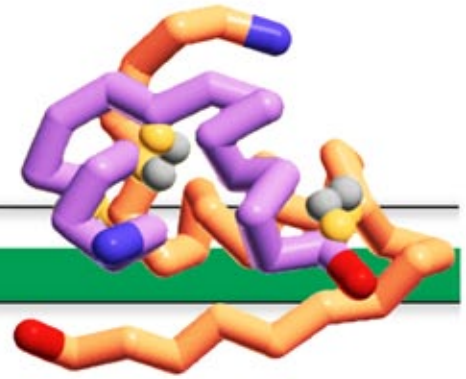


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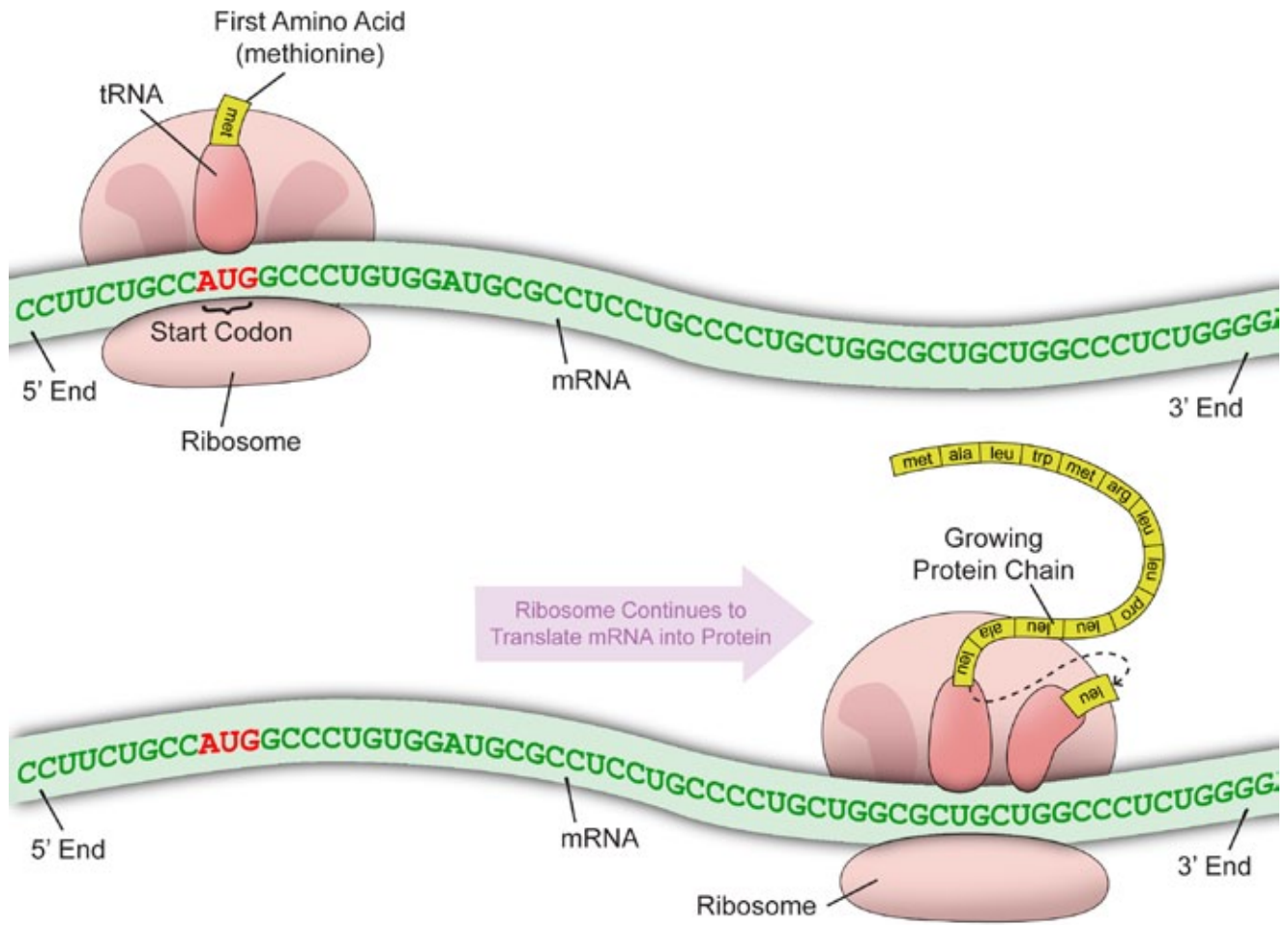






## Translating mRNA Into Protein

### 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?

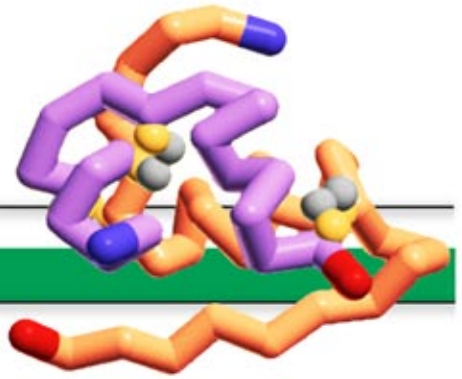
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b. How many amino acids are in the insulin protein?

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## 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

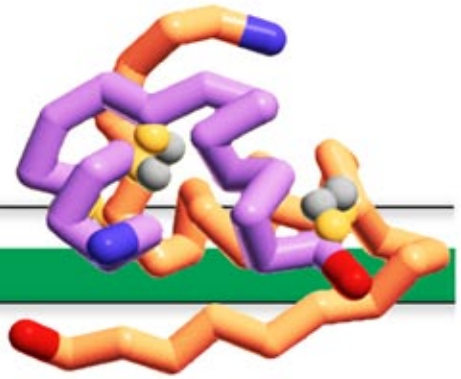
- a. Referring to the Standard Genetic Code table, *categorize* the chemical properties of each of the 24 amino acids that make up the ER Signal Peptide (hydrophobic, hydrophilic, positive charge, or negative charge). What is notable about the chemical properties of the amino acids that make up the ER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

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## 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?

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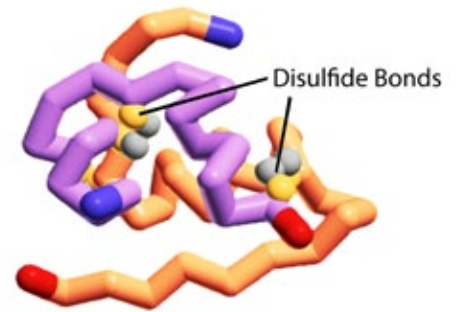
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As with many secreted proteins that must function in the harsh environment outside the cell, insulin is stabilized by two covalent disulfide bonds that join the B-chain to the A-chain. Each chain contributes one cysteine amino acid (Cys, C) to each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

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

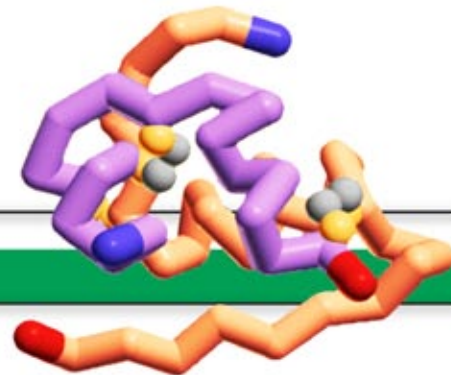
Cys19 of the B-chain forms a disulfide bond with Cys20 of the A-chain.

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.

T G C I R R G H Q A D H C P S A M A L W M R L L  
 I G C C C U G U G A U G C G C C C U G C C C C U G C C C C U G C C C C U G C C C C U G C C C C U G G G A C C U G A C C C A G C C G C A G



## 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.

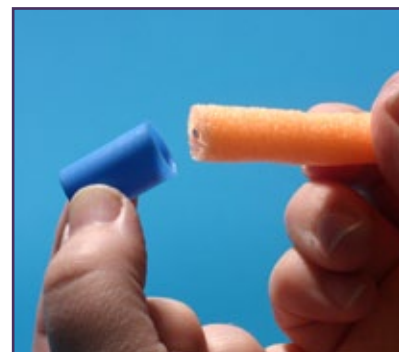
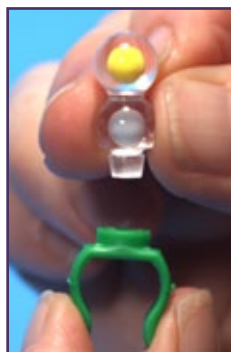
1. Gather all of the parts you need (see contents photo on page 2).

- 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

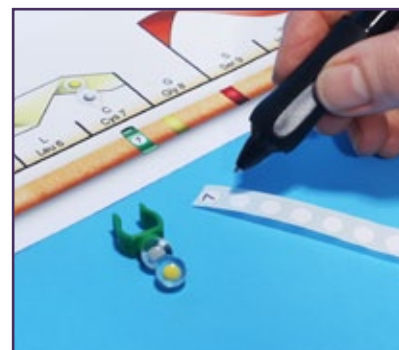
As you proceed with the directions (2) through (6) below you can work with the two chains at the same time or you can complete the B-chain (orange mini toober) and then repeat with the A-chain (purple mini toober).

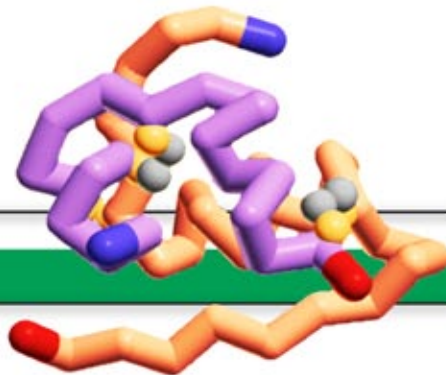
2. Insert each cysteine into a green plastic clip

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.



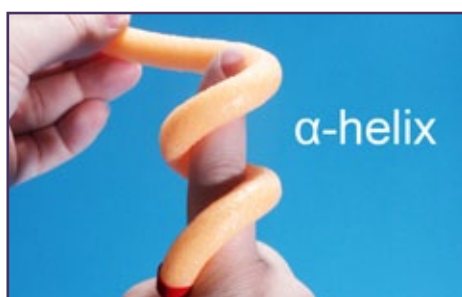
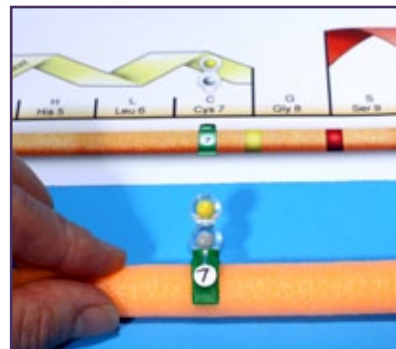
4. Using the map, locate the cysteine amino acids on each protein chain. Write the number of each of the six cysteines on the white dots and add these numbered dots to six plastic clips.





## Folding the Physical Model Of Insulin (continued)

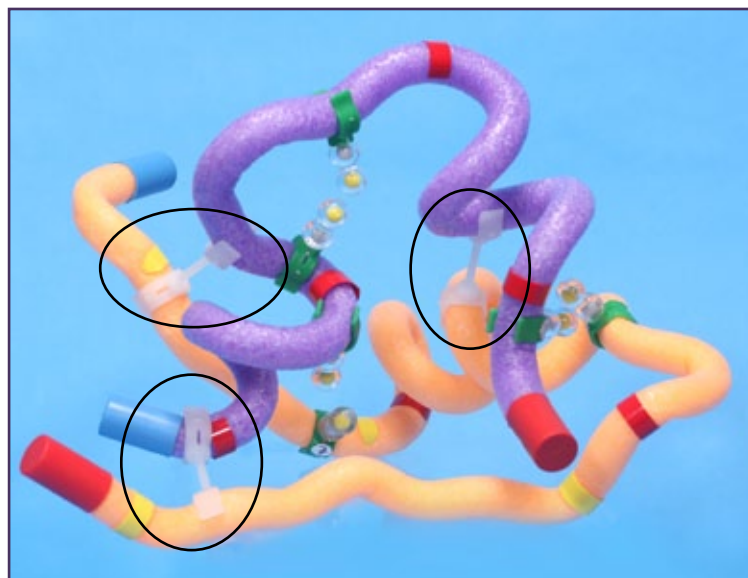
- Carefully align each mini toober with the corresponding chain on the Insulin Mini Toober Folding Map matching the end caps to the images of the end caps on the map. Add the appropriately numbered plastic clips to the mini toober. The plastic clips represent the alpha-carbon of each cysteine amino acid.
- Indicate where the  $\alpha$ -helicies are on each protein chain by placing the red plastic markers at the beginning and the end of each  $\alpha$ -helix. Indicate where the  $\beta$ -sheets are on each protein chain – by placing the yellow plastic markers on the mini-toober at the beginning and the end of each  $\beta$ -sheet shown on the map.



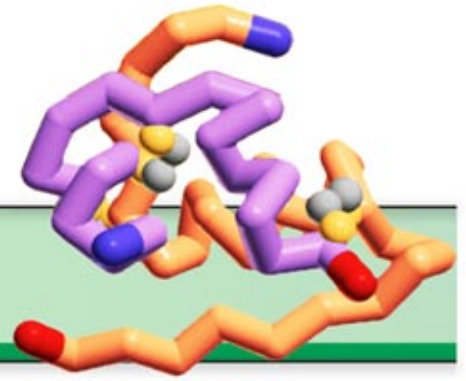
- Fold the mini toobers to create the  $\alpha$ -helicies (right-handed) and the  $\beta$ -sheet strands (extended zig-zag) in each protein chain. See photos above.

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

- 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.