HHS Public Access

Author manuscript

Annu Rev Genet. Author manuscript; available in PMC 2016 November 23.

Published in final edited form as:

Annu Rev Genet. 2015 November 23; 49: 603–625. doi:10.1146/annurev-genet-112414-054813.

General Stress Signaling in the Alphaproteobacteria

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Abstract

The Alphaproteobacteria uniquely integrate features of two-component signal transduction and alternative σ factor regulation to control transcription of genes that ensure growth and survival across a range of stress conditions. Research over the past decade has led to the discovery of the key molecular players of this general stress response (GSR) system, including the sigma factor σ^{EcfG}, its anti-σ factor NepR, and the anti-anti-σ factor PhyR. The central molecular event of GSR activation entails aspartyl phosphorylation of PhyR, which promotes its binding to NepR and thereby releases σ^{EcfG} to associate with RNAP and direct transcription. Recent studies are providing a new understanding of complex, multilayered sensory networks that activate and repress this central protein partner switch. This review synthesizes our structural and functional understanding of the core GSR regulatory proteins and highlights emerging data that are defining the systems that regulate GSR transcription in a variety of species.

Keywords

HisKA_2 and HWE kinases; signal transduction; PhyR; ECF sigma factor; partner switch; two-component signaling

INTRODUCTION

Bacteria employ suites of transcriptional regulatory proteins to facilitate growth and survival in fluctuating environments (Figure 1). Although many of these proteins control narrow transcriptional responses to specific chemical or physical signals, bacteria often encode **general stress response (GSR)** regulators that modulate gene expression at a global level and enable cell growth under a range of adverse conditions. Bacterial GSRs are typically mediated by an alternative **sigma (σ) factor** that, when active, competes directly with the primary σ factor for binding to core RNA polymerases (RNAPs) and initiates transcription of a gene set that broadly supports growth and survival across many different nonoptimal conditions.

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DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

The GSR systems of *Escherichia coli* and *Bacillus subtilis*, regulated by σ^S and σ^B respectively, are well studied. σ^S is conserved across the Gamma, Delta, and Beta classes of Proteobacteria (20). A multigene network regulates σ^S at the transcriptional, posttranscriptional and posttranslational levels in response to myriad stress and cellular growth signals, including starvation, UV exposure, hyperosmotic stress, low pH, and heat shock (9, 47). σ^B is a GSR σ factor in select Gram-positive phyla (43) that is regulated posttranslationally by a partner switching mechanism involving the anti-σ^B factor RbsW and the anti-anti-σ^B factor RsbV (102, 107). Acute environmental stress signals, including heat shock and low pH, and internal signals tied to the metabolic/energetic state of the cell coordinately regulate RsbV phosphorylation, which in turn controls σ^B activity.

The Alphaproteobacteria are a metabolically and ecologically diverse class that encompasses free-living soil and aquatic microbes, host-associated symbionts and pathogens, and obligate endosymbionts (10, 28). However, species in this clade do not encode orthologs of σ^S (*rpoS*) or σ^B (*sigB*) and, until recently, it was not known whether this class possessed a GSR system. Research over the past decade has shown that Alphaproteobacteria do indeed encode a GSR system that displays regulatory parallels to σ^B of Gram-positive bacteria, although the components of these systems are evolutionarily distinct.

The core of the alphaproteobacterial GSR system encompasses three cytoplasmic proteins: (*a*) an **extracytoplasmic function (ECF)** σfactor, EcfG; (*b*) its anti-σ factor, NepR; and (*c*) an anti-anti-σ factor, PhyR (Figure 2). PhyR is a unique multidomain protein that bridges the **two-component signal transduction (TCST)** and alternative σ factor regulatory paradigms; it contains an N-terminal σ-like domain and a C-terminal TCST receiver domain. Aspartyl phosphorylation of the PhyR receiver domain promotes binding of the σ-like domain to NepR, thereby releasing σ^{EcfG} to associate with RNAP and direct transcription (34) (Figure 2). Thus, a phosphorylation-dependent protein partner switch also regulates the alphaproteobacterial GSR.

The three core GSR proteins involved in the regulatory partner switch are broadly conserved in Alphaproteobacteria. Across the clade, however, additional regulatory proteins and mechanisms are integrated into the core GSR system. These include distinctive sensory proteins that activate and repress PhyR activity, **paralogs** of the core regulators, and posttranslational regulatory mechanisms that modulate GSR transcriptional output by affecting PhyR stability. Moreover, the transcriptional output of GSR signaling is not highly conserved. Our emerging understanding of the alphaproteobacterial GSR provides evidence that the environmental inputs, transcriptional outputs, and regulatory dynamics of this system reflect the evolutionary histories and lifestyles of the particular species. In this review, we describe the key components and define molecular and structural features of the alphaproteobacterial GSR system. We further synthesize recent studies demonstrating variation and complexity in the signaling inputs controlling this important environmental response system.

ENVIRONMENTAL SIGNALS AND PHYSIOLOGICAL RESPONSES ACROSS THE ALPHAPROTEOBACTERIAL CLADE

An intact GSR system enables survival across an array of ecological niches and adverse environmental conditions. In the laboratory, a number of defined stress conditions have been identified under which GSR regulators are activated and provide protection. These include hyperosmolarity (ionic or nonionic), low or high pH, peroxide, stationary phase, UV exposure, temperature shifts, and desiccation. Given the diversity of environments exploited by Alphaproteobacteria, it is not surprising that the stresses for which the GSR system is protective vary between species (35). Even closely related strains of the same species show differences in stress sensitivities in mutants lacking GSR regulators [e.g., *Methylobacterium extorquens* PA1 and *Methylobacterium extorquens* AM1 (38, 79)]. In this section, we briefly outline how the GSR system regulates growth and survival of Alphaproteobacteria that inhabit a diverse range of environments.

Intracellular Pathogens

Brucella spp. are intracellular pathogens that persist in the soil and establish a long-term residence within mammalian cells. PhyR, the general stress σ factor (σ^{E1}), and the PhyR activating kinase (LovhK) are required for *Brucella abortus* and *Brucella melitensis* to establish and maintain chronic infection and also to survive in vitro stresses, such as low pH or peroxide exposure, that mimic aspects of the intracellular environment (26, 67, 68). *Bartonella* spp. are insect-transmitted intracellular pathogens that alternate between life in the gut of the insect vector and a mammalian host. GSR-dependent transcription is activated and required for survival of *Bartonella quintana* in high-hemin and low-temperature environments, which reflect conditions in the insect gut (1).

Plant-Associated Species

Species that inhabit the phyllosphere (i.e., plant surfaces above the soil), such as *M. extorquens* and *Sphingomonas melonis*, require GSR regulators for competitive epiphytic survival and also for resistance to thermal, UV, and desiccation stresses (38, 39, 63, 79). Similarly, *Methylosinus* sp. B4S requires an intact GSR system for survival of high temperatures and UV exposure, but it can grow in the phyllosphere in the absence of elevated temperatures or UV (59). Thus, the GSR may not be explicitly required for association with the plant, but rather for survival under conditions typically encountered on plant surfaces. Soil-dwelling plant symbionts, such as *Rhizobium etli*, *Bradyrhizobium japonicum*, and *Sinorhizobium meliloti*, exist in free-living forms or in symbiotic root nodules as differentiated, nitrogen-fixing bacteroids. Similar to microbes in the phyllosphere, these soil microbes are subject to extremes of temperature, water, and nutrient availability, and they encounter complex chemical signals from plants and other microbes. The *S. meliloti* general stress σ factor (σ^{E2}) facilitates survival in the face of many adverse conditions that correlate with soil environments, including heat shock, osmotic and oxidative stress, and nutrient limitation (30, 31, 92). Similarly, *R. etli* requires σ^{EcfG1} for tolerance of elevated temperatures and high osmolarity (75, 101). *B. japonicum* σ^{EcfG} and PhyR are induced by desiccation and exposure to plant flavonoids that activate expression of genes

involved in symbiosis (24, 25). Furthermore, *B. japonicum* lacking either *ecfG* or *phyR* are defective in development of symbiotic nodules (40).

Free-Living Species

For other free-living organisms, the ecologically relevant environmental stress conditions are more difficult to predict. Most of the Rhodobacterales and Caulobacterales species occupy oligotrophic marine or freshwater environments. The freshwater species *Caulobacter crescentus* relies on its GSR regulators for protection from osmotic and oxidative stresses (3, 51, 71). Notably, genes under the control of *C. crescentus* σ^{EcfG}, also annotated as σ^{T}, are temporally regulated in a cell cycle–dependent manner under seemingly nonstress conditions (78). Neither the activating signal nor the adaptive advantage of this cell cycle regulation is understood. However, cells lacking σ^{T} exhibit cell cycle defects under acute carbon starvation (11). It is not known whether cell cycle control of the GSR regulon is a regulatory feature conserved throughout the Alphaproteobacteria.

General Stress Response Transcriptional Output

Many transcriptomic experiments have identified genes under the control of PhyR, NepR, σ^{EcfG}, or GSR related kinases (4, 11, 33, 34, 38, 40, 60, 67, 68, 71, 75, 92). Not surprisingly, GSR-regulated genes vary from species to species and include a large number of genes of unknown function. Nevertheless, some general patterns have emerged from these studies. First, genes with known functions in stress protection, including genes involved in DNA protection during starvation, *dps* (13), the broadly conserved but uncharacterized stress response protein, *csbD* (2), antioxidants such as catalase (*katE, katC, katG*) and superoxide dismutase (*sodA*), heat shock regulators (*rpoH*) and chaperones, and osmoprotectants such as *osmC* and trehalose synthesis genes, are often identified. Second, the GSR regulons typically include a large number of genes encoding predicted inner and outer membrane proteins, suggesting that activation of the GSR leads to significant modulation of the bacterial envelope. Third, other regulatory proteins, including two-component regulators, transcription factors, and other alternative σ factors, are common in GSR regulons across species, suggesting multiple layers of control in these systems.

ORGANIZATION OF THE GENERAL STRESS RESPONSE CHROMOSOMAL LOCUS: *PHYR*, *NEPR*, *ECFG*, AND KINASES

The organization of the GSR chromosomal locus has conserved features, although there is notable variability among alphaproteobacterial orders (Figure 3). The anti-anti-σ factor *phyR* is usually immediately adjacent to *nepR*, *ecfG*, and genes encoding sensor histidine kinases of the **HWE or HisKA_2** families (95). In orders Rhodobacterales and Caulobacterales, the arrangement of the GSR locus is highly conserved; *phyR* is encoded adjacent, but in the opposite orientation, to *nepR*, *ecfG*, and a HisKA_2-family histidine kinase bearing a periplasmic sensory domain. Species that deviate from this organization include those that contain a second copy of the entire GSR locus (*Roseobacter denitrificans*) or of *phyR* (*Hyphomonas neptunium*), or that lack a GSR locus altogether (e.g., *Silicibacter pomeroyi*, *Maricaulis maris, Roseobacter* sp. *MED193*). In order Rhodospirillales, the three core GSR genes are rearranged such that *nepR* is positioned between one or two copies of *ecfG* and a

transmembrane HisKA_2-family kinase gene. Several genera in Rhodospirillales lack *phyR* and *ecfG* homologs, including *Acidophilium* and *Magnetospirillum*.

HWE and HisKA_2 kinases: Nomenclature and Key Characteristics

HisKA_2 and HWE are unusual histidine kinase families that share features of primary structure in both the dimerization and histidine phosphotransfer (DHp) domain, and in the catalytic ATPase (CA) domain (Figure 5). The shared ExxHRxxN motif at the site of histidine phosphorylation (41, 66) has led to the collective moniker HRxxN kinases to represent these two groups (64, 65). The HWE superfamily, Cl06527 (previously called the HisKA_2 superfamily), also encompasses these related DHp domains (29).

The CA domains of HisKA_2 kinases are typically annotated as HATPase_c, whereas the CA domains of HWE kinases are not readily captured in the HATPase_c domain model and thus not usually annotated. Nevertheless, both HisKA_2 and HWE possess an atypical glutamate in the ATP binding N-box (Figure 5c; 41, 66), which is required for autophosphorylation (89). HWE kinases are further distinguished by a histidine in the N-box and a WxE motif in the G-box region, which gave rise to the name HWE (66). The HWE CA domain, not represented by a defined sequence model in Pfam or MiSTdb (29, 99), is included in COG3920, which spans both domains of HisKA_2 and HWE kinases (69).

Species in the order Rhizobiales largely maintain the typical *ecfG, nepR, phyR* arrangement, although rearrangements involving the associated histidine kinase genes are common. The Sphingomonadales exhibit significant variability in GSR locus organization with respect to both associated kinases and core GSR gene order. Notably, the anti-σ factor *nepR* and its cognate σ factor *ecfG* are spatially decoupled in the chromosomes of several species (see Figure 3).

THE GENERAL STRESS RESPONSE TRANSCRIPTIONAL REGULATOR: σ^{ECFG}

ECF σ factors constitute a broad class of proteins that regulate transcriptional responses to environmental stresses (45, 96). These proteins are pared-down versions of primary σ^{70} factors that contain only σ regions 2 and 4, which determine interaction with core RNAP and recognition of −35 and −10 box promoter elements. ECF σ factors typically autoregulate their transcription, are coexpressed with their cognate anti-σ factor, and are sequestered by their anti-σ factor in the absence of an appropriate environmental signal. The mechanism by which anti-σ factors are inactivated in response to environmental cues varies (77, 96).

The GSR of Alphaproteobacteria is controlled by ECF σ factors classified as ECF15 (96). Their phylogenetic restriction to Alphaproteobacteria and frequent genomic proximity to *phyR* and *nepR* are defining features of this σ family. Vorholt, Mascher, and colleagues have proposed the name *ecfG* (G for GSR) to unify and clarify the nomenclature of this family (96). Variable annotations of functionally characterized ECF15 (EcfG)-family σ factors include *sigT* in *C. crescentus* (4), *rpoE1* in *B. abortus* (67), *rpoE2* in *S. meliloti* (92), and

rpoE4 in *R. etli*, which was recently renamed *ecfG1* in an effort to unify nomenclature (76) (60).

A motif contained in the −35 and −10 boxes of σ^{EcfG}-regulated promoters has been identified in species across Alphaproteobacteria, including *S. meliloti* (92), *M. extorquens* (38), *B. japonicum* (40), *R. etli* (60, 75), *C. crescentus* (4, 78), *B. abortus* (67), *B. quintana* (1), *Rhodopseudomonas palustris* (70), *S. melonis* FR1 (63), *Methylosinus* sp. B4S (59), and *Erythrobacter litoralis* (21). This EcfG motif has a consensus sequence of GGAAC-$N_{16–17}$-CGTT (Figure 4*b*) and is upstream of *ecfG* operons, consistent with observed *ecfG* positive autoregulation (1, 4, 34, 40, 59, 60, 63, 70, 75, 92).

Paralogy in the ecfG Family

Although *ecfG* is most often present in a single genomic copy, many species encode multiple **paralogs** (95, 96, 99) (Figure 3). For example, in the order Rhodospirillales, select species encode tandem copies of *ecfG*-family genes at the GSR locus. In *C. crescentus* and *S. melonis*, orphan *ecfG* paralogs (*sigU* and *ecfG2*, respectively) are transcriptional targets of the primary σ^{EcfG} encoded from the GSR locus. Neither of these orphan copies interacts with NepR (63, 71), and their deletion does not result in acute stress survival defects (4, 63, 71). However, under carbon starvation conditions, a *C. crescentus* Δ*sigU* null mutant exhibits a cell differentiation defect that is similar to the Δ*sigT* strain (11). This finding suggests that in certain environmental conditions, *ecfG* paralogs may coordinately regulate specific functions. Within the *C. crescentus* and *S. melonis* genomes, the paralogous copies of *ecfG* are more similar to each other than to orthologs from related species (60). Other sequenced species in the genus *Caulobacter* or *Sphingomonas* bear only a single *ecfG*-family σ factor (99). These data provide evidence that recent duplication events have generated these secondary *ecfG* paralogs.

Rhizobium, *Agrobacterium*. and *Sinorhizobium* species bear two copies of *ecfG* that are apparently a result of an older gene duplication event (60). Experiments in *R. etli* suggest that these two paralogs, *ecfG1* and *ecfG2* (previously called *rpoE4*, respectively) (75, 101), have diverged to function in parallel rather than in series. Each gene autoregulates its expression as well as unique, although overlapping, sets of downstream genes. Moreover, expression of *ecfG1* and *ecfG2* is activated by different conditions (101). These results are consistent with the distinct stress phenotypes associated with individual deletion of each gene (60, 101). In *R. etli*, the primary *ecfG1* is located proximal to *phyR* and *nepR*. The promoter motif identified in σ^{EcfG1}-regulated genes matches the conserved σ^{EcfG} consensus binding site (75) (Figure 4b). There is not an apparent motif upstream of σ^{EcfG2}-regulated genes (60).

Species in the genus *Methylobacterium* have an unusual abundance of *ecfG*-family σ factors (4–10 copies per species) (99). The functional relationship between these paralogs remains largely undefined. In *M. extorquens*, the experimentally defined σ^{EcfG1} regulon is significantly smaller than the set of genes under direct or indirect control of PhyR and NepR (34), raising the possibility that PhyR and NepR may regulate multiple EcfG paralogs. Furthermore, *M. extorquens* strains lacking *ecfG1* do not exhibit a stress survival defect

(34), which is consistent with a model in which multiple *ecfG* paralogs have overlapping functions in *M. extorquens* GSR.

Sigma Factor Utilization

When considering the mechanism of GSR regulation in cells, it is important to note that this system actually entails multiple protein-partner switching events. In addition to stress activated titration of NepR from σ^{EcfG} to PhyR, σ^{EcfG} switches between binding NepR and competing with σ^{70} and other alternative σ factors for binding to a limited pool of core RNAP (Figures 1*c* and 2). Although the PhyR-NepR-EcfG partner switch is the focus of this review and most of the work on alphaproteobacterial GSR, other cellular factors, such as the starvation second messenger guanosine tetraphosphate (ppGpp), can modulate the affinity of σ factor binding to core RNAP and thus affect usage of alternative σ factors under particular environmental conditions (62, 72, 81). Control of σ factor usage by ppGpp may explain starvation-dependent phenotypes and starvation-induced cross protection in the alphaproteobacterial GSR system (11, 34, 38, 40, 60, 101).

THE ANTI-σ FACTOR: NEPR

Anti-σ factors are proteins that interact with σ factors and inhibit their ability to associate with core RNAP and/or DNA. These proteins are therefore key regulators involved in a broad set of developmental programs and physiological adaptations, including sporulation, flagellar biogenesis, virulence, and general and specific stress responses (12, 44, 45, 56, 83, 96).

The small (≈70 aa) soluble protein NepR (*ne*gative regulator of *p*hyR response) is the anti-σ factor of σ^{EcfG} (34). Consistent with its function as a negative regulator of σ^{EcfG}, overexpression of *nepR* results in transcriptional and stress survival defects that match strains harboring deletions of *ecfG* or *phyR* (8, 34, 40, 50, 63, 67, 71, 92). As with the σ^{B}/RsbW σ/anti-σ GSR system of Gram-positive bacteria (42, 87), deletion of the anti-σ factor *nepR* is incompatible with cell growth (71, 92) because of either uncontrolled transcription of genes in the GSR regulon or overtitration of the core RNAP from the essential RNAP-σ^{70} holocomplex. The apparent essential nature of *nepR* is determined by *ecfG; nepR* can be readily deleted from the chromosome in a background lacking *ecfG* (52, 63, 67, 92).

Co-transcription of anti-σ factors with their cognate σ factor is typical. Such an arrangement enables proportional expression of both proteins. Indeed, it is most often the case that *nepR* is encoded directly adjacent to *ecfG* (see Figure 3). However, there are instances in which *nepR* and *ecfG* have been spatially decoupled on the chromosome. For example, in *Methylobacterium* species, *nepR* is encoded adjacent to *phyR*, whereas *ecfG* is expressed from a distal chromosomal locus (34) (Figure 3). In *S. meliloti*, two *nepR* paralogs (named RsiA1 and RsiA2) function as negative regulators of σ^{RpoE2} (an EcfG-family σ factor) (8); one is cotranscribed with and regulated by σ^{RpoE2}, whereas the other *nepR* paralog is adjacent to a second *phyR* paralog and regulated independently of σ^{RpoE2}. The control properties conferred on the *S. meliloti* GSR system by this particular regulatory topology have not been fully explored.

Anti-σ factors typically interact with their cognate σ factors in one of two ways: (*a*) the anti-σ is wedged between regions σ2 and σ4 of the σ factor, or (*b*) the anti-σ is wrapped around the outer surface of the σ factor. In either case, the interaction inhibits the σ factor from interacting with RNAP core enzyme and DNA by inducing structural changes in the organization of the σ factor regions or by occluding RNAP binding surfaces (14, 16). NepR wraps around the PhyR σ-like domain competing for the same interaction interface as the PhyR receiver domain (15, 51) (Figure 4*c*). On the basis of these NepR structures with PhyR, and the structure of the **ECF** σ factor CnrH bound to its anti-σ factor, CnrY (73), NepR is predicted to wrap around the outer surface σ^{EcfG}, competing for the same interaction interface as RNAP (Figure 4*a*) (14, 15, 51).

NepR has simple structural features composed of two central α-helices joined by a small linker, and two unstructured regions at the N and C termini that flank the central helices (Figure 4*d*). In the absence of a binding partner, *S. melonis* NepR is disordered in solution (15). When bound to the PhyR σ-like domain, NepR adopts a more structured α-helical conformation (15). The NepR helical core is the most conserved region of the sequence and is essential for interaction with both σ^{EcfG} and PhyR (15, 51, 52). The flanking regions are variable both in sequence and in length (15, 51, 96), a feature that complicates prediction of the *nepR* translation start site (8, 52). Although it is poorly conserved, the N-terminal flanking region (FR1 in Figure 4*d*) plays an important role in NepR partner interactions. Specifically, in *S. meliloti*, the length of the RsiA1 (one of two NepR paralogs) N terminus affects its efficacy as a negative regulator of σ (8). Similarly, *C. crescentus* NepR lacking the flanking regions does not function as a repressor of σ activity (52). These data suggest that the unconserved, disordered N-terminus is required for NepR function as an anti-σ^{EcfG} factor. Although the molecular foundations of these observations are not fully understood at the present, the nonconserved termini NepR play an important role in the regulatory interactions with its binding partners, PhyR and σ^{EcfG}.

PHYR: A CENTRAL REGULATOR IN GENERAL STRESS RESPONSE SIGNAL TRANSDUCTION

PhyR, first described in *M. extorquens* as phyllosphere-induced regulator (39), is a central GSR regulatory protein in Alphaproteobacteria. The juxtaposition of a σ-like domain and a **TCST** receiver domain in this protein composes a unique sequence family in the Conserved Domain Database (74). Although widely distributed throughout the Alphaproteobacteria, *phyR* is phylogenetically restricted to this class. Other than species within the order Rickettsiales, which have highly reduced genomes, very few Alphaproteobacteria lack the *phyR* gene (Figure 3). In instances in which *phyR* is absent from a genome, the other core genetic components of the GSR system are also absent (95) (Figure 3).

Typically, Alphaproteobacteria encode a single copy of *phyR*. A handful of species encode two *phyR* paralogs, which appear to be have arisen from recent gene duplication or horizontal gene transfer (Figure 3). In the case of *S. meliloti*, each PhyR paralog (RsiB1 and RsiB2) can interact with each NepR paralog (RsiA1 and RsiA1) and function to regulate transcription in response to environmental stress (8). The adaptive advantage of this redundant regulatory topology in *S. meliloti* GSR signaling remains poorly understood.

Like ECF σ factors, the σ-like domain of PhyR can be subdivided into two regions, σ2 and σ4 (15, 50, 51) (Figure 4*c*,*e*,*f*). However, the PhyR σ regions are missing key primary structures required for interaction with DNA. PhyR region σ2 is incomplete, lacking the sequence involved in binding the −10 box. σ4 is degenerate in region 4.2, which is involved in binding the −35 box (34, 96). Thus, PhyR appears incapable of directly regulating transcription. Rather, this domain functions as a σ factor mimic that titrates an anti-σ factor and indirectly regulates GSR transcription (34).

The PhyR receiver domain integrates information about the state of the internal and external environment by acting as a substrate for one or more sensory kinases or phosphatases in the cell. A range of experimental approaches provides evidence that PhyR phosphorylation increases its affinity for NepR and is required for GSR activation (1, 15, 21, 34, 40, 51, 63, 67, 71). Qualitative assays from a number of species fail to demonstrate strong interactions between NepR and PhyR, and identify interactions between NepR and σ^{EcfG}, PhyR-σ-like domain, or phosphorylated PhyR (PhyR~P). Quantitative measurements of binding kinetics indicate that affinities between homologous partners can range by orders of magnitude between species. Nevertheless, within a species, affinities between pairs confirm a hierarchy consistent with the model that PhyR~P, but not PhyR, can titrate NepR from σ^{EcfG} (15, 51, 67). *B. abortus* presents an interesting variation on this model which is discussed below.

Structural Basis of PhyR Activation

Phosphorylation of the PhyR receiver domain triggers protein conformational changes that expose the high-affinity NepR binding site on the σ-like domain. Two key changes in PhyR structure facilitate NepR binding to the σ-like domain (Figure 4*e*,*f*). First, the α11-β5 loop of the receiver domain, which stabilizes the interaction between the σ-like and receiver domains (15, 50, 79), is destabilized and mobilized upon phosphorylation (51). Second, the small α4 helix connecting the σ-like and receiver domains undergoes a major rearrangement between the structures of the closed full-length PhyR and σ-like domain bound to NepR (Figure 4*c*,*e*) (15, 50, 51). In the NepR bound state, α4 is postulated to act as a doorstop that prevents the receiver domain from displacing NepR and rebinding the σ-like domain (51). Functionally, the dynamic helix α4 is required for high affinity PhyR~P/NepR binding and for efficient activation of GSR transcription (15, 51).

Notably, NepR strongly enhances PhyR phosphorylation in vitro by acetyl phosphate or PhyR kinases (52, 64). Thus, there is interesting reciprocal regulation in this two-protein system: PhyR phosphorylation promotes its association with NepR, and NepR/PhyR association stabilizes a PhyR conformation that is more amenable to phosphorylation.

Regulation of PhyR Concentration

Binding between protein interaction partners depends on the kinetics of association and dissociation, and on protein concentration. NepR partner switching between PhyR and σ^{EcfG} can be accomplished by regulating any of these parameters. Experiments in different species provide evidence that PhyR concentration is a regulated feature of GSR control systems. In *S. melonis*, quantitative western blots show that PhyR and σ^{EcfG} are present at an approximately 1:1 ratio (15). Thus, when NepR is not in excess, the fraction bound to each

partner is directly proportional to the affinity between NepR and each binding partner, PhyR, PhyR~P and σ^{EcfG}. In *S. meliloti*, which encodes two paralogs of PhyR, genetic studies suggest that PhyR concentration in the cell is limiting; higher expression of PhyR enables more robust transcription of GSR targets (8). Limiting the concentration of PhyR likely reduces spurious NepR binding in unstressed cells and inappropriate activation of the GSR, and controls the amplitude of GSR transcriptional output.

B. abortus has notable features when compared with other alphaproteobacterial systems and presents an example of posttranslational regulation of steady-state PhyR levels in the cell. Unlike other studied species, *B. abortus* NepR binds unphosphorylated PhyR and its cognate σ^{EcfG} factor (RpoE1) with similar high affinities (≈50 nM) in vitro (67). Thus, under conditions in which PhyR, NepR, and σ^{EcfG} concentrations are equal, one may expect that GSR transcription is partially active. However, *B. abortus* PhyR carries a three amino acid sequence (VAA) at its C-terminus that resembles an *ssrA*-like **degron** sequence for ClpX-dependent protein degradation (32), and PhyR protein is basally degraded in cells (67). Mutation of this C-terminal sequence stabilizes PhyR and derepresses GSR transcription (67). As in other systems, phosphorylation of *B. abortus* PhyR strongly enhances its affinity for NepR. The adaptive advantage of two layers of posttranslational PhyR regulation (i.e., phosphorylation and proteolysis) in *B. abortus* remains undefined. This C-terminal *ssrA*-like degron sequence on PhyR is not unique to *Brucella* species; terminal VAA or LAA sequences are found in several species in the orders Rhizobiales and Sphingomonadales (dotted species in Figure 3), suggesting that proteolytic control of PhyR concentration may be a regulatory strategy employed by multiple species.

REGULATORY KINASES: SIGNALING COMPLEXITY IN GENERAL STRESS RESPONSE

The sensory kinases that regulate PhyR phosphorylation vary across species, and these components are not as fully defined as the proteins that compose the core GSR partner switching system. Nevertheless, several recent studies in species with different evolutionary histories and lifestyles have brought new understanding to this important layer of GSR regulation. Although data remain limited, general principles that govern GSR sensory transduction in the Alphaproteobacteria are beginning to emerge. Specifically, there is evidence that multiple kinases and phosphatases serve as inputs that regulate the phosphorylation state of PhyR. **Single domain response regulators (SDRR)**, i.e., standalone receiver domains without an output domain, contribute additional layers of negative and positive control to GSR transcription. Together these upstream sensory proteins compose complex environmental sensory transduction networks that control the phosphorylation state of PhyR.

HWE and HisKA_2 Kinases as Positive and Negative General Stress Response Regulators

Early studies of alphaproteobacterial GSR reported that sensory kinases of the HWE_HK superfamily, which encompases the related HisKA_2 and HWE protein families, are associated with the chromosomal locus encoding the core GSR regulators (38, 95). These kinase families, defined by related regions of primary structure in their **DHp domains** (see

sidebar, HWE and HisKA_2 kinases: Nomenclature and Key Characteristics and Figure 5*a*), are generally uncommon (constituting <3% of kinases in Pfam), yet are highly enriched in the Alphaproteobacteria; 55% of HisKA_2 and 95% of predicted HWE domains are contained in class Alphaproteobacteria (Pfam v 27.0; 29). Genomes that encode PhyR also encode at least one HWE or HisKA_2 kinase (Figure 3) (95). The coconservation of HWE kinase superfamily and the GSR system makes these proteins attractive candidate PhyR kinases. Indeed, the known kinase regulators of PhyR/GSR transcription belong to this atypical kinase group (21, 33, 63, 64, 68, 71, 91). Eight of the nine HisKA_2 or HWE sensors in the *S. melonis* FR1 genome directly regulate PhyR phosphorylation, positively or negatively, generally supporting the hypothesis that GSR regulation is a key role of these atypical kinase families (63, 64).

Sensory histidine kinases can phosphorylate or dephosphorylate their cognate receiver domains. In the handful of species in which GSR sensor kinases have been investigated, multiple proteins function coordinately to activate and repress PhyR phosphorylation. *C. crescentus* PhyK and *B. abortus* LovhK appear to function as the primary GSR-activating kinases under the conditions tested (68, 71). Furthermore, genetic evidence indicates that these species each possess at least one additional sensory protein that acts as a bifunctional or negative GSR regulator (*C. crescentus* LovK and *B. abortus* BAB1_1673) (33, 68). Similarly, in *S. meliloti*, an activating kinase, SMc0322, and a bifunctional kinase, RsiC, coordinately regulate PhyR phosphorylation and GSR transcription (91). At least two *E. litoralis* kinases, EL346 and EL368, specifically phosphorylate PhyR in vitro and modulate the affinity of PhyR for NepR (21). The picture is increasingly complex in *S. melonis*, where seven PhyR-activating kinases (PakA-G) phosphorylate PhyR in vitro and have overlapping functions in GSR transcription and stress survival (64). These Pak kinases function in conjunction with PhyP, a GSR repressor described below. The sensory landscape is expected to be similarly complex in *M. extorquens*, where screens for GSR activators have failed to reveal sensory kinase genes, presumably because of redundancy (79). All of these activating or bifunctional kinases bear highly conserved H- and N-box motifs (Figure 5).

Distinct groups of sensors function as essential negative regulators of GSR. These include PhyP from *S. melonis* FR1 (63), Bab1_1673 from *B. abortus* 2308 (68), and Rpal_04707 from *R. palustris* TIE-1 (70). All these regulators are encoded at the GSR locus and are essential in wild-type cells. However, PhyP and Bab1_1673 are essential only in the context of an intact GSR system; they can be deleted from cells lacking PhyR, σ^{EcfG}, or an activating kinase(s) (63, 64, 68). It is reasonable to predict that this genetic feature is true of Rpal_04707 as well. Notably, *E. coli* membrane fractions containing heterologously expressed PhyP exhibit PhyR phosphatase activity (63). Together, data support a model in which these negative regulators function as PhyR phosphatases that prevent lethal hyperactivation of GSR, reduce spurious or promiscuous PhyR phosphorylation, and/or reset the system when the stress conditions have been alleviated.

The negative GSR regulators and their close relatives have degenerate sequence in either the conserved H-box or N-box regions. PhyP orthologs have a highly degenerate **CA (catalytic ATPase) domain**, which lacks portions of the N-box (63; Figure 5c). Similarly, Rpal_04707 orthologs form a discrete phylogenetic group in which the conserved asparagine is a

histidine (Figure 5c). N-box asparagines are required for kinase, but not phosphatase, activity (27, 55, 104). Thus, the sequences of PhyP- and Rpal_4707-related sensors are consistent with their predicted functions as dedicated phosphatases that lack kinase activity. Although sensors related to Bab1_1673 appear to have intact N-boxes, their H-boxes deviate from the HWE and HisKA_2 consensus (Figure 5c). Residues in the H-box are critical for autophosphorylation and for downstream *trans*-phosphorylation/dephosphorylation interactions with the receiver domain (18, 19, 57, 86, 93, 103, 104). Mutations in the H-box region can selectively disable kinase functions without affecting phosphatase functions (7, 54, 58, 103). These examples suggest that degeneration of key sequence features of HisKA_2/HWE domains may be a predictor of dedicated GSR phosphatases.

General Stress Signals and Signal Detection

Sensory kinases that regulate GSR display a range of periplasmic or cytoplasmic sensory input domains aminoterminal to their kinase domains. Membrane-anchored GSR sensors typically possess CHASE or CHASE3 (5, 80, 108) domains, although the input domains of transmembrane sensors are often not classified by a defined family (e.g., Bab1_1673, PhyK, Smc00322, PhyP). Cytoplasmic GSR sensors largely utilize PAS (*P*er-*A*rnt-*S*im) and/or GAF (c*G*MP-specific phosphodiesterases, *a*denylyl cyclases and *F*hlA) sensory domains (e.g., LovhK, LovK, EL346, EL368, RisC, PakA-E). GAF and PAS domains are widespread and structurally related signal transduction domains capable of sensing a broad range of small molecule signals, redox, and/or light (6, 48, 53). Some GSR kinases possess N-terminal two-component REC domains, which permit integration of signals from other sensory kinase(s). An example is the PhyR kinase PakF, which is activated by phosphorylation of its N-terminal receiver domain by the HisKA-family kinase KipF (65). Finally, some HisKA_2 family kinases such as *S. melonis* PakG lack signal input domains altogether (64). Alhough PakG phosphorylates PhyR and activates GSR transcription, it is not clear how such kinases are regulated or how they contribute to global regulation of the GSR.

Although the sensory domains contained in GSR kinases are relatively easy to identify, the identity of the activating physical or chemical signals and the mechanisms of signal perception remain largely undefined. A conserved periplasmic cysteine is important for the function of *C. crescentus* PhyK as a GSR sensor (71), but it is not clear whether this residue has a generic role in protein structure or whether it is directly involved in signal perception. Similarly, Kaczmarczyk and colleagues identified a small motif in the CHASE3 domain of *S. melonis* KipF that is important for the salt sensing but not temperature sensing functions of this kinase (65). LOV domains, a subset of PAS domains capable of sensing blue light and/or redox (49), are signal input domains for GSR kinases in a number of species, including *C. crescentus*, *B. abortus*, and *E. litoralis* (21, 33, 68). Blue light is a ubiquitous environmental signal that regulates each of these kinases in vitro (21, 88, 97). Although the molecular basis of LOV domain photoperception is understood in structural detail (23, 90), the physiological role of blue light as a GSR signal is not fully understood. It is noteworthy that *Brucella* LovhK has two sensory input domains, LOV and PAS, that are important for survival in distinct conditions. A conserved cysteine in the LOV domain is required for photosensing and necessary for intracellular survival in mammalian macrophages but is

dispensable for responding to acute oxidative stress in vitro. Conversely, there are residues in the LovhK PAS domain that are dispensable for photosensing and intracellular survival but required for acute oxidative stress survival (68). This result suggests that GSR kinases may, in some cases, integrate different environmental signals through different sensory input domains.

SINGLE DOMAIN RESPONSE REGULATORS; ADDITIONAL REGULATORY COMPLEXITY

Recent studies have shown that SDRRs provide an additional layer of GSR control, both as activators and as repressors. SDRRs, which lack an output domain, constitute approximately one in six annotated **REC domains** (36, 37). These single domain proteins can function in two-component phosphorelays (100), as phosphorylation-dependent allosteric regulators of other proteins (61) or as phosphosinks that remove phosphoryl modifications from a signaling pathway (94). Although the regulatory mechanisms employed by SDRRs involved in GSR are not entirely clear, current data suggest they likely function either as allosteric regulators of GSR proteins or as phosphosinks.

Genetic screens for GSR activators in *M. extorquens* and *S. melonis* FR1 identified genes encoding SDRR; *Mext_0407* and *sdrG*, respectively (64, 79). Both are required for GSR transcriptional activation and stress survival, and both function either upstream or coordinately with PhyR in a phosphorylation-dependent manner. *S. melonis* Pak kinases phosphorylate SdrG in vitro, and some Pak kinases require SdrG for activation of GSR (64). Genetic experiments suggest these SDRR regulators are not part of a phosphorelay. Rather, they are proposed to either function as allosteric regulators of Pak kinases or to somehow modulate the PhyR-NepR partner switch.

SDRRs encoded adjacent to a HisKA_2 or HWE kinase typically function as negative GSR regulators. For example, in *S. melonis*, four of the seven Pak kinases are encoded adjacent to an SDRR gene. At least two of these, *prkC* and *prkF*, are genetic repressors of GSR; this repressive function requires their adjacent kinases, PakC and PakF, respectively (64). PrkC is hypothesized to function either as an allosteric negative regulator of PakC or as a phosphosink that competes with PhyR for phosphorylation. A recent report shows that PrkF is not efficiently phosphorylated by PakF in vitro. Rather, PrkF is phosphorylated by the PakF kinase KipF (65). Thus, in *S. melonis* there is evidence that SDRRs can act at multiple levels to control GSR sensory transduction.

In *C. crescentus*, the SDRR LovR functions together with its adjacent kinase, LovK, to repress GSR transcription (33). As with PrkC and PrkF, the repressive activity of LovR requires both LovK and the core GSR regulators PhyR andσ. These data are consistent with a model in which LovR functions as a phosphosink and are complementary to in vitro biochemical data from related proteins in *E. litoralis*. In *E. litoralis*, purified LOV kinases EL368 and EL346 phosphorylate both EL_PhyR and EL_LovR. Moreover, when combined, EL_LovR promotes dephosphorylation of EL_PhyR~P via EL368 (21). EL_LovR~P has a short half-life, rapidly losing its phosphoryl group to water (82). Rapid dephosphorylation in this system is consistent with a phosphosink model in which the SDRR EL_LovR depletes

the kinase of phosphoryl groups and thereby stimulates phosphatase activity of the kinase EL368 and dephosphorylation of PhyR.

CONCLUSION

Our current understanding of alphaproteobacterial GSR started with the discoveries that (*a*) PhyR is required for *M. extorquens* to colonize leaf surfaces (39) and that (*b*) an EcfG homolog and its adjacently encoded anti-σ factor regulate the GSR in *S. meliloti* (92). Since then, a large body of work from a number of laboratories has emerged and has led to a more complete understanding of general stress regulation across the clade. It is now known that PhyR phosphorylation facilitates its association with the anti-σ factor NepR, which releases the alternative σ factor σ^{EcfG} to drive transcription. This phosphorylation-dependent partner switch is the central molecular event of the alphaproteobacterial GSR. However, there are a number of genetic components and biochemical features of the system that permit the cell to tune the central partner switch. In some cases, multiple paralogous core GSR regulators function together, although the role of redundancy and paralogy in the GSR is not fully understood.

There remains much to be learned about how signal inputs are perceived and integrated to regulate GSR transcription. GSR-regulating kinases or phosphatases have been identified in only a handful of species. Unlike standard histidine to aspartate phosphorelays, which typically involve linear signaling pathways, PhyR acts as a central node that integrates the activity of multiple protein sensors that can detect signals inside and outside the cell. In several species, there is evidence for negative feedback mechanisms in the regulation of GSR. Negative feedback involves either a dedicated phosphatase or a bifunctional kinase, which can control the amplitude of GSR transcriptional output or reset the system when the cell is no longer stressed. Notably, SDRRs have emerged as important activators and repressors of the alphaproteobacterial GSR. Developing a system-level understanding of how these multiple layers of GSR regulatory interactions ensure cellular growth and survival in a shifting physicochemical landscape is one frontier in the study of alphaproteobacterial GSR.

Acknowledgments

S.C acknowledges support for this work from NIH grants 1R01GM087353 and 1R01AI107159.

ACRONYMS AND DEFINITIONS

−35 and −10 box	σfactor promoter binding regions, denoted by their distance from the transcriptional start site
CA domain	catalytic ATPase domain
Degron	a specific amino acid sequence that directs protein degradation

Dimerization and histidine phosphorylation domain (DHp domain)	a two helix bundle containing the phosphorylated histidine and phosphotransfer specificity residues
Extra cytoplasmic function (ECF)	alternative sigma factors involved in redirecting transcription in response to envelope and other environmental stresses
General stress response (GSR)	a reversible global transcriptional response that facilitates survival in a range of adverse conditions
HWE and HisKA_2 kinases	related atypical histidine kinase families
Paralog	related copies of a gene within a genome that are derived from an ancestral gene
REC domain	TCST phosphoreceiver domain
RNAP	RNA polymerase
SDRR	single domain response regulator
Sigma (σ) factor	a variable subunit of the RNA polymerase holoenzyme that determines promoter specificity and enables transcription initiation
TCST	two-component signaling transduction

LITERATURE CITED

1. Abromaitis S, Koehler JE. The *Bartonella quintana* extracytoplasmic function sigma factor RpoE has a role in bacterial adaptation to the arthropod vector environment. J Bacteriol. 2013; 195:2662–74. [PubMed: 23564167]
2. Akbar S, Lee SY, Boylan SA, Price CW. Two genes from *Bacillus subtilis* under the sole control of the general stress transcription factor σB. Microbiology. 1999; 145(Pt. 5):1069–78. [PubMed: 10376822]
3. Alvarez-Martinez CE, Baldini RL, Gomes SL. A *Caulobacter crescentus* extracytoplasmic function sigma factor mediating the response to oxidative stress in stationary phase. J Bacteriol. 2006; 188:1835–46. [PubMed: 16484194]
4. Alvarez-Martinez CE, Lourenco RF, Baldini RL, Laub MT, Gomes SL. The ECF sigma factor σ^T is involved in osmotic and oxidative stress responses in *Caulobacter crescentus*. Mol Microbiol. 2007; 66:1240–55. [PubMed: 17986185]
5. Anantharaman V, Aravind L. The CHASE domain: a predicted ligand-binding module in plant cytokinin receptors and other eukaryotic and bacterial receptors. Trends Biochem Sci. 2001; 26:579–82. [PubMed: 11590000]
6. Aravind L, Ponting CP. The GAF domain: an evolutionary link between diverse phototransducing proteins. Trends Biochem Sci. 1997; 22:458–59. [PubMed: 9433123]
7. Atkinson MR, Ninfa AJ. Mutational analysis of the bacterial signal-transducing protein kinase/phosphatase nitrogen regulator II (NRII or NtrB). J Bacteriol. 1993; 175:7016–23. [PubMed: 7901195]
8. Bastiat B, Sauviac L, Bruand C. Dual control of *Sinorhizobium meliloti* RpoE2 sigma factor activity by two PhyR-type two-component response regulators. J Bacteriol. 2010; 192:2255–65. [PubMed: 20154128]

9. Battesti A, Majdalani N, Gottesman S. The RpoS-mediated general stress response in *Escherichia coli*. Annu Rev Microbiol. 2011; 65:189–213. [PubMed: 21639793]
10. Batut J, Andersson SG, O'Callaghan D. The evolution of chronic infection strategies in the Alphaproteobacteria. Nat Rev Microbiol. 2004; 2:933–45. [PubMed: 15550939]
11. Britos L, Abeliuk E, Taverner T, Lipton M, McAdams H, Shapiro L. Regulatory response to carbon starvation in *Caulobacter crescentus*. PLOS ONE. 2011; 6:e18179. [PubMed: 21494595]
12. Brown KL, Hughes KT. The role of anti-sigma factors in gene regulation. Mol Microbiol. 1995; 16:397–404. [PubMed: 7565101]
13. Calhoun LN, Kwon YM. Structure, function and regulation of the DNA-binding protein Dps and its role in acid and oxidative stress resistance in *Escherichia coli*: a review. J Appl Microbiol. 2011; 110:375–86. [PubMed: 21143355]
14. Campagne S, Allain FH, Vorholt JA. Extra cytoplasmic function sigma factors, recent structural insights into promoter recognition and regulation. Curr Opin Struct Biol. 2015; 30C:71–78. [PubMed: 25678040]
15. Campagne S, Damberger FF, Kaczmarczyk A, Francez-Charlot A, Allain FH, Vorholt JA. Structural basis for sigma factor mimicry in the general stress response of Alphaproteobacteria. PNAS. 2012; 109:E1405–14. [PubMed: 22550171]
16. Campbell EA, Westblade LF, Darst SA. Regulation of bacterial RNA polymerase sigma factor activity: a structural perspective. Curr Opin Microbiol. 2008; 11:121–27. [PubMed: 18375176]
17. Capra EJ, Laub MT. Evolution of two-component signal transduction systems. Annu Rev Microbiol. 2012; 66:325–47. [PubMed: 22746333]
18. Casino P, Miguel-Romero L, Marina A. Visualizing autophosphorylation in histidine kinases. Nat Commun. 2014; 5:3258. [PubMed: 24500224]
19. Casino P, Rubio V, Marina A. Structural insight into partner specificity and phosphoryl transfer in two-component signal transduction. Cell. 2009; 139:325–36. [PubMed: 19800110]
20. Chiang SM, Schellhorn HE. Evolution of the RpoS regulon: origin of RpoS and the conservation of RpoS-dependent regulation in bacteria. J Mol Evol. 2010; 70:557–71. [PubMed: 20506020]
21. Correa F, Ko WH, Ocasio V, Bogomolni RA, Gardner KH. Blue light regulated two-component systems: enzymatic and functional analyses of light-oxygen-voltage (LOV)-histidine kinases and downstream response regulators. Biochemistry. 2013; 52:4656–66. [PubMed: 23806044]
22. Crooks GE, Hon G, Chandonia JM, Brenner SE. WebLogo: a sequence logo generator. Genome Res. 2004; 14:1188–90. [PubMed: 15173120]
23. Crosson S, Moffat K. Photoexcited structure of a plant photoreceptor domain reveals a light-driven molecular switch. Plant Cell. 2002; 14:1067–75. [PubMed: 12034897]
24. Cytryn EJ, Sangurdekar DP, Streeter JG, Franck WL, Chang WS, et al. Transcriptional and physiological responses of *Bradyrhizobium japonicum* to desiccation-induced stress. J Bacteriol. 2007; 189:6751–62. [PubMed: 17660288]
25. da Silva Batista JS, Hungria M. Proteomics reveals differential expression of proteins related to a variety of metabolic pathways by genistein-induced *Bradyrhizobium japonicum* strains. J Proteomics. 2012; 75:1211–19. [PubMed: 22119543]
26. Delory M, Hallez R, Letesson JJ, De Bolle X. An RpoH-like heat shock sigma factor is involved in stress response and virulence in *Brucella melitensis* 16M. J Bacteriol. 2006; 188:7707–10. [PubMed: 16936018]
27. Dutta R, Inouye M. Reverse phosphotransfer from OmpR to EnvZ in a kinase/phosphatase +mutant of EnvZ (EnvZ. N347D), a bifunctional signal transducer of *Escherichia coli*. J Biol Chem. 1996; 271:1424–29. [PubMed: 8576133]
28. Ettema TJ, Andersson SG. The Alphaproteobacteria: the Darwin finches of the bacterial world. Biol Lett. 2009; 5:429–32. [PubMed: 19324639]
29. Finn RD, Bateman A, Clements J, Coggill P, Eberhardt RY, et al. Pfam: the protein families database. Nucleic Acids Res. 2014; 42:D222–30. [PubMed: 24288371]
30. Flechard M, Fontenelle C, Blanco C, Goude R, Ermel G, Trautwetter A. RpoE2 of *Sinorhizobium meliloti* is necessary for trehalose synthesis and growth in hyperosmotic media. Microbiology. 2010; 156:1708–18. [PubMed: 20223801]

31. Flechard M, Fontenelle C, Trautwetter A, Ermel G, Blanco C. *Sinorhizobium meliloti* rpoE2 is necessary for H_2O_2 stress resistance during the stationary growth phase. FEMS Microbiol Lett. 2009; 290:25–31. [PubMed: 19025578]
32. Flynn JM, Neher SB, Kim YI, Sauer RT, Baker TA. Proteomic discovery of cellular substrates of the ClpXP protease reveals five classes of ClpX-recognition signals. Mol Cell. 2003; 11:671–83. [PubMed: 12667450]
33. Foreman R, Fiebig A, Crosson S. The LovK-LovR two-component system is a regulator of the general stress pathway in *Caulobacter crescentus*. J Bacteriol. 2012; 194:3038–49. [PubMed: 22408156]
34. Francez-Charlot A, Frunzke J, Reichen C, Ebneter JZ, Gourion B, Vorholt JA. Sigma factor mimicry involved in regulation of general stress response. PNAS. 2009; 106:3467–72. [PubMed: 19218445]
35. Francez-Charlot A, Kaczmarczyk A, Fischer HM, Vorholt JA. The general stress response in Alphaproteobacteria. Trends Microbiol. 2015; 23:164–71. [PubMed: 25582885]
36. Galperin MY. Structural classification of bacterial response regulators: diversity of output domains and domain combinations. J Bacteriol. 2006; 188:4169–82. [PubMed: 16740923]
37. Galperin MY. Diversity of structure and function of response regulator output domains. Curr Opin Microbiol. 2010; 13:150–59. [PubMed: 20226724]
38. Gourion B, Francez-Charlot A, Vorholt JA. PhyR is involved in the general stress response of *Methylobacterium extorquens* AM1. J Bacteriol. 2008; 190:1027–35. [PubMed: 18024517]
39. Gourion B, Rossignol M, Vorholt JA. A proteomic study of *Methylobacterium extorquens* reveals a response regulator essential for epiphytic growth. PNAS. 2006; 103:13186–91. [PubMed: 16926146]
40. Gourion B, Sulser S, Frunzke J, Francez-Charlot A, Stiefel P, et al. The PhyR-sigma(EcfG) signalling cascade is involved in stress response and symbiotic efficiency in *Bradyrhizobium japonicum*. Mol Microbiol. 2009; 73:291–305. [PubMed: 19555458]
41. Grebe TW, Stock JB. The histidine protein kinase superfamily. Adv Microb Physiol. 1999; 41:139–227. [PubMed: 10500846]
42. Haldenwang WG. The sigma factors of *Bacillus subtilis*. Microbiol Rev. 1995; 59:1–30. [PubMed: 7708009]
43. Hecker M, Pane-Farre J, Volker U. SigB-dependent general stress response in *Bacillus subtilis* and related Gram-positive bacteria. Annu Rev Microbiol. 2007; 61:215–36. [PubMed: 18035607]
44. Helmann JD. Anti-sigma factors. Curr Opin Microbiol. 1999; 2:135–41. [PubMed: 10322161]
45. Helmann JD. The extracytoplasmic function (ECF) sigma factors. Adv Microb Physiol. 2002; 46:47–110. [PubMed: 12073657]
46. Helmann, JD. Regulation by alternative sigma factors. In: Storz, G.; Hengge, R., editors. Bacterial Stress Responses. 2. Washington, DC: ASM Press; 2011. p. 31-43.
47. Hengge R. Proteolysis of σ^S (RpoS) and the general stress response in *Escherichia coli*. Res Microbiol. 2009; 160:667–76. [PubMed: 19765651]
48. Henry JT, Crosson S. Ligand-binding PAS domains in a genomic, cellular, and structural context. Annu Rev Microbiol. 2011; 65:261–86. [PubMed: 21663441]
49. Herrou J, Crosson S. Function, structure and mechanism of bacterial photosensory LOV proteins. Nat Rev Microbiol. 2011; 9:713–23. [PubMed: 21822294]
50. Herrou J, Foreman R, Fiebig A, Crosson S. A structural model of anti-anti-sigma inhibition by a two-component receiver domain: the PhyR stress response regulator. Mol Microbiol. 2010; 78:290–304. [PubMed: 20735776]
51. Herrou J, Rotskoff G, Luo Y, Roux B, Crosson S. Structural basis of a protein partner switch that regulates the general stress response of Alpha-proteobacteria. PNAS. 2012; 109:E1415–23. [PubMed: 22550172]
52. Herrou J, Willett JW, Crosson S. Structured and dynamic disordered domains regulate the activity of a multi-functional anti-sigma factor. 2015 Under review.
53. Ho YS, Burden LM, Hurley JH. Structure of the GAF domain, a ubiquitous signaling motif and a new class of cyclic GMP receptor. EMBO J. 2000; 19:5288–99. [PubMed: 11032796]

54. Horstmann N, Sahasrabhojane P, Saldana M, Ajami NJ, Flores AR, et al. Characterization of the Effect of the Histidine Kinase CovS on Response Regulator Phosphorylation in Group A *Streptococcus*. Infect Immun. 2015; 83:1068–77. [PubMed: 25561708]
55. Hsing W, Russo FD, Bernd KK, Silhavy TJ. Mutations that alter the kinase and phosphatase activities of the two-component sensor EnvZ. J Bacteriol. 1998; 180:4538–46. [PubMed: 9721293]
56. Hughes KT, Mathee K. The anti-sigma factors. Annu Rev Microbiol. 1998; 52:231–86. [PubMed: 9891799]
57. Huynh TN, Noriega CE, Stewart V. Conserved mechanism for sensor phosphatase control of two-component signaling revealed in the nitrate sensor NarX. PNAS. 2010; 107:21140–5. [PubMed: 21078995]
58. Huynh TN, Stewart V. Negative control in two-component signal transduction by transmitter phosphatase activity. Mol Microbiol. 2011; 82:275–86. [PubMed: 21895797]
59. Iguchi H, Sato I, Yurimoto H, Sakai Y. Stress resistance and C1 metabolism involved in plant colonization of a methanotroph *Methylosinus* sp B4S. Arch Microbiol. 2013; 195:717–26. [PubMed: 24037422]
60. Jans A, Vercruysse M, Gao S, Engelen K, Lambrichts I, et al. Canonical and non-canonical EcfG sigma factors control the general stress response in *Rhizobium etli*. Microbiologyopen. 2013; 2:976–87. [PubMed: 24311555]
61. Jenal U, Galperin MY. Single domain response regulators: molecular switches with emerging roles in cell organization and dynamics. Curr Opin Microbiol. 2009; 12:152–60. [PubMed: 19246239]
62. Jishage M, Kvint K, Shingler V, Nystrom T. Regulation of sigma factor competition by the alarmone ppGpp. Genes Dev. 2002; 16:1260–70. [PubMed: 12023304]
63. Kaczmarczyk A, Campagne S, Danza F, Metzger LC, Vorholt JA, Francez-Charlot A. Role of *Sphingomonas* sp strain Fr1 PhyR-NepR-σEcfG cascade in general stress response and identification of a negative regulator of PhyR. J Bacteriol. 2011; 193:6629–38. [PubMed: 21949070]
64. Kaczmarczyk A, Hochstrasser R, Vorholt JA, Francez-Charlot A. Complex two-component signaling regulates the general stress response in Alphaproteobacteria. PNAS. 2014; 111:E5196–204. [PubMed: 25404331]
65. Kaczmarczyk A, Hochstrasser R, Vorholt JA, Francez-Charlot A. Two-tiered histidine kinase pathway involved in heat shock and salt sensing in the general stress response of *Sphingomonas melonis* Fr1. J Bacteriol. 2015; 197:1466–77. [PubMed: 25666137]
66. Karniol B, Vierstra RD. The HWE histidine kinases, a new family of bacterial two-component sensor kinases with potentially diverse roles in environmental signaling. J Bacteriol. 2004; 186:445–53. [PubMed: 14702314]
67. Kim HS, Caswell CC, Foreman R, Roop RM 2nd, Crosson S. The *Brucella abortus* general stress response system regulates chronic mammalian infection and is controlled by phosphorylation and proteolysis. J Biol Chem. 2013; 288:13906–16. [PubMed: 23546883]
68. Kim HS, Willett JW, Jain-Gupta N, Fiebig A, Crosson S. The *Brucella abortus* virulence regulator, LovhK, is a sensor kinase in the general stress response signalling pathway. Mol Microbiol. 2014; 94:913–25. [PubMed: 25257300]
69. Kristensen DM, Kannan L, Coleman MK, Wolf YI, Sorokin A, et al. A low-polynomial algorithm for assembling clusters of orthologous groups from intergenomic symmetric best matches. Bioinformatics. 2010; 26:1481–87. [PubMed: 20439257]
70. Kulkarni G, Wu CH, Newman DK. The general stress response factor EcfG regulates expression of the C-2 hopanoid methylase HpnP in *Rhodopseudomonas palustris* TIE-1. J Bacteriol. 2013; 195:2490–98. [PubMed: 23524612]
71. Lourenco RF, Kohler C, Gomes SL. A two-component system, an anti-sigma factor and two paralogous ECF sigma factors are involved in the control of general stress response in *Caulobacter crescentus*. Mol Microbiol. 2011; 80:1598–612. [PubMed: 21564331]
72. Magnusson LU, Farewell A, Nystrom T. ppGpp: a global regulator in *Escherichia coli*. Trends Microbiol. 2005; 13:236–42. [PubMed: 15866041]
73. Maillard AP, Girard E, Ziani W, Petit-Hartlein I, Kahn R, Coves J. The crystal structure of the anti-sigma factor CnrY in complex with the sigma factor CnrH shows a new structural class of

anti-sigma factors targeting extracytoplasmic function sigma factors. J Mol Biol. 2014; 426:2313–27. [PubMed: 24727125]

74. Marchler-Bauer A, Derbyshire MK, Gonzales NR, Lu S, Chitsaz F, et al. CDD: NCBI's conserved domain database. Nucleic Acids Res. 2015; 43:D222–26. [PubMed: 25414356]
75. Martinez-Salazar JM, Salazar E, Encarnacion S, Ramirez-Romero MA, Rivera J. Role of the extracytoplasmic function sigma factor RpoE4 in oxidative and osmotic stress responses in *Rhizobium etli*. J Bacteriol. 2009; 191:4122–32. [PubMed: 19376852]
76. Martinez-Salazar JM, Sandoval-Calderon M, Guo X, Castillo-Ramirez S, Reyes A, et al. The *Rhizobium etli* RpoH1 and RpoH2 sigma factors are involved in different stress responses. Microbiology. 2009; 155:386–97. [PubMed: 19202087]
77. Mascher T. Signaling diversity and evolution of extracytoplasmic function (ECF) sigma factors. Curr Opin Microbiol. 2013; 16:148–55. [PubMed: 23466210]
78. McGrath PT, Lee H, Zhang L, Iniesta AA, Hottes AK, et al. High-throughput identification of transcription start sites, conserved promoter motifs and predicted regulons. Nat Biotechnol. 2007; 25:584–92. [PubMed: 17401361]
79. Metzger LC, Francez-Charlot A, Vorholt JA. Single-domain response regulator involved in the general stress response of *Methylobacterium extorquens*. Microbiology. 2013; 159:1067–76. [PubMed: 23596318]
80. Mougel C, Zhulin IB. CHASE: an extracellular sensing domain common to transmembrane receptors from prokaryotes, lower eukaryotes and plants. Trends Biochem Sci. 2001; 26:582–84. [PubMed: 11590001]
81. Nystrom T. Growth versus maintenance: a trade-off dictated by RNA polymerase availability and sigma factor competition? Mol Microbiol. 2004; 54:855–62. [PubMed: 15522072]
82. Ocasio VJ, Correa F, Gardner KH. Ligand-induced folding of a two-component signaling receiver domain. Biochemistry. 2015; 54:1353–63. [PubMed: 25629646]
83. Osterberg S, del Peso-Santos T, Shingler V. Regulation of alternative sigma factor use. Annu Rev Microbiol. 2011; 65:37–55. [PubMed: 21639785]
84. Overbeek R, Begley T, Butler RM, Choudhuri JV, Chuang HY, et al. The subsystems approach to genome annotation and its use in the project to annotate 1000 genomes. Nucleic Acids Res. 2005; 33:5691–702. [PubMed: 16214803]
85. Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, et al. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic Acids Res. 2014; 42:D206–14. [PubMed: 24293654]
86. Podgornaia AI, Laub MT. Determinants of specificity in two-component signal transduction. Curr Opin Microbiol. 2013; 16:156–62. [PubMed: 23352354]
87. Price, CW. General stress response in *Bacillus subtilis* and related Gram-positive bacteria. In: Storz, G.; Hengge, R., editors. Bacterial Stress Responses. 2. Washington, DC: ASM Press; 2011. p. 301-18.
88. Purcell EB, Siegal-Gaskins D, Rawling DC, Fiebig A, Crosson S. A photosensory two-component system regulates bacterial cell attachment. PNAS. 2007; 104:18241–46. [PubMed: 17986614]
89. Rivera-Cancel G, Ko WH, Tomchick DR, Correa F, Gardner KH. Full-length structure of a monomeric histidine kinase reveals basis for sensory regulation. PNAS. 2014; 111:17839–44. [PubMed: 25468971]
90. Salomon M, Eisenreich W, Durr H, Schleicher E, Knieb E, et al. An optomechanical transducer in the blue light receptor phototropin from *Avena sativa*. PNAS. 2001; 98:12357–61. [PubMed: 11606742]
91. Sauviac L, Bruand C. A putative bifunctional histidine kinase/phosphatase of the HWE family exerts positive and negative control on the *Sinorhizobium meliloti* general stress response. J Bacteriol. 2014; 196:2526–35. [PubMed: 24794560]
92. Sauviac L, Philippe H, Phok K, Bruand C. An extracytoplasmic function sigma factor acts as a general stress response regulator in *Sinorhizobium meliloti*. J Bacteriol. 2007; 189:4204–16. [PubMed: 17400745]
93. Skerker JM, Perchuk BS, Siryaporn A, Lubin EA, Ashenberg O, et al. Rewiring the specificity of two-component signal transduction systems. Cell. 2008; 133:1043–54. [PubMed: 18555780]

94. Sourjik V, Schmitt R. Phosphotransfer between CheA, CheY1, and CheY2 in the chemotaxis signal transduction chain of *Rhizobium meliloti*. Biochemistry. 1998; 37:2327–35. [PubMed: 9485379]
95. Staron A, Mascher T. General stress response in Alphaproteobacteria: PhyR and beyond. Mol Microbiol. 2010; 78:271–27. [PubMed: 20979331]
96. Staron A, Sofia HJ, Dietrich S, Ulrich LE, Liesegang H, Mascher T. The third pillar of bacterial signal transduction: classification of the extracytoplasmic function (ECF) sigma factor protein family. Mol Microbiol. 2009; 74:557–81. [PubMed: 19737356]
97. Swartz TE, Tseng TS, Frederickson MA, Paris G, Comerci DJ, et al. Blue-light-activated histidine kinases: two-component sensors in bacteria. Science. 2007; 317:1090–93. [PubMed: 17717187]
98. Ulrich LE, Koonin EV, Zhulin IB. One-component systems dominate signal transduction in prokaryotes. Trends Microbiol. 2005; 13:52–56. [PubMed: 15680762]
99. Ulrich LE, Zhulin IB. The MiST2 database: a comprehensive genomics resource on microbial signal transduction. Nucleic Acids Res. 2010; 38:D401–7. [PubMed: 19900966]
100. Varughese KI. Conformational changes of Spo0F along the phosphotransfer pathway. J Bacteriol. 2005; 187:8221–27. [PubMed: 16321923]
101. Vercruysse M, Fauvart M, Jans A, Beullens S, Braeken K, et al. Stress response regulators identified through genome-wide transcriptome analysis of the (p)ppGpp-dependent response in *Rhizobium etli*. Genome Biol. 2011; 12:R17. [PubMed: 21324192]
102. Vijay K, Brody MS, Fredlund E, Price CW. A PP2C phosphatase containing a PAS domain is required to convey signals of energy stress to the σ^B transcription factor of *Bacillus subtilis*. Mol Microbiol. 2000; 35:180–88. [PubMed: 10632888]
103. Willett JW, Kirby JR. Genetic and biochemical dissection of a HisKA domain identifies residues required exclusively for kinase and phosphatase activities. PLOS Genet. 2012; 8:e1003084. [PubMed: 23226719]
104. Willett JW, Tiwari N, Muller S, Hummels KR, Houtman JC, et al. Specificity residues determine binding affinity for two-component signal transduction systems. mBio. 2013; 4:e00420–13. [PubMed: 24194534]
105. Williams KP, Sobral BW, Dickerman AW. A robust species tree for the Alphaproteobacteria. J Bacteriol. 2007; 189:4578–86. [PubMed: 17483224]
106. Xue B, Dunbrack RL, Williams RW, Dunker AK, Uversky VN. PONDR-FIT: a meta-predictor of intrinsically disordered amino acids. Biochim Biophys Acta. 2010; 1804:996–1010. [PubMed: 20100603]
107. Yang X, Kang CM, Brody MS, Price CW. Opposing pairs of serine protein kinases and phosphatases transmit signals of environmental stress to activate a bacterial transcription factor. Genes Dev. 1996; 10:2265–75. [PubMed: 8824586]
108. Zhulin IB, Nikolskaya AN, Galperin MY. Common extracellular sensory domains in transmembrane receptors for diverse signal transduction pathways in bacteria and archaea. J Bacteriol. 2003; 185:285–94. [PubMed: 12486065]

SUMMARY POINTS

1. A protein partner switch triggered by PhyR phosphorylation controls the activity of the alternative σ factor σ^{EcfG} and mediates the alphaproteobacterial GSR.
2. A hierarchy of differential binding affinities between the anti-σ factor, NepR and its partners, σ^{EcfG} and PhyR, determines the regulatory partner switch.
3. PhyR combines features of two-component signal transduction and alternative σ factor regulation in one polypeptide.
4. GSR is required for survival of bacteria in real-life environments, including the interior of mammalian cells during chronic infection (*B. abortus*), the surfaces of plants (*S. melonis* and *M. extorquens*), and the interior of root nodules (*B. japonicum*).
5. Multiple sensory kinases and bifunctional kinases/phosphatases coordinately control PhyR phosphorylation and control GSR transcription.
6. SDRRs function both as activators and repressors of PhyR phosphorylation, and provide an additional layer of regulatory control of GSR.

FUTURE ISSUES

1. What are the molecular signals that are perceived by kinases and phosphatases that control PhyR phosphorylation?
2. How are multiple signals integrated through the GSR sensory machinery to control σ^{EcfG}-dependent transcription?
3. What is the role of SDRRs in controlling GSR transcriptional output?
4. What are the regulatory implications and adaptive advantages of redundancy and paralogy in the GSR system?

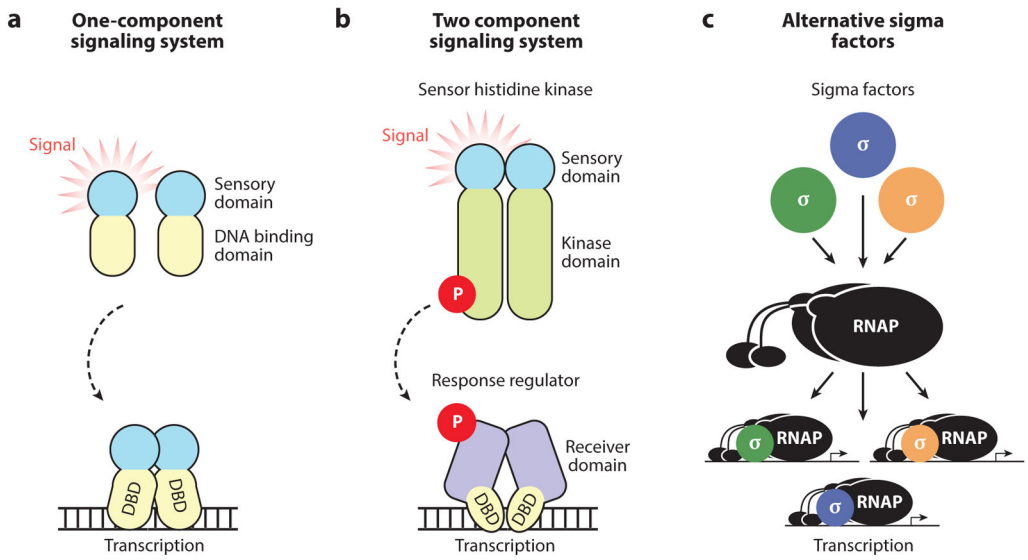

Figure 1.

Transcriptional regulation in response to environmental change. *a* Simple and abundant, one-component regulators consist of a sensory input domain that regulates its adjacent DNA binding domain (DBD) (98). *b* Two-component signaling systems involve a sensory histidine kinase and a response regulator, the latter of which typically consists of a phosphoreceiver domain and a DNA binding output domain. The sensory domain of the histidine kinase regulates the autophosphorylation activity. The phosphoryl group is transferred from a histidine on the kinase to an aspartate on the receiver domain, which regulates the DBD output domain (17). *c* σ factors bind core RNA polymerase (RNAP) and confer promoter specificity to the transcriptional machinery. A host of regulatory mechanisms are employed to control alternative σ factors (46).

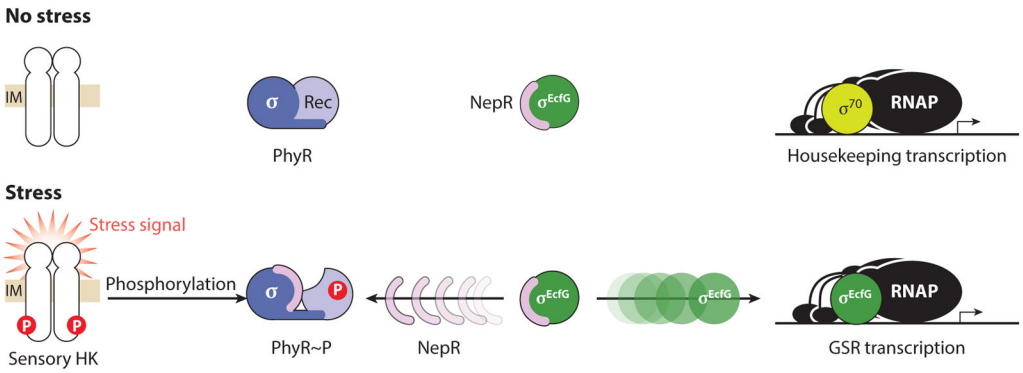

Figure 2.

Alphaproteobacterial general stress response (GSR) partner switching. Under inactivating conditions, σ^{EcfG} (*green*) is sequestered by the anti-σ factor NepR (*pink*) and cannot compete with the housekeeping σ^{70} for binding to core RNAP (*black*). PhyR (*purple*) is unphosphorylated, in a closed confirmation, and has a low affinity for NepR. Under stress conditions, the receiver domain of PhyR (*light purple*) can by phosphorylated by a sensor histidine kinase(s) (*white*) often associated with the inner membrane (tan bar), destabilizing the interaction interface between the σ-like domain (*dark purple*) and the receiver domain. This open PhyR conformation reveals the NepR binding interface and thereby increases the affinity of PhyR for NepR. PhyR~P-NepR binding releases σ^{EcfG} to interact with RNAP and initiate transcription of the GSR regulon.

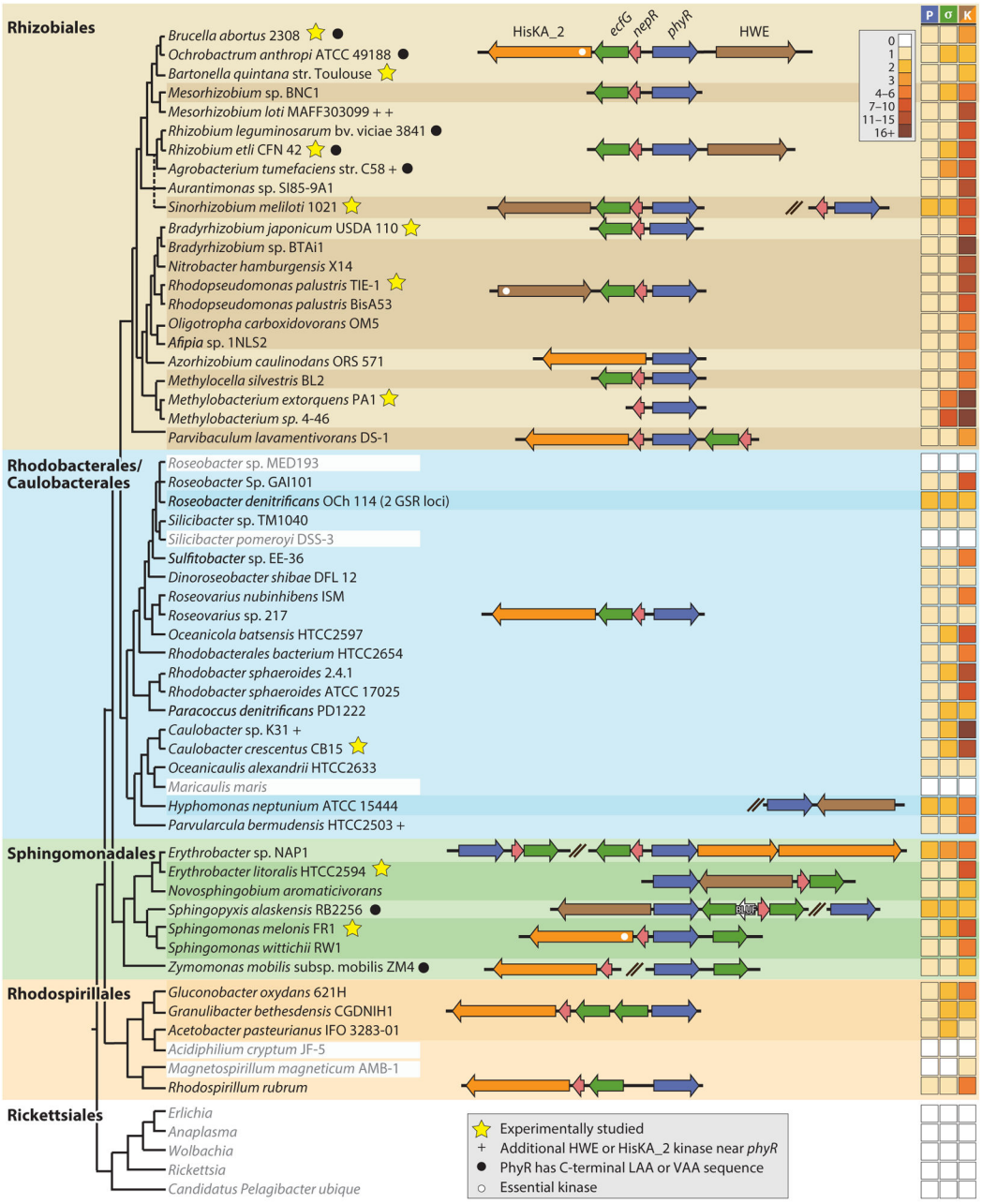

Figure 3.

Phylogenetic distribution and organization of general stress response (GSR) loci. Organization of genomic regions surrounding *phyR* is overlayed on a phylogenetic tree based on analyses in Reference 105. PhyR-encoding loci were identified in all genomes in the SEED viewer database (using an Evalue cutoff of $1e^{-30}$) (84, 85). Pinned regions (16,000 bp) surrounding *phyR* were extracted to identify conserved genes in the *phyR* vicinity. *nepR* genes are not always annotated and were confirmed by manual inspection. Genes are colored as labeled at top. Closely related genomes were collapsed to a single representative. Species in a shared background color block have the same GSR locus organization. Species boxed in white lack *phyR* and *ecfG*. The color heat map on the right

indicates the number of *phyR*, *ecfG*, and HisKA_2 or HWE kinase genes in each genome based on hits in SEED for *phyR*, annotated ECF15 σ factors in MiSTDb (99), and the sum of HisKA_2 and HWE_HK domain kinases in Pfam (29). The small size of *nepR* impedes reliable annotation, thus *nepR* paralogs were not tallied. Key indicates other annotations.

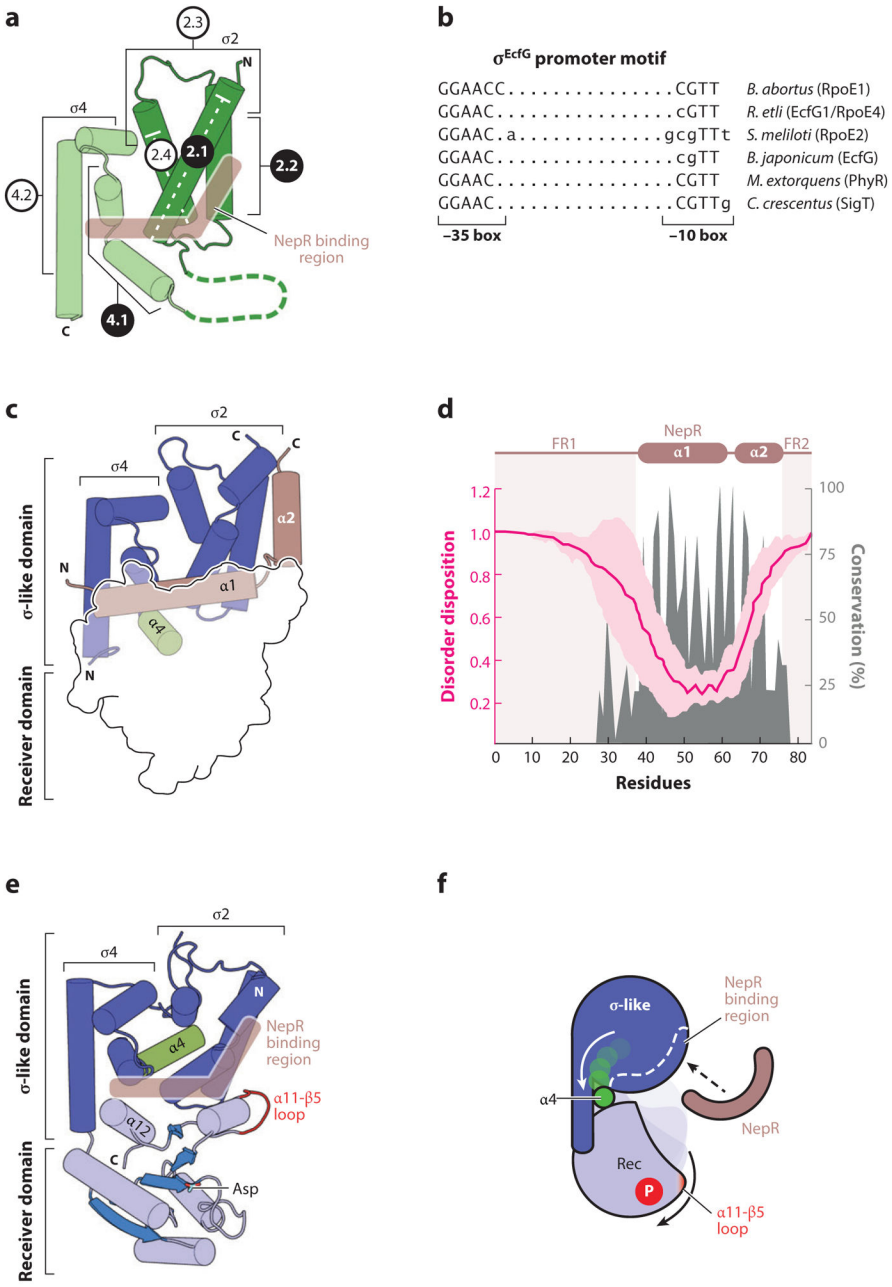

Figure 4.

Structural features of σ^{EcfG}, NepR, and PhyR. (*a*) Structural model of σ^{EcfG} built using CnrH (PDB: 4CXF) as a template. The σ2 and σ4 regions are depicted in dark and light green, respectively. Subregions that interact with RNAP (2.1, 2.2, and 4.1) are indicated with black circles; regions involved in promoter recognition and binding (2.3, 2.4, and 4.2) are in white circles. The predicted NepR binding site (*pink*) overlaps with the conserved RNAP binding interface. (*b*) Promoter motifs identified upstream of genes regulated by EcfG or PhyR are highly conserved (4, 38, 40, 60, 67, 75, 78, 92). Dots represent nonconserved bases; sequences −35 and −10 base pairs from the transcriptional start site are indicated. (*c*) Structural representation of NepR (*pink*) bound across the σ2 and σ4 regions of

the PhyR σ-like domain (*dark purple*; PDB: 3T0Y). NepR and the PhyR-receiver domain (*modeled in white*) compete for binding to the same surface on the σ-like domain. When NepR is bound, PhyR helix α4 (*green*) is repositioned and likely inhibits the receiver domain from displacing NepR. (*d*) NepR amino acid sequences from *Caulobacter crescentus*, *Brucella abortus*, *Methylobacterium extorquens*, *Bradyrhizobium japonicum*, *Sinorhizobium meliloti*, *Sphingomonas melonis*, and *Rhodopseudomonas palustris* were aligned based on the conserved core. Conservation is shown on the right (*gray*). Disorder disposition was predicted for each sequence using PONDR-Fit (http://www.disprot.org/pondr-fit.php) (106); the average (*red*) ± standard deviation (*green*) is plotted on the left. (*e*) Structural representation of PhyR in the closed conformation (PDB: 3N0R). The receiver domain (*light purple*) is packed against the σ-like domain (*dark purple*) blocking the NepR binding site (*pink*). Helix α4 (*green*) is positioned against the surface of the σ-like domain. (*f*) Graphical representation of the key features of PhyR that regulate partner switching.

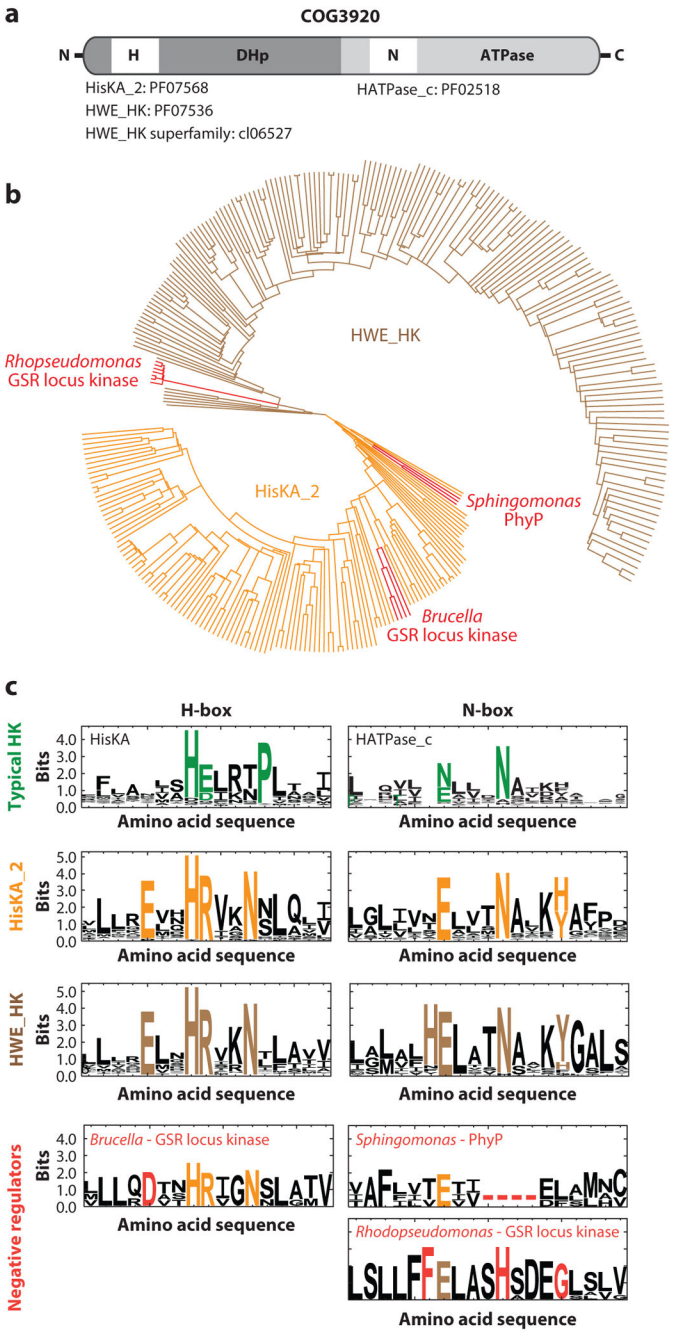

Figure 5.

Characteristics of the atypical HisKA_2 and HWE kinase families. (*a*) COG3920 encompasses the DHp (dimerization and histidine phosphorylation) and catalytic domains of HWE and HisKA_2 kinases. The Pfam domains for each kinase family domain are indicated (29). The position of the H- and N-boxes are shown as white boxes. (*b*) Unrooted circular tree based on alignment of COG3920 domains from kinases found in the species shown in Figure 3. Sensory input domains are not included. Tree includes 127 HisKA_2 kinases and 177 HWE kinases. Sequences were downloaded and analyzed using Geneious (http://

www.geneious.com). Branches including known negative regulators or essential GSR kinases are red with the experimental species indicated. *Sphingomonas* PhyP branch includes the GSR locus kinases from *Sphingomonas melonis* FR1, *Sphingomonas wittichii* RW1, and *Zymomonas mobilis* subsp. *mobilis* ZM4. The *Brucella* branch includes the GSR locus HisKA_2 kinases from *Brucella abortus* 2308, *Ochrobactrum anthropi* ATTC 49188, *Bartonella quintana* str. Toulouse, and *Parvibaculum lavamentivorans* DS-1. The *Rhodopseudomonas* branch includes the GSR locus kinases from *Bradyrhizobium* sp. BTAi1, *Nitrobacter hamburgensis* X 14, *Rhodopseudomonas palustris* TIE-1, *R. palustris* BisA53, *Oligotropha carboxidovorans* OM5, *Afipia* sp. 1NLS2, and a kinase from *Bradyrhizobium japonicum* USDA110 encoded distal from the GSR locus. (*c*) Weblogos generated from the alignment of the H-box or N-box regions from the 127 HisKA_2 and 177 HWE alphaproteobacterial kinases displayed in panel *b*, and alignment of the 265 HisKA or 659 HATPase_c sequences in the SEED database, downloaded from Pfam (22, 29). Key conserved residues are indicated in color. Weblogos for negative regulators were generated from sequences related to known negative regulators or essential GSR kinases (*red branches in panel* b). For each group, only the motif that deviates from the consensus is shown; divergent residues are red.