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REVIEW ARTICLE

The three-component signalling system HbpS–SenS–SenR as an example of a redox sensing pathway in bacteria

Darío Ortiz de Orué Lucana · Matthew R. Groves

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Abstract The two-component system SenS–SenR and the extracellular HbpS protein of the cellulose degrader *Streptomyces reticuli* have been shown to act in concert as a novel system which detects redox stress. In vivo and in vitro experiments have led to the hypothesis that HbpS binds and degrades heme, communicating the extracellular presence of heme and oxidative stress to the membrane-embedded sensor histidine kinase SenS via a bound iron. The response regulator SenR would then up-regulate downstream signalling cascades, leading to the appropriate gene expression levels for bacterial survival in an oxidative environment. Sequence analysis has shown that homologs of HbpS and SenS–SenR exist in a number of ecologically and medically relevant bacterial species, suggesting the existence of a previously undescribed bacterial oxidative stress-response pathway common to both Gram-negative and Gram-positive bacteria. The presented report reviews the current knowledge of the function of this novel protein family consisting of an accessory protein and its cognate two-component system, which could be more properly described as a three-component system.

D. Ortiz de Orué Lucana (✉)
FB Biologie/Chemie, Angewandte Genetik der Mikroorganismen, Universität Osnabrück, Barbarastr. 13, 49069 Osnabrück, Germany
e-mail: ortiz@biologie.uni-osnabrueck.de

M. R. Groves
EMBL Hamburg Outstation, c/o DESY, Building 25A, Notkestrasse 85, 22603 Hamburg, Germany

Introduction

Two-component signal transduction systems (TCS) are one of the most important mechanisms by which bacteria sense, respond and adapt to changes in environmental or intracellular state. TCS signalling pathways have been shown to be involved in the mediation of responses to a wide range of signals and stimuli, including cellular redox state, changes in osmolarity, quorum signalling and the presence of antibiotics (Lindemann et al. 2007; Jubelin et al. 2005; Novick and Geisinger 2008; Rietkötter et al. 2008). The central importance of TCS is demonstrated by the fact that most bacterial genomes encode for 10–50 TCS, with some bacterial genomes encoding for up to 200 TCS (Laub and Goulian 2007). TCS are also present in some eukaryotes, such as fungi and plants, in which they also play an important role in processes such as the induction of pathogenicity or hormone signalling (Nemecek et al. 2006; Klein and Tebbets 2007; To and Kieber 2008).

A typical TCS consists of an autophosphorylating histidine kinase (HK) and its cognate response regulator (RR) (Hoch and Varughese 2001). Extracellularly stimulated autophosphorylation of HKs occurs either through the stimulus of an extracellular input domain or via transmembrane regions and short extracellular loops; intracellular signals are detected through cytoplasmic regions (Mascher et al. 2006). In addition to the N-terminal input domain, HKs contain a C-terminal region that plays the role of a transmitter module, with several blocks of amino acid residues conserved amongst these kinases. Autophosphorylation of a typical HK usually takes place at a conserved histidine residue; the phosphoryl group of the HK is then subsequently transferred to a conserved aspartic acid residue within the receiver domain of the RR. This modification of the RR impacts on the interaction of a

C-terminal DNA binding domain of the RR with the cognate regulatory region of its target gene(s) or operons (Zapf et al. 2000). Generally, the signalling pathway includes a phosphatase that dephosphoylates the RR, returning the response regulator to the inactivated state. This phosphatase can exist either as distinct protein, or it may be integrated as a domain within the response regulator or kinase. Such kinase/phosphatase feedback mechanisms are common (e.g. MAPK signalling) and the opposition between them ensure a tight regulation of cellular response (Yoshida et al. 2002).

While a large number of bacterial TCS have been identified to date, the nature of the response signal has been identified for only a few of these. For instance, the Nar system of *E. coli* consists of two sensor kinases and two response regulators that recognize nitrate and nitrite independently (Lee et al. 1999). The LuxN/LuxO system of *Vibrio harveyi* detects the autoinducer AI-1 (Timmen et al. 2006). In the PmrA/PmrB system of several bacteria, including species of *Salmonella*, *Klebsiella* and *E. coli*, the recognition of Fe^{3+}-ions is provided by the periplasmic sensing domain of the HK PmrB (Wösten et al. 2000; Nishino et al. 2006). Iron is the fourth most abundant element in the earths crust and is an essential trace mineral for nearly all life on the planet. The presence of oxygen and highly reactive transition metal ions, such as iron, frequently leads to redox stress and the production of toxic free radicals. Thus, bacteria and other organisms have to ensure that while enough iron is present for essential biochemical reactions they also have to avoid or mitigate the potentially damaging effects of redox stress (Baker et al. 2003; Rudolph et al. 2006). The same is true for iron-containing compounds such as the porphyrin heme, which is a cofactor for a variety of proteins [e.g. oxygen carriers (hemoglobin), redox enzymes (bc1 complex) and regulatory proteins (e.g. ChrS) (Panek and O'Brian 2002)]. However, at higher concentrations hemin (the Fe^{3+} oxidized form of heme) is highly toxic due to its ability to catalyze the formation of free radicals (Baker et al. 2003). Redox sensing systems are often related to oxygen sensing mechanisms and are widely distributed along bacteria, yeast and metazoans (Cash et al. 2007). In *E. coli* the TCS ArcA/ArcB was shown to sense anoxic or low redox conditions dependent upon on the redox state of its cysteine residues, and the VicR/VicK system of *Streptococcus mutans* was found to be responsible for protection against oxidative stress (Malpica et al. 2004; Deng et al. 2007). The TCS ChrS/ChrA from *Corynebacterium diphteriae* and the HssS/HssR system of *Staphylococcus aureus* are also involved in the sensing of heme-mediated signals (Schmitt 1999; Stauff et al. 2007). The exact mechanisms by which ArcB, VicK, ChrS and HssS sense the corresponding signals are to date still unknown. *Mycobacterium tuberculosis* possesses several sensor kinases with bound heme groups for detection of oxidative stress. One of them is DosS a redox sensor which has reduced autokinase activity upon oxidation and the other one is DosT a hypoxia sensor that displays highest autophosphorylation activity in its deoxy form (Kumar et al. 2007). Recognition of hypoxia and nitric oxide by the sensor kinase DevS of the DevS/DevR system also requires heme, which is bound to one of its aminoterminal GAF domains (Ioanoviciu et al. 2007).

Recently, the influence of accessory proteins during signal recognition and thereby on the activity of HKs has been reported (Table 1). For example, the YycG HK of *Bacillus subtilis* is subjected to a complicated activity-control circuit involving two other proteins with N-terminal transmembrane helices, YycH and YyvI (Szurmant et al. 2008). In *Rhodobacter capsulatus* the cytoplasmic H_2-sensing HupUV protein was shown to interact specifically with the cytoplasmic domain of the sensor kinase HupT and to modulate its activity during recognition of H_2. HupT subsequently modulates the activity of the response regulator HupR (Elsen et al. 2003). The lipoproteins CseA [*S. coelicolor* A3(2)] and NlpE (*E. coli*) have been suggested to modulate the activity of the corresponding HK (Hutchings et al. 2006; DiGiuseppe and Silhavy 2003) through a currently unknown mechanism.

Streptomyces are Gram-positive and G + C-rich bacteria with a complex developmental life cycle. Germination

Table 1 Characterized and deduced accessory proteins for bacterial TCS

Accessory proteins	TCS	Organism	References
Characterized			
HbpS	SenS–SenR	*S. reticuli*	Bogel et al. (2008)
YycH and YyvI	YycF–YycG	*B. subtilis*	Szurmant et al. (2008)
FixT	FixJ–FixL	*S. meliloti*	Garnerone et al. (1999)
HupUV	HupT–HupR	*R. capsulatus*	Elsen et al. (2003)
Deduced			
CseA	CseB–CseC	*S. coelicolor* A3(2)	Hutchings et al. (2006)
NlpE	CpXA–CpxR	*E. coli*	DiGiuseppe and Silhavy (2003)
LpqB	MtrA–MtrB	*S. coelicolor* A3(2)	Hoskisson and Hutchings (2006)

of spores and subsequent elongation of germ tubes lead to a network of vegetative hyphae. In response to nutritional stress and extracellular signalling aerial hyphae develop, in which spores mature (Flardh 2003). They produce a wide repertoire of medically relevant antibiotics, antifungal and cytostatics compounds, as well as many extracellular hydrolytic enzymes and enzyme inhibitors (Challis and Hopwood 2003; Baltz 2006). *Streptomyces reticuli* (*S. reticuli*), for example, produces an unusual cellulase (Avicelase), which is solely sufficient to degrade crystalline cellulose (Schlochtermeier et al. 1992). Cellulose is the main polymeric component of the plant cell wall, the most abundant polysaccharide on Earth, and an important renewable resource. Recently, reviewed data (Baldrian and Valášková 2008) discuss the fact that also natural formed reactive oxygen species (ROS) are involved in the degradation of biopolymers. The production of ROS has been suggested to be also induced by bactericidal antibiotics to kill bacteria in a complex pathway, in which iron-ions and the Fenton reaction play a role (Kohanski et al. 2007). It was additional shown, that mistranslation and misfolding of membrane proteins are central to bactericidal antibiotics-induced oxidative stress and cell death. Signalling through the envelope stress-response CpxA–CpxR two-component system is found to be a key player in this process (Kohanski et al. 2008).

As soil-dwelling organisms streptomycetes need to respond to highly variable conditions and the range of environmental stimuli to which a bacterium can respond is expected to correlate with the number of functional HKs and RRs. These are assumed to have evolved by selection pressure for different ecophysiological properties of the different strains (Alm et al. 2006). Sequence analysis of the *Streptomyces coelicolor* A3(2) genome revealed 84 HK- and 80 RR-genes, respectively (Hutchings et al. 2004) whereas that of *Streptomyces avermitilis* revealed 67 HK- and 68 RR-genes (Wei et al. 2007). In comparison, an analysis of the complete sequence of the *B. subtilis* chromosome revealed the presence of 36 genes for HK and 34 genes encoding RR (Fabret et al. 1999). The genome of the Gram-negative bacterium *E. coli* encodes 32 HK and 23 RR and five hybrid HK (Mizuno 1997). The larger number of different two-component systems within *Streptomyces* species appears to reflect their ability to respond to a wide range of environmental stimuli in their rapidly changing natural habitat.

The HbpS–SenS–SenR system from *Streptomyces reticuli*

We have previously identified the TCS SenS–SenR from the cellulose degrader *S. reticuli* (Ortiz de Orué Lucana et al. 2005), in which the HK SenS comprises a predicted membrane protein composed of five transmebrane regions. SenS has been shown to autophosphorylate at a conserved histidine residue and is able to transfer the phosphate group to its cognate response regulator, SenR, which possesses a C-terminal domain with a predicted helix–turn–helix (HTH) DNA binding motif. Upon phosphorylation SenR binds with high affinity to the upstream regions of the *senS–senR* operon and the gene *hbpS*, which encodes for a secreted heme-binding protein (HbpS). The phosphatase activity of SenS leads to the dephosphorylation of SenR, which binds upstream of the *furS–cpeB* operon encoding for the redox regulator (FurS) and the mycelia associated catalase-peroxidase (CpeB), respectively (Bogel et al. 2007). In addition, physiological and biochemical studies have been shown that the SenS–SenR system participates in the sensing of redox signals mediated by iron (Ortiz de Orué Lucana et al. 2005; Bogel et al. 2008).

HbpS is an accessory protein of SenS–SenR

HbpS is an extracellular oligomer-forming protein, which has been shown to be secreted in a Tat (twin-arginine translocation) dependent manner (Ortiz de Orué Lucana et al. 2004; Berks et al. 2000; Schaerlaekens et al. 2001). Mutational analyses revealed that the presence of HbpS increases the synthesis of the highly active catalase-peroxidase CpeB in vivo. Therefore, it was proposed that HbpS interacts with an extracellular, membrane-associated or membrane-integrated protein(s) involved in a signal transduction cascade regulating *cpeB* transcription. Protein–protein interaction studies revealed that both native *S. reticuli* HbpS and recombinantly produced HbpS interact specifically with the iron-sensing histidine kinase SenS in vitro (Ortiz de Orué Lucana et al. 2005). In addition, it was shown that the SenS N-terminal domain (containing predicted extracytoplasmic and transmembrane regions) is essential for the interaction with HbpS (Bogel et al. 2008). Phosphorylation analyses of SenS in the presence of HbpS revealed that high quantities of heme-free HbpS inhibit the autophosphorylation of the sensor kinase under non-oxidative stress conditions in vitro. Thus, an interaction with heme-free HbpS is predicted to lead to an inactive conformation of SenS. However, HbpS significantly enhanced SenS autokinase activity in the presence of HbpS and heme alone or heme in combination with DTT or H_2O_2. $FeCl_2$ and H_2O_2 are precursors of the Fenton reaction leading to the formation of hydroxyl radicals and Fe^{3+} (Fenton 1986). Additional autokinase activity tests in the presence of $FeCl_2$ (or $FeCl_3$) alone or combined with DTT or H_2O_2 showed that HbpS was able to enhance the autokinase activity of SenS. Interestingly, the greatest enhancement

took place in the presence of HbpS and $FeCl_3$ (Bogel et al. 2008).

Recently, we have elucidated the high resolution crystal structures (1.6 and 2.3 Å) of HbpS crystallized in the presence or absence of hemin (Fe^{3+}-oxidized form of heme) (Ortiz de Orué Lucana et al. 2009). Electron density analysis from the crystal structure obtained in the presence of hemin revealed the presence of a bound iron. Subsequently, spectroscopic and biochemical studies demonstrated that HbpS can bind and degrade heme through a nonenzymatic H_2O_2-dependent mechanism known as "coupled oxidation", leading to free iron. Coupled oxidation has previously been demonstrated for other heme-binding proteins, such as myoglobin (Nagababu and Rifkind 2004). These data have resulted in the hypothesis that that HbpS not only binds heme, but that HbpS supports coupled oxidation of the bound heme and then captures the released iron atom. The captured iron is coordinated by a lysine residue (at position 108, Lys108) of HbpS that is located on the surface of the protein. This surface displayed iron could then perturb the interaction between HbpS and the extracellular domain of SenS, triggering the start of the signalling cascade. This hypothesis would allow the extracellular presence of heme and/or iron-based oxidative stress conditions to be communicated across the cell membrane of *S. reticuli* via the HbpS-SenS–SenR system (Fig. 1).

Previous in vivo comparative analyses using *S. reticuli* wild-type and *hbpS* mutant strains demonstrated that *hbpS* mutants are more sensitive to higher concentrations of hemin, which is highly toxic due to its ability to catalyze free radical formation (Baker et al. 2003). Additionally, further studies revealed that the production of HbpS is highly enhanced in vivo during the cultivation of *Streptomyces* in the presence of high amounts of hemin (Ortiz de Orué Lucana et al. 2004). Thus, the heme-binding and heme-degrading activity of HbpS (Ortiz de Orué Lucana et al. 2009) described above strongly supports the hypothesis that HbpS directly participates in the degradation of hemin and in the protection of *S. reticuli* against the formation of hazardous free radicals.

While previous biochemical studies have demonstrated that HbpS forms a variety of oligomers in vivo and in vitro (interpreted as monomeric, dimeric and tetrameric, etc.; Ortiz de Orué Lucana et al. 2004; Zou et al. 2008), both crystal structures revealed an octomeric assembly of HbpS. A detailed analysis of the crystal structure of HbpS and subsequent biophysical measurements allowed us to identify two residues (Ser26 and His28) that are essential for the oligomeric assembly of HbpS. Further studies demonstrated that this assembly is a required for efficient interaction with SenS and modulation of its phosphorylation state (Ortiz de Orué Lucana et al. 2009).

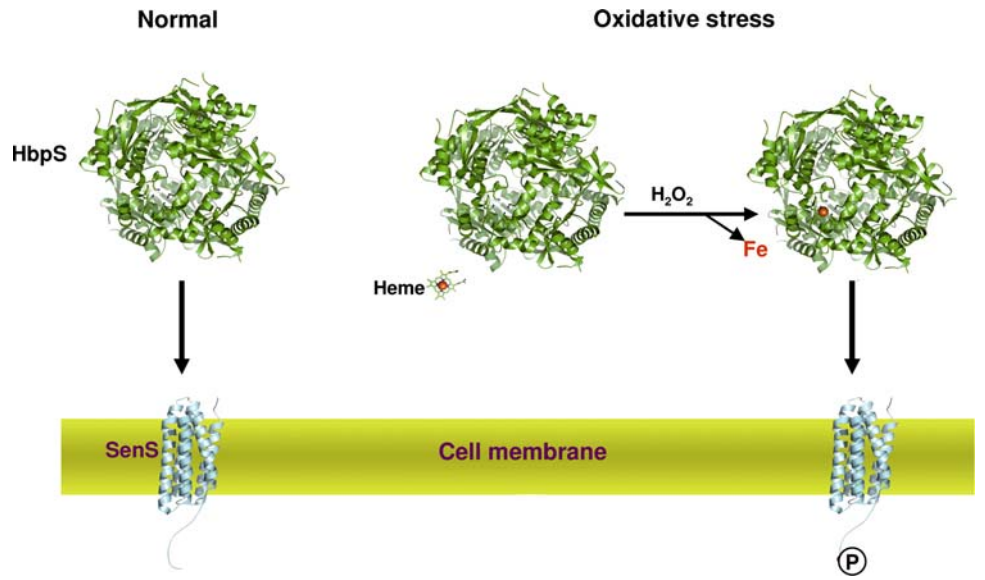

Fig. 1 Model showing the sensing of heme-based oxidative stress signals through HbpS and SenS. Under non-stressing conditions (Normal, *left*) HbpS inhibits SenS autophosphorylation activity. After the binding of heme (*middle*), HbpS degrades it in H_2O_2-dependent manner (Oxidative stress, *right*). The liberated iron is then coordinated by lysine residues of HbpS that are located on the surface of the protein. This surface displayed iron could then perturb the interaction between HbpS and the extracellular domain of SenS, inducing the phosphorylation of the sensor kinase and hence the start of the signalling cascade. The cartoon representation of the octomeric HbpS assembly as well as the structure model of SenS was produced using PyMol and povray (http://www.povray.org/). The position of the iron atom on the surface of HbpS is shown in stick (*right*, *red*) representation

Regulation of redox proteins

In addition to the autoregulation of HbpS, other proteins have been shown to be under the control of HbpS–SenS–SenR:

1. *The catalase-peroxidase CpeB* a mycelium-associated heme-containing enzyme which uses H_2O_2 to oxidize a number of substrates via an attached heme group or in a haem-independent reaction, coupled with Mn(II)/(III) peroxidation (Zou and Schrempf 2000). The additional heme-dependent catalase activity of CpeB leads to the disosication of H_2O_2 to O_2. Thus, CpeB plays an important role in detoxifying H_2O_2 and in minimizing the effect of reactions caused by highly reactive oxygen species arising from interaction of H_2O_2 with certain divalent metal ions (Fe^{2+} and others). CpeB shows a high degree of amino acid identity (62% identity, 73% similarity) to the catalase-peroxidase KatG from *Mycobacterium tuberculosis*. The front-line anti-tuberculosis drug isoniazid (isonicotinic acid hydrazide) requires KatG activation before exerting a lethal effect (Sherman et al. 1996) and isoniazid resistant strains of *M. tuberculosis* have been shown to possess no detectable levels of KatG, although they acquire a compensatory mutation resulting in an up regulation of expression of an alkyl hydroperoxide reductase protein, Ahp18. Sherman and co-workers have suggested that KatG would confer protection against H_2O_2-mediated damage even in the absence of adequate catalase and peroxidase activities, thus promoting survival of the organism in the environment of the phagocyte oxidative burst.
2. *The redox regulator FurS* the thiol-reduced (SH) form of the zinc-containing metalloregulator FurS represses the transcription of the *furS–cpeB* operon after binding of an operator located upstream of *furS*. However, under oxidative stress conditions an internal S–S bridge is formed within FurS with a concomitantl loss of the bound zinc. The S–S form of FurS is not able to bind the operator, leading to an inability to block the transcription of *furS–cpeB* which in turn leads to a high production of CpeB under oxidative stress conditions (Ortiz de Orué Lucana et al. 2003). FurS contains six C-terminally located cysteine residues, four of them contained within two C–X–X–C motifs. Such a motif can be found in a number of redox-active proteins, including thioredoxin, glutaredoxins and thiol-disulfide oxidoreductases (Rietsch and Beckwith 1998). FurS is closely related to the *M. tuberculosis* FurA, which regulates *katG* transcription (Sala et al. 2003) and is considerably divergent from the global *E. coli* Fur regulator (Hantke 2001).
3. *Deduced extracellular tyrosinase and xylanase* comparative physiological and biochemical studies have revealed two proteins, which are upregulated under iron-based oxidative stress conditions in the presence of an intact SenS–SenR TCS system (Bogel et al. 2008). One of these proteins shows significant amino acid identity to xylanase B from *Streptomyces lividans*, the other to a tyrosinase from *Streptomyces galbus*. Xylanases have been found to be important soil redox effectors as xylan can modify the redox potential of the soil by degrading the linear polysaccharide β-1,4-xylan (hemicellulose), which is a major component of the cell wall of plants), into xylose. Additionally, xylanases are used industrially during the production of paper (Pidello et al. 1996; Polizeli et al. 2005). Tyrosinases are copper-binding monooxygenases that catalyse the oxidation of *O*-Diphenols to *O*-Quinones during the production of melanin using tyrosine as substrate (Claus and Decker 2006).

Gene organization and conservation

Analysis of the HbpS primary sequence using BlastP (Altschul et al. 1997) and Pfam (Sonnhammer et al. 1997) yielded a number of putative homologues encoded within gram-positive and gram-negative bacterial genomes (Zou et al. 2008; Ortiz de Orué Lucana et al. 2004). Further detailed analysis of the adjacent regions of the corresponding *hbpS*-like genes allowed us to distribute them into two classes. One represents the majority and encompasses *hbpS*-like genes which are located in the direct vicinity of genes encoding for sensor kinases and response regulators. While some of them are clustered with the *hbpS*, *senS* and *senR* genes in the same relative transcriptional orientation others have a different arrangement (Fig. 2). The second class represents *hbpS*-like genes that are not clustered with two-component system encoding genes (data not shown). All these homologues have been identified in a number of ecologically relevant bacteria [including *S. coelicolor* A3(2), *S. kasugaensis*, *Arthrobacter aurescens*, *Pseudomonas fluorescens*, *Pseudomonas putida* and *Sphingomonas aromaticivorans*] and medically relevant bacteria (including *Vibrio cholera*, *Yersinia enterolitica*, *Leifsonia xyli*, *Acinetobacter baumani*, *Photorabdus luminiscens* and *Bordetella avium*). Interestingly, each of the deduced HbpS-like proteins showed a predicted domain with unknown function termed "DUF336". This domain is also present in other uncharacterised sequences, including several GlcG proteins and contains many conserved motifs that are suggestive of cofactor binding and enzymatic activity. These deduced structural similarities emphasize the hypothesis for a common functional role of the HbpS-like protein family.

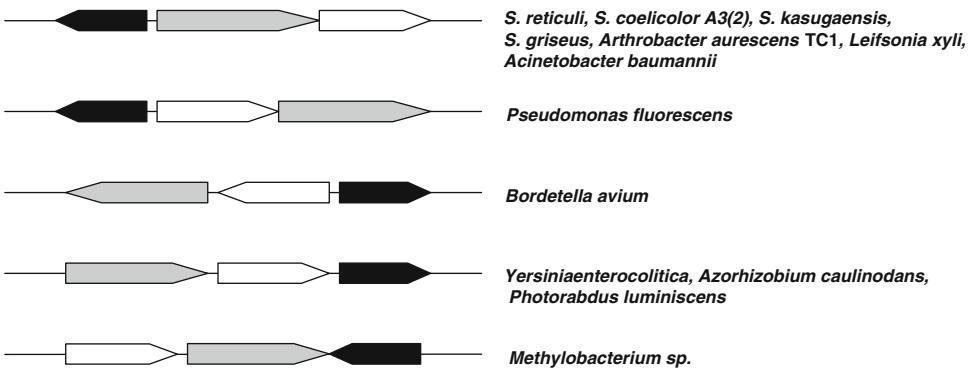

Fig. 2 Relative location and transcriptional orientation of *hbpS* and *hbpS*-like genes on different bacterial genomes. Sequence analysis revealed the presence of *hbpS* and *hbpS*-like genes (*black boxes*) in a number of ecologically and medically relevant bacteria (some examples are indicated). They are clustered with genes encoding for histidine sensor kinases (*grey boxes*) and response regulators (*white boxes*), respectively

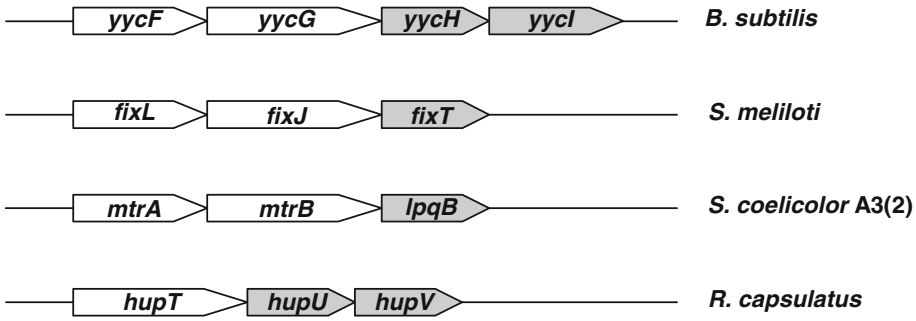

Fig. 3 Relative location and transcriptional orientation of genes encoding accessory proteins on indicated bacterial genomes. Genes for accessory proteins for the indicated histidine sensor kinases and response regulators are indicated in grey background

The deduced SenS shares some similar features with known sensor kinases. Among the characterised sensor kinases, ChrS (from the *Corynebacterium diphtheria* TCS ChrS–ChrA; Schmitt 1999) is most related (Ortiz de Orué Lucana et al. 2005). ChrS–ChrA is involved in sensing of heme signals, by an unknown mechanism, and in the regulation of a heme oxygenase (*hmuO*) gene. HmuO catalyzes the oxygen dependent degradation of heme leading to a free iron, indicating a common functional activity for SenS–SenR and ChrS–ChrA.

Other TCS with accessory proteins

To date one of the best-studied TCS with accessory proteins is YycFG, which has been shown to be essential for cell viability. It is highly conserved and specific to low-G + C content bacteria, such as *B. subtilis*, *Streptococcus pneumoniae*, *S. aureus*, and *Enterococcus faecalis* (Szurmant et al. 2005). The activity of the HK YycG from *B. subtilis* is modulated by two other proteins with N-terminal transmembrane helices, YycH and YyvI. Interestingly, all corresponding four genes are clustered and located in the same operon (Fig. 3; Szurmant et al. 2008). Another example of a characterized system with an accessory protein is FixT–FixJ–FixL from *Sinorhizobium meliloti*, which is conserved among several rhizobial species. The sensorkinase FixL is regulated by FixT through direct binding of FixT to the cytoplasmic catalytic domain of FixL resulting in a reduced level of autophosphorylation of the sensorkinase. The corresponding genes *fixT*, *fixJ and fixL* are clustered (Fig. 3; Garnerone et al. 1999; Crosson et al. 2005). In *Rhodobacter capsulatus* the cytoplasmic HupUV protein was identified to interact specifically with the cytoplasmic domain of the sensor kinase HupT and to modulate its activity. Whereas *hupT* and *hupUV* form an operon (Fig. 3), the corresponding response regulator gene *hupR* is located anywhere else on the genome (Elsen et al. 2003). The role as accessory protein has been recently postulated for the lipoprotein (LpqB) and the TCS MtrA–MtrB, which together might form an actinobacterial three-component system. The genes *mtrA*, *mtrB* and *lpqB* are also clustered and located as one transcriptional unit (Fig. 3; Hoskisson and Hutchings 2006).

Conclusion and perspectives

The extracellular HbpS protein and its interaction partner, the histidine sensor kinase SenS of the TCS SenS–SenR,

are involved in the detection of redox stress-mediated signals. In these processes, it is proposed that the sensing of heme-based signals is mediated by HbpS, which not only binds heme but also degrades it in H_2O_2-dependent manner. The liberated iron is then coordinated by lysine residues of HbpS that are located on the surface of the protein. This surface displayed iron could then perturb the interaction between HbpS and the extracellular domain of SenS, triggering the start of the signalling cascade (Fig. 1).

Among different bacteria with ecological and/or medically relevance HbpS-like proteins are widespread. The majority of the corresponding *hbpS*-like genes are located in the direct vicinity of genes encoding two-component systems (Fig. 2). The structural clustered organization is similar and relatively conserved for all of them. As reported for other systems with an accessory protein (Fig. 3), such gene organization has high relevance for the functioning of the corresponding signalling system. Therefore, a detailed analysis of DNA-regions adjacent to the numerous noted TCS genes should be performed in order to identify and characterize additional accessory proteins.

Despite the fact that the function of HbpS and SenS–SenR has been well characterized, other interesting aspects merit further study. In particular, the molecular mechanism encompassing possible conformational changes of HbpS and SenS during their interaction and signal-sensing remains poorly understood. It will also to be important to analyse these processes in vivo using reporter proteins (green fluorescence protein and red fluorescence protein, respectively). The identification of further through HbpS and SenS–SenR regulated genes should deepen the characterization of this system. Finally, the analysis of other HbpS–like proteins and their putative cognate TCS should give insights into their physiological role.

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