

The following information applies to all parts.

2. Eukaryotic cells use small interfering RNA (siRNA) molecules that regulate the expression of certain genes by binding to complementary sequences in target mRNAs. This binding causes the protein AGO2 to cleave the target mRNAs. As a result of the cleavage, the mRNAs are not translated.

Part A

Describe one location where ribosomes are found in eukaryotic cells.

The following information applies to parts B, C, and D.

To investigate the role of AGO2 in the cleavage of mRNA transcribed from two genes, Gene *G* and Gene *H*, scientists determined the amount of Gene *G* and Gene *H* mRNA produced in cells with three different *AGO2* genotypes:

- Homozygous for the wild-type AGO2 protein (*AGO2*^{+/+})
- Heterozygous for the allele encoding a nonfunctional AGO2 protein (*AGO2*^{+/-})
- Homozygous for the allele encoding a nonfunctional AGO2 protein (*AGO2*^{-/-})

The scientists then calculated the amount of Gene *G* and Gene *H* mRNA produced from cells of each genotype relative to that in *AGO2*^{+/+} cells. These results are shown in the table.

Relative Average Amounts of Gene *G* and Gene *H* mRNA in Cells with Different *AGO2* Genotypes

	mRNA in <i>AGO2</i> ^{+/+} Cells ± SE _{$\bar{x}$}	mRNA in <i>AGO2</i> ^{+/-} Cells ± SE _{$\bar{x}$}	mRNA in <i>AGO2</i> ^{-/-} Cells ± SE _{$\bar{x}$}
Gene <i>G</i>	1.0 ± 0.1	0.9 ± 0.4	1.5 ± 0.5
Gene <i>H</i>	1.0 ± 0.1	2.0 ± 0.1	3.0 ± 0.4

Part B

- Using the template in the space provided for your response, **construct** a bar graph that represents the data in the table. Your graph should be appropriately plotted and labeled.
- Based on the table, **determine** all the cells in which the amount of Gene *G* mRNA produced is statistically the same as the amount in the cells with the *AGO2*^{+/+} genotype.

Part C

- Based on the table, **describe** the relationship between the number of wild-type copies of the *AGO2* gene and the amount of Gene *H* mRNA in the cells.
- Based on the data in the table for Gene *H*, **calculate** the percent by which the average amount of mRNA in *AGO2*^{-/-} cells increases compared with that in *AGO2*^{+/+} cells.

Part D

- The scientists claim that siRNA binding and AGO2 cleavage of mRNAs play a greater role in the regulation of Gene *H* expression than in the regulation of Gene *G* expression. Based on the table, **support** the scientists' claim.
- Anaphase I of meiosis is blocked in mice with the *AGO2*^{-/-} genotype. Scientists hypothesize that the block in meiosis occurs because the continued presence of a spindle-fiber stabilizing protein prevents the chromosomes from separating. Based on the table, **explain** how the *AGO2*^{-/-} genotype could cause this effect on the dividing cells.

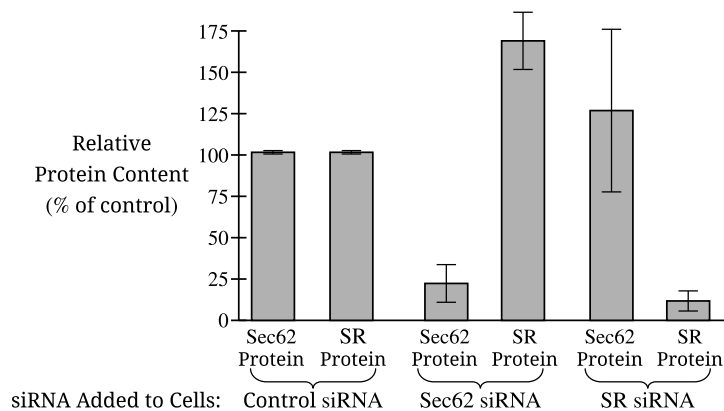
1. Most proteins that are secreted from a cell must be transported to the endoplasmic reticulum (ER) either during translation or after translation.

A. Describe the function of ribosomes.

For proteins transported during translation, this process begins in the cytosol and pauses when a specific sequence of amino acids is translated. The translation complex is then transported to the surface of the ER where translation continues. Proteins that are transported after translation are translated entirely in the cytosol and then transported to the ER. In both instances, the translated proteins enter the ER through a protein channel in the membrane of the ER.

Researchers studying the two types of protein transport identified that the ER membrane protein SR is necessary for transport during translation, while the ER membrane protein Sec62 is necessary for transport after translation. To investigate which transport mechanism is used for different proteins, researchers first created small interfering RNAs (siRNAs) that reduce expression of either SR or Sec62. They then treated groups of cells with either the SR siRNA or the Sec62 siRNA and determined the relative amount of SR and Sec62 protein in each group of cells compared with cells treated with a control siRNA (Figure 1).

Figure 1. Average relative amounts of Sec62 and SR proteins in cells treated with control siRNA, Sec62 siRNA, or SR siRNA. Error bars represent $\pm SE_{\bar{x}}$.

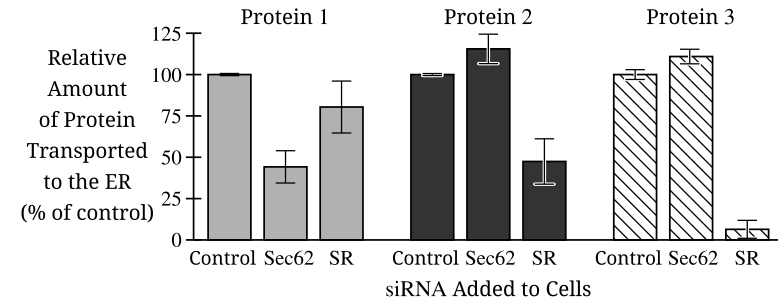


B.

- Identify** the dependent variable in the experiments shown in Figure 1.
- Justify** why the researchers included the control of measuring the relative amounts of both Sec62 and SR proteins in cells that were treated with Sec62 siRNA only (data shown in Figure 1).
- Based on Figure 1, **describe** the effect on the production of SR protein when cells are treated with Sec62 siRNA.

The researchers then measured the amount of each of three different proteins that was transported to the ER in cells treated with Sec62 siRNA or SR siRNA. The researchers calculated the percent transported relative to the cells treated with control siRNA (Figure 2).

Figure 2. Average relative amounts of three proteins that were transported to the ER when treated with control siRNA, Sec62 siRNA, or SR siRNA. Error bars represent $\pm SE_{\bar{x}}$.



C.

- Identify** the independent variable in the researchers' second experiment (data shown in Figure 2).
- Based on Figure 2, **identify** the protein(s) that when treated with Sec62 siRNA showed an increase in percent transport to the ER compared with the control.
- Protein 1 is encoded by 234 nucleotides, while protein 2 is encoded by 495 nucleotides. Assuming all nucleotides for both proteins encode amino acids, **calculate** the difference in the number of amino acids between the two proteins.

D.

- Researchers claim that protein 1 is the only tested protein that is transported to the ER following its complete translation in the cytosol. Using data from Figure 2, **support** the researchers' claim.
- For any protein that enters the ER, researchers claim that amino acids close to the protein's amino terminus determine how likely the protein is to pass through the protein channel within the ER membrane. **Justify** the researchers' claim based on your understanding of factors that affect the transport of proteins across membranes.

2. Elevated levels of CO₂ increase the rate of photosynthesis and growth in plants. Scientists studying the mechanisms involved in these increases examined a variety of species and found that when plants are exposed to elevated levels of CO₂, there is an increase in the number of chloroplasts per cell. To investigate whether the elevated levels of CO₂ have a similar effect on the number of mitochondria in plant cells, the scientists then selected six of these species to quantify the number of mitochondria per cell when the plants were exposed to both normal and elevated levels of CO₂ (Table 1).

TABLE 1. AVERAGE NUMBER OF MITOCHONDRIA IN PLANTS EXPOSED TO NORMAL AND ELEVATED LEVELS OF CO₂

Species	Mitochondria at Normal CO ₂ (per 100 μm ² of cell area) ±2SE _{$\bar{x}$}	Mitochondria at Elevated CO ₂ (per 100 μm ² of cell area) ±2SE _{$\bar{x}$}
1	1.0 ± 0.10	1.6 ± 0.10
2	0.4 ± 0.05	0.9 ± 0.08
3	0.5 ± 0.07	0.9 ± 0.10
4	0.3 ± 0.03	0.6 ± 0.06
5	0.7 ± 0.06	1.5 ± 0.22
6	1.3 ± 0.15	2.4 ± 0.22

- (a) **Describe** the role of the inner mitochondrial membrane in cellular respiration.
- (b) Using the template in the space provided for your response, **construct** an appropriately labeled graph that represents the data in Table 1. **Determine** which species show(s) a difference in the number of mitochondria between normal and elevated levels of CO₂.
- (c) Based on the data in Table 1, **describe** the relationship between the level of CO₂ and the average number of mitochondria per unit area of a cell.
- (d) The leaves of a particular plant species are typically green, but scientists notice a plant in which the leaves have white stripes. They determine that the stripes result from a mutation in mitochondrial DNA that interferes with the development of chloroplasts. The scientists crossed plants using pollen from the plant with white-striped leaves and ovules from a plant with green leaves. **Predict** the phenotype(s) of the leaves of offspring produced from this cross. Provide reasoning to **justify** your prediction. **Explain** why plants with the same genotype are able to differ in the structure and/or number of certain organelles in response to changes in atmospheric levels of CO₂.

Write your responses to this question only on the designated pages in the separate Free Response booklet.

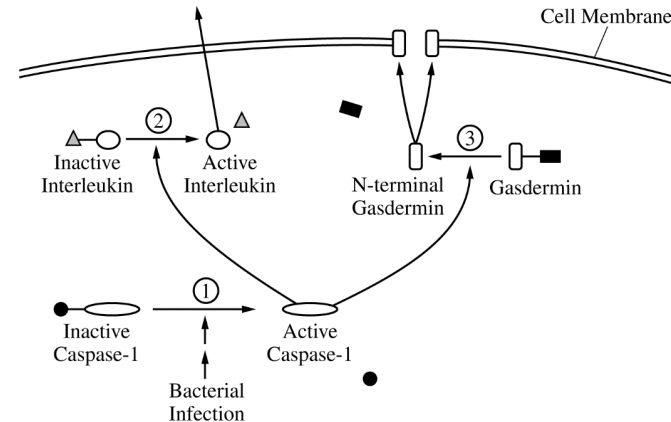


Figure 1. Cellular response to infection by pathogenic bacteria

2. Some pathogenic bacteria enter cells, replicate, and spread to other cells, causing illness in the host organism. Host cells respond to these infections in a number of ways, one of which involves activating particular enzymatic pathways (Figure 1). Cells normally produce a steady supply of inactive caspase-1 protein. In response to intracellular pathogens, the inactive caspase-1 is cleaved and forms an active caspase-1 (step 1). Active caspase-1 can cleave two other proteins. When caspase-1 cleaves an inactive interleukin (step 2), the active portion of the interleukin is released from the cell. An interleukin is a signaling molecule that can activate the immune response. When caspase-1 cleaves gasdermin (step 3), the N-terminal portions of several gasdermin proteins associate in the cell membrane to form large, nonspecific pores.

Researchers created the model in Figure 1 using data from cell fractionation studies. In the experiments, various parts of the cell were separated into fractions by mechanical and chemical methods. Specific proteins known to be located in different parts of the cell were used as markers to determine the location of other proteins. The table below shows the presence of known proteins in specific cellular fractions.

CELL FRACTIONS CONTAINING DIFFERENT CELLULAR PROTEINS

	Aconitase (Krebs cycle protein)	DNA polymerase	GAPDH (glycolytic protein)	Sodium- potassium pump	NF-κB (Immune response protein)
Whole cell sample	+	+	+	+	+
Fraction 1	+				
Fraction 2		+			+
Fraction 3			+		+
Fraction 4				+	

+ = presence of protein

- (a) **Describe** the effect of inhibiting step 3 on the formation of pores AND on the release of interleukin from the cell.
- (b) **Make a claim** about how cleaving inactive caspase-1 results in activation of caspase-1. A student claims that preinfection production of inactive precursors shortens the response time of a cell to a bacterial infection. **Provide ONE reason** to support the student's claim.
- (c) A student claims that the NF- κ B protein is located in the cytoplasm until the protein is needed for transcription. **Justify** the student's claim with evidence. **Identify TWO** fractions where N-terminal gasdermin would be found in cells infected with pathogenic bacteria.
- (d) **Describe** the most likely effect of gasdermin pore formation on water balance in the cell in a hypotonic environment.
- (e) **Explain** how gasdermin pore formation AND interleukin release contribute to an organism's defense against a bacterial pathogen.
6. Cystic fibrosis is a genetic condition that is associated with defects in the CFTR protein. The CFTR protein is a gated ion channel that requires ATP binding in order to allow chloride ions (Cl^-) to diffuse across the membrane.
- (a) In the provided model of a cell, **draw** arrows to describe the pathway for production of a normal CFTR protein from gene expression to final cellular location.
- (b) **Identify** the most likely cellular location of the ribosomes that synthesize CFTR protein.
- (c) **Identify** the most likely cellular location of a mutant CFTR protein that has an amino acid substitution in the ATP-binding site.

