Thiol-based redox switches in prokaryotes

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Review

Melanie Hillion and Haike Antelmann*

Thiol-based redox switches in prokaryotes

Abstract: Bacteria encounter reactive oxygen species (ROS) as a consequence of the aerobic life or as an oxidative burst of activated neutrophils during infections. In addition, bacteria are exposed to other redox-active compounds, including hypochloric acid (HOCl) and reactive electrophilic species (RES) such as quinones and aldehydes. These reactive species often target the thiol groups of cysteines in proteins and lead to thiol-disulfide switches in redox-sensing regulators to activate specific detoxification pathways and to restore the redox balance. Here, we review bacterial thiol-based redox sensors that specifically sense ROS, RES and HOCl via thiol-based mechanisms and regulate gene transcription in Gram-positive model bacteria and in human pathogens, such as *Staphylococcus aureus* and *Mycobacterium tuberculosis*. We also pay particular attention to emerging widely conserved HOCl-specific redox regulators that have been recently characterized in *Escherichia coli*. Different mechanisms are used to sense and respond to ROS, RES and HOCl by 1-Cys-type and 2-Cys-type thiol-based redox sensors that include versatile thiol-disulfide switches (OxyR, OhrR, HypR, YodB, NemR, RclR, Spx, RsrA/RshA) or alternative Cys phosphorylations (SarZ, MgrA, SarA), thiol-S-alkylation (QsrR), His-oxidation (PerR) and methionine oxidation (HypT). In pathogenic bacteria, these redox-sensing regulators are often important virulence regulators and required for adapation to the host immune defense.

Keywords: HypT; MarR; NemR; OhrR; OxyR; RclR; RshA; RsrA; SarA; Spx; Thiol-redox switches.

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Introduction

Bacteria have to cope in their natural environment or during bacterial infection in association with the host immune system to reactive oxygen species (ROS) that are known to cause an oxidative stress response and affect the reduced state of the cytoplasm. ROS are produced in microorganisms as the unavoidable consequence of the aerobic life, by incomplete reduction of molecular oxygen during respiration (Imlay, 2003, 2008, 2013). Beside ROS, bacteria have to cope with many other redox-active compounds, including antimicrobials, antibiotics and environmental xenobiotics, which can act as reactive electrophilic species (RES) and affect the cellular redox status (Marnett et al., 2003; Jacobs and Marnett, 2010). ROS and RES cause specific post-translational thiol-modifications in redox-sensing transcription factors which lead to conformational changes and activate or inactive the transcriptional regulator. As consequence, specific detoxification pathways are up-regulated to destroy the reactive species or to repair the resulting damage (Antelmann and Helmann, 2011; Vazquez-Torres, 2012; Imlay, 2013). With the discovery of the peroxide sensor OxyR of *Escherichia coli*, it became evident that ROS-sensing by thiol-disulfide switches represents an important regulatory device in bacteria (Zheng et al., 1998; Choi et al., 2001; Kim et al., 2002). However, during the last decade this classical thiol-disulfide-switch model for redox regulation has been expanded by different reversible and irreversible thiol-modifications, such as S-thiolation, Cys phosphorylation or thiol-S-alkylation that are employed by thiol-based redox sensors to regulate expression of specific antioxidant enzymes and virulence mechanisms. In addition to thiol-redox switches, redox sensors can also use methionine oxidation switches, His-oxidation or flavin cofactors, iron and iron-sulfur clusters, heme centers either directly or indirectly for redox-sensing. Here, we review the currently known thiol-based ROS, RES and HOCl-specific redox sensors that have been characterized in Gram-positive model bacteria and human pathogens as well as in *E. coli*.

Thiol chemistry of ROS, RES and HOCl

Reactive oxygen species (ROS) include superoxide anion ($O_2\cdot^-$), hydrogen peroxide (H_2O_2) and the highly reactive hydroxyl radical (OH·) that are generated during aerobic respiration by the incomplete stepwise reduction of O_2 (Imlay, 2003, 2008). The highly toxic hydroxyl radical (OH·) is produced in the Fenton reaction by H_2O_2 and free ferrous iron (Fe^{2+}) (Imlay, 2003, 2008). Upon infection, the oxidative burst from activated neutrophils generates $O_2\cdot^-$, H_2O_2, nitric oxide (NO) and hypochloric acid (HOCl) with the aim to kill invading pathogenic bacteria (Forman and Torres, 2001; Winterbourn and Kettle, 2012). Reactive electrophilic species include quinones, aldehydes, epoxides, diamide and α,β-unsaturated dicarbonyl compounds that have electron-deficient centers (Antelmann and Helmann, 2011). RES can arise endogenously as secondary reactive intermediates from oxidation products of amino acids, lipids or carbohydrates (Marnett et al., 2003; Rudolph and Freeman, 2009). The dicarbonyl compound methylglyoxal is produced as a byproduct during the glycolysis from tri-ose-phosphate intermediates (Ferguson et al., 1998; Booth et al., 2003; Kalapos, 2008). Formaldehyde is encountered by bacteria as intermediate in the C_1-metabolism of methanotrophic and methylotrophic bacteria. Thus, bacteria have evolved redox sensors and conserved detoxification pathways for the natural RES formaldehyde and methylglyoxal.

The thiol group of cysteine is the main target for ROS, RES and HOCl and is subject to reversible and irreversible post-translational thiol-modifications. The thiol group can be reversibly oxidized to protein disulfides or irreversibly overoxidized to sulfinic or sulfonic acids by ROS or S-alkylated by RES (Antelmann and Helmann, 2011). ROS lead first to oxidation of protein thiols to Cys sulfenic acids (R-SOH) that rapidly react further to form intramolecular, intermolecular disulfides or mixed disulfides with LMW thiols (termed as S-thiolations) (Figure 1). Hypochloric acid (HOCl) is a strong two-electron oxidant and chlorinating agent that targets the sulfur-containing amino acids cysteine and methionine with the second-order rate constants of $k=3\times10^7$ M^{-1}s^{-1} (Hawkins et al., 2003). HOCl first chlorinates the thiol group to form the unstable sulfenyl-chloride intermediate that reacts further with another thiol group to form disulfides. In the absence of another Cys residue, the chlorinated thiol group is overoxidized very rapidly to sulfinic or sulfonic acids (Hawkins et al., 2003; Gray et al., 2013a) (Figure 1). RES, like quinones, can react with Cys thiols via thiol-disulfide switches or thiol-S-alkylation. During the incomplete one-electron reduction
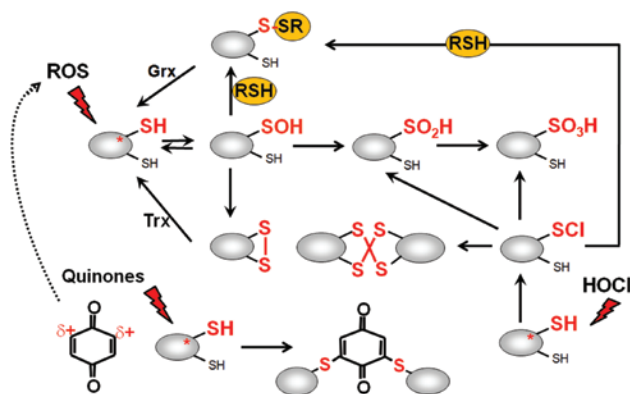

Figure 1: Thiol chemistry of ROS, RES and HOCl with redox-sensing regulators.
Reversible thiol-oxidation by ROS leads first to a Cys sulfenic acid intermediate (R-SOH) that is unstable and reacts further to form intramolecular and intermolecular disulfides or mixed disulfides with LMW thiols, such as glutathione, bacillithiol, cysteine or CoASH, termed as S-thiolations. The Cys sulfenic acid can be also overoxidized to Cys sulfinic and sulfonic acids. Reactive electrophilic species (RES) such as quinones have been shown to act via the S-alkylation and oxidation mode with quinone-sensing redox regulators. HOCl causes first chlorination of Cys thiol goups to the unstable sulfenylchloride, which react further to form protein disulfides and S-thiolations in the presence of proximal thiols. In the absence of another thiol the sulfenylchloride rapidly forms irreversible Cys sulfinic or sulfonic acids (Hawkins et al., 2003).

of quinones the highly reactive semiquinone radical is produced that leads to subsequent reduction of O_2 and the production of $O_2\cdot^-$. The electrophilic reaction of quinones involves the 1,4-reductive Michael-type addition of thiols to quinones (Marnett et al., 2003). Toxic quinones lead to irreversible thiol-S-alkylation and protein aggregation to deplete protein thiols in the proteome in vivo (Liebeke et al., 2008). However, non-toxic quinones cause disulfide formation in RES-sensing redox regulators, such as YodB or NemR to up-regulate quinone detoxification pathways (Chi et al., 2010a; Gray et al., 2013b; Lee et al., 2013).

Thiol-based redox sensors for ROS, RES and HOCl in bacteria

OxyR as thiol-based redox sensor for peroxides and NO in *E. coli* and Actinomycetes

OxyR is a redox sensor for peroxides and NO in *Salmonella* Typhimurium and *E. coli* and was the first discovered

redox-sensitive regulator that is activated by a thiol-disulfide-switch model (Storz et al., 1990b; Zheng et al., 1998). OxyR belongs to the LysR-family of transcription factors that acts both as transcriptional activator of per-oxide detoxification pathways and repressor of its own transcription and binds as tetramer to operator sequences (Figure 2, Table 1). Gisela Storz has shown that OxyR oxidation occurs by H_2O_2 at the conserved Cys199 that is oxidized to a sulfenic acid and subsequently forms an intramolecular disulfide with Cys208 in each of the four subunits of the OxyR tetramer (Storz et al., 1990b; Zheng et al., 1998). The OxyR tetramer binds to the operator sequences in the reduced and oxidized forms, but the interaction of reduced and oxidized OxyR with the DNA is different (Toledano et al., 1994). Oxidized OxyR recognizes a motif comprised of four ATAGnt elements spaced at 10 bp intervals and binds to this motif in four adjacent major grooves on one face of the DNA. Reduced OxyR binds to the DNA at two pairs of adjacent major grooves separated by on helical turn. The two modes of binding are essential for OxyR to function as both an activator and a repressor *in vivo* (Toledano et al., 1994). Oxidized OxyR induces the cooperative binding of the RNAP to activate transcription (Kullik et al., 1995a,b).

The crystal structures of reduced and oxidized OxyR and thiol-trapping assays confirmed the Cys199-Cys208 disulfide-switch model both *in vitro* and *in vivo*. The redox-sensing Cys199 is located in a loop between the α-helix and the β8 strand and is 17 Å apart from C208. OxyR oxidation to the Cys199-Cys208 intramolecular disulfide results in unwinding of the α-helix and movement of the α-helix/β8 loop causing large structural changes in the oligomeric interfaces and relative rotation among the OxyR subunits (Choi et al., 2001; Barford, 2004; Lee et al., 2004). Disulfide formation leads to the rearrangement of the N-terminal DNA-binding domains relative to the DNA to facilitate proper DNA-binding of oxidized OxyR to the four adjacent major grooves to induce the cooperative interactions with the RNAP required for transcriptional activation of peroxide detoxification genes.

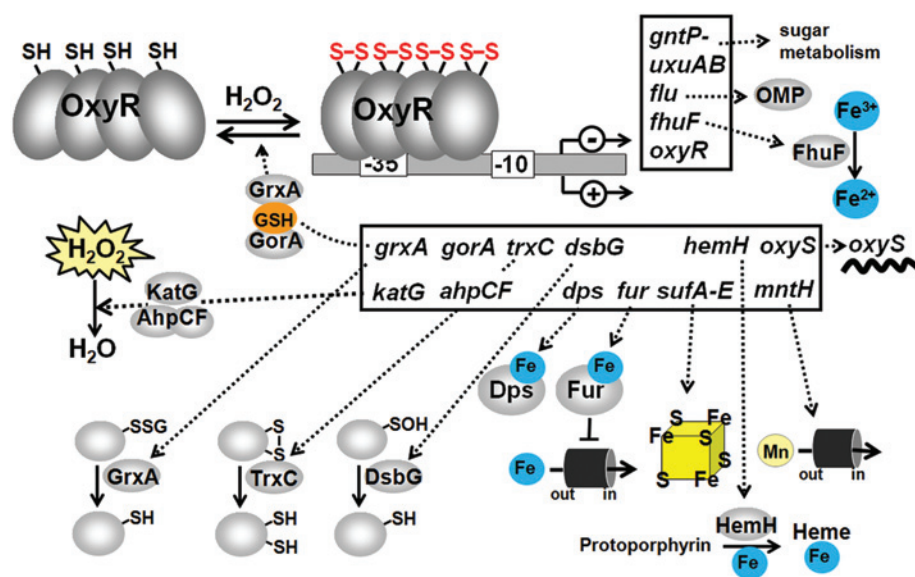However, this thiol-disulfide-switch model was questioned by the group of Jonathan Stamler, because

Figure 2: The thiol-disulfide-switch model of *E. coli* OxyR and functions of the OxyR regulon.
OxyR responds to hydrogen peroxide (H_2O_2) in *E. coli* and other bacteria. The conserved C199 and C208 residues of OxyR are essential for redox-sensing of OxyR. C199 is initially oxidized to the sulfenic acid intermediate that rapidly reacts further to form an intramolecular disulfide with C208. Oxidized OxyR binds as a tetramer to promoter regions of target genes and activates transcription of peroxide detoxification genes by contact with αCTD of RNA polymerase. OxyR positively controls genes for peroxide detoxification, such as catalase and peroxiredoxin (*katG, ahpCF*), Fe-storage miniferritin (*dps*), glutaredoxin, thioredoxin and glutathione reductase (*grxA, trxC, gor*), sulfenic acid oxidoreductase (*dsbG*), ferric uptake regulator (*fur*), Fe-S-cluster assembly machinery (*sufABCDE*), ferrochelatase (*hemH*), manganese import (*mntH*) and the small RNA (*oxyS*). OxyR negatively regulates its own expression and that of the genes for the ferric ion reductase (*fhuF*), the outer membrane protein (*flu*), the mannonate hydrolase (*uxuAB*) and gluconate permease (*gntP*). OxyR is regenerated by the glutaredoxin/GSH/Gor system upon return to non-stress conditions. Examples for OxyR regulon genes and their functions are also listed in Table 1.

Table 1: The LysR-family OxyR-type peroxide-sensing transcriptional regulators.

Redox sensor	Organism	Signal	Redox-sensing mechanism	Regulon genes	Regulon functions	References
OxyR	*Escherichia coli*	H_2O_2	C199*-C208 intramolecular disulfide	*ahpCF*	Peroxiredoxin	Storz et al. (1990b)
				katG	Catalase	Zheng et al. (1998)
				fur	Fe-uptake repressor	Choi et al. (2001)
				dps	Miniferritin	
		H_2O_2	Cys199-SOH	*mntH*	Mn-uptake	
		NO	Cys199-SNO	*sufABCDE*	Fe-S cluster assembly	Kim et al. (2002)
		GSSG	Cys199-SSG	*trxC*	Thioredoxin	
				grxA	Glutaredoxin	
				gor	GSH reductase	
				dsbG	Cys-SOH reduction	
				hemH	Ferrochelatase	
				oxyR	LysR-type regulator	
				fhuF	Fe³⁺ reductase	
				flu	antigen 43 outer membrane protein	
				uxuAB	Mannonate hydrolase	
				gntP	Gluconate permease	
OxyS	*Mycobacterium tuberculosis*	H_2O_2	C25* peroxide sensing	*katG*	Catalase	Domenech et al. (2001) Li and He (2012)
OxyR	*Deinococcus radiodurans*	H_2O_2	C210*-SOH	*katE*	Catalase	Chen et al. (2008)
				drb0125	Iron(III) dicitrate-uptake	
				feoB	Fe²⁺ uptake	
				dps	Miniferritin	
				mntH	Mn-uptake	
OxyR	*Corynebacterium glutamicum*	H_2O_2	C206* and C215 conserved	*katA*	Catalase	Teramoto et al. (2013)
				dps, *ftn*	Miniferritins	Milse et al. (2014)
				cydABCD	cytochrome bd oxidase	
				hemH	ferrochelatase	
				cg1292	Flavin-monooxygenase	
				sufABCDE	Fe-S-cluster assembly	
				proP	Proline-ectoine transporter	
				oxyR	LysR-type regulator	
				sufR	FeS-cluster regulator	
				ripA	Regulator of iron-proteins	

mutational analyses suggested that only Cys199 is required for redox-sensing and transcriptional activation of OxyR. Different post-translational thiol-modifications were introduced at Cys199 of OxyR, including sulfenic acid formation, *S*-nitrosylation or *S*-glutathionylation that were sufficient for OxyR activation *in vitro* (Kim et al., 2002). These different OxyR modifications resulted in different OxyR activation states. The *S*-glutathionylated OxyR conferred non-cooperative DNA-binding while the sulfenic acid and *S*-nitrosylated forms of OxyR provoke cooperative binding to the DNA.

Interestingly, *S*-nitrosylation of OxyR occurred specifically under conditions of anaerobic nitrate respiration. Moreover, OxyR controls widespread endogenous protein *S*-nitrosylation and the expression of a different anaerobic OxyR regulon during nitrate respiration (Seth et al., 2012). The anaerobically OxyR-controlled *hcp* gene, encoding a hybrid cluster protein, was shown to be specifically activated by *S*-nitrosylated OxyR. The *oxyR* mutant showed a growth defect with nitrate and Hcp was required for protection against endogenous nitrosative stress under anaerobic nitrate respiration. This indicates that OxyR can be activated by different post-translational thiol-modifications, including the thiol-disulfide-switch under H_2O_2 stress and *S*-nitrosylation under anaerobic nitrate respiration to activate distinct regulons for protection against peroxide and nitrosative stress (Seth et al., 2012).

OxyR is conserved in Gram-negative and Gram-positive bacteria and has been studied in *Proteobacteria*, *Bacteroidetes* and *Actinomycetes* (Chiang and Schellhorn,

2012). In many bacteria, OxyR controls catalases and peroxiredoxins while the size of the OxyR regulon varies. In *E. coli*, OxyR positively regulates genes for the peroxide scavenging peroxiredoxin (*ahpCF*) and catalase (*katG*), the iron-uptake regulator (*fur*), the miniferritin (*dps*), the Mn-importer (*mntH*), the FeS-cluster assembly machinery (*sufABCDE*), the ferrochelatase for ferrous ion incorporation into heme (*hemH*), thioredoxins (*trxC*), glutaredoxins (*grxA*), glutathione reductase (*gor*) and the periplasmic sulfenic acid oxidoreductase (*dsbG*) (Storz et al., 1990a) (Figure 2, Table 1). OxyR is both an activator and repressor and controls negatively its own transcription and that of the genes for the ferric ion reductase (*fhuF*), the antigen 43 outer membrane protein (*flu*), the mannonate hydrolase and oxidoreductase (*uxuAB*), the gluconate permease (*gntP*) and some unknown function proteins (Zheng et al., 2001). Oxidized OxyR is reduced by the glutaredoxin (GrxA)/GSH/Gor reducing system upon return to non-stress conditions. The OxyR regulon genes confer peroxide resistance in *E. coli*, but protect cells also against heat, UV, singlet oxygen, lipid peroxides and neutrophil killing (Chiang and Schellhorn, 2012).

OxyR homologs have been studied in Gram-positive Actinomycetes, such as *Mycobacteria* and *Corynebacteria* where they control catalases and peroxiredoxins. Interestingly, in *Mycobacterium tuberculosis* the catalase KatG activates the anti-tuberculosis pro-drug isoniazid (INH) upon treatment of *M. tuberculosis* infections (Zhang et al., 1992). However, *katG* expression is not regulated by OxyR and the *oxyR* gene has acquired several non-sense mutations and is non-functional in *M. tuberculosis* (Deretic et al., 1997). These non-sense *oxyR* mutations are conserved among most *Mycobacteria*, except for *Mycobacterium leprae* and *Mycobacterium avium* which encode functional *oxyR* genes (Sherman et al., 1995). Expression of *katG* is regulated by the ferric uptake regulator FurA in *M. tuberculosis* and *M. smegmatis* (Milano et al., 2001; Pym et al., 2001; Zahrt et al., 2001). The *furA* gene is located upstream of *katG* and the *furA-katG* operon was induced by oxidative stress in a FurA-dependent manner (Milano et al., 2001). However, in a fast-growing *Mycobacterium* sp. strain JC1 DSM 3803, *katG* was induced FurA-independently under oxidative stress (Lee et al., 2010). This peroxide-inducible expression of *katG* was shown to be controlled by the OxyR-homolog OxyS in *M. tuberculosis* (Domenech et al., 2001; Li and He, 2012). The *oxyR* homologous *oxyS* gene is located in the *M. tuberculosis* cosmid T919 and is highly conserved among *Mycobacteria*. OxyS is a repressor of *katG* transcription and overexpression of OxyS resulted in stronger repression of *katG* transcription and increased susceptibility to H_2O_2 stress in

M. smegmatis (Domenech et al., 2001; Li and He, 2012). The operator sequence for OxyS binding was identified as GC-rich T-N(11)-A motif within the *katG* promoter region. OxyS has 4 Cys residues: Cys113, Cys124 and Cys293 are in the LysR-substrate-binding domain and Cys25 is located in the N-terminal DNA-binding domain. Cys25 of mycobacterial OxyS is required for peroxide-sensing, which is conserved also among enteric OxyR proteins but absent from OxyR of *M. leprae* and *M. avium* (Domenech et al., 2001). The DNA-binding activity of OxyS was inhibited by H_2O_2 *in vitro* in gel-shift assays while the OxySC25A mutant did not respond to H_2O_2 *in vitro* and *in vivo*. These results suggest that OxyS regulation involves oxidation of the single Cys25 in the DNA-binding domain under oxidative stress, but the thiol-modification that inactivates OxyS is unknown (Li and He, 2012).

Similar to mycobacterial OxyS, a 1-Cys-type OxyR redox sensor was characterized in *Deinococcus radiodurans* that is oxidized at the conserved single Cys210 to a sulfenic acid under peroxide stress. OxyR of *Deinococcus radiodurans* activates transcription of genes for the catalase (*katE*), the ferrous iron transporter (*feoB*) and the iron(III)dicitrate transporter (*drbO125*), but also operates as repressor of *dps* and *mntH* transcription to control antioxidant functions and Mn/Fe ion homeostasis (Chen et al., 2008). This indicates, that 1-Cys-type OxyR homologs are also present in other bacteria, including OxyS of Mycobacteria and OxyR of *D. radiodurans* that might sense peroxide stress by alternative thiol-modifications similar as has been described for OxyR of *E. coli* (Kim et al., 2002).

In *Corynebacterium glutamicum* and *Corynebacterium diphtheriae*, OxyR is functional as transcriptional repressor of the catalase-encoding gene. In both species, disruption of *oxyR* led to derepression of the catalase gene that conferred a H_2O_2 resistance phenotype (Kim and Holmes, 2012; Teramoto et al., 2013). DNaseI-footprinting analyses revealed the OxyR binding region that is ~50 bp long with multiple T-N$_{11}$-A motifs but no sequence similarities, characteristic for operators recognized by LysR-type regulators. Reduced OxyR binds specifically to this operator sequence in different OxyR target gene promoters (Teramoto et al., 2013). However, DNA-binding activity of OxyR was not inhibited after oxidation and even non-specific binding of oxidized OxyR was observed. This suggests that alleviation of OxyR repression by peroxides might be the result of decreased strength of its interaction with the DNA. In genome-wide transcriptome analyses, the OxyR regulon of *C. glutamicum* was characterized after peroxide stress (Milse et al., 2014). OxyR acts as transcriptional repressor and negatively regulates expression of 23 genes that belong to 12 transcriptional units. DNA-binding assays

confirmed specific binding of OxyR to the 12 target promoters. In total, the OxyR regulon consists of genes encoding the catalase (*katA*), two miniferritins that function in iron homeostasis (*dps* and *ftn*), cytochrome bd oxidases (*cydABCD*), the heme biosynthesis enzyme ferrochelatase (*hemH*), a flavin-monooxygenase (*cg1292*), the FeS-cluster biosynthesis machinery (*suf* operon), the proline-ectoine transporter (*proP*) and several unknown function genes (Table 1) (Milse et al., 2014). In addition, transcriptional regulators were regulated by OxyR, such as *oxyR*, *sufR* and *ripA*. OxyR of *C. glutamicum* shares with *E. coli* OxyR the conserved redox-sensing Cys199 and Cys206 residues indicating a similar thiol-disulfide-switch model for OxyR of *C. glutamicum*.

PerR as Fur-family metal-based peroxide sensor in Firmicutes bacteria

In *B. subtilis*, the Fur-family protein PerR functions as the main peroxide sensor. PerR is a dimeric repressor that binds to the PerR box (TTATAATNATTATAA) as heptameric 7-1-7 inverted repeat in the promoter region of its target genes (Fuangthong and Helmann, 2003). PerR is inactivated by H_2O_2 stress, leading to derepression of the PerR regulon genes. The PerR regulon includes the genes for the peroxiredoxin (*ahpCF*), the catalase (*katA*), the miniferritin (*mrgA*), the heme biosynthesis operon (*hemAXCDBL*), the iron-uptake repressor (*fur*), and the Zn-uptake system (*zosA*) (Fuangthong et al., 2002; Helmann et al., 2003) (Figure 3, Table 2). Two overlapping PerR boxes are present in the *perR* upstream region indicating that PerR is autoregulated (Fuangthong et al., 2002). The derepression of the PerR regulon genes under peroxide stress conditions leads to peroxide resistance as adaptive response in *B. subtilis* (Faulkner and Helmann, 2011). In *S. aureus*, the PerR regulon is also peroxide-inducible and includes genes for catalase, peroxiredoxins and bacterioferritin comigratory protein (*katA*, *ahpCF, bcp*), two miniferritins (*mrgA*, *ftn*) and thioredoxin reductase (*trxB*) that are required for virulence (Horsburgh et al., 2001). In addition, PerR negatively regulates its own transcription and that of the gene for the ferric uptake regulator (*fur*). PerR is required for full virulence in a murine skin abscess model of infection. It was further shown that both *katA* and *ahpC* have compensatory roles in peroxide resistance and mediate environmental persistence and nasal colonization in *S. aureus* (Cosgrove et al., 2007).

The regulatory mechanism for peroxide-sensing of PerR has been shown by the Helmann group in *B. subtilis* (Lee and Helmann, 2006). PerR contains two metal
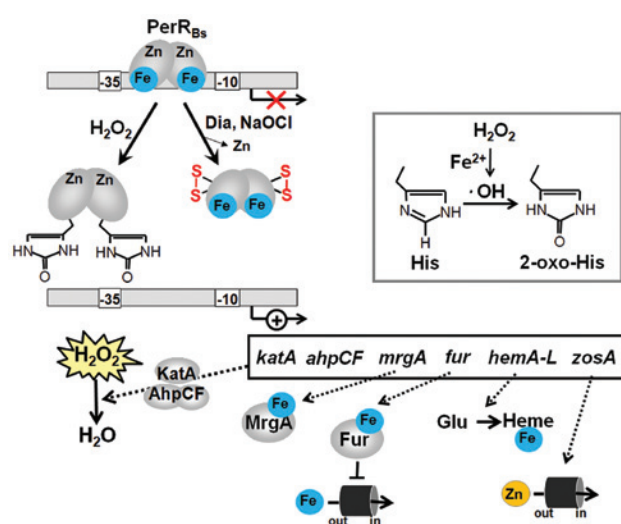

Figure 3: Redox regulation of *B. subtilis* PerR$_{Bs}$ by peroxides (metal-catalyzed histidine oxidation) and by diamide stress (intramolecular disulfides) and functions of the PerR regulon members.
PerR has a regulatory Fe^{2+} or Mn^{2+}-binding site with Asp and His residues as ligands and a structural Zn^{2+}-binding site coordinated by four cysteine residues. Reaction of PerR-Fe with H_2O_2 leads to a Fenton reaction generating HO· with subsequent oxidation of His37 and His91 to the 2-oxo-His derivatives that inactivate the PerR repressor under H_2O_2 stress leading to up-regulation of the PerR regulon genes. Under disulfide-stress conditions provoked by diamide and NaOCl, PerR is inactivated by intramolecular disulfide formation in the Zn-binding site that also lead to derepression of the PerR regulon genes. The PerR regulon includes genes with antioxidant functions, such as the catalase and peroxiredoxin (*katA*, *ahpCF*), Fe-storage miniferritin (*mrgA*), ferric uptake regulator (*fur*), heme biosynthesis enzymes (*hemAXCDBL*) and zinc uptake systems (*zosA*). Examples for PerR regulon genes of *B. subtilis* and their functions are also listed in Table 2.

binding sites, a structural Zn^{2+} binding site coordinated by four cysteine residues (Cys96, Cys99, Cys136, Cys139) in the C-terminal domain and a regulatory Fe^{2+} or Mn^{2+} binding site with three histidine and two aspartic acid residues as ligands (Lee and Helmann, 2006). Both Mn^{2+} and Fe^{2+} bind competitively to the PerR regulatory site, but only iron-bound PerR is sensitive to metal-catalyzed oxidation (Mongkolsuk and Helmann, 2002). Exposure to H_2O_2 leads to oxidation of Fe^{2+} in the regulatory site by a Fenton reaction generating HO· which causes oxidation of His37 and His91 to 2-oxo-histidine and inactivation of PerR (Figure 3) (Lee and Helmann, 2006; Traore et al., 2009; Duarte and Latour, 2010). Although the 2-oxo-His37 still had affinity for the regulatory metal, no metal binding with 2-oxo-His91 was possible and PerR fails to retain the close conformation for DNA-binding (Traore et al., 2009; Duarte and Latour, 2010). Thus, in contrast to OxyR which is activated by a thiol-disulfide redox switch, the

Table 2: The Fur/PerR-family of peroxide-specific redox regulators.

Redox sensor	Organism	Signal	Redox-sensing mechanism	Regulon genes	Regulon functions	References
PerR	*Bacillus subtilis*	H_2O_2	2-oxo-His37 2-oxo-His91 His93 Conserved Fe-site	*ahpCF* *katA* *mrgA* *hemAXCDBL* *fur*	Peroxiredoxin Catalase Miniferritin Heme biosynthesis Fe-uptake repressor	Fuangthong et al. (2002) Lee and Helmann (2006)
		Diamide NaOCl	C96, C99 C136-C139 intramolecular disulfide Conserved Zn-site	*zosA*	Zn-uptake	Chi et al. (2011)
PerR	*Staphylococcus aureus*	H_2O_2	His43, His97, His99 Conserved Fe-site C102,C105 C142,C145 Conserved Zn-site	*ahpCF* *katA* *mrgA* *ftn* *bcp* *trxA* *fur* *perR*	Peroxiredoxin Catalase Miniferritin Ferritin Bacterioferritin comigrating protein Thioredoxin Fe-uptake repressor Peroxide repressor	Horsburgh et al. (2001)
PerR	*Clostridium acetobutylicum*	O_2 H_2O_2	His32, His86, His88 Conserved Fe-site C91,C94, C131,C134 Conserved Zn-site	*rbr3A*, *rbr3B* *bcp* *tpx* *bsa2* *grx* *CAC2452* *dfx* *fprA2* *rb* *nror* *ofrAB* *gapN*	Reverse ruberythrins Bacterioferritin comigrating protein Thiol peroxidase Glutathione peroxidase Glutaredoxin flavodoxin Desulfoferredoxin Flavodiiron protein Rubredoxin NADH-rubredoxin oxidoreductase 2-oxoglutarate ferredoxin oxidoreductase NADP-dep. GapDH	Hillmann et al. (2009a) Hillmann et al. (2009b) Riebe et al. (2009)

PerR transcription factor senses peroxide stress by metal-catalyzed histidine oxidation. However, the PerR regulon genes are also induced under disulfide conditions, such as diamide and hypochlorite in *B. subtilis* (Antelmann et al., 2008; Chi et al., 2011). Thus, the response of PerR to disulfide stress could involve thiol-redox switches in the structural Zn-site of PerR, leading to inactivation of its repressor function (Figure 3). In support of this thiol-based mechanism, an intramolecular C136-C139 disulfide in the Zn-binding site of PerR was identified by mass spectrometry in hypochlorite-stressed *B. subtilis* cells *in vivo* (Chi et al., 2011). In addition, a thiol-redox switch was identified as peroxide-sensing mechanism of the Fur-family PerR homolog of *Streptomyces coelicolor* CatR that controls expression of the catalase gene *catA* (Hahn et al., 2000a,b). In CatR two CXXC motifs are present that coordinate Zn in the reduced state. In response to peroxide stress, intramolecular disulfides are formed in the Zn-site of CatR that inactivate CatR's repressor function

resulting in derepression of *catA*. Thus, it is likely that PerR homologs respond also via thiol-based mechanisms under certain disulfide-stress conditions that are different from peroxide stress.

The PerR homolog that senses peroxides and O_2 has been identified in the strict anaerobic Gram-positive bacterium *Clostridium acetobutylicum* as defense mechanism against O_2 toxicity (Hillmann et al., 2008). PerR inactivation conferred aerotolerance to *C. acetobutylicum*, enabled aerobic growth and O_2 consumption and conferred resistance to H_2O_2. The PerR regulon includes genes for the reverse ruberythrins as major peroxidases for H_2O_2 reduction (*rbr3A*, *rbr3B*), the peroxiredoxin (*bcp*), the thiol peroxidase (*tpx*), the glutathione peroxidase (*bsa2*), the glutaredoxin (*grx*), the flavodoxin (*CAC2452*), the superoxide-reducing desulfoferrodoxin (*dfx*), the oxygen-reducing flavodiiron proteins (*fprA2*), rubredoxins (*rd*) as intermediates to regenerate the reductases FprA2, Dfx and revRbr, the NADH-dependent rubredoxin oxidoreductase
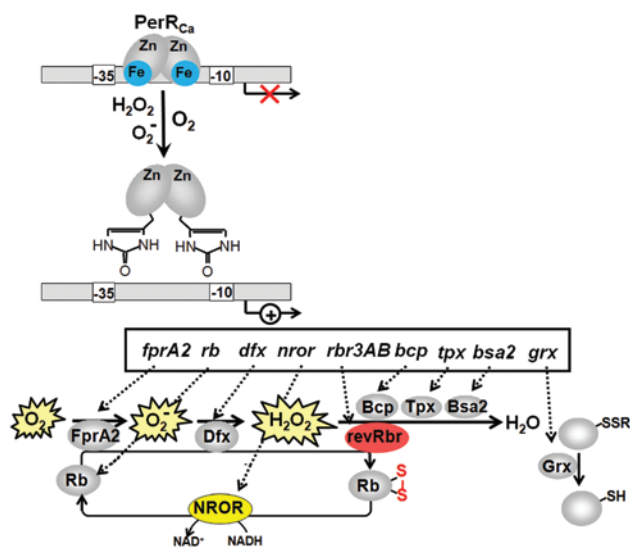
Figure 4: Proposed redox regulation of PerR$_{Ca}$ in the strict anaerobe *Clostridium acetobutylicum* by oxygen, peroxides, superoxide and functions of the PerR regulon members.
The PerR repressor is proposed to sense O_2, O_2^- and H_2O_2 by oxidation of two His residues in the conserved regulatory Fe-binding site leading to 2-oxo-His generation. This causes PerR inactivation and derepression of the PerR regulon genes. The PerR regulon controls genes for an anaerobic oxygen and ROS detoxification pathway, including the oxygen-reducing flavodiiron proteins (*fprA2*), reverse rubrerythrins and peroxidases (*rbr3A*, *rbr3B*), peroxiredoxin (*bcp*), thiol peroxidase (*tpx*), glutathione peroxidase (*bsa2*), glutaredoxin (*grx*), superoxide-reducing desulfoferrodoxin (*dfx*), rubredoxin (*rd*) and the NADH-dependent rubredoxin oxidoreductase (*nror*). Examples for PerR regulon genes of *C. acetobutylicum* and their functions are also listed in Table 2.

(*nror*) to provide electrons for rubredoxin reduction, the 2-oxoglutarate ferredoxin oxidoreductase (*ofrAB*) and the NADPH-dependent non-phosphorylating GapDH (*gapN*) (Hillmann et al., 2009a,b; Riebe et al., 2009)(Figure 4, Table 2). These PerR regulon genes are all induced under O_2 and peroxide stress and function collectively as anaerobic O_2 and ROS detoxification pathways to promote the survival of the strict anaerobe *C. acetobutylicum* under short-time microaerophilic conditions.

The MarR/OhrR-family regulators as sensors of organic hydroperoxides

The MarR/OhrR-family regulators in *B. subtilis* and *Xanthomonas campestris*

MarR or Multiple antibiotics resistance-type regulators are characterized by winged helix-turn-helix (HTH) DNA-binding motifs and control genes that confer resistance

to antibiotics, organic solvents, detergents, ROS and RES. Several MarR-family members are important for the regulation of virulence (Ellison and Miller, 2006). Among the MarR-family regulators, the MarR/OhrR subfamily responds to organic hydroperoxides (OHP). OHP can be derived from peroxidation of unsaturated fatty acids of eukaryotic membrane lipids. Ohr-like peroxiredoxins catalyze the reduction of OHPs to their corresponding alcohols (Atichartpongkul et al., 2001; Fuangthong et al., 2001). *B. subtilis* has two *ohr* paralogs: *ohrA* and *ohrB* (Fuangthong et al., 2001). The *ohrA* gene is regulated by the redox-sensing OhrR repressor and *ohrB* is controlled by the σ^B alternative sigma factor in *B. subtilis* (Völker et al., 1998).

The OhrR repressor acts as a dimeric repressor that binds to inverted repeat sequences in the *ohrA* promoter, thereby inhibiting transcription (Fuangthong et al., 2001). OhrR harbors a conserved redox-sensing Cys residue in its N-terminal region that senses OHPs via different redox-switch mechanisms. Thiol-oxidation of OhrR results in dissociation of the protein from the operator and derepression of *ohrA* transcription. Based on the number of Cys residues, the OhrR-family can be divided into two subfamilies: the one-Cys-type with the prototype of *B. subtilis* OhrR$_{Bs}$ (Lee et al., 2007) and the two-Cys-type with the example of *X. campestris* OhrR$_{Xc}$ (Figure 5, Table 3) (Panmanee et al., 2006; Antelmann and Helmann, 2011). In the two-Cys type OhrR$_{Xc}$, Cys22 is oxidized by OHPs to a sulfenic acid intermediate, which reacts further with Cys127 in the opposing subunit forming an intersubunit disulfide (Panmanee et al., 2006). Oxidation inactivates OhrR$_{Xc}$ and releases the protein from the promoter DNA. X-ray crystallography reveals that disulfide formation causes a large rotation of the DNA-binding domain that is not compatible with DNA-binding (Newberry et al., 2007; Antelmann and Helmann, 2011). In contrast, the one-Cys type OhrR$_{Bs}$ of *B. subtilis* is oxidized at Cys15 to the sulfenic acid that reacts further to a mixed disulfide with BSH (*S*-bacillithiolated OhrR) in response to OHPs (Lee et al., 2007) (Figure 5). *B. subtilis* OhrR$_{Bs}$ can be also converted from a one-Cys-type to a two-Cys regulator by introduction of a C-terminal Cys at a position equivalent to Cys127 of OhrR$_{Xc}$ (Soonsanga et al., 2008).

We could show that OhrR responds also to NaOCl stress and thus, OhrR is a redox sensor for OHPs and NaOCl (Chi et al., 2011). Transcriptome analysis revealed that the *ohrA* gene was the most strongly up-regulated gene (220-fold) under NaOCl stress in *B. subtilis*. Mass spectrometry identified the *S*-bacillithiolation of OhrR as thiol-redox switch mechanism for OhrR inactivation. Phenotype analyses showed that the OhrA peroxiredoxin and BSH protect cells against hypochlorite stress as the growth of *ohrA*
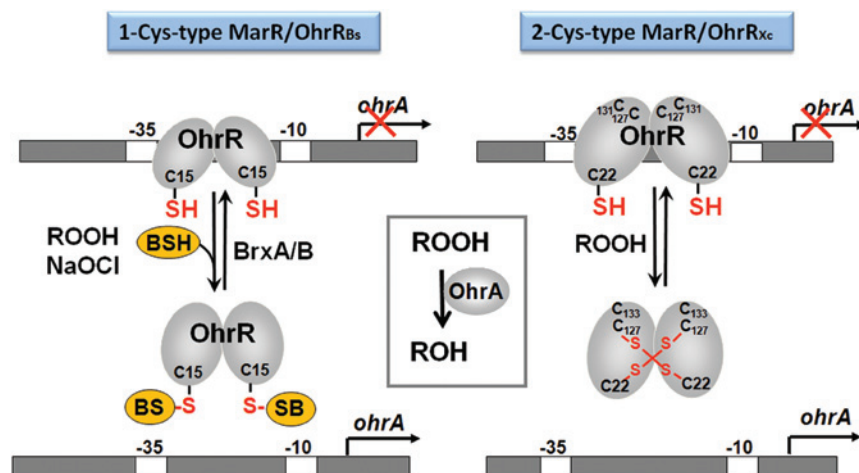
Figure 5: Thiol-based redox-sensing of organic hydroperoxides by 1-Cys and 2-Cys-type MarR/OhrR regulators.
OhrR controls the OhrA thiol-dependent peroxiredoxin and contains one conserved Cys15 residue in *B. subtilis* and three Cys residues (C22, C127 and C131) in *X. campestris*. The 1-Cys OhrR protein of *B. subtilis* is initially oxidized by CHP and NaOCl to the Cys sulfenic acid, which reacts further with bacillithiol (BSH) to form *S*-bacillithiolated OhrR. The 2-Cys OhrR protein of *X. campestris* is regulated by intersubunit disulfide formation between the redox-sensing C22 and C127 of opposing subunits. These different thiol-disulfide switches inactivate OhrR proteins, leading to derepression of the peroxidase OhrA that functions in detoxification of organic hydroperoxides. Regeneration of *S*-bacillithiolated OhrR involves the bacilliredoxins BrxA and BrxB in *B. subtilis* (Gaballa et al., 2014).

and *bshA* mutants was strongly impaired by NaOCl stress (Chi et al., 2011). Thus, we hypothesize that OhrA could be involved in NaOCl detoxification. Because hypochlorite is produced by activated neutrophils, it could be also a more physiologically oxidant for the OhrR-homologs SarZ and MgrA in the human pathogen *S. aureus*.

The *B. subtilis* OhrR repressor is redox-controlled by *S*-bacillithiolation which was recently shown to be reversed by bacilliredoxins (BrxA/B), which function as glutaredoxin-like enzymes in the reduction of BSH mixed protein disulfides to regenerate and re-activate the OhrR repressor in *B. subtilis in vitro* (Gaballa et al., 2014).

The MgrA/SarZ/SarA family of virulence and antibiotic regulators

In *S. aureus*, two homologs of the MarR/OhrR 1-Cys-type repressor are present, including the MgrA and SarZ global regulators for antibiotic resistance and virulence (Figure 6, Table 3) (Truong-Bolduc et al., 2005, 2008; Kaito et al., 2006; Ballal et al., 2009; Chen et al., 2009; Poor et al., 2009). The Multiple gene regulator MgrA of *S. aureus* regulates more than 300 genes that are involved in virulence, autolysis, antibiotic resistance, biofilm formation and cell wall biosynthesis (Luong et al., 2006). MgrA controls genes for virulence factors that include enzymes for capsule polysaccharide biosynthesis (*cap5(8)*-locus), α-toxin (*hla*), coagulase (*coa*), protein A (*spa*), extracellular serine
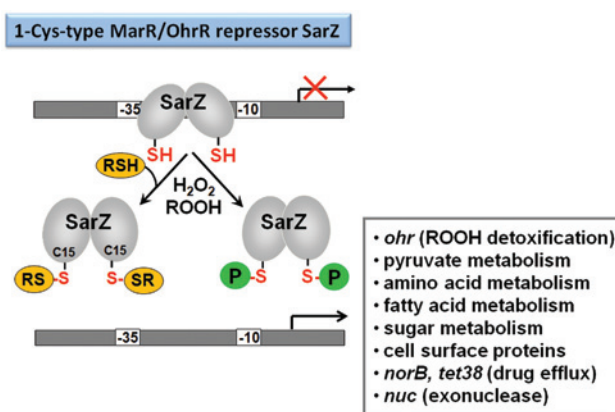

Figure 6: Redox-sensing by the MarR/OhrR-family regulator SarZ of *S. aureus*.
In *S. aureus*, the MarR/OhrR-family regulator SarZ functions as global regulator for ROS detoxification, antibiotic resistance and virulence functions and contains a single Cys13 required for redox-sensing. The DNA-binding activity of SarZ was shown to be reversibly redox-regulated by *S*-thiolation with a synthetic benzene thiol (Poor et al., 2009) and by cysteine phosphorylation *via* the eukaryotic-like serine/threonine kinase (Stk1) and phosphatase (Stp1) (Sun et al., 2012). SarZ controls genes for the *ohr* peroxiredoxin, cell surface proteins, antibiotic resistance efflux pumps (*norB, tet38*), amino acid, sugar, fatty acid and anaerobic metabolism (*pflAB*). Examples for SarZ regulon genes are listed in Table 3.

proteases (*splABCDEF*) and nuclease (*nuc*). In addition, genes encoding autolysis factors (*lytM* and *lytN*), multidrug efflux pumps (*norA, norB* and *tetAB)* and regulatory genes (*agr, lytRS, arlRS, sarS* and *sarV*) are members of the MgrA

Table 3: The MarR-type redox regulators of the OhrR/SarA/DUF24-subfamilies.

Redox sensor	Organism	Signal	Redox-sensing mechanism	Regulon genes	Regulon functions	References
OhrR	*Bacillus subtilis*	ROOH NaOCl	C15*-SSB	*ohrA*	2-Cys-peroxiredoxin	Fuangthong et al. (2001) Lee et al. (2007) Chi et al. (2011)
OhrR	*Xanthomonas campestris*	ROOH	C22*-C127 Intermolecular disulfide	*ohrA*	2-Cys-peroxiredoxin	Panmanee et al. (2006) Newberry et al. (2007)
MgrA (OhrR)	*Staphylococcus aureus*	H_2O_2 ROOH	C12*-SOH C12*- Phosphate	*cap5(8)* *hlgABC, pvl, lukED, lukMF-PV* *hla* *coa* *spa* *splABCDEF* *nuc* *lytM, lytN* *norA, norB, tetAB* *agr, lytRS, arlRS, sarS and sarV*	Capsule polysaccharides Leukotoxins α-hemolysin Coagulase, Protein A Serine proteases Nuclease Autolysis factors Multidrug efflux pumps Virulence regulators	Luong et al. (2006) Chen et al. (2006) Chen et al. (2009) Sun et al. (2012)
SarZ (OhrR)	*Staphylococcus aureus*	H_2O_2 ROOH	C13*-SOH C13*-SSR C13*- Phosphate	*ohr* *acs*, *pflAB pckA* *argGH, ilvD, lysC, hisC* *fabG* *gntRK, lacD, malA, treC, isdC, epiEF, lrgB, efb, fib, tcaA* *norB, tet38* *nuc*	2-Cys peroxiredoxin pyruvate metabolism Amino acid metabolism Fatty acid synthesis Sugar metabolism Cell surface proteins Drug efflux pumps Exonuclease	Ballal et al. (2009) Chen et al. (2009) Poor et al. (2009) Sun et al. (2012)
SarA (MarR)	*Staphylococcus aureus*	H_2O_2 Diamide	C9* redox-sensitive C9*- Phosphate	*sodA* *trxB* *hla* *spa* *fnb* *cna* *sec* *icaRA, bap* *ssp*, *aur* *rot, agr, sarS, sarV, sarT*	Superoxide dismutase Thioredoxin reductase α-hemolysin Protein A Fibronectin-binding Collagen-binding Enterotoxin C Biofilm formation Proteases Virulence regulators	Ballal and Manna (2009) Ballal and Manna (2010) Sun et al. (2012)
MosR (OhrR)	*Mycobacterium tuberculosis*	H_2O_2 ROOH	C10-C12 Intramolecular disulfide	*rv1050* *mosR*	Exported oxidoreductase MarR/OhrR-like repressor	Brugarolas et al. (2012)
RosR (MarR)	*Corynebacterium glutamicum*	H_2O_2	C92* redox-sensitive	*rosR* *narKGHJI* *cg2329* *cg3085* *cg1848* *cg3084* *cg1150* *cg1850* *cg1426* *cg1322*	MarR-type repressor Nitrate/nitrite transporter Luciferase-like monooxygenases Flavin-containing monooxygenases FMN reductases Glutathione-*S*-transferases Polyisoprenoid-binding protein	Bussmann et al. (2010)

(Table 3: Continued)

Redox sensor	Organism	Signal	Redox-sensing mechanism	Regulon genes	Regulon functions	References
HypR (MarR/ DUF24)	*Bacillus subtilis*	Quinone Diamide NaOCl	C14*-C49 Intermolecular disulfide	*hypR* *hypO*	MarR/DUF24-activator FMN-nitroreductase	Palm et al. (2012)
YodB (MarR/ DUF24)	*Bacillus subtilis*	Quinone Diamide NaOCl	C6*-C101 Intermolecular disulfide	*yodB* *yodC* *azoR1* *catDE*	MarR/DUF24-repressor Nitroreductase Azoreductase Dioxygenase	Leelakriangsak et al. (2008) Chi et al. (2010a) Chi et al. (2010b)
CatR (MarR/ DUF24)	*Bacillus subtilis*	Quinone Diamide NaOCl	C7*redox-sensitive	*catDE*	Dioxygenase	Chi et al. (2010b)
QsrR (MarR/ DUF24)	*Staphylococcus aureus*	Quinone	C5*-S-alkylation	*SAV0340 (azoR1)* *SAV2033 (yodC)* *SAV0338* *SAV2522 (catE)*	FMN-dependent quinone reductase Nitroreductase Glyoxalase Dioxygenase	Ji et al. (2013)
QorR (MarR/ DUF24)	*Corynebacterium glutamicum*	Quinone Diamide	C17*-C17 Intermolecular disulfide	*qorA*	Quinone reductase	Ehira et al. (2009a)

regulon (Ingavale et al., 2005; Kaatz et al., 2005; Truong-Bolduc et al., 2005, 2008; Luong et al., 2006) (Table 3). Hence, MgrA confers resistance to the antibiotics fluoroquinone, tetracycline, vancomycin and penicillin. MgrA is also required for virulence in murine abscess, septic arthritis and sepsis models. MgrA shares with OhrR$_{Bs}$ the single conserved Cys12 and uses a thiol-based oxidation sensing mechanism to control virulence and antibiotic resistance (Chen et al., 2006). Cys12 can be oxidized by CHP, H$_2$O$_2$ and superoxide anion to Cys-SOH that leads to dissociation of MgrA from the operator DNA *in vitro* and induction of antibiotic resistance in *S. aureus in vivo* (Chen et al., 2006, 2009). However, it was shown recently that the DNA-binding activity of MgrA can be also reversibly regulated by cysteine phosphorylation *via* the eukaryotic-like serine/threonine kinase (Stk1) and phosphatase (Stp1)(Sun et al., 2012). Cys phosphorylation was detected as posttranslational modification also in other regulatory proteins, including the MarR-family proteins SarZ and SarA and the cysteine biosynthesis regulator CymR. Moreover, Stk1 was required for full virulence and resistance to the antibiotic vancomycin by controlling Cys-phosporylation of MgrA, SarZ and SarA. Increased Cys phosphorylation of the virulence regulators in a *stp1* mutant led to decreased virulence in a mouse abscess model. Interestingly, like the

previously shown thiol-oxidation mechanism (Chen et al., 2006, 2009) Cys phosphorylation was also DTT-reversible, but the mechanism is still unknown (Sun et al., 2012).

The second MarR/OhrR-type regulator of *S. aureus* is SarZ, which is also a pleiotropic virulence regulator (Kaito et al., 2006). SarZ controls the *ohr* peroxiredoxin, genes involved in virulence, autolysis, cell wall metabolism, antibiotic resistance, intermediary, amino acid, sugar, fatty acid and anaerobic metabolism, such as the pyruvate-formate lyase genes *pflA* and *pflB* (Figure 6, Table 3). SarZ is also transcriptionally activated by MgrA (Ballal et al., 2009; Chen et al., 2009). SarZ was shown to use a thiol-based oxidation sensing mechanism via the conserved lone Cys13 residue (Chen et al., 2009). The crystal structure of SarZ was resolved in the reduced, sulfenic acid and mixed disulfide form (Poor et al., 2009). SarZ is oxidized at Cys13 to sulfenic acid that still retains DNA-binding activity. Further oxidation of SarZ with an external synthetic thiol (benzene thiol) leads to *S*-thiolated SarZ. These mixed SarZ disulfides cause steric clashes that contribute to an allosteric conformational change of the DNA-binding domains and release of SarZ from the operator DNA (Poor et al., 2009).

SarZ is also controlled by Cys phosphorylation via the Stk1/Stp1 kinase/phosphatase pair (Sun et al., 2012). However, it remains yet to be shown if *S*-bacillithiolation

can control DNA-binding activity of SarZ or MgrA *in vivo* also in *S. aureus*. Future studies should elucidate if there is a possible cross-talk between Cys phosphorylation and thiol-oxidation in these MarR-family virulence regulators of *S. aureus*.

The Staphylococcal accessory regulator (SarA) is another global redox-sensing regulator of the MarR-family that contains a single Cys9 residue in the dimer interface. SarA positively regulates many virulence factors including fibronectin and fibrinogen binding proteins (*fnb*), hemolysins (*hla*), enterotoxins (*sec*), toxic shock syndrome toxin 1, and genes involved in biofilm formation (*icaRA*, *bap*) (Cheung et al., 2008; Tamber and Cheung, 2009). SarA negatively regulates the transcription of proteases (*ssp*, *aur*), protein A (*spa*), and collagen-binding proteins (*cna*). Many virulence regulators are members of the SarA-regulon of *S. aureus*, such as *rot*, *agr*, *sarS*, *sarV*, *sarT* resulting in pleiotropic phenotypes of the *sarA* mutant. Furthermore, SarA was shown to control oxidative stress-related genes, such as superoxide dismutase (*sodA*) and thioredoxin reductase (*trxB*) (Ballal and Manna, 2009; Ballal and Manna, 2010) (Table 3). The redox-sensitivity of Cys9 in SarA has been analyzed in the wild type and a SarAC9G mutant *in vivo* and *in vitro*, which revealed that oxidation of Cys9 by H_2O_2 and diamide reduced the DNA-binding activity of SarA to the *trxB* promoter (Ballal and Manna, 2010). SarA has been also shown to sense oxidative stress by Cys phosphorylation *in vitro* (Sun et al., 2012). However, the detailed thiol-switch mechanism for SarA redox regulation *in vivo* has yet to be elucidated.

The MarR/OhrR-family regulators MosR and RosR in Actinomycetes

The MarR/OhrR-family of redox regulators is also conserved among Actinomycetes. In *M. tuberculosis* the OhrR-family regulator MosR has been characterized as transcriptional repressor and sensor for peroxides that shares 28% sequence identity with *B. subtilis* OhrR and *S. aureus* MgrA (Brugarolas et al., 2012). Reduced MosR binds to a specific operator sequence (GTGTAnnTACAC) in its target promoters and represses its own transcription and that of the adjacent *rv1050* gene, encoding an exported oxidoreductase of unknown function (Brugarolas et al., 2012). The *rv1050* gene was most strongly induced by H_2O_2 and 352-fold derepressed in the *mosR* mutant. Rv1050 was also up-regulated during infection in INF-γ-activated macrophages, suggesting a role in the host immune defense (Schnappinger et al., 2003). In addition, arachidonic acid and linoleic acid were found to induce *rv1050*, indicating that Rv1050 could also function in fatty acid metabolism in macrophages.

MosR contains four Cys residues (Cys10, 12, 96, 147), but only Cys12 is conserved. The Cys10 and Cys12 residues are oxidized to intramolecular disulfides by peroxides and both Cys residues are essential for redox-sensing as C10S and C12S mutant proteins were non-responsive to H_2O_2 in gel-shift assays (Brugarolas et al., 2012). The structures of reduced and oxidized MosR proteins were resolved to reveal the structural mechanism for the inactivation of MosR's repressor function upon oxidation. Disulfide formation between Cys10 and Cys12 breaks the hydrogen bond of Cys12 to Asn37′ and causes new hydrogen bonds of Arg16 and Ser41′. This rearrangement of hydrogen bonds results in a movement of α2 which pushes α3 ~ 4.5 Å towards α4. This causes rotation of α4 and α4′ that prevent them to fit into consecutive major grooves resulting in the release from the operator DNA. Consequently, MosR oxidation leads to rearrangements of hydrogen bonds, resulting in large conformational changes and MosR dissociation from the DNA (Brugarolas et al., 2012).

Corynebacterium glutamicum encodes the redox-sensitive MarR-type repressor RosR that responds to peroxide stress (Bussmann et al., 2010). RosR controls positively expression of the *narKGHJI* operon encoding a nitrate/nitrite transporter and the dissimilatory nitrate reductase complex. RosR acts as repressor of its own transcription and represses several genes that encode luciferase-like monooxygenases (*cg1848*, *cg2329*, *cg3085*), flavin-containing monooxygenases (*cg3084*), FMN reductases (*cg1150*, *cg1850*), glutathione-*S*-transferases (*cg1426*) and a polyisoprenoid-binding protein (*cg1322*). The polyisoprenoid-binding protein was important under peroxide stress as the mutant showed increased H_2O_2 sensitivity (Bussmann et al., 2010). Reduced RosR binds to an 18-bp inverted repeat with the consensus sequence TTGTTGAYRYRT-CAACWA in its target promoters. The DNA-binding activity of RosR was inhibited by H_2O_2 and restored by DTT in gel-shift assays *in vitro*. RosR contains three Cys residues (Cys64, 92, 151), but only Cys92 is conserved among RosR homologs of other *Corynebacteria*. Cys92 was most important for redox-sensing because the DNA-binding activity of the C92S mutant was not inhibited by H_2O_2 *in vitro*. In contrast, other single Cys mutants behaved like the wild type RosR, although in double and triple Cys mutants the DNA-binding activity was also affected in response to H_2O_2 *in vitro* (Bussmann et al., 2010). This suggests that RosR might be inactivated by formation of inter- or intramolecular disulfides by peroxide stress which remains to be demonstrated.

The MarR/DUF24-family regulators as sensors for RES (quinone, diamide)

The MarR/DUF24-family regulators YodB, CatR, HypR and HxlR of *B.subtilis*

The MarR/DUF24 family of transcription factors is conserved among Gram-positive bacteria (Antelmann and Helmann, 2011). In *C. glutamicum*, the MarR/DUF24-type regulator QorR was first characterized as a transcriptional repressor that senses diamide and H_2O_2 and controls the quinone oxidoreductase QorA (Ehira et al., 2009a). Inactivation of QorR involves intersubunit disulfide formation between the conserved single Cys17 residues of both subunits (Ehira et al., 2009a). *B. subtilis* encodes eight MarR/DUF24-family proteins: HxlR, HypR, YodB, CatR, YdeP, YdzF, YkvN, and YtcD. HxlR was identified as activator of the formaldehyde-inducible *hxlAB* operon that encodes enzymes of the ribulose monophosphate pathway (Yurimoto et al., 2005). HypR was characterized as positive regulator of the nitroreductase HypO that is induced by NaOCl, diamide and quinones and confers NaOCl-resistance (Palm et al., 2012) (Table 3). HypR is a two-Cys MarR/DUF24-type regulator with a redox-sensing Cys14

and a second Cys49 that are 8 Å apart in the reduced HypR structure. Both Cys14 and Cys49 are essential for activation of *hypO* transcription by disulfide stress. HypR is activated by Cys14-Cys49′ intersubunit disulfide disulfide formation under diamide and NaOCl stress. Disulfide bond formation breaks the H-bonds of Cys14 and moves the α4 and α4′ helices of HypR ~4 Å towards each other (Palm et al., 2012).

The MarR-type regulators YodB, CatR and MhqR control specific detoxification pathways that confer resistance to quinones and diamide, such as azoreductases (AzoR1 and AzoR2), nitroreductases (YodC and MhqN), and thiol-dependent dioxygenases (CatE, MhqA, MhqE, MhqO) (Töwe et al., 2007; Antelmann et al., 2008; Leelakriangsak et al., 2008; Chi et al., 2010b). Azoreductases and nitroreductases reduce quinones and diamide to hydroquinones and dimethylurea, respectively (Figure 7, Table 3). Dioxygenases catalyze the ring-cleavage reaction of quinone-*S*-adducts. The azoreductase AzoR1 is controlled by YodB and expression of the catechol-2,3-dioxygenase CatE and oxidoreductase CatD are regulated by both YodB and CatR (Leelakriangsak et al., 2008; Chi et al., 2010b). The promoter region of the *catDE* operon contains two inverted repeat sequences overlapping the
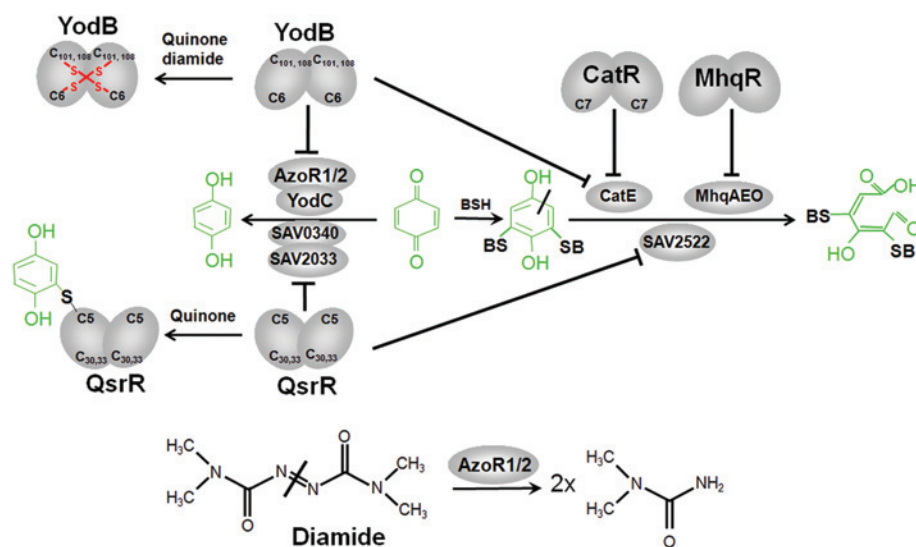

Figure 7: Redox-sensing of RES by the MarR/DUF24-regulators YodB in *B. subtilis* and QsrR in *S. aureus*.
Exposure of *B. subtilis* to quinones induces quinone detoxification regulons controlled by the MarR-type repressors MhqR, YodB and CatR. In *S. aureus*, the homologous quinone-sensing YodB (QsrR) repressor responds to quinones and controls paralogous quinone reductases, dioxygenases and nitroreductases. The redox-sensing YodB and CatR repressors are inactivated by the oxidative mode of quinone leading to disulfide formation that involves the conserved Cys6 or Cys7 residues (Antelmann et al., 2008; Antelmann and Helmann, 2011; Chi et al., 2010a,b; Towe et al., 2007). In *S. aureus*, YodB (QsrR) with mutated C-terminal Cys residues senses quinones by thiol-*S*-alkylation of Cys5, leading to up-regulation of the dioxygenase SAV2522, the quinone reductase SAV0340 and the nitroreductase SAV2033 (Ji et al., 2013). The thiol-dependent dioxygenases MhqA, MhqE, MhqO, CatE of *B. subtilis* and SAV2522 of *S. aureus* are involved in specific ring-cleavage of quinones-*S*-adducts. The quinone reductases AzoR1, AzoR2 of *B. subtilis* and SAV0340 of *S. aureus* and the nitroreductases YodC, MhqN of *B. subtilis* and SAV2033 of *S. aureus* catalyze the reduction of quinones to redox stable hydroquinones.

-35 promoter region (BS1) and the transcription start point (BS2) that are the operator sites for CatR and YodB. Both YodB and CatR are inactivated in response to quinone and diamide. YodB is inactivated by a two-Cys-type redox-switch mechanism and oxidized to intersubunit disulfides between Cys6 of one subunit and Cys101 or Cys108 of the other subunit by diamide and quinones *in vivo* and *in vitro* (Chi et al., 2010a). The conserved Cys7 is essential for redox-sensing of quinones and diamide in CatR, but its redox-sensing mechanism has yet to be explored.

The MarR/DUF24-family regulator QsrR of *S. aureus*

The redox-sensing mechanism of the quinone-sensing MarR/DUF24-family regulator YodB (QsrR) has also been characterized in *S. aureus*, which shares 38% sequence identity with the YodB repressor of *B. subtilis* (Ji et al., 2013). QsrR contains the conserved N-terminal Cys5 and two further Cys30 and Cys33 residues. QsrR and YodB control both homologous genes involved in quinone reduction and ring-cleavage that confer resistance to benzoquinone. The QsrR regulon includes genes for the FMN-dependent quinone reductase (*SAV0340* or *azoR1*), the nitroreductase (*SAV2033* or *yodC*), the glyoxalase (*SAV0338*) and the thiol-dependent dioxygenase (*SAV2522* or *catE*). Hence, QsrR controls homologous quinone reductases and dioxygenases in *S. aureus* that are controlled by YodB and CatR in *B. subtilis* (Figure 7, Table 3). The quinone resistance QsrR regulon conferred resistance to killing by macrophages in a phagocytosis assay indicating its crucial role for virulence regulation in *S. aureus*. The conserved Cys5 of QsrR was shown to sense quinones by a thiol-*S*-alkylation mechanism. The QsrR structure shares strong similarities with the HypR structure of *B. subtilis* (Palm et al., 2012; Ji et al., 2013). HypR and QsrR are both dimers that have in common the wHTH motif composed of α3, α4, β1 and β2. The wHTH motifs bind to the major and minor grooves of the DNA double helix and the wing is much larger compared to OhrR-like regulators. To elucidate the structural changes upon quinone-binding at Cys5, the menadione-bound QsrR structure was resolved for the QsrRC30,33S mutant (Ji et al., 2013). Menadione-binding at Cys5 causes a shift in the distance and rotation between the α4 and α4′ helices from 29.9 Å distance with 106° rotation in reduced QsrR to 39.1 Å distance and 117° rotation in the menadione-bound form. These structural changes lead to dissociation of QsrR from the operator DNA. In contrast to QsrR, YodB and HypR sense diamide and quinones by intersubunit disulfide

bond formation (Palm et al., 2012). However, the *in vivo* mechanism of quinone-sensing by wild type QsrR has yet to be explored.

Emerging thiol-based redox sensors for RES (quinones, aldehydes) and HOCl

The TetR-family regulator NemR as redox sensor for RES (N-ethylmaleimide, quinones, aldehydes) and HOCl

The thiol-based TetR-family NemR regulator is conserved across Gram-negative and Gram-positive bacteria and has been characterized in *E. coli* as redox sensor for RES and HOCl (Gray et al., 2013b; Lee et al., 2013; Ozyamak et al., 2013) (Table 4). NemR negatively controls transcription of the *nemRA* operon and the *gloA* gene, which function in detoxification of electrophiles. The *nemA* gene was previously shown to be strongly induced by thiol-alkylating compounds, such as N-ethylmaleimide (NEM) and iodoacetamide and shown to function as NEM reductase (Umezawa et al., 2008). The FMN-dependent reductase NemA belongs to the old-yellow-enzyme family and has a broad substrate spectrum to reduce several quinones (ubiquinone, menaquinone) and aldehydes, (glyoxal, methylglyoxal and glycolaldehyde) *in vitro* (Lee et al., 2013). GloA is the glyoxalase-I enzyme involved in methylglyoxal detoxification and was revealed as main methylglyoxal protection mechanism (MacLean et al., 1998). Hence, the NemR repressor responds to quinones and aldehydes like methylglyoxal and is inactivated via thiol-based redox switches, which lead to up-regulation of the *nemRA* operon and *gloA* that both confer resistance to methylglyoxal and quinones in *E. coli* (Lee et al., 2013; Ozyamak et al., 2013). Moreover, the *nemRA* operon and *gloA* were most strongly induced by methylglyoxal in a transcriptome analyses supporting the major role of GloA and NemA as protection mechanism (Ozyamak et al., 2013). However, NemR was also shown to sense reactive chlorine species, such as HOCl and N-chlorotaurine, which leads to derepression of *nemRA* and *gloA* that both confer HOCl resistance as the *nemA* and *gloA* mutants displayed HOCl sensitive phenotypes (Gray et al., 2013b). Exposure of cells to HOCl stress caused increased methylglyoxal production suggesting that detoxification of methylglyoxal is an important bacterial HOCl defense mechanism. It remains to be shown if NemA could also confer HOCl resistance by reduction of reactive chlorines as direct substrates.

NemR was shown to sense reactive electrophiles and HOCl via thiol-based oxidation mechanisms that involve

Table 4: The NemR, RclR and HypT redox sensors for RES and HOCl in *E. coli*.

Redox sensor	Organism	Signal	Redox-sensing mechanism	Regulon genes	Regulon functions	References
NemR (TetR-type)	*Escherichia coli*	Quinones Glyoxal Methylglyoxal N-ethylmaleimide Iodoacetamide HOCl	C106* conserved C21-C116 intersubunit disulfide	*nemR* *nemA* *gloA*	TetR-type repressor FMN-dep. reductase for aldehydes, quinones and NEM glyoxalase-I	Umezawa et al. (2008) Gray et al. (2013) Lee et al. (2013) Ozyamak et al. (2013)
RclR (AraC-type)	*Escherichia coli*	HOCl N-chlorotaurine	C21-C89 intramolecular disulfide	*rclA* *rclB* *rclC*	Flavoprotein disulfide reductase Periplasmic protein quinone-binding membrane protein	Parker et al. (2013)
HypT (LysR-type)	*Escherichia coli*	HOCl	Met123-SO Met206-SO Met230-SO C4-C4 intersubunit disulfide (*in vitro*) C4: HypT dodecamer formation C150: HypT stability	*metB/K*/N *cysH/K/N*, *cysPUW*, *sbp*, *sufA* *entC*, *entH*, *fecABCDE*, *fecR*, *fepCD*, *ryhB*, *tonB*, *yncE*	Sulfur, Cys and Met biosynthesis and metabolism Fur-regulon genes involved in iron homeostasis	Gebendorfer et al. (2012) Drazic et al. (2013a) Drazic et al. (2013b)

intersubunit disulfides which lead to inactivation of its repressor function. NemR possesses 6 Cys residues, but only Cys106 is conserved among bacteria. However, the Cys106S mutant still responds to HOCl and forms intersubunit disulfides both *in vitro* and *in vivo*, suggesting that other Cys residues are involved in redox regulation of NemR which can substitute for the absence of the conserved Cys106 (Gray et al., 2013b). In another study, the glyoxal sensitivity and effect on *nemRA* expression was analyzed for various NemR Cys double mutants, revealing significant lower responsiveness to glyoxal in the Cys21,116S double mutant both *in vivo* and *in vitro,* but no difference to the wild type in any other Cys single and double mutant (Ozyamak et al., 2013). Cys21 is located in the DNA-binding domain and Cys116 is in the dimer interface. Both Cys21 and Cys116 were involved in intersubunit disulfide formation and oligomerization of NemR. This indicates that *E. coli* NemR is inactivated by intersubunit disulfide formation in response to RES and HOCl to up-regulate quinone and glyoxal detoxification enzymes. This thiol-disulfide-switch model of NemR redox regulation resembles that of other quinone-sensing redox regulators, such as QorR, YodB and HypR suggesting that the alternative thiol-*S*-alkylation as shown for QsrR *in vitro* might be rather the exception for thiol-based quinone-sensing regulators.

RclR as AraC-family HOCl-specific thiol-based redox sensor in *E. coli*

Recently, novel HOCl-specific redox regulators have been discovered in *E. coli* that are specific for chlorine species, such as HOCl, but do not respond to ROS, electrophiles or other thiol-reactive compounds. RclR (formerly YkgD) is widely conserved among Gram-negative bacteria and Actinobacteria and was characterized as redox-sensing transcriptional activator of the AraC family, which uses a thiol-based oxidation mechanism for redox-sensing of HOCl (Parker et al., 2013). The redox-sensing mechanism of RclR involves both conserved Cys residues, Cys21 and Cys89 which likely form an intramolecular disulfide that stabilizes the active RclR protein *in vivo*. Both Cys21 and Cys89 residues are required for redox-sensing of the HOCl-response *in vivo,* while only Cys21 is essential for redox-sensing *in vitro*. Oxidation of RclR by HOCl leads to specific activation of transcription of the *rclABC* operon, which is important for survival of HOCl and N-chlorotaurine. Mutants in each single gene of the *rclABC* operon are sensitive to HOCl suggesting that this operon is an important HOCl-protection determinant (Parker et al., 2013). However, the functions of the RclABC proteins for HOCl-protection are still unknown, which resemble a flavoprotein

disulfide reductase, periplasmic protein and possible quinone-binding membrane protein (Table 4).

HypT as LysR-family HOCl-specific Met-oxidation switch in *E. coli*

The LysR-type regulator HypT has been discovered as another HOCl-specific redox sensor and transcriptional activator of *E. coli* (Gebendorfer et al., 2012). HypT belongs like OxyR to the LysR-family of transcriptional regulators, which often form dimers or tetramers (OxyR) (Maddocks and Oyston, 2008), but HypT was shown to form unusual large dodecameric ring-like structures *in vitro* that serve as a storage form of HypT (Drazic et al., 2014). These HypT dodecamers dissociate into smaller oligomers (dimers, tetramers) in the presence of DNA *in vitro* and tetramers are also found *in vivo* in HOCl-exposed *E. coli* cells. Specifically, the HypT tetramers were revealed as activation-competent DNA-binding species of HypT (Gebendorfer et al., 2012; Drazic et al., 2014).

The DNA-binding activity of HypT was activated specifically by HOCl and HypT was required for the survival under HOCl stress conditions *in vivo* (Gebendorfer et al., 2012). HypT was shown to control positively genes that function in sulfur, Cys and Met biosynthesis (*metB*, *metK*, *metN*, *cysH*, *cysK*, *cysN*, *cysPUW*, *sbp*, *sufA*) while genes of the Fur-regulon are negatively regulated by HypT that function in iron homeostasis (*entC*, *entH*, *fecABCDE*, *fecR*, *fepCD*, *ryhB*, *tonB*, *yncE*) (Table 4). As Met is rapidly oxidized by HOCl to methionine sulfoxide (Met-SO), it is suggested that HypT activates Met biosynthesis to restore the pool of reduced Met (Gebendorfer et al., 2012). Interestingly, HypT uses a reversible methionine oxidation switch model for transcriptional activation (Drazic et al., 2013a) while the Cys residues are important for stability and oligomerization of HypT (Drazic et al., 2013b). HypT activation involves oxidation of three Met residues (Met123, 206, 230) to their Met-SO forms which were identified in HOCl-activated HypT *in vitro*. Mutations of the three Met to glutamines mimics the oxidized Met-SO form of HypT and resulted in constitutively active HypT *in vivo*, while the Met-to-Ile mutation resulted in inactive HypT as revealed by the transcriptional studies of the target genes and HOCl survival assays (Drazic et al., 2013a). Furthermore, inactivation of oxidized HypT required the Met-SO reductases MsrA and MsrB both *in vivo* and *in vitro*, revealing the reversibility of this Met-oxidation switch model for HypT.

Surprisingly, HypT was only activated *in vivo* in *E. coli* cells by HOCl stress, but *in vitro* HypT rapidly lost its DNA-binding activity when treated with HOCl (Drazic et al., 2013b). HypT possesses five non-conserved Cys residues and all Cys residues are required for HypT activity *in vivo* and HypT stability *in vitro*. HypT oxidization by HOCl *in vitro* leads to intermolecular Cys4-Cys4 disulfides resulting in HypT inactivation. Furthermore, Cys150 was required for HypT stability and Cys4 involved in oligomerization of HypT to dodecamers (Drazic et al., 2013b). The thiol-switch model of HypT was suggested as check point in the activation of HypT preventing unwanted HypT interaction with its target promoters under oxidative stress conditions that are not sufficient to activate HypT. In conclusion, the HOCl-specific regulator HypT represents an important HOCl-protection mechanism and is activated by a reversible Met-oxidation switch to up-regulate Met biosynthesis in *E. coli*. It will be interesting to explore if other bacteria also use Met-oxidation switches as defense mechanisms against HOCl or ROS.

The Spx disulfide-stress redox sensors in Gram-positive Firmicutes bacteria

SpxA and MgsR as paralogous thiol-redox sensors for disulfide and general stress conditions in B. *subtilis*

The thiol-based redox sensor SpxA is an unusual transcription factor without typical DNA-binding domains that responds to different redox stress conditions in Gram-positive bacteria (Zuber, 2004, 2009; Nakano et al., 2005; Antelmann and Helmann, 2011). SpxA is an arsenate reductase (ArsC) family protein with a CXXC redox switch motif in its N-terminus that is essential for redox-sensing and transcriptional activation. ROS, RES and HOCl lead to oxidation of the CXXC motif to an intramolecular disulfide to activate SpxA (Figure 8). Oxidized SpxA interacts with the C-terminal domain (CTD) of the α subunit of the RNA polymerase (RNAP) to recognize promoter regions of the SpxA regulon genes and thereby activates transcription (Nakano et al., 2003, 2005; Zuber, 2004, 2009). SpxA positively regulates the expression of genes that maintain the thiol-redox balance in *B. subtilis*, including the genes for thioredoxin/thioredoxin reductases (*trxAB*), thiol peroxidase (*tpx*), FMN-dependent oxidoreductases (*nfrA, yugJ*), methionine sulfoxide reductase (*msrA*), cysteine biosynthesis and cystine transporters (*yrrT* operon, *cysK*, *tcyABC*) and bacillithiol biosynthesis (*bshA, bshB1, bshB2* and *bshC*) (Zuber, 2009; Antelmann and Helmann, 2011; Gaballa et al., 2013) (Table 5). Genome-wide chromatin immunoprecipitation (ChIP-chip) analysis of RNAP-SpxA complexes revealed 275 genes that are directly controlled
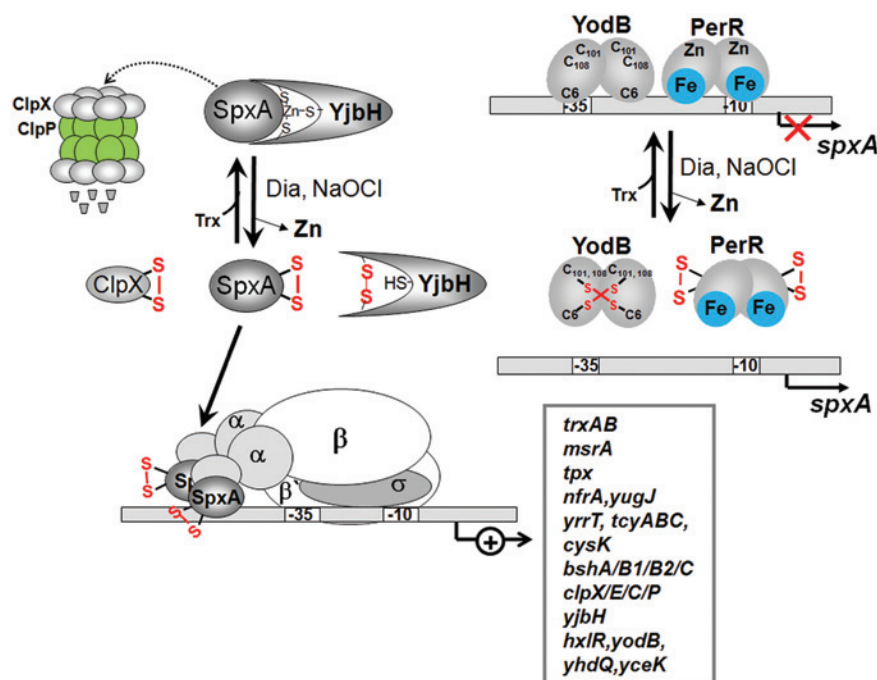
Figure 8: Post-translational and transcriptional control of SpxA by disulfide stress in *B. subtilis*.
Under non-stressed conditions, SpxA is unstable and targeted by the YjbH adaptor protein to the ClpXP machinery for proteolytic degradation. The stability of SpxA is increased by diamide because of oxidation of SpxA, YjbH and ClpX that prevents SpxA degradation. SpxA is oxidized in its redox-active CXXC motif and binds to the αCTD of RNAP, resulting in transcriptional activation of the SpxA disulfide-stress regulon. Transcription of *spxA* is regulated by the repressors YodB and PerR that are oxidized and inactivated under diamide stress, leading to increased *spxA* transcription. SpxA controls a large regulon of genes encoding thioredoxin/thioredoxin reductase (*trxAB*), thiol peroxidase (*tpx*), FMN-dependent oxidoreductases (*nfrA, yugJ*), methionine sulfoxide reductase (*msrA*), cysteine biosynthesis enzymes (*yrrT*-operon, *cysK, tcyABC*), bacillithiol biosynthesis enzymes (*bshA, bshB1, bshB2* and *bshC*), the protein quality control Clp machinery (*clpX, clpE, clpC, clpP, yjbH*) and several thiol-based redox regulators (*hxlR, yodB, yhdQ, yceK*). Examples for SpxA regulon genes and their functions are listed in Table 5.

by SpxA. An extended -35 box was identified within SpxA-controlled promoters in which the -43/-44 positions correlated with the activation by SpxA (Rochat et al., 2012). Additional targets for SpxA control were identified among the Clp machinery as ATPase subunits (*clpX*, *clpE* and *clpC*) and the proteolytic subunit (*clpP*) and the adpaptor for ClpXP proteolysis (*yjbH*). Furthermore, SpxA controls thiol-based redox regulators (*hxlR*, *yodB*, *yhdQ*, *yceK*) and many other transcription factors that respond to diamide and SpxA was required for the basal level of 32 genes under non-stress conditions (Rochat et al., 2012) (Table 5).

Expression of SpxA is controlled at transcriptional and post-translational levels. Transcription of *spxA* is initiated from at least four different promoters that are recognized by three forms of RNAP containing σ^A, σ^B and σ^M (Leelakriangsak and Zuber, 2007). SpxA is transcriptionally regulated in response to disulfide stress provoked by diamide and NaOCl by the PerR and YodB repressors. Both PerR and YodB repressors are oxidized under disulfide stress conditions, which inactivates their repressor function leading to derepression of *spxA* transcription (Zuber, 2009). The YodB repressor is oxidized to intermolecular disulfides between C6 and one of the C-terminal Cys residues leading the *spxA, azoR1* and *yodC* derepression. PerR is oxidized to form intramolecular disulfides in the Zn-binding structural site that possibly lead to PerR inactivation and *spxA* transcription by diamide and NaOCl. The second level is the post-translational control of SpxA protein stability by proteolysis via the ClpXP proteases. The *spxA* gene was discovered as <u>S</u>uppressor of *clp<u>P</u>* and *clp<u>X</u>* mutations and it was shown that the absence of ClpXP leads to stabilization of SpxA responsible for the pleiotropic phenotypes of *clpXP* mutants (Nakano et al., 2001). Under non-stress conditions, SpxA is targeted to the ClpXP system with the help of the adaptor YjbH for rapid degradation of SpxA (Nakano et al., 2002; Larsson et al., 2007). The YjbH adaptor protein contains a His-Cys-rich N-terminal region that is oxidized by diamide resulting in loss of YjbH adaptor activity and SpxA stabilization. However, mutational analyses showed that the Cys and

Table 5: The Spx disulfide-stress-sensing redox regulators of Firmicutes bacteria.

Redox sensor	Organism	Signal	Redox-sensing mechanism	Regulon genes	Regulon functions	References
SpxA	*Bacillus subtilis*	Quinone Diamide NaOCl H_2O_2	C10*-C13 Intramolecular disulfide	*trxAB* *tpx* *nfrA, yugJ* *msrA* *yrrT, cysK* *tcyABC* *bshA,bshB1,* *bshB2, bshC* *clpX, clp, clpC* *clpP* *yjbH* *hxlR, yodB,* *yhdQ, yceK*	Thioredoxin/thioredoxin reductase Thiol peroxidase FMN-dependent oxidoreductases methionine sulfoxide reductase Cysteine biosynthesis Cystine transporter Bacillithiol biosynthesis ATPase subunit of Clp Protease subunit of Clp Adaptor for ClpXP Thiol-redox regulators	Nakano et al. (2003) Nakano et al. (2005) Zuber (2004) Zuber (2009) Rochat et al. (2012)
MgsR	*Bacillus subtilis*	Heat NaCl ethanol	C10*-C13 Intramolecular disulfide	*ydbD* *ykuV* *ydaD, yhdF,* *yhxC, yhxD*	Mn-catalase Thiol-disulfide oxidoreductase Short-chain oxidoreductases	Reder et al. (2008) Reder et al. (2012b)
Spx	*Staphylococcus aureus*	Diamide H_2O_2	C10*-C13 conserved	*trxAB* *icaABCD* *trfA*	Thioredoxin/thioredoxin reductases Biofilm formation β-lactam resistance	Pamp et al. (2006) Wang et al. (2010) Jousselin et al. (2013)
SpxA1/ SpxA2	*Bacillus anthracis*	Diamide H_2O_2	C10*-C13 conserved	*trxAB, ytpP* *BA1263,* *BA0774* *bshA,bshB1,* *bshB2, bshC* *ypdA* *yphP, ytxJ* *uvrC, uvrD* *exoA* *BA1951* *BA0838,* *BA2647,* *BA3438* *yjbH*	Thioredoxin/thioredoxin reductase CoASH disulfide reductases (CoADR) Bacillithiol biosynthesis NADPH-disulfide oxidoreductase Bacilliredoxins DNA damage repair Spore outgrowth and germination Putative oxidoreductase Alcohol, aldehyde, quinone dehydrogenases Adaptor for ClpXP	Barendt et al. (2013)
SpxA/ SpxB SpxA1/ SpxA2	*Streptococcus mutants* *Streptococcus suis*	Diamide H_2O_2	C10*-C13 conserved	*trxB* *dpr* *sod* *ahpCF* *gor* *nox* *tpx*	Thioredoxin reductase Peroxide resistance Superoxide dismutase Peroxiredoxin Glutathione reductase NADH oxidase Thiol peroxidase	Kajfasz et al. (2010) Zheng et al. (2014)

His residues of YjbH are not required for SpxA stability *in vivo* (Chan et al., 2012). Studies with the YjbH$_{Gt}$ ortholog of *Geobacillus thermodenitrificans* without the Cys-His redox motif revealed that the C-terminus of YjbH$_{Gt}$ is required for stabilization of SpxA (Chan et al., 2012). The ATPase ClpX contains a Cys-rich Zn finger motif that also functions as redox switch and is oxidized under disulfide-stress

conditions (Zhang and Zuber, 2007). Therefore, SpxA of *B. subtilis* is controlled at multiple levels, at the transcriptional level by PerR and YodB and at post-translational levels via three redox switches in SpxA itself, YjbH and ClpX that together lead to up-regulation, stabilization and activation of SpxA (Zuber, 2009; Antelmann and Helmann, 2011) (Figure 8). The mechanism of how the SpxA-RNAP

complex activates transcription is unknown, but a cis-acting element in the -10 promoter sequences of *trxB* and *trxA* was identified required for activation of transcription (Reyes and Zuber, 2008; Nakano et al., 2010).

While SpxA interacts with RNAP containing σ^A, a SpxA paralog MgsR was identified as member of the σ^B general stress regulon (Reder et al., 2008). Thus, *B. subtilis* contains Spx paralogs that interact with RNAP holoenzymes containing different sigma factors. MgsR is a Modulator of the σ^B general stress response and controls a sub-regulon within the σ^B regulon that functions in protection against secondary oxidative stress caused by ethanol, heat, salt stress (Reder et al., 2008). MgsR controls positively about 50 genes including 18 σ^B-dependent genes that were up-regulated in the *mgsR* mutant. Antioxidant and protective functions can be attributed to the genes encoding the manganese-containing catalase (*ydbD*), the thiol-disulfide oxidoreductase (*ykuV*) and paralogous short-chain oxidoreductases (*ydaD*, *yhdF*, *yhxC* and *yhxD)* that are postulated to function in NADPH production as electron source for cellular Trx/TrxR reducing systems (Reder et al., 2008) (Table 5). The mechanism of MgsR control resembles in part that of SpxA and involves a positive autoregulatory loop to increase *mgsR* transcription, and post-translational control of MgsR stability via ClpXP and ClpCP proteolysis (Reder et al., 2012b). In addition, ethanol stress leads to a redox switch and causes intramolecular disulfides in the CXXC motif of MgsR as activation mechanism. However, the detailed mechanism of MgsR interaction with the RNAP containing σ^B and activation of transcription remains to be elucidated.

The σ^B general stress regulon is induced after exposure to heat, salt and ethanol stress which causes non-specific resistance to secondary oxidative stress in *B. subtilis* (Mols and Abee, 2011; Reder et al., 2012b). The involvement of σ^B in the protection against secondary oxidative stress has been demonstrated by the role of the σ^B-dependent miniferritin Dps in stationary-phase-induced peroxide resistance (Antelmann et al., 1997). Moreover, several genes of the primary PerR-, OhrR- and SpxA-controlled oxidative stress response (*katA*, *mrgA, ohrA, spxA*) have paralogs within the σ^B regulon (*katE, katX, ydbD, dps, ohrB, mgsR*) conferring non-specific secondary oxidative stress resistance (Zuber, 2009). Other redox-stress-related genes like *trxA* are controlled by both, SpxA and σ^B or by specific regulators, such as *clpC* that is controlled by σ^B and CtsR (Zuber, 2009). Moreover, phenotype screening of 94 mutants in σ^B-controlled genes identified 62 mutants with increased sensitivity towards paraquat or peroxides (Reder et al., 2012a). Thus, *B. subtilis* employs PerR, OhrR

and SpxA as specific antioxidant control mechanisms to cope with ROS and MgsR and σ^B for protection against primary and secondary generated oxidative stress (Mols and Abee, 2011; Reder et al., 2012b).

Spx as thiol-redox sensor for oxidative stress in the pathogens *Bacillus anthracis*, *S. aureus* and *Streptococcus*

Spx homologs are highly conserved in low GC Gram-positive bacteria, such as *Bacillus*, *Staphylococcus*, *Streptococcus*, *Lactobacillus* and *Listeria* species where they play important functions in the oxidative stress resistance and virulence (Zuber, 2004; Pamp et al., 2006; Kajfasz et al., 2010; Wang et al., 2010). In *S. aureus*, Spx functions as global regulator of genes that maintain the thiol-redox balance, such as the Trx/TrxR system. The *spx* mutant displays growth defects and is hypersensitive to peroxide and disulfide-stress, heat shock and osmotic stress (Pamp et al., 2006; Wang et al., 2010). Spx controls biofilm development in *S. aureus* and *S. epidermidis* through control of the *icaABCD* operon. The post-translational proteolytic control of Spx by the ClpXP system is similar like in *B. subtilis*, shown by the increased stability of Spx in *S. aureus clpP* and *clpX* mutants (Pamp et al., 2006). In *S. aureus*, YjbH functions as adaptor for ClpXP-mediated proteolysis of Spx although there is only 30% sequence identity between YjbH homologs of *S. aureus* and *B. subtilis* (Engman et al., 2012). The Cys-His rich N-terminal domain is not conserved in *S. aureus* YjbH and instead a CxC motif is present at another location. The *B. subtilis yjbH* mutant could be complemented with *S. aureus yjbH* to restore the Spx level and diamide susceptability to that of wild type cells (Gohring et al., 2011; Engman et al., 2012). Hence YjbH controls Spx proteolysis like in *B. subtilis*. The *yjbH* mutant further shows growth-impaired phenotypes and increased pigmentation (Engman et al., 2012), resistance to oxacillin and other β-lactam antibiotics, glycopeptides and sensitivity to desiccation stress (Charbonnier et al., 2005; Chaibenjawong and Foster, 2011; Gohring et al., 2011). This higher resistance to β-lactam antibiotics might be caused by the higher PBP4 level and increased peptidoglycan cross-linking in *yjbH* mutants, but the Cys residues were not required for the antibiotic resistant phenotype (Gohring et al., 2011). This indicates that YjbH regulates Spx proteolysis and antibiotic resistance mechanisms in *S. aureus*. Spx also controls *trfA*, the *B. subtilis mecA* homolog and *trfA* mutants are sensitive to oxacillin and glycopeptide antibiotics (Jousselin et al., 2013). Expression of *trfA* was constitutively up-regulated in glycopeptide-intermediate *S. aureus* (GISA) derivatives of methicillin-suscepti-ble or methicillin-resistant in *S. aureus* (MRSA) clinical or

laboratory isolates (Jousselin et al., 2013). This indicates that the up-regulation of *trfA* in the *yjbH* mutant is responsible for the β-lactam antibiotic resistance. In summary, *S. aureus* YjbH regulates Spx proteolysis and the Spx-dependent *trfA* confers antibiotic resistance in *S. aureus*. The role of YjbH for virulence was shown in *Listeria monocytogenes* where *yjbH* and *clpX* mutants had hypohemolytic phenotypes indicating that YjbH and ClpX are required for the function of the pore-forming toxin listeriolysin as virulence factor (Zemansky et al., 2009).

Bacillus anthracis encodes two *spx* paralogs, *spxA1* and *spxA2*. Mutants lacking *spxA1* displayed increased peroxide sensitivity but only the *spxA1spxA2* double mutant was hypersensitive to diamide stress suggesting overlapping roles of SpxA1 and SpxA2 in disulfide-stress resistance (Barendt et al., 2013). Microarray analyses identified many genes involved in the thiol-redox homeostasis that were up-regulated when stabilized protease-resistant forms of SpxA1DD or SpxA2DD were produced. These SpxA1 and SpxA2-controlled disulfide-sress related genes encode for thioredoxins and thioredoxin reductases (*trxA*, *trxB*, *ytpP*), two CoASH disulfide reductases (*BA1263*, *BA0774*) to keep CoASH in its reduced state and genes for bacillithiol biosynthesis (*bshA*, *bshB1*, *bshB2*, *bshC*), bacilliredoxins (*ytxJ*, *yphP*) and the putative BSH reductase (*ypdA*) (Table 5). In addition, the Spx paralogs control genes involved in DNA damage repair (*uvrC*, *uvrD*), spore outgrowth and germination (*exoA*), detoxification of alcohols, aldehydes, and quinones (*BA0838*, *BA2647*, *BA3438*), unknown oxidoreductase functions (*BA1951*) and adapter for ClpXP proteolysis (*yjbH*) (Barendt et al., 2013). Furthermore, both Spx paralogs control also their own subregulon. Interestingly, *spxA2* was shown to be up-regulated in phagocytosis assays with infected macrophages (Bergman et al., 2007). The expression of *spxA2* is negatively controlled by the Rrf2 family regulator SaiR that is conserved among the *Bacillus cereus* group. The Rrf2-family regulators include also IscR and NsrR with 3 conserved Cys residues that coordinate an FeS-cluster. SaiR shares C89 and C96 with IscR and NsrR and C96 was required for SaiR repressor activity and redox regulation of *spxA2*. Repression of *spxA2* is alleviated under NaOCl stress and in infected macrophages probably by thiol-oxidation of SaiR leading to its dissociation from the *spxA2* promoter (Nakano et al., 2014). However, the role of the Cys residues and the possible involvements as ligands are still unknown in the regulation of SaiR.

Streptococci also encode two *spx* paralogs: *spxA* and *spxB* in *Streptococcus mutans* (Kajfasz et al., 2010); *spxA1* and *spxA2* in *Streptococcus pneumoniae* (Turlan et al., 2009), *Streptococcus suis* (Zheng et al., 2014) and *Streptococcus sanguinis* (Chen et al., 2012). These Spx paralogs share the conserved redox-sensing CXXC motif and also the Gly residue required for Spx-RNAP interaction. However, only SpxA and SpxA1 contain the RPI motif involved in modulation of the reactivity of the CXXC motif. In SpxB and SpxA2, the arginine is replaced by a serine residue and this may affect the sensory function (Newberry et al., 2005; Turlan et al., 2009; Kajfasz et al., 2010; Chen et al., 2012; Zheng et al., 2014). These Spx proteins are involved in the oxidative stress response and in the virulence of streptococci as demonstrated by the use of murine, rabbit or insect infection models (Kajfasz et al., 2010; Chen et al., 2012; Zheng et al., 2014). Both Spx paralogs control the expression of genes for the thioredoxin reductase (*trxB*), the peroxide resistance protein (*dpr*), the superoxide dismutase (*sod*), the peroxiredoxin (*ahpCF*), the glutathione reductase (*gor*), the NADH oxidase (*nox*) and thiol peroxidase (*tpx*) in *S. mutans* and *S. suis* (Kajfasz et al., 2010; Zheng et al., 2014) (Table 5). In *S. sanguinis*, SpxA1 regulates the genes for the pyruvate oxidase (*spxB*) and the NADH oxidase (*nox*) that are both responsible for the high production of H_2O_2 (Chen et al., 2012). Thus, the *spxA1* mutant is more sensitive to H_2O_2 but produces also lower amounts of cytoplasmic H_2O_2 as defense mechanism against bacterial competitors.

ECF sigma factors and their cognate redox-sensitive zinc-associated anti-sigma factors (RsrA/SigR in *Streptomyces*; RshA/SigH in *C. glutamicum* and *M. tuberculosis*)

In Actinomycetes, the disulfide-stress response is controlled by ECF sigma factors and their redox-sensitive cognate zinc-containing anti-sigma factors (ZAS) that share a conserved HX(3)CX(2)C (HCC) motif (Jung et al., 2011). In addition, the region K(33)FEHH(37)FEEC(41) SPC(44)LEK(47) that includes the conserved HCC motif was identified as redox-sensitive determinant in ZAS factors. In another model, alternative Zn-binding sites were identified in redox-sensitive ZAS factors that might increase the susceptibility of zinc-coordinating cysteine residues to oxidation (Heo et al., 2013). Redox-sensitive ZAS factors that are involved in the disulfide-stress response include RsrA of *S. coelicolor* and its homologs RshA of *M. tuberculosis* and *C. glutamicum*. RsrA of *S. coelicolor* is the best studied ZAS factor that coordinates zinc and sequesters its cognate sigma factor SigR under reducing conditions forming a RsrA/SigR complex (Paget et al., 2001). Three of the seven cysteines in RsrA (C11, C41 and C44) are

essential for anti-sigma factor activity and redox-sensing (Kang et al., 1999) (Cherney et al., 2003; Zdanowski et al., 2006). Upon disulfide stress, the redox-sensitive Cys11 and Cys44 form an intramolecular disulfide resulting in zinc release and conformational changes in RsrA that lead to free SigR (Kang et al., 1999; Bae et al., 2004) (Figure 9). Free SigR interacts with the RNAP to direct transcription of the SigR disulfide-stress regulon.

The SigR-regulon consists of more than 100 genes as revealed by DNA microarrays and genome-wide ChIP-chip analyses (Kim et al., 2012). The majority of SigR-controlled genes function in the thiol-redox homeostasis and encode for thioredoxins and thioredoxin reductase (*trxAB*, *trxC*), enzymes for mycothiol biosynthesis and recycling (*mshA*, *mca*, *mtr*), putative glutaredoxin-like mycoredoxins (*mrxA*, *mrxB*) and methionine sulfoxide reductase (*msrA*, *msrB*) (Paget et al., 1998, 2001; Newton and Fahey, 2008; Park and Roe, 2008). Targets for SigR control are also involved in protein quality control (*pepN*, *ssrA*, *clpP1P2*, *clpX*, *clpC*, *lon*), in the prokaryotic ubiquitin-like protein-conjugation and proteasomal degradation pathway (*pup*, *pafD*, *mpa*, *prcAB*), guanine biosynthesis (*guaB*, *guaB1*) and ribosome-associated functions (*rpmE*, *rpmI*, *rplT*, *relA*, *engA*,

obgE, *era*, *truB*, *rbfA*, *infA*, *infB*) (Kallifidas et al., 2010; Kim et al., 2012). The SigR-regulon includes further genes that encode Fe-S assembly components (*sufA*, *sufU*), Fe-S containing enzymes (*nadA*, *lipA*), biosynthesis enzymes for redox-sensitive sulfur-containing cofactors, such as Fe-S, folate, CoASH and lipoic acid (*moeB*, *coaE*, *folE*, *lipA*), UV damage repair enzymes (*uvrA*) and the major housekeeping sigma factor (*hrdB*) (Figure 9, Table 6). This core SigR-regulon and the SigR consensus sequence are conserved across 42 Actinomycetes revealing a robust adaptation strategy to oxidative and disulfide-stress conditions in different natural environments (Kim et al., 2012).

MSH and TrxA were shown to reduce oxidized RsrA, allowing to switch-off of the SigR-dependent stress response upon return to non-stress conditions (Kang et al., 1999; Park and Roe, 2008). SigR and an unstable SigR′ protein with an N-terminal extension are positively autoregulated under diamide stress (Kim et al., 2009). This unstable SigR′ protein is rapidly degraded by the induced ClpP1/P2 proteases which represents another negative feedback loop.

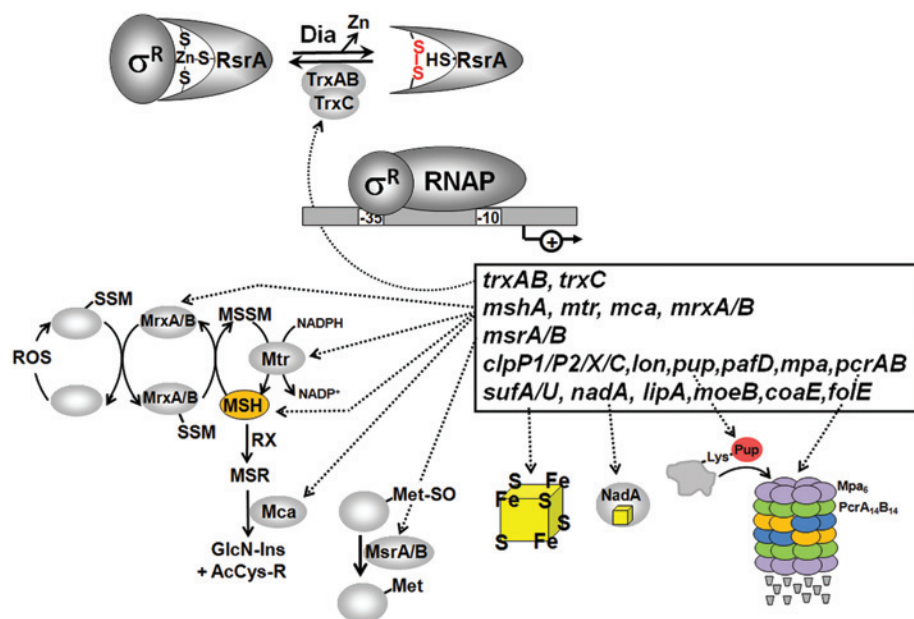The ECF sigma factor SigH of *M. tuberculosis*, an ortholog of SigR, plays an important role in the survival

Figure 9: Redox regulation of the ZAS factor RsrA and its cognate sigma factor SigR in *S. coelicolor* and role of the SigR-regulon. RsrA is a redox-sensitive zinc-binding anti-sigma (ZAS) factor in *S. coelicolor* that sequesters its cognate sigma factor SigR under reducing conditions. Diamide stress leads to intramolecular disulfide formation in RsrA, resulting in Zn release and relief of SigR. Free SigR activates transcription of the SigR-regulon that functions to restore the thiol-redox balance. The SigR-regulon includes genes for thioredoxins and thioredoxin reductase (*trxAB, trxC*), enzymes for mycothiol biosynthesis and recycling (*mshA, mca, mtr*), mycoredoxins (*mrxA, mrxB*), methionine sulfoxide reductase (*msrA, msrB*), protein quality control machinery (*pepN, ssrA, clpP1P2, clpX, clpC, lon*), ubiquitin-like protein-conjugation pathway and proteasomal components (*pup, mpa, pafD, prcAB*), Fe-S assembly components (*sufA, sufU*) and Fe-S containing enzymes (*nadA, lipA*), biosynthesis enzymes for the cofactors Fe-S, folate, CoASH and lipoic acid (*moeB, coaE, folE, lipA*). Examples for SigR-regulon genes and their functions are also listed in Table 6.

Table 6: The RsrA/RshA zinc-associated anti-sigma factors (ZAS) for disulfide stress.

Redox sensor	Organism	Signal	Redox-sensing mechanism	Regulon genes	Regulon functions	References
RsrA (ZAS of SigR)	*Streptomyces coelicolor*	Diamide RES	C11, C41,C44 Redox-sensitive, Zn-site of ZAS	*sigR-rsrA*	RsrA/SigR	Paget et al. (1998) Paget et al. (2001) Park and Roe (2008) Kim et al. (2012)
				trxAB, trxC	Thioredoxin/thioredoxin reductase	
				mshA, mca	Mycothiol biosynthesis and recycling	
				mtr	MSH reductase	
				mrxA, mrxB	Mycoredoxins	
				msrA,msrB	Methionine sulfoxide reductase	
				pepN, ssrA, clpP1P2, clpX, clpC, lon	Protein quality control	
				pup, mpa, pafD, prcAB	Ubiquitin-like protein-conjugation	
				guaB, guaB1	GMP biosynthesis	
				rpmE, rpmI, rplT, relA, engA, obgE, era, truB, rbfA, infA, infB	Ribosome-associated functions	
				sufA, sufU	Fe-S assembly machinery	
				nadA, lipA	Fe-S enzymes	
				moeB, coaE, folE, lipA	Cofactor synthesis (Fe-S, folate, CoASH, lipoic acid)	
				hrdB	Housekeeping sigma factor	
				uvrA, uvrD	UV damage repair	
RshA (ZAS of SigH)	*Coryne-bacterium glutamicum*	Diamide NaOCl	C23, C53, C56 Redox-sensitive Zn-site of ZAS	*sigH-rshA*	RshA/SigH	Ehira et al. (2009b) Busche et al. (2012) Toyoda et al. (2014)
				trxB, trxB1, trxC	Thioredoxin/thioredoxin reductase	
				mshC, mca	Mycothiol biosynthesis and recycling	
				mtr	MSH reductase	
				cg2838, *cg2661*	*dsbA*-like thiol-disulfide oxidoreductases	
				msrA, msrB	methionine sulfoxide reductases	
				pup	Ubiquitin-like protein-conjugation	
				cg0378	Phage-associated	
				cg1397	tRNA-(5-methylaminomethyl-2-thiouridylate)-methyltransferase	
				sigM, hspR, cglR, sufR, whcA, whcE	Stress-responsive regulators	
				uvrA, uvrD3	UV damage repair	

against oxidative stress. SigH is activated upon entry of *M. tuberculosis* into macrophages and has a key role in the response to oxidative stress as *sigH* mutants of *M. tuberculosis* and *M. smegmatis* are highly sensitive to peroxide stress (Fernandes et al., 1999; Manganelli et al., 2002). SigH is sequestered by its cognate redox-sensitive ZAS factor RshA under reduced conditions. Oxidative stress leads to RshA oxidation and release of SigH to activate transcription of its regulon (Song et al., 2003). SigH controls transcription of approximately 30 genes that include the Trx system, but also other ECF sigma factors (SigE and SigB) (Manganelli et al., 2002). Despite the presence of a ZAS motif, RshA has weak affinity for zinc and the cysteines were shown to coordinate an Fe-S cluster. However, neither the cysteines nor the Fe-S cluster in RshA were essential for RshA/SigH interaction, which is mediated by salt bridge suggesting another regulatory mechanism (Kumar et al., 2012). The serine-threonine kinase PknB that is essential for survival within the host, was shown to phosphorylate both

SigH and RshA. Phosphorylation of RshA results in disruption of its interaction with SigH leading to increased activity of SigH (Park et al., 2008). These observations suggest an important role of PknB in mycobacterial adaptation to oxidative stress by activating SigH via RshA phosporylation.

SigE is another important ECF sigma factor required for *M. tuberculosis* survival under oxidative stress and within infected macrophages (Manganelli et al., 2001, 2004a,b). Its expression is regulated by SigH and the two-component system MprAB, which responds to polyphosphate stress and is implicated in the persistence of *M. tuberculosis in vivo* (He et al., 2006). SigE activity depends on interaction with its anti-sigma factor RseA, a ZAS protein that senses ROS and disulfide stress. This interaction requires Cys70 and Cys73 of the ZAS motif, which is disrupted under oxidative stress conditions. The release of SigE leads to transcriptional activation of *sigB* and *clgR*, that controls the ClpC1/P2 system. SigE regulates also itself in a positive feedback loop (Barik et al., 2010).

In *C. glutamicum,* the ECF sigma factor SigH is involved in the thiol-specific oxidative stress and heat shock response (Kim et al., 2005; Ehira et al., 2009b). SigH is sequestered by its redox-sensitive ZAS factor RshA, which responds to diamide and NaOCl stress (Busche et al., 2012; Chi et al., 2014). The SigH-regulon includes 83 genes that are up-regulated in the *rshA* mutant most of which are involved in the thiol-redox balance (Busche et al., 2012). SigH transcribes genes for the Trx/TrxR system (*trxB, trxB1, trxC*), for MSH biosynthesis and recycling (*mshC*, *mca*, *mtr*), *dsbA*-like thiol-disulfide oxidoreductases (*cg2838*, *cg2661*), methionine sulfoxide reductases (*msrA*, *msrB*), phage-associated functions (*cgO378*), the tRNA-(5-methylaminomethyl-2-thiouridylate)-methyltransferase (*cg1397*), the ubiquitin-like proteasomal conjugation pathway (*pup*) and DNA damage repair enzymes (*uvrA, uvrD3*) (Ehira et al., 2009b; Busche et al., 2012) (Table 6). SigH controls expression of its own *sigH-rshA* operon and that of genes for other stress-responsive regulators (*sigM, hspR, cglR, sufR, whcA* and *whcE*) (Ehira et al., 2009b). ChIP-chip analysis of the *rshA* mutant identified 75 SigH-dependent promoters that included 39 novel promoters not identified in previous transcriptome analyses (Toyoda et al., 2014). In addition, internal σ^H-dependent promoters within operons were identified that are involved in the pentose phosphate pathway, riboflavin biosynthesis, and Zn-uptake. This resulted in increased riboflavin production and Zn overload phenotypes in the *rshA* mutant expanding the roles of the SigH-Regulon in *C. glutamicum* (Toyoda et al.,

2014). In addition to SigH, SigM is also involved in the oxidative stress response. Its transcription is increased after disulfide and peroxide stresses and the *sigM* mutant displayed a high sensitivity to oxidative stress. SigM controls expression of genes encoding the Trx/TrxR systems, the Fe-S-cluster assembly machinery and the disulfide-stress response (Nakunst et al., 2007). These genome-wide studies have revealed an integrated network of disulfide-stress specific ECF sigma factors that can partially replace each other in functions and maintain together the thiol-redox balance among Actinomycetes. In conclusion, the functions of the SigR/SigH regulons in Actinomycetes are very similar to that of the disulfide-stress responsive Spx regulons among Firmicutes bacteria.

Concluding remarks and future challenges

We have provided an overview about thiol-based redox regulators in Gram-positive bacteria and emerging redox sensors in Gram-negative bacteria that belong to different transcription factor families, including LysR(OxyR,HypT), Fur(PerR), MarR(OhrR/SarA/DUF24), TetR(NemR), AraC(RclR), Spx and ZAS factors (RsrA/RshA). Many of these redox sensors employ conserved cysteine residues for redox-sensing of ROS, HOCl or RES that are characterized by their lower Cys pK$_a$ value and hence are in the thiolate anion state and susceptable for versatile post-translational thiol-modifications. The redox-sensing mechanisms of many ROS, HOCl- and RES-sensing redox regulators are often variations of this classical disulfide-switch model of *E. coli* OxyR. The 1-Cys and 2-Cys-type models of the OhrR-family regulators have also been widely confirmed for the widespread MarR/DUF24-family regulators of *Firmicutes* bacteria and for NemR and RclR as main sensors for RES and HOCl. However, different *in vitro* models have shown that thiol-based MarR-type regulators can also be inactivated by Cys phosphorylation and Cys alkylation as new themes of thiol-based redox regulation, yet the reversibility of these modifications still needs to be demonstrated. Another question is to what extent thiol-disulfide switches or alternative Cys phosphorylations contribute to the *in vivo* model of redox regulation. The burgeoning field of redox proteomics coupled with mass spectrometry and new chemical probes to analyze different types of redox-modifications, such as sulfenic acids, *S*-glutathionylations, *S*-bacillithiolations, protein disulfides, *S*-alkylations, *S*-sulfhydrations, etc., allows

a perspective to analyze global thiol-oxidations in the proteome as well as for specific redox regulators under both normal and stress conditions *in vivo* (Leonard and Carroll, 2011; Thamsen and Jakob, 2011; Paulsen and Carroll, 2013; Zhang et al., 2014). While significant progress has been made to characterize protection mechanisms and redox sensors for reactive electrophiles (quinones and aldehydes) and reactive chlorines (HOCl), the mechanisms for the specificity of the emerging HOCl and RES-sensing redox regulators still remains a future challenge and requires more structural information.

Thiol-oxidations play also an important role in pathogens as they have to cope with ROS in the defense against the host immune system. The correlation between thiol-switches and regulation of virulence and antibiotic resistance is well established for many redox sensors of important human pathogens, including PerR, SarZ, MgrA, SarA, QsrR and Spx of *S. aureus* and OxyS, MosR and RshA of *M. tuberculosis*. Particularly, several Spx paralogs have been characterized among pathogenic *Firmicutes* bacteria that contribute to virulence and to the ROS defense. While the knowledge about virulence regulation by thiol-based redox switches is emerging, the importance of *S*-thiolations in redox regulation of virulence functions is still unknown and represents an important challenge for future research in pathogenic bacteria.

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