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Positive feedback in cellular control systems

Alexander Y. Mitrophanov and **Eduardo A. Groisman***
Howard Hughes Medical Institute, Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO.

Abstract

Summary—Feedback loops have been identified in a variety of regulatory systems and organisms. While feedback loops of the same type (negative or positive) tend to have properties in common, they can play distinctively diverse roles in different regulatory systems, where they can affect virulence in a pathogenic bacterium, maturation patterns of vertebrate oocytes and transitions through cell cycle phases in eukaryotic cells. This review focuses on the properties and functions of positive feedback in biological systems, including bistability, hysteresis and activation surges.

Introduction

A biochemical control system comprises a set of components (molecules, genes, etc), and a set of regulatory interactions.(1–4) An interaction is designated positive if activation or accumulation of a component leads to activation or accumulation of another component, and negative if activation or accumulation of a component leads to deactivation or depletion of another component.(1,4) If the structure of a system is such that a certain component influences its own activity and/or levels, then this component is said to regulate itself via a feedback loop (Fig. 1) (see Box 1 for a Glossary).

Box 1. Glossary

Feedback: the property of a control system to use its output as (a part of) its input.

Positive feedback: the type of feedback when a deviation in the controlled quantity is further amplified by the control system.

Negative feedback: the type of feedback when a deviation in the controlled quantity is counterbalanced by the control system.

Deterministic system: a system with exactly predictable (non-random) behavior; this term is often applied to systems that can be described by differential equations.

Stable steady state: the state of a deterministic systems such that all trajectories that start from a certain domain in the state space converge to this state.

Bistability: the property of a deterministic system to have two stable steady states.

*Correspondence to: Eduardo A. Groisman, Howard Hughes Medical Institute, Department of Molecular Microbiology, Washington University School of Medicine, Campus Box 8230, 660 South Euclid Avenue, St. Louis, MO 63110. E-mail: groisman@borcim.wustl.edu.

Bimodality: the property of a probability distribution to have two distinct maxima.

Signal–response curve: the curve reflecting the dependency of the output (response) of a deterministic system on the incoming signal (represented by a parameter).

Hysteresis: dependency of the steady-state response curve of a deterministic system on the direction of the parameter change (increase or decrease).

Ultrasensitivity: the property of a system to generate a sharp, switch-like response, resembling that of a positively cooperative enzyme; this type of response is typically described by a sigmoidal signal–response curve.

The notion of feedback was first introduced in cybernetics to denote the ability of a control system to adjust itself using its output as (a part of) its input (Fig. 1A).(5,6) The output constitutes the specific property that the system controls. In systems with negative feedback, a deviation in the output results in changes in the direction opposite to the original deviation. By contrast, in systems with positive feedback, a deviation in the output causes the output to change even more in the direction of the original deviation.(5) As a result, negative feedback generally serves to stabilize the state of the controlled system, whereas positive feedback amplifies deviations and triggers state changes.(1)

Positive and negative feedback loops may consist of a single component that activates and represses directly its own activity, respectively (Fig. 1B,C); or they may include several components and involve indirect interactions (Fig. 1D–G). The overall sign of a complex feedback loop (i.e. positive or negative) depends on the constituting elementary interactions (Fig. 1D–G).(1) For example, two mutually repressing components form a positive feedback loop (PFL, also termed “double-negative feedback loop”) (Fig. 1D). This is also true of circular regulatory cascades consisting only of positive regulators or having an even number of negative regulators. By contrast, a circular cascade consisting of an odd number of negative regulators forms a negative feedback loop (NFL).

In this review, we discuss the behaviors promoted by positive feedback in the regulation of cellular processes. Perhaps the earliest example of biological feedback control was end-product inhibition in enzymatic pathways whereby the final product of a biochemical pathway inhibits the activity of an enzyme operating early in the pathway. For example, the biosynthesis of L-isoleucine requires L-threonine deaminase, the activity of which is inhibited by L-isoleucine.(7) Later, it became evident that feedback also plays a critical role in gene regulation.

The extensively investigated *lac* operon of *Escherichia coli*(8) and the lysis–lysogeny decision circuit of phage lambda(9) exemplify systems with positive feedback. The main component of the feedback circuit of the *lac* operon is the repressor LacI, which, upon binding the inducer allolactose, loses the ability to bind to the *lac* operator and repress transcription of the *lacZYA* operon. Accumulation of allolactose thus leads to elevated expression of the transport protein LacY, which in turn increases the rate of lactose intake and its conversion into allolactose. While this circuit constitutes a multi-component PFL, there is also a NFL because LacZ metabolizes allolactose thus decreasing its availability to LacI, which would eventually lead to repression of the lacZYA operon.(10) The circuit governing the lysis–lysogeny decision of phage lambda includes the main regulator CI that directly activates its own expression. However, at very high levels, CI represses its own transcription, thus preventing CI over-expression.(9) CI also represses the cro gene, whose product is a repressor of both the *cI* and *cro* genes, which results in additional feedback loops.(9)

The type of feedback in which proteins directly regulate their own expression—termed "autogenous regulation"—received special attention in the mid-1970s.(11–13) A theoretical study argued against the existence of positive autogenous regulation because such circuits were expected to be disadvantageous with respect to a number of functional criteria.(12) To provide empirical support for this argument, the study indicated that the only known examples of self-regulating transcription factors in enteric bacteria were repressors. However, as the information about bacterial control systems accumulated, it has become clear that a considerable number of regulatory proteins directly activate transcription of their own genes. Indeed, several of the 49 autoregulated transcription factors in *E. coli* promote their own expression.(14) The wide occurrence of positive autoregulation raises questions of the biological roles of this mode of control and of positive feedback in general.

Positive feedback can provide an efficient switching mechanism

Regulatory systems allow living cells to alter biochemical processes or gene expression programs in response to changes in the intracellular and/or extracellular environments. Then, what advantages does positive feedback provide in terms of a system's switching efficiency? How does the presence of a PFL enhance the cell's ability to respond to environmental signals?

Mathematical modeling demonstrates that positive feedback contributes to the efficiency of a transcriptional regulatory system (Box 2; Fig. 2). Consider the case when the autogenous regulator must be activated (e.g. phosphorylated) to exert its biological activity, such as the ability to bind to a promoter and mediate gene transcription (Fig. 1G). This mode of positive regulation is typical of a variety of bacterial signal transduction pathways,(15) such as those governing sporulation in the Gram-positive *Bacillus subtilis*(16) and virulence in the Gram-negative pathogen *Salmonella enterica*.(17) In the absence of an activating signal, the rate of dephosphorylation of the regulatory protein will be higher than the phosphorylation rate so that the existing regulator molecules will be largely unphosphorylated. The production rate and, therefore, the levels of the regulatory protein are low, because there is very little induction in the absence of phosphorylation (Fig. 2). Having more protein would not increase a cell's capacity to activate genes, because the regulator molecules would still be unphosphorylated and therefore inactive. Thus, by limiting the production of unphosphorylated regulator, the cell avoids wasting its resources, and may also circumvent the possibility of having the regulatory protein phosphorylated by a non-physiological partner in response to a non-physiological signal.(18)

When a signal promotes phosphorylation of an autogenous regulator, the number of phosphorylated regulator molecules goes up, which, due to positive feedback, leads to further increases in the total regulator levels, as well as the levels of the phosphorylated form (Fig. 2, solid lines). By contrast, if the inducible promoter is replaced by a constitutive promoter, the total levels of the regulator will not depend on the phosphorylation or dephosphorylation rates (Fig. 2, green dashed line). Therefore, under non-activating conditions such a system will contain many unphosphorylated, and thus inactive, regulator molecules. Indeed, under such conditions, the total regulator level (Fig. 2, green dashed line) is significantly higher than the phosphorylated regulator level (Fig. 2, blue dashed line). While the total regulator level would be lower in a system with a weaker constitutive promoter, this would also decrease the level of phosphorylated regulator (Fig. 2, blue dash-dotted line), which may limit the number of targets that the phosphorylated regulator can control effectively.

Box 2. Mathematical model of gene regulation with positive feedback

In a simple transcriptional regulatory circuit, a regulator in its activated (e.g. phosphorylated) form directly induces transcription of its own gene (Fig. 1G). A prototypical system of this type is the PhoP/PhoQ system of *S. enterica*, in which the *phoPQ* operon has a constitutive promoter and a promoter that can be induced by phosphorylated PhoP (Fig. 6).(93) The dynamics of gene regulation can be quantitatively described using the formalism of ordinary differential equations.(63,101–103) Our model consists of the following two equations:

$$\frac{dA}{dt} = k_a P - (k_{-a} + k_d) A;$$
$$\frac{dP}{dt} = k_1 + k_2 \frac{KA^H}{1 + KA^H} + k_{-a} A - (k_a + k_d) P.$$

In these equations, A and P are the concentrations of the active (phosphorylated) and inactive (unphosphorylated) forms of the regulatory protein, respectively; k_a and k_{-a} are the phosphorylation and dephosphorylation rates for the regulatory protein. The parameter k_d is its degradation/dilution rate for the unphosphorylated and phosphorylated forms of the regulator. Whereas in the general case these two forms can decay at different rates, here we make the simplifying assumption that these rates are equal. k_1 is the rate of protein synthesis due to the constitutive promoter (assumed to be weak), and k_2 is the protein synthesis rate due to the inducible promoter. k_a and k_{-a} are also the control parameters for the circuit: by adjusting the cell regulates the levels of the active regulator. The parameter K is the association constant for regulator–promoter interactions. H is the so-called Hill coefficient;(65,70,102,104,105) it describes cooperativity of binding of the regulator to its own promoter. If the regulator protein binds DNA as a dimer, which is frequently the case in bacterial signal transduction, then $H = 2$.(106) The equations describing the absence of feedback can be obtained in the limit $K \to \infty$: in this case, there are two constitutive promoters with protein production rates k_1 and k_2. We can also model feedback disruption by setting $k_2 = 0$, which is equivalent to inactivation of the inducible promoter.

The computed dynamics of the active form of the regulator, as well as its total levels, are shown in Figs 2 and 3. Fig. 2 was generated with the following default parameter values: k_a=5 min^{-1}, k_{-a}=20 min^{-1}, k_1=0.01 μM·min^{-1}, k_2=0.3 μM·min^{-1}, k_d=0.08 min^{-1}, K = 5 μM^{-2} H=2. These parameter values were chosen to be close to biologically significant values. In the case of two constitutive promoters (K = ∞), the total regulator concentration (Fig. 2, green dashed line) remains at a constant level that is close to the steady-state level for the system with feedback (Fig. 2, blue dashed line). This agrees with experimental observations for the transcriptional regulator PhoP of *S. enterica*.(17) If the inducible promoter is disrupted ($k_2 = 0$), then the modeling results show a substantial decrease in the total regulator levels (Fig. 2, brown dash-dotted line), which also agrees with experimental results for PhoP (D. Shin and E. A. Groisman, unpublished). The ability of the model to reproduce the above experimental results is robust with respect to parameter variations. Fig. 3A was generated with the following default parameter values: k_a=25 min^{-1}, k_{-a}=20 min^{-1}, k_1=0.001 μM·min^{-1}, k_2=0.3 μM·min^{-1}, k_d=0.08 min^{-1}, K=5 μM^{-3}, H=3. Fig. 3B was generated with the following parameter values: k_a=25 min^{-1}, k_{-a}=20 min^{-1}, k_1=0.01755 μM·min^{-1}, k_2=0.3 μM·$^{-1}$, k_d=0.08 min^{-1}, K=5 μM^{-3}, H=3.

Systems with positive feedback can display a slower response to an environmental signal when compared to those that produce a regulatory protein constitutively (Fig. 2).(12,19,20)

Activation delays are due to the need to synthesize more regulator molecules upon activation, while in the case of constitutive production, the molecules have already been synthesized and just need to be phosphorylated. The extent of the activation delay, however, will depend on the kinetic parameters of a system and may be negligible. On the other hand, medium to large delays can sometimes be beneficial as they can provide a means to order in time the action of cellular response mechanisms.(21–23)

Many eukaryotic cellular control systems contain a fast and a slow PFL.(24,25) The presence of several interlinked feedback loops can enhance switching performance. While single PFLs tend to amplify noise(19) and to slow down activation, a combination of a slow and a fast PFL increases robustness of the ON-state in the presence of noise, and promotes a quick turn-on and a slow turn-off.(24) Analysis of a systems having two (fast and slow) PFLs and a slow NFL demonstrated that the two PFLs confer rapid activation, persistence of the system's ON-state, and insensitivity to noise, whereas the NFL is responsible for efficient deactivation in the absence of signal.(25) PFL cascades and combinations of PFLs and NFLs can also promote excitability, which is the ability of a system to become activated in response to relatively small perturbations in the input signal, and then exit the ON-state spontaneously.(26,27) This behavior may be advantageous when it is desirable to limit the time that the system spends in the ON-state; this is the case, for instance, in bacterial competence control.(27–29)

Positive feedback can promote bistability and hysteresis

Perhaps the most-studied dynamic feature of control circuits with positive feedback is bistability(1,16,30–59) (for reviews, see Refs 10,60), which is intrinsic to sporulation(16) and competence(48,57) in the bacterium *B. subtilis*, the control of the eukaryotic cell cycle(51–53) and the maturation of frog oocytes.(37,60) A system is called bistable if it has two stable steady states. The term “bistability” is used to characterize a system that can be described by a set of variables whose values change over time as deterministic (non-random) functions. In a biochemical setting, such variables usually correspond to the concentrations of the key molecular species in the system. Deterministic descriptions are valid for the traditional “batch culture” biological experiments, where averaging over large populations of cells masks random variations of intracellular concentrations of the relevant chemical species. Typically, these concentrations converge to certain values over large periods of time, as long as the system is not perturbed externally. The values that characterize the state of the system “when nothing happens” correspond to stable steady states. Convergence to one of the two stable steady states in a bistable system depends on the initial conditions (Box 3; Fig. 3). One of the two steady states generally corresponds to low activity of the system, and the other one to high activity. For example, in the *E. coli* lactose utilization system (mediated by the *lac* operon), β-galactosidase activity can converge to one of two steady states depending on the initial concentrations of the system's components.(61) Likewise, the cell cycle control systems of eukaryotes has two stable states, interphase and mitosis, that are characterized by low and high activity of the kinase Cdc2, respectively.(53,62,63)

The property of bistability is closely associated with the property of distributional bimodality for clonal cell populations. At the single-cell level, a biochemical system can be described by the distributions of its biochemical components (Fig. 4). Such distributions show how many cells in a population have a particular number of molecules (or a particular concentration) of a biochemical component. If we average the distributions, we will obtain a set of mean values that can be viewed as the state of a deterministic system (i.e. the batch-culture approach). Such a system can possess the property of bistability. If it is bistable with respect to a biochemical component, then the intracellular levels of this component in a

clonal population are likely characterized by a bimodal distribution (Fig. 4B).(32,49,64) Because bimodality is relatively easy to study experimentally, some researchers have adopted the term "bistability" as a synonym for distributional bimodality of the biochemical component of interest or relevant reporter molecules (Box 3).(10,35,48,57,58)

The strong connection between positive feedback and bistability arises from the fact that a PFL is necessary, but not sufficient for bistability.(2,40,60) Therefore, it should be expected that the key regulator(s) of a bistable circuit will be involved in a PFL. This principle can be used as a guideline to identify candidate regulators for experimental studies when the detailed architecture of the bistable control circuit is unknown.(4)

All bistable systems are expected to display some degree of hysteresis,(38,60) which is the ability to produce different steady-state signal–response curves for the cases of increasing and decreasing stimulus intensity (Fig. 5A,B).(10,60) Bistable systems are also characterized by an abrupt transition from a low to a high steady state after the activating signal passes a certain threshold value (Fig. 5A,B). Although a similar phenomenon can be observed for monostable systems with very steep signal–response curves (Fig. 5C),(65) monostable systems do not demonstrate hysteresis. An extreme manifestation of hysteresis is irreversibility, which takes place when changing the signal intensity from high to low fails to bring the system back to the initial inactive state (Fig. 5B).(60,66,67) This occurs when two stable steady states, high and low, coexist for arbitrary small signal intensities. The irreversibility implies that, after the stimulus has been removed, the system will be trapped in the activated state, unable to return to the basal level of activity.

Box 3. Conditions and attributes of bistability

In addition to positive feedback (a necessary condition), requirements for bistability usually include the presence of a functional element with a sigmoidal signal–response curve within the feedback loop.(10,38,60) Sigmoidality guarantees a sharp, threshold-like ("ultrasensitive") response to the activating stimulus. In mathematical modeling studies, sigmoidal functions are typically approximated using Hill functions with Hill coefficient >1 (see Box 2).(65,70,102,104,105) Increases in the Hill coefficient tend to favor bistability, but changes in other parameters can make the system monostable (Fig. 3A). An example of molecular mechanism leading to sigmoidal response is cooperative binding of a regulator protein to DNA, or binding in the form of a multimer.(106) It should be noted, however, that other, not necessarily sigmoidal, signal–response curves can result in bistability.(107) While a system with a PFL can have at most two stable steady states, the presence of several PFLs can result in multistability.(4) Some monostable systems can demonstrate quasi-bistable behavior: over large periods of time, the system appears to be "stuck" in one of two states, and the convergence from these states to the unique steady state is very slow (Fig. 3B). This seemingly bistable behavior can have biological roles, because the true unique steady state might not be reachable over biologically reasonable time scales.

If the concentrations of components in a biochemical system are low, stochastic fluctuations in the concentration values cannot be neglected; this phenomenon is one of the major factors contributing to physiological heterogeneity of populations of genetically identical cells.(64,72,108,109) Bimodal distributions for (bio)chemical systems are often associated with bistability of the deterministic model of the system, which describes the temporal dynamics of average concentrations.(32,49,64) In rigorous terms, bistability is not equivalent to distributional bimodality.(110) In relatively simple chemical systems with mass action kinetics, bistability is neither necessary nor sufficient for bimodality; however, when the system is large enough, the two properties tend to be present simultaneously.(64) This raises the possibility that the same general rule will

apply to complex biochemical systems. It should also be mentioned that, while bistability characterizes steady states, experimentally observed bimodality does not necessarily correspond to a steady state of the system.(48)

The best-characterized examples of a PFL promoting distributional bimodality are synthetic transcriptional regulatory circuits implemented in model organisms.(32,39) A synthetic system was engineered in *Saccharomyces cerevisiae* where the main component was the tetracycline-responsive transactivator (rtTA), which assumes an active form and binds DNA in the presence of doxycycline.(32) In this strain, rtTA was fused to the green fluorescent protein (GFP), making it possible to monitor the levels of the regulator expressed from an rtTA-activated promoter. For a range of doxycycline concentrations, the distribution of GFP fluorescence in the system was bimodal, with the peak corresponding to high expression being more pronounced for higher doxycycline concentrations.(32) Bistability has been demonstrated not only for the relatively simple synthetic regulatory circuits, but also for the well-studied *lac* operon of *E. coli*(49) and in the lysis–lysogeny circuit of phage lambda.(46,68)

Biological roles of bistability and hysteresis

Genetic, metabolic and signaling regulatory systems with bistable behavior (or, more precisely, bimodal distributions of reporter signal intensity) have been found both in prokaryotes(10,16,35,48,69) and eukaryotes.(30,31,33,50,58,59,70,71) While all these systems are known (or expected) to contain PFLs, they differ in the feedback loop structure, regulatory elements and their overall complexity. Wide occurrence of bistability among different types of biological regulatory systems raises the question of the biological significance of bistability.

Cell population heterogeneity: bacterial sporulation and competence control

Heterogeneity of a clonal cell population, which is promoted by the intrinsic randomness of molecular mechanisms of gene expression,(29,71,72) can often be attributed to bistable regulation.(31,37,57,69,73) Therefore, whenever associated with survival advantages, heterogeneity itself could be viewed as a biological role of positive feedback. This appears to be the case in the complex sporulation control system of *B. subtilis*.(10,16,35) When this soil bacterium experiences nutrient-limiting conditions, it undergoes dramatic morphological and physiological changes to generate a dormant spore. Only a percentage of cells in a *B. subtilis* population sporulate in response to nutrient limitation, which indicates bistable behavior.(10) Commitment to sporulation is modulated by the master regulator Spo0A which directly activates its own expression(16) and participates in a complex phosphorelay that constitutes a multicomponent feedback loop promoting Spo0A activation. Positive autogenous regulation of Spo0A is necessary for coexistence of two (sporulating and nonsporulating) subpopulations.(16) Phenotypic heterogeneity may be a mechanism that increases the chances of the population's survival in a randomly changing environment.(35) Sporulation is an expensive and irreversible process; therefore, having only a subpopulation of cells committed to sporulation appears to be a clever strategy in an environment where harsh conditions can be reversed, thus alleviating the necessity to sporulate.(35)

Genetic competence is the property of bacterial cells to directly uptake DNA from their surroundings.(28) The competence regulation circuit of *B. subtilis* displays characteristics of bistable behavior; a cell population in the late exponential phase typically consists of subpopulations of competent and non-competent cells.(10,48,57,74) Similarly to Spo0A, the key regulator of competence, ComK, directly activates its own expression, and also participates in additional feedback loops. Auto-activation of ComK is necessary for

heterogeneity.(57) The size of the competent subpopulation is typically small: the proportion of competent cells in laboratory strains is only ~10%, and for wild isolates ~1%.(35,48) Although enriching the genome with foreign DNA can confer new traits to a population, the prolonged semidormancy that accompanies the competent state can pose a challenge for survival.(48,75) In addition, efficient genetic exchange can be detrimental, especially in the case of interspecies exchange.(28) Thus, this heterogeneity-generating mechanism of *B. subtilis* might have evolved in a way that maximizes benefit-to-risk ratio by triggering competence only in a small subpopulation of cells.(48)

Eukaryotic cell fate determination

Bistability is used by eukaryotes as a mechanism of cell fate determination. (37,45,47,54,59,60) For example, maturation of *Xenopus laevis* oocytes is controlled by a protein kinase cascade (termed the Mos-Mek-MAPK cascade) which is activated by the hormone progesterone, and demonstrates bistable behavior.(37,59,60) Treatment of immature oocytes with progesterone causes sequential accumulation and/or activation of the kinases Mos, MEK, and p42 MAPK. The MAPK promotes activation of the complex of cyclin B with the cyclin-dependent kinase Cdc2, which in turn triggers maturation. MAPK also stimulates Cdc2-mediated Mos accumulation; therefore, the cascade is a multicomponent PFL. By probing individual progesterone-treated oocytes, it was demonstrated that a group of oocytes consisted of a maturating subpopulation (high levels of phosphorylated MAPK kinase, or MAPK-P) and a non-maturating subpopulation (with no detectable MAPK-P).(37) For low concentrations of progesterone, the majority of the oocytes did not maturate, whereas high progesterone caused most of the oocytes to maturate. For intermediate concentrations of progesterone, the two sub-populations had comparable size, but no oocyte had an intermediate MAPK-P level. The presence of distinct coexisting oocyte subpopulations reflects the normal logic of cell fate determination: an oocyte should either maturate or not. Notably, after the removal of progesterone, mature oocytes did not de-mature, which is indicative of irreversible behavior.(59) Inhibition of protein synthesis abrogated distributional bimodality of MAPK-P levels and irreversibility of oocyte maturation by disrupting the protein synthesis-dependent multicomponent positive feedback loop.(59,70)

Eukaryotic cell cycle oscillations

Hysteretic behavior is an aspect of bistable systems with particular significance for biochemical oscillations.(51,52) As an inherent property of bistable systems, hysteresis can be used to detect bistability. Thus, hysteresis was used to distinguish between two alternative models of abrupt switching between high and low Cdc2 activity levels in the course of mitosis in cell-free *X. laevis* egg extracts.(53) The protein kinase Cdc2 is a key player in mitosis. The activity of Cdc2 is modulated by another protein, cyclin B, whose concentration oscillates as the cell repeatedly goes through the phases of the cell cycle. The basic mechanisms of Cdc2 activity control have been understood by studying a system where the synthesis of endogenous cyclin B was blocked, and a non-degradable cyclin B (Δcyclin B) was used instead (which ensured constant total cyclin B levels).(52,53,76) The Δcyclin B–Cdc2 system did not exhibit sustained oscillations, but reached a steady state that depended on the levels of Δcyclin B. Hysteretic behavior in the Δcyclin B–Cdc2 system was predicted using a mathematical model of the cell cycle.(62,77) Hysteresis implies that the cyclin B level threshold to enter mitosis is higher than the corresponding threshold to exit mitosis.(53,78) Experimental measurements showed a notable difference between these thresholds, thus confirming the hysteretic behavior of the system;(52,53) similar results have been obtained for cell cycle control in budding yeast.(34,79)

The role of a bistable circuit in mitosis control is efficient toggling between two distinct states—interphase and early mitosis—excluding the possibility of the cell resting in an intermediate state.(52,62) A central component of the mitosis control system is the positive feedback loop in which the active Cdc2–cyclin B complex inactivates its inhibitors, but there are also other positive feedback loops and a negative feedback loop in the system.(51,52,60) The circuit thus exemplifies a regulatory design that promotes sustained oscillations through a combination of positive and negative feedback.(51,52,60) Oscillators of this type control a variety of cellular processes(34,79–81) (including circadian cycles),(82–84) and possess advantageous features such as robustness, noise resistance and synchronizability.(41,82,84,85)

Positive feedback in two-component signal transduction systems

Two-component systems constitute the most prevalent form of bacterial signal transduction.(86,87) A two-component signal transduction system consists of two proteins, a sensor kinase and a response regulator.(15) The sensor kinase is a transmembrane kinase (which can also display phosphatase activity), and the response regulator is typically a transcription factor. In response to an environmental signal, the sensor kinase autophosphorylates from ATP and then transfers the phosphoryl group to the response regulator. This increases the ability of the regulator to bind DNA and modulate gene expression. Besides eubacteria, two-component systems are found in archaea and some cell-wall-containing eukaryotes.(15,88) Positive feedback is a frequent property of two-component systems because many response regulators activate their own expression.(17,22,89–92) There are several functional roles of positive autoregulation in two-component signal transduction, including the generation of activation surges, transcriptional memory and hierarchical organization of a regulon.

Insights into the possible roles of such regulation were obtained in the studies of the two-component system PhoP/PhoQ, which is a critical regulator of S. enterica virulence.(17,93) The *phoP* and *phoQ* genes are parts of a bi-cistronic operon, which is transcribed from a constitutive promoter and from a PhoP-activated promoter; thus, the expression of PhoP is regulated via a PFL. Shifting *S. enterica* from repressing to inducing conditions for the PhoP/PhoQ system results in a transcription surge: the mRNA levels of PhoP-activated genes peak at 20–30 minutes upon activation, and then decrease to reach a steady state by ~60 minutes (Fig. 6).(17) This overshoot behavior is not due to PhoP synthesis and subsequent decay because the total level of PhoP increases monotonically upon activation. Temporal changes in the PhoP-dependent mRNA levels reflect the changes in the level of phosphorylated PhoP, which is also characterized by a surge, and correlate with PhoP binding to the promoters of PhoP-activated genes (Fig. 6). Although positive autoregulation of the PhoP/PhoQ system is normally required for the surge observed in wild-type *Salmonella*, a surge has also been observed when the *phoPQ* operon was expressed from a heterologous promoter.(17)

The autoregulation-dependent surge is essential for S. enterica virulence, because the mutant strain with disrupted PhoP autoregulation lost the ability to cause a lethal infection in mice even though it produced the same steady-state levels of PhoP-activated mRNAs as the strain with the wild-type autoregulated promoter.(17) The transient increase in the PhoP activity may allow the establishment of a new phenotypic state, which enables the bacterium to react adequately to the environment that triggered PhoP/PhoQ activation. The transition to this new state might be promoted by the expression of PhoP-activated genes exceeding some threshold value at their peak intensities. The steady-state expression levels would then guarantee that the new phenotypic state is maintained as long as the PhoP/PhoQ system is active.(17)

Transient surge-like activation patterns have also been observed for several other two-component systems that activate their own expression. These include the PmrA/PmrB system of *S. enterica*,(17) the CusR/CusS(92) and KdpE/KdpE(91) systems of *E. coli*, the VanR/VanS system of *Streptomyces coelicolor*(90) and the ComE/ComD system of *Streptococcus pneumoniae*,(89) which control different physiological functions and include Gram-positive and Gram-negative bacterial species. Thus, surge generation is not limited to virulence activation in *S. enterica* and may characterize a large class of positive feedback systems.

Positive autoregulation of the response regulator in a two-component system can lead to "learning behavior" in gene regulation.(94,95) Activation of an autoregulated system will result in increased levels of the sensor kinase and response regulator after the signal is removed. Thus, subsequent reactivation of a system will be characterized by a shorter activation time. This has been demonstrated for the PhoB/PhoR system of *E. coli*,(95) where disruption of autoregulation abolished the activation speed-up.(95) A similar phenomenon of "galactose memory" has been reported for the yeast GAL gene cluster, whose activation system contains PFLs.(96) Transcriptional memory reflects the intrinsic activation delay that characterizes positive feedback because the delays are caused by the need to synthesize more regulator, pre-synthesis of the regulator abolishes the delay.

The hierarchical organization of expression is also determined by activation delays, but here the delays correspond to the activation times of different genes co-regulated by a given system.(94) These activation times are defined by the locations and affinities of the binding sites for the response regulator. As a result of the differences in these promoter features, different genes are activated at different regulator levels, which leads to a temporal pattern of gene expression, such as the one observed for the *bvg* virulence regulon of *Bordetella pertussis*.(97) Indeed, elimination of autoregulation in the BvgA/BvgS system leads to a serious disruption in the sequence of phenotypic states associated with the expression pattern displayed by wild-type *B. pertussis*.(22)

Conclusions

Positive feedback is a general control principle frequently encountered in the regulation of molecular processes in living cells. It is found in both bacteria and eukaryotes organisms, and controls diverse processes ranging from bacterial virulence to eukaryotic cell fate determination. Positive feedback can be implemented at the level of transcriptional control, as in bacterial two-component signal transduction, or at the level of protein–protein interactions, as in the regulation of eukaryotic cell cycle. The complexity of systems employing positive feedback ranges from single one-component PFLs to large systems containing multiple interacting PFLs and NFLs.

The most-studied functional feature of positive feedback is promotion of bistability together with the related properties of hysteresis and heterogeneity of clonal cell populations. While, in a number of situations, bistability appears to be a desirable property, many positive feedback systems do not demonstrate bistable (or quasi-bistable) behavior under usual circumstances. In such cases, possible biological roles of positive feedback include efficient switching behavior, robustness in the presence of noise, and tunability.

Bistability is essentially a steady-state property of regulatory systems. It is becoming increasingly clear that positive feedback is crucial for intrinsically transient properties of cellular control circuits, such as excitability and oscillations. These properties are frequently found in multipart systems with more than one feedback loop. However, even the simplest

single-PFL systems can display transient surge-like behavior that is critical for such an important biological property as bacterial virulence.

The significant roles played by positive feedback, together with its ubiquity, make it one of the major functional elements of regulatory pathways, along with negative feedback,(5,13,65) feedforward control(19,98) and cascade-like regulation.(99,100) Combinations of these elements often display qualities that the separate components do not possess, thus providing the foundation for the diversity of cellular control processes. Understanding of the properties of PFLs and their interactions with other regulatory elements will help to improve our ability to predict the behavior of regulatory circuits in living cells and to construct biomolecular systems with desired characteristics.

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Abbreviations

PFL positive feedback loop

NFL negative feedback loop.

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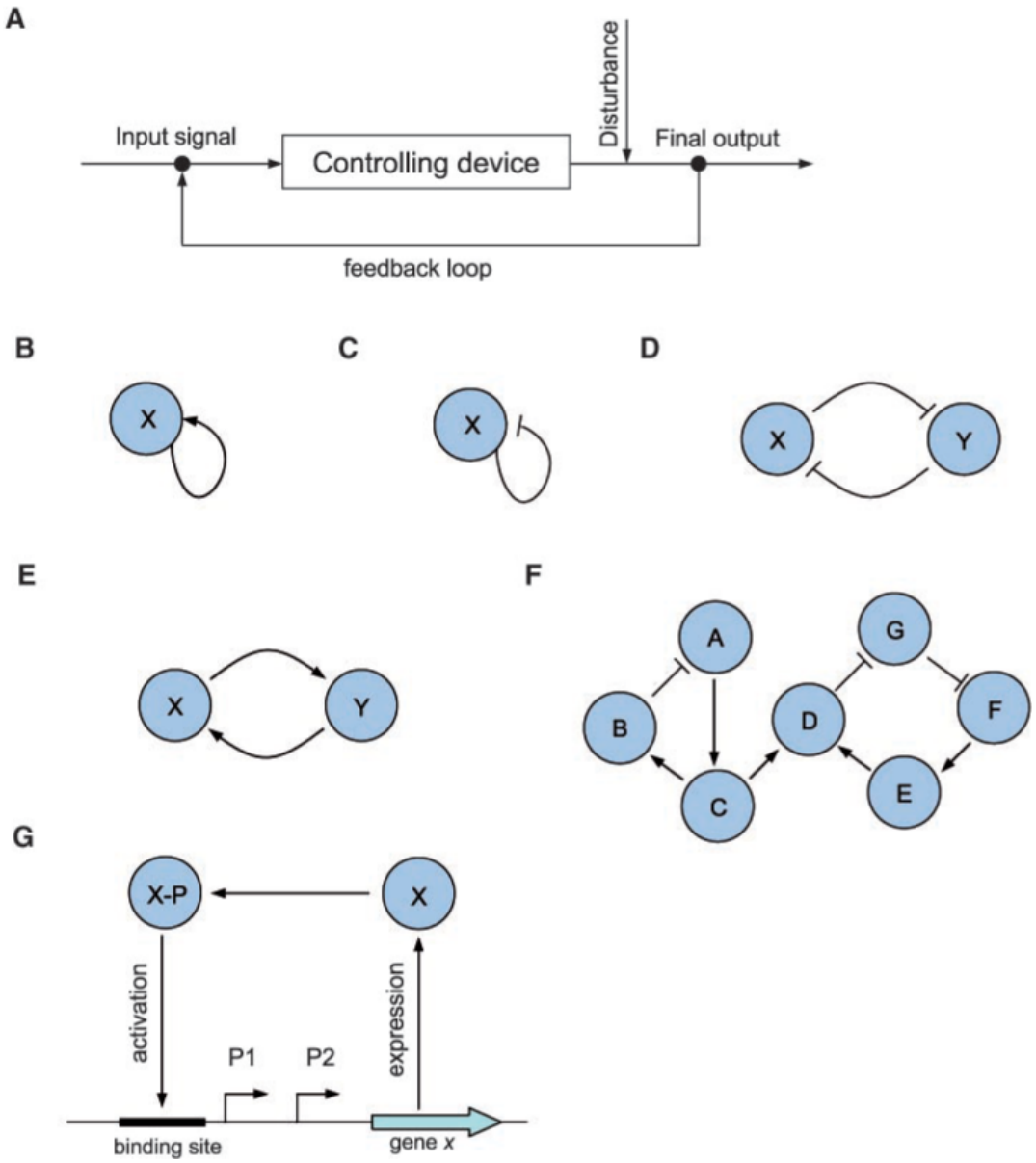

Figure 1.

Regulatory circuits with feedback. **A:** General schematic of a system with feedback. **B–G:** Possible architectures of circuits with feedback. Circles represent regulatory components, arrows denote activation, and T-shaped pointers denote repression. **B:** Positive feedback system with one component. **C:** Negative feedback system with one component. **D:** Double-negative feedback loop. **E:** Positive feedback loop with two mutual activators. **F:** Regulatory system containing a negative feedback loop (A–C–B) and a positive feedback loop (D–G–F–E). **G:** Positive autogenous transcriptional regulation involving activation of the regulator X by phosphorylation. The gene *x* encoding the regulator X is transcribed from two promoters: P1, which is inducible by the phosphorylated form of the regulator X, and P2, which is constitutive.

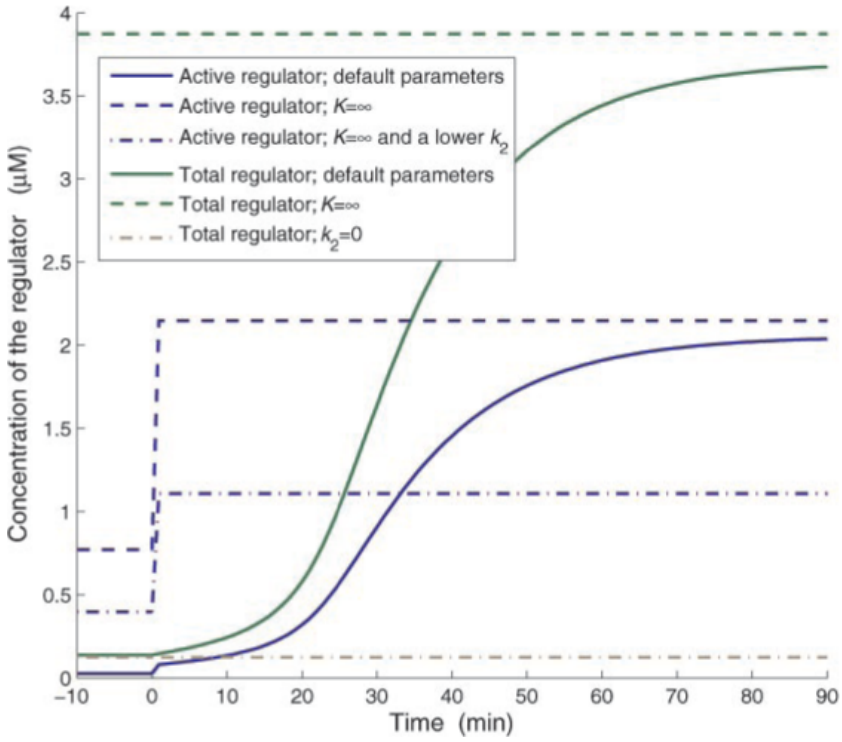

Figure 2.

Activation dynamics for the positive autoregulation model described in Box 2. The model reflects the presence of an inducible promoter, which gives rise to a positive feedback loop, and a constitutive promoter (Fig. 1G). The assignment $K = \infty$ in the model renders the inducible promoter constitutively active, whereas the assignment $k_2 = 0$ makes the inducible promoter inactive; both of these assignments result in constitutive synthesis of the regulator (no feedback). Regardless of the presence of feedback, the initial (pre-activation) state of the system is its steady state under non-activating conditions. This was implemented in the simulations by solving the algebraic equations for the steady state of the model under non-inducing conditions. These equations had a unique real solution which was used to define the state of the system before and at the time of activation. When the system is activated (in our example, at 0 minutes), it experiences an instantaneous 5-fold increase in the regulator phosphorylation rate (k_a). The post-activation temporal dynamics was simulated by numerically solving the differential equations given in Box 2.

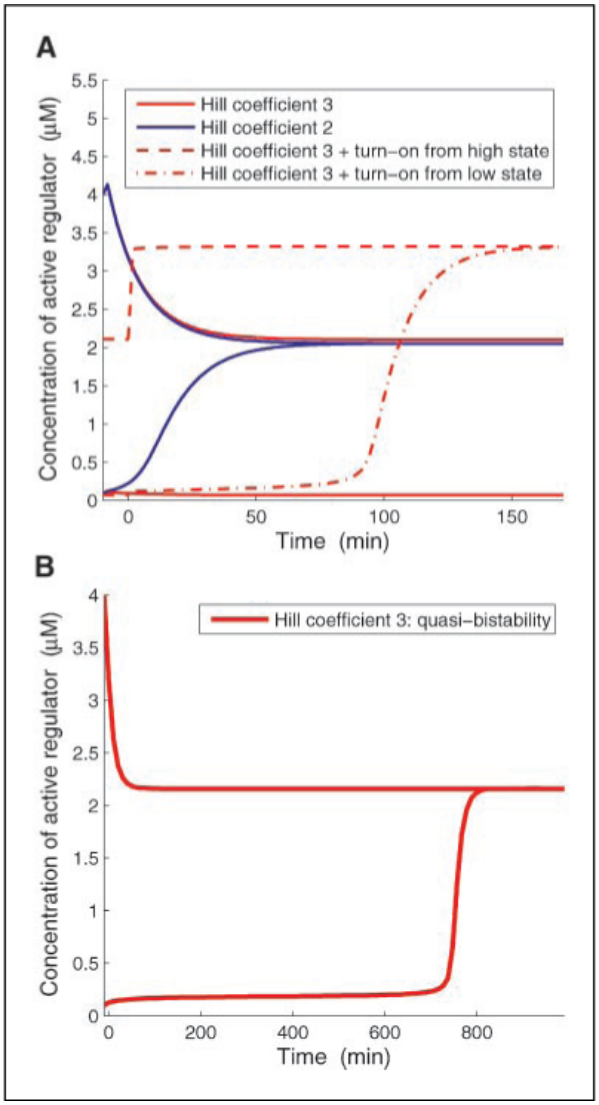

Figure 3.

Dynamical regimes of the positive autoregulation model described in Box 2. **A:** System dynamics in the monostable and bistable regimes. Hill coefficient reflects the degree of cooperativity in binding of DNA by the activator (see Box 2). For Hill coefficient 3, the model demonstrates bistable behavior (solid red lines): the system converges to a low-activity steady state if the initial level of the active regulator is low, and it converges to a high–activity steady state if the initial active regulator level is high. The system is monostable for Hill coefficient 2 (blue lines) and Hill coefficient 3 after activation (dashed and dash-dotted red lines). For the trajectories depicted by solid lines, the two distinct initial states are (0.1, 0.1) and (4, 4), where the two numbers in parentheses represent the concentrations of the phosphorylated and unphosphorylated regulator. The dashed and dash-dotted lines illustrate the dynamics of activation, simulated in a similar way to the results shown in Fig. 2. The initial state of the system is its steady state under non-inducing conditions. The system is activated (in our example, at time 0) via an instantaneous 5-fold increase in the regulator phosphorylation rate (k_a; see Box 2), which can occur as a result of

binding of an inducing ligand to the sensor kinase phosphorylating the regulator. **B:** Pseudo-bistable behavior of the model (see Box 3); the initial states are (0.1, 0.1) and (4, 4). Although the model possesses a unique steady state (evident from the large-time dynamics), on biologically realistic time intervals (50–200 minutes) the model behaves like a bistable system.

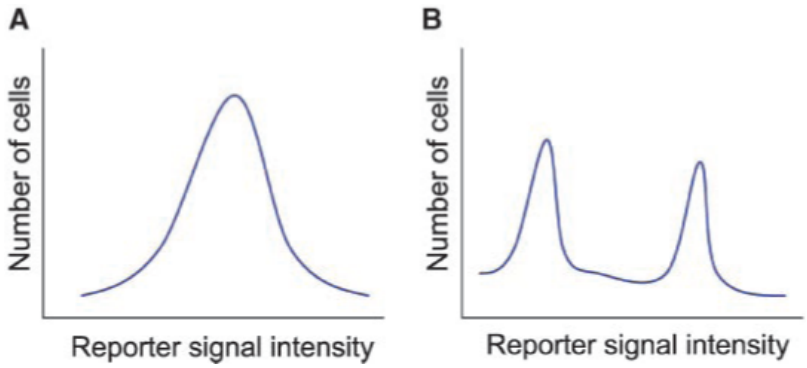

Figure 4.
Schematic representations of distributional monomodality **(A)** and bimodality **(B)**. Reporter signal intensity is measured for every cell in a cell population. The signal intensity represents the number of molecules of a chemical species present in a single cell.

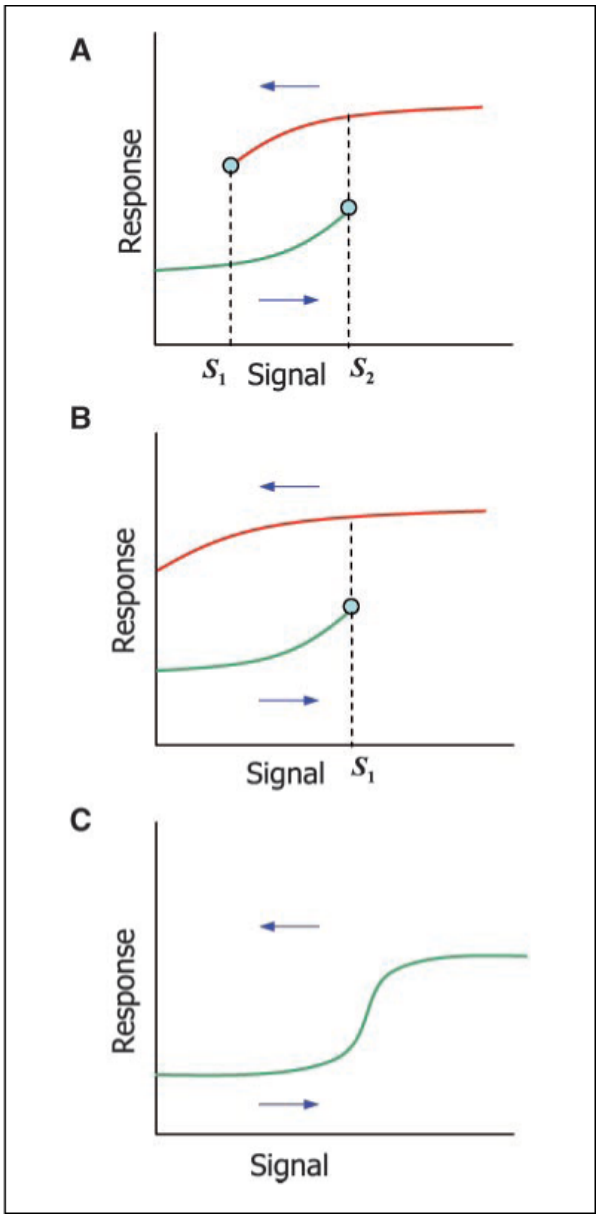

Figure 5.

Schematics showing the steady-state signal–response dependency for bistable and monostable systems. **A:** Hysteresis in a bistable system. When the signal intensity is less than S_1 or greater than S_2, there is only one stable steady state; two stable steady states coexist when the signal intensity is in the interval (S_1, S_2). Depending on the direction of change in the signal intensity (indicated by the arrows), the system will be characterized by different signal–response curves (red and green). At S_1 and S_2, the system undergoes abrupt transitions from the high response level state to the low response level state and vice versa, respectively. **B:** Irreversibility in a bistable system. At S_1, the system undergoes an abrupt transition from the low response level state to the high response level state. However, when the signal intensity shifts from high to low, the system remains in the high response level

state. **C:** Sigmoidal signal–response curve of a monostable system. Regardless of the direction of change in the signal intensity, the signal– response relationship is uniquely defined.

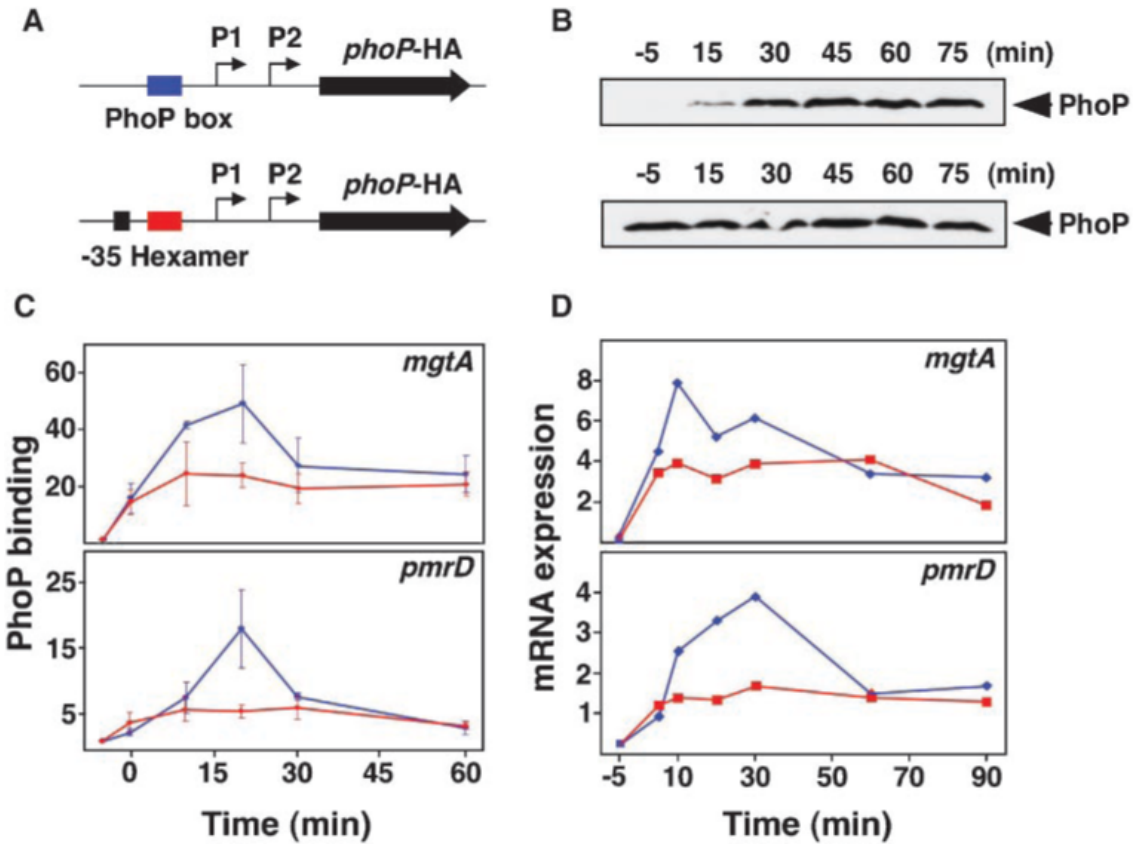

Figure 6.

The positive feedback loop of the *phoPQ* operon is necessary for the surge in activity of the PhoP/PhoQ system in *Salmonella enterica*. **A:** Schematic representation of the *phoPQ* promoter in two isogenic strains. One strain (top) harbors the wild-type P1 promoter, which is positively autoregulated by the PhoP protein, and the constitutive P2 promoter. The other strain (bottom) harbors a consensus –35 hexameric sequence (red square) in place of the PhoP box (blue square). The black square indicates the "scar" sequence generated during the construction of the strains. **B:** The levels of total PhoP protein in extracts from equivalent numbers of the wild-type (top) and mutant (bottom) cells after switching from repressing (high Mg^{2+}) to inducing (low Mg^{2+}) conditions. The levels of promoter occupancy by the PhoP protein (**C**) and mRNA expression (**D**) of the PhoP-activated *mgtA* and *pmrD* genes were determined in wild-type (blue) and mutant (red) strains that were shifted from repressing to inducing conditions. (Reproduced from Ref. 17 with permission.)