Antioxidants and Redox Signaling

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Comprehensive Invited Review

Redox-based transcriptional regulation in prokaryotes: revisiting model mechanisms

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Abstract

The successful adaptation of microorganisms to ever-changing environments depends to a great extent on their ability to maintain redox homeostasis. To effectively maintain the redox balance, cells have developed a variety of strategies mainly coordinated by a battery of transcriptional regulators through diverse mechanisms. Redox-responsive transcriptional regulation is an intricate process since identical signals may be sensed and transduced by different transcription factors, which often interplay with other DNA-binding proteins with or without regulatory activity. This review focuses on the main mechanisms used by major redox-responsive regulators in prokaryotes and their relationship with the different redox signals received by the cell. An overview of the corresponding regulons is also provided. Taking into account the complexity of some regulators which may contain several sensing domains, we have classified them in three main groups. The first group contains one-component or direct regulators, whose sensing and regulatory domains are in the same protein. The second group comprises the classical two-component systems involving a sensor kinase that transduce the redox signal to its DNA-binding partner. The third group encompasses a heterogeneous group of flavin-based photosensors whose mechanisms are not always fully understood and are often involved in more complex regulatory networks. This review provides an overall insight into redox-based transcriptional regulation in bacteria, highlighting recent advances and challenges for future applications of these pivotal regulators in biotechnology and medicine.

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I. Introduction

Aerobic metabolism provides significant advantages in energy production, detoxification of xenobiotics and virulence of bacterial cells. However, reactive intermediates produced in the reduction of oxygen by electron transfer systems can damage all cellular components. In addition to these reactive oxygen species (ROS), other by-products of metabolism including reactive nitrogen species (RNS) and xenobiotics challenge cellular redox homeostasis. Among the main targets of ROS during oxidative stress are iron-containing proteins which become severely damaged due to the ability of H_2O_2 and O_2^- to oxidize the iron present in exposed iron-sulfur clusters and other cofactors (164, 170). Furthermore, the release of Fe^{2+} from these centers promotes the Fenton reaction, which produces even more reactive hydroxyl radicals with deleterious consequences for cells (394).

As a response to this scenario, bacteria have developed a set of redox-responsive proteins that trigger the appropriate inducible response according to the level of stress. Redox sensing by the cell is a complex process that integrates diverse stimuli such as O_2 tension, nutrient availability, light intensity, RNS and ROS, among other parameters. Transduction of these redox signals is frequently carried out by transcriptional regulatory proteins through a variety of mechanisms (237, 334, 364). Due to the tight relationship between iron metabolism and redox homeostasis, the activity of many major regulators relies on iron, either as an ion cofactor assembled in iron-sulfur clusters or as heme-based sensors (80, 121, 134, 274, 288, 291). Other essential metal ions such as zinc or manganese also play important roles in reestablishing the redox balance (71, 234) and act as cofactors in transcriptional regulation (101, 200, 297, 325). Besides their role in zinc metalloregulation, zinc ions often prevent the oxidation of redox-sensitive cysteines that work as thiol-based redox switches in numerous transcriptional regulators (149, 200).

As will be highlighted in further sections, multiple transcriptional regulatory mechanisms as response to oxidants are widespread strategies in most prokaryotes. Another interesting issue is the diversity of mechanisms developed by the cell to detect and detoxify the same signal. Thus, in *Escherichia coli*, nitrosative stress is sensed by several transcription factors, such as NorR, FNR, Fur, MetR and, to a lesser extent, SoxR and OxyR (84, 351). In cyanobacteria, in addition to Fur and PerR, a prominent role for histidine kinases in the perception and signal transduction of H_2O_2 has been reported in

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Synechocystis sp. PCC 6803 (32, 181). In *Salmonella spp.*, peroxide is sensed by OxyR, SoxR and the zinc finger motif DksA (68, 97). Therefore, as a strategy for a more efficient adaptation, the same redox signal may induce different transduction mechanisms, allowing a finer tuning of the cell response.

In order to better understand redox regulation beyond the identification of the antioxidant defensive genes, numerous mechanistic studies of the diverse regulators that convert redox signals into regulatory outputs have been carried out in recent decades. In this comprehensive review we revisit the main groups of redox-responsive transcriptional regulators with a particular emphasis on recent findings concerning the structural and mechanistic basis of their regulatory functions.

II. Direct redox-sensing and regulation

Most of the major direct redox sensors monitorize the redox state of the cell through oxidant-sensitive metal-sulfur clusters or non-metallated thiol-based switches. Other relevant mechanisms include those used by heme-based sensor proteins. A summary of the direct-redox sensors discussed in this section can be found in Tables 1 and 2.

A. Regulation involving oxidant-sensitive iron-sulfur clusters

Iron-sulfur clusters function as cofactors of a wide range of transcriptional regulators that exploit the redox and coordination properties of these clusters to act as sensors of environmental conditions.

1. SoxR

SoxR is a conserved regulator in Enterobacteriaceae that belongs to the MerR family of transcriptional regulators. MerR homologues share similar N-terminal winged helix-turn-helix (wHTH) DNA binding regions, while C-terminal effector binding regions appear specific to the effector recognised (37). Most members of the family respond to stress signals, such as oxidative stress, heavy metals or antibiotics. SoxR was initially identified as an O_2^- stress sensor (221, 370), though further studies showed the activation of SoxR by nitric oxide and a variety of endogenous and xenobiotic redox-cycling agents (267, 412). Additionally, SoxR becomes activated though DNA-mediated oxidation by guanine radicals

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which are produced in the early stages of oxidative stress (217). In *E. coli*, the SoxRS response involves around 100 genes whose transcription is modulated by SoxR through activation of its SoxS partner, an AraC-type regulator (135). Most genes composing this extensive regulon are involved in minimizing oxidative damage caused by free radicals, including destruction of superoxide (*sodA*), reduction of iron-sulfur clusters (*fpr*), DNA repair (*nfo*) and NADPH production (*zwf*), among others. Induction of the SoxR regulon also confers resistance to a variety of antibiotics due to the reduction in OmpF and S6A levels (78, 135). Conversely, since nonenteric bacteria lack SoxS, SoxR directly controls a small regulon of key genes involved not only in the detoxification of redox active compounds but also in antibiotic resistance and quorum-sensing which in many cases are essential for full virulence of mammalian pathogens (248, 260, 279).

The mechanism of action of SoxR in enteric bacteria has been extensively investigated. In solution, SoxR is a homodimer that exhibits one [2Fe-2S] cluster per monomer coordinated by the four cysteines in the conserved sequence ($CysX_2CysXCysX_5Cys$) near the carboxyl terminus. In the absence of oxidative stress, SoxR with the reduced [2Fe-2S] cluster may bind to DNA but it is inactive for transcription initiation (Fig. 1A). As a MerR-like regulator, SoxR controls the expression of genes whose promoters contain suboptimal 19-bp spacers between their -35 and -10 elements, which are not recognized by the sigma factor of RNA polymerase (RNAP) (37). SoxR activates transcription of its counterpart *soxS* through the change in the oxidation state of the [2Fe-2S] cluster from $[2Fe\text{-}2S]^{+1}$ to the $[2Fe\text{-}2S]^{+2}$ form. Upon oxidation, untwisting the *soxS* promoter allows remodelling of -35 and -10 elements enabling its recognition by RNAP and in turn the *soxS* transcription, which increases around 100-fold (147). Notably, SoxR is the only regulator able to modulate its transcriptional activity undergoing a single change in the cluster redox state (64). The large conformational change of SoxR and the target promoter triggered just by cluster oxidation could be elicited by the remarkable asymmetric environment of the [2Fe-2S] cluster observed in the structural analysis of *E. coli* SoxR bound to DNA (390). Moreover, the [2Fe-2S] cluster is completely solvent-exposed enabling fast electron transfer to several redox partners, as well as direct modification through nitrosylation. The completely conserved Arg55 and Trp91 interacting cysteine residues are important for SoxR activity. Direct interaction of the cluster-binding domain with the DNA-binding domain occurs through the

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highly conserved residues Arg55′ and Trp91′ (390) (Fig. 1B). Previous electrochemical studies suggested that upon binding to DNA targets, the reduction potential of SoxR [2Fe-2S] undergone a shift from -285 mV (free regulator) to +200 mV (complexed to DNA) (80). Those results suggested that only strong oxidants would be able to oxidize the cluster to the +2 state and were not in good agreement with the ability of SoxR to react with some redox-cycling drugs and other weak oxidants. However, a recent study using DNA-modified electrodes concluded that DNA binding causes a moderate shift in the reduction potential of SoxR, namely -320 mV of the SoxR bound to DNA versus -293 mV of the free protein versus NHE (normal hydrogen electrode), in better concordance with the cognate *E. coli* SoxR signals (193).

The absence of SoxS in *Pseudomonas aeruginosa* and *Streptomyces coelicolor*, as well as their lower SoxR sensitivity to superoxide, one order of magnitude smaller than that of the *E. coli* homologue, raised the question of SoxR functionality in non-Enterobacteriaceae (194, 279, 284, 343). In *P. aeruginosa* and *S. coelicolor*, unlike in *E. coli*, SoxR is not oxidized by redox-cycling agents and superoxide, but rather is activated by endogenous redox-active pigments, namely pyocyanin and actinorhodin, to directly regulate a set of targets encoding enzymes likely to be involved in the modification and transport of small molecules, such as antibiotics (79, 338). Another interesting issue is the different selectivity against redox-cycling drugs of SoxR regulators from diverse species. Mutational studies demonstrated that small alterations in the SoxR structure can lead to the evolution of proteins with distinct specificities for redox-active small molecules (335). In addition, a series of physicochemical and mutational studies evidenced the importance of two lysine residues in the vicinity of the [2Fe-2S] cluster, namely Lys89 and Lys92 in *E. coli* SoxR crystal structure (Fig. 1B), which are substituted by alanine in non-enteric bacteria (108). Furthermore, the presence of the three-residue hydrophilic motif (Arg127Ser128Asp129) near the [2Fe-2S] cluster in *E. coli* SoxR, which is not conserved in non-enteric bacteria, also contributed to SoxR sensitivity to redox-active molecules (108). All these results gave valuable information about the molecular basis of functional differences between SoxR proteins and provide new insights into how species-specific residues could tune SoxR sensitivity to different oxidants.

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2. IscR

IscR belongs to the Rrf2 family of wHTH transcription factors. Members of the Rrf2 superfamily are widespread in bacteria and respond to different signals, such as nitric oxide (NsrR), iron limitation (RirA), cysteine availability (CymR) or O_2 (RsrR) (93, 146, 167, 256). IscR senses the iron-sulfur cluster status in the cell and it is an outstanding example of the integration of redox, sulfur and iron availability signals. IscR was first identified in *E. coli* and isolated in anaerobiosis as a $[2Fe\text{-}2S]^{1+}$-repressor of the *iscRSUA-hscBA-fdx* operon involved in Fe-S cluster assembly (323). In a feed-back loop, IscR senses iron-sulfur homeostasis through the occupancy level of its own [2Fe-2S] cluster (118, 316). When iron-sulfur clusters are scarce, apo-IscR dissociates from DNA, derepressing transcription of the cluster biogenesis pathway. Under conditions of low iron, oxidative stress or disruption of the sulfur metabolism, apo-IscR activates transcription of the *suf* operon involved in iron-sulfur biogenesis (118, 402). Therefore, IscR can be active in both holo and apo forms, whose ratio is determined by iron availability, redox status and O_2 tension. Consequently, IscR regulation and activity is directly or indirectly influenced by other master transcriptional regulators, such as FNR (fumarate nitrate reductase regulator), IHF (Integration host factor), OxyR, or Fur (ferric uptake regulator) (Fig. 2). An excellent review describing the roles, regulation and structural details of Isc proteins is available (316). Due to the prominent role of [Fe-S] clusters in metabolism, IscR is considered a master regulator that controls more than 40 genes of 20 predicted operons in the *E. coli* genome (118). IscR can recognize two different binding motifs. Type I promoters such as those in *iscR*, *yadR* and *yhgY* genes are targets for holo-IscR, while type II binding motifs deduced from *hyaA*, *ydiU* and *sufA* promoter regions exhibit a different consensus and may recruit holo and apo-IscR (118, 262). This dual activity enables IscR to control two different regulons and, in turn, coordinately regulate iron-sulfur cluster homeostasis. IscR also plays a critical function in *P. aeruginosa,* which lacks the SUF machinery. *P. aeruginosa* IscR controls the *isc* operon, and contributes to iron homeostasis and resistance to oxidants (311, 347). Furthermore, IscR controls the ferredoxin-$NADP^+$ reductase *fprB* that in *P. aeruginosa* is involved in [4Fe-4S] cluster biogenesis and tolerance to several stresses (312). The relationship between FprB and IscR suggests that *Pseudomonas* IscR activity could contribute to the modulation of the $NADP^+$/NADPH ratio through *frpB* regulation.

Since IscR is essential in the multiple stress response and pathogenesis of several organisms (248), it has been proposed as a potential therapeutic target for novel drugs. Although some aspects of the molecular mechanism of IscR remain unknown, significant advances in the understanding of IscR-ligand interaction have been made through the characterization of the IscR [2Fe-2S] cluster from *E. coli* (103), and with the resolution of free and DNA-bound structures of apo-IscR from *E. coli* and *Thermincola potens* (299, 315). Mössbauer spectroscopy analysis showed that, *in vivo*, the [2Fe-2S] cluster was predominantly reduced. Interestingly, the affinity of IscR for its binding site was not affected by partial cluster oxidation upon anaerobic isolation of the regulator, suggesting that the cluster oxidation state is not important for the regulation of DNA binding (103). Coordination of the [2Fe-2S] cluster takes place through three conserved cysteine residues at the C-terminus (Cys92, Cys98, and Cys104 in the *E. coli* regulator) and the highly conserved His107 residue. An exception to this 3Cys-1His-coordination is the IscR protein from the facultative phototrophic bacterium *Rhodobacter sphaeroides* whose single-Cys residue is not involved in cluster coordination (304). The three-dimensional structure of apo-IscR proteins exhibits an overall architecture similar to Rrf2 regulators harboring a wHTH DNA-binding motif and a dimerization domain mainly composed of helix α5 in monomer 1 and helix α6 in the adjacent unit that stabilize dimer formation mainly by hydrophobic interactions. Analysis of the apo-IscA-DNA interface (PDB ID: 4CHU), together with sequence alignments of the DNA-binding domains, led to the identification of relevant residues for specific DNA recognition and highlights the role of Glu43 as a selectivity filter in apo-IscR to discriminate against type-1 binding motifs (316). Moreover, the characterization of IscR orthologs from different organisms suggests a high conservation of this unique mechanism of sequence discrimination, unveiling a similar regulation of [2Fe-2S] cluster biogenesis to maintain a perfect balance between favorable and adverse conditions (316).

3. NsrR

NsrR (nitric oxide sensitive repressor) is the master regulator of nitrosative stress response in most β and γ *Proteobacteria*, with the exceptions of Pseudomonales, Pasteurellaceae and *Vibrio cholera*e (309). Under NO stress imposed by macrophages or as a by-product of

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denitrification, NsrR derepresses the transcription of genes involved in NO detoxification. The most conserved member of the NsrR regulon is the flavohaemoglobin gene *hmp*. The main activity of this enzyme consists of the oxidation of NO to nitrate (66, 372).

NsrR is a member of the Rrf2 family that can harbor either a [2Fe-2S] or a [4Fe-4S] cluster, depending on the organism and the purification conditions (167, 371, 407). In *E. coli,* NsrR mediates the adaptive response to NO together with NorR (28, 156), and controls a regulon with more than 60 genes including targets involved in iron-sulfur cluster repair, motility and biofilm development (28). Usually, NsrR works as a transcriptional repressor, recognizing and binding as a dimer to a conserved A/T-rich 11-3-11-bp inverted repeat sequence. Upon nitrosylation of the sulfo-ferric cluster, NsrR releases from DNA. However, it has been reported that NsrR can activate virulence gene expression in *Salmonella typhimurium* and in the enterohemorrhagic *E. coli* (35, 183). Moreover, in *Bacillus subtilis* two different types of regulation by NsrR have been described (Fig. 3A). The so-called class I promoters, such as those upstream of the *hmp* and *nasR* genes, are controlled by [4Fe-4S]-NsrR in response to NO (407). Class II promoters are upstream of other genes of the NsrR regulon. DNA binding to class II regulatory sites is weaker, NO insensitive and involves apo-NsrR (196, 197). Class II sites are abundant in the NsrR regulon and many of those genes are controlled by multiple transcription regulators, such as ResD, AbrB, Rok and Fur. Further work evidenced the importance of combinatorial transcriptional control by NsrR, Fur and ResD in *B. subtilis* anaerobic gene regulation (59). NsrR has been shown to work coordinately with other transcription factors in several organisms. In *S. typhimurium*, NsrR controls a set of genes with overlapping binding sites for Fur and FNR (367), while in *E. coli* NsrR participates in the regulation of the *sufABCDSE* operon together with IscR and Fur (Fig. 2) (50, 213).

The observation of active NsrR with [2Fe-2S] and [4Fe-4S] clusters has raised some controversy about the physiologically relevant structure of the iron-sulfur cluster in this regulator. Inactivation of aerobically purified [2Fe-2S] NsrR regulators from *S. coelicolor* and *Neisseria gonorrhoeae* only occurred upon nitrosylation of iron in the [2Fe-2S] cluster, suggesting that O_2 does not affect the redox-sensing module (167, 371). However, anaerobically isolated NsrR from *S. coelicolor* and *B. subtilis* harbors [4Fe-4S] clusters that are also stable in aerobic cultures. It has been proposed that the [4Fe–4S] cluster in

aerobic cultures of *B. subtilis* could be stabilized by glutathione and low-molecular weight thiols, such as bacillithiol (407). In contrast, in NsrR from *S. coelicolor*, low molecular weight thiols dramatically reduce the O_2 stability of the [4Fe-4S] cluster, leading to a fast stoichiometric conversion to the [2Fe-2S] form (66).

Resolution of the crystal structure of [4Fe-4S] NsrR from *S. coelicolor* (Fig. 3B) revealed an unusual, asymmetric cluster coordination by three conserved cysteine residues (Cys93, Cys99 and Cys105) from one of the monomers and the Asp8 residue from the other, that is displaced by NO as a cluster ligand (385). Nitrosylation [4Fe-4S] disrupts several H-bonds causing the displacement of the DNA recognition helix and preventing apo-NsrR binding (385). This unique coordination of the redox center in holo-ScNsrR suggests that the breaking of both inter-monomer Asp8-[4Fe–4S] bonds, caused by their substitution with NO, will initiate both cluster degradation and structural changes.

4. RsrR

The Rrf2 regulator RsrR (Sven6563) was initially annotated as a NsrR homolog in *Streptomyces venezuelae*. However, comparative *in vivo* mapping of RsrR binding sites in *S. venezuelae* and a *ΔrsrR* mutant indicates that this regulator controls a large set of genes with different functions than NsrR (256). Targets of RsrR exhibit either an 11-3-11 bp inverted repeat motif (class I genes) or a single repeat/half site (class II genes). Class I genes represent around 2.7% of RsrR targets, including the bidirectional promoter located between *rsrR* and *nmrA* and other genes mainly involved in signal transduction and NAD(P)H metabolism. Class II targets comprise more than 600 genes with diverse functions, including 21 putative transcriptional regulators, genes involved in *S. venezuelae* metabolism, RNA/DNA replication and modification, small molecule-transporters and proteases, among others (256).

RrsR DNA-binding activity is controlled by the status of its [2Fe-2S] cluster that works as a redox switch in a manner similar to SoxR. Under anaerobic conditions, RsrR is a dimer with each monomer containing a reduced $[2Fe\text{-}2S]^{+1}$ cluster that is rapidly oxidized to $[2Fe\text{-}2S]^{+2}$ by O_2, increasing *in vitro* DNA-binding activity. This redox transition controls the affinity of RrsR for its DNA targets, while apo-RrsR in inactive in DNA binding. Since RrsR mechanism and target genes differ of those from NsrR, it has been proposed that RsrR displays a novel

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sensing domain for an Rrf2 protein and therefore represents a new member of this superfamily (256).

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5. FNR

FNR is a widespread sensor of environmental O_2 that switches the transition between aerobic and anaerobic respiration. Identified in *E. coli* in the 70´s (204), FNR is a member of the CRP family of transcriptional regulators. Its structure comprises a sensory domain at the N-terminus with a β-roll motif and a long α-helix involved in subunit dimerization, and a C-terminal DNA binding domain that contains a HTH motif. Unlike CRP, FNR holds an N-terminal extension that contains four cysteines involved in the coordination of the iron-sulfur cluster, which functions as a direct sensor of O_2 (133). In anaerobic conditions, FNR contains one $[4Fe\text{-}4S]^{2+}$ cluster per monomer that in the presence of O_2 is rapidly converted into a $[2Fe\text{-}2S]^{2+}$ form through a $[3Fe\text{-}4S]^{1+}$ intermediate, releasing Fe^{2+} and O_2^- with the subsequent DNA damage (63, 64). The conversion of the cubic $[4Fe\text{-}4S]^{2+}$ center to planar $[2Fe\text{-}2S]^{2+}$ requires a series of rearrangements in the proximity of the cluster that, in turn, modifies the dimerization interface, causing dimer dissociation and release from DNA (64). This process may be reverted under low O_2 tension. However, if O_2 persists in the environment, the $[2Fe\text{-}2S]^{2+}$ clusters are slowly degraded to produce inactive apo-FNR that, depending of the redox status in the cell, will either work as a scaffold for the incorporation of novel $[4Fe\text{-}4S]^{2+}$ clusters or will be degraded by the ClpXP protease (Fig. 4) (104, 245). The active dimeric $[4Fe\text{-}4S]^{2+}$-FNR is also sensitive to NO, that generates a mixture of monomeric and dimeric dinitrosyl-iron-cysteine complexes suppressing its ability to bind DNA (69). Therefore, the behavior of FNR differs considerably from that of most CRP family members which are stable homodimers and bind DNA upon activation by their corresponding effectors. In contrast, a dimer-monomer transition driven by ligation of one $[4Fe\text{-}4S]^{2+}$ cluster per subunit and/or O_2 tension is critical for precise FNR activity. Exceptions to this general working model are the FNR regulators from *Bacillus* spp. FNR from *B. subtilis* is a permanent dimer activated by the ligation of one $[4Fe\text{-}4S]^{2+}$ per cluster, coordinated by three cysteine residues and one aspartate (136). Conversely, *B. cereus* apo-FNR appeared active in DNA-binding in both dimeric and monomer forms. Although further work should be done to fully understand the mechanism used by *B.*

cereus FNR, it has been shown that the interaction with ResD and PicR of both the holo-form and the cluster-free FNR is involved in the control of enterotoxin production by this pathogen (91).

FNR can work as either a repressor or an activator of a different set of genes. For positive regulation, FNR contains three individual activating regions that mediate contacts with RNA polymerase depending on the promoter architecture (324). Class II promoters, whose FNR binding site is around 41.5-bp upstream of the TSS, predominate over class I with the recognition site at -61.5-bp. Different studies have unveiled the complexity of the FNR regulon in *E. coli*. Although FNR can bind up to 207 sites across the *E. coli* chromosome, the *in vivo* FNR occupancy is restricted by nucleoid-binding proteins, as well as by the larger number of other regulators bound at FNR-regulated promoters. Thus, changes in accessibility of FNR would occur under the appropriate growth conditions, resulting in a highly ductile gene regulation (258). The core of the FNR regulon appears to be conserved across many facultative anaerobes and, typically, contains operons associated with anaerobic respiration (*e.g.*, *nar*, *dms* and *frd*), including glycolytic and fermentative enzymes, whose transcription is activated by FNR. On the other hand, FNR represses a set of genes encoding several aerobic respiratory enzymes, such as cytochrome oxidase and NADH dehydrogenase, among others. Most of these FNR-repressed genes in *E. coli* are co-regulated by ArcA and other FNR-regulatory networks which may involve the pyruvate sensing PdhR and the GadE regulators (244, 258). Furthermore, as part of the strategy to overcome changes in O_2 tension suffered during the course of infection, FNR triggers virulence gene expression during host colonization and infection in many facultative anaerobic pathogens (134, 248).

Prior to the resolution of the FNR structure from *Aliivibrio fischeri* (384), a CRP-based model from the *E. coli* FNR was used in numerous studies to understand the mechanism of this regulator. Thus, the characterization of a series of site-directed mutants has provided valuable information about the conformational alterations driven by O_2 that mediate FNR activity. In particular, some of those involving mutations with altered O_2-sensing mechanisms or mutants with miss-regulated FNR dimerization deserve to be highlighted (Fig. 4). While the Leu28His variant showed increased resistance to O_2, substitution of Ser24 for different amino acids enhanced the aerobic activity of FNR *in vivo* (20, 172).

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Furthermore, residues Asp154 and Ile151 were critical for proper monomer-dimer transition (252, 253). A detailed comparison between the structural information provided by *A. fischeri* crystals in relation to these results obtained from FNR mutants can be found in a recent review by Mettert and Kiley (244). The authors dissect the protein in four main regions, namely cluster-binding, dimerization, DNA binding and interaction with RNA polymerase. The N-terminal region, which contains the four cysteine ligands of the [4Fe-4S]$^{2+}$ cluster, exhibits high conformational flexibility and is more disordered than the rest of the protein. Cluster assembly seems to organize the FNR N-terminal region eliciting O_2 accessibility to the redox center. A network of hydrophobic interactions proximal to the redox cluster that involves residues of the A, B, and C α-helices would serve as a signaling relay between O_2-mediated cluster oxidation and dimer dissociation (244, 384). Residue Asp154, which has an inhibitory effect on dimerization, together with Glu150 form a negatively charged pocket in holo-FNR, proximal to Ile151. In contrast to what was previously proposed, Ile151 does not shelter Asp154 to afford dimerization, but establishes inter-subunit van der Waals contacts that are critical for dimer stability. Another important amino acid is Arg140, which enables the O_2 sensitivity of the FNR monomer-dimer equilibrium through the formation of a salt bridge with Asp130 belonging to the αB helix of the opposite subunit (244).

Previous studies, as well as a comparison with the structure of the FNR-homolog $FixK_2$ from *Bradyrhizobium japonicum* in a complex with DNA point to Glu209, Ser212 and Arg213, located in the αF helix of the HTH motif, as key residues involved in FNR-DNA interaction (29, 244). Furthermore, it is well established that activating regions denoted as AR1 and AR3 have predominant roles in the interaction of FNR with RNA polymerase. The Arg184 residue located in AR1 stabilizes FNR in a conformation optimal for interaction with RNA polymerase allowing AR1 to hasten RNAP isomerization from a closed to an open complex (392). Notably, the region consisting of residues 183 to 186 is in the vicinity of the [4Fe-4S]$^{2+}$ cluster binding domain, suggesting that this close proximity could permit communication between the cluster binding domain and AR1 upon cluster ligation (244). Furthermore, Ile81, Gly85 and Asp86 were found to be relevant residues for proper interaction of AR3 with σ^{70} (205).

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The variability across species of the residues composing the cluster-binding domain in the vicinity of the four conserved cysteines likely determines cluster sensitivity to O_2 in each organism, according to the environment (86, 244, 396). Moreover, the occurrence of multiple FNR proteins in several organisms, such as *Pseudomonas putida* and *Burkholderia,* exhibiting different reactivities extends the range of O_2-responsive gene expression within a single bacterium (158). More complex is the situation in *B. japonicum* and other bacterial species that use nitrate as a respiratory substrate, which need to adapt their respiratory pathways not only to O_2 tension but also to the available sources of nitrogen (242).

6. WhiB/Wbl

WhiB and Wbl (WhiB-like) are a family of multifunctional proteins exclusive to actinomycetes. Wbl proteins play diverse roles in morphogenesis, cell division, metabolism, virulence and antibiotic production. WhiB was first discovered in *Streptomyces* as an essential regulator of sporulation (76). Further studies expanded this family, which in *Mycobacterium tuberculosis* consists of seven WhiB paralogs (24, 169). Anaerobically isolated Wbl proteins contain a C-terminal DNA-binding domain and a [4Fe-4S]$^{1+}$ redox cluster at the N-terminus. The DNA-binding domain contains a Trp/Gly-rich motif, predicted to form a β-turn, followed by two positively charged amino acid motifs with different degrees of similarity with the DNA-binding motif known as AT-hook (11, 300, 314, 345). The [4Fe-4S]$^{1+}$ cluster is NO sensitive, though in some paralogs it also becomes oxidized upon exposure to O_2. The redox cluster is coordinated by four conserved cysteines, two of them in a CysXXCys motif, commonly found in the thioredoxin fold and in oxido-reductases.

Nitrosylation of the [4Fe-4S]$^{1+}$ cluster is a multistep process that consumes up to 8 NO molecules and if the stress persists is followed by complete loss of the cluster (67). Depending on the environment, the coordinating cysteine residues may then remain as –SH, or establish intramolecular disulfide bridges. Unlike other transcriptional regulators containing iron-sulfur clusters, nitrosylated and apo-Wbl strongly bind DNA. The oxidized, disulfide-containing apo-protein exhibits the highest DNA-binding affinity. In contrast, DNA interaction with the holo-form is very weak or null (314). Therefore, besides gene regulation mediated by the redox status of the cluster, changes in the redox state of the

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cysteines provide Wbl with an additional layer of regulation. Moreover, most Wbl proteins present disulfide-reductase activity representing a novel redox system in *M. tuberculosis* (4).

The increasing knowledge of this intriguing family of proteins has revealed significant differences among them, possibly related to their functional diversity. The chromosome of *S. coelicolor* contains 11 *wbl* genes. Nevertheless, not all of them are $[4Fe\text{-}4S]^{2+}$ transcription factors and the functions of some Wbl proteins still remain controversial (3, 105, 186). A recent *Streptomyces* genome-wide chromatin immunoprecipitation sequencing analysis evidenced that WhiA and WhiB cooperatively control the expression of a common set of WhiAB target genes (40). Characterization of the holo and the cluster-free forms of WhiD identified a ROS sensitive [4Fe-4S] cluster whose disassembly was partially protected by low molecular weight thiols. Unlike other Wbl proteins, WhiD did not show disulfide-reductase activity (62). Functional and mechanistic analyses of WhiB proteins from *M. tuberculosis* reveal a variety of roles for the seven paralogs (Table 1).

Several important differences may account for the functional divergence among them. First, the variability in cluster environments and exposure to oxidants indicates differences in the redox potentials, consistent with the unequal O_2 sensitivity among WhiB paralogs (5). Their similar behavior against NO is supported by mechanistic studies evidencing that nitrosylation takes place through a common mechanism in phylogenetically unrelated regulatory proteins (65). Secondly, unconserved amino acid residues between the key CysXXCys motifs will certainly result in different redox potentials for each WhiB paralog and, therefore, different disulfide-reductase activities. As an exception, the WhiB2 paralog lacks disulfide reductase activity and, instead, has a chaperone-like function (Table 1) (198). Besides, a comparative study of the thermal stability of all seven WhiB proteins from *M. tuberculosis* evidences important structural differences among them (5). Moreover, the variability in the sequences of their AT-hook motifs results in different DNA-binding patterns (24). Furthermore, the function of some members, namely WhiB3 and WhiB7 depends on their direct interaction with SigA (38, 353). Finally, the different responses upon induction with CRP as part of the diversity of factors influencing their regulation under different redox environments (115, 209, 411) endow *M. tuberculosis* with a robust,

versatile redox-responsive system to successfully infect and survive in hostile environments.

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B. Redox-sensing by thiol-based switches

The activity of transcriptional regulators with thiol-based switches builds on the oxidation state of cysteine thiol groups. Thus, –SH groups can be reversibly oxidized upon exposure to redox active compounds so that a chemical signal is transformed into a biological signal through a conformational change in the regulator that modifies its DNA binding affinity. Several classes of structural changes undergone by the regulator can be observed: major reorganization of the polypeptide backbone in association with disulfide redox-activity, order/disorder transitions, changes in the quaternary structure or disulfide oxidation following the expulsion of metals (95). A comprehensive review of a large number of bacterial thiol-based redox sensors that specifically sense ROS, reactive electrophile species (RES) and HOCl via thiol-based mechanisms and regulate gene transcription is available (149). In the next section, an overview of how these proteins are structurally influenced by the formation of disulfide bonds or other oxidative modifications is provided.

1. Sensors involving cysteine-zinc clusters

1.1. DksA

Prolonged oxidative stress leads to the inactivation of key enzymes of the central metabolism, leading to nutritional starvation. As a response, a transcriptional program known as the stringent response provides bacteria with survival advantages and efficient environmental adaptation (295). The major regulatory component of the stringent response are hyperphosphorilated guanines ((p)ppGpp) whose accumulation in the bacterial cell modifies the transcriptional profile through the binding to RNA polymerase, inducing a large-scale restructuration of metabolic gene expression (366). Together with the alarmone (p)ppGpp, the global regulator DksA is critical for the stringent response of most Gram-negative bacteria. Without binding to DNA, as a consequence of amino acid limitation, DksA binds to the RNA polymerase secondary channel, destabilizing the open

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promoter complex and impairing transcriptional initiation. In this way, bacteria quickly reprogram transcription in response to changes in nutrient availability (287).

Beyond its participation in the stringent response and independently of (p)ppGpp, DksA works as a redox-dependent global regulator that has different organism-specific functions (Fig. 5). In *Legionella pneumophila*, DksA is required for pathogenicity and is critical for differentiation, flagellar gene activation, lysosome avoidance, and macrophage resistance (74). DksA also contributes to efficient symbiosis between *Sinorhizobium meliloti and Medicago sativa (395)*. In *P. aeruginosa*, DksA is required for the secretion of extracellular virulence factors through posttranscriptional control of *las* and *rhl* quorum-sensing systems (176). In *Salmonella enterica* DksA plays a central role in the coordination of antioxidant defences through the modulation of glutathione biosynthetic genes and the central metabolism (138). In this way, DksA controls the NAD(P)H/NAD(P)$^+$ redox balance that, in turn, fuels downstream antioxidant enzymatic systems essential for adaptation to nutrient starvation (138).

In most Gram-negative bacteria, DksA consists of a coiled-coil domain separated from the C-terminal α-helix by a hinge region containing a 4-cysteine zinc finger motif (290). The coiled-coil domain presents an AspXXAspXAla motif in the loop at its tip that is essential for the protein function (220), while the relationship between the presence of Zn^{2+} and the functionality of the protein seems more complex. It has been established that thiols in the 4-cysteine zinc finger motif sense oxidative and nitrosative stresses by releasing the zinc ion, independently of the second messenger ppGpp (Fig. 5) (139). Zn^{2+} release causes an evident loss in α-helicity of the protein, likely due to disulfide bond formation, since it can be reverted by DTT. This oxidation, which has been suggested to occur in the complex DksA-RNA polymerase, converts DksA into a stronger repressor of down-regulated genes or, alternatively, fails in the transcriptional activation of DksA-activated promoters, increasing the threshold of the stringent response. These results are consistent with a role for Zn^{2+}-bound thiolates as redox sensors of nitrosative and/or oxidative stress and evidence the ability of 4-cysteine DksA to rapidly integrate nutritional, oxidative and nitrosative signals into a coordinated transcriptional response (139, 162).

Interestingly, under conditions of zinc limitation, some organisms express DksA paralogues that do not contain zinc, such as DksA2 from *P. aeruginosa,* which is functional in

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regulating RNA polymerase and successfully complements a *dksA* deletion mutant in *E. coli* (109). In Pseudomonadales, DksA2 is also involved in zinc homeostasis under the control of the Zur regulator (25). Both paralogues from *Pseudomonas* have been used to complement a *S. enterica* strain defective in *dksA* in order to gain more insights into the relationship between zinc content in DksA and redox homeostasis (68). This study shows that both four-cysteine, zinc bound and two-cysteine, zinc-free DksA proteins are functional in mediating the stringent control in *S. enterica* and conserve the ability to sense reactive species via thiol oxidation. However, zinc-containing DksA proteins were more tolerant to oxidative or nitrosative stresses than cysteine-free DksA homologues, revealing a redox-active sensory function for DksA. Following these observations, the authors proposed that zinc would work as an antioxidant, dampening cysteine reactivity against moderate levels of reactive species.

In addition to DksA2 from *Pseudomonas*, other atypical DksAs lacking two or three of the four cysteines making up the zinc-finger motif have been described in the alpha-proteobacteria *Caulobacter crescentus*, the rhizobia *S. meliloti* and some strains of the purple bacterium *R. sphaeroides*, among others (139, 219, 395). However, all these DksA proteins contained a highly conserved cysteine, namely C114, surrounded by several charged and hydrophobic residues that stabilize the thiolate form and are usually involved in thiol-mediated sensing of reactive species, as in the cases of thiol-based redox sensing of OhrR and OxyR (379), and cyanobacterial FurA, reviewed in the following section (33).

1.2. TraR

TraR is a 73-amino acid protein that exhibits 29% sequence identity with the C-terminal half of DksA (26). TraR is encoded in the *E. coli* F element and appears to be ubiquitous in bacteria even in phyla distant from the Proteobacteriaceae. In spite of its smaller size, expression of TraR compensates for *dksA* activities in vivo, even in the absence of the alarmone ppGpp. TraR contains one zinc ion coordinated by 4 cysteines that are located in positions equivalent to those of DksA (130). Unlike DksA, TraR variants with single cysteine to alanine substitutions of residues corresponding to the zinc binding motif in DksA, were unable to complement a Δ*dksA* strain for growth in minimal medium, suggesting that zinc

coordination is essential for TraR activity. Whether the TraR zinc finger motif can be involved in redox sensing, remains to be investigated.

1.3. RsrA

RsrA functions as an σ^R-specific zinc-associated anti-sigma factor (ZAS) that inhibits σ^R-directed transcription under reducing conditions, regulating the response to thiol oxidative stress in the cytoplasm of *Actinobacteria* (7, 73, 277). *S. coelicolor* RsrA contains seven cysteines, being three of them, Cys11, Cys41 and Cys44, essential for redox sensing *in vivo* and *in vitro*. All three cysteines, along with His37 coordinate a single zinc ion in both, RsrA and the RsrA-σ^R complex [168, 169]. Disulfide stress-inducing compounds lead to the formation of a degenerate trigger disulfide bond between Cys11 and either Cys41 or Cys44 that displaces the zinc and causes a dramatic structural change. As a result, σ^R dissociates from RsrA, thereby allowing σ^R-dependent transcription (Fig. 6) [170]. RsrA utilizes its hydrophobic core to bind to the sigma factor σ^R preventing its association with RNA polymerase. Zinc plays a central role in maintaining this high-affinity complex. The system can be reset by the reduction of RsrA by cellular thiol-disulfide oxidoreductases such as thioredoxins, whose transcription is activated by σ^R [171]. Several σ^R target genes have been identified. Their products include thioredoxin systems (TrxBA, TrxC), the protein MshA involved in mycothiol synthesis, mycoredoxin-1 (Mrx-1), proteolytic components (Lon, PepN, ClpX), UV resistance components (UvrA system) and proteins involved in cysteine production (CysM), methionine sulfoxide reduction (MsrA, MsrB), guanine synthesis (GuaB), ribosome-associated function (RpmE, RelA) or detoxification of electrophiles (179, 182, 189). Apart of the control of σ^R by the antisigma factor RsrA, SigR activity is also controlled at the translation level (99).

2. Non-metallated thiol-based switches

2.1. OxyR

OxyR was the first transcriptional regulator discovered to have the ability to sense ROS. It regulates the expression of defensive genes against the harmful effect of H_2O_2 such as those encoding catalases (*katG*), alkyl hydroperoxide reductases (*ahpCF*) or superoxide dismutases (*sod*) (58), as well as others involved in iron homeostasis, including the master

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regulator Fur (*fur*) and the mini-ferritin Dps (*dps*) (413). Furthermore, genes related to quorum sensing (*rsaL*), protein synthesis (*rpsL*), oxidative phosphorylation and respiration (*cyoA* and *snr1*) also belong to the OxyR regulon (387, 391) (Table 2). OxyR from *E. coli* is activated in response to peroxide stress via an intramolecular disulfide bond between the conserved cysteines Cys199 and Cys208 (211) (Fig. 7). However, in some bacteria OxyR functions as a repressor in its reduced form by binding to a more extended region of the target promoters than in its oxidized state, occluding RNA polymerase binding. In particular, it has been demonstrated that the catalase expression in *Corynebacterium glutamicum* follows a negative regulation by OxyR (365) (Fig. 7C) and a repression/activation mechanism of catalase control by OxyR has been reported in *P. aeruginosa* PA14 and *Neisseria meningitidis* (141, 159) (Fig. 7D). As other members of the LysR family of transcriptional regulators, the OxyR fold consists of two domains: the N-terminal DNA binding domain containing a helix-turn-helix (HTH) motif, and a C-terminal regulatory domain (319, 363). In reduced OxyR, the regulatory domain consists of two α/β domains that exhibit a similar folding pattern. The redox active Cys199 resides between two α/β domains whereas Cys208 is located at the lower part of one of domains separated from Cys199 by ~17 Å (Fig. 7B). Upon disulfide bond formation between Cys199 and Cys208, the short helix formed by residues 199-203 in the reduced structure uncoils leading to a significant rearrangement of the secondary structure of the domain that allocates Cys208 (55). The oxidation of OxyR involves changes in the orientation of monomers in the dimer, compared to the reduced form, that affect the inter-dimer orientation in the tetramer and eventually the binding to DNA (Fig. 7A). Oxidation of OxyR by H_2O_2 proceeds in a two-step mechanism that involves selective oxidation of Cys199 to form sulfenic acid (Cys199-SOH) and its subsequent reaction with Cys208, resulting in an intramolecular disulfide bond (211). The structure of a *P. aeruginosa* OxyR mutant in which the peroxidatic cysteine (Cys199) was substituted by an aspartate to mimic the sulfinic acid moiety contained an H_2O_2 molecule near the mutated aspartic acid residue, suggesting that deprotonation of Cys199 and the donation of the proton to H_2O_2 are coupled. According to this mechanism and the results of susceptibility tests to H_2O_2 of different *P. aeruginosa* OxyR variants *in vivo*, it is required that H_2O_2 binds to the catalytic pocket in order to react with Cys199-SH (174). Moreover, the crystal structure of the full-length *P.*

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aeruginasa OxyR shows that it has a tetrameric arrangement assembled via two distinct dimerization interfaces. Thus, the OxyR tetramer consists of two compact subunits and two extended subunits. Each subunit is composed of a DNA binding domain and a regulatory domain which are connected by a short hinge region. Four DNA binding domains are arranged in the bottom of the tetramer. Polar interactions between the DNA binding domain and the regulatory domain occur in the compact subunits, whereas none were observed between those in the extended subunits. The dimeric interface at the DNA binding domain is formed by hydrophobic interactions together with some polar interactions, suggesting that the DNA binding domain dimers are relatively stable even upon structural changes in the regulatory domains.(174). In some pathogenic bacteria *oxyR* is found in an operon with *regG* helicase gene. For some of them, such as *P. aeruginosa*, *P. putida* and *E. coli* it has been reported that purified RecG binds to the promoters of many OxyR controlled genes and that expression of these genes is not induced under conditions of oxidative stress in RecG mutants suggesting that induction of the OxyR regulon might require unwinding palindromic DNA by RecG for transcription (403).

However, the OxyR thiol-disulfide switch model appears to be more complex since this regulator can be activated by different post-translational thiol modifications. In this way, anaerobic respiration on nitrate of *E. coli* cells revealed that S-nitrosylation of OxyR induced transcription from a regulon that is distinct from the regulon induced by OxyR oxidation. Interestingly, the expression of those anaerobically controlled genes was found to protect against S-nitrosothiols (329). Also, cysteine overoxidation has emerged as a mechanism of regulation of OxyR1 and OxyR2 from *Vibrio vulnificus*. Both, OxyR1 and OxyR2 are 2-Cys OxyRs that show different sensitivity to H_2O_2 and induce expression of two different peroxidases (Prx1 and Prx2) in defense to oxidative stress (190). Unlike OxyR1, OxyR2 exhibits limited sequence similarity to other OxyR proteins and is more sensitive to H_2O_2. Structural data suggest that a glutamic acid (Glu204), (position occupied by glycine in other OxyR proteins) in the vicinity of the peroxidatic cysteine (Cys206) is important to provide in that region the rigidity necessary for different H_2O_2 sensing (175). According to mass spectrometry data, high levels of H_2O_2 lead to the overoxidation of Cys206 to S-sulfonated cysteine (Cys-SO_3H) *in vitro* and *in vivo,* deterring *prx2*

transcription. In this way, the production of useless Prx2 under inactivating levels of H_2O_2 is avoided (18).

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2.2. PpsR/CrtJ

PpsR, directly or indirectly controls the synthesis of all the different components of the photosystem in purple bacteria. It belongs to the LuxR family of transcriptional regulators and is redox sensitive through the formation of an intramolecular disulfide bond (171). Unlike OxyR, formation of this disulfide bond in PpsR is insensitive to the addition of H_2O_2 but is sensitive to O_2 indicating a different mechanism of disulfide formation from that used by OxyR where disulfide bond formation is stimulated by trace amounts of hydrogen peroxide even under reducing conditions (Fig. 8) (12).

The PpsR proteins have been mainly characterized in two related species *Rhodobacter capsulatus* and *R. sphaeroides*, although in the latter the PpsR orthologue is named CrtJ. PpsR and CrtJ have similar behavior. Under oxidizing conditions, both proteins bind to a palindromic ($TGTN_{12}ACA$) motif and block transcription of bacteriochlorophyll, carotenoid, light harvesting, or respiratory gene expression (*puf* and *puhA* operons) (357). In addition to photosystem genes, direct targets of PpsR repression are genes involved in the early steps of tetrapyrrole biosynthesis (*hemC* and *hemE*) in *R. sphaeroides* (254). *R. capsulatus* active site titration data support an octameric PpsR species for DNA binding (Fig. 8) (393). PpsR and CrtJ share 53% amino acid identity and the presence of a HTH DNA binding motif placed at the C-terminal region. In PpsR from *R. sphaeroides*, the redox dependent DNA binding response relies on the formation of an intramolecular disulfide bond between Cys251 and Cys424 (49, 240). However, direct evidence of the formation of this disulfide bond between homologous Cys249 and Cys420 in *R. capsulatus* CrtJ has not yet been obtained. A comparison with other PpsRs amino acid sequences indicates that only the cysteine residue located in the HTH domain is conserved so that a general scheme for modulating PpsRs includes, apart from disulfide bond formation, the change of the redox state of that thiol into diverse derivatives (sulfenic, sulfinic or sulfonic acid). Thus, alteration of the redox state of Cys420, beyond disulfide bond formation, is a major contributor to redox regulation of CtrJ DNA activity (49). *In vivo* labeling with 4-(3-azidopropyl)cyclohexane-1,3-dione indicates that Cys420 is *in vivo* modified and forms

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sulfenic acid when cells are exposed to O_2. Moreover, the substitution of Cys420 by serine, an amino acid that mimics a cysteine sulfenic acid, results in a ~4-fold increase of DNA binding activity. However, Cys420 to alanine mutation that leads to a ~60-fold reduction of DNA binding activity. Since mutations of Cys249 to alanine and serine behave as the wild type strain, it can be assumed that the stimulation of DNA binding activity is not only achieved by disulfide bond formation between both cysteines (49), suggesting that different homologues are regulated by different types of oxidizing situations. The DNA binding mechanism of the PpsR proteins from other organisms show variations. *Bradyyrhizobium* and *Rhodopseudomonas palustris* regulation of photosystem synthesis depends on the light quality and O_2 tension conditions. It involves the unexpected dual action of two different regulators, PpsR1 and PpsR2, which have a strong similarity with PpsR/CtrJ from the *Rhodobacter* species in their predicted architectures, DNA recognition sequences, and photosynthesis target genes. However, they show fundamental differences with the PpsR/CtrJ family of regulators. In particular, PpsR1 is a redox sensitive activator through the formation of a disulfide bond that unlike PpsR is intermolecular. Furthermore, oxidation of PpsR1 remains very limited in response to O_2. Unlike PpsR1, PpsR2 does not contain cysteine residues and is not redox sensitive. Therefore, the DNA binding affinity of PpsR2 is redox independent (171). Regulation of this type of regulator can undergo further modulation by its association with other proteins. In *R. sphaeroides* the light-sensing anti-repressor AppA inhibits DNA binding of PpsR by two mechanisms. One mechanism involves AppA-mediated reduction of the disulfide bond in PpsR. The second mechanism entails the formation of a stable AppA-$Ppsr_2$ complex that prevents PspR binding to DNA (Fig. 8) (239). In contrast to PpsR from *R. sphaeroides*, the repressive activity of CtrJ from *R. capsulatus* is not antagonized by AppA.

2.3. OhrR

The OhrR family of regulators sense organic hydroperoxides (OHP) and other ROS by oxidation of a critical and highly conserved cysteine residue. OhrR belongs to the MarR (Multiple antibiotic resistance-type regulators) superfamily of transcriptional regulators. It primarily regulates the expression of organic hydroperoxide reductase (Ohr) but also genes related to the detoxification of peroxides (antioxidant enzymes and thiol-reducing

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systems), the degradation of the aromatic moiety of the model compound cumene hydroperoxide and genes involved in the protection against other secondary stresses (DNA repair, heat shock, iron limitation, and nitrogen starvation responses) or virulence (113, 298, 328). Ohr also responds to NaOCl stress since transcriptome studies indicated that *ohrA* gene was the most strongly up-regulated gene in NaOCl stressed *B. subtilis* (52). Two OhrR subfamilies have been described based on their peroxide sensing mechanism (Fig. 9): the single cysteine class, represented by *B. subtilis* OhrR, whose DNA binding activity is modulated by cysteine oxidation and the second class, represented by *Xanthomonas campestris* OhrR that requires the reversible formation of a disulfide bridge between two cysteines, either intersubunit or mixed, to modulate the repressor function (149). In both 1-Cys and 2-Cys OhrR subfamilies, the initial step leading to transcription derepression mediated by peroxide involves oxidation of a sensing cysteine to sulphenic acid (Cys-SOH) that is not sufficient to derepress transcription. In the first case (Fig. 9A), *B. subtilis* Ohr has a single, conserved cysteine (Cys15) that is ionized at physiological pH (151, 216). According to *in vitro* studies, exposure of OhrR to model organic hydroperoxides results in oxidation of Cys15 to sulfenic acid (107). The subsequent reaction of the Cys15 sulfenate with a low molecular weight thiol, to generate a mixed disulfide or with the backbone of the protein, to generate a sulfenamide derivative correlates with transcription derepression (216). In this sense, *in vivo* changes in the transcriptome and redox proteome of *B. subtilis* caused by the strong oxidant hypochloric acid identified OhrR as a S-bacillithiolated protein, indicating that OhrR forms mixed disulfides with the redox buffer bacillithiol leading to inactivation of the OhrR repressor and up-regulation of the thiol-dependent OhrA peroxiredoxin to protect the cells against organic hydroperoxides and NaOCl (52). OhrR homologues that contain a single cysteine have been characterized in *S. coelicolor* (270) or *Mycobacterium smegmatis*, where OhrR is induced by organic hydroperoxides in the intracellular environment upon ingestion of the bacteria by macrophages (113). In the 2-Cys OhrR-type described in *X. campestris* OhrR (Fig. 9B), a reactive cysteine (Cys22) located in the N-terminus is oxidized by OHP to a sulphenic acid intermediate and undergoes the rapid formation of an intermolecular disulfide bond with residue Cys127 of the other subunit in the homodimer, leading to major structural change (263, 281). Disulfide-linked dimer formation induces the dissociation of OhrR from DNA

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and the expression of a peroxidase that reduces OHP to their corresponding alcohols (263, 281). A 2-Cys OhrR-type homologue has been reported in *P. aeruginosa* (13, 14). Therefore, depending on their amino acid content OhrR proteins isolated from different bacteria can exhibit varied DNA binding properties. Even oligomerization can be important in controlling OhrR activity. *Burkholderia thailandesis* OhrR forms oligomeric species by virtue of reversible disulfide bonds formed between redox-active cysteines on treatment with organic and inorganic oxidants. These disulfide bonds involve conformational changes that result in attenuated OhrR DNA binding in the presence of oxidants (280). Furthermore, binding of the small molecule 2-aminophenol to *Burkholderia xenovorans* LB400, which lacks oxidizing properties, attenuates the regulator affinity for its DNA operator sequence by promoting a conformational change in the regulator. In this case, 2-aminophenol functions in *B. xenovorans* as a typical effector molecule. It plays a role in the response to ROS by this organism because 2-aminophenol is an intermediate in tryptophan or nitrobenzene metabolism and can be metabolized to generate ROS but it does not affect the redox state of cysteines (264).

In *Staphylococcus aureus*, two homologues of the MarR/OhrR 1-Cys-type repressor are present, the MgrA and SarZ global regulators that confer antibiotic resistance and virulence (17, 178, 369). The MgrA regulon includes a battery of genes involved in virulence (*cap5(8)*-locus, *hla*, *coa*, *spa*, *splABCDEF*, *nuc*), autolysis (*lytM*, *lytN*), antibiotic resistance (*norA*, *norB*, *tetAB*), as well as virulence regulators (*agr*, *lytRS*, *arlRS*, *sarS*, *sarV*) (232). According to MgrA homodimer crystal structure, a unique cysteine residue located at the protein dimer interface can be oxidized by H_2O_2 and OHPs leading to dissociation of MgrA from DNA (47). MgrA activity can also be reversibly regulated by cysteine phosphorylation (355). In the same way, SarZ can be controlled by cysteine phosphorylation (355). SarZ is a global transcriptional regulator that uses a single cysteine (Cys13) to sense peroxide stress and control genes involved in hydroperoxide resistance (*ohr*, *hla*, *agr*), hemolysin production and virulence regulation in *S. aureus* and even can be involved in biofilm formation as reported in *Staphylococcus epidermidis* (48, 178, 389). Structural data indicate that protein with Cys13 sulphenic acid modified is competent to bind to DNA. A further reaction with an external thiol is necessary to disrupt SarZ DNA

binding ability (294). It has been suggested that *S. aureus* MgrA and SarZ could be controlled by *S*-bacillithiolation (163).

Besides MarR/OhrR family of redox sensors there are other MarR-type regulators that belong to the MarR/DUF24 subfamily, conserved in Gram-positive bacteria (7). Unlike OhrR, in *B. subtilis* DUF24 family regulators respond specifically to RES (diamide, quinones, aldehydes) instead to ROS (6). In particular, YodB regulator (renamed QsrR) controls the azoreductase AzoR1 and also regulates the expression of the *catDE* operon (catechol-2,3-dioxygenase CatE and oxidoreductase CatD), that belong to detoxification pathways that confer resistance to quinones and diamide (53, 218). YodB contains three cysteine residues (Cys6, Cys101 and Cys108) that are involved in its mechanism of inactivation. The accepted model of functioning indicates that upon treatment with diamide and quinones YodB is inactivated by formation of a Cys6-Cys101 intersubunit disulfide both *in vitro* and *in vivo* (51). *B. subtilis* also contains HypR (formerly YybR), another MarR/ DUF24 protein. It is activated by Cys14-Cys49 intersubunit disulfide formation, entailing reorientation of the monomers and repositioning of α-helices that are involved in major groove recognition (278). HypR is a positive regulator of the nitroreductase HypO that confers NaOCl resistance and is induced by NaOCl, diamide and quinones (278).

2.4. NemR

The NemR repressor (formerly named YdhM) belongs to the TetR family of transcriptional regulators and responds to cysteine-modifying electrophiles, alkylation and reactive chlorine species (RCS) (375). The oxidation of cysteine residues by RCS is a reversible process that leads to a decrease in NemR DNA binding affinity and the consequent derepression of transcription of the NemR-controlled genes *gloA* and *nemA*. The *gloA* gene encodes glyoxalase I (GlxI), the first enzyme of the glyoxalase system for the conversion of toxic alpha ketoaldehydes into non-toxic 2-hydroxycarboxilic acids. The *nemA* gene encodes N-ethylmaleimide reductase, an enzyme involved in reductive degradation of N-ethylmaleimide (NEM) and other nitrous compounds (132, 375). Both gene products contribute to detoxification of toxic compounds that can be reused as nitrogen sources. In fact, phenotypic studies suggest that deletion of *gloA* and *nemA* increases the HOCl sensitivity of *E. coli* cells since both contribute to increased bleach resistance in *E. coli* by

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detoxifying reactive electrophiles produced during RCS stress (132). However, the response in NemR to RCS does not depend on any commonly known oxidative cysteine modifications. According to crystal structure data of *E. coli* NemR, RCS treatment of NemR results in the formation of a reversible Cys106-Lys175 sulfenamide bond that is favored by the inherent structural flexibility within the EF loop. In this case, the formation of the bond allows control of gene expression while the overall architecture of the protein is maintained (131).

2.5. FurA

Fur is the master regulator of iron homeostasis in most heterotrophic bacteria, where it works as a classical repressor. In a simplified model of regulation, Fur binds to its target sequences using Fe^{2+} as co-repressor to block the transcription of an ample regulon (92, 101). Fur belongs to a superfamily including homologues that control processes intimately linked to redox homeostasis, such as PerR (response to peroxide stress) and Zur (control of zinc homeostasis). Remarkably, peroxide transduction by PerR is carried out through a completely different mechanism than that described by Fur (discussed in the next section). Both PerR and Zur can work with Fur to coordinately regulate a set of genes involved in the response to oxidative stress or virulence, among other important processes (152, 325, 368). Furthermore, anaerobiosis affects the gene expression programs of Fur and the small RNA regulator RyhB in *E coli* K-12. The impact of O_2 availability on the Fur regulon suggests a change in the set point for iron homeostasis and evidences the relationship between Fur and redox regulation (21, 22). This link has also been reported for *Helicobacter pylori* where Fur mediates the response to oxidative stress by an allosteric regulatory mechanism that specifically targets iron inducible apo-Fur repressed genes (289).

Usually, Fur proteins contain a structural Zn^{2+} ion that is absent in the cyanobacterial regulator (142). Thus, the lack of structural Zn^{2+} in FurA from *Anabaena* sp. PCC 7120 elicits a redox-response controlled by thiol-disulfide interconversion mediated by cysteines belonging to CysXXCys motifs which usually are involved in the coordination of Zn^{2+} in the regulators from heterotrophic bacteria (41, 101, 383). In this way, FurA couples iron homeostasis and the response to oxidative stress with major physiological processes in cyanobacteria (125, 126). The cyanobacterial FurA regulon contains genes that belong to

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diverse functional categories including iron homeostasis, photosynthesis and respiration, heterocyst differentiation, oxidative stress defense and light-dependent signal transduction mechanisms, among others (125, 127-129). FurA contains five cysteine residues, four of them arranged into two active CysXXCys redox motifs (Cys101XXCys104 and Cys141XXCys144) located in the C-terminal domain of the protein (dimerization domain). FurA needs not only metal but also reducing conditions to remain fully active *in vitro* (143), and both CysXXCys motifs display disulfide reductase activity (31). Notably, Cys141 is also part of a CysPro heme regulatory motif (HRM) (discussed in section E). Moreover, FurA is mainly a monomer with a single free cysteine in the cytoplasm of *Anabaena* sp. PCC 7120 at the stationary phase, suggesting the ability of this regulator to form two disulfide bonds. A mutational study of single cysteines introduced in FurA revealed that Cys101 and its particular redox state is critical for the coordination of the metal co-repressor which ultimately controls the FurA ability to bind to DNA *in vitro*. When Cys101 is oxidized, FurA loses the metal and dissociates from the DNA. Taking into account that the redox status of Cys101 varies with the presence or absence of Cys133 or Cys104 from the Cys101XXCys104 redox motif, the environments of these cysteines are apparently mutually interdependent suggesting a mechanism of FurA activation/inactivation based on a thiol/disulfide redox switch that involves these cysteines and controls the redox state of Cys101 which coordinates the co-repressor metal. Accordingly, Cys133 would be responsible for maintaining Cys104 in the oxidized state to avoid Cys101-Cys104 disulfide bond formation and consequent inactivation of the protein (Fig. 10). This thiol-disulfide exchange of FurA responds to the alteration of the cellular redox potential (33). Apparently, this mechanism is specific for cyanobacterial Fur homologues since it relies on Cys133, a residue conserved in cyanobacterial Fur homologues but absent in Fur homologues from heterotrophic bacteria. The FurA redox switch resembles that described for RsrA in the previous section. In both cases, a disulfide bond between both cysteines of a CysXXCys motif controls the redox state of a third cysteine that coordinates the metal ion. However, whereas in RsrA the coordination of Zn^{2+} keeps cysteines in a reduced state determining oxidation kinetics of this regulator, in FurA the Fe^{2+} that binds to the reduced cysteine plays a role as co-repressor metal, coordinating in this way iron homeostasis and redox responses.

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C. Regulation by metal-catalyzed oxidation: PerR The metal-catalyzed oxidation of histidine to 2-oxo-histidine is an important marker of oxidative stress commonly associated to the regulation of enzyme activity (322). Unlike other Fur paralogs that regulate their target genes in response to the availability of different metals, PerR activity is based on metal-catalyzed oxidation of a histidine residue located in the conserved HisHisHisXHisX2CysX2Cys motif positioned at the hinge between the metal-sensing C-terminus and the DNA-binding domain of Fur proteins (173). Oxidation of PerR leads to loss of the iron cofactor and its dissociation from DNA to derepress transcription of genes involved in the antioxidant response. The mechanistic differences between Fur and PerR from heterotrophic bacteria have recently been reviewed (291). PerR may function as both activator and repressor of gene expression. Targets include antioxidant enzymes, virulence genes and other regulators (36, 306, 377).

D. Methionine oxidation-based transcriptional regulation: HypT

The *E.coli* hypochlorite-responsive transcription factor HypT (formerly YjiE) positively and negatively regulates the expression of several genes in response to HOCl oxidation (83). HypT belongs to the LysR family of transcriptional regulators (319), and is activated through the oxidation of three methionine residues (Met123, Met206 and Met230) to methionine sulfoxide. Most of the genes that are positively regulated are involved in the biosynthesis of cysteine and methionine, whereas most of the genes that are negatively regulated are involved in iron acquisition and homeostasis (114). This could indicate that the intracellular pool of cysteine and methionine must be replenished in response to oxidative damage, whereas the intracellular concentration of iron, which could enhance the production of superoxide and hydroxyl radicals, must be kept at low levels (94). HypT has different multimeric forms. On binding to DNA, the dodecameric ring-like structure of HypT dissociates into an active tetrameric form that acts as a transcriptional activator. The current model proposes that the oxidation of methionine residues promotes the transition of the inactive dodecameric form of HypT to the active tetrameric form (83). Methionine sulfoxide reductase A (MsrA) and MsrB are required to reverse the oxidation state of the oxidized methionine residues, thus inactivating the activity of HypT1 (83).

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E. Redox-sensing by heme-based sensor proteins

Bacterial heme-based sensor proteins exploit the redox chemistry of heme to sense environmental gases (e.g., O_2, CO, NO) (96) and the intracellular redox state of the bacterium. These particular sensor proteins typically contain two distinct domains, a heme-containing regulatory domain and a catalytic domain. Gas binding to the heme-containing regulatory domain regulates the catalytic domain function, including binding to DNA (153).

1. CooA

CooA (bacterial CO oxidation transcriptional activator) is a heme-binding protein that controls the expression of a regulon allowing anaerobic growth of *Rhodospirillum rubrum* upon CO oxidation (331). It belongs to the CAP/CRP superfamily and is distantly related to the O_2 sensor FNR and the denitrification regulator/nitric oxide reductase regulator (DnrD/NNR) group of NO sensors. Its heme-binding is an example of a heme-containing regulatory domain where the intracellular redox status, heme and DNA-binding activity are related. CooA is a homodimer and each monomer contains a *b*-type heme as the active site for sensing CO. The structure of CO-free Fe^{2+} CooA (inactive for DNA binding) has been solved and although the structure of the CO-bound Fe^{2+} CooA (active for DNA binding) has not yet been determined, experimental data and comparisons with the crystal structure of the active form of the CRP homologue bound to DNA have enabled a model of operation to be proposed (10, 206). Apparently, this protein exists in the cell in three general heme states (Fig. 11). Under oxidizing conditions low-spin Fe^{3+} heme is axially coordinated by Cys75 and Pro2, a residue located in the N-terminus of each protein monomer. In this situation, the protein is unable to associate to CO and consequently to bind to specific DNA sequences efficiently. In reducing conditions, Fe^{2+} heme is obtained and Cys75 is replaced by His77 as an axial ligand of ferrous iron. Therefore, a redox-dependent axial ligand exchange between Cys75 (ferric form) and His77 (ferrous form) occurs on reduction of the heme iron (332). After exposure of RrCooA to CO under anaerobic conditions CO binds to Fe^{2+} heme and displaces the Pro2 iron ligand (Fig. 11A) (9). Displacement of Pro2 entails a conformational change leading to a reposition of the heme exposing the CO-bound heme to the long α-helices (C-helices) that extend along the homodimer interface

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(Fig. 11B) (188, 406). This interaction stabilizes an alternative conformation of the domain containing these C-helices that alters the hinge region separating the DNA- and CO-binding domains. When the geometry of the hinge region alters, the inactive form of CooA destabilizes and the active form stabilizes (405). Reorientation of CooA DNA-binding domains is necessary to produce a transcriptionally active state (157). A semi-apo state of the CooA dimer, with a heme-bound monomer in a CO-bound form of *Carboxydothermus hydrogenformans* CooA structures reveals a heme and C-helix displacement that support this model (30). The reduction midpoint potential of ferric CooA with cysteine thiolate as an axial ligand is -320 mV whereas the heme having histidyl imidazole as an axial ligand shows an oxidation midpoint potential of -260 mV (259). The difference between the reduction and oxidation midpoint potentials seems to be caused by the redox-controlled ligand exchange of the heme between Cys75 and His77. Since only ferrous CooA binds CO, the low oxidation potential of CooA would facilitate the oxidation of the heme in order to prevent CooA activation *in vivo*, once O_2 is present in the cells. Therefore, RrCooA is an example wherein the binding of O_2 leads to the oxidation of heme iron Fe^{3+} and the inactivation of the protein function under normoxic conditions. This oxidation links the redox state of the cell to the sensing capability of CooA. Oxidized CooA must be reduced upon anoxia in order to bind CO (96).

CooA modulates the expression of the *coo* regulon, which allows the CO-dependent anaerobic growth of *R. rubrum* (405). The CO oxidation system is encoded by two CO-regulated transcriptional units, *cooMKLXUH* and *cooFSCTJ*. The key products of this regulon are the O_2-sensitive CO dehydrogenase CooS, the CooS-associated Fe-S protein CooF, and the CO-tolerant hydrogenase CooH.

2. RcoM

RcoM (regulator of CO metabolism) is a CO-sensing transcription factor that undergoes a redox-mediated ligand switch and may utilize redox active heme to sense the redox state of the cell. It couples an N-terminal PAS fold (like the mammalian NPAS2 CO sensor) to a C-terminal DNA-binding LytTR domain, but its particular heme ligation characteristics, DNA binding modules, and organization of domains are different from those reported for mammalian sensors (187). In different organisms, it appears to regulate *coo* (encoding

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proteins of the CO-oxidizing H_2-producing enzymatic complex) and *cox* (encoding the aerobic CO oxidation system) gene expression. RcoM1 and RcoM2 from *B. xenovorans* are the most extensively characterized RcoM proteins. Both activate the expression of the *coxM* gene according to *in vivo* reporter assays where *lacZ* is fused to the *coxM* promoter (187). Similar to the CO sensing *R. rubrum* CooA, *B. xenovorans* RcoM-2 undergoes redox-dependent ligand switching and CO-induced ligand displacement. In the Fe^{3+} oxidation state, the heme is low-spin and six-coordinate with a cysteine (thiolate) as one of the two ligands. The sixth ligand is a histidine (His74), which is present in all states of the protein. Reduction to the Fe^{2+} oxidation state results in replacement of the cysteine (thiolate) with a neutral thioether ligand, Met104. CO binds to the Fe^{2+}*Bx*RcoM-2 heme opposite the histidine ligand. Thus, coordination state changes involve redox-dependent loss of a cysteine (thiolate) ligand and displacement of a relatively weakly bound axial ligand by the effector gas molecule (236). Electronic absorption, resonance Raman and electron paramagnetic resonance spectroscopies have revealed that Cys94 is the distal Fe^{3+} heme ligand in *Bx*RcoM-2 (344).

In the aforementioned heme-proteins, heme exists as a stable and essential prosthetic group. However, recent studies reveal that the function of some proteins is acutely modulated by the reversible binding of heme that acts as a cellular signaling messenger (257). The association/dissociation of the heme iron complex to/from the protein regulates(s) its functions, including catalytic reactions (kinase and proteolysis) or DNA binding. In contrast to heme-based gas sensors where gas molecules (O_2, CO) bind only to the heme Fe^{2+} complex, proteins that are regulated by the reversible binding of heme associate/dissociate to the heme Fe^{3+} complex. Under reducing conditions, a heme-responsive heme sensor can be converted into a heme-based gas sensor (337).

3. Heme sensing by thiol-based switch sensors

Some of the previously described regulators whose mechanism of action relies on a thiol-based switch have also shown the ability to bind heme through redox sensing cysteines affecting their DNA-binding activity. In these cases, the reversible binding of heme plays a pivotal role in up- and down-regulation of transcription factors. Heme-responsive proteins of this type display fast, easy heme association and dissociation. They contain a HRM

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characterized by the presence of a CysPro sequence. The cysteine of the CysPro motif is an axial ligand of Fe^{3+}-heme, but upon reduction a redox-dependent ligand switch occurs and the cysteine of the CysPro sequence is no longer a ligand of Fe^{+2}-heme (160).

It has been demonstrated for both PpsR and FurA that the binding of heme alters their DNA-binding pattern and inhibits their ability to form higher-order complexes with DNA. Cyanobacterial FurA binds heme in the micromolar concentration range and this interaction negatively affects its *in vitro* DNA binding ability in a concentration-dependent fashion (144). Cys141, within a CysPro motif or HRM (409), is an axial ligand of the Fe^{3+} high-spin heme but it does not bind the Fe^{2+} heme centre, suggesting a redox-dependent ligand switch (288). FurA exhibits the typical physicochemical characteristics just described for a heme sensor protein (160, 161). In the case of PpsR, the Cys424 present in its DNA binding domain is critical for heme interaction. The binding of heme changes PpsR-DNA binding pattern, inhibiting the formation of higher order PpsR-DNA complexes and inducing increased transcription of several PpsR regulated genes. This interaction seems to provide a mechanism for bacteria to react to the unbound tetrapyrrole products since in this way excess heme can quickly change the state of photosynthetic gene expression from inhibition to activation (404). Oxidation of Cys424 in the absence of heme stimulates the DNA binding of PpsR. This redox regulation is also observed while heme is present. It appears that Cys424 could be a versatile target for different types of modification such as forming/breaking disulfide-bridges (239), coordinating heme and potentially being oxidized into different oxidation states. One notable difference with FurA is that an Ile residue follows Cys424 instead of Pro, as would be expected for a typical heme sensing protein. However, PpsR utilizes cysteine as the critical axial residue as is the case for FurA and other HRM-containing heme sensors. Thus it is feasible that Cys coordinated to Fe^{2+}/Fe^{3+} in heme is a conserved strategy for heme sensing (404).

F. Sensors of the NAD^+/NADH balance: Rex

Beyond working as a cofactor for oxidoreductase enzymes, NAD^+/NADH serves as a substrate for a wide range of proteins and provides a direct link between the cellular redox status and the control of signalling and transcriptional events. The ratio of NAD^+ to NADH inside the cell is mainly affected by substrate availability and O_2 tension, driving a set of

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responses aimed at maintaining redox homeostasis. Modification of this ratio is often used in industrial processes in order to improve the productivity of certain metabolites (225, 333, 410).

The main actor that converts the redox signal provided by the NAD^+/NADH balance into a regulatory input is the transcriptional regulator Rex, whose ability to bind DNA is modulated specifically by the NAD^+/NADH ratio (Fig. 12) (133). Rex has been identified and characterized in archaea and eubacteria regardless of their O_2 requirements (154). Overall, Rex is a transcriptional repressor that remains bound to its DNA targets when the NAD^+/NADH ratio is sufficiently high. Under microaerobic or anoxic conditions, NADH competitively binds to the Rex C-terminal domain, causing a conformational change of the Rex homodimer and subsequent release from its recognition sites on DNA, allowing transcription of downstream genes. An estimated value of this ratio for transcription derepression has been calculated in *S. coelicolor*, where the level of NADH has to rise by around 2% to impair the binding of Rex to DNA ~50% (133). Further characterization studies of the *B. subtilis* repressor show that its affinity for NAD^+ is 20.000 times lower than that for NADH. Interestingly, the affinity for NAD^+ increased around 30 fold upon DNA binding, suggesting that there is a positive allosteric coupling between DNA binding and NAD^+ binding (388). The available crystal structures of Rex in complex with DNA and/or NADH (PDB code 2VT3) indicate that Rex comprises an N-terminal wHTH-fold domain interacting with DNA and a C-terminal Rossmann-fold domain binding NADH and mediating subunit dimerization (241, 339, 388).

Key domains involved in DNA-binding and NAD-sensing are broadly conserved in Rex orthologs identified in the phyla *Firmicutes*, *Thermotogales*, *Actinobacteria*, *Chloroflexi*, *Deinococcus-Thermus*, and *Proteobacteria* (302). Similarly, the DNA-binding motifs harbor the conserved consensus TTGTGAANNNNTTCACAA. In anaerobic bacteria, a different Rex regulation mechanism has been proposed (414), which could be the result of subtle variations in the NAD^+/NADH binding motifs of the Rex-family, such as the substitution of Tyr98 by histidine in the regulator characterized from anaerobes. The Tyr98 residue, highly conserved in aerobic bacteria, has been proposed to play a key role in the switching mechanism between open and closed dimers though the interaction with a conserved Asp residue that is essential for binding DNA. Furthermore, the DNA-binding motifs recognized

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by anaerobic Rex-family members exhibit consistent deviations from the consensus established for aerobic bacteria (302, 414). These substitutions in the operator motifs seem essential for optimal protein-DNA interaction and a settling factor for the different structural bases for NAD^+/NADH sensing between aerobic and anaerobic Rex proteins.

Rex regulons have been identified in several bacteria, highlighting the importance of this protein in the control of redox homeostasis, central metabolism or hydrogen production, among other processes (207, 302). In *Clostridium kluyveri* Rex has been described as a global redox-sensing transcriptional regulator (154). In *S. aureus*, Rex acts as a central regulator of anaerobic metabolism leading to anaerobic NAD^+ regeneration. Its regulon comprises at least 19 genes, some of them involved in lactate, formate, and ethanol fermentation (*adh1, adhE, lctP, ldh1, pflBA*) and nitrate respiration (*narG, nirC, nirR*) (276). In *Streptomyces avermitilis*, in addition to regulating aerobic metabolism, Rex also controls avermectin production and morphological differentiation (226). However, a bioinformatic reconstruction of the sets of Rex-regulated genes in 119 genomes from 11 taxonomic groups also revealed remarkable variations in the functional repertoires of candidate Rex-regulated genes in various microorganisms (302), most of them being lineage specific.

III. Two-component systems: redox control of sensor kinase regulation

One of the main mechanisms that allow bacteria to overcome changes in the physicochemical parameters of natural environments is the regulation mediated by two-component systems (199). These systems are usually composed of a sensory protein called sensor kinase or histidine kinase (HK) and a response regulator (RR) protein. The sensor kinase is able to sense the signal and as a consequence the protein performs autophosphorylation of a conserved histidine residue located in the histidine kinase domain. The phosphate is then transferred to an aspartate residue of the response regulator through a process called transphosphorylation. The phosphorylation of the response regulator causes its activation and then the response regulator binds to promoters of target genes modulating their transcription. However, sometimes the response regulator promotes other cellular responses in the cell different to transcriptional modulation. For example, some response regulators contain catalytic

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domains that once activated by phosphorylation are able to carry out enzymatic activities (111). Canonical two-component systems perform transphosporylation in one step, while phosphorelays work in multistage processes to allow a fine-tune regulation of the system. Phosphorelays are found in non-orthodox two-component systems and in hybrid sensor kinases (199).

Sensor kinases may sense a wide variety of environmental signals including single molecules, such as nitrate or citrate, and more complex signals such as light or gas molecules (O_2, CO or NO) (34, 121, 184, 210). In the present review, we focus on sensor kinases that perceive redox signals and respond to these signals modulating the transcriptional regulation of the cell. A summary of the two-component systems reviewed in this work can be found in Table 3.

A. Indirect redox-sensing based in PAS/GAF domains

The widespread Per-Arnt-Sim (PAS) domain functions as a transduction module acting as a sensor of environmental stimuli such us light, redox state, respiration, O_2 and overall energy level of the cells, among others (364). PAS domains are present in both one-component and two-component systems, as well as in many other proteins that may contain an ample range of different domains simultaneously.

The PAS domains have a highly conserved three-dimensional structure, although they exhibit low sequence homology. They consist of approximately 300 amino acid-region with several imperfect repeats, sometimes associated with a PAS-associated C-terminal motif. These motifs contain a conserved sequence of about 40 amino acids at the C-terminal of PAS domains, contributing to the correct structure and folding of the PAS (140).

The highly versatile and multipurpose PAS scaffold can bind a broad range of redox ligands, including heme, flavins and metal ions (140). PAS domains may also determine the specificity of transcriptional factors in modulating the expression of target genes. Some proteins, such as cGMP-specific phosphodiesterases, adenylyl cyclases and FhlA (GAF) contain domains with a very similar fold to PAS, known as GAF domains (150).

Classification of the PAS/GAF proteins is difficult because tandem and multiple PAS domains are common in individual proteins, and often many other domains are also present; about one third of PAS proteins contain two or more PAS domains (140). PAS-

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proteins may include simultaneously PAS domains that bind heme, flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), 4-hydroxycinnamic acid, C3-C4 carboxylic acids (malonate, malate and succinate), C6 carboxylic acids (citrate), and divalent metal cations. It has also been suggested that fatty acids may play a role as a PAS ligand (192). In any case, PAS domains have evolved as multifunction protein modules and they have very diverse functions, including redox sensing.

1. Redox control of sensor kinase regulation involving metal clusters

Sensor kinases are able to sense the presence of signal molecules by using sensor domains (*e.g.*, TodS or CitA) (39, 303), transmembrane domains (*e.g.*, DesK) (72) or even through accessory proteins (*e.g.*, CheA) (273). Redox-sensing is conducted mainly by PAS or GAF domains containing cofactors such as heme, iron-sulfur clusters or FAD and FMN, or using mechanisms based on the oxidation or reduction of cysteine residues. These redox-sensing mechanisms are described in detail below using some model sensor kinases.

1.1. PAS domain-heme: FixL-FixJ two-component system

The FixL-FixJ two-component system is involved in the regulation of nitrogen fixation genes that are tightly controlled by O_2 availability. This system has been widely studied in symbiotic bacteria *S. meliloti* and *B. japonicum* (121, 318). Once the activation of FixL sensor kinase occurs in *S. meliloti*, the response regulator FixJ activates the transcription of two transcriptional factors, NifA and FixK, which induce the expression of *nif* and *fix* genes involved in nitrogen fixation (2, 75, 110, 305). The expression of these genes must be induced in the developing nodule of symbiotic bacteria when the concentration of O_2 remains below 50 µM (349). In *B. japonicum*, FixJ activates the transcription of the FixK2 transcriptional regulator whose targets are *fixNOPQ* and *fixGHIS* operons (296), heme biosynthetic genes (*hemA*, *hemB*, *hemN$_1$*, *hemN$_2$*) (43, 102, 275), denitrification genes (*napEDABC*, *nirK*, *norCBDQ* and *nosRZDFYLX*) (77, 243, 308, 380, 381), and some hydrogen oxidation genes (*hup*) (85).

FixL in *S. meliloti* (*Sm*FixL) contains four transmembrane regions in the N-terminal domain followed by a PAS domain, a HK domain and an ATPase domain (Fig. 13A) (120, 228). In contrast, its ortholog in *B. japonicum Bj*FixL apparently does not contain transmembrane

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regions (8), but holds two PAS domains and the HK domain (Fig. 13B). *Sm*FixL contains a heme moiety located inside its PAS domain (Fig. 13A) and *Bj*FixL inside its PAS2 domain (Fig. 13B). In *Sm*FixL, heme is non-covalently attached to the His194 of the PAS domain (251). Biochemical studies revealed that changes in the spin-state of Fe inside the heme moiety can modulate the regulatory effect mediated by the sensor domain (123). The authors proposed that under oxygen-limiting conditions, no O_2 was bound to the sensory domain and the heme assumed a high-spin state. In this configuration the auto-phosphorylation of FixL was allowed, after which FixL phosphorylated FixJ that in turn activated the transcription of *nifA* and *fixK* genes (Fig. 13C). Conversely, when O_2 was bound to heme, the auto-phosphorylation of FixL was inhibited (Fig. 13C) (122). More recently, a model of an allosteric transduction pathway for *Sm*FixL has been proposed. The authors suggest that the PAS domain undergoes structural changes in the presence of O_2 that are transmitted to the HK domain. In this model, changes in the Tyr201 residue when O_2 is dissociated from FixL trigger conformational changes that increase kinase activity and initiate the signalling cascade (400, 401).

1.2. GAF domain-heme: DosS-DosR two-component system

DosS-DosR is a two-component system involved in the dormancy process of *M. tuberculosis*. The dormant state of this pathogenic bacterium has been related to anaerobic conditions and CO or NO presence, since these conditions are found in infected macrophages (202). DosS (also known as DevS) histidine kinase contains two GAF domains called GAF-A and GAF-B, followed by a HK domain and an ATPase domain (Fig. 14A). The GAF-A domain contains a heme moiety and GAF-B seems to be essential to the folding of GAF-A in the conformation that allows the inhibition of kinase activity (408). The GAF-A heme domain is able to bind different gas molecules such as O_2, NO and CO (203, 215, 317). It has been reported that the hydrogen-bonding network is a key factor in gas-molecule recognition. Two amino acids, Tyr171 and Glu87, seem to play an important role in gas discrimination (19). DosS shows kinase activity in Fe^{2+}deoxy, Fe^{2+}-CO and Fe^{3+}-NO forms and has little activity in the Fe^{3+} and Fe^{2+}-O_2 forms (Fig. 14B) (165, 166). The formation of Fe^{2+}-O_2 complex has been described, but other groups have described the oxidation of Fe^{2+} to Fe^{3+} when it is exposed to O_2 (54, 165, 203, 285, 350). In view of these

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contradictory reports, the detailed mechanism of O_2 sensing remains unknown. On the other hand, it has been reported that DosS can act as a bifunctional enzyme, showing both kinase and phosphatase activities (185). In response to the availability of O_2, NO, CO and ascorbic acid, the DosS-DosR two-component system induces the expression of approximately 50 genes (202, 282, 336, 362). The role of DosR in the regulation of *devRS*, *hspX*, *narK2* and *tgs1* gene expression, among others, has been investigated (Fig. 14B) (44-46).

1.3. PAS Domain-Fe-S cluster: NreB-NreC two-component system

Some sensor kinases use FeS-containing PAS domains to sense changes in O_2 tension. NreB sensor kinase is the cognate partner of the NreC response regulator, both present in *Staphylococcus carnosus* and in *S. aureus*. The NreB/NreC two-component system is able to regulate nitrate/nitrite respiration under O_2-limiting conditions (98). *S. carnosus* grows preferentially by aerobic respiration but nitrate can be used as a terminal electron acceptor in the electron transport chain under anaerobic conditions. Nitrate and nitrite reduction are catalyzed by a membrane-bound nitrate reductase NarG (*narGHJI* genes) and a cytoplasmic nitrite reductase, respectively (98, 321). The expression of both operons is controlled by the NreB/NreC two-component system (98). NreB is a classical sensor kinase containing a PAS domain followed by a HK domain and an ATPase domain (Fig. 15A). Under anoxic conditions, the PAS domain holds an $[4Fe\text{-}4S]^{2+}$ iron-sulfur cluster coordinated by four conserved cysteines (180, 255). In this configuration it performs auto-phosphorylation and transphosphorylation of its response regulator NreC. Once activated, this response regulator achieves transcriptional regulation (Fig. 15B). In the presence of O_2, the $[4Fe\text{-}4S]^{2+}$ cluster is converted into $[2Fe\text{-}2S]^{2+}$ which is unstable and becomes degraded (Fig. 15B). Recently, it has been reported that a third protein called NreA interacts with NreB and is involved in the regulation of the NreB phosphorylation level. The NreA crystal structure shows that the protein binds one molecule of nitrate at its GAF domain. The authors suggested a nitrate/O_2 co-sensing by NreA/NreB system as part of what is called NreABC system (265, 266).

1.4. GAF-domain-Fe-S cluster: AirS-AirR two-component system

The AirS-AirR two-component system (formerly called YhcS-YhcR) contains an [Fe-S] cluster inside a GAF domain. This system has been described in *S. aureus* and responds to redox signals. AirR regulates directly or indirectly the expression of the Agr two-component system involved in quorum sensing, the two-component system SaeRS implicated in virulence, stress associated factors (RsbU and RsbW), as well as virulence factors (Cap5A, Spa and HlgC) (356). Recently the AirSR system was reported to be involved in the transcriptional regulation of staphyloxanthin production (137).

AirS contains an N-terminal domain that holds the GAF domain followed by the HK domain and the ATPase domain (Fig. 16A). The GAF domain holds a Fe-S cluster-binding motif with four conserved cysteines (Cys-X_7-CysXCys-X_{17}-Cys). Experiments performed by Sun and co-workers, suggested that the iron-sulfur cluster was critical for AirS autophosphorylation (356). The model proposes that oxidized $[2Fe\text{-}2S]^{2+}$-AirS is the active form that is autophosphorylated and then phosphorylates AirR, stimulating the transcriptional response. Thus the oxidation of $[2Fe\text{-}2S]^{+}$ to $[2Fe\text{-}2S]^{2+}$ seems to be the signal that initiates the signalling cascade. However, the prolonged exposure to O_2 or the presence of strong oxidants such as H_2O_2 as well as the presence of NO inhibits the auto-kinase activity of AirS (Fig. 16B). The authors suggested that these situations may cause over-oxidation and loss of the Fe-S cluster, thus inhibiting AirS activity (Fig. 16B) (356).

2. Sensing by NAD-binding PAS domains: KinA-KinE-Spo0A system

The KinA-KinE-Spo0A system has been described in Gram-positive bacteria such as *Bacillus* and *Clostridium spp.* (155, 212, 249, 352, 382). This complex system is composed of five HKs (KinA-KinE), two intermediary proteins (Spo0F and Spo0B) and one response regulator (Spo0A). The regulon of Spo0A comprises more than 100 genes. Among these genes, those deeply implicated in sporulation, colony morphology and biofilm development can be found (250).

KinA is a cytoplasmic sensor kinase containing three PAS domains called PAS-A, PAS-B and PAS-C. It has been shown that PAS-A was able to bind NAD^+. This binding allowed KinA to sense the intracellular NADH/NAD^+ ratio (195). The authors proposed that KinA is inhibited by NAD^+ when respiration levels are high. Conversely, when the NAD^+/NADH ratio

decreases (low respiration levels), the kinase is activated stimulating the Spo0B phosphorylation (195).

3. Flavin-binding PAS based histidine kinase sensors: MmoS-MmoQ two-component system.

Flavin-binding PAS domains are abundant in signalling proteins. The redox state of FAD and FMN cofactors senses the redox state of cytoplasm, the electron transfer chains or the visible light perception, and allows a response to readapt the metabolism to new conditions. In the past few years, several FAD/FMN-containing primary redox sensors have been described. These sensors transmit the redox signal to a secondary downstream effector domain or protein. The MmoS-MmoQ system constitutes a suitable model for the study of sensor kinases containing a FAD-binding PAS domain.

MmoS is a sensor kinase that regulates the expression of a soluble methane monooxygenase (sMMO) in a process that depends on copper availability. sMMO catalyzes the oxidation of methane to methanol. This enzyme is found in methanotrophs such as *Methylococcus capsulatus* (Bath) (222). In this bacterium, the *mmoS* gene forms an operon with its cognate response regulator gene *mmoQ* that is divergently transcribed to *mmoR*. The working model proposes that MmoS sensor kinase phosphorylates MmoQ, which does not contain DNA-binding domains and it is able to phosphorylate MmoR that finally binds to the target promoters and regulates gene transcription (Fig. 17). Another gene located next to *mmoQ* called *mmoG* encodes a putative chaperonine that seems to facilitate the folding of MmoR and/or the sMMO complex (70).

MmoS is a non-orthodox sensor kinase that contains two PAS domains (PAS-A and PAS-B) and a GAF domain followed by a HK domain, two receiver domains and a histidine phosphotransfer domain (Fig. 17A). MmoR is activated by MmoS at low copper levels, activating in turn the expression of *mmoXYBZ* genes that encode structural genes of sMMO. The PAS domains of MmoS appear not to contain copper ions so that a redox sensing mechanism has been proposed. This mechanism is based on the idea of MmoS holding a reduced $FADH_2$ at low copper levels. This configuration triggers auto-phosphorylation in sensor kinase and in turn the phosphorylation of MmoR that finally induces the expression of sMMO genes (Fig. 17B). At high concentrations of copper, $FADH_2$

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is oxidized to FAD and a conformational change inhibits the phosphotransfer to MmoR and then the activation of sMMO expression (70, 373). The crystal structure of MmoS was resolved by Ukaegbu and Rosenzweig in 2009 (374). A single FAD molecule was found in the PAS-A domain, which is in agreement with the model proposed by the same authors in 2006. Nowadays the redox signal sensed by MmoS remains unknown. Several hypotheses have been proposed such as MmoS direct sensing of copper reduction or indirect sensing *via* the quinone pool or the copper chelator methanobactin (16). Recent studies suggest that methanobactin together with a polypeptide called MmoD are involved in the copper-switch of methanotrophs. This last model proposed that MmoD regulates the transcription of the methanobactin synthesis gene cluster, MmoR and MmoG. Then methanobactin, MmoR and MmoG interact to induce the expression of the MmoX operon (Fig. 17B). When copper is present, it binds to MmoD preventing the expression of the methanobactin gene cluster (81, 327).

4. Signal modulation by disulfide bond formation: ArcB-ArcA two-component system

Sometimes sensor kinases sense changes in O_2 availability indirectly. In these cases, they are able to sense the redox status of elements that make up part of the electron transport chain such as quinones or cytochrome oxidases and in turn activate or repress the transcription. These redox molecules/proteins are able to oxidize or reduce some cysteines in sensor kinases, generating the redox switch. An increasing number of sensor kinases which perform this type of indirect redox sensing are being described in the literature. The best characterized examples are ArcB and RegB sensor kinases. ArcB senses the redox state of the quinone pool by using a complex and delicate mechanism whereas RegA kinase seems to be able to sense the redox state of the cell by using two different mechanisms. ArcB is addressed in detail below since its redox sensing mechanism involves PAS domains. However, RegB sensor kinase is included in section III.B entitled “Non-PAS domain redox sensing based on disulfide bond formation” because although RegB uses a cysteine-based switch, these cysteines are not held in a PAS domain. Indeed, neither RegB nor its ortholog PrrB include PAS domains in their architecture.

ArcB is a non-orthodox sensor kinase since it contains a histidine phosphotransfer domain after the receiver domain (Fig. 18A). ArcB also holds a PAS domain in the N-terminal region

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containing two cysteines, Cys180 and Cys241, which are responsible for auto-phosphorylation. ArcB has a crucial role in the adaptation of *E. coli* to anaerobic environments (168). This complex transition from aerobic to anaerobic environments in *E. coli* is coordinated with the FNR, SoxRs and OxyR redox regulators previously described in the present review. The genes regulated by the ArcB/ArcA system are mainly involved in respiratory metabolism such as enzymes of the TCA cycle, the glyoxylate shunt and terminal oxidases (227). As already stated, anaerobic conditions are sensed by ArcB indirectly. Auto-phosphorylation of ArcB is inhibited by oxidized ubiquinone-0 and menadione (117). These molecules are soluble analogs of ubiquinone-8 and menaquinone-8. The authors proposed that under anaerobic conditions when the quinone pool was mainly formed by oxidized ubiquinone and menaquinone, the activity of ArcB was silenced. In contrast, when O_2 became limited the quinone pool was transformed into ubiquinol and menaquinol and the auto-phosphorylation was triggered (117, 233). Afterwards, a more sophisticated mechanism of regulation was proposed in which ArcB is able to respond to the redox state of the ubiquinone/ubiquinol pool and the menaquinone/menaquinol pool depending on O_2 availability (23). The authors suggested that after a transition from anaerobic (0% O_2) to low aerobiosis conditions (20% O_2) the menaquinone pool is oxidized resulting in the inactivation of ArcB. Upon a shift from low aerobiosis conditions (20% O_2) to high aerobiosis conditions (80% O_2), the total ubiquinone pool increases and therefore ubiquinol reduces disulfide bonds and activates ArcB (Fig. 18B). In aerobic conditions the quinone pool decreases, the oxidation of cysteines occurs and the inactivation of ArcB takes place (Fig. 18B) (23). Recently, it has been reported that a third type of quinone (demethyl-menaquinone) is involved in ArcB phosphorylation modulation. It seems that demethyl-menaquinone is also able to oxydize ArcB and that demethyl-menaquinol is able to reduce ArcB (376).

In recent years, several sensor kinases such as EvgS, TodS and HskA have been found that also respond to the redox state of quinone pool although the detailed mechanisms are not as well understood as for ArcB (27, 330, 340).

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5. Atypical signal transduction PAS/GAF-based mechanisms: NifL-NifA system

The NifL protein in nitrogen-fixing organisms senses both the redox and fixed nitrogen status to regulate nitrogen fixation by controlling the activity of the transcriptional activator NifA (82, 148). In *Azotobacter vinelandii*, NifL contains two N-terminal PAS domains and a C-terminal transmitter region containing a conserved histidine residue (H domain) and a GHKL (Gyrase, Hsp90, Histidine Kinase, MutL) nucleotide binding domain corresponding to the catalytic core of the histidine kinases. Despite these similarities, NifL does not exhibit kinase activity and regulates its partner NifA by direct protein-protein interactions rather than phosphorylation (223). The amino terminal PAS1 domain of NifL from *A. vinelandii* accommodates a redox-active FAD group; the elevation of cytosolic O_2 concentrations results in FAD oxidation and a concomitant conformational re-arrangement that is relayed via a short downstream linker to the second PAS domain, PAS2. At PAS2, the signal is amplified and passed on to effector domains generating the 'on' (inhibitory) state of the protein (224).

The NifA protein from *A. vinelandii* belongs to a family of enhancer binding proteins that activate transcription by RNA polymerase containing the sigma factor σ^{54}. These proteins have conserved AAA+ domains that catalyse ATP hydrolysis to drive the conformational changes necessary for open complex formation by σ^{54}-RNA polymerase (235). The activity of the NifA protein is highly regulated in response to redox and fixed nitrogen through interaction with the antiactivator protein NifL. Binding of NifL to NifA inhibits the ATPase activity of NifA, and this interaction is controlled by the amino-terminal GAF domain of NifA that binds 2-oxoglutarate (348).

B. Non-PAS domain redox sensing based on disulfide bond formation

1. RegB-RegA two-component system

RegB/RegA in *R. capsulatus* is a two-component system that responds to redox signals and regulates important cellular processes such as carbon and nitrogen fixation, electron transport chain configuration, photosynthesis and aerotaxis (87, 320, 358, 397). RegB protein is a classical sensor kinase that contains five transmembrane regions, a HK domain and an ATPase domain (Fig. 19A). RegB sensor kinase is able to auto-phosphorylate in the presence of specific redox signals and then phosphorylates its cognate response regulator

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RegA that binds its target promoters activating or inhibiting the transcription. RegB senses redox signals by using the complex mechanism shown in Figs. 19B and 19C. The RegB HK domain holds a highly conserved quinone binding site GlyGlyXXAsnProPhe and a conserved cysteine in position 265. At high O_2 conditions the Cys265 forms an intermolecular disulfide bond that inactivates the auto-phosphorylation activity of RegB. This molecular bond converts the RegB dimers into inactive tetramers (Fig. 19B). In addition, ubiquinone molecules are able to bind to the quinone binding site of RegB inhibiting RegB activity *in vitro* (Fig. 19C) (359, 398). This second mechanism is independent of Cys265 because a protein mutant lacking this residue maintains the ability to respond to redox changes in the presence of ubiquinone (398). Both mechanisms contribute then to the redox sensing. Moreover, it has also been reported that the sulfenic acid modification at Cys265 as a consequence of high O_2 tension led to inactivation of RegB kinase (399).

2. PrrB-PrrA two-component system

A homolog system of RegB/RegA in *R. capsulatus* is the PrrB/PrrA system in *R. sphaeroides*, a purple non-sulfur photosynthetic bacterium with a versatile metabolism, since it is able to grow aerobically, anaerobically, photosynthetically, fermentatively and lithotrophically (90). The PrrB/PrrA two-component system has a pivotal role controlling the expression of photosynthetic genes (89, 214), but also regulates directly or indirectly 25% of the total genes present in *R. sphaeroides,* suggesting that it is a global regulator system (90). PrrB is a redox sensor whose model of action is based on the Cbb3-1 terminal oxidase redox state, although the underlying mechanism that controls HK activity is unknown (191). The model suggests that Cbb3-1 oxidase generates an inhibitory signal on PrrB sensor kinase under aerobic conditions (268, 269). This inhibition of PrrB triggers a silencing of genes related to photosynthesis. Other HKs among the family of RegB and PrrB sensor kinases are ActS in *S. meliloti* (88) and *Agrobacterium tumefaciens* (15), and RoxS in *P. aeruginosa and P. putida* (60, 100). However, in these cases the redox sensing mechanisms of both ActS and RoxS sensor kinases remain unknown.

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IV. Redox Photosensors based on flavins

There are three main classes of flavin-based photosensors attending to domain receptor type, namely light-oxygen-voltage sensing (LOV) domains, blue light sensors using FAD (BLUF) domains and cryptochromes. They regulate many physiological responses to blue-light. Changes induced by the light promote conversion between different redox and protonation states of the flavin, which are then coupled to conformational or other changes that signal physiological responses (61). For both the photolyase/cryptochrome and LOV domain photosensors, photoexcitation leads to changes to the flavin that are common in flavin-dependent enzyme reactions: an alteration in the oxidation state of the flavin or the formation of a covalent adduct (119). However, it is not yet clear if there is a mechanism linking flavin excitation to photoreceptor activation in the BLUF protein family. The formation of a flavin adduct is not involved in BLUF domain activation, therefore the role of electron transfer and accompanying changes in the flavin redox state remains a subject of controversy (119). A summary of the redox photosensors reviewed in this work can be found in Table 4

A. LOV-domains, a special class of PAS-domains

LOV domains are ubiquitous regulators of phototropic responses, described as a class of PAS domains that binds FMN or FAD noncovalently (57). LOV domains are present in both chemotrophic and phototrophic bacterial species. They have been found in regulatory domains of sensor histidine kinases, diguanylate cyclases-phosphodiesterases, DNA-binding domains and regulators of RNA polymerase sigma factors (145). LOV proteins exhibit the typical PAS fold, with a globular α/β-fold (LOV core) flanked by variable and often helical N- and C-terminal extensions (140). This kind of domain may be found as a single or multiple domain or associated with additional sensor domains such as GAF, cyclases and HKs-associated sensory extracellular (CHASE) domains, or other PAS domains (145).

As a consequence of the light signal and changes in the flavin state, a conserved cysteine residue in the LOV domain forms a flavin adduct. Details of the photocycle of this kind of photosensors are extensively discussed in an excellent recent review (231). The LOV domain signalling involves the generation of conformational changes triggered by the

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conserved photochemistry of the LOV core (61). The mechanism depends on the oligomeric state, structural changes and the type of effector domain (61). In these processes, blue light induces the unfolding of flanking helices, dimerization and rotation of the LOV modules (61).

Two major groups of LOV proteins have been described (145). The first, LOV-HKs, correspond to approximately 50% of bacterial LOV proteins. The second group comprises the so-called LOV-GGDEF-EAL proteins that contain conserved GlyGlyAsp/GluAsp/GluPhe and GluAlaLeu motifs. These domains could participate in metal binding and might form the phosphodiesterase active site. LOV-GGDEF-EAL proteins are predicted to regulate the synthesis and hydrolysis of cyclic di-GMP and constitute ~20% of bacterial LOV proteins. Other, less common LOV signalling proteins include LOV STAS (sulphate transporter anti-σ antagonist) proteins (~10%), LOV HTH proteins (~3.5%) and the LOV SpoIIE (sporulation stage II protein E) proteins (~2%). A small number of LOV proteins with a globin domain, a CheB or CheR chemotaxis domain, or a cyclase 4 domain have also been reported (230).

Several LOV proteins have a specific DNA binding domain, activated by changes in the flavin state. In bacteria, a certain number of LOV proteins with a HTH effector domain have been identified in recent years. However, no bacterial LOV proteins have been described with a zinc-finger DNA binding motif similar to the *Neurospora crassa* white collar complex. Neither have been described aureochromes in bacteria, with LOV and a C-terminal leucine zipper domain for DNA binding. To date, aureochromes have been detected only in a single group of algae, photosynthetic stramenopiles, but not in any other prokaryotic or eukaryotic organisms (201). However, constructs using this type of domains, LOV and DNA-binding motifs have been engineered for design experimental approaches based on light-inducible gene regulation (292, 293).

1. LOV/Helix-Turn-Helix DNA-binding proteins

Among the light-regulated transcriptional regulators bearing the LOV domain, there is an interesting class in which the LOV domain is fused to a HTH DNA-binding domain. While these proteins have been well described in plants and animals, the information for bacteria is scarce. They have been described in the alphaproteobacterium *Erythrobacter*

litoralis (Q2NB98) (307, 415), while others have been identified at the genomic level (http://www.orthodb.org/).

In the light-activated EL222 transcription factor from *E. litoralis*, blue light drives conformational changes of the LOV sensor domain and the signal is transmitted to the DNA-binding effector domain (LuxR-type HTH domain) to allow photoactivation of gene transcription (261). The mechanism occurs through changes that induce dimerization, and the dimer then recognizes specific promoters, affecting the transcription of target genes (415). EL222 acts as a light-dependent transcriptional factor. The photochemical reactions of EL222 and the light sensing properties of the LOV domain have been investigated. Concentration dependent experiments revealed that the EL-LOV domain is in equilibrium between the dimer and the monomer in the dark state, and the main photoreaction is the dimerization reaction between a monomer in the ground state and that in the excited state (360). Utilization of light-driven allosteric changes are interesting tools to control gene expression or biochemical activities. LOV/HTH proteins have also been proposed as good candidates for the design of light-controlled systems (415).

2. Short-LOV proteins

Several LOV proteins have been identified in bacteria and fungi as so-called “short” LOV proteins composed of a conserved LOV core and N- and/or C-terminal helical extensions. Due to the absence of fused effector domain(s), the next step in signal propagation in short LOV proteins is expected to involve partner proteins, which remain unidentified (310). Interestingly, the genes encoding PpSB1-LOV (Q88E39) and PPSB2-LOV (Q88JB0) from *P. putida* are contiguous to putative DNA-binding proteins, and they could perhaps represent their molecular partners *(230)*. In *R. sphaeroides* the short-LOV protein, RsLOV, lacking a C-terminal output domain, similarly to PpSB2 in *P. putida,* has been shown to be responsible for controlling the expression of photosynthetic genes (247). "Short" LOV proteins could represent suitable building blocks for the design of genetically encoded photoswitches (*i.e.*, LOV-based optogenetic tools) (301).

3. Phototropins: YtvA (PfyP) and stress response

Phototropins are membrane-associated LOV-proteins that usually possess two N-terminal

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photoactive LOV domains (LOV1 and LOV2) and a C-terminal serine/threonine kinase domain (229). Blue light photoexcitation of the LOV domains results in the receptor auto-phosphorylation and initiation of the phototropin signalling (56). Phototropins are ubiquitous and they are especially abundant in plants.

YtvA from *B. subtilis* is one of the more widely-studied bacterial phototropines, involved in transcriptional regulation*. B. subtilis* YtvA acts as a positive regulator of the general stress transcription factor σ^B, *sigB.* The N-terminal LOV domain is followed by a STAS, carrying a nucleoside triphosphate binding site (42, 229). It is noticeable that the blue-light receptor YtvA from *B. subtilis* is permanently incorporated into the stressosome independent of the illumination state (177). This is consistent with the data observed in *Listeria monocytogenes* YtvA-like photoreceptor where blue light induces responses *via* the upregulation of σ^B. The effect depends on blue light induced generation of ROS in the medium (231, 272).

B. Non-PAS domain photosensors

1. Blue Light sensors Using FAD (BLUF domains)

BLUF domains are light-triggered switches that control enzyme activity or gene expression in response to blue light, remaining activated for seconds or even minutes after stimulation (283). BLUF was initially described in purple bacteria for its role in photosynthetic gene expression (238). Well-studied BLUF domains are present in proteins such as AppA, PAC-a/PAC-b, BlsA, BlrB, BlrP (YegF) and PixD (61).

The secondary structure of the BLUF domain is dissimilar to those of the PAS domains or DNA photolyases. Furthermore, no significant similarity was found between the fold of its FAD-binding region and those from other FAD-binding protein families (124). For this reason, the BLUF domain was considered a novel FAD-binding domain involved in blue-light- or redox-dependent sensory transduction, with a novel FAD-binding fold (124).

BLUF proteins are unique in being the only family of photoreceptors known to show photo-induced proton-coupled electron transfer (283). The photoresponse of BLUF sensing depends on interactions of the flavin with several conserved residues of the domain. Using ultrafast time resolved infra-red spectroscopy to investigate the primary photophysics of the BLUF domain of the light activated anti-repressor AppA, Laptenok and co-workers

established that the electron donor is the Trp104 residue (208). The photocycle is initiated by light causing an electron and then a proton to transfer from the conserved tyrosine to the flavin, yielding a bi-radical (283). The reaction is not photo-reversible, and within 10 ns the photo-excited state falls back to the signalling state with recombination of the bi-radical (283).

AppA, a light-and O_2-sensor anti-repressor from *R. sphaeroides* is the best characterized BLUF protein (124, 239). It carries a C-terminal sensor containing heme instead of cobalamin (SCHIC) responsible for O_2-sensing domain that senses redox conditions. AppA interacts with the transcription repressor PpsR in the dark and AppA modulates DNA-binding of PpsR in a ternary complex (393). Previously, Masuda and Bauer (239) suggested that AppA could convert PpsR from an active DNA-binding tetramer to an inactive dimer by reducing a disulfide bond in the PpsR tetramer. Blue light inactivates the DNA-binding activity of the complex so gene expression occurs only under suitable conditions of light and redox potential (283, 393). Crystal structures and hydrogen/deuterium exchange of AppA complexed with PpsR suggested that blue light dissociated multimeric AppA/PpsR complex from DNA but did not appreciably alter the affinity of the two protein components. Fig. 8 summarizes the current model for the mechanism of the AppA/PpsR system (393).

BLUF domains are abundant in cyanobacteria, and several AppA homologues are annotated in the cyanobase (http://genome.microbedb.jp/cyanobase). The PixD-PixE system is the best studied, as for instance PixD (*slr1694*) from *Synechocystis* sp. PCC6803 (119, 271). In this case, PixD lacks the SCHIC C-terminal domain present in AppA, and in fact is a short-BLUF protein, with its partner PixE bearing the response regulatory domain (http://prosite.expasy.org/cgi-bin/prosite). The light-induced change in the PixD–PixE interaction is a crucial part of the early signal transduction process, but the downstream signalling, involving gene expression changes, remains largely unresolved (361). Genome-wide sequencing of mutants has revealed that an uncharacterized *Synechocystis* gene product (*sll2003*) may be involved in the signal transduction of phototaxis in response to light intensity (354).

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2. Cryptochromes

Cryptochromes are blue light/UVA photoreceptors involved in regulatory processes. They are closely related to photolyases (involved in DNA repair functions, they use light to repair UV-damaged DNA), and form the cryptochrome–photolyase family. Cryptochromes and photolyases bear a conserved N-terminal α/β domain and an α-helical domain which non-covalently binds a FAD in their catalytic centre. The catalytic activity of photolyases requires the FAD to be in its two-electron reduced active state as $FADH^-$ (386). The N-terminus might bind other antenna chromophores. Cryptochromes are a relatively heterogeneous group found in different types of organisms, and they are not as well characterized as the photolyases. Cryptochromes act as photoreceptors and transcriptional regulators, depending on the type of organisms. The classical cryptochromes show high sequence similarity to photolyases, but they lack DNA repair activity and only act as signalling molecules, regulating the circadian clock, growth or development. However, a distinct group of the classical cryptochromes has been identified. Its homologues were found in diverse organisms (*Drosophila sp.*, *Arabidopsis sp.*, *Synechocystis sp.*, and *Homo sapiens*) and named cry-DASH (378). The most prominent member and initiator of the new group was described in the cyanobacteria *Synechocystis* (Syn-CRY, encoded by the *sll1629* gene), which was the first cryptochrome to be identified from bacteria.

Very few cryptochromes from bacteria have been characterized, and most of them also show photolyase activity. In addition to the Syn-CRY, CryB of *R. sphaeroides,* was first described as a cryptochrome that affects light-dependent and singlet oxygen-dependent gene expression (106). Also, *V. cholerae* cryptochrome, VcCry1, has been described as a DASH cryptochome (326). RsCryB exhibits repair activity of (6-4) photoproducts (386), suggesting a dual character combining the functions of cryptochromes and photolyases. Moreover, RsCryB is a close homologue of the photolyase PhrB from *A. tumefaciens.* Based on structural data from RsCryB, Geisselbrecht and co-workers defined a new class of cryptochromes, called CryPro (116). This contains two cofactors only conserved in the CryPro subfamily: 6,7-dimethyl-8-ribityl-lumazine in the antenna-binding domain and a [4Fe-4S] cluster within the catalytic domain (116). The key feature of the CryPro subfamily of cryptochromes is a [4Fe-4S] cubane cluster in the C-terminal domain, considered

characteristic of the bacterial cryptochromes. *cryB* transcription of *R. sphaeroides* increases in response to singlet O_2 and *Rs*CryB itself affects the regulation of photosynthesis related genes. Electron paramagnetic resonance spectroscopy has shown that the [4Fe-4S] cluster of *Rs*CryB can readily be oxidized, and thus *Rs*CryB might itself act as a sensor for ROS as a result of photooxidative stress. Oxidative changes of the [4Fe-4S] cluster could trigger structural changes of the C-terminal nucleotide-binding domain (116).

V. Concluding remarks

The regulation of redox homeostasis is of paramount importance for the survival of free-living bacteria and species infecting a host. Thus, their ecological success is strongly dependent on the correct performance of a range of transcription factors that trigger the appropriate genetic program in response to different redox signals. Understanding the mechanisms of the different redox-responsive regulators has been hindered by the instability of the redox centres and prosthetic groups that are essential for their activities. It is likely that some of them still remain to be discovered. Moreover, cross-talk among several regulators and the diversity of responses displayed against the same signal complicate the identification of their direct gene targets. Fortunately, in recent decades the development of novel biophysical tools together with the resolution of the crystal structures of several redox regulators have provided a wealth of knowledge about their response mechanisms. Furthermore, high-throughput transcriptomic analyses have allowed researchers to complete the cross-roads of regulatory networks in numerous bacteria. All these data furnish researchers with valuable information that may allow the development of novel drugs and other applications in microbial biotechnology.

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Abbreviations

BLUF: blue light sensors using FAD

DASH: *Drosophila-Arabidopsis-Synechocystis-Homo*

FAD: flavin adenine dinucleotide

FNR: fumarate nitrate reductase regulator

FMN: flavin mononucleotide

GAF: cGMP-specific phosphodiesterases, adenylyl cyclases and FhlA

HRM: heme regulatory motif

HTH: helix-turn-helix

IHF: integration host factor

HK: histidine kinase

LOV: light-oxygen-voltage sensing

NHE: normal hydrogen electrode

OHP: organic hydroperoxides

PAS: Per-Arnt-Sim

PDB: Protein Data Bank

ppGpp: guanosine tetraphosphate

pppGpp: guanosine pentaphosphate

RCS: reactive chlorine species

RES: reactive electrophile species

RNS: reactive nitrogen species

ROS: reactive oxygen species

SCHIC: sensor containing heme instead of cobalamin

sMMO: soluble methane monooxygenase

SUF: sulfur mobilization

TCA: tricarboxylic acids

wHTH: winged helix-turn-helix

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Table 1. Main properties of Wbl paralogs from *M. tuberculosis*

Paralog	Roles	Stability of the [4Fe-4S] cluster	Disulfide reductase activity	Regulation	References
WhiB1	Essential for growth and dormancy NO-sensing transcription factor. Transcriptional reprograming in host environment Reduces GlyB	O_2-stable Very sensitive to NO (reaction 10^4 fold faster than with O_2)	yes	Upregulated by cAMP. Autorepressed by Apo-Whi1	(1, 112, 209, 345, 346)
WhiB2	Proposed chaperone-like function Node in drug resistance Essential for growth	Sensitive to O_2	no	Activator. Activated upon exposure to antibiotics. Moderate upregulation under prolongued hypoxia. Upregulated by cAMP. Inhibited by WhiB4.	(5, 198, 209, 313, 346)
WhiB3	Sensor of oxidative stress. Control of virulence. Metabolic regulator of	Sensitive to O_2	yes	Activator. Interacts with SigA. Activated under acid stress, hypoxia	(5, 209, 314, 341,

	virulence associated lipids. Maintenance of redox homeostasis during infection. Maintenance of cell shape and size	Sensitive to NO		and NO	342, 353)
WhiB4	Redox balance. Virulence	Sensitive to O_2 and NO. Resistant to chaotropic agents	yes	Moderate upregulation under prolongued hypoxia. Upregulated by cAMP	(4, 5, 209)
WhiB5	Reactivation. Virulence	Sensitive to O_2	yes	Activator	(5)
WhiB6	Virulence. Stress resistance	O_2-stable Stable versus reduced glutathion	yes	Upregulated under prolongued hypoxia and NO. Moderate upregulation by cAMP	(5, 209)
WhiB7	Activates transcription of genes involved in drug resistance. Redox balance	O_2-stable Stable versus reduced glutathion	yes	Activator, redox-sensitive. Autoregulated. Interacts with SigA. Activated by low iron or antibiotics. Moderate upregulation by cAMP	(5, 38, 209, 314)

Table 2. Summary of representative regulators involved in direct redox-sensing and regulation

Regulator	Family	Redox signal	Transduction mechanism	PDB code	Function/some target genes	Ref.
SoxR	MerR	O_2^-, nitric oxide, natural redox-cycling molecules and drugs, guanine radicals	Homodimer with 2 [2Fe-2S] clusters. Activates transcription through oxidation of the $[2Fe\text{-}2S]^{+1}$ to a $[2Fe\text{-}2S]^{+2}$ form of the cluster	2ZHH 2ZHG	Redox sensor. In enteric bacteria oxidized SoxR activates transcription of SoxS which in turn activates transcription of a regulon of around 100 genes including *sodA, fumC and fpr*, among others	(78, 135, 248, 260, 279)
IscR	Rrf2	Senses [2Fe-2S] homeostasis which depends on O_2 tension, redox status and	[2Fe-2S] occupancy. Works as a repressor.	4ClC 4HF0 4CHU 4HF1	Integration of iron availability, O_2 tension and redox signals. Works as holo and apo-forms. Targets of holo-IscR incudes *iscRSUA-hscBA-fdx, yadR, yhgY,* and *sufA*. Holo and apo-IscR modulates *hyaA, ydiU,* and *sufA*.	(118, 248, 311, 323, 402)

		iron availability				
NsrR	Rrf2	Nitric oxide (nitrosative stress)	[2Fe-2S] or [4Fe-4S] cluster. Usually works as repressor though activation of virulence genes has been described in some cases.	5N07 5N08	Genes involved in nitric oxide metabolism and detoxification. Some main targets are *hmp*, *ytfE, nasR, fliA, msqR*	(28, 183, 286, 372)
RsrR	Rrf2	O_2, other oxidants may also be important *in vivo*	Senses redox via its [2Fe-2S] cluster. RsrR becomes activated for DNA binding through oxidation and inactivated through reduction.	N.R.	Its regulon includes several regulators, such as NmrA, genes required for glutamine synthesis, NADH/NAD(P)H metabolism, as well as general DNA/RNA and amino acid/protein turnover.	(256)
FNR	Crp/Fnr	O_2, secondary role in nitric oxide sensing	Senses O_2 via its $[4Fe\text{-}4S]^{2+}$ cluster that in aerobiosis is converted into a $[2Fe\text{-}2S]^{2+}$ releasing Fe^{2+} and O_2^- and dissociating from DNA. Can	5E44	Global regulator, its core includes operons associated with anaerobic respiration, such as *nar, dms, frd*, as well as glycolytic and fermentative enzymes	(248, 258)

			work as repressor or as activator depending of the targets.			
DksA/ TraR	TraR	Oxidative and nitrosative stresses	4-cysteine zinc finger motif releases zinc ion under oxidative and nitrosative stresses, triggering conformational changes in the regulator	1TJL 4IJJ	Beyond its function in the stringent response, DksA is also involved in modulation of several pathogenicity associated processes such as differentiation, flagellar gene activation, lysosome avoidance, quorum-sensing, antioxidant defences; but also central metabolism and zinc homeostasis.	(25, 26, 74, 138)
RsrA	TetR	Oxidative stress	Zinc ion coordinated by cysteines is released under oxidative stress, triggering conformational changes in the regulator	5FRH 5FRF	Anti-sigma factor. Inactivation of RsrA via forming disulphide bonds under oxidative stress activates expression of extra-cytoplasmic function (ECF) sigma factor SigR and its regulon, including thioredoxin system and several other putative thiol-disulphide oxidoreductases.	(179, 182, 189)

OxyR	LysR	Oxidative stress, H_2O_2	Disulfide bond formation between conserved cysteines may activate or inactivate the regulator, which can function as activator but also as repressor of transcription. S-nitrosylation and cysteine overoxidation appear as other mechanisms of regulation.	1I69 1I6A 3HO7 3JV9 4X6G 5X0V	Regulates the expression of antioxidant enzymes including catalases, superoxide dismutases, peroxidases, alkyl hydroperoxide reductases; but also modulates expression of Fur, Dps proteins, etc.	(58, 190, 391, 413)
PpsR/ CrtJ	LuxR	O_2	Disulfide bonds between conserved cysteines	4HH2	Modulates the synthesis of photosystem components in purple bacteria, including bacteriochlorophyll, carotenoids, light harvesting proteins, but also tetrapyrrole biosynthesis genes.	(254, 357)
OhrR/ MgrA/ SarZ	MarR	Organic hydroperoxides (OHP) and other ROS	Two sensing mechanisms: (1) single cysteine oxidation, and (2) disulfide bond formation between two cysteines. Both	2PFB 1Z9C 2BV6	OhrR modulates expression of organic hydroperoxide reductase (Ohr) and other antioxidant enzymes and thiol-reducing systems. MgrA and SarZ control genes	(17, 113, 178, 232, 298, 369, 389)

			cause inactivation of repressor and transcription of target genes.	3HSE	involved in antibiotic resistance and virulence.	
NemR	TetR	Cysteine-modifying electrophiles, alkylation and reactive chlorine species (RCS).	Cysteine oxidation by RCS causes reversible inactivation of repressor	4YZE	NemR modulates the expression of enzymes involved in detoxification processes including glyoxalase I (*gloA*) and N-ethylmaleimide reductase (*nemA*).	(132, 375)
FurA	Fur	Senses iron availability (Fe^{2+}) and redox status	Thiol/disulfide redox switch	N.R.	Targets involved in iron homeostasis, oxidative stress defences, photosynthesis, respiration, heterocyst differentiation, tetrapyrrole biosynthetic pathway, virulence, etc.	(101, 125-129)
PerR	Fur	Peroxide	Metal-catalyzed oxidation of histidine to 2-oxo-histidine	3F8N 4I7H	PerR may function as activator and repressor of gene expression. Targets include antioxidant enzymes, virulence genes and	(36, 306, 377)

					other regulators.	
HypT	LysR	HOCl	Methionine oxidation to methionine sulfoxide triggers its activation	N.R.	Acts as transcriptional activator for genes involved in amino acids (Met, Cys) biosynthesis. Negatively regulates expression of genes involved in iron homeostasis.	(114)
CooA	Crp/Fnr	Carbon monoxide	CO binds to Fe^{2+} heme promoting a redox-mediated ligand switch that leads conformational changes which activate the regulator	2FMY 1FT9	Modulates the expression of the *coo* regulon, which allows the CO-dependent anaerobic growth of *R. rubrum*.	(405)
RcoM	LytR/AlgR	Carbon monoxide	CO binds to Fe^{2+} heme promoting a redox-mediated ligand switch that leads conformational changes which activate the regulator	N.R.	Regulates both aerobic (*cox*) and anaerobic (*coo*) CO oxidation systems.	(187)
Rex	Rex	$NADH/NAD^{+}$ ratio	Under microaerobic or anoxic conditions, NADH	2VT3	Rex is a transcriptional repressor that remains bound to its DNA targets when the	(154, 207, 226, 276,

			competitively binds to the Rex C-terminal domain, causing a conformational change in the regulator which decrease affinity for DNA		NAD^+/NADH ratio is sufficiently high. Rex regulons comprise genes involves in redox homeostasis, anaerobic and aerobic metabolism, lactate and ethanol fermentation, nitrate respiration, avermectin production, etc. Some examples are *hemZ, lctP-ldh, ndh, roxS, yjlC, ywcJ* and the operons *alsS-alsD and cydA-cydB-cydC-cydD.*	302)

N.R. Not resolved

Table 3. Summary of two-component systems involved in redox sensing

System	Redox signal	Domain/mechanism involved in redox sensing	PDB code	Function/some target genes	Ref.
FixL-FixJ	O_2	PAS domain-heme	1DP6	Nitrogen fixation genes *(fix)* heme biosynthetic genes *(hem),* denitrification genes *(napEDABC*, *nirK*, *norCBDQ* and *nosRZDFYLX)* and some hydrogen oxidation genes (*hup*)	(85, 275, 296, 308)
DosS-DosR	O_2, NO and CO	GAF domain-heme	2W3D	*devRS*, *hspX*, *narK2* and *tgs1* genes.	(44-46)
NreB-NreC	O_2	PAS domain-Fe-S cluster	N.R.	Nitrate reductase genes (*narGHJI*), nitrite reductase genes (*nirDB*)	(98, 321)
AirS-AirR	O_2	GAF domain-Fe-S cluster	N.R.	*saeRS* genes, genes encoding stress associated factors (*rsbU* and *rsbW*) and virulence factors (*cap5A*, *spa* and *hlgC*)	(137, 356)
KinA-KinE-Spo0A	NADH/NAD^+ ratio	PAS-A domain	2VLG	Genes implicated in sporulation, colony morphology and biofilm development	(249)

MmoS-MmoQ	Unknown	PAS domain-FAD	3EWK	Soluble methane monooxygenase (sMMO)	(70)
ArcB-ArcA	Redox state of quinone pool	PAS domain-disulfide bond formation	N.R.	Genes involved in the TCA cycle, glyoxylate shunt and terminal oxidases.	(227)
NifL-NifA	$FADH_2$/FAD ratio	PAS domain-FAD	2GJ3	Nitrogen fixation genes *(nif* genes*)*	(82, 148)
RegB-RegA	O_2/Ubiquinone	Disulfide bond formation	N.R.	Genes involved in carbon and nitrogen fixation, electron transport chain, photosynthesis and aerotaxis	(320)
PrrB-PrrA	Redox state of Cbb3-1 oxydase	Disulfide bond formation	N.R.	Photosynthetic genes	(90)

N.R. Not resolved

Table 4. Summary of redox photosensors

Protein	Signal	Domain/family involved in redox sensing	PDB code	Function/some target genes	Ref.
EL222	Blue-light	LOV-domain	3P7N	Radical SAM protein putative pyrimidine dimer lyase (*ELI_05380*), putative indoleamine 2,3-dioxygenase (*ELI_06040*) and NAD synthetase (*ELI_08405*), among others.	(307)
PpSB1	Blue-light	LOV-domain	5J3W	Photosynthetic genes	(247)
YtvA	Blue-light	LOV-domain	2MWG	General stress transcription factor *sigB*	(231, 272)
AppA	Blue-light and O_2	BLUF-domain	1YRX	Photosynthetic genes	(246)
SynCry	Blue-light and UVA	Cry-DASH	1NP7	Genes involved in PSII repair	(378)

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Figure legends

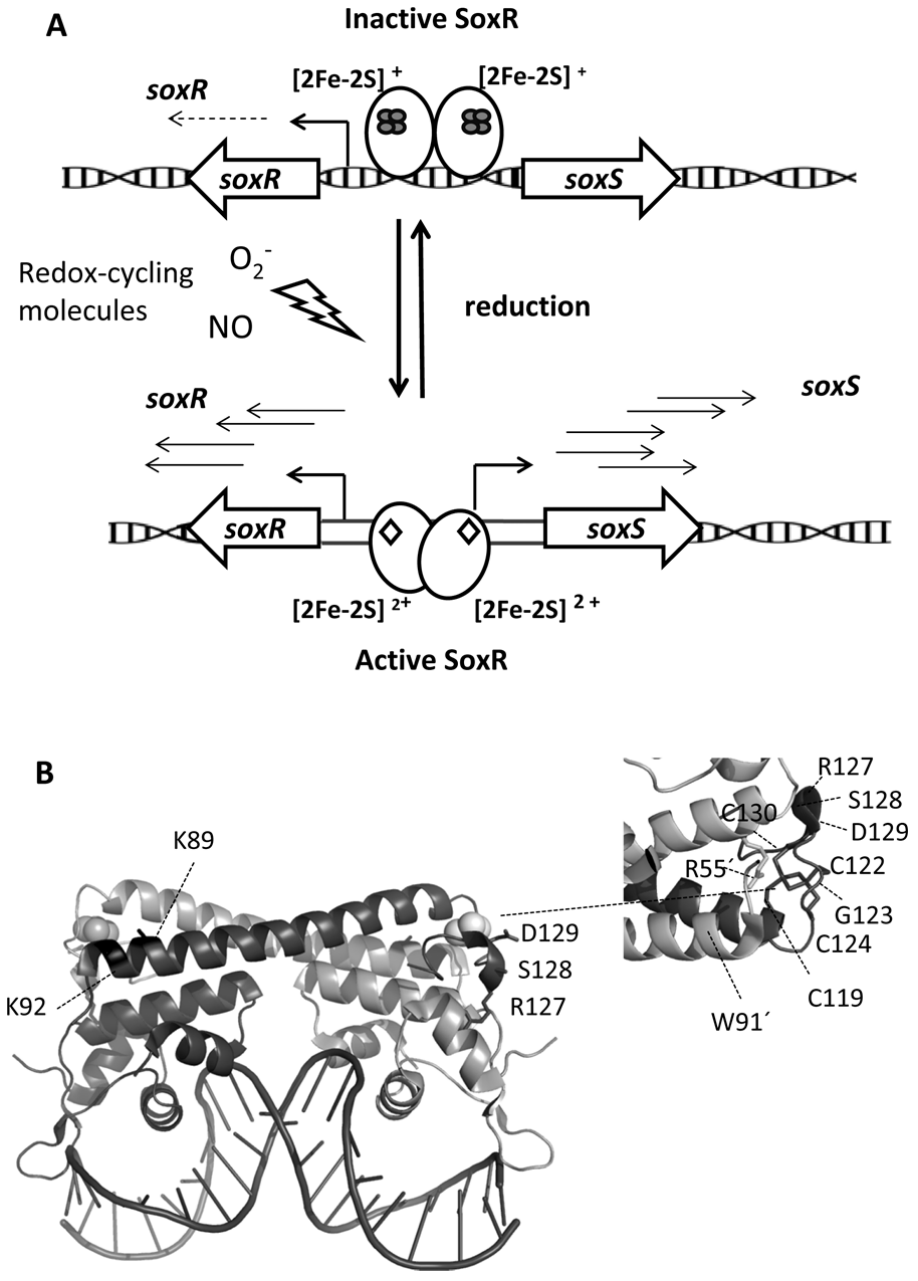

Figure 1. Scheme of the SoxR sensing mechanism. A. Oxidative challenge results in SoxR activation through reversible oxidation of the sulfo-ferric cluster and untwisting of the *soxS* promoter allowing its transcription. B. Structure of the SoxR-*soxS* promoter complex showing relevant amino acids for the SoxR redox-sensing mechanism. The figure is based on the structure from PDB with code 2ZHG and was produced with PyMol.

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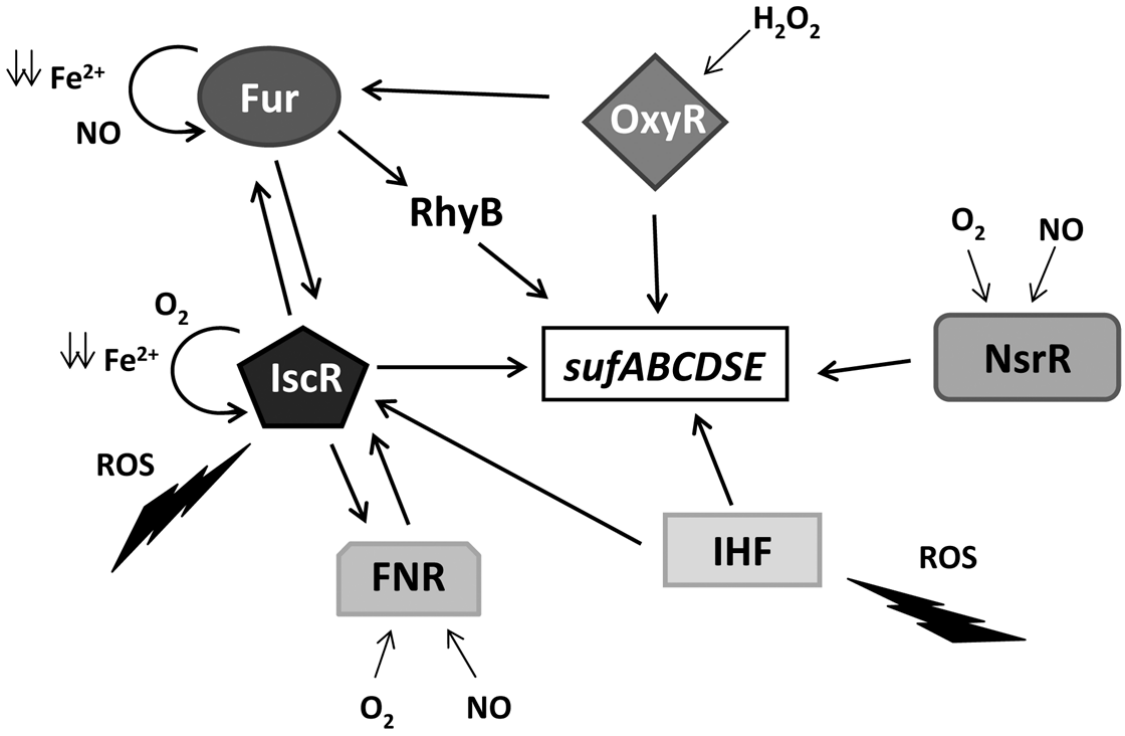

Figure 2. Factors influencing the regulation and DNA-binding activity of IscR in *E. coli* and their relationship with the *suf* operon.

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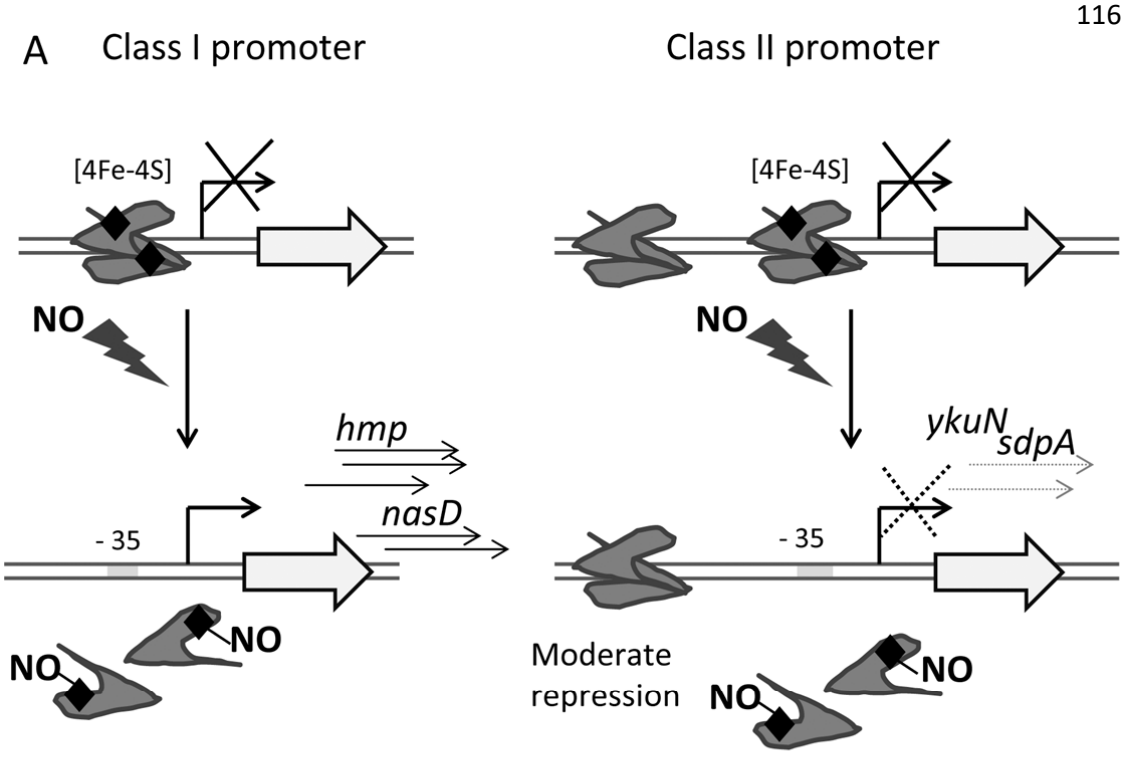

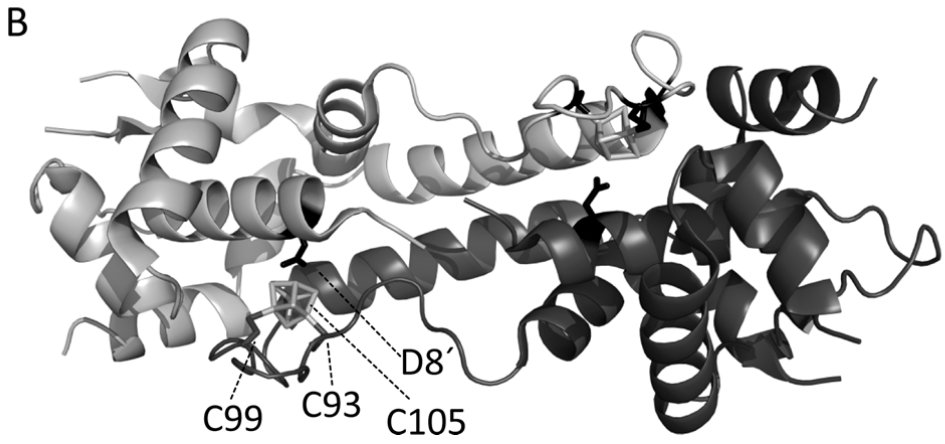

Figure 3. A. Model of the two types of regulation by NsrR reported in *B. subtilis*. While type I promoters are controlled by holo-NsrR in response to NO, type II promoters may recruit several transcription factors including holo- and apo-NsrR, being the later insensitive to NO. B. Model of a *S. coelicolor* NsrR dimer showing the assymetric environment of the [4Fe-4S] cluster. The structure was taken from PDB (code 5N07) and was produced with PyMol.

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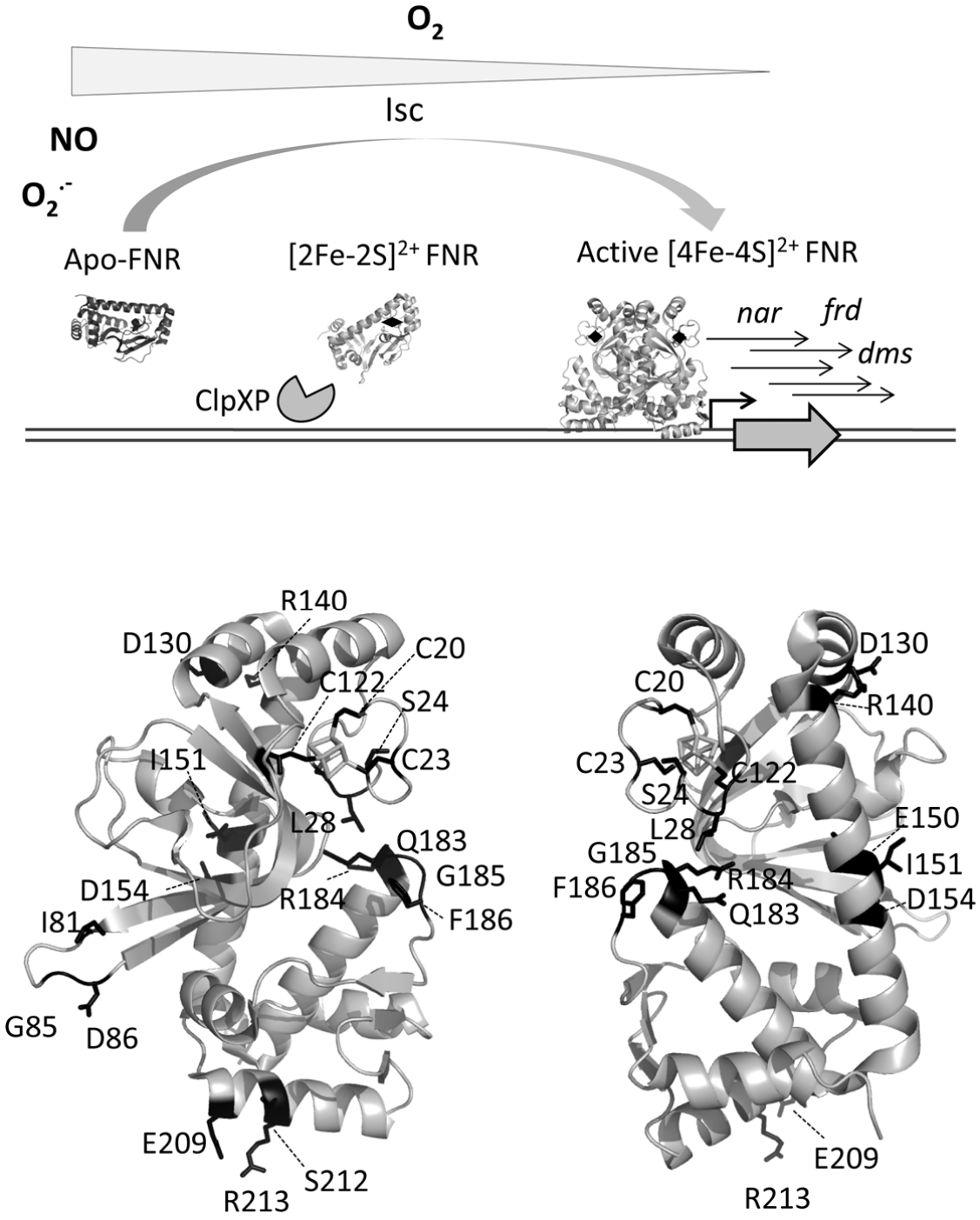

Figure 4. Upper panel. Redox response of FNR in *E. coli* depending of O_2 tension. The lower panel shows a model of the *E. coli* FNR monomer in two different orientations. Relevant amino acids for its sensing mechanism according to reference [244] are indicated. The figure is based on the structure of FNR from *A. fischeri* (PDB code 5E44) and was produced with PyMol.

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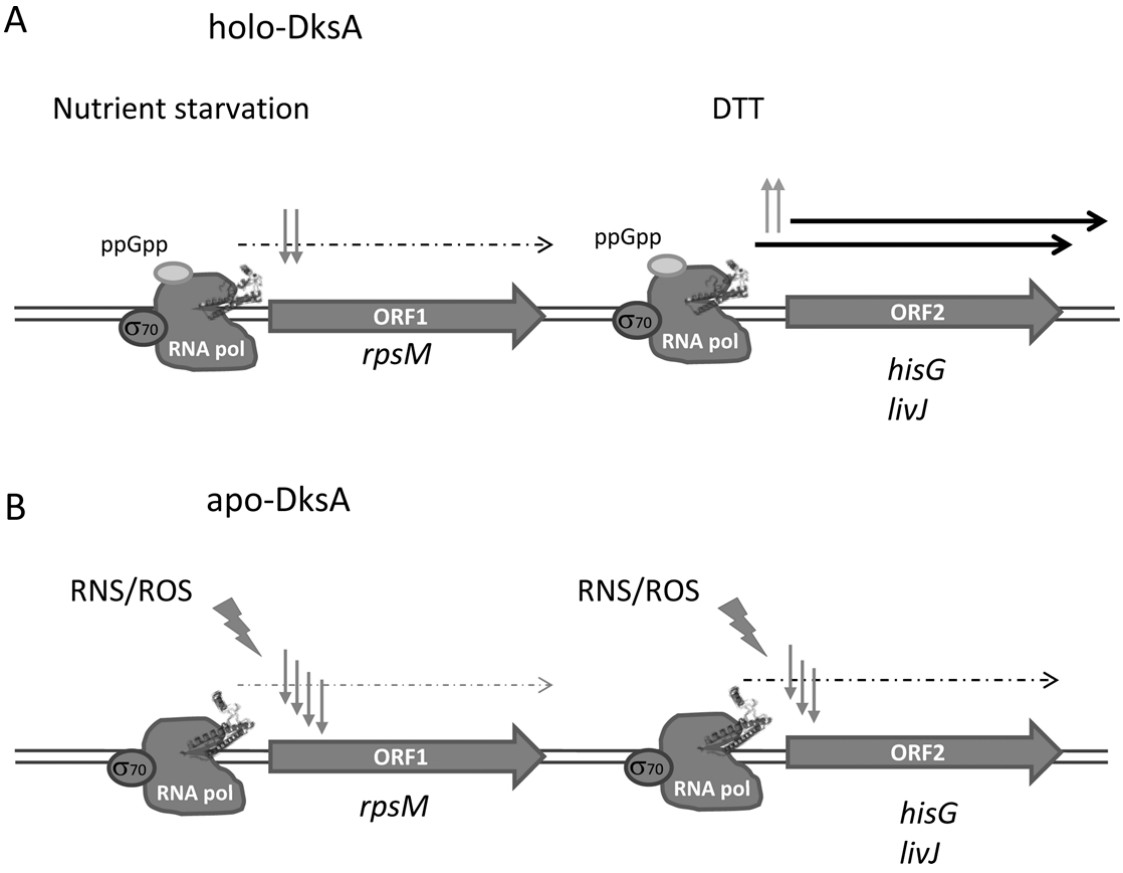

Figure 5. Scheme summarizing the action of holo- and apo-DksA under different stresses in *Salmonella*, based in reference [138]. A. (left) The oxidative stress associated with starvation leads to the downregulation of *rpsM* by ppGpp-bound holo-DksA. Conversely, holo-DksA reduced with DTT supports the activation of *livJ* and *hisG* (right). B. DksA responds to ROS and NRS independently of ppGpp. Oxidative and nitrosative stress releases Zn^{2+} from the 4-cysteine zinc-finger motif of DksA. Then, the free cysteines serve as a thiol switch able of increasing repression of *rpsM (left).* Oxidized apo-DksA also prevents the activation of *livJ* and *hisG*, elicited by reduced holo-DksA (A, right).

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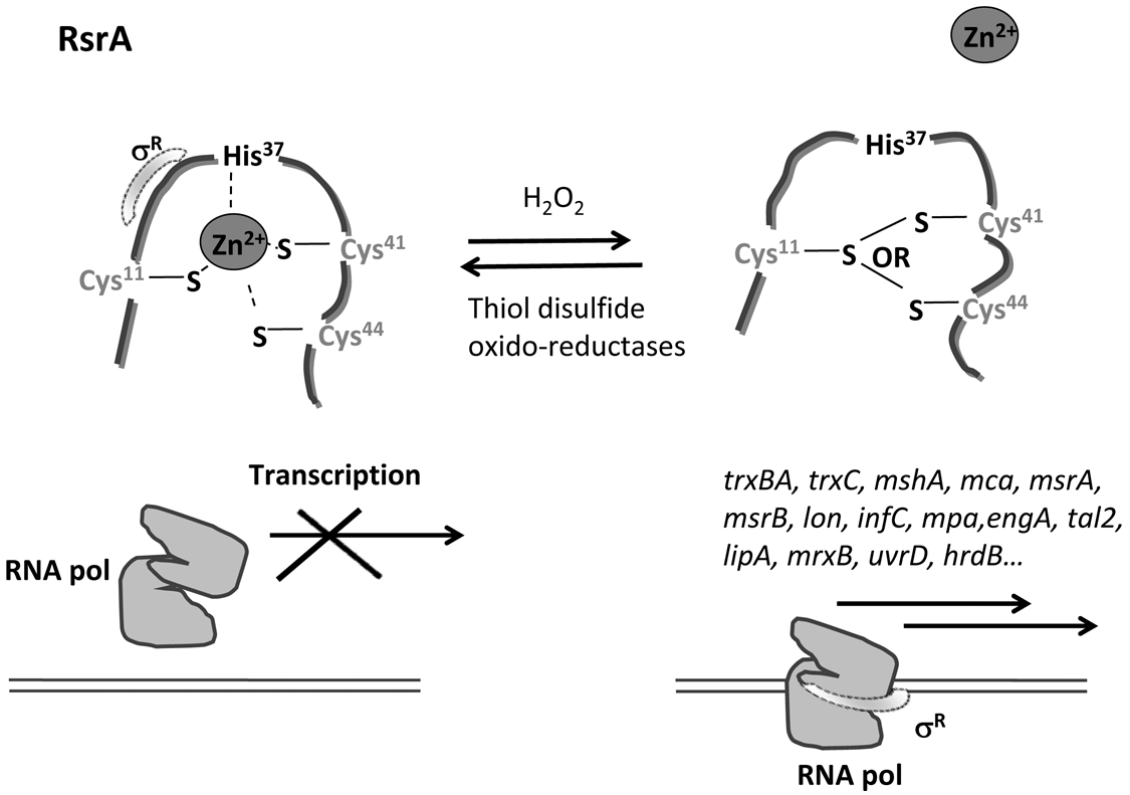

Figure 6. Model of metal action in RsrA activity. A. Under reducing conditions, Zn^{2+} coordination by H_{37} and reduced C_{11}, C_{41} and C_{44} activates RsrA repressor state by sequestering its cognate sigma factor SigR forming a RsrA/SigR complex. Oxidation of either residue C_{41} or residue C_{44} of the $C_{41}XXC_{44}$ motif by formation of a disulfide bond with residue C_{11} releases Zn^{2+} and inactivates RsrA. The regulator undergoes a dramatic change in its 3-D structure that sets SigR free. Once released, SigR can interact with RNA polymerase to trancribe the SigR target genes. B. Comparison of the structures of reduced (left) and oxidized (right) RrsA from *S. coelicolor* (PDB codes 5FRF and 5FRH) produced with PyMol.

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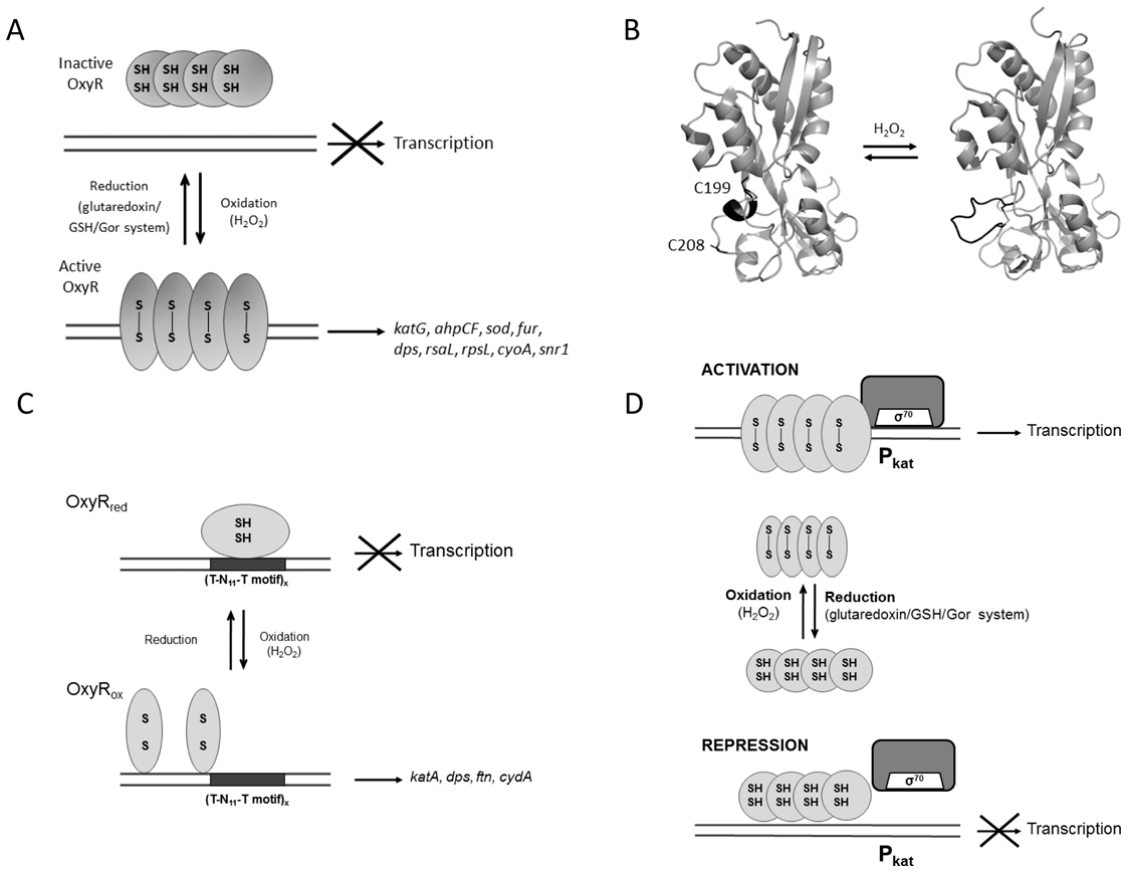

Figure 7. OxyR-mediated oxidative stress response via intramolecular disulfide formation. A. *E. coli* OxyR has a tetrameric arrangement assembled via two distinct dimerization interfaces. The oxidation of a sensing cysteine residue to sulfenic acid in inactive OxyR, followed by formation of an intramolecular disulfide bond with the resolving cysteine, involves a conformational change that affects DNA binding affinity stimulating gene expression. Oxidized OxyR is reduced by reduced glutathione via glutaredoxin/glutathione reductase system, using reducing equivalents supplied by NADPH. B. Structures of reduced and oxidized OxyR from *E. coli* (PDB codes: 1l69 and 1l6A). In the reduced state (left), the redox active residue C199 is separated from residue C208 by ~ 17 Å. A short helix formed by residues C199-C208 is highlighted in black. Once C199 is oxidized to sulfenic acid intermediate (C199-SOH), rapidly reacts with C208 to form an intramolecular disulfide bond. During oxidation the short helix disappears rendering a flexible region that increases the chance of C199-SOH and C208 meeting to form the disulfide bond. The final result upon disulfide bond formation is a significant rearrangement of the secondary structure

(right). C. *Proposed model for Corynebacterium glutamicum* transcriptional repression by OxyR. OxyR binding site overlaps the transcription start site and -10 region of *katA*, but in *dps* promoter overlaps -10 and -35 regions. This suggests that once OxyR binds to its target site prevents interaction of RNA polymerase with these promoters leading to transcriptional repression. Transcriptional repression by OxyR is alleviated under oxidative stress in a titration mechanism due to the decrease in specificity in its DNA-binding activity. D. *Neisseria meningitidis kat* gene repression/activation by OxyR. *N. Meningitidis* activates the catalase gene as response to H_2O_2 increase. After been oxidized, it binds to a region overlapping the -35 hexamer of the single σ^{70}-dependent promoter P_{kat}. As a result, a fast and strong activation of the transcription initiation occurs, possibly through direct contact to RNA polymerase. Once the redox state of OxyR is reversed the reduced form of OxyR represses again transcription.

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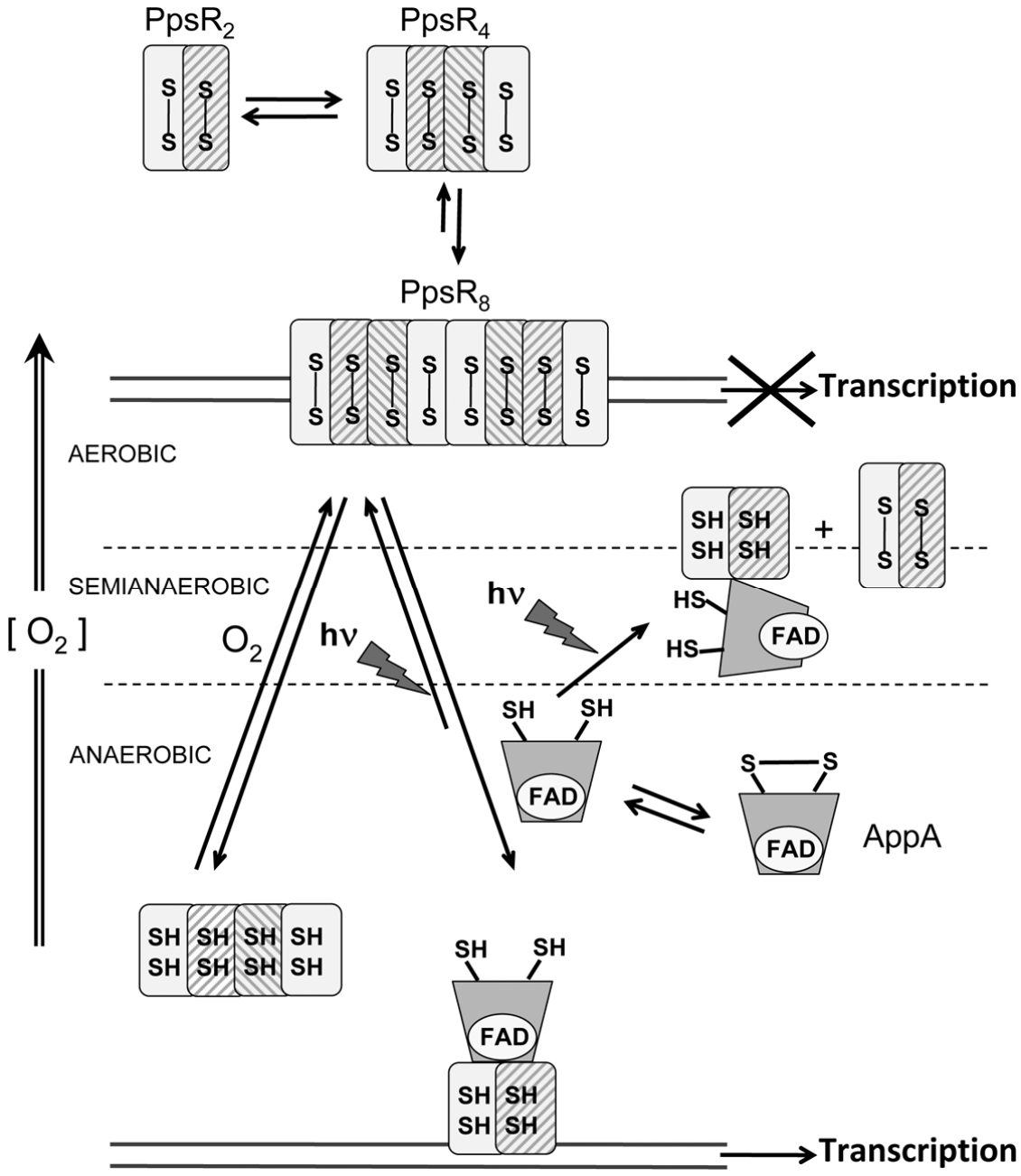

Figure 8. Schematic model of PpsR and AppA control of gene expression in response to O_2 and light. A conserved cysteine in the DNA binding domain of PpsR undergoes oxidation in the presence of O_2. Cysteine oxidation induces binding of PpsR to DNA as an octamer that represses controlled genes. Reduced PspR and reduced AppA form an AppA-$PpsrR_2$ complex to enable light- and oxygen-dependent regulation. Photon absorption by AppA BLUF domain induces an allosteric structural change in AppA-$PspR_2$ complex that reduces its affinity for DNA. AppA-$PpsR_2$-DNA complex prevents the repressing effect of $PpsR_8$-DNA

and promotes gene expression depending on relative AppA/PspR concentrations. An excess of PpsR competes with AppA-$PpsR_2$ for promoters under light but cannot replace the ternary complex in the dark. The levels of AppA and PpsR are inversely regulated by O_2. When the concentration of O_2 increases, $PpsR_8$-DNA is favored with the consequent repression of photosynthetic genes [171].

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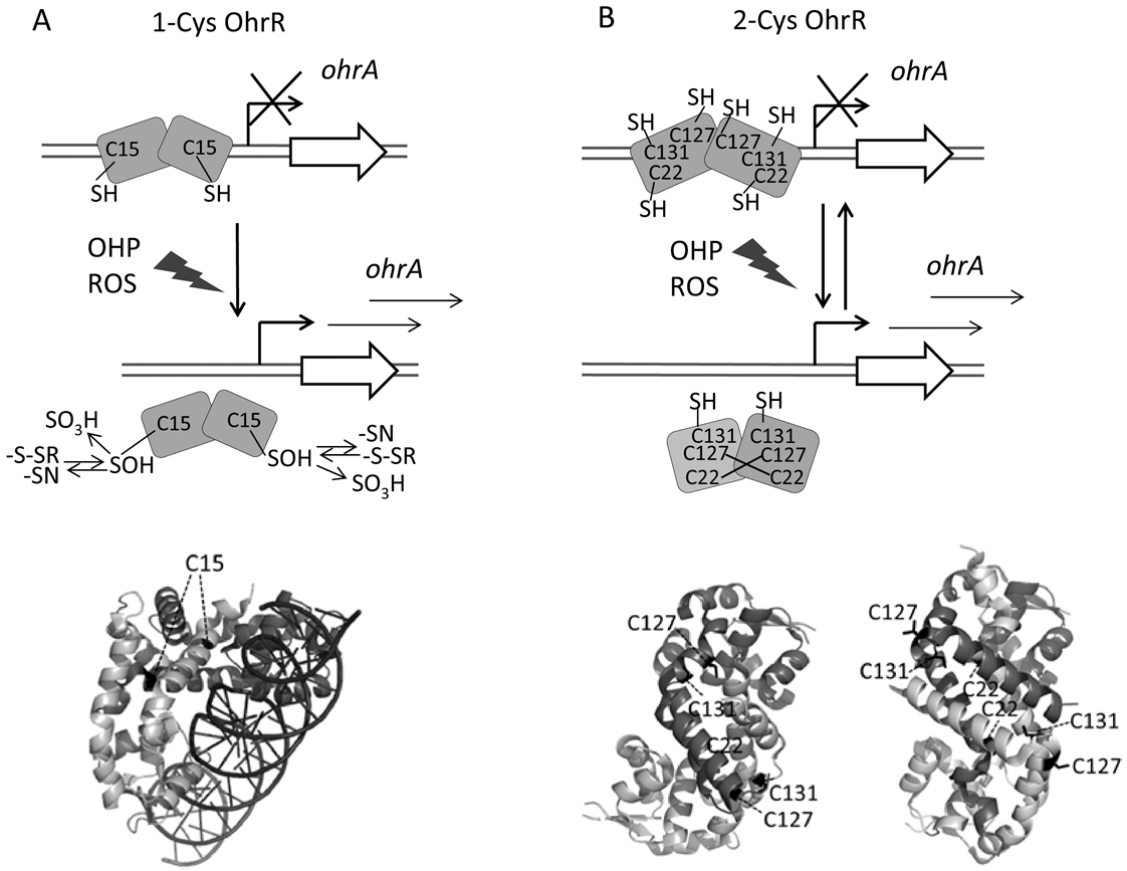

Figure 9. Scheme of the redox control of the *ohrA* peroxidase by OhrR. A. In the 1-Cys OhrR from *B. subtilis* peroxides cause the oxidation of the conserved Cys_{15} to sulfenic acid that in turn may undergo reversible S-thiolation or be irreversibly overoxidized in the presence of strong oxidants. The lower panel shows the location of Cys_{15} in a model based on the PDB structure with code 1Z9C. B. In the *X. campestris* 2-Cys OhrR oxidants lead to intersubunit disulfide formation between Cys_{22} and $Cys_{127'}$ that results in a major structural change of the regulator. The lower panel shows this structural rearrangement, as well as the location of the three conserved cysteines of *X. campestris* in the oxidized 2-Cys OhrR (left) and the reduced form (right). The structures were produced with PyMol according to the PDB files with codes 2PFB and 2PEX.

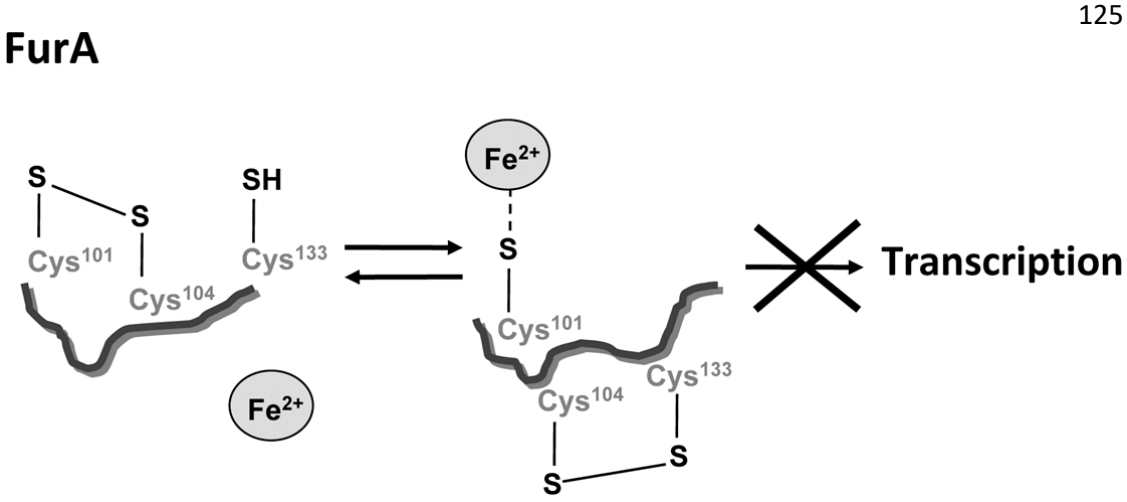

Figure 10. Model of metal involvement in the activity of cyanobacterial FurA. The Cys_{101}-Cys_{104} disulfide bridge of the $Cys_{101}XXCys_{104}$ motif keeps the residue Cys_{101} in the oxidized state and therefore, unable to coordinate the metal co-repressor, rendering inactive FurA.

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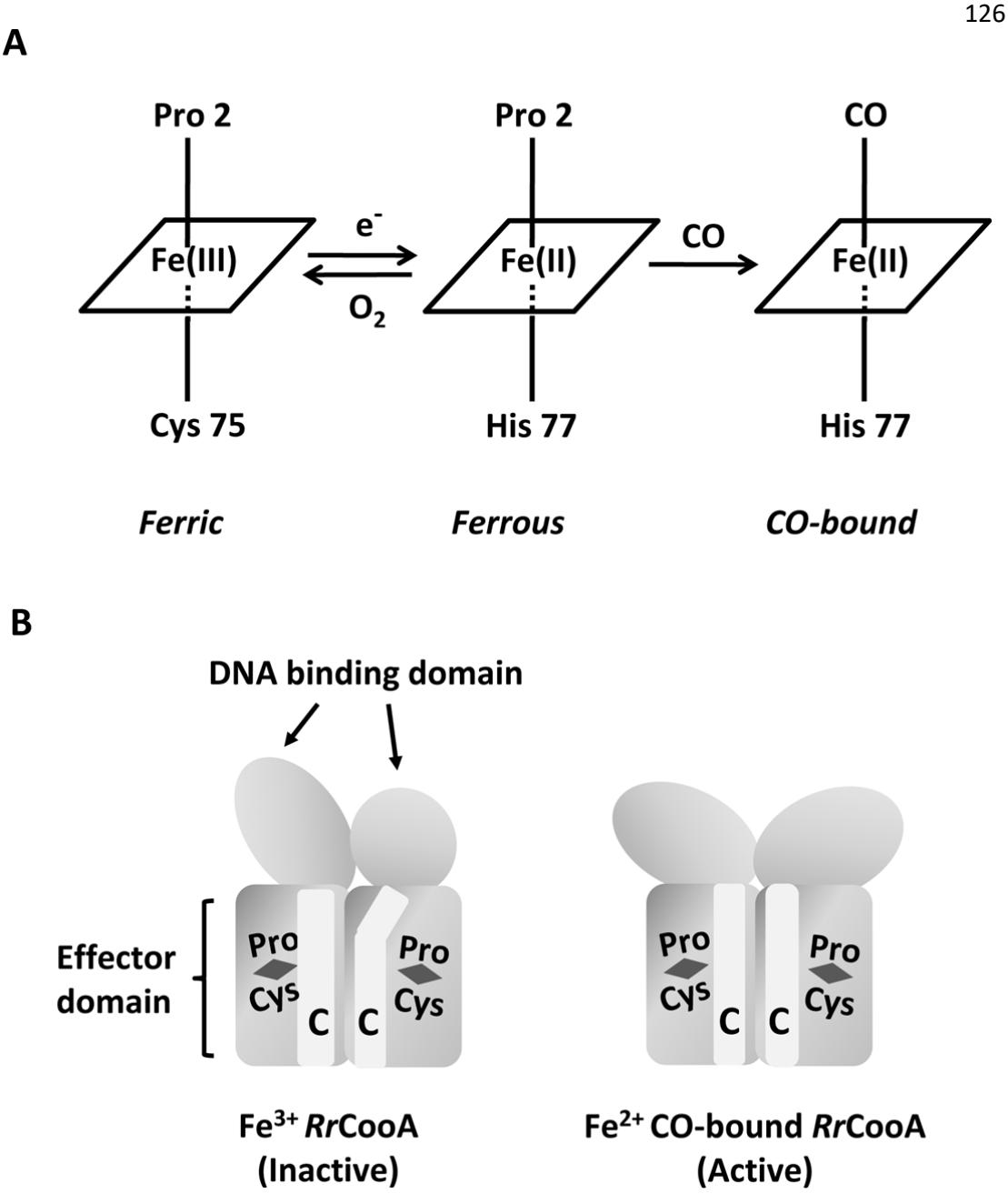

Figure 11. Features of *Rr*CooA redox and CO mediated activation. A) One of the axial ligands of the heme ferric state undergoes a redox-mediated ligand switch upon reduction. The displacement of the axial ligand in the reduced form by CO apparently causes a conformational change that induces *Rr*CooA to bind its target site in a site-specific manner. B) Schematic models of oxidized *Rr*-CooA and CO-bound *Rr*CooA. The C-helix is shown as a light grey rectangular box.

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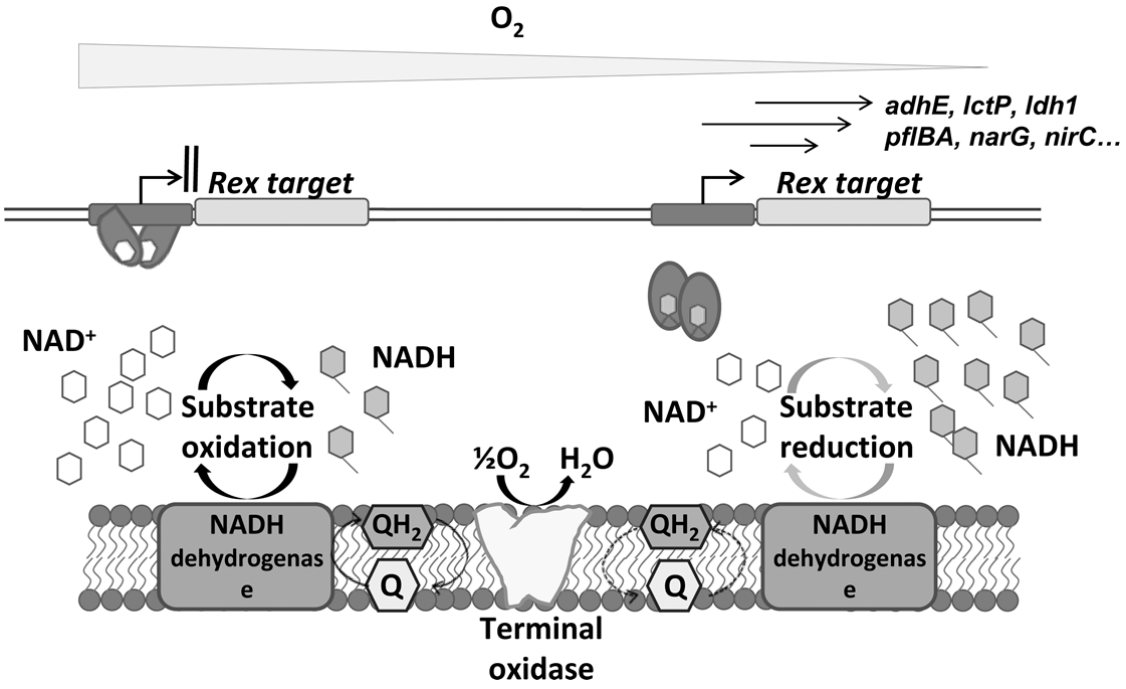

Figure 12. Modulation of Rex DNA-binding activity in response to the ratio of NADH/NAD^+ and its relationship with the respiratory chain (adapted from reference [133]). Under aerobic conditions NADH is rapidly re-oxidized and the concentration of NAD^+ is higher than that of NADH and Rex becomes activated by the binding of NAD^+, blocking the transcription of target genes. In contrast, when O_2 availability decreases, NADH > NAD^+ and Rex repression is relieved, leading to the transcription of its regulon. QH_2: reduced quinone. Q: oxidized quinone

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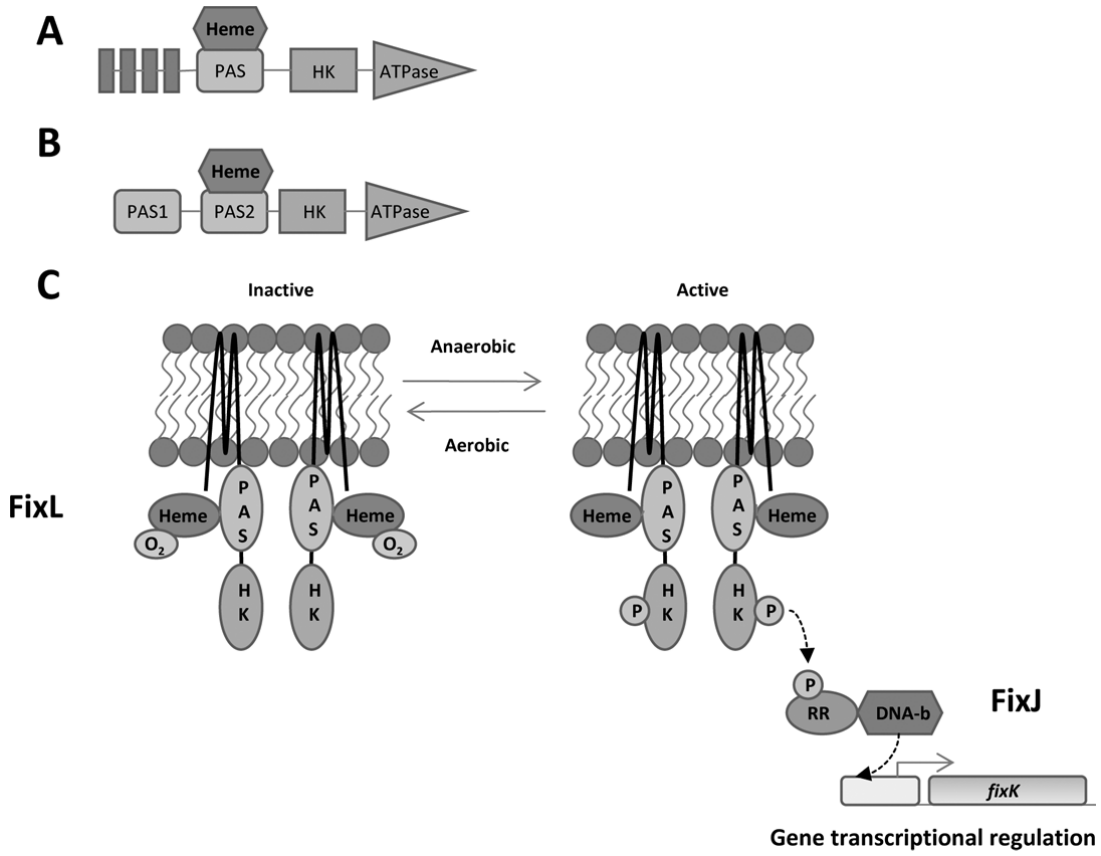

Figure 13. *S. meliloti* and *B. japonicum* FixL domain architecture and proposed model of action of the FixL-FixJ two-component system in *S. meliloti*. A. Domain organization in the sensor kinase FixL in *S. meliloti* and B. in *B. japonicum*. C. Redox-dependent phosphotransfer mechanism in the *S. meliloti* FixL-FixJ two-component system. RR: receiver domain, DNA-b: DNA binding domain.

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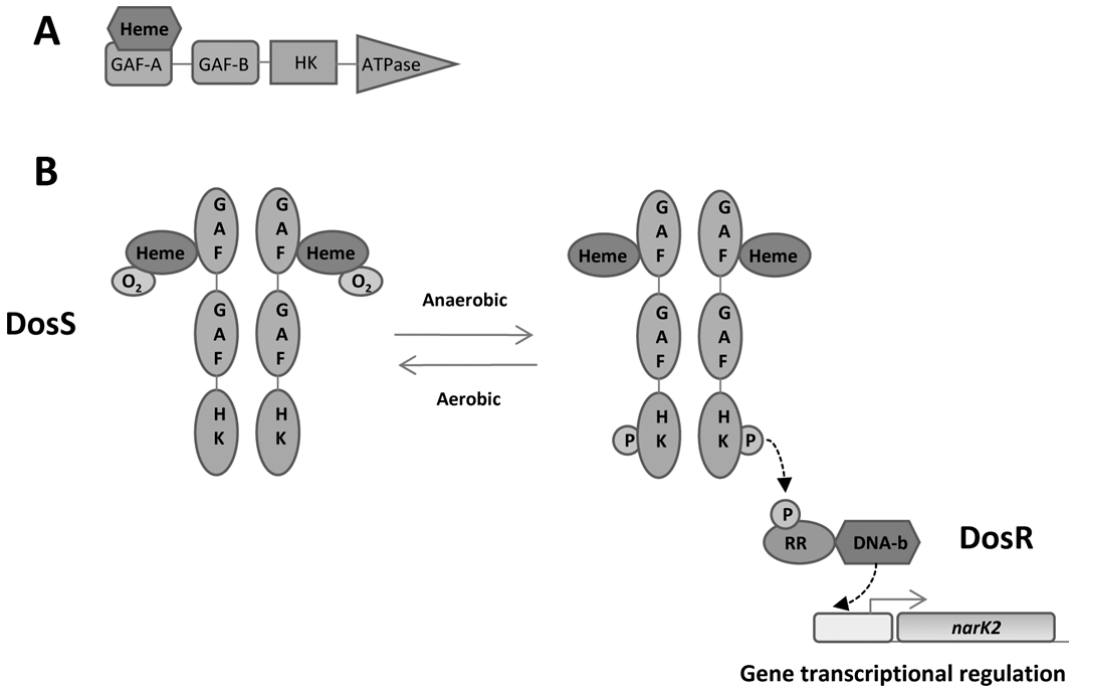

Figure 14. *M. tuberculosis* DosS domain architecture and proposed model of action of the DosS-DosR two-component system. A. DosR domain organization B. Redox-dependent phosphotransfer mechanism in the DosS-DosR two-component system from *M. tuberculosis*.

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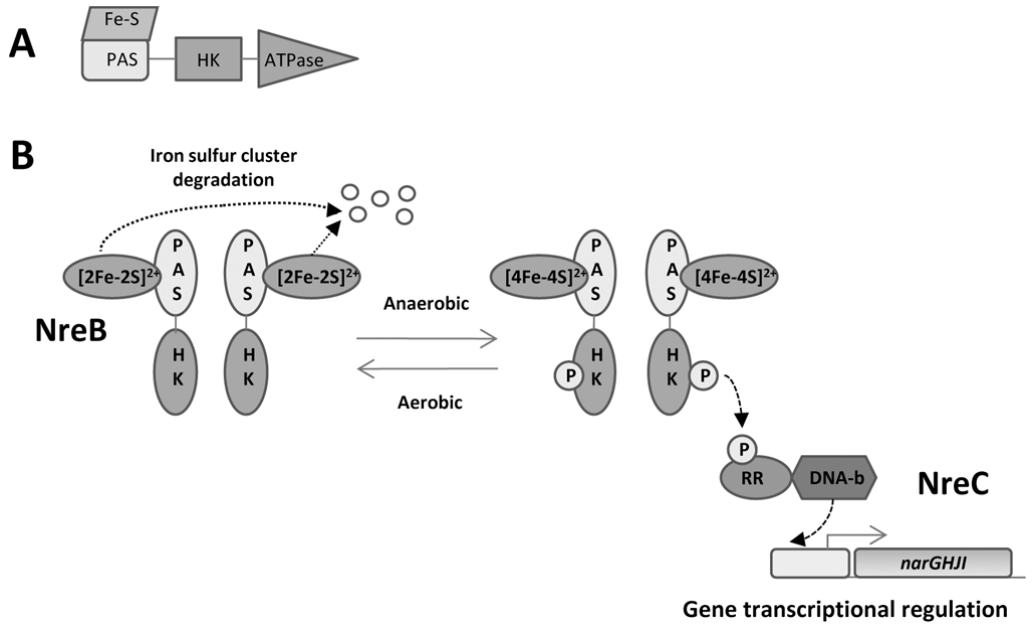

Figure 15. *S. carnosus* NreB domain architecture and proposed model of action of the NreB-NreC two-component system. A. NreB domain organization B. Redox-dependent phosphotransfer mechanism in the *S. carnosus* NreB-NreC two-component system. RR: receiver domain, DNA-b: DNA binding domain.

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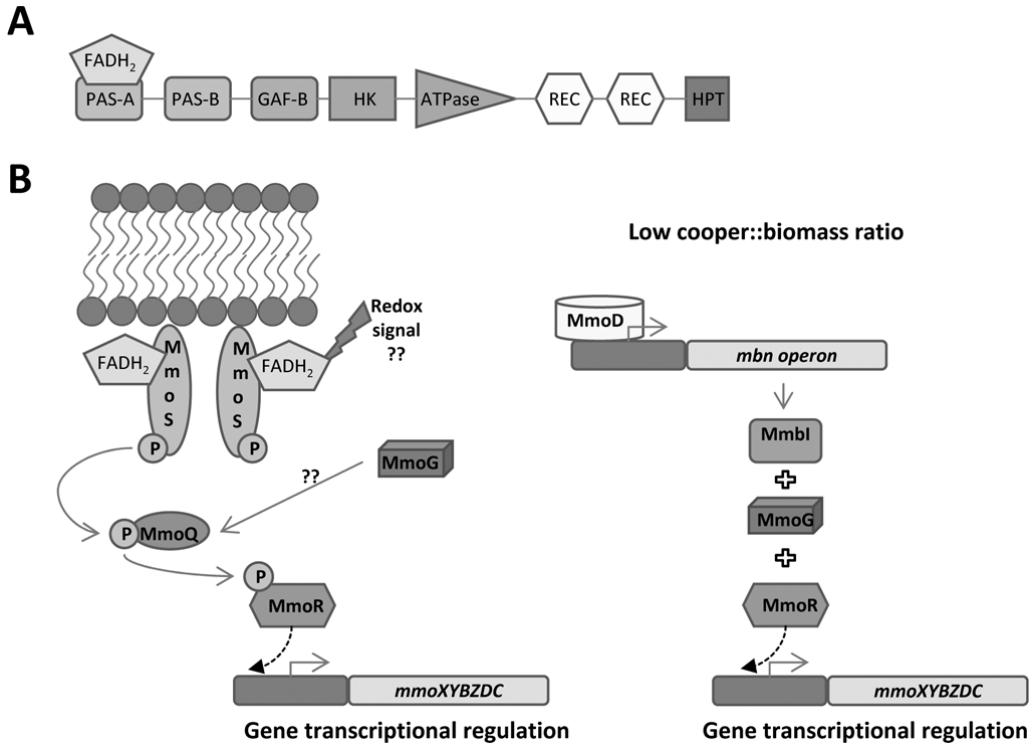

Figure 16. *S. aureus* AirS domain architecture and proposed model of action of the AirS-AirR two-component system. A. AirS domain organization B. Redox-dependent phosphotransfer mechanism in the *S. aureus* AirS-AirR two-component system. This figure is adapted from Sun et al. [356].

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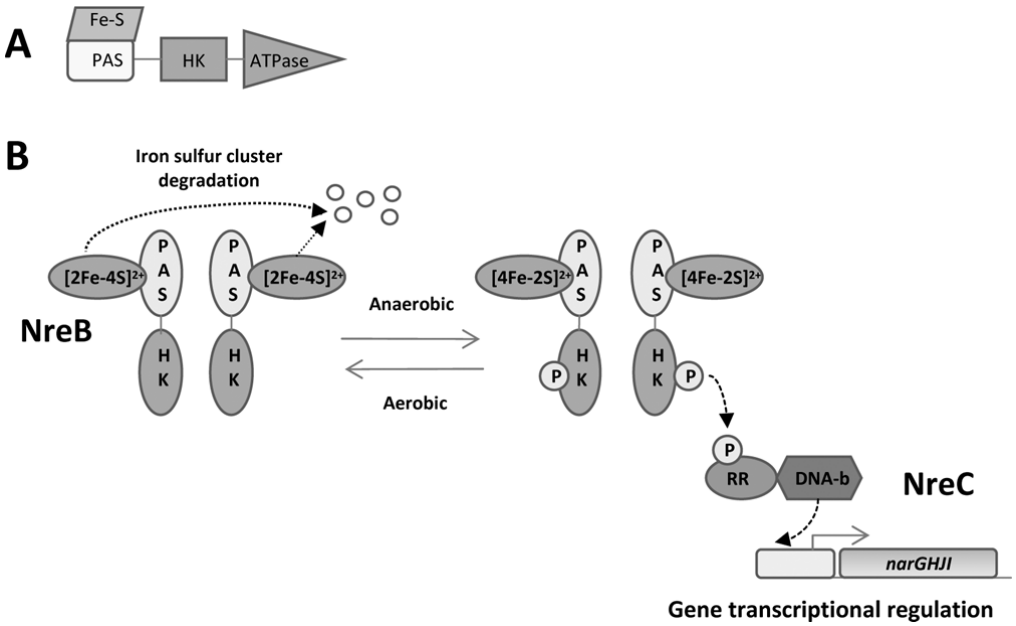

Figure 17. *M. capsulatus* (Bath) MmoS domain architecture and proposed model of action of the MmoS-MmoQ two-component system. A. MmoS domain organization B. Redox-dependent phosphotransfer mechanism in the *M. capsulatus* MmoS-MmoQ two-component system. ATPase: ATPase domain, REC: receiver domain, HPT: histidine phosphotransfer domain.

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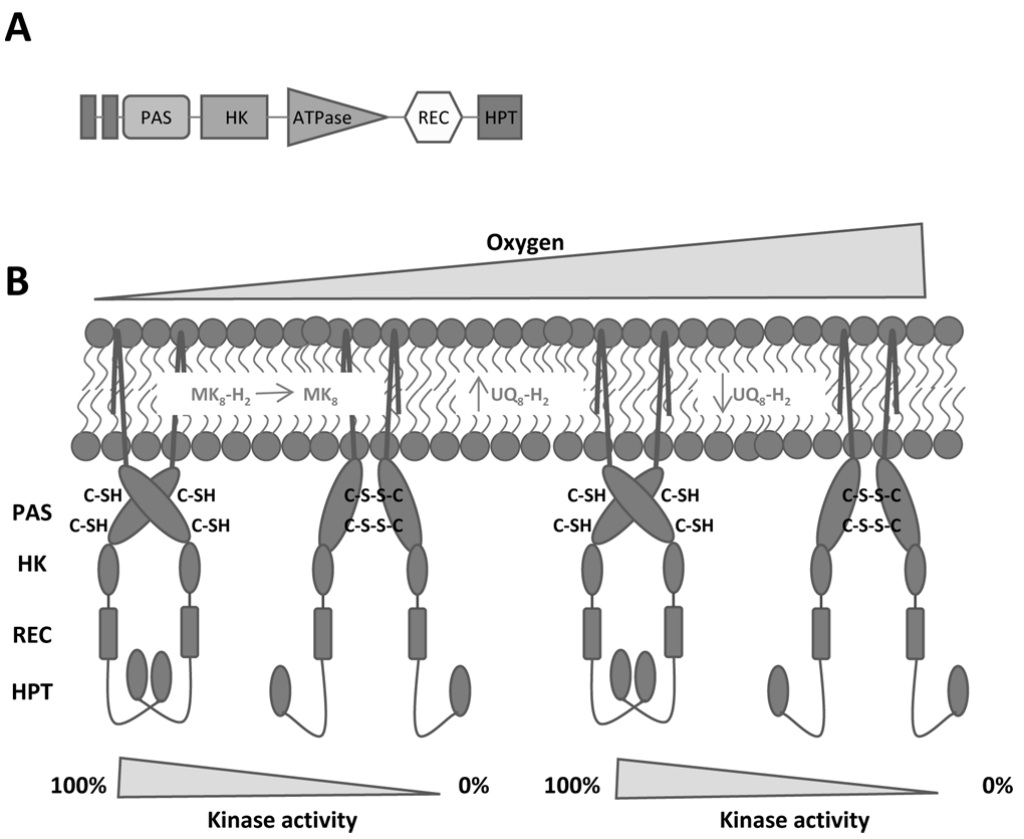

Figure 18. *E. coli* ArcB domain architecture and proposed model of action of the ArcB-ArcA two-component system. A. ArcB domain organization B. Redox-dependent phosphotransfer mechanism by ArcB in *E. coli*. REC: receiver domain, HPT: histidine phosphotransfer domain, MK8: menaquinone, UQ8: ubiquinone. This figure is adapted from Bekker et al. [23].

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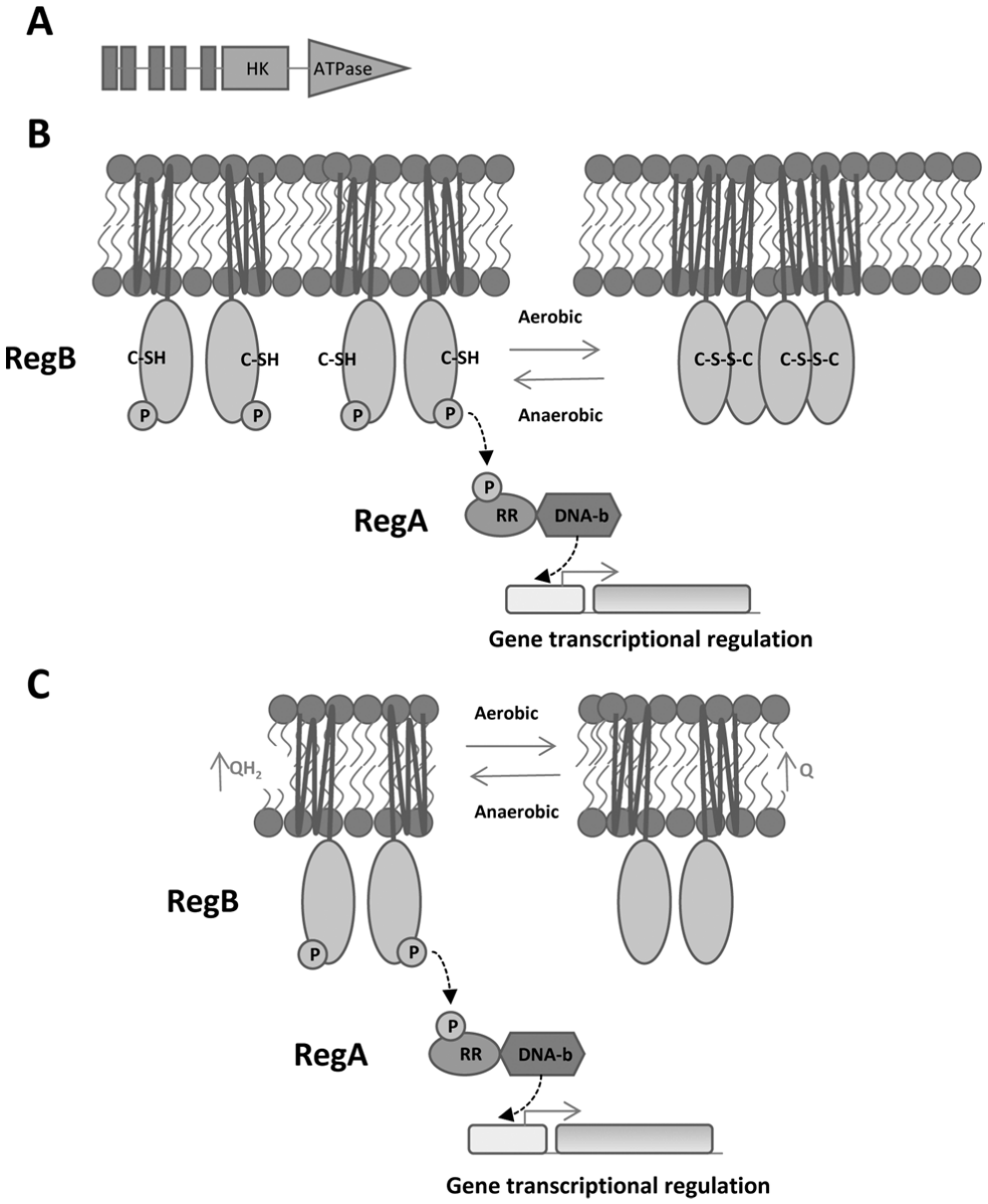

Figure 19. *R. capsulatus* RegB domain architecture and proposed model of action of the RegB-RegA two-component system. A. RegB domain organization B. and C. Redox-dependent phosphotransfer mechanism in the *R. capsulatus* RegB-RegA two-component system. Q: quinone. This figure is adapted from Wu et al. [398].