AP Bio FRQ Fridays

2023 #1
Gene Expression & Cell Communication

FRQ Friday #29

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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.

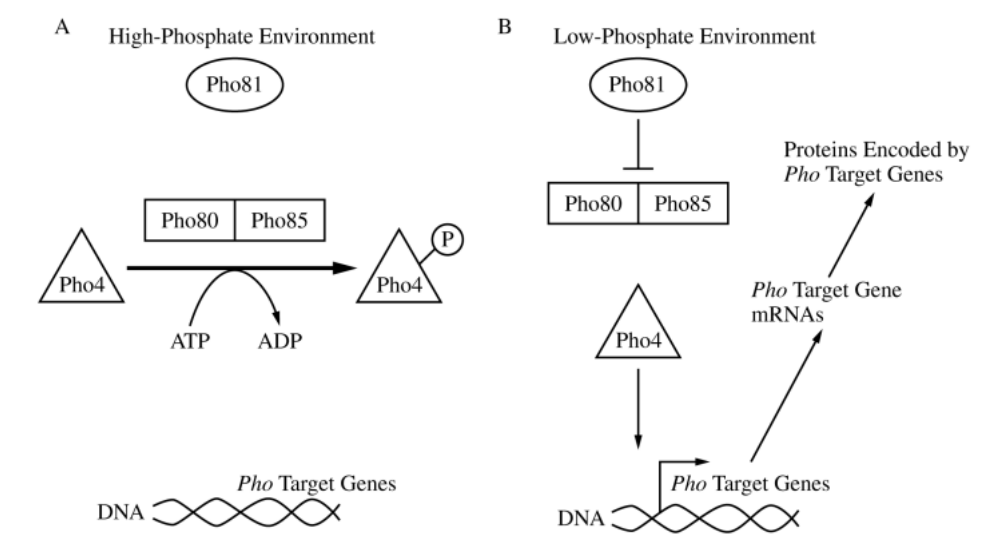

Figure 1. A simplified model of the regulation of expression of *Pho* target genes in (A) a high-phosphate (high-Pi) environment and (B) a low-phosphate (low-Pi) environment

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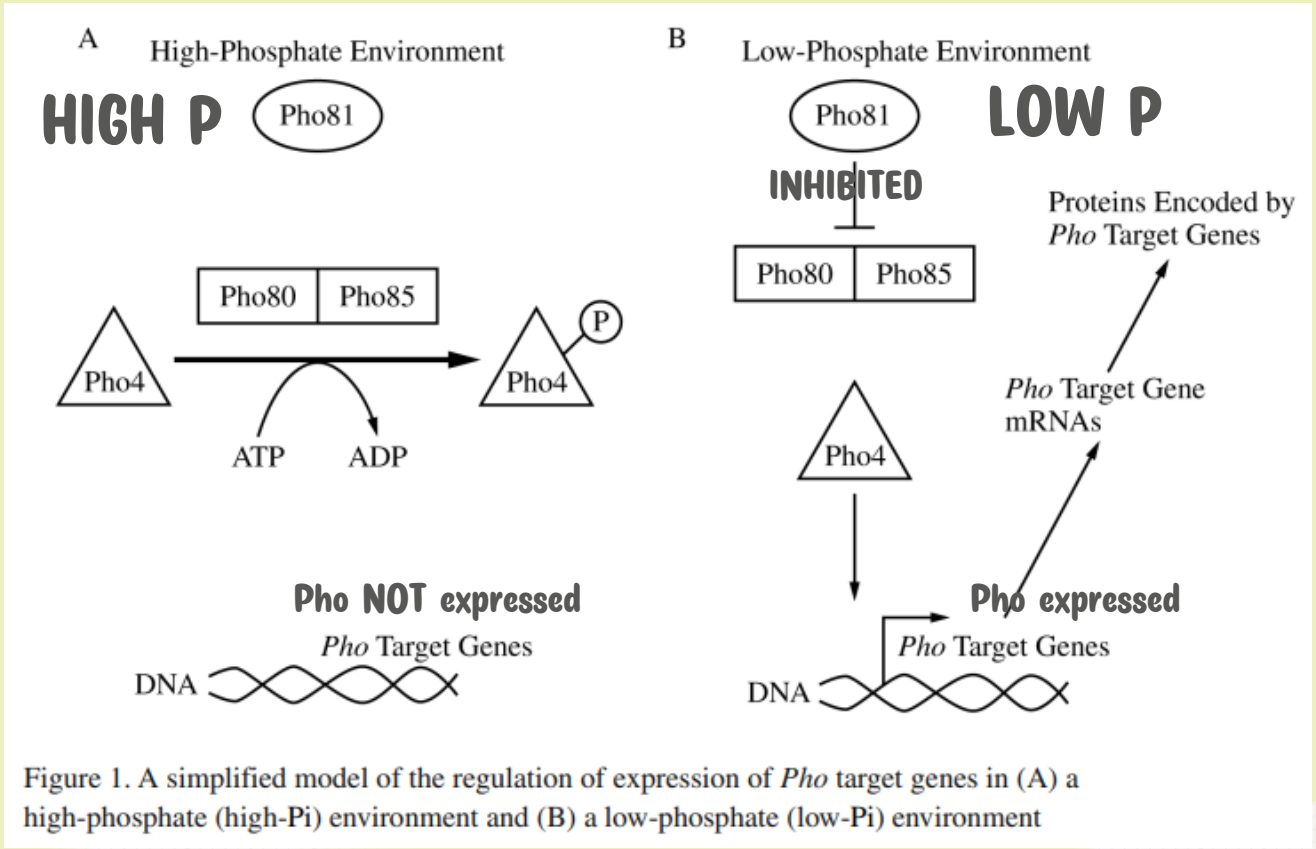

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To study the role of the different proteins in the PHO pathway, researchers used a wild-type strain of yeast to create a strain with a mutant form of Pho81 (*pho81mt*) and a strain with a mutant form of Pho4 (*pho4mt*). In each of these mutant strains, 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 $\pm 2SE_{\bar{x}}$
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(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.

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- It changes the structure/shape of the protein.

a. The addition of a charged phosphate group likely changes the tertiary folding structure of a protein, and since structu determines function, a change in its folding due to the phosphate group could cause the protein to become inactive. A

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

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

Signal is amplified when there is a chain reaction, likely a phosphorylation cascade. One reaction leads to another, often increasing the size and impacts of the reactions as they continue or pass along.

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(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.

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

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

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).
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APase. In using a wild-type strain to create the mutant strain, the researchers can be sure that the only mutation is the one they induced and all other genes are consistant with the wild type. Further,

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(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.

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- Wild-type yeast in a low-Pi environment

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$$percent\ change = \frac{final - inital}{initial} x100 = \frac{17.3 - 0.5}{0.5} x100 = \frac{16.3}{0.5} x100 = 33.6\ x\ 100 = 3360\%$$

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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

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%]

The wild-type yeast in the low-Pi environment had the highest relative amount of PHO1 mRNA.

Percent change: $\frac{17.3 - .5}{.5} \times 100 =$ 3360%

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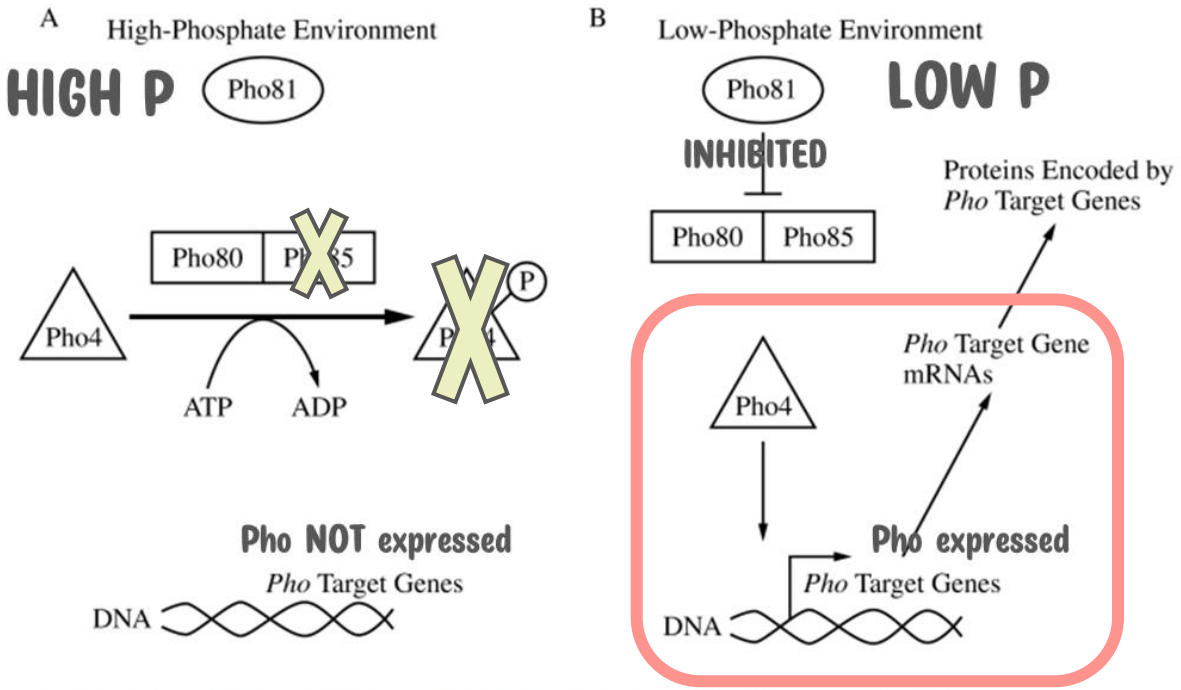
(d) In a follow-up experiment, researchers created a strain of yeast with a mutation that resulted in a nonfunctional Pho85 protein. Based on Figure 1, **predict** the effects of this mutation on *PHO1* expression in the mutant strain in a high-Pi environment. Provide reasoning to **justify** your prediction.



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- It/*PHO1*/Target genes will be expressed.

Provide reasoning to **justify** your prediction.

- (In a high-Pi environment) a nonfunctional Pho85 will be unable to phosphorylate/inhibit Pho4.

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d. A mutation resulting in nonfunctional Pho85 would allow transcription of the Pho target gene despite a High Pi environment. This is because Pho85 would not be able to form the Pho80-Pho85 complex and phosphorylize Pho4. Therefore, Pho4 will transcribe the Pho target genes even when it should not, leading to increased PHO1 expression.