The following information applies to all parts.

---

1. Molecules known as dinucleoside polyphosphates, a type of nucleotide, are signaling molecules that accumulate in plant cells during dry or stressful conditions.

Part A

Describe the three structural components of a nucleotide.

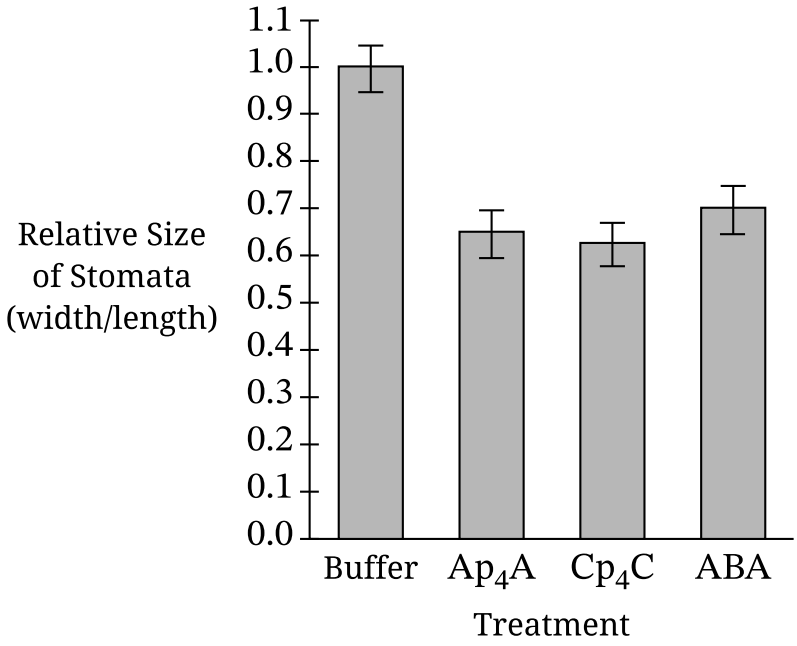
The following information applies to parts B, C, and D.

Stomata are pores on the surface of leaves that open when exposed to light and regulate the flow of carbon dioxide, oxygen, and water vapor in and out of a plant. In response to dry environmental conditions, many plants close their stomata, which reduces water loss.

Scientists hypothesized that the dinucleoside polyphosphates Ap_4A and Cp_4C bind to DORN1 receptors on the plasma membrane of plant cells and initiate signaling cascades that cause stomata to close. The scientists exposed plants to light to open the stomata and then placed samples of the leaves in buffer alone or in buffer containing one of three different compounds: Ap_4A, Cp_4C, or abscisic acid (ABA), a molecule that causes stomata to close.

The scientists measured the size of the stomata in each sample and calculated their sizes relative to the size of the stomata in the sample kept in buffer alone, as shown in Figure 1.

Figure 1. Effect of Ap_4A, Cp_4C, and ABA on Relative Stomatal Size (Error Bars Represent $\pm SE_{\bar{x}}$)



Part B

i. **Identify** the dependent variable in the scientists' first experiment.

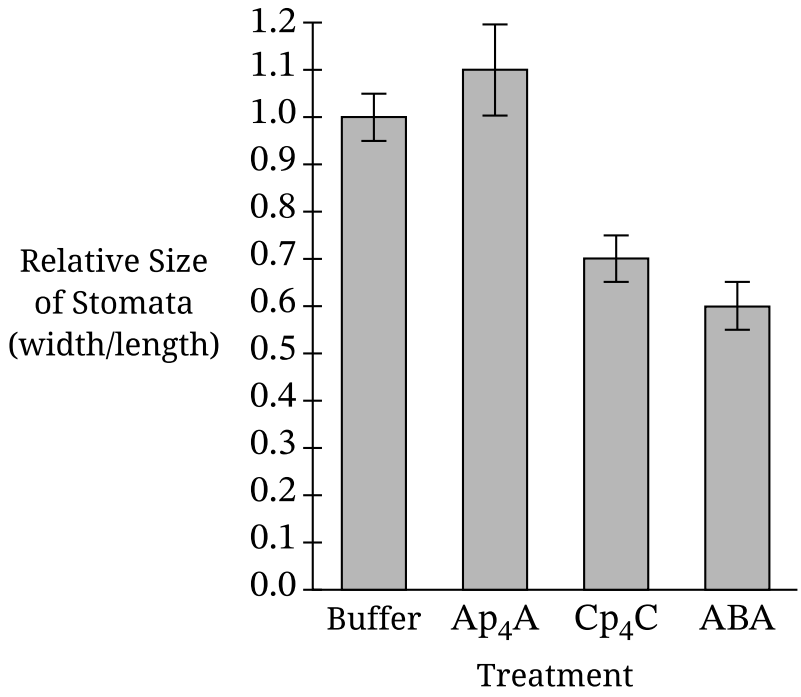
ii. Based on Figure 1, **describe** the relative size of the stomata in the Cp_4C treatment group as compared with the size of the stomata in the group treated with buffer alone.

iii. **Justify** the use of buffer alone as a control in the scientists' experiments.

Part C

In a second experiment, the scientists used plants that were identical to those in the first experiment except that the gene encoding the DORN1 receptor was mutated. The scientists repeated the procedure of the first experiment. Their results are shown in Figure 2.

Figure 2. Effect of Ap_4A, Cp_4C, and ABA on Relative Stomatal Size in DORN1 Mutants (Error Bars Represent $\pm SE_{\bar{x}}$)



i. **Justify** the scientists' treating one sample of leaves with ABA in the experiments.

ii. Based on Figure 2, **describe** the difference between the effects of Ap_4A and Cp_4C treatments on the size of stomata in plants with mutated DORN1 receptors.

iii. Activation of DORN1 receptors induces plant cells to produce certain molecules that cause the stomata to close. Based on the data in Figures 1 and 2 for cells treated with Ap_4A, **predict** the relative production of the molecules by DORN1-mutated cells in comparison to the production by nonmutated cells.

Part D

The DORN1 receptor transmits signals that result in an increase in the transcription of genes involved in plant defenses. The scientists then developed DORN1-mutant plants in which the DORN1 receptor lacked most of its intracellular domain.

i. In cells homozygous for this mutation, **predict** the effect of Ap_4A treatment on transcription of the genes involved in plant defenses relative to the effect of Ap_4A on nonmutated cells.

ii. **Justify** your prediction.

2. Many insects rely on pheromones (chemical signals) that are released by the females to find mating partners. Scientists hypothesize that, in a certain type of moth, the behavior of male moths in response to pheromones is regulated by the extracellular signaling molecule 20E.

A. Many receptors are embedded in the plasma membrane. **Describe** the polarity of the portion of the receptor that is inside the membrane.

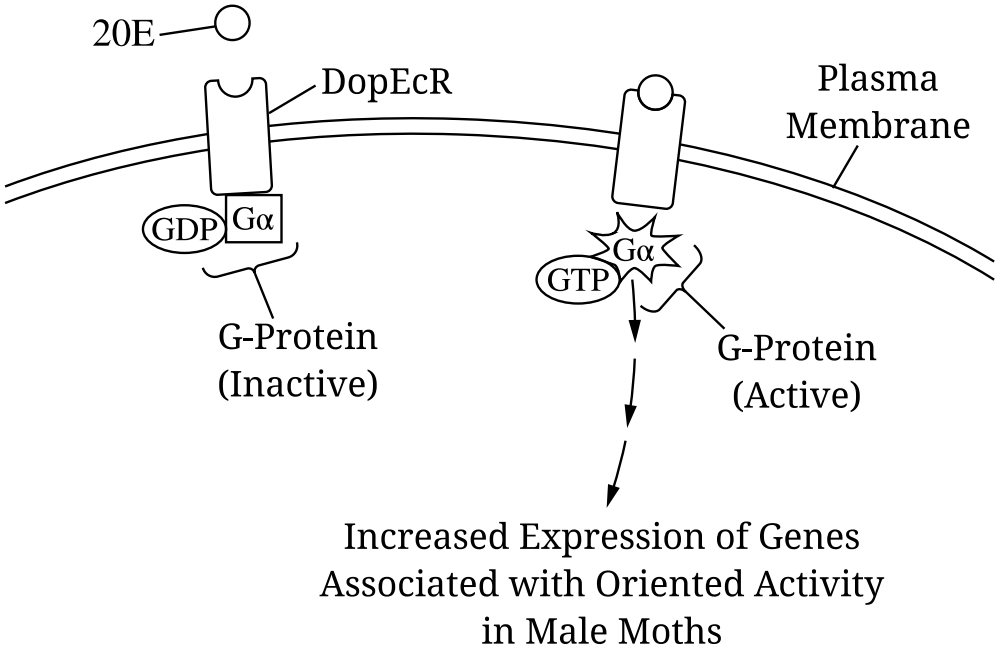
To investigate whether the binding of 20E to its receptor, DopEcR, affects behavior in moths, scientists injected male moths with saline (control solution) or with small interfering RNA molecules (siRNAs) that inhibit the expression of the gene encoding DopEcR. The scientists then exposed the moths to the pheromone and determined the percent of total time observed that the moths engaged in general activity, defined as movement in any direction. The scientists also determined the percent of the general activity time that the moths spent in oriented activity, defined as movement toward an area of high pheromone concentration (Table 1).

Table 1. Average General and Oriented Activity in Male Moths Injected With Saline or siRNA Molecules

Treatment	General Activity (percent of total time observed, average $\pm 2SE_{\bar{x}}$)	Oriented Activity (percent of general activity, average $\pm 2SE_{\bar{x}}$)
Male moths injected with saline (control solution)	95 ± 5	60 ± 4
Male moths injected with siRNAs that inhibit expression of the gene encoding DopEcR	90 ± 8	25 ± 6

DopEcR is a G protein-coupled receptor. When 20E binds to DopEcR, GTP displaces the GDP bound to the G protein, and a signaling pathway is activated. The scientists hypothesize that this leads to the transcription of genes associated with the oriented activity observed in the male moths (Figure 1).

Figure 1. A simplified model of a signaling pathway activated by the binding of 20E to its receptor, DopEcR



B.

i. Using the template in the space provided for your response, **construct** an appropriate type of graph that represents the data in Table 1. Your graph should be appropriately plotted and labeled.

ii. Based on the data in Table 1, **determine** the type of activity that was affected by inhibiting the expression of the DopEcR receptor.

C.

i. Based on Table 1, **identify** the treatment group in which the oriented activity was greater than 50% of the general activity.

ii. The scientists studied some moths with a mutation in the gene encoding the G protein. The mutation prevents GTP from displacing the GDP bound to the G protein. Based on Figure 1, **predict** the effect of this mutation on the oriented activity in male moths exposed to the pheromone.

Expression of the gene encoding DopEcR is low in the male moths during their first few days as adults, when they are sexually immature. Gene expression rapidly increases as the moths reach sexual maturity. The scientists claim that this increase in gene expression increases the likelihood of males finding females with whom to mate.

D.

i. Use evidence from the information provided to **support** the scientists' claim.

ii. Based on Figure 1, **explain** how an inhibitor of the DopEcR pathway might serve as an effective chemical to protect crops from moth damage.

1. In eukaryotic microorganisms, the PHO signaling pathway regulates the expression of certain genes. These genes, *Pho* target genes, encode proteins involved in regulating phosphate homeostasis. When the level of extracellular inorganic phosphate (Pi) is high, a transcriptional activator Pho4 is phosphorylated by a complex of two proteins, Pho80–Pho85. As a result, the *Pho* target genes are not expressed. When the level of extracellular Pi is low, the activity of the Pho80–Pho85 complex is inhibited by another protein, Pho81, enabling Pho4 to induce the expression of these target genes. A simplified model of this pathway is shown in Figure 1.

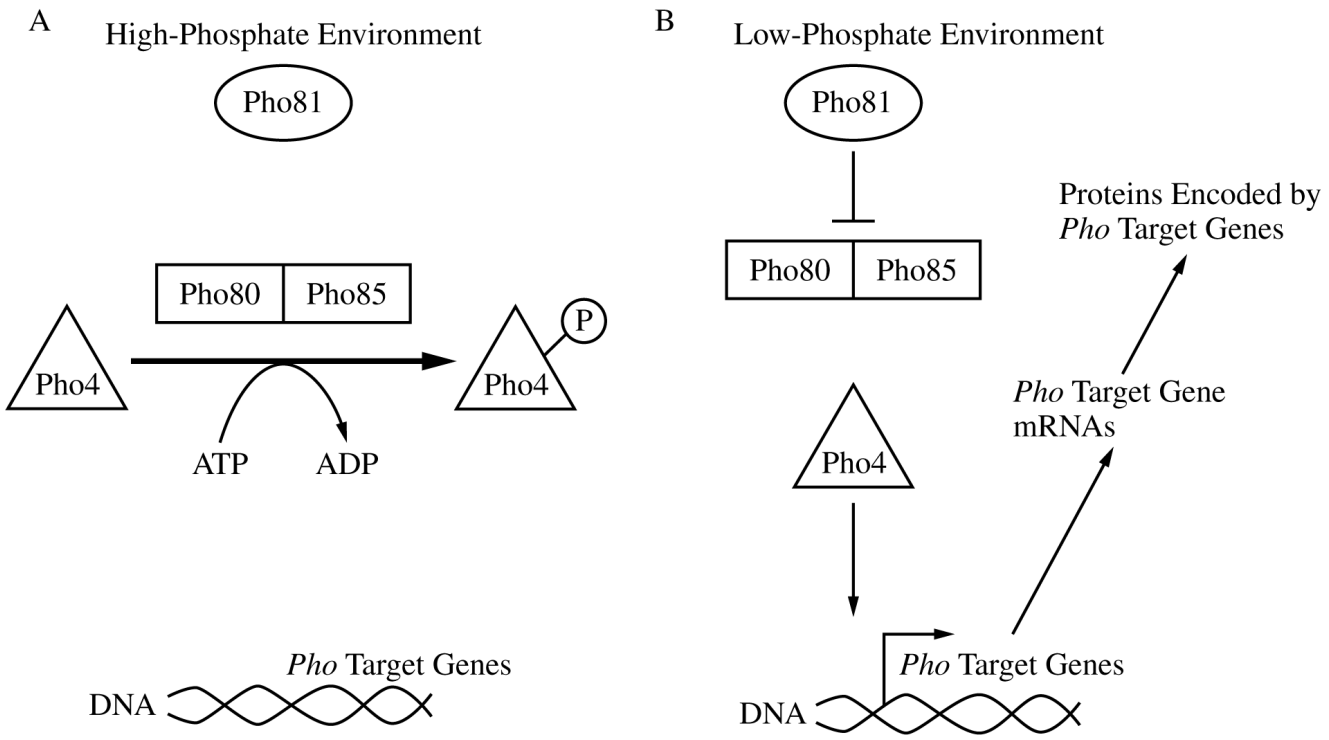


Figure 1. A simplified model of the regulation of expression of *Pho* target genes in (A) a high-phosphate (high-Pi) environment and (B) a low-phosphate (low-Pi) environment

To study the role of the different proteins in the PHO pathway, researchers used a wild-type strain of yeast to create a strain with a mutant form of Pho81 (*pho81mt*) and a strain with a mutant form of Pho4 (*pho4mt*). In each of these mutant strains, researchers measured the activity of a particular enzyme, APase, which removes phosphates from its substrates and is encoded by *PHO1*, a *Pho* target gene (Table 1). They then determined the level of *PHO1* mRNA relative to that of the wild-type yeast strain, which was set to 10.

TABLE 1. APase ACTIVITY AND RELATIVE AMOUNTS OF *PHO1* mRNA IN WILD-TYPE AND MUTANT STRAINS OF YEAST IN HIGH- AND LOW-PHOSPHATE ENVIRONMENTS

Yeast Strain	Mutation	APase Activity in High-Pi Environment (mU/mL/OD_{600}) $\pm 2SE_{\bar{x}}$	APase Activity in Low-Pi Environment (mU/mL/OD_{600}) $\pm 2SE_{\bar{x}}$	Relative Amounts of *PHO1* mRNA in High-Pi Environment $\pm 2SE_{\bar{x}}$	Relative Amounts of *PHO1* mRNA in Low-Pi Environment $\pm 2SE_{\bar{x}}$
Wild-type	None	0.5 ± 0.1	17.3 ± 0.9	0.1 ± 0.0	10 ± 2.0
pho81mt	Nonfunctional Pho81	0.4 ± 0.1	0.6 ± 0.1	0.7 ± 0.2	0.9 ± 0.8
pho4mt	Nonfunctional Pho4	0.5 ± 0.0	0.8 ± 0.2	0.6 ± 0.4	0.3 ± 0.1

(a) **Describe** the effect that the addition of a charged phosphate group can have on a protein that would cause the protein to become inactive. **Explain** how a signal can be amplified during signal transduction in a pathway such as the PHO signaling pathway.

(b) Based on Table 1, **identify** a dependent variable in the researchers' experiment. **Justify** the researchers' using the wild-type strain for the creation of the mutant strains. **Justify** the researchers' using mutant strains in which only a single component of the pathway was mutated in each strain.

(c) Based on the data in Table 1, **identify** the yeast strain and growth conditions that lead to the highest relative amount of *PHO1* mRNA. **Calculate** the percent change in APase activity in wild-type yeast cells in a high-Pi environment compared with that of wild-type cells in a low-Pi environment.

(d) In a follow-up experiment, researchers created a strain of yeast with a mutation that resulted in a nonfunctional Pho85 protein. Based on Figure 1, **predict** the effects of this mutation on *PHO1* expression in the mutant strain in a high-Pi environment. Provide reasoning to **justify** your prediction.

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

1. The binding of an extracellular ligand to a G protein-coupled receptor in the plasma membrane of a cell triggers intracellular signaling (Figure 1, A). After ligand binding, GTP replaces the GDP that is bound to Gsα, a subunit of the G protein (Figure 1, B). This causes Gsα to activate other cellular proteins, including adenylyl cyclase that converts ATP to cyclic AMP (cAMP). The cAMP activates protein kinases (Figure 1, C). In cells that line the small intestine, a cAMP-activated protein kinase causes further signaling that ultimately results in the secretion of chloride ions (Cl^-) from the cells. Under normal conditions, Gsα hydrolyzes GTP to GDP, thus inactivating adenylyl cyclase and stopping the signal (Figure 1, A).

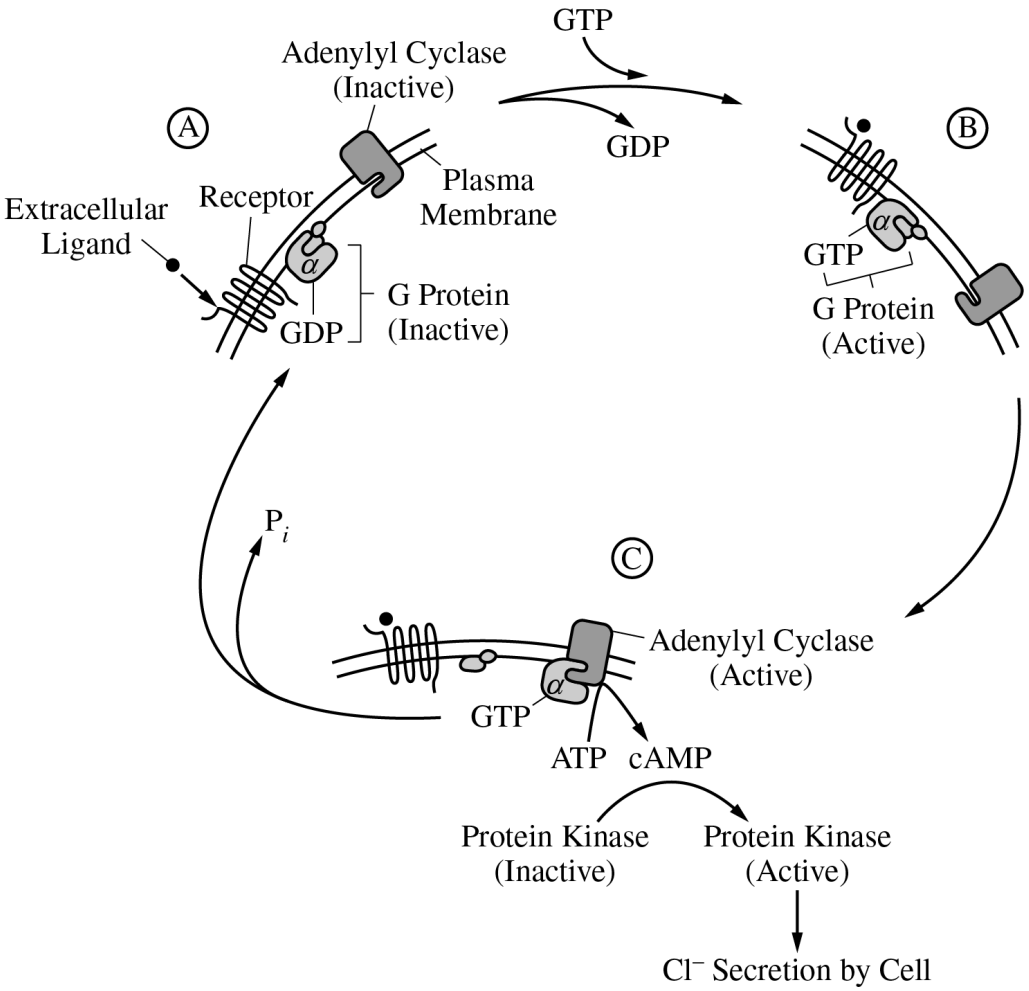


Figure 1. Under normal conditions, ligand binding to a G protein-coupled receptor results in chloride ion transport from an intestinal cell.

Individuals infected with the bacterium *Vibrio cholerae* experience severe loss of water from the body (dehydration). This is due to the effects of the bacterial cholera toxin that enters intestinal cells. Scientists studied the effects of cholera toxin on four samples of isolated intestinal cell membranes containing the G protein-related signal transduction components shown in Figure 1. GTP was added to samples II and IV only; cholera toxin was added to samples III and IV only. The scientists then measured the amount of cAMP produced by the adenylyl cyclase in each sample (Table 1).

TABLE 1. AMOUNT OF cAMP PRODUCED FROM INTESTINAL CELL MEMBRANES IN THE ABSENCE OR PRESENCE OF CHOLERA TOXIN

Sample	GTP	Cholera Toxin	Rate of cAMP Production (pmol per mg adenylyl cyclase per min)
I	−	−	0.5
II	+	−	10.0
III	−	+	0.5
IV	+	+	127.0

present, +; absent, −

(a) **Describe** one characteristic of a membrane that requires a channel be present for chloride ions to passively cross the membrane. **Explain** why the movement of chloride ions out of intestinal cells leads to water loss.

(b) **Identify** an independent variable in the experiment. **Identify** a negative control in the experiment. **Justify** why the scientists included Sample III as a control treatment in the experiment.

(c) Based on the data, **describe** the effect of cholera toxin on the synthesis of cAMP. **Calculate** the percent change in the rate of cAMP production due to the presence of cholera toxin in sample IV compared with sample II.

(d) A drug is designed to bind to cholera toxin before it crosses the intestinal cell membrane. Scientists mix the drug with cholera toxin and then add this mixture and GTP to a sample of intestinal cell membranes. **Predict** the rate of cAMP production in pmol per mg adenylyl cyclase per min if the drug binds to all of the toxin. In a separate experiment, scientists engineer a mutant adenylyl cyclase that cannot be activated by Gsα. The scientists claim that cholera toxin will not cause excessive water loss from whole intestinal cells that contain the mutant adenylyl cyclase. **Justify** this claim.

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

1. Polycystic kidney disease (PKD) is an inherited disease that causes water loss from the body and affects cell division in the kidneys. Because water movement across cell membranes is related to ion movement, scientists investigated the role of the Na^+/K^+ATPase (also known as the sodium/potassium pump) in this disease. Ouabain, a steroid hormone, binds to the Na^+/K^+ATPase in plasma membranes. Individuals with PKD have a genetic mutation that results in an increased binding of ouabain to the Na^+/K^+ATPase. The scientists treated normal human kidney (NHK) cells and PKD cells with increasing concentrations of ouabain and measured the number of cells (Figure 1) and the activity of the Na^+ / K^+ATPase (Figure 2) after a period of time. The scientists hypothesized that a signal transduction pathway that includes the protein kinases MEK and ERK (Figure 3) may play a role in PKD symptoms.

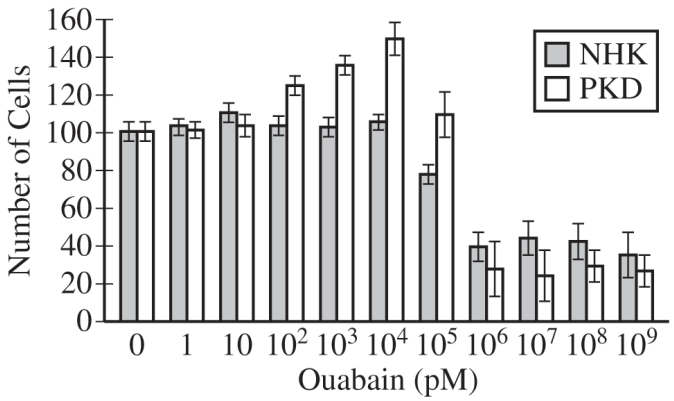


Figure 1. Cell number compared with the number of cells at 0 pM ouabain. Normal human kidney (NHK) cells and polycystic kidney disease (PKD) cells were treated with increasing concentrations of ouabain. Error bars represent $\pm 2SE_{\bar{x}}$.

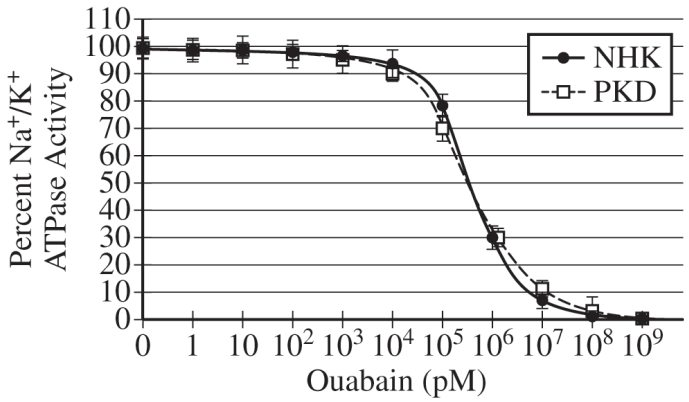


Figure 2. Percent Na^+/K^+ATPase activity of NHK and PKD cells treated with increasing concentrations of ouabain. Error bars represent $\pm 2SE_{\bar{x}}$.

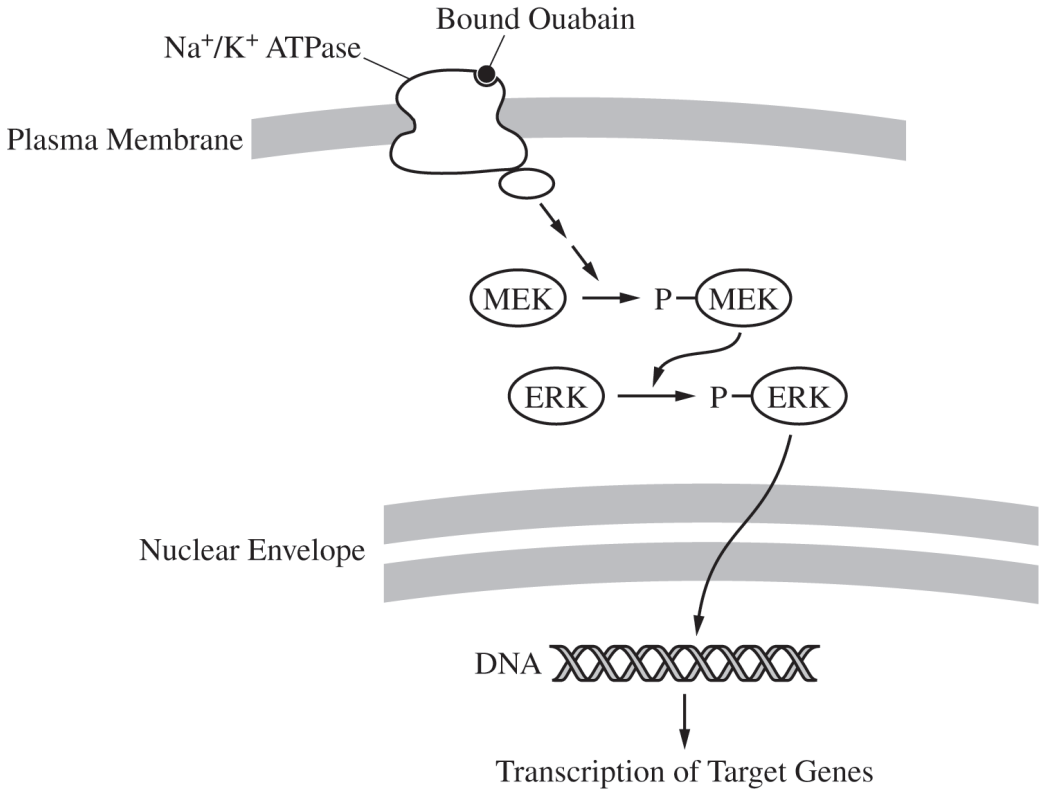


Figure 3. Signal transduction pathway hypothesized to play a role in the increased number of PKD cells

(a) **Describe** the characteristics of the plasma membrane that prevent simple diffusion of Na^+ and K^+ across the membrane. **Explain** why ATP is required for the activity of the Na^+ / K^+ATPase.

(b) **Identify** a dependent variable in the experiment represented in Figure 1. **Justify** the use of normal human kidney (NHK) cells as a control in the experiments. **Justify** the use of a range of ouabain concentrations in the experiment represented in Figure 1.

(c) Based on the data shown in Figure 2, **describe** the relationship between the concentration of ouabain and the Na^+ / K^+ ATPase activity both in normal human kidney (NHK) cells AND in PKD cells. The scientists determined that Na^+ / K^+ATPase activity in PKD cells treated with 1 pM ouabain is 150 units of ATP hydrolyzed/sec. **Calculate** the expected Na^+ / K^+ATPase activity (units/sec) in PKD cells treated with 10^6 pM ouabain.

(d) In a third experiment, the scientists added an inhibitor of phosphorylated MEK (pMEK) to the PKD cells exposed to 10^4 pM ouabain. Based on Figure 3, **predict** the change in the relative ratio of ERK to pERK in ouabain-treated PKD cells with the inhibitor compared with ouabain-treated PKD cells without the inhibitor. Provide reasoning to **justify** your prediction. Using the data in Figure 1 AND the signal transduction pathway represented in Figure 3, **explain** how the concentration of cyclin proteins may increase in PKD cells treated with 10^4 pM ouabain.

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

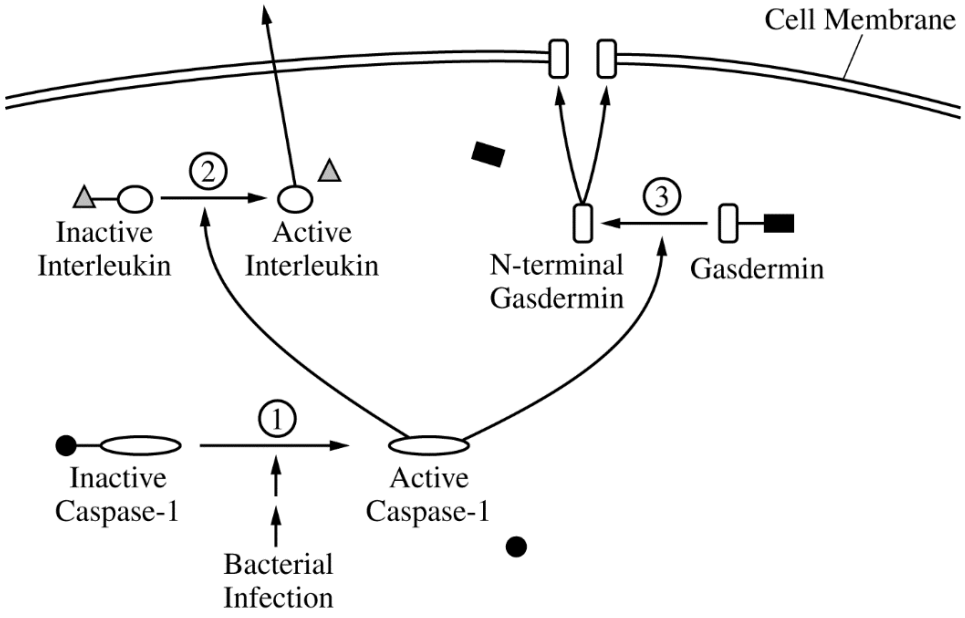


Figure 1. Cellular response to infection by pathogenic bacteria

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-κB 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.

8. Acetylcholine receptor (AChR) proteins are found at the synapse between neurons and skeletal muscle cells. Acetylcholine released from neurons binds to a specific site on the receptor proteins, which causes an ion channel in the receptors to open and allow sodium ions (Na^+) to enter muscle cells. The resulting depolarization of muscle cells initiates muscle contractions. Another molecule, nicotine, can also bind to certain types of AChR proteins and activate the receptors.

   A researcher is investigating two different types of AChR proteins: type 1 and type 2. To determine which stimuli activate the receptors, the researcher exposes muscle cells expressing the different types of receptor proteins to stimuli and observes the results indicated in Table 1.

   TABLE 1. RESPONSE OF AChR PROTEINS TO DIFFERENT STIMULI

AChR Protein Type	Acetylcholine	Nicotine
Type 1	+	+
Type 2	+	–

   + indicates activation
   – indicates no activation

   (a) **Describe** the difference in the structure AND function between AChR type 1 and AChR type 2.

   (b) Acetylcholinesterase is an enzyme that breaks down acetylcholine in the synapse. **Describe** the effect of inhibiting acetylcholinesterase on the muscle cells with AChR type 2.

STOP

END OF EXAM