Past-paper walk-through

Signal Transduction Pathways ▪ 4.3

eXercise

Questions in this set

- 2026 Q1
- 2025 Q2
- 2023 Q1
- 2022 Q1
- 2021 Q1
- 2018 Q2
- 2018 Q8

Reading the mark scheme

- **B1** a mark that stands on its own – the point is either made or not.
- **M1** a *method* mark: the right approach, even if the number is wrong.
- **A1** an *answer* mark, and it usually needs its M mark first.
- **C1** a working mark on the way to the answer.
- // separates answers the examiner accepts equally.
- **ecf** = error carried forward: a later part is marked on your own earlier answer.
- **owtte** = "or words to that effect" – the wording need not match.

Question 1

2026 ▪ Q1

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

Question 1

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 $\pm SE_{\bar{x}}$)
[Bar graph; y-axis "Relative Size of Stomata (width/length)" from 0.0 to 1.1 in increments of 0.1; x-axis "Treatment" with four bars: Buffer, Ap_4A, Cp_4C, ABA. Approximate bar heights with error bars: Buffer $\approx 1.0 \pm 0.05$; $Ap_4A \approx 0.65 \pm 0.05$; $Cp_4C \approx 0.62 \pm 0.05$; ABA $\approx 0.70 \pm 0.05$.]
(a) Describe the three structural components of a nucleotide.

Question 1

2026 ▪ Q1

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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.

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(b)(i) Identify the dependent variable in the scientists' first experiment.

(b)(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.

(b)(iii) Justify the use of buffer alone as a control in the scientists' experiments.

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2026 ▪ Q1

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Question 1

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(c)(i) 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 $\pm SE_{\bar{x}}$)

Question 1

[Bar graph; y-axis "Relative Size of Stomata (width/length)" from 0.0 to 1.2 in increments of 0.1; x-axis "Treatment" with four bars: Buffer, Ap_4A, Cp_4C, ABA. Approximate bar heights with error bars: Buffer $\approx 1.0 \pm 0.05$; $Ap_4A \approx 1.1 \pm 0.1$; $Cp_4C \approx 0.70 \pm 0.05$; ABA $\approx 0.60 \pm 0.05$.]

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

(c)(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.

(c)(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.

Question 1

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(d)(i) 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.

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.

Question 1

(d)(ii) Justify your prediction.

Question 2

2025 ▪ Q2

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

Question 2

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 $\pm 2\mathrm{SE}_{\bar{x}}$)	Oriented Activity (percent of general activity, average $\pm 2\mathrm{SE}_{\bar{x}}$)
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).

Question 2

Figure 1. A simplified model of a signaling pathway activated by the binding of 20E to its receptor, DopEcR
[Diagram of a signaling pathway across a plasma membrane. On the left: a molecule labeled "20E" (circle) approaches a membrane receptor labeled "DopEcR" embedded in the "Plasma Membrane" (drawn as two parallel wavy lines). Below the receptor inside the membrane, an inactive "G-Protein (Inactive)" complex is shown as an oval labeled "Gα" bound to "GDP". On the right: after 20E binds DopEcR, a second receptor is shown with an active "G-Protein (Active)" complex, labeled "Gα" bound to "GTP" (shown as a jagged/starburst shape indicating activation), with an arrow pointing down to the text "Increased Expression of Genes Associated with Oriented Activity in Male Moths".]

Question 2

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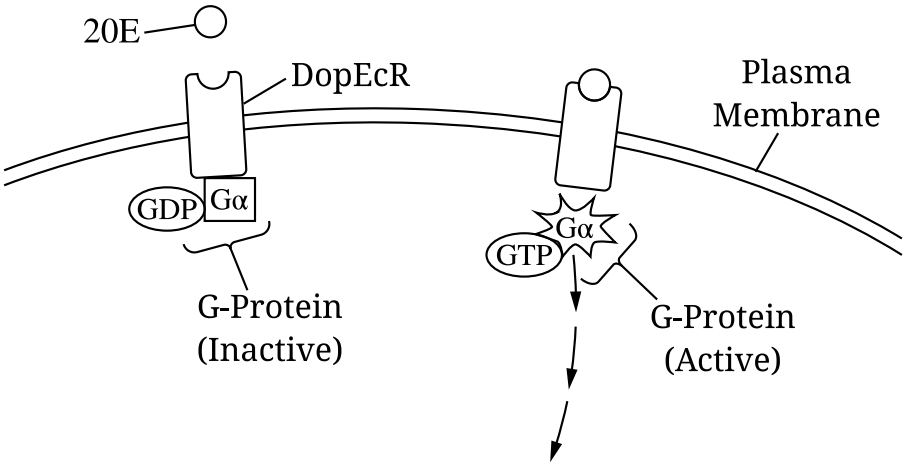
Question 2


20E
DopEcR
Plasma
Membrane
GDP
Gα
G-Protein
(Inactive)
Gα
GTP
G-Protein
(Active)

Question 2

2025 ▪ Q2

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.

(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.

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

Question 2

2025 ▪ Q2

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.

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

(c)(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.

Question 2

2025 ▪ Q2

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.

(d)(i) 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. Use evidence from the information provided to support the scientists' claim.

(d)(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.

Solution 2

(a)

Mark scheme

- The portion of the receptor inside the membrane is nonpolar/hydrophobic, consistent with the hydrophobic interior of the phospholipid bilayer it spans.

Solution 2

(b)(i) Mark scheme

- Construct an appropriate graph of the Table 1 data; 3 points total, one for each of: (1) choosing an appropriate graph type — a bar graph or modified/grouped bar graph showing General Activity and Oriented Activity (percent) for both the saline/control and siRNA treatment groups; (2) accurately plotting the data with error bars — Saline: General 95 ± 5, Oriented 60 ± 4; siRNA: General 90 ± 8, Oriented 25 ± 6 (using the table's $\pm 2SE_{\bar{x}}$ values); (3) appropriately labeling the graph — axis titles/units (e.g., percent), a legend or clear grouping distinguishing treatment and activity type. Grade a student's described or drawn graph against these three criteria.

Solution 2

(b)(ii)

Mark scheme

- Oriented activity was the type of activity affected by inhibiting DopEcR expression (it dropped from 60% to 25% of general activity), while general activity was comparatively unaffected (95% vs 90%).

Solution 2

(c)(i)

Mark scheme

- The control/saline-treated group (male moths injected with saline/control solution): oriented activity (60%) was greater than 50% of general activity (95%) in that group, whereas the siRNA group's oriented activity (25%) was well under 50% of its general activity (90%).

Solution 2

(c)(ii)

Mark scheme

- The moths will show decreased oriented activity. Because the mutation prevents GTP from displacing GDP on the G protein, the G protein cannot become active, so the downstream signaling pathway that increases expression of genes associated with oriented activity is not activated.

Solution 2

(d)(i)

Mark scheme

- Any one line of evidence is sufficient: increased DopEcR expression will increase oriented activity (and thus the moths' ability to find a mate); an increase in DopEcR expression will enable binding of 20E to more receptors; an increase in DopEcR expression will make males more sensitive to pheromones released by females — all consistent with higher DopEcR expression at sexual maturity improving mate-finding.

Solution 2

(d)(ii)

Mark scheme

- An inhibitor of the DopEcR pathway will reduce oriented activity / reduce expression of genes associated with oriented activity, and therefore decrease mating success and population growth of the moths — reducing crop damage by lowering the pest population.

Question 1

2023 ▪ Q1

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, two panels side by side, showing a simplified model of the regulation of expression of *Pho* target genes. Panel A - "High-Phosphate Environment": An oval labeled "Pho81" sits above and unconnected to the rest of the diagram. A rectangle divided into two boxes labeled "Pho80" and "Pho85" sits above a horizontal arrow.

Question 1

The arrow points from a triangle labeled "Pho4" to another triangle labeled "Pho4" with a circled "P" attached (phosphorylated Pho4). Below the arrow, a curved arrow goes from "ATP" to "ADP", indicating ATP is consumed to phosphorylate Pho4. Below that, a DNA double helix is drawn (labeled "DNA") leading into an X-shaped region labeled "*Pho* Target Genes" (genes not being transcribed). Panel B - "Low-Phosphate Environment": An oval labeled "Pho81" sits above a "T"-shaped inhibition symbol (flat bar) pointing down to the "Pho80 | Pho85" rectangle, indicating Pho81 inhibits the Pho80-Pho85 complex. Below that, a triangle labeled "Pho4" (unphosphorylated) has a downward arrow to a bent arrow ("promoter" symbol) pointing into the DNA/"*Pho* Target Genes" region, indicating Pho4 activates transcription. From the *Pho* Target Genes region, a diagonal arrow labeled "*Pho* Target Gene mRNAs" points up and to the right to another diagonal arrow labeled "Proteins Encoded by *Pho* Target Genes".]

Question 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.

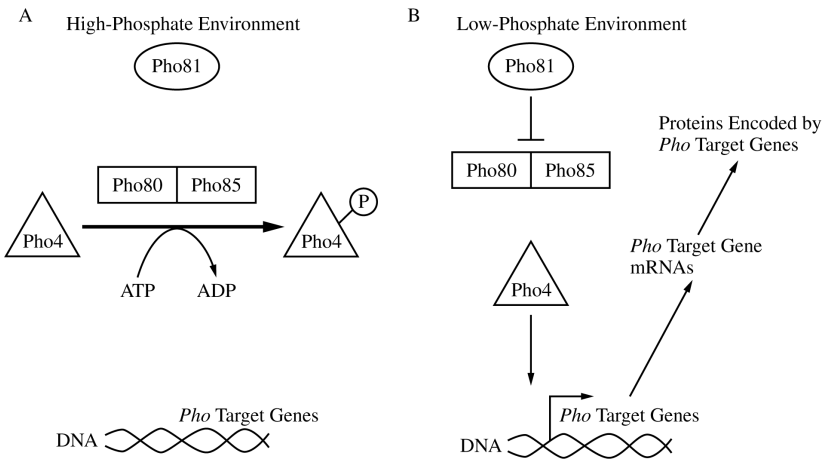
TABLE 1. APase ACTIVITY AND RELATIVE AMOUNTS OF *PHO1* mRNA IN WILD-TYPE AND MUTANT STRAINS OF YEAST IN HIGH- AND LOW-PHOSPHATE ENVIRONMENTS

Question 1

| 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.

Question 1


A
High-Phosphate Environment
Pho81
Pho80
Pho85
Pho4
P
Pho4
ATP
ADP
DNA
Pho Target Genes
B
Low-Phosphate Environment
Pho81
Pho80
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Pho4
Pho Target Genes
DNA

Question 1

2023 ▪ Q1

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Question 1

(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.

Question 1

2023 ▪ Q1

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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

Question 1

(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.

Question 1

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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, two panels side by side, showing a simplified model of the regulation of expression of *Pho* target genes. Panel A - "High-Phosphate Environment": An oval labeled "Pho81" sits above and unconnected to the rest of the diagram. A rectangle divided into two boxes labeled "Pho80" and "Pho85" sits above a horizontal arrow. The arrow points from a triangle labeled "Pho4" to another triangle labeled "Pho4" with a circled "P" attached (phosphorylated Pho4). Below the arrow, a curved arrow goes from "ATP" to "ADP", indicating ATP is consumed to

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phosphorylate Pho4. Below that, a DNA double helix is drawn (labeled "DNA") leading into an X-shaped region labeled "*Pho* Target Genes" (genes not being transcribed). Panel B - "Low-Phosphate Environment": An oval labeled "Pho81" sits above a "T"-shaped inhibition symbol (flat bar) pointing down to the "Pho80 | Pho85" rectangle, indicating Pho81 inhibits the Pho80-Pho85 complex. Below that, a triangle labeled "Pho4" (unphosphorylated) has a downward arrow to a bent arrow ("promoter" symbol) pointing into the DNA/"*Pho* Target Genes" region, indicating Pho4 activates transcription. From the *Pho* Target Genes region, a diagonal arrow labeled "*Pho* Target Gene mRNAs" points up and to the right to another diagonal arrow labeled "Proteins Encoded by *Pho* Target Genes".]
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

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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

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(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.

Solution 1

(a)

Mark scheme

- Effect of a charged phosphate group on a protein: adding the charged phosphate changes the structure/shape (conformation) of the protein, which is what causes it to become inactive. Signal amplification: in a signal-transduction pathway such as PHO signaling, each enzyme (e.g., a kinase) in the pathway can act on many copies of the next protein/substrate, so a single upstream signaling event is converted into activation of many downstream molecules — this stepwise, one-to-many catalysis at each relay step is how the signal is amplified.

Solution 1

(b) Mark scheme

- Dependent variable: APase activity, or the relative amount of PHO1 mRNA (either is acceptable). Justify using the wild-type strain as the source for creating the mutant strains: this ensures any observed differences in experimental results between strains are due to the introduced mutation itself and not to other, pre-existing genetic differences — i.e., the strains are genetically identical except for the one introduced mutation, giving a valid comparison. Justify using strains in which only a single pathway component was mutated in each strain: this allows the researchers to test the effect of each mutation separately, so they can determine which specific pathway component is responsible for any observed differences (avoids confounding effects of multiple simultaneous mutations).

Solution 1

(c)

Mark scheme

- Strain/conditions with highest relative PHO1 mRNA: wild-type yeast grown in a low-Pi environment (value of 10, per Table 1). Percent change in APase activity in wild-type cells, high-Pi vs. low-Pi: using the values 0.5 (high-Pi) and 17.3 (low-Pi) mU/mL/OD600, percent change $= (17.3 - 0.5)/0.5 \times 100\% \approx 3{,}360\%$ increase going from high-Pi to low-Pi (equivalently, a change of about -97% going from low-Pi to high-Pi: $(0.5 - 17.3)/17.3 \times 100\% \approx -97\%$). Credit either direction of calculation provided the arithmetic and setup are correct.

Solution 1

(d)

Mark scheme

- Prediction: in a high-Pi environment, a strain with nonfunctional Pho85 will still express PHO1 (Pho target genes will be expressed), unlike wild type where they are normally off in high-Pi. Justification: Pho85 is part of the Pho80–Pho85 complex that normally phosphorylates and inactivates Pho4 when Pi is high; a nonfunctional Pho85 cannot phosphorylate/inhibit Pho4, so Pho4 remains active and continues to induce transcription of PHO1 and other Pho target genes even when extracellular Pi is high.

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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: A cyclic diagram showing G protein-coupled receptor signaling in an intestinal cell membrane, with three stages labeled A, B, C connected in a cycle. Stage A: an "Extracellular Ligand" binds to a "Receptor" in the "Plasma Membrane"; the

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receptor is coupled to an inactive "G Protein" (with GDP bound) next to inactive "Adenylyl Cyclase"; GTP is shown converting the complex forward, releasing GDP, moving to stage B. Stage B: the "G Protein (Active)" is shown bound to GTP, moving to stage C. Stage C: "Adenylyl Cyclase (Active)" converts ATP to cAMP; GTP is bound to the G protein alpha subunit; cAMP activates "Protein Kinase (Inactive)" to become "Protein Kinase (Active)", which leads to "Cl^- Secretion by Cell"; a P_i arrow returns from stage C back to stage A, representing hydrolysis of GTP to GDP that inactivates adenylyl cyclase and restarts the cycle at 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

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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.

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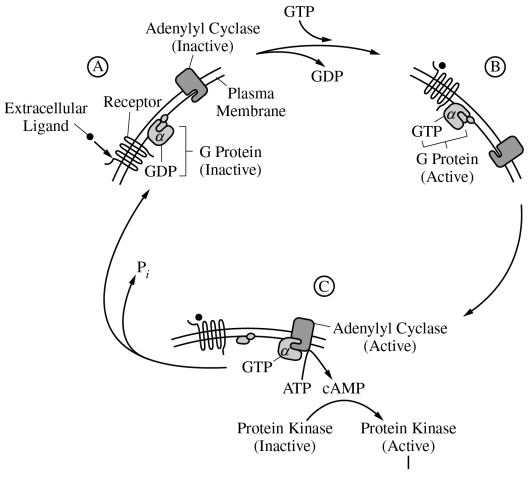
GTP
Adenylyl Cyclase
(Inactive)
GDP
A
B
Plasma
Membrane
Extracellular
Ligand
Receptor
G Protein
(Inactive)
GDP
GTP
G Protein
(Active)
P_i
C
Adenylyl Cyclase
(Active)
GTP
ATP
cAMP
Protein Kinase
(Inactive)
Protein Kinase
(Active)

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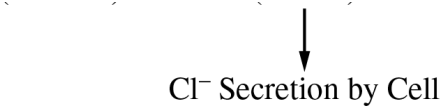

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Figure 1. Under normal conditions, ligand binding to a G protein-coupled receptor results in chloride ion transport from an intestinal cell.

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present, +; absent, −

(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.

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present, +; absent, −

(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.

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(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.

Solution 1

(a)

Mark scheme

1 pt A channel is required because the interior of the membrane (phospholipid tail) is nonpolar / not charged / hydrophobic, so charged chloride ions cannot passively cross the lipid bilayer without a channel. [1 pt] Movement of Cl- out of intestinal cells makes the extracellular space hypertonic/hyperosmotic (lower water potential) compared with the cytoplasm, so water moves out of the cells by osmosis, causing water loss (dehydration).

Solution 1

(b) Mark scheme

1 pt Independent variable: the presence or absence of cholera toxin (or presence/absence of GTP). [1 pt] Negative control: the sample lacking both cholera toxin and GTP (Sample I) - or equivalently, the samples lacking cholera toxin (I and II), the sample lacking cholera toxin but containing GTP (Sample II), or the samples lacking GTP (I and III). [1 pt] Justification for including Sample III as a control: Sample III (no GTP, + toxin) is compared with Sample IV (+GTP, + toxin) to evaluate whether cholera toxin's effect on cAMP production requires GTP, i.e. acts via the G-protein pathway.

Solution 1

(c)

Mark scheme

1 pt Cholera toxin increases cAMP production in the presence of GTP (Sample IV = 127.0 vs Sample II = 10.0); cholera toxin has no effect on cAMP production in the absence of GTP (Sample III = 0.5 vs Sample I = 0.5). [1 pt] Percent change = [(127-10)/10] x 100 = 11.7 x 100 = 1,170% increase in the rate of cAMP production due to cholera toxin (Sample IV compared with Sample II).

Solution 1

(d)

Mark scheme

1 pt If the drug binds all of the toxin, the toxin is neutralized, so the predicted rate of cAMP production equals the GTP-only rate (Sample II) = 10 pmol per mg adenylyl cyclase per min. [1 pt] Justification for the mutant-adenylyl-cyclase claim: even in the presence of cholera toxin, cAMP will not be produced via this pathway (because the mutant adenylyl cyclase cannot be activated by Gs-alpha), so the protein kinases will not be activated and Cl- ions will not be secreted, so less water will leave the intestinal cells (no excessive water loss).

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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.

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[Figure 1: Bar graph, "Number of Cells" (y-axis, 0 to 160) vs. "Ouabain (pM)" (x-axis: 0, 1, 10, 10^2, 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9), grouped bars for NHK (grey) and PKD (white). Approximate values: at 0 pM both ~100; NHK stays roughly 100-110 across most concentrations dropping to ~30-40 at 10^6-10^9 pM; PKD rises to ~110 at 10^2 pM, ~125 at 10^3 pM, peaks ~148 at 10^4 pM, ~110 at 10^5 pM, then drops to ~35-45 at 10^6-10^9 pM. Error bars represent $\pm 2SE_{\bar{x}}$. Caption: "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: Line graph, "Percent Na^+/K^+ATPase Activity" (y-axis, 0 to 110) vs. "Ouabain (pM)" (x-axis: 0, 1, 10, 10^2, 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9), two curves (NHK filled circles, PKD open squares) that overlap closely: both start near 95-100% at low concentrations, stay high (~90-95%) through 10^3 pM, begin declining at 10^4

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pM (~80%), drop steeply through 10^5 pM (~30%), reaching near 0% by 10^7-10^9 pM. Error bars represent $\pm 2SE_{\bar{x}}$. Caption: "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: Diagram of a signal transduction pathway. Bound Ouabain sits on the Na^+/K^+ ATPase embedded in the Plasma Membrane. An arrow leads from the receptor down to MEK, which is converted to P-MEK (phosphorylated MEK); P-MEK activates ERK, converting it to P-ERK (phosphorylated ERK). An arrow from P-ERK crosses the Nuclear Envelope to DNA (shown as a double helix), leading to "Transcription of Target Genes." Caption: "Figure 3. Signal transduction pathway hypothesized to play a role in the increased number of PKD cells."]

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(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.

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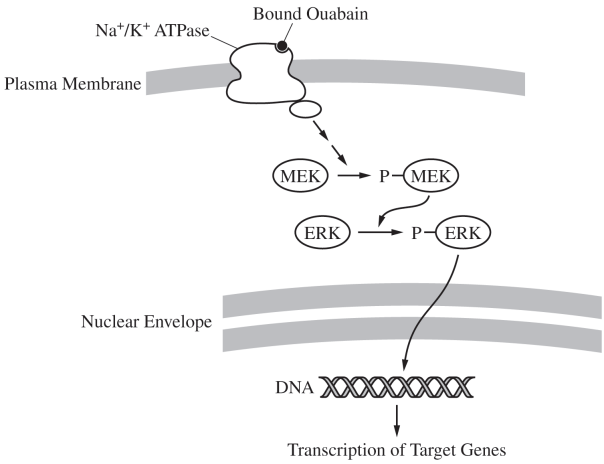
Bound Ouabain
Na+/K+ ATPase
Plasma Membrane
MEK
P–MEK
ERK
P–ERK
Nuclear Envelope
DNA
Transcription of Target Genes

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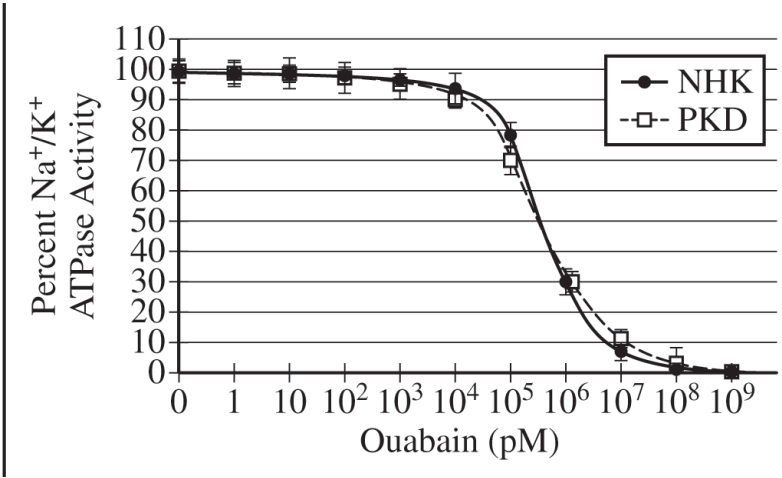
Percent Na^+/K^+ ATPase Activity
110
100
90
80
70
60
50
40
30
20
10
0
0
1
10
10^2
10^3
10^4
10^5
10^6
10^7
10^8
10^9
Ouabain (pM)
NHK
PKD

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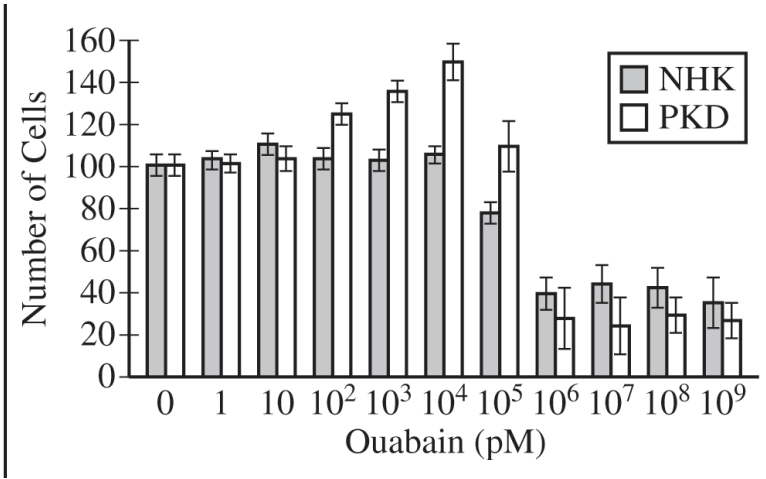
Number of Cells
160
140
120
100
80
60
40
20
0
NHK
PKD
0
1
10
10^2
10^3
10^4
10^5
10^6
10^7
10^8
10^9
Ouabain (pM)

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concentrations dropping to ~30-40 at 10^6-10^9 pM; PKD rises to ~110 at 10^2 pM, ~125 at 10^3 pM, peaks ~148 at 10^4 pM, ~110 at 10^5 pM, then drops to ~35-45 at 10^6-10^9 pM. Error bars represent $\pm 2SE_{\bar{x}}$. Caption: "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 $\pm 2SE_{\bar{x}}$."]

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(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.

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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: Bar graph, "Number of Cells" (y-axis, 0 to 160) vs. "Ouabain (pM)" (x-axis: 0, 1, 10, 10^2, 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9), grouped bars for NHK (grey) and PKD (white). Approximate values: at 0 pM both ~100; NHK stays roughly 100-110 across most

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concentrations dropping to ~30-40 at 10^6-10^9 pM; PKD rises to ~110 at 10^2 pM, ~125 at 10^3 pM, peaks ~148 at 10^4 pM, ~110 at 10^5 pM, then drops to ~35-45 at 10^6-10^9 pM. Error bars represent $\pm 2SE_{\bar{x}}$. Caption: "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: Line graph, "Percent Na^+/K^+ATPase Activity" (y-axis, 0 to 110) vs. "Ouabain (pM)" (x-axis: 0, 1, 10, 10^2, 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9), two curves (NHK filled circles, PKD open squares) that overlap closely: both start near 95-100% at low concentrations, stay high (~90-95%) through 10^3 pM, begin declining at 10^4 pM (~80%), drop steeply through 10^5 pM (~30%), reaching near 0% by 10^7-10^9 pM. Error bars represent $\pm 2SE_{\bar{x}}$. Caption: "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: Diagram of a signal transduction pathway. Bound Ouabain sits on the Na^+/K^+ ATPase embedded in the Plasma Membrane. An arrow leads from the receptor down to MEK,

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which is converted to P-MEK (phosphorylated MEK); P-MEK activates ERK, converting it to P-ERK (phosphorylated ERK). An arrow from P-ERK crosses the Nuclear Envelope to DNA (shown as a double helix), leading to "Transcription of Target Genes." Caption: "Figure 3. Signal transduction pathway hypothesized to play a role in the increased number of PKD cells."]

(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.

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(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.

Solution 1

(a)

Mark scheme

- Describe (1 pt): Accept any one — the interior of the plasma membrane is hydrophobic/nonpolar; the phospholipid tails are hydrophobic/nonpolar; the exterior of the plasma membrane is hydrophilic/polar; the phospholipid heads are hydrophilic/polar. Na+ and K+ are charged ions, so they cannot cross the hydrophobic membrane interior by simple diffusion. Explain (1 pt): The Na+/K+ ATPase pumps ions (Na+ out, K+ in) against their concentration gradients; this active transport requires an input of (metabolic) energy, which ATP hydrolysis provides.

Solution 1

(b) Mark scheme

- Identify (1 pt): A dependent variable in the Figure 1 experiment is the number of cells. Justify NHK control (1 pt): Accept either — using normal human kidney (NHK) cells allows the scientists to determine the effect of PKD on the cells' responses to (various concentrations of) ouabain; or it allows them to compare the responses of PKD cells and normal cells (to ouabain). Justify range of ouabain concentrations (1 pt): Accept either — the scientists needed to determine whether different concentrations have different effects on cell numbers; or the scientists did not know at which concentration of ouabain there would be an effect.

Solution 1

(c)

Mark scheme

- Describe relationship (1 pt): Accept either — increasing concentrations of ouabain result in decreasing ATPase activity (in both types of cells); or there is an inverse relationship/negative correlation between the concentration of ouabain and the ATPase activity (in both types of cells). Calculate (1 pt): Given 150 units/sec at 1 pM ouabain, the expected Na+/K+ ATPase activity at 10^6 pM ouabain is 45 units/sec (accept between 40 and 50), read from the plateau/low end of the Figure 2 dose-response curve (~30% of the 1 pM baseline activity).

Solution 1

(d) Mark scheme

- Predict (1 pt): Accept either — Option 1: the ratio of ERK to pERK will increase in the cells with the (pMEK) inhibitor; or Option 2: the ratio of ERK to pERK will stay the same in the cells with the inhibitor. Justify (1 pt): Must indicate the pMEK inhibitor blocks further phosphorylation of ERK AND one of the following. For Option 1: the amount of pERK will not increase as it does without the inhibitor; or the amount of ERK will not decrease as it does without the inhibitor; or the cell continues to synthesize ERK; or phosphorylated ERK is being dephosphorylated to ERK. For Option 2: no additional ERK is synthesized/pERK is not being dephosphorylated. Explain cyclin increase (1 pt): Cell number (Figure 1) increases to a maximum at 10^4 pM ouabain. The ouabain-triggered signaling

Solution 1

pathway (Figure 3) stimulates transcription of genes involved in cell division; the target genes likely include those for cyclins, because cyclins regulate the cell cycle.

Question 2

2018 ▪ Q2

Question 2

[Figure 1: Diagram of cellular response to infection by pathogenic bacteria. At the top is a double-line "Cell Membrane" with two small vertical channel/pore segments embedded in it, arrows converging up into the pore from below (labeled with circled "3"), and one arrow pointing up and out through the membrane at the far left (from the Active Interleukin). Below the membrane, left region: "Inactive Interleukin" (drawn as a triangle attached to an oval) is converted via a step labeled circled "2" into "Active Interleukin" (an oval with a triangle nearby, arrow pointing up out of the membrane, showing the active portion is released from the cell). Middle-right region: "N-terminal Gasdermin" (a small rectangle/bracket shape) sits just below the membrane pore; "Gasdermin" (a rectangle attached to a filled black square) is converted via a step labeled circled "3" into N-terminal Gasdermin, which is shown assembling into the membrane pore. Bottom-left region: "Inactive Caspase-1" (a filled circle attached to an oval) is converted via a step labeled circled "1", triggered by "Bacterial Infection" (arrow pointing up into step 1), into "Active Caspase-1" (an oval with a filled circle nearby); Active

Question 2

Caspase-1 then has arrows pointing to both step 2 (activating Interleukin) and step 3 (activating Gasdermin). Caption: "Figure 1. Cellular response to infection by pathogenic bacteria."]

Question 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.

Question 2

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 poly-merase**	**GAPDH (glycolytic protein)**	**Sodium-potassium pump**	**NF-κB (Immune response protein)**
Whole cell sample	+	+	+	+	+
Fraction 1	+				
Fraction 2		+			+
Fraction 3			+		+
Fraction 4				+	

Question 2

- = 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.

Question 2

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Fraction 2		+			+
Fraction 3			+		+
Fraction 4				+	
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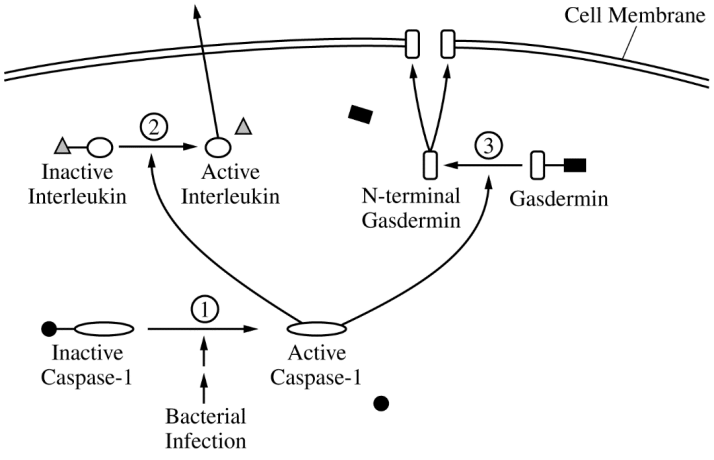
Question 2


Cell Membrane
2
Inactive
Interleukin
Active
Interleukin
3
N-terminal
Gasdermin
Gasdermin
1
Inactive
Caspase-1
Active
Caspase-1
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Infection

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Fraction 4				+	