Review

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The general stress response in Alphaproteobacteria

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The general stress response (GSR) is a widely conserved response that allows bacteria to cope with a multitude of stressful conditions. In the past years the PhyR–NepR–σ^{EcfG} cascade was identified as the core pathway regulating the GSR in Alphaproteobacteria, in which it also plays an important role in bacteria–host interactions. The regulatory system is composed of the extracytoplasmic function sigma factor σ^{EcfG}, its anti-sigma factor NepR (for negative regulator of the PhyR response), and the anti-sigma factor antagonist PhyR (phyllosphere regulator). The three proteins function via a partner-switching mechanism that is triggered by PhyR phosphorylation, termed 'sigma factor mimicry'. This review will cover core features of the pathway, its physiological role, and summarize recent advances towards understanding of the partner-switching mechanism and of the two-component signaling pathways controlling the GSR.

The GSR and its regulation

The GSR is defined as a reversible and preventive response to multiple stresses. Pioneering work in the Gammaproteobacterium *Escherichia coli* and the Firmicute *Bacillus subtilis* indicated that a single central regulator – σ^S or σ^B, respectively – controls this global response. Although not orthologous, these regulators are in both cases a sigma factor, the subunit of the RNA polymerase responsible for promoter recognition [1]. Following the discovery of the central players, complex regulatory pathways have been identified in *E. coli* and *B. subtilis*, which allow integration of multiple signals and fine-tuning of the response ([2–5] for review; Box 1). While the regulatory systems seem conserved in closely related strains, the master regulators governing the GSR are not ubiquitous, indicating that there are alternative systems that control the GSR in other bacteria.

Discovery of the PhyR–NepR–σ^{EcfG} system in Alphaproteobacteria and the partner switch triggering the GSR

The identification of the molecular basis of the regulatory system involved in the GSR and its significance came from independent studies in different Alphaproteobacteria. PhyR (for phyllosphere regulator), a response regulator essential for plant colonization in *Methylobacterium extorquens* [6], was shown to be required for resistance to various stresses and to control several stress-related genes [6,7]. In *Sinorhizobium meliloti* and *Caulobacter crescentus*, the σ^{EcfG}-orthologous sigma factors RpoE2 and SigT, respectively, were analyzed and shown to induce large regulons in response to various stressful conditions [8,9]. A possible anti-sigma factor encoded upstream of *rpoE2* was identified [9]. Interestingly, PhyR, σ^{EcfG}, and the putative anti-sigma factor are exclusively present in Alphaproteobacteria [6,7,10,11]. The fact that all three regulators are encoded at the same locus in many members of this class (see below and Figure 1) [7,9–12], and that PhyR- and σ^{EcfG}-regulated genes share a common promoter motif, suggested they act in the same pathway. This assumption was confirmed by experimental data from *M. extorquens*: NepR (for negative regulator of the PhyR response) and PhyR were shown to act as an anti-sigma factor and an anti-sigma factor antagonist, respectively, to control the activity of the sigma factor σ^{EcfG} in a partner switch [13] (Box 2). In the proposed model, NepR sequesters σ^{EcfG} under non-inducing conditions; in response to stress, the response regulator PhyR becomes phosphorylated and interacts with NepR, releasing σ^{EcfG} which can then associate with the RNA polymerase and activate its regulon. Because the domain of PhyR interacting with NepR resembles σ^{EcfG}, the term 'sigma factor mimicry' was coined [13]. This review will cover our current knowledge of the PhyR–NepR–σ^{EcfG} system and the important aspects of bacterial lifestyle it controls, and highlight recent progress made towards the understanding of the partner-switching mechanism and of the signal transduction pathways controlling the GSR.

Phenotypes and regulons associated with the GSR

In the Alphaproteobacteria analyzed so far, common phenotypes of *ecfG* or *phyR* mutants unable to mount a GSR include increased sensitivity towards different types of stresses as well as loss of cross-protection, in other words resistance to one stress elicited by exposure to another unrelated stress. The precise spectrum of inducing conditions and the degree to which mutants are sensitive to a particular stress differ between species (Table 1 for examples). Besides resistance to stresses under laboratory conditions, the GSR also plays an

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Box 1. Regulation of GSR sigma factor activity

***Escherichia coli*: σ^S**

The activity of σ^S is primarily dictated by its protein level, and this is regulated at essentially all possible levels – transcription, mRNA stability, translation, and protein degradation. Different stresses affect different levels of regulation, and one inducing condition can also affect multiple levels. Generally, σ^S accumulation in post-exponential and stationary phase is controlled by transcriptional and translational mechanisms, whereas rapid σ^S accumulation in response to more severe conditions is achieved by inhibition of σ^S proteolysis. σ^S activity can also be regulated at the level of RNA polymerase (RNAP) holoenzyme formation ([3,4] for review).

***Bacillus subtilis*: σ^B**

The activity of σ^B is mainly controlled by a partner-switching mechanism involving the anti-sigma factor RsbW and its antagonist RsbV. RsbW is an atypical serine/threonine kinase that, in unstressed conditions, sequesters σ^B and phosphorylates its own antagonist RsbV, keeping it inactive. Upon sensing stress, RsbV is dephosphorylated by one of two PP2C phosphatases (RsbP and RsbU), thereby allowing RsbV to inhibit the anti-sigma factor RsbW, releasing σ^B to associate with the RNAP core enzyme and activating the GSR. There are two basic branches of signal sensing, environmental sensing through RsbU, and energy status sensing through RsbP. For the environmental pathway, signal integration occurs in a large multiprotein complex called the stressosome that modulates the phosphatase activity of RsbU ([2,5] for review).

Alphaproteobacteria: σ^{EcfG}

The activity of σ^{EcfG} is controlled by a partner switch that involves the anti-sigma factor NepR and the anti-sigma factor antagonist PhyR (see text). Although similar to σ^B in that a partner switch controls σ^{EcfG} activity, in the latter case the partner switch follows a different mechanism because the anti-sigma factor antagonist PhyR uses a sigma factor-like domain to bind to the anti-sigma factor NepR, hence mimicking the true sigma factor σ^{EcfG}. Signals are sensed and transduced by histidine kinases and phosphatases that control PhyR phosphorylation.

important role in bacterial physiology under environmental conditions. For example, the plant commensals *M. extorquens* and *Sphingomonas melonis* Fr1 (previously *Sphingomonas* sp. Fr1) are impaired in leaf colonization and show reduced competitiveness in the phyllosphere, respectively [6,14]. The GSR also plays a crucial role in bacteria that establish intimate relationships with their eukaryotic hosts. *Brucella* spp., the causative agents of brucellosis, invade and replicate in phagocytes of their human and animal hosts (for review [15]). Mutants lacking RpoE1 (σ^{EcfG} ortholog) or PhyR are attenuated in several infection models including long-term survival in a mammalian host during chronic infection [16,17]. Another example is the nitrogen-fixing symbiont of soybean, *Bradyrhizobium japonicum*, which replicates in plant cells of dedicated organs termed nodules. Many nodules elicited by *B. japonicum ecfG* or *phyR* mutants are aberrant and develop ectopically emerging roots [18]. While these examples illustrate that the GSR is essential to survive in natural environments and can be crucial for a balanced bacteria–host interaction, sustained overactivation – for example by means of *nepR* deletion or *ecfG* overexpression – can interfere with normal growth and result in a lethal phenotype [9,14,19,20]. At the moment, however, it is unknown whether this lethality is caused by an unspecific effect, such as sigma factor competition or toxicity of target gene overexpression, or whether there is a dedicated mechanism that would slow down or stop growth under severe stress conditions.

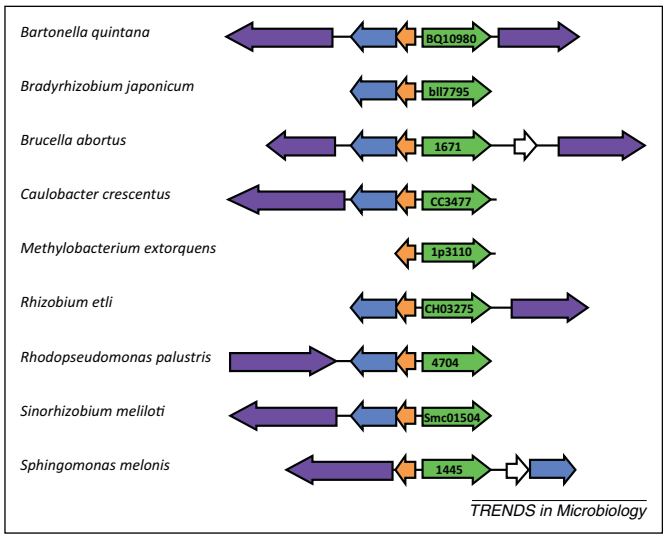

Figure 1. Genetic organization of *phyR*, *nepR*, and *ecfG* in selected Alphaproteobacteria. The color code used is as follows: blue for *ecfG*, orange for *nepR*, green for *phyR*, and purple for histidine kinase/phosphatase-encoding genes. Gene labels are given for *phyR*. The full locus tags of *phyR* of *Brucella abortus*, *Methylobacterium extorquens*, *Rhizobium etli*, *Rhodopseudomonas palustris*, and of *Sphingomonas melonis* are Bab1_1671, MexAM1_META1p3110, RHE_CH03275, Rpal_4704, and Sphme2DRAFT_1445, respectively.

Regulons of σ^{EcfG} and PhyR have been determined in several alphaproteobacterial species using transcriptomics or proteomics approaches [6–9,13,17,18,20–25]. The regulons include genes encoding proteins involved in defense against oxidative stress (e.g., catalase, peroxiredoxin, and DNA protection and repair proteins), in osmoprotection (trehalose synthesis), the synthesis of storage compounds (glycogen and polyhydroxybutyrate metabolism enzymes), and regulation (e.g., sigma factors, one- and two-component regulatory systems). A remarkably high proportion of genes in the σ^{EcfG} regulons have unknown functions in some species (e.g., >75% in *B. japonicum* [18]) pointing towards so far undiscovered adaptation mechanisms to environmental conditions. Future studies on those genes might establish novel stress-defense functions and might also help to identify other aspects of bacterial physiology controlled by the alphaproteobacterial GSR, which is probably not restricted to multiple stress resistance, similarly to the GSR in *E. coli* [4].

phyR, *nepR*, and *ecfG*: genetic organization

PhyR, NepR, and σ^{EcfG} (Box 2) are conserved in almost all Alphaproteobacteria, but are not present in members that have experienced extreme genome reduction such as obligate intracellular *Rickettsia*. The encoding genes are generally found at the same locus, with *nepR* and *ecfG* forming an operon divergently transcribed from *phyR* (Figure 1). The two transcriptional units are usually under control of σ^{EcfG}-type promoters (consensus sequence GGAAC-$N_{16–17}$-C/GGTT) [7,9,11,14,18]. Occasionally, for example in *S. melonis*, a bidirectional promoter can drive divergent expression of the two transcriptional units, whereby the −35 box of each promoter overlaps the −10 box of the other promoter on the opposite strand [14]. In theory, the autoregulation of all