REVIEW

Signal integration in bacterial two-component regulatory systems

Alexander Y. Mitrophanov and Eduardo A. Groisman[1]

Department of Molecular Microbiology, Howard Hughes Medical Institute, Washington University School of Medicine, St. Louis, Missouri 63110, USA

Two-component systems (TCSs) and phosphorelays are key mediators of bacterial signal transduction. The signals activating these systems promote the phosphorylated state of a response regulator, which is generally the form that carries out specific functions such as binding to DNA and catalysis of biochemical reactions. An emerging class of proteins—termed TCS connectors—modulate the output of TCSs by affecting the phosphorylation state of response regulators. TCS connectors use different mechanisms of action for signal integration, as well as in the coordination and fine-tuning of cellular processes. Present in both Gram-positive and Gram-negative bacteria, TCS connectors are critical for a variety of physiological functions including sporulation, competence, antibiotic resistance, and the transition to stationary phase.

Free-living organisms modulate their gene expression patterns in response to environmental cues. This modulation requires sensors to detect chemical and/or physical signals, and regulators to bring about changes in the levels of gene products. Certain cellular processes require the integration of multiple signals into the decision to promote or inhibit the expression of a given gene product, which raises questions about the mechanisms used by different organisms to connect signal transduction pathways and genetic regulatory circuits.

In bacteria, extracellular signals are transduced into the cell predominantly by two-component systems (TCSs) (Hoch 2000; Stock et al. 2000; Mascher et al. 2006; Gao et al. 2007). The prototypical TCS consists of a sensor kinase that responds to specific signals by modifying the phosphorylated state of a cognate response regulator (i.e., the second component) (Fig. 1). Sensor kinases are usually integral membrane proteins that autophosphorylate from ATP at a conserved histidine residue and then transfer the phosphoryl group to a conserved aspartate in the response regulator. Phosphorylation of a response regulator changes the biochemical properties of its output domain, which can participate in DNA binding and transcriptional control, perform enzymatic activities, bind RNA, or engage in protein–protein interactions (Gao et al. 2007). In addition to serving as phosphoryl donors, certain sensor kinases display phosphatase activity toward their cognate phosphorylated regulators.

Phosphorelays are a more complex version of the TCS in which a sensor kinase first transfers the phosphoryl group to a response regulator possessing the domain with the conserved aspartate but no output domain (Appleby et al. 1996; Perraud et al. 1999). The response regulator subsequently transfers the phosphoryl group to a histidine-containing phosphotransfer protein, and it is the latter protein that serves as a phosphodonor to the terminal response regulator, which possesses an output domain mediating a cellular response (Fig. 1). In some phosphorelays, the sensor kinase and the response regulator lacking the output domain (and sometimes also the histidine-containing phosphotransfer protein) are fused in a single polypeptide (Appleby et al. 1996).

The vast majority of response regulators are active only when phosphorylated (Hoch 2000; Gao et al. 2007). Therefore, any condition or product that affects the phosphorylated state of a response regulator will impact its ability to exert its biological functions. Consequently, the output of a response regulator is determined not only by the presence of the specific signals sensed by its cognate sensor kinase but also by gene products that stimulate or inhibit its phosphorylation. Such products can, in principle, target any one of the various steps leading to phosphorylation of the response regulator, including sensor kinase autophosphorylation, phosphotransfer to the response regulator, dephosphorylation of a phosphorylated response regulator, and the activity of the output domain. The presence of multiple stages in a phosphorelay provides additional potential targets for control.

TCS connectors (which for the sake of brevity will also be called connectors) are an emerging group of proteins that modulate the activity of sensor kinases and response regulators at the post-translational level. Because connector proteins are typically synthesized in response to signals that are different from those sensed by the

[*Keywords*: Gene regulation; phosphorelay; sensor; two-component system]
[1]Corresponding author.
E-MAIL groisman@borcim.wustl.edu; FAX (314) 747-8228.
Article is online at http://www.genesdev.org/cgi/doi/10.1101/gad.1700308.

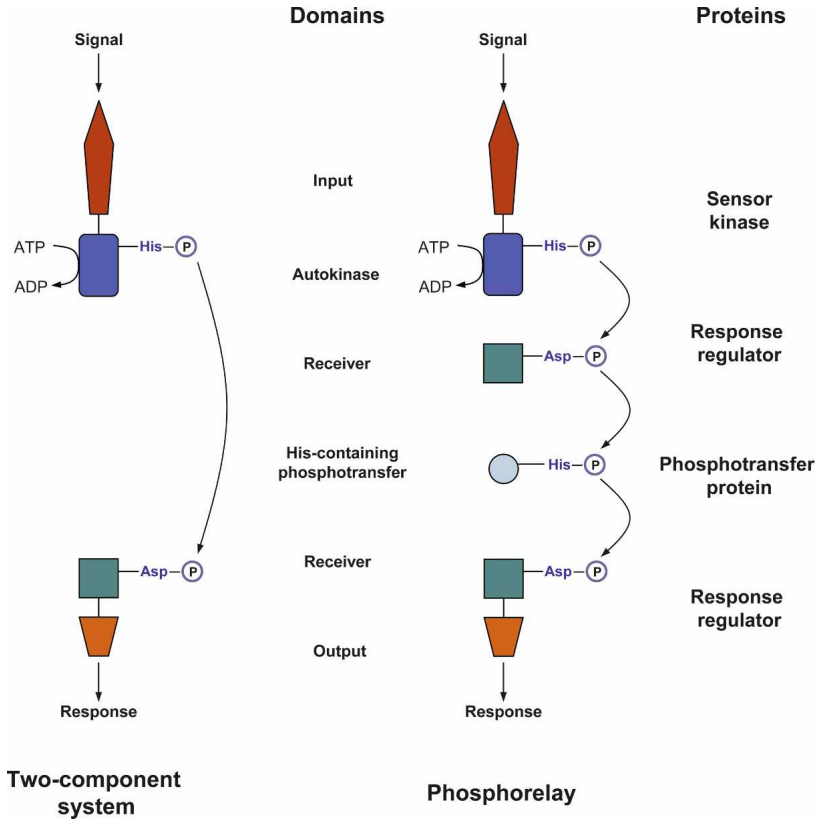

Figure 1. Schematics of the proteins and domains that constitute TCSs (*left*) and phosphorelays (*right*). The input domain of a sensor kinase responds to its signal by activating the autokinase domain, which autophosphorylates from ATP at a conserved histidine residue. The phosphorylated sensor kinase interacts with the receiver domain of the response regulator, which catalyzes the phosphoryl transfer to a conserved aspartate residue. Phosphorylation of the response regulator activates its output domain, which performs a specific biochemical function such as transcriptional regulation. A phosphorelay contains, besides the sensor kinase and the terminal response regulator, an intermediate response regulator lacking an output domain and a His-containing phosphotransfer protein. In some phosphorelays, the phosphotransfer protein and/or the intermediate response regulator is fused with the sensor kinase in a single polypeptide.

cognate sensor, they often establish regulatory links between otherwise independent signal transduction pathways (in other words, they "connect" a TCS to the signal(s) controlling a different regulatory system). Here we describe the critical roles played by bacterial TCS connectors in a variety of cellular functions, including the adaptation to nutrient-limiting conditions, sporulation, competence, antibiotic resistance, and the transition to stationary phase. We also discuss the distinct dynamic properties of the regulatory circuits in which connectors participate, and we examine how dissimilarities in the sequences of connectors or their genes' promoters can result in phenotypic differences among closely related bacterial species.

TCS connectors use a variety of mechanisms to alter response regulator output

Connector proteins modulate the levels of the active form of response regulators by affecting the various possible steps that determine their phosphorylation state or their activity. Below, we present the various mechanisms of action adopted by connectors in the context of their physiological functions.

Inhibiting sensor kinase autophosphorylation

The Gram-positive soil bacterium *Bacillus subtilis* forms a dormant spore when it experiences nutrient-limiting conditions (Piggot and Hilbert 2004). Because sporulation is an energy-consuming process that becomes irreversible at an early stage (Dubnau and Losick 2006), commitment to sporulation is tightly regulated and coordinated with other physiological functions. *B. subtilis* sporulation is governed by a phosphorelay whereby five different sensor kinases—termed KinA, KinB, KinC, KinD, and KinE—serve as phosphoryl donors for the single-domain response regulator Spo0F (Piggot and Hilbert 2004). Spo0F then transfers the phosphoryl group to the histidine-containing phosphotransfer protein Spo0B, which in turn transfers it to the response regulator Spo0A, a DNA-binding protein that controls the expression of several genes, including those involved in sporulation (Fig. 2; Piggot and Hilbert 2004).

The Sda and KipI proteins modulate the levels of phosphorylated Spo0A (Spo0A-P), which constitutes the output of the *B. subtilis* phosphorelay, by blocking autophosphorylation of the sensor kinase KinA (Fig. 2; Wang et al. 1997; Burkholder et al. 2001). KinB is a possible second target for inhibition by Sda (Burkholder et al. 2001). The *sda* gene is under transcriptional control of the key replication initiation factor DnaA, and mutation of the *dnaA* gene leads to overexpression of Sda and inhibition of sporulation (Burkholder et al. 2001). It was proposed that conditions that affect replication initiation alter the level of active DnaA, thereby regulating *sda* through DnaA (Burkholder et al. 2001). Thus, the

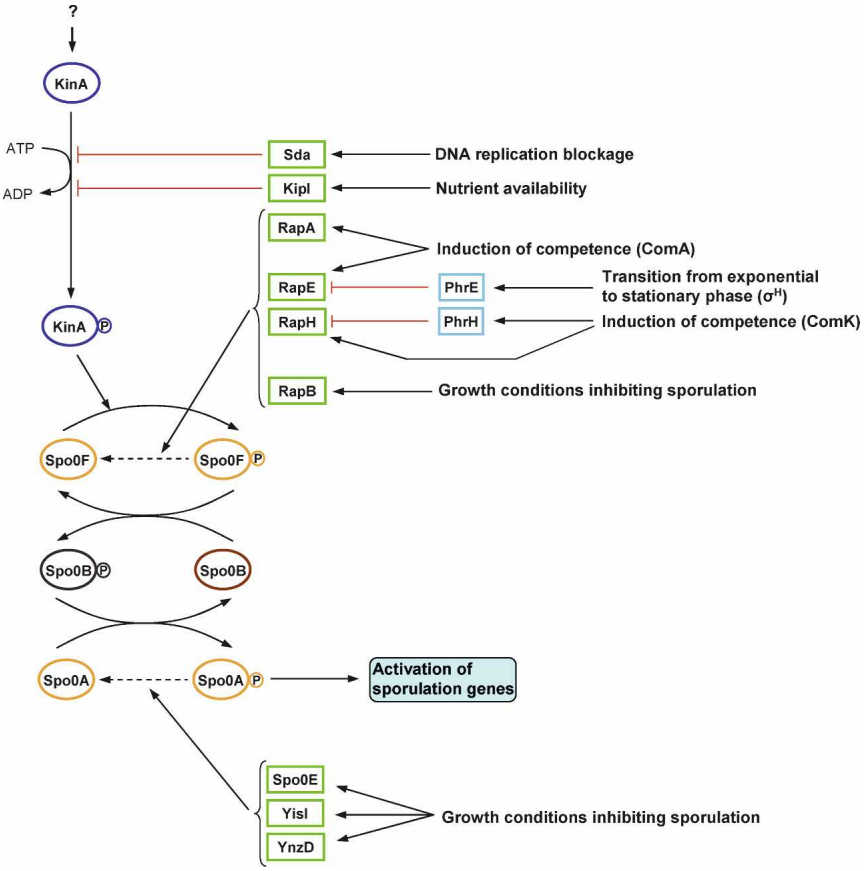

Figure 2. TCS connectors can inhibit sensor autophosphorylation or promote dephosphorylation of phosphorylated response regulators. In the *B. subtilis* phosphorelay, the sensor kinase KinA is activated by an unknown signal, which results in autophosphorylation from ATP and subsequent phosphotransfer to the response regulator Spo0F. Spo0F passes on the phosphoryl group to the His-containing protein Spo0B, which in turn transfers it to the terminal acceptor, the response regulator Spo0A. The phosphorylated form of Spo0A acts as a transcription factor, being the key activator of sporulation genes. The connectors Sda and KipI block activation of the phosphorelay by inhibiting KinA autophosphorylation. The connectors RapA, RapB, RapE, and RapH promote dephosphorylation of the response regulator Spo0F-P; the connectors Spo0E, YisI, and YnzD act in a similar way on Spo0A-P. The expression of connectors is controlled by factors such as growth conditions, status of the DNA replication machinery, and development of competence (through the action of ComA and ComK—the key regulators of competence genes).

Sda-mediated control circuit could prevent *B. subtilis* from entering the sporulation process if the DNA replication machinery, necessary for successful completion of sporulation, is impaired. The KipI protein links sporulation to the availability of certain nutrients (Wang et al. 1997). Because expression of the *kipI* gene is promoted in the presence of sugars (Wang et al. 1997), the KipI protein likely plays a role in sporulation blockage in nutrient-rich environments, which are antithetic to sporulation.

The inhibition of sensor kinase autophosphorylation has also been adopted by the Gram-negative bacterium *Sinorhizobium meliloti* to regulate nitrogen fixation in the root nodules of its plant symbiont, alfalfa. Nitrogen fixation is a process that allows certain bacteria to use atmospheric nitrogen to synthesize nitrogen-containing compounds (Dixon and Kahn 2004). The micro-oxic conditions prevailing in the nodule and necessary for nitrogen fixation are sensed by the FixJ/FixL TCS (Fischer 1994). The activity of the sensor kinase FixL is inhibited by FixT (Garnerone et al. 1999), which is post-translationally controlled by the product of the *asnO* gene (Bèrges et al. 2001). It has been suggested that AsnO and FixT establish a link between the oxygen-responding FixJ/FixL system and the nitrogen status of the bacterium (Bèrges et al. 2001).

Promoting dephosphorylation of phosphorylated response regulators and His-containing phosphotransfer proteins

In the *B. subtilis* phosphorelay, stimulatory and inhibitory signals are combined to determine the levels of Spo0A-P, the key activator of sporulation genes (Burbulys et al. 1991; Wang et al. 1997; Sonenshein 2000). In addition to the Sda and KipI proteins, which exert their inhibitory action "at the top" of the phosphorelay (Fig. 2), several other proteins prevent the accumulation of Spo0A-P by targeting the response regulators Spo0F and Spo0A (Fig. 2). The Rap protein family members RapA, RapB, RapE, and RapH trigger dephosphorylation of Spo0F-P, whereas the Spo0E family members Spo0E, YisI, and YnzD promote dephosphorylation of Spo0A-P (Perego and Brannigan 2001; Smits et al. 2007). The Rap and Spo0E proteins appear to function by stimulating the intrinsic autodephosphorylation activity of Spo0F-P and Spo0A-P, respectively, which is exerted upon binding to these connector proteins (Perego and Brannigan 2001).

The production and functioning of the connector proteins controlling the *B. subtilis* phosphorelay is dependent on the presence of signals indicating the physiological state of the bacterium (Fig. 2). For example, transcription of the *rapA* and *rapE* genes is induced by ComA, the response regulator of the ComA/ComP TCS (Mueller et

al. 1992; Jiang et al. 2000). This system, in turn, responds to the extracytoplasmic peptide ComX, a quorum-sensing signal that triggers the development of competence, a physiological state characterized by the ability of a bacterial cell to take up DNA from the surrounding environment (Claverys et al. 2006). The genes *rapB*, *spo0E*, *yisI*, and *ynzD* are expressed under conditions that promote vegetative growth (Jiang et al. 2000; Perego 2001; Perego and Brannigan 2001). Hence the Rap and Spo0E phosphatases prevent *B. subtilis* from committing to sporulation in environments that stimulate an active lifestyle and are therefore contrary to sporulation.

The RapA, RapE, and RapH proteins are also controlled at the post-translational level, which provides further possibilities for modulating the levels of active Rap proteins. RapA is regulated by the pentapeptide inhibitor PhrA, which specifically binds to RapA and suppresses its dephosphorylation activity (Perego and Hoch 1996; Perego 1997); in a similar way, RapE and RapH are inhibited by the pentapeptides PhrE and PhrH, respectively (Jiang et al. 2000; Smits et al. 2007). Expression of the *phrE* gene is, in turn, activated by Spo0H (σ^H), an alternative σ-factor that modulates a gene expression program when *B. subtilis* transitions from exponential growth to stationary phase (McQuade et al. 2001). σ^H itself is under complex multilevel control and responds to a number of conditions including pH and availability of nutrients (Britton et al. 2002). Therefore, RapE and PhrE link the sporulation phosphorelay to factors that regulate the shift between growth phases.

The SixA protein from *Escherichia coli* inhibits the ArcA/ArcB phosphorelay by stimulating dephosphorylation of the His-containing phosphotransfer domain of the sensor kinase ArcB (Ogino et al. 1998). SixA functions in a manner analogous to that of the *B. subtilis* Rap proteins in that it prevents the normal phosphoryl flow in a phosphorelay.

Inhibiting response regulator dephosphorylation

For those response regulators that regulate cellular functions by binding to particular DNA regions in bacterial genomes, the phosphorylated form typically has a higher affinity for its target promoters than the unphosphorylated form (Hoch 2000). Indeed, only the phosphorylated form of certain response regulators binds DNA in vivo when produced at physiological levels (Piggot and Hilbert 2004; Shin and Groisman 2005). Therefore, mechanisms enhancing the phosphorylated state of a response regulator will result in the induction of genes activated by such a regulator.

The 85-amino-acid-long PmrD protein from *Salmonella enterica* connects the PhoP/PhoQ and PmrA/PmrB TCSs, thereby enabling PmrA-dependent genes to be expressed under the conditions that activate the PhoP/PhoQ system (Fig. 3A; Kox et al. 2000; Kato et al. 2003; Kato and Groisman 2004). The sensor kinase PmrB responds to the presence of extracellular Fe^{3+}, Al^{3+} (Wösten et al. 2000), or mild acid pH (Perez and Groisman 2007) by promoting transcription of PmrA-activated genes, most likely by enhancing the levels of PmrA-P. In the absence of its inducing signals, however, PmrB acts primarily as a phosphatase for PmrA-P. The low Mg^{2+} signal activates the PhoP/PhoQ system, which triggers transcription of the *pmrD* gene. The synthesized PmrD protein binds to PmrA-P and protects it from dephosphorylation by PmrB, thus allowing PmrA to regulate its target promoters. Therefore, PmrD expands the scope of environments that promote the expression of PmrA-activated genes by incorporating the environments distinguished by the signals sensed by the noncognate sensor PhoQ. As PmrA governs the expression of products mediating resistance to the antibiotic polymyxin B, the PmrD protein enables *S. enterica* to exhibit polymyxin B resistance not only in environments characterized by the

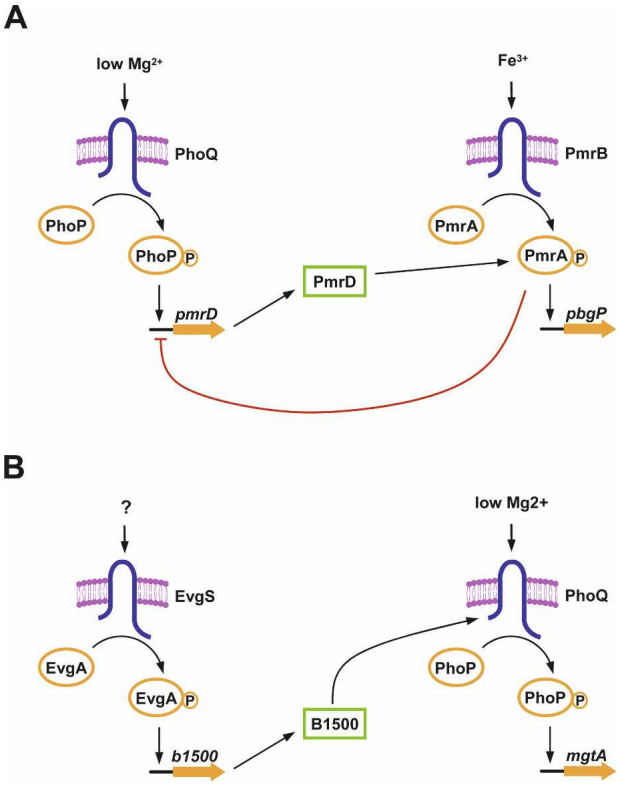

Figure 3. TCS connectors can promote activation of response regulators and sensor kinases. (*A*) The connector-mediated pathway from *S. enterica*. The low Mg^{2+} signal activates the PhoP/PhoQ TCS, which triggers the expression of the connector PmrD. PmrD binds to the phosphorylated form of the response regulator PmrA, thereby protecting it from dephosphorylation. PmrA-P binds to DNA and regulates its target promoters. PmrA-P represses transcription of the *pmrD* gene, thus establishing a negative feedback loop controlling PmrD expression. The PmrA/PmrB TCS can be activated directly by the Fe^{3+} signal. (*B*) The B1500 protein from *E. coli* connects the EvgA/EvgS and PhoP/PhoQ TCSs. In response to an unknown signal, the EvgA/EvgS system triggers the expression of B1500, which interacts with the sensor kinase PhoQ, thereby promoting activation of PhoP and resulting in transcription of PhoP-activated genes.

Fe^{3+} signal but also in those of low Mg^{2+} (Kox et al. 2000). The mode of action of PmrD is reminiscent of that of eukaryotic 14–3–3 proteins, a highly conserved group of polypeptides that have the ability to bind to phosphorylated proteins, thereby protecting them from dephosphorylation and enhancing their physiological activities (Tzivion and Avruch 2002; Kato and Groisman 2004).

The connector proteins that promote response regulator phosphorylation integrate signals by acting as the Boolean OR gate because the response regulator can be activated either through its cognate sensor kinase *or* by the signal stimulating the synthesis of the connector protein (Kato and Groisman 2004). By contrast, connectors such as KipI, Sda, and the Rap proteins, which control the *B. subtilis* phosphorelay, function as the Boolean AND gate because the phosphorelay response regulators will be phosphorylated only if the cognate sensor proteins perform their kinase activity and if the synthesis of the connectors is inhibited.

Activating a sensor kinase

Connector proteins can act as Boolean OR gates by modulating the activity of a sensor kinase. This mode of regulation is exemplified by B1500, a protein that establishes a functional link between the TCSs EvgA/EvgS and PhoP/PhoQ in *E. coli* (note that the EvgA/EvgS system is absent from the closely related species *S. enterica*) (Eguchi et al. 2007). Activation of the sensor kinase EvgS results in transcription of the *b1500* gene promoted by EvgS's cognate response regulator EvgA. B1500 is a 65-amino-acid-long inner membrane peptide that interacts with the sensor kinase PhoQ, enhancing PhoP activity (Fig. 3B). As a result, PhoP-activated genes are transcribed even when *E. coli* experiences high Mg^{2+}, which is a repressing condition for the PhoP/PhoQ system. This allows expression of PhoP-activated genes not only under low Mg^{2+} but also under conditions that activate the sensor EvgS, which are presently unknown.

Inhibiting DNA binding by a response regulator

Genetic competence is a complex phenotypic state experienced by *B. subtilis* during late exponential/early stationary phase. Competence induction results in the expression of DNA-binding, DNA-uptake, and recombination genes (Hamoen et al. 2003). Like sporulation, competence is a response aimed at increasing the cells' ability to survive in hostile environments. However, sporulation and competence are two mutually exclusive responses (Smits et al. 2007). While the development of competence is controlled at multiple levels (Hamoen et al. 2003), there is a central regulator known as ComK whose level is enhanced upon activation of the response regulator ComA (Claverys et al. 2006). ComA, in turn, is post-transcriptionally regulated by the Rap protein family members RapC, RapF, and RapH. When the latter proteins bind to ComA, they prevent ComA from interacting with DNA (Core and Perego 2003; Bongiorni et al. 2005; Smits et al. 2007), thereby inhibiting competence development (Fig. 4). Thus, RapH acts differently on the Spo0F and ComA proteins. In a manner reminiscent of that described above for the control of the *B. subtilis* Kin–Spo0F–Spo0B–Spo0A phosphorelay (which controls sporulation), three pentapeptides—PhrC, PhrF, and PhrH—specifically bind to their respective targets, RapC, RapF, and RapH, thus preventing them from deactivating ComA (Lazazzera et al. 1999; Core and Perego 2003). The levels of PhrC and PhrF are directly linked to the transition between growth phases because the genes encoding the protein precursors of PhrC and PhrF are regulated by the alternative σ-factor σ^H (Fig. 4; Lazazzera et al. 1999; McQuade et al. 2001).

Inhibiting recruitment of RNA polymerase to promoters

Binding of RNA polymerase to a gene promoter is the critical step to initiate bacterial gene transcription (Browning and Busby 2004). Transcriptional activators often function by recruiting RNA polymerase to target operators, where they establish effective interactions with different subunits of RNA polymerase. Such interactions can be disrupted by certain connector proteins (Ansaldi et al. 2004; Zuber 2004). For example, the connector Spx from *B. subtilis* can bind to the C-terminal domain of the α-subunit of the promoter-bound RNA polymerase, which interferes with the ability of RNA polymerase to interact with the response regulators ComA and ResD (Fig. 4; Nakano et al. 2003b). As discussed above, ComA is one of the key activators of competence development in *B. subtilis*, whereas ResD is part of the ResD/ResE TCS, which is necessary for the induction of oxygen-limitation response genes (Nakano et al. 1996). Transcription of the *spx* gene is induced by σ^M, the extracytoplasmic function σ-factor that is activated by envelope stress (Eiamphungporn and Helmann 2008); other factors promoting *spx* transcription include ethanol stress and phosphate limitation (Antelmann et al. 2000; Thackray and Moir 2003). The activity of the Spx protein is induced under the conditions of disulfide stress (Nakano et al. 2003a). Thus, Spx may function as a global repressor of development- and growth-promoting processes under a variety of stress conditions (Nakano et al. 2003b).

The inhibition of RNA polymerase recruitment is used by *E. coli* to regulate anaerobic respiration controlled by the response regulator TorR, which is activated via a phosphorelay (Ansaldi et al. 2004). In this case, the TorI protein interacts directly with the C-terminal (effector) domain of TorR, thus preventing TorR from recruiting RNA polymerase and initiating gene transcription. The *torI* and *torR* genes are transcribed independently from one another, raising a possibility that TorI acts as a connector by modulating the activity of TorR in response to signals that may be different from those activating the phosphorelay.

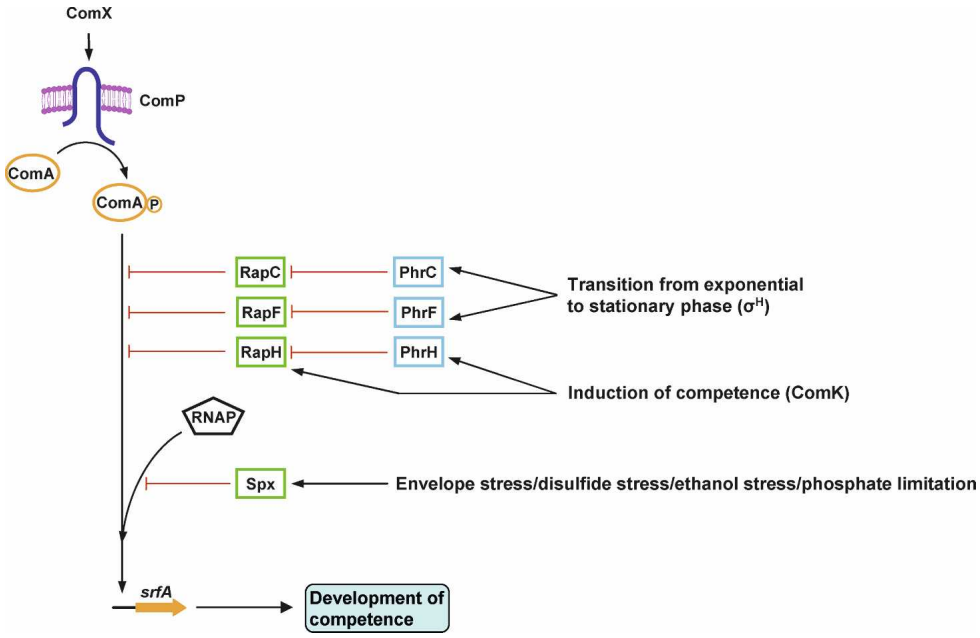

Figure 4. TCS connectors can inhibit binding of activated response regulators to DNA or prevent RNA polymerase from interacting with a response regulator. The ComA/ComP TCS from *B. subtilis* responds to the extracellular quorum-sensing signal, the peptide ComX. Upon activation, ComA promotes transcription of the *srf* operon, which leads to development of the competent state. Binding of ComA to DNA is inhibited by the connectors RapC, RapF, and RapH. These connectors, in turn, are deactivated upon binding to the corresponding Phr peptides. The action of ComA is also inhibited by the connector Spx, which disrupts the interaction between ComA and RNA polymerase.

Sequestering adaptor proteins that promote protein degradation

Many regulatory proteins are subjected to proteolytic degradation by cellular proteases. Proteolysis is often performed by multiprotein complexes containing proteases and ATP-hydrolyzing chaperones (Gottesman 2003; Jenal and Hengge-Aronis 2003). Delivery of a protein targeted for degradation to the protease complex may require an additional adaptor protein (Jenal and Hengge-Aronis 2003). For example, degrading the general stress response σ-factor σ^S (RpoS) of *E. coli* by the ClpXP protease requires the adaptor protein RssB (also referred to as SprE in *E. coli* and MviA in *S. enterica*) (Hengge-Aronis 2002). This adaptor is a response regulator that consists of the conserved N-terminal domain, which harbors the aspartate site of phosphorylation, and the C-terminal domain, which is required for interaction with RpoS and delivery of RpoS to the ClpXP protease. Under conditions of nutrient abundance or exponential growth, the RpoS protein is degraded. However, RpoS degradation is inhibited when bacteria experience deprivation for a variety of nutrients such as phosphate and carbon sources, or when they are exposed to environments that trigger growth arrest.

The inhibition of RpoS degradation is mediated by anti-adaptor proteins that bind to the adaptor RssB. Because RssB is in short supply in the bacterial cell (Hengge-Aronis 2002), if RssB is bound to an anti-adaptor, then it is not available for binding to RpoS, which can reprogram RNA polymerase to transcribe RpoS-dependent promoters. For example, the 86-amino-acid IraP protein is an anti-adaptor that is produced when *E. coli* experiences low phosphate levels (Bougdour et al. 2006). The 130-amino-acid IraD protein acts on RssB in a similar way to IraP, but it is activated as a result of hydrogen peroxide oxidation, which causes DNA damage (Zheng et al. 2001; Bougdour et al. 2008). Likewise, the 107-amino-acid IraM protein, whose gene is induced by the PhoP/PhoQ system under the low Mg^{2+} conditions, has the ability to bind to RssB and/or RpoS, thereby preventing RpoS degradation (Bougdour et al. 2008). Thus, different connector proteins convey the nutritional and/or stress status of the cell to the RssB protein that controls RpoS levels. Similar mechanisms govern RpoS degradation in *S. enterica* (Tu et al. 2006; Bougdour et al. 2008). Notably, the IraP protein, just like the PmrD connector, is transcriptionally controlled by the PhoP/PhoQ system (Fig. 3A), which allows *S. enterica* to use distinct connectors to activate different regulatory systems in response to one signal—low extracellular Mg^{2+} (Kox et al. 2000; Tu et al. 2006).

Quantitative features of connector-mediated gene control

Connectors endow regulatory circuits with distinct quantitative and kinetic properties, which determine the intensity and timing of the output of connector-mediated circuits. In the case of transcriptional control, such properties can be elucidated by comparing the gene tran-

scription levels promoted by a connector-mediated mechanism with those of a direct regulation circuit, in which a transcription factor binds to the promoter of the target gene and activates its transcription. In comparison with a direct regulation circuit, the PmrD-mediated pathway connecting the PhoP/PhoQ and PmrA/PmrB systems (Fig. 3A; Kox et al. 2000; Kato et al. 2003; Kato and Groisman 2004) displays heightened mRNA induction ratios (i.e., mRNA levels normalized relative to those observed under repressing conditions) (Kato et al. 2007). Mathematical modeling results imply that this signal amplification is an intrinsic feature of the connector-mediated pathway architecture, rather than a consequence of a particular combination of kinetic parameters (Kato et al. 2007).

The connector-mediated pathway promotes small transcription activation delays in comparison with the direct regulation circuit; such delays could be attributed to the necessity to transcribe the *pmrD* gene and translate the *pmrD* mRNA (Kato et al. 2007). In addition, the connector-mediated pathway promotes large deactivation delays (i.e., persistence of expression when the organism goes from inducing to repressing environments) because any remaining PmrD will continue to bind to PmrA-P, thereby affecting PmrA-dependent gene transcription (Kato et al. 2007). PmrA stimulates modifications in the outer membrane that protect *S. enterica* from toxic agents, such as metal ions and the antibiotic polymyxin B; thus, persistence of expression could be advantageous for survival in fluctuating environments (Kato et al. 2007). The lability of the phosphorylated state of response regulators allows for rapid regulatory changes without new protein turnover. Therefore, connectors that protect phosphorylated states can confer stability upon a signaling system. Mathematical modeling suggests that noticeable activation delays and large deactivation delays are inherent properties of the connector-mediated pathway architecture (Mitrophanov et al. 2008). However, because PmrD stability affects persistence of expression (Kato et al. 2007), it is anticipated that as the stability of a connector decreases the length of the deactivation delays will decrease as well. This can happen when the connector itself is a protease substrate or when the connector's activity is modified post-translationally (e.g., in a similar way to the Rap–Phr interaction).

TCS connectors that function as anti-adaptors (e.g., IraP) regulate cellular processes by binding to adaptor proteins (e.g., RssB), thereby preventing them from recruiting their targets for degradation (Tu et al. 2006; Bougdour et al. 2008). Such regulation could confer advantageous quantitative properties in comparison with other regulatory mechanisms. Indeed, degradation of a protein can sometimes be quickly inhibited, in which case the concentration of this protein will increase much faster than in the case of transcriptional control (Jenal and Hengge-Aronis 2003). In stress response, time-efficient regulatory reactions appear to be particularly important (Jenal and Hengge-Aronis 2003).

Biological consequences of connector-mediated regulation

TCS connector proteins can expand the spectrum of signals that influence the activity of a response regulator. The signals can be integrated by connector-dependent OR gates, in which any one of the incoming signals is sufficient for circuit activation. Alternatively, connector-based mechanisms can act as AND gates, whereby circuit activation requires the presence of all the signals, or the presence of one signal and the absence of another. Signal integration facilitates coordination and fine-tuning of cellular processes. Furthermore, connector proteins can promote responses with specific quantitative properties. Here, we discuss characteristics attributable to connectors.

Temporal coordination of complex processes

Connector proteins determine when and for how long response regulators will be active. The connectors that regulate sporulation in *B. subtilis* affect the levels of phosphorylated Spo0A, the central transcriptional regulator of sporulation. The dynamics of the Spo0A-P levels is critical for sporulation, because gradual accumulation of Spo0A establishes temporal activation order for the sporulation gene promoters that differ in their affinity for Spo0A-P (Fujita and Losick 2005; Fujita et al. 2005). This suggests that a prominent role of connectors in sporulation control is to facilitate proper timing and coordination of sporulation with other cellular functions. Indeed, the connector Sda causes a delay in the initiation of sporulation in the case of transient replication blocks or DNA damage, thus giving the cell an opportunity to repair the damage (Ruvolo et al. 2006). The connectors RapA, RapE, and RapH prevent simultaneous occurrence of two alternative physiological processes—competence and sporulation—thus carrying out time coordination of the two distinct responses (Mandicmulec et al. 1995; Perego and Brannigan 2001; Smits et al. 2007). Such time coordination is well illustrated by the action of RapH, which is required to prevent temporal overlaps between the expression of late competence genes and sporulation genes under conditions that promote both phenotypic states (Smits et al. 2007).

Intricate control of the activity of Rap proteins is carried out by their cognate Phr peptides (Perego 1997). Existing evidence suggests that the protein precursors of the Phr peptides are exported outside of the cell via the Sec-dependent pathway, a ubiquitous bacterial mechanism for protein export (Perego and Hoch 1996; Perego 1997; Lazazzera et al. 1999; Lazazzera 2001; Perego and Brannigan 2001). These precursors are subsequently processed by extracellular enzymes, and then re-enter the cell via the oligopeptide permease. Each of the Phr production steps can be controlled by different cellular mechanisms. Thus, Phr transport and processing events have been suggested to represent regulatory checkpoints coordinating the timing and rate of Spo0A-P accumulation with other physiological functions (Perego 1997; Perego and Brannigan 2001).

Promoting heterogeneity in cell populations

Gene expression patterns or responses to environmental changes often characterize only a part of a bacterial cell population (Smits et al. 2006). Regulation by connectors is critical for the heterogeneity of genetically identical *B. subtilis* cells with respect to their ability to sporulate. Under sporulation-promoting conditions, there exist two cell subpopulations that consist of sporulating and nonsporulating cells, respectively (Dubnau and Losick 2006; Smits et al. 2006). The two subpopulations differ in the levels of Spo0A-P, and sporulation is triggered in the cells whose Spo0A-P level exceeds a certain threshold (Fujita et al. 2005; Veening et al. 2005; Smits et al. 2006). If the environmental conditions change so that nutrients become plentiful, the nonsporulating cells will resume their normal activities. Thus, heterogeneity is believed to be an advantageous property allowing the population to avoid commitment to the irreversible and energy-consuming sporulation in environments where harsh conditions can be easily reversed (Dubnau and Losick 2006). The connectors RapA and Spo0E, which regulate the *B. subtilis* phosphorelay, are necessary for Spo0A-associated cell heterogeneity, because deletions of the *rapA* and *spo0E* genes abolish heterogeneity by inducing Spo0A activity in the vast majority of cells in the population (Veening et al. 2005).

Specificity of connectors

Specificity of biochemical mechanisms is a means to avoid undesired cross-talk between regulatory pathways (Bardwell et al. 2007). At the same time, regulatory interactions between pathways that do not normally intersect can serve as means of signal integration or generating distinct outputs in response to a single input. Even though a few examples of such cross-talk have been reported in two-component signal transduction, the kinetic preference of a sensor protein for its cognate response regulator makes cross-talk extremely rare (Laub and Goulian 2007). Likewise, the specificity of TCS connectors for their targets is a key factor contributing to the fidelity in two-component signal integration. For example, the PmrD connector protein of *S. enterica* can inhibit dephosphorylation of PmrA-P, but not of its nearest homolog, the response regulator YgiX (Kato and Groisman 2004). Furthermore, the activity of the Phr peptides, RapA, RapB, RapE proteins, and Spo0E phosphatases is also highly specific (Perego and Brannigan 2001). The connector KipI appears to act exclusively toward its target (KinA), and so do the known anti-adaptors of Gram-negative bacteria. The action of the connector protein Sda is specific in the sense that both of its targets, KinA and KinB, phosphorylate Spo0F.

Whereas target specificity seems to be predominant in connector-mediated regulation, a few connectors affect more than one target. For example, RapH inhibits the DNA-binding ability of ComA and promotes dephosphorylation of Spo0F-P, thereby acting as a bifunctional connector that controls competence and sporulation (Smits et al. 2007). The connector Spx prevents RNAP from binding to DNA and initiating gene transcription. This ability allows Spx to inhibit DNA binding of two response regulators—ComA and ResD. In addition, Spx can induce or repress the transcription of a considerable number of genes (Nakano et al. 2003a,b, 2005; Zuber 2004; Choi et al. 2006; Zhang and Zuber 2007) and can enhance the interaction between ComK and the protease ClpC (Nakano et al. 2002).

Feedback in connector-mediated regulation

Many TCS connectors participate in feedback loops, which influence their level and/or activity. For example, transcription of the *pmrD* gene, which encodes the protein that stabilizes the activated state of the response regulator PmrA, is inhibited by PmrA (Kato et al. 2003). Further examples of connectors that participate in or interfere with negative feedback loops include KipI (Wang et al. 1997), FixT (Foussard et al. 1997), RapC (Lazazzera et al. 1999), and RapF (Bongiorni et al. 2005). The adaptor RssB, which recruits RpoS for degradation and is regulated by multiple anti-adaptors (Bougdour et al. 2008), is involved in a negative feedback loop because *rssB* transcription is RpoS-dependent (Ruiz et al. 2001). Thus, accumulation of RpoS stimulates expression of the RssB protein that, by delivering RpoS to the ClpXP protease, will promote RpoS degradation, resulting in stabilization of the RpoS levels. Connector-mediated feedback can be positive. For instance, RapE promotes the dephosphorylation of Spo0F-P in the *B. subtilis* phosphorelay, resulting in lower levels of Spo0A-P. The activity of RapE, in turn, is counteracted by PhrE (Perego and Brannigan 2001), which is under positive transcriptional control of σ^H (McQuade et al. 2001), whose gene is indirectly activated by Spo0A-P (Britton et al. 2002).

Feedback loops confer special dynamic properties on the systems that they regulate (Thomas and D'Ari 1990; Mitrophanov and Groisman 2008). They speed up or slow down the response of a regulatory circuit (Rosenfeld et al. 2002; Mitrophanov and Groisman 2008), which can result in delays contributing to temporal fine-tuning of connector-mediated processes. Simple negative feedback loops exemplified by the PmrD regulation by PmrA-P can protect the cell from the accumulation of PmrA-P that would result from overexpression of PmrD. It is also possible that such feedback could serve the purpose of shaping the dynamic curve of the *pmrD* mRNA expression levels upon induction of the PhoP/PhoQ system. Indeed, the specific shape of the dynamic curve describing response regulator phosphorylation and mRNA expression is critical for certain phenotypic features, such as bacterial virulence (Shin et al. 2006).

Evolution of connectors

The differences in structure and physiological functions of connectors suggest that the evolutionary origins of

connectors may be diverse. Indeed, database searches for close homologs demonstrate that some connectors are highly conserved, while others have none or very few hits in the databases. Notably, even the connectors that are tightly functionally linked can have drastically different conservation properties. For example, the Rap proteins from *B. subtilis* are highly conserved among Gram-positive endospore-forming species (Perego and Brannigan 2001), but their Phr partners typically have no hits or just one hit (to a *Bacillus licheniformis* protein) when searched using BLASTP in the NCBI database. Furthermore, Sda and KipI perform the same function in *B. subtilis*—inhibition of KinA autophosphorylation—but display different phylogenetic distributions with Sda being well-conserved only among *Bacillus* and *Geobacillus* species, and KipI homologs found in a wide range of Gram-positive and Gram-negative bacteria. These data suggest that some connectors may have been acquired laterally (Ochman et al. 2000), which is supported by the phage origin of the gene for the connector TorI in *E. coli* (Ansaldi et al. 2004).

Allelic differences between orthologous connectors in closely related species can drastically affect their functional properties. While the PmrD proteins within natural *S. enterica* isolates and natural *E. coli* isolates are highly similar in sequence, the PmrD proteins between *E. coli* and *S. enterica* isolates are only 53.5%–56.6% identical (Winfield and Groisman 2004). This is in contrast to the target of PmrD, the response regulator PmrA, which is highly conserved between *E. coli* and *S. enterica*. Statistical analysis of substitution frequencies has demonstrated that the evolution of the PmrD protein is nonneutral (driven by selection) (Winfield and Groisman 2004). As a consequence of the allelic differences in the PmrD protein, *E. coli* does not generally have a connection between the PhoP/PhoQ and PmrA/PmrB TCSs, while *S. enterica* does (Winfield and Groisman 2004). These results raise the possibility that the divergence of the PmrD-mediated regulatory connection between *S. enterica* and *E. coli* contributes to the distinct lifestyles of these two species.

Connector proteins can preserve their functional properties despite substantial sequence divergence, if they constitute a part of a regulatory module conserved in related bacteria. The FixT protein of *Caulobacter crescentus* is only 25% identical to the connector FixT from *S. meliloti* but is transcriptionally controlled by the regulator FixK and inhibits the activity of the sensor kinase FixL, in a similar way to the rhizobial FixT that regulates nitrogen fixation (Garnerone et al. 1999; Crosson et al. 2005). While *C. crescentus* does not fix nitrogen, respire anaerobically, or metabolize hydrogen, it harbors the FixJ/FixL–FixK–FixT regulatory module, which is structurally and functionally similar to that of *S. meliloti* (Fischer 1994; Bèrges et al. 2001; Crosson et al. 2005).

Related species can have nonorthologous genes performing the same physiological function. The nonorthologous replacement (i.e., displacement) (Koonin et al. 1996) of connectors is exemplified by the IraP protein of *S. enterica* and the IraM protein of *E. coli*. Both of these anti-adaptors prevent the adaptor RssB (MviA) from recruiting the stationary phase σ-factor RpoS for degradation by ClpXP, and both the *iraP* and *iraM* genes are activated by the PhoP/PhoQ system in response to the low Mg^{2+} signal (Tu et al. 2006; Bougdour et al. 2008). Yet, the IraP and IraM proteins do not share substantial sequence identity. *E. coli* does have an IraP ortholog, but unlike the *Salmonella* IraP, it promotes RpoS stabilization primarily under phosphate starvation conditions.

Concluding remarks

TCS connectors endow bacterial cells with the ability to connect signal transduction pathways by modulating the output of two-component regulatory systems. Apart from signal integration, they confer distinct quantitative properties on signal transduction pathways. The sporadic phylogenetic distribution of connector-encoding genes suggests that they are continuously being invented and/or acquired via horizontal gene transfer. Connectors usually display very low or no amino acid sequence identity, which makes it difficult to identify novel connectors solely on the basis of their primary structure. Therefore, it is possible that regulatory proteins lacking a DNA-binding domain but affecting the output of a TCS may function as connectors (Zhan and Leigh 1990; Keating 2007).

Acknowledgments

We thank the reviewers for their thoughtful comments on the original version of the manuscript. Our research is supported by grants from the National Institutes of Health. E.A.G. is an investigator of the Howard Hughes Medical Institute.

References

Ansaldi, M., Theraulaz, L., and Mejean, V. 2004. TorI, a response regulator inhibitor of phage origin in *Escherichia coli*. *Proc. Natl. Acad. Sci.* **101:** 9423–9428.

Antelmann, H., Scharf, C., and Hecker, M. 2000. Phosphate starvation-inducible proteins of *Bacillus subtilis*: Proteomics and transcriptional analysis. *J. Bacteriol.* **182:** 4478–4490.

Appleby, J.L., Parkinson, J.S., and Bourret, R.B. 1996. Signal transduction via the multi-step phosphorelay: Not necessarily a road less traveled. *Cell* **86:** 845–848.

Bardwell, L., Zou, X.F., Nie, Q., and Komarova, N.L. 2007. Mathematical models of specificity in cell signaling. *Biophys. J.* **92:** 3425–3441.

Bèrges, H., Checroun, C., Guiral, S., Garnerone, A.-M., Boistard, P., and Batut, J. 2001. A glutamine-amidotransferase-like protein modulates FixT antikinase activity in *Sinorhizobium meliloti*. *BMC Microbiol.* **1:** 6. doi: 10.1186/1471-2180-1-6.

Bongiorni, C., Ishikawa, S., Stephenson, S., Ogasawara, N., and Perego, M. 2005. Synergistic regulation of competence development in *Bacillus subtilis* by two Rap-Phr systems. *J. Bacteriol.* **187:** 4353–4361.

Bougdour, A., Wickner, S., and Gottesman, S. 2006. Modulating RssB activity: IraP, a novel regulator of as stability in *Esch-*

erichia coli. Genes & Dev. **20:** 884–897.
Bougdour, A., Cunning, C., Baptiste, P.J., Elliott, T., and Gottesman, S. 2008. Multiple pathways for regulation of σ^S (RpoS) stability in *Escherichia coli* via the action of multiple anti-adaptors. *Mol. Microbiol.* **68:** 298–313.
Britton, R.A., Eichenberger, P., Gonzalez-Pastor, J.E., Fawcett, P., Monson, R., Losick, R., and Grossman, A.D. 2002. Genome-wide analysis of the stationary-phase σ factor (σ-H) regulon of *Bacillus subtilis. J. Bacteriol.* **184:** 4881–4890.
Browning, D.F. and Busby, S.J.W. 2004. The regulation of bacterial transcription initiation. *Nat. Rev. Microbiol.* **2:** 57–65.
Burbulys, D., Trach, K.A., and Hoch, J.A. 1991. Initiation of sporulation in *Bacillus subtilis* is controlled by a multicomponent phosphorelay. *Cell* **64:** 545–552.
Burkholder, W.F., Kurtser, I., and Grossman, A.D. 2001. Replication initiation proteins regulate a developmental checkpoint in *Bacillus subtilis. Cell* **104:** 269–279.
Choi, S.Y., Reyes, D., Leelakriangsak, M., and Zuber, P. 2006. The global regulator Spx functions in the control of organosulfur metabolism in *Bacillus subtilis. J. Bacteriol.* **188:** 5741–5751.
Claverys, J.P., Prudhomme, M., and Martin, B. 2006. Induction of competence regulons as a general response to stress in Gram-positive bacteria. *Annu. Rev. Microbiol.* **60:** 451–475.
Core, L. and Perego, M. 2003. TPR-mediated interaction of RapC with ComA inhibits response regulator–DNA binding for competence development in *Bacillus subtilis. Mol. Microbiol.* **49:** 1509–1522.
Crosson, S., McGrath, P.T., Stephens, C., McAdams, H.H., and Shapiro, L. 2005. Conserved modular design of an oxygen sensory/signaling network with species-specific output. *Proc. Natl. Acad. Sci.* **102:** 8018–8023.
Dixon, R. and Kahn, D. 2004. Genetic regulation of biological nitrogen fixation. *Nat. Rev. Microbiol.* **2:** 621–631.
Dubnau, D. and Losick, R. 2006. Bistability in bacteria. *Mol. Microbiol.* **61:** 564–572.
Eguchi, Y., Itou, J., Yamane, M., Demizu, R., Yamato, F., Okada, A., Mori, H., Kato, A., and Utsumi, R. 2007. B1500, a small membrane protein, connects the two-component systems EvgS/EvgA and PhoQ/PhoP in *Escherichia coli. Proc. Natl. Acad. Sci.* **104:** 18712–18717.
Eiamphungporn, W. and Helmann, J.D. 2008. The *Bacillus subtilis* σ^M regulon and its contribution to cell envelope stress responses. *Mol. Microbiol.* **67:** 830–848.
Fischer, H.M. 1994. Genetic regulation of nitrogen fixation in Rhizobia. *Microbiol. Rev.* **58:** 352–386.
Foussard, M., Garnerone, A.M., Ni, F., Soupene, E., Boistard, P., and Batut, J. 1997. Negative autoregulation of the *Rhizobium meliloti* fixK gene is indirect and requires a newly identified regulator, FixT. *Mol. Microbiol.* **25:** 27–37.
Fujita, M. and Losick, R. 2005. Evidence that entry into sporulation in *Bacillus subtilis* is governed by a gradual increase in the level and activity of the master regulator Spo0A. *Genes & Dev.* **19:** 2236–2244.
Fujita, M., Gonzalez-Pastor, J.E., and Losick, R. 2005. High- and low-threshold genes in the Spo0A regulon of *Bacillus subtilis. J. Bacteriol.* **187:** 1357–1368.
Gao, R., Mack, T.R., and Stock, A.M. 2007. Bacterial response regulators: Versatile regulatory strategies from common domains. *Trends Biochem. Sci.* **32:** 225–234.
Garnerone, A.M., Cabanes, D., Foussard, M., Boistard, P., and Batut, J. 1999. Inhibition of the FixL sensor kinase by the FixT protein in *Sinorhizobium meliloti. J. Biol. Chem.* **274:** 32500–32506.
Gottesman, S. 2003. Proteolysis in bacterial regulatory circuits. *Annu. Rev. Cell Dev. Biol.* **19:** 565–587.
Hamoen, L.W., Venema, G., and Kuipers, O.P. 2003. Controlling competence in *Bacillus subtilis*: Shared use of regulators. *Microbiology* **149:** 9–17.
Hengge-Aronis, R. 2002. Signal transduction and regulatory mechanisms involved in control of the σ^S (RpoS) subunit of RNA polymerase. *Microbiol. Mol. Biol. Rev.* **66:** 373–395.
Hoch, J.A. 2000. Two-component and phosphorelay signal transduction. *Curr. Opin. Microbiol.* **3:** 165–170.
Jenal, U. and Hengge-Aronis, R. 2003. Regulation by proteolysis in bacterial cells. *Curr. Opin. Microbiol.* **6:** 163–172.
Jiang, M., Grau, R., and Perego, M. 2000. Differential processing of propeptide inhibitors of Rap phosphatases in *Bacillus subtilis. J. Bacteriol.* **182:** 303–310.
Kato, A. and Groisman, E.A. 2004. Connecting two-component regulatory systems by a protein that protects a response regulator from dephosphorylation by its cognate sensor. *Genes & Dev.* **18:** 2302–2313.
Kato, A., Latifi, T., and Groisman, E.A. 2003. Closing the loop: The PmrA/PmrB two-component system negatively controls expression of its posttranscriptional activator PmrD. *Proc. Natl. Acad. Sci.* **100:** 4706–4711.
Kato, A., Mitrophanov, A.Y., and Groisman, E.A. 2007. A connector of two-component regulatory systems promotes signal amplification and persistence of expression. *Proc. Natl. Acad. Sci.* **104:** 12063–12068.
Keating, D.H. 2007. *Sinorhizobium meliloti* SyrA mediates the transcriptional regulation of genes involved in lipopolysaccharide sulfation and exopolysaccharide biosynthesis. *J. Bacteriol.* **189:** 2510–2520.
Koonin, E.V., Mushegian, A.R., and Bork, P. 1996. Non-orthologous gene displacement. *Trends Genet.* **12:** 334–336.
Kox, L.F.F., Wösten, M.M.S.M., and Groisman, E.A. 2000. A small protein that mediates the activation of a two-component system by another two-component system. *EMBO J.* **19:** 1861–1872.
Laub, M.T. and Goulian, M. 2007. Specificity in two-component signal transduction pathways. *Annu. Rev. Genet.* **41:** 121–145.
Lazazzera, B.A. 2001. The intracellular function of extracellular signaling peptides. *Peptides* **22:** 1519–1527.
Lazazzera, B.A., Kurtser, I.G., McQuade, R.S., and Grossman, A.D. 1999. An autoregulatory circuit affecting peptide signaling in *Bacillus subtilis. J. Bacteriol.* **181:** 5193–5200.
Mandicmulec, I., Doukhan, L., and Smith, I. 1995. The *Bacillus subtilis* SinR protein is a repressor of the key sporulation gene Spo0A. *J. Bacteriol.* **177:** 4619–4627.
Mascher, T., Helmann, J.D., and Unden, G. 2006. Stimulus perception in bacterial signal-transducing histidine kinases. *Microbiol. Mol. Biol. Rev.* **70:** 910–938.
McQuade, R.S., Comella, N., and Grossman, A.D. 2001. Control of a family of phosphatase regulatory genes (*phr*) by the alternate σ factor σ-H of *Bacillus subtilis. J. Bacteriol.* **183:** 4905–4909.
Mitrophanov, A.Y. and Groisman, E.A. 2008. Positive feedback in cellular control processes. *Bioessays* **30:** 542–555.
Mitrophanov, A.Y., Jewett, M.W., Hadley, T.J., and Groisman, E.A. 2008. Evolution and dynamics of regulatory architectures controlling polymyxin B resistance in enteric bacteria. *PLoS Genetics* (in press).
Mueller, J.P., Bukusoglu, G., and Sonenshein, A.L. 1992. Transcriptional regulation of *Bacillus subtilis* glucose starvation-inducible genes—Control of GsiA by the ComP–ComA signal transduction system. *J. Bacteriol.* **174:** 4361–4373.
Nakano, M.M., Zuber, P., Glaser, P., Danchin, A., and Hulett, F.M. 1996. Two-component regulatory proteins ResD–ResE are required for transcriptional activation of *fnr* upon oxygen

limitation in *Bacillus subtilis*. *J. Bacteriol.* **178:** 3796–3802.
Nakano, M.M., Nakano, S., and Zuber, P. 2002. Spx (YjbD), a negative effector of competence in *Bacillus subtilis*, enhances ClpC–MecA–ComK interaction. *Mol. Microbiol.* **44:** 1341–1349.
Nakano, S., Kuster-Schock, E., Grossman, A.D., and Zuber, P. 2003a. Spx-dependent global transcriptional control is induced by thiol-specific oxidative stress in *Bacillus subtilis*. *Proc. Natl. Acad. Sci.* **100:** 13603–13608.
Nakano, S., Nakano, M.M., Zhang, Y., Leelakriangsak, M., and Zuber, P. 2003b. A regulatory protein that interferes with activator-stimulated transcription in bacteria. *Proc. Natl. Acad. Sci.* **100:** 4233–4238.
Nakano, S., Erwin, K.N., Ralle, M., and Zuber, P. 2005. Redox-sensitive transcriptional control by a thiol/disulphide switch in the global regulator, Spx. *Mol. Microbiol.* **55:** 498–510.
Ochman, H., Lawrence, J.G., and Groisman, E.A. 2000. Lateral gene transfer and the nature of bacterial innovation. *Nature* **405:** 299–304.
Ogino, T., Matsubara, M., Kato, N., Nakamura, Y., and Mizuno, T. 1998. An *Escherichia coli* protein that exhibits phosphohistidine phosphatase activity towards the HPt domain of the ArcB sensor involved in the multistep His-Asp phosphorelay. *Mol. Microbiol.* **27:** 573–585.
Perego, M. 1997. A peptide export–import control circuit modulating bacterial development regulates protein phosphatases of the phosphorelay. *Proc. Natl. Acad. Sci.* **94:** 8612–8617.
Perego, M. 2001. A new family of aspartyl phosphate phosphatases targeting the sporulation transcription factor Spo0A of *Bacillus subtilis*. *Mol. Microbiol.* **42:** 133–143.
Perego, M. and Brannigan, J.A. 2001. Pentapeptide regulation of aspartyl-phosphate phosphatases. *Peptides* **22:** 1541–1547.
Perego, M. and Hoch, J.A. 1996. Cell–cell communication regulates the effects of protein aspartate phosphatases on the phosphorelay controlling development in *Bacillus subtilis*. *Proc. Natl. Acad. Sci.* **93:** 1549–1553.
Perez, J.C. and Groisman, E.A. 2007. Acid pH activation of the PmrA/PmrB two-component regulatory system of *Salmonella enterica*. *Mol. Microbiol.* **63:** 283–293.
Perraud, A.L., Weiss, V., and Gross, R. 1999. Signalling pathways in two-component phosphorelay systems. *Trends Microbiol.* **7:** 115–120.
Piggot, P.J. and Hilbert, D.W. 2004. Sporulation of *Bacillus subtilis*. *Curr. Opin. Microbiol.* **7:** 579–586.
Rosenfeld, N., Elowitz, M.B., and Alon, U. 2002. Negative autoregulation speeds the response times of transcription networks. *J. Mol. Biol.* **323:** 785–793.
Ruiz, N., Peterson, C.N., and Silhavy, T.J. 2001. RpoS-dependent transcriptional control of *sprE*: Regulatory feedback loop. *J. Bacteriol.* **183:** 5974–5981.
Ruvolo, M.V., Mach, K.E., and Burkholder, W.F. 2006. Proteolysis of the replication checkpoint protein Sda is necessary for the efficient initiation of sporulation after transient replication stress in *Bacillus subtilis*. *Mol. Microbiol.* **60:** 1490–1508.
Shin, D. and Groisman, E.A. 2005. Signal-dependent binding of the response regulators PhoP and PmrA to their target promoters in vivo. *J. Biol. Chem.* **280:** 4089–4094.
Shin, D., Lee, J., Huang, H., and Groisman, E.A. 2006. A positive feedback loop promotes transcription surge that jump-starts *Salmonella* virulence circuit. *Science* **314:** 1607–1609.
Smits, W.K., Kuipers, O.P., and Veening, J.W. 2006. Phenotypic variation in bacteria: The role of feedback regulation. *Nat. Rev. Microbiol.* **4:** 259–271.
Smits, W.K., Bongiorni, C., Veening, J.W., Hamoen, L.W., Kuipers, O.P., and Perego, M. 2007. Temporal separation of distinct differentiation pathways by a dual specificity Rap-Phr system in *Bacillus subtilis*. *Mol. Microbiol.* **65:** 103–120.
Sonenshein, A.L. 2000. Control of sporulation initiation in *Bacillus subtilis*. *Curr. Opin. Microbiol.* **3:** 561–566.
Stock, A.M., Robinson, V.L., and Goudreau, P.N. 2000. Two-component signal transduction. *Annu. Rev. Biochem.* **69:** 183–215.
Thackray, P.D. and Moir, A. 2003. SigM, an extracytoplasmic function σ factor of *Bacillus subtilis*, is activated in response to cell wall antibiotics, ethanol, heat, acid, and superoxide stress. *J. Bacteriol.* **185:** 3491–3498.
Thomas, R. and D'Ari, R. 1990. *Biological feedback*. CRC Press, Boca Raton, FL.
Tu, X., Latifi, T., Bougdour, A., Gottesman, S., and Groisman, E.A. 2006. The PhoP/PhoQ two-component system stabilizes the alternative σ factor RpoS in *Salmonella enterica*. *Proc. Natl. Acad. Sci.* **103:** 13503–13508.
Tzivion, G. and Avruch, J. 2002. 14–3–3 proteins: Active cofactors in cellular regulation by serine/threonine phosphorylation. *J. Biol. Chem.* **277:** 3061–3064.
Veening, J.W., Hamoen, L.W., and Kuipers, O.P. 2005. Phosphatases modulate the bistable sporulation gene expression pattern in *Bacillus subtilis*. *Mol. Microbiol.* **56:** 1481–1494.
Wang, L., Grau, R., Perego, M., and Hoch, J.A. 1997. A novel histidine kinase inhibitor regulating development in *Bacillus subtilis*. *Genes & Dev.* **11:** 2569–2579.
Winfield, M.D. and Groisman, E.A. 2004. Phenotypic differences between *Salmonella* and *Escherichia coli* resulting from the disparate regulation of homologous genes. *Proc. Natl. Acad. Sci.* **101:** 17162–17167.
Wösten, M.M.S.M., Kox, L.F.F., Chamnongpol, S., Soncini, F.C., and Groisman, E.A. 2000. A signal transduction system that responds to extracellular iron. *Cell* **103:** 113–125.
Zhan, H.J. and Leigh, J.A. 1990. Two genes that regulate exopolysaccharide production in *Rhizobium meliloti*. *J. Bacteriol.* **172:** 5254–5259.
Zhang, Y. and Zuber, P. 2007. Requirement of the zinc-binding domain of CIPX for Spx proteolysis in *Bacillus subtilis* and effects of disulfide stress on ClpXP activity. *J. Bacteriol.* **189:** 7669–7680.
Zheng, M., Wang, X., Templeton, L.J., Smulski, D.R., LaRossa, R.A., and Storz, G. 2001. DNA microarray-mediated transcriptional profiling of the *Escherichia coli* response to hydrogen peroxide. *J. Bacteriol.* **183:** 4562–4570.
Zuber, P. 2004. Spx–RNA polymerase interaction and global transcriptional control during oxidative stress. *J. Bacteriol.* **186:** 1911–1918.

Signal integration in bacterial two-component regulatory systems

Alexander Y. Mitrophanov and Eduardo A. Groisman

Genes Dev. 2008, **22:**
Access the most recent version at doi:10.1101/gad.1700308