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COMMENTARY

Learning from Adversity?

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ABSTRACT Many two-component regulatory systems, including *Escherichia coli* PhoRB, are positively autoregulated, so stimuli result in an increase in the concentration of signaling proteins. When the quantity of signaling proteins depends on exposure history, how do past conditions affect future responses to stimuli? Hoffer et al. (J. Bacteriol. 183: 4914–4917, 2001, https://doi.org/doi:10.1128/JB.183.16.4914-4917.2001) previously reported that *E. coli* bacteria "learn" from phosphate starvation and respond more rapidly to subsequent episodes of starvation. Gao et al. (J. Bacteriol. 199:e00390-17, 2017, https://doi.org/doi:10.1128/JB.00390-17) describe another aspect of hysteresis in the PhoRB regulon. Phosphate starvation also leads to a global decline in transcription, counteracting the effects of positive autoregulation and resulting in a similar net *pho* response (homeostasis), regardless of exposure history.

KEYWORDS homeostasis, hysteresis, PhoB, PhoR, positive autoregulation, two-component systems

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A striking feature of many bacterial species is the ability to thrive under diverse environmental conditions. For the sake of efficiency, these versatile capabilities are not constitutively expressed but are instead turned on in a "just in time" manner when actually needed. Thus, bacteria continuously monitor properties of interest in their environment (e.g., nutrients, pH, the presence of other organisms, etc.) and respond to the challenges and opportunities posed by changing conditions.

Positive autoregulation of two-component systems can lead to hysteresis. Two-component regulatory systems are used by most bacteria to sense and respond to environmental change. In the basic scheme (1), transmembrane sensor kinases monitor specific environmental conditions and record that information in the form of phosphoryl groups covalently attached to a cytoplasmic domain. The phosphoryl groups are then transferred to partner response regulator proteins, which modulate appropriate adaptive responses (most commonly, changes in gene expression) based on their phosphorylation state. Loss of phosphoryl groups from the response regulators usually terminates activation of the response, although the products of the response may persist for some time.

The manner in which the basic elements of a two-component system are functionally connected contributes to the information-processing properties of the circuit (2). For example, many two-component systems positively autoregulate expression of the genes encoding their sensor kinase and response regulator (3). Basal levels of sensor proteins monitor the environment at minimal energetic cost. When conditions warrant a response, a positive-feedback loop substantially increases the population of sensor kinase and response regulator proteins available to detect and respond to the situation, which in turn affects the magnitude and kinetics of the response. A question concerning hysteresis then arises—how do the responses of bacteria to fluctuating conditions depend on their history of exposure? (I use the term "hysteresis" in the broad sense that the responses of a system to the same input differ depending on system history, rather than the commonly used but narrower sense that approaching a condition from opposite directions yields different responses [4], such as occurs in thermal hysteresis, where the melting and freezing points of a solution are different [5].)

Can *Escherichia coli* "remember" and "learn" from phosphate starvation? As a case in point, the *E. coli* PhoRB two-component system governs assimilation of the essential nutrient phosphorus. As inorganic phosphate (P_i) sensed via PhoR becomes depleted, PhoB activates transcription of genes in the *pho* regulon in a hierarchical manner, sequentially implementing increasingly desperate measures to obtain phosphorus (6). In 2001, Hoffer et al. (7) reported an interesting consequence of PhoRB autoregulation. If cells initially starved of P_i are exposed to excess P_i and subsequently starved of P_i again, they respond to the second starvation event more rapidly than cells that had not previously experienced starvation. The increased levels of PhoRB generated by positive autoregulation during the initial starvation event allow bacteria to "learn" from their experience and respond more quickly when starved for a second time. However, the "memory" of starvation fades as the duration of the intervening period of P_i abundance increases. Rong Gao, Ann Stock, and colleagues have revisited the phenomenon of P_i starvation and hysteresis in more detail, with intriguing results (8). The present work is the latest in a series of studies by Gao and Stock (6, 9–11) that combine clever analyses of experimental data with computer modeling to explore the *in vivo* performance of PhoRB.

Starting with a yellow fluorescent protein reporter expressed from the alkaline phosphatase promoter, Gao et al. (8) used the time derivative of fluorescence to measure PhoRB-regulated promoter activity. The authors first provided a simple explanation for the results of Hoffer et al. (7) by demonstrating that the amounts of PhoRB in the cell could account for creation and loss of "memories" of P_i starvation as expected. (i) Changing the basal level of PhoRB via an inducible promoter altered the kinetics of response to P_i starvation (more PhoRB resulted in a faster response). (ii) When PhoRB was not being synthesized, the concentration of existing PhoB declined at the rate expected for dilution through cell growth and division.

The stress of phosphate starvation apparently leads to response homeostasis. A thorough investigation of the cellular response to cycles of P_i starvation and abundance revealed surprising results. Although cells grown in abundant P_i for shorter times between P_i starvation events responded more rapidly to the second starvation (as expected from conditions that included a lower dilution of PhoRB), promoter activity declined after the initial response. The decline was greater for cells that experienced shorter recovery times between starvation events. Counterbalancing effects of positive autoregulation of PhoRB and negative regulation of promoter activity resulted in similar levels of reporter expression (reflecting the net response to a given level of P_i starvation) regardless of history (Fig. 1). This remarkable, but not perfect, homeostasis of Pho system output strongly suggests that the observed response was nearly optimal. The convergence of Pho responses from different starting points is particularly striking because Gao and Stock previously also demonstrated that the autoregulated amounts of PhoRB signaling proteins generated under conditions of various degrees of P_i starvation provide optimal fitness (9).

Additional experiments provided important clues to the mechanism of reduced promoter activity. The history-dependent decline in promoter activity was not a consequence of PhoRB autoregulation. All tested promoters were affected similarly, including a promoter not regulated by PhoB. Because P_i starvation is known to induce stress responses (12, 13), a plausible explanation for the observed decline in promoter activity is simple competition for RNA polymerase between the housekeeping σ^{70} and the stationary-phase σ^S proteins. In this scheme, the negative "memory" of stress caused by P_i starvation dilutes during growth in excess P_i in a similar manner to the positive "memory" embodied in PhoRB. A mutant lacking σ^S did not support homeostasis, and a computer simulation incorporating the sigma factor competition hypothesis faithfully reproduced all features of the experimental data. Nevertheless, stress responses are complex and the mechanism(s) actually responsible for negative regulation of promoter activity remains to be determined.

What's next? In addition to its use in deciphering the mechanism(s) of promoter inactivation, the PhoRB system appears to be rich with opportunities for future inves-

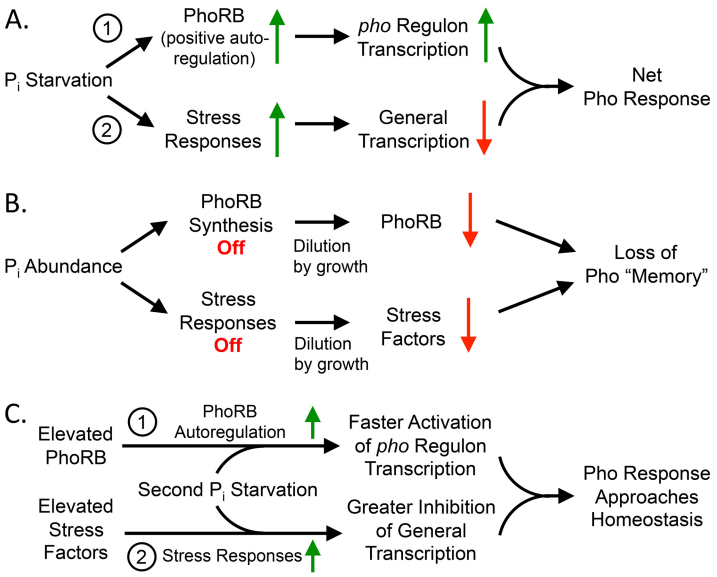

FIG 1 Model of hysteresis and homeostasis in the *E. coli* PhoRB system. (A) P_i starvation activates PhoRB, resulting in more PhoRB and increased transcription of the *pho* regulon (path 1). P_i starvation also causes stress, which diminishes global transcription (path 2). The sum of these two effects determines the net Pho response. (B) When P_i is abundant, additional PhoRB and stress factors are not synthesized. The concentrations of PhoRB and stress factors remaining from previous episodes of P_i starvation decrease exponentially due to cell division, erasing the "memories" of past P_i starvation events. (C) History affects the kinetics of *pho* output (hysteresis). Cells that previously experienced P_i starvation consequently contain increased amounts of PhoRB. If subsequent growth in P_i abundance is too brief to eliminate the excess PhoRB, then such cells initially respond more rapidly to a second P_i starvation event (path 1), because they are starting from a higher baseline of PhoRB than naive cells. Similarly, previously starved cells also have accumulated stress factors and so more extensively diminish general transcription (path 2) when starved again. Together, these two effects result in similar final *pho* outputs (homeostasis) regardless of history.

tigation. The existing computer model may provide a head start. *In silico* studies allow rapid and convenient predictions regarding system behavior(s) under many permutations of conditions such as the duration and magnitude of P_i starvation. Investigators can then choose to experimentally test the most informative predictions. (i) For example, what happens when more than two episodes of P_i starvation occur? Is the history completely erased during intervening periods of P_i abundance or does it continue to accumulate? (ii) The present work focused on activity of the *phoA* promoter. Does the near-homeostasis of the Pho response apply to all levels of the *pho* regulon hierarchy? (iii) What happens under conditions of intermediate rather than extreme levels of P_i limitation? In the absence of full starvation for P_i, stress responses might not be sufficient to cause the negative regulation of global transcription that led the Pho response to approach homeostasis. (iv) Positive autoregulation can lead to bistable circuit conditions and mixed populations of "on" and "off" cells, if conditions are such that some cells cross the threshold for autoamplification whereas others do not. The *pho* response exhibits just such heterogeneity in the artificial circumstance represented by cells that lack PhoR and activate PhoB by inefficient cross talk from a nonpartner sensor kinase (14). On the other hand, when *E. coli* bacteria with the wild-type PhoRB system are starved once for P_i, the population response is homogeneous and bistability is not seen (9). What happens with a more complex exposure history? The experiments reported by Gao et al. (8) reasonably examined populations rather than single cells. Could circumstances in which cycles of starvation and abundance lead to bistability have been overlooked, particularly if the positive and negative regulatory effects were not precisely balanced?

What is bacterial memory? There is a fascinating literature on phenomena that may be interpreted as bacterial memory, learning, or anticipation (15–19). However, it is much easier to design experiments to determine the mechanism of a system than to

deduce its purpose, and data are subject to multiple interpretations. For example, positive autoregulation has been alternately regarded as a mechanism for memory and learning (7) or as a means to smooth out responses to a fluctuating environment (20). Nevertheless, there are unambiguous examples of bacterial memory. The methylation status of chemoreceptors provides short-term memory of chemoeffector exposure that extends several seconds into the past (21, 22). By comparing current conditions to the immediate past, bacteria decide whether to continue on the same path or change direction, thus accomplishing chemotaxis. CRISPR (clustered regularly interspaced short palindromic repeat) provides a long-term memory of exposure to foreign genetic elements, written into the genetic material of the cell to ensure transmission to future generations (23). Regardless of whether the phenomena exhibited by PhoRB are widely accepted to constitute "memory," the work of Gao et al. suggests that *E. coli* has learned the value of an optimal response.

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