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.

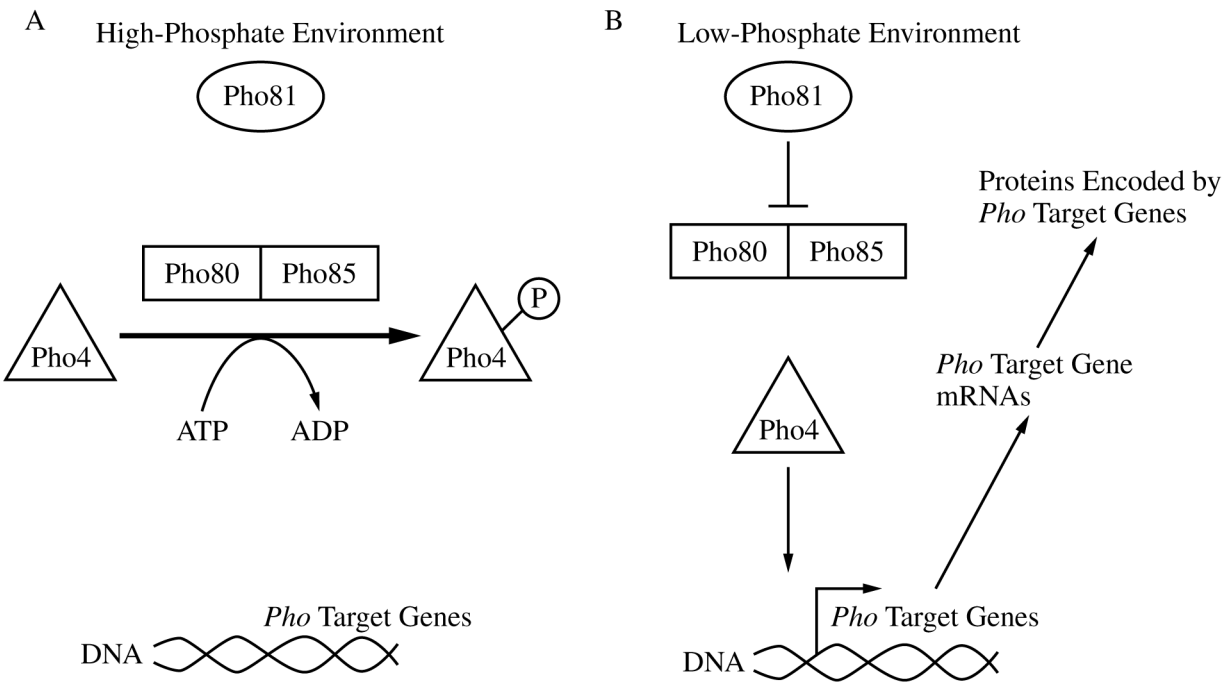

Figure 1. A simplified model of the regulation of expression of *Pho* target genes in (A) a high-phosphate (high-Pi) environment and (B) a low-phosphate (low-Pi) environment

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.

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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.

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
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pho4mt	Nonfunctional Pho4	0.5 ± 0.0	0.8 ± 0.2	0.6 ± 0.4	0.3 ± 0.1

(a) **Describe** the effect the addition of a charged phosphate group can have on a protein that would cause the protein to become inactive. **1 point**

- It changes the structure/shape of the protein.

Explain how a signal can be amplified during signal transduction in a pathway such as the PHO signaling pathway. **1 point**

- Each enzyme (in a signal transduction pathway) can act on many copies of a protein.

Total for part (a) 2 points

(b) Based on Table 1, **identify** a dependent variable in the researchers' experiment. **1 point**

Accept one of the following:

- APase activity
- (Relative) amount of *PHO1* (mRNA)

Justify the researchers' using the wild-type strain for the creation of the mutant strains. **1 point**

Accept one of the following:

- It ensures that any observed differences (in experimental results) between the strains are due to the introduced mutations (and not to other genetic differences between the yeast strains).
- It ensures that the strains are genetically identical except for the introduced mutations.

Justify the researchers' using mutant strains in which only a single component of the pathway was mutated in each strain. **1 point**

Accept one of the following:

- It allows them to test the effect of each mutation separately.
- It allows them to (better) determine which component is responsible for any observed differences.

Total for part (b) 3 points

(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. • Wild-type yeast in a low-Pi environment	**1 point**
	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. Accept one of the following: • 3,360% [(17.3–0.5)/0.5 × 100%] • -97% [(0.5–17.3)/17.3 × 100%]	**1 point**
	Total for part (c)	**2 points**
(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. • It/*PHO1*/Target genes will be expressed.	**1 point**
	Provide reasoning to **justify** your prediction. • (In a high-Pi environment) a nonfunctional Pho85 will be unable to phosphorylate/inhibit Pho4.	**1 point**
	Total for part (d)	**2 points**
	Total for question 1	**9 points**