Published in final edited form as:

Curr Opin Microbiol. 2010 April ; 13(2): 184–189. doi:10.1016/j.mib.2010.01.009.

Two-Component Signaling Circuit Structure and Properties

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Summary

Various modeling and experimental studies have analyzed the reactions, interconnections, and motifs in two-component systems, with an eye towards understanding their physiological implications and the differences between alternative designs. Examples where recent progress has been made include aspects of autoregulation, signal integration in branched pathways, cross-talk suppression, and cross-regulation via connector proteins.

Introduction

Studies of two-component systems have revealed a diversity of designs that control the flow of information within and between circuits. Despite the variety and increasing complexity of these systems, some aspects are amenable to simple modeling. This type of analysis, when closely coupled with experiments, bolsters one's intuition, gives insights into the functioning of these circuits, and provides a starting point for analyzing the larger networks within which two-component systems are embedded. This review will cover some of the simple design features that are commonly found in two-component systems and that have been explored through modeling and experiment. The emphasis will not be on the methods for modeling regulatory circuits but rather on the conclusions reached as a result of modeling.

Phosphorylation and dephosphorylation

Single-step phosphotransfer

The pathways of phosphorylation and dephosphorylation are at the heart of two-component signaling. In some circuits, the histidine kinase plays a role only in response regulator phosphorylation. The best-studied example is the histidine kinase CheA in chemotaxis circuits, where response regulator dephosphorylation is controlled by a separate phosphatase (see [1] for a recent review). In many two-component systems, however, the histidine kinase participates in the phosphorylation and dephosphorylation of its partner response regulator. For these bifunctional histidine kinases, the input stimulus controls the ratio of kinase to phosphatase activity, by modulating one or both of these reactions, and thereby setting the response regulator phosphorylation level [2]. A simple kinetic model of this cycle of phosphorylation and dephosphorylation predicts that the steady state output of the circuit—the level of phosphorylated response regulator—is robust or insensitive to fluctuations in the concentrations of histidine kinase and response regulator proteins [3]. The analysis assumed that the histidine kinase is in low abundance relative to the response regulator, which is

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consistent with measurements for at least three two-component systems in *E. coli* (EnvZ/OmpR, KdpD/KdpE, PhoQ/PhoP) [4-6]. The concentration robustness predicted by the model has been observed experimentally for the *E. coli* EnvZ/OmpR [3], PhoQ/PhoP [6], and CpxA/CpxR [7] systems. The model also predicts that for sufficiently high expression of the histidine kinase, a significant amount of response regulator will be complexed with the histidine kinase, with a concomitant decrease in response regulator phosphorylation. These predictions are consistent with experiments overexpressing the histidine kinase EnvZ, which show a decrease in OmpR-regulated transcription [3] and, in cells expressing an OmpR-GFP fusion, an increase in fluorescence at the cell periphery [8], consistent with OmpR-GFP binding to the transmembrane histidine kinase.

A related model was recently analyzed, which predicts a stronger form of robustness with respect to the regulatory proteins [9]. As with the model in [3], the response regulator is assumed to be dephosphorylated by the histidine kinase. However an additional assumption is that the major pathway for histidine kinase dephosphorylation is through phosphotransfer to the response regulator; the contributions from histidine kinase auto-dephosphorylation or reverse phosphotransfer to ADP are assumed to be sufficiently small to be negligible. If phosphoryl groups are removed from the histidine kinase only through phosphotransfer to the response regulator then at steady state, the rate of histidine kinase autophosphorylation must equal the rate of response regulator dephosphorylation by the histidine kinase. The concentration of histidine kinase cancels from the two sides of the equation for this rate balance. The end result is that the output (the concentration of phosphorylated response regulator) becomes strictly independent of the concentration of histidine kinase. The output is also independent of the total amount of response regulator down to a threshold value, below which all of the response regulator is phosphorylated.

Another recent modeling study considered the behavior of systems where a dead-end complex can form between the unphosphorylated histidine kinase and response regulator [10]. Under certain conditions this system can show bistability, in which the output has two different stable steady states (corresponding to two different levels of phosphorylated response regulator) for the same input. This would translate to bimodal cell populations and switch-like responses to increasing stimulus (e.g. [11]). The bistability requires not only the dead end complex, but also that the dominant mode of response regulator dephosphorylation is histidine kinase-independent [10]. Therefore, these predictions are not in conflict with observations of uniform, rather than bimodal, cell populations for several two-component systems with bifunctional histidine kinases [6,12].

Phosphorelays

The two-component systems considered above involve a single phosphotransfer step. Phosphorelays, on the other hand, employ multiple phosphotransfers: following histidine kinase autophosphorylation, the phosphoryl group is passed to an aspartate residue on a receiver domain, then to a histidine on an HPt domain, and finally to an aspartate on the receiver domain of a response regulator. The various phosphorylated intermediates can be separate proteins, as in the sporulation phosphorelay of *Bacillus subtilis* [13], or they can be fused in “hybrid” proteins. For many of these systems, response regulator dephosphorylation proceeds by reverse phosphotransfer [14-16]. The general properties of phosphorelay architectures are relatively unexplored and it remains a mystery why some signaling systems make use of single-step phosphotransfers and others phosphorelays. One possibility is that the intermediate steps in phosphorelays provide additional points of control. This is the case for the sporulation phosphorelay [13]. Several phosphatases act at various points to provide additional inputs and multiple levels of regulation to control the switch between sporulation and alternative cell fates [17-22]. However, among the many other well-studied phosphorelays, there are few reports of

additional regulators acting at points along the phosphotransfer pathway. It may be that the additional regulators simply have not yet been uncovered in these systems. It is also possible that phosphorelays have other important properties. One modeling study considered hybrid kinases that contain the first three sites of phosphorylation of the phosphorelay [23]. By scanning through ranges of parameter sets it was argued that these systems are likely to show activation kinetics that is a sigmoidal function of time, and to filter noise in the input stimulus. These predictions have yet to be explored experimentally.

Autoregulation

It is not uncommon to find that a phosphorylated response regulator activates transcription of its own gene and also the gene encoding its partner histidine kinase. This autogenous control is intuitively associated with positive feedback. The nature of this feedback, however, depends on the effects of histidine kinase and response regulator expression level on response regulator phosphorylation. For systems with a bifunctional histidine kinase, an application of the modeling described above suggested that the positive feedback associated with autoregulation can be strongly dependent on input stimulus [6]. Indeed, such behavior was observed for the PhoQ/PhoP system in *E. coli*, where the steady state response was unaffected by autoregulation over a wide range of input stimulus levels (magnesium concentrations). However when cells were grown in sufficiently high stimulus conditions (growth-limiting levels of magnesium), autoregulation amplified the output. In contrast, a strain with a mutated PhoQ that lacks phosphatase activity, and therefore functions as a monofunctional protein, showed strong amplification from autoregulation and considerable cell-to-cell variability, irrespective of the stimulus conditions [6].

The effects of autoregulation on steady-state behavior were also explored in the *Bordetella bronchiseptica* BvgS/BvgA system [24], which involves a phosphorelay through the hybrid sensor kinase BvgS. It was found that autoregulation modulates the sensitivity of the system to applied stimulus. The properties of the phosphorelay that give rise to this shift in sensitivity have not yet been established.

Positive autoregulation may also have significant effects on the kinetics of activation and inactivation of two-component systems. A study of the *E. coli* PhoR/PhoB system demonstrated that autoregulation produces a memory or “learning” behavior in the activation kinetics [25], a result of the stability of the histidine kinase and response regulator proteins. This is likely to be a relatively short-term memory, with a decay time on the order of the cell generation time. The temporal control of Bvg-regulated gene expression is also affected by autoregulation [24]. In this case, autoregulation produces a gradual increase in BvgA phosphorylation, which may be important for sustained expression of genes that are transcribed only under intermediate levels of activation. A similar role for autoregulation in controlling the temporal progression of gene expression has been proposed for the *B. subtilis* sporulation phosphorelay [26].

Autoregulation can also lead to a transient overshoot or surge in response regulator phosphorylation, which subsequently relaxes to a lower steady-state level [27]. The requirement of autoregulation for this behavior was shown explicitly for the *Salmonella* PhoQ/PhoP system. Similar surges have also been observed for several other autoregulated two-component systems [5,27-29]. A detailed kinetic model of the KdpD/KdpE system did not predict this overshoot [5]. This discrepancy between modeling and experiment suggests it may be necessary to include additional reaction steps or possibly additional regulators in the model to understand the origin of the overshoot.

Negative autoregulation, in which the response regulator represses its own expression, appears to be less common, at least among two-component systems that have been studied to date.

However, a few examples have been reported. In the CovS/CovR system, found in *Streptococcus pyogenes*, phosphorylated CovR represses transcription of the *covRcovS* operon [30]. Modeling of this system suggests that autorepression may give rise to oscillatory behavior [31]. Examples of mixed positive and negative autoregulation have also been observed in some systems. For example transcription of the gene encoding the response regulator CtrA in *Caulobacter crescentus* is both activated and repressed by phosphorylated CtrA. The resulting feedback loops are part of a complex phosphorelay network that functions as an oscillator to control the *Caulobacter* cell cycle [32,33]. In at least a few cases a response regulator will repress its own expression, irrespective of its phosphorylation status. Two examples are the response regulators TorR [34] and LuxO [35]. The effects of these phosphorylation-independent negative feedback loops on the regulatory circuits have not yet been established.

It is also important to note that in circuits where auxiliary proteins interact with the histidine kinase or response regulator to modulate signal transduction (discussed below), autoregulation may affect the system by controlling the relative concentrations of the interacting proteins.

Signal Integration and Distribution Among Phosphotransfer Pathways

Branched Pathways

In some two-component systems, the phosphorylation pathway is branched, with more than one source or target of phosphotransfer [36], Fig. 1A,B. Such branched pathways can either be many-to-one, in which multiple phosphodonors phosphorylate a single protein, or “one-to-many” in which the phosphodonor phosphorylates multiple targets. A well-studied example of “one-to-many” branched regulation is found in bacterial chemotaxis, where the histidine kinase CheA phosphorylates the two response regulators CheY and CheB [1].

An interesting example of “many-to-one” regulation is found in the quorum sensing network of *Vibrio harveyi*, where three different phosphorelays converge on the same HPt-domain containing protein LuxU [16]. The three sensor kinases, LuxN, LuxQ, and CqsS, detect three distinct autoinducer signals AI-1, AI-2, and CAI-1, respectively. The signal integrating properties of the LuxN and LuxQ branches have been studied in detail. Remarkably, it was found that the output of this circuit is very well fit by a simple linear combination of the contributions from LuxN and LuxQ [37]. This is surprising since, given the combined kinase and phosphatase activities of the two converging pathways, one would have expected some form of nonlinear integration of the two inputs. From a simple model of this system, it was shown that this linearity can be neatly explained if the phosphatase activity of LuxN and LuxQ are constitutive and the input signals only modulate the kinase activities of these receptors [37]. The convergence of the AI-1, AI-2, and CAI-1 signaling branches suggests the autoinducers will interfere with each other and limit the ability of the circuit to separately detect individual concentrations of the three different signals. A recent study, which used information theory to explore the limitations imposed by this interference, found that the optimal condition for the circuit to acquire information about the concentrations of both AI-1 and AI-2 is obtained when the kinase rates of the active LuxN and LuxQ receptors are comparable [38] – a condition that was observed to be the case experimentally [37]. It was also shown that modifications of the signaling circuit, through feedback or modulation of autoinducer production, can improve the information transmission [38]. The basic limitation arising from interference of multiple input signals may account for the use of alternative pathway integration architectures, discussed below, rather than convergent phosphorylation pathways in many systems.

Cross-talk

In principle, one might imagine cross-phosphorylation between otherwise distinct signal transduction pathways, or cross-talk, could be an effective means to integrate signals to control

multiple outputs. However few examples of bona-fide cross-talk have been reported in the literature (reviewed in [36]). Such cross-talk is ultimately controlled by the specificity of interactions ([36,39]), but the design features of many two-component systems also play a role in suppressing phosphorylation from non-partner histidine kinases or alternative phosphodonors. In particular, bifunctional histidine kinases can buffer against cross-phosphorylation [7,40,41]. In this case, a high flux of phosphorylation and dephosphorylation will render the system insensitive to weak sources of phosphorylation from other histidine kinases or alternative phosphodonors. A second mechanism that limits cross-talk from a histidine kinase is mediated by the partner response regulator. This cross-talk suppression likely results from the partner response regulator out competing non-partner response regulators for interaction with the histidine kinase [7,42].

Cross-regulation via Auxiliary Proteins

Despite the relatively rare appearance of cross-talk, two-component systems do not always function independently. Indeed, there are many examples of one two-component system regulating the activity of another [19,43-48]. In these cases, cross-regulation is achieved by controlling the expression of an auxiliary protein that modulates the activity of a histidine kinase or response regulator of a second system Fig. 1C,(see [49] for a recent review). Such proteins, which effectively connect two signaling pathways, have been named “connectors” [50]. Some connectors also act in feedback loops [19,48,49,51] and are part of a growing list of auxiliary proteins that mediate positive and negative feedback in two-component systems by acting on histidine kinases or response regulators [52-56].

The protein PmrD, which connects the PhoQ/PhoP and PmrB/PmrA path ways in *Salmonella* has been studied in some detail. Transcription of *pmrD* is activated by the PhoQ/PhoP system. PmrD binds to the phosphorylated form of the response regulator PmrA, preventing dephosphorylation by the histidine kinase PmrB [57]. Using a combination of modeling and experimental analysis, this architecture for indirect regulation of a gene by PhoQ/PhoP via PmrD and PmrA was compared with alternative scenarios in which regulation is directly controlled by PhoP or in which both forms of regulation were present (a feedforward connector loop) [50,58]. The PmrD connector-mediated circuit resulted in higher levels of activation upon PhoQ stimulation, and a more sustained level of target gene transcription following a decrease in PhoQ stimulus, when compared with the direct pathway. This latter property can be understood as arising from the persistence of PmrD protein in the cell after *pmrD* transcription has stopped. Interestingly, for the regulation of the gene *pbgP* by PhoQ/PhoP and PmrB/PmrA, the three different architectures (connector-mediated, direct pathway, and feedforward connector loop) are found in three different species (*Salmonella enterica*, *Yersinia pestis*, *Klebsiella pneumoniae*) [58].

Concluding Remarks

Studies of phosphotransfer-based signaling circuits continue to reveal new layers of complexity for these systems. Indeed, there are fewer and fewer examples of signal transduction pathways that really are made up of only “two components”. To provide some level of organization and understanding for this subject, there is a growing need to characterize the properties of different circuit architectures and, wherever possible, the general principles that govern their function, through modeling and experiment. Some examples of open questions and future research directions are listed in Box 1. Most of the properties of two-component signaling circuits covered above were derived from considerations of one or a few systems and therefore the conclusions may not readily generalizable in all cases. However, one of the benefits of a tight interplay between theory and experiment is that identifying the exceptions, deviations, and violations will be even more informative and help to formulate a circuit science of bacterial cell signaling.

Box 1

There are numerous open questions concerning the design and organization of two-component signaling systems. Some questions and future research directions that follow from the results reviewed here include:

- What are the functional differences between simple single-step two-component systems and the various phosphorelays?
- What are the limitations imposed by noise and interference for different circuit architectures? Are there specific designs that effectively proofread or increase fidelity?
- What are the general principles that distinguish branched phosphotransfer pathways, various connectors, or other mechanisms for integrating signals and that may account for the use of one architecture over another?
- Progress in addressing these and many other questions will likely depend on the development of new methods to follow histidine kinase and response regulator activity *in vivo*.
- Future research directions will also likely include an increasing focus on the interactions between two-component systems and other regulatory networks in the cell.

Acknowledgments

The research from the Goulian lab that was covered in this review was supported by grants from the National Science Foundation and National Institutes of Health.

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Fig. 1.

Three different architectures that extend two-component systems beyond simple linear pathways

A) One-to-many branched pathway. One histidine kinase phosphorylates two response regulators.

B) Many-to-one branched pathway. Two histidine kinases phosphorylate the same response regulator.

C) Connector-mediated pathway. The HK1/RR1 two-component system activates expression of a connector protein X, which modulates the activity of the HK2/RR2 system. X may act on the histidine kinase or response regulator and may increase or decrease RR2 phosphorylation.