REVIEW

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An intimate link: two-component signal transduction systems and metal transport systems in bacteria

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ABSTRACT Bacteria have evolved various strategies to contend with high concentrations of environmental heavy metal ions for rapid, adaptive responses to maintain cell viability. Evidence gathered in the past two decades suggests that bacterial two-component signal transduction systems (TCSTSs) are intimately involved in monitoring cation accumulation, and can regulate the expression of related metabolic and virulence genes to elicit adaptive responses to changes in the concentration of these ions. Using examples garnered from recent studies, we summarize the cross-regulatory relationships between metal ions and TCSTSs. We present evidence of how bacterial TCSTSs modulate metal ion homeostasis and also how metal ions, in turn, function to control the activities of these signaling systems linked with bacterial survival and virulence.

Bacterial interactions with transition metal ions present a dual challenge: while many metal ions are biologically necessary at low levels, they can also be toxic at high concentrations. Bacteria use metal ions as cofactors for the function of several critical enzymes involved in electron transport and/or cell metabolism [1–4]. Accumulation of metal ions can impose deleterious effects on metabolic and cellular pathways thus compromising cell survival: different metal ions in the cytoplasm can tend to displace the metal cofactors at the active site(s) of enzymes ultimately leading to their inactivation [2,5]. For instance, when cellular metal homeostasis is disrupted, metals at the upper end of the Irving–Williams series – Mn(II) < Fe(II) < Co(II) < Ni(II) < Cu(II) > Zn(II) – have the potential to displace enzymatic metal cofactors at the lower end, thus rendering the proteins inactive [5,6]. In other cases, metal ions can also affect cell growth and viability by disrupting the structure of nucleic acids, phospholipid membranes and enzyme function [7,8]. Therefore, bacteria have developed complex mechanisms to monitor cellular metal ion levels and simultaneously maintain the homeostasis of multiple cations within a cell [9,10].

Bacteria use various strategies to regulate heavy metal homeostasis, which include the use of metal efflux pumps, channels, cation-specific metalloregulatory proteins, small noncoding RNAs and two-component signal transduction systems (TCSTSs) [2]. The intracellular import of metal ions is often facilitated by ATP-binding cassette transporters and Nramp transporters, whereas their export is usually carried out by cation diffusion facilitators [11], P-type ATPases [12,13] and tripartite resistance-nodulation-cell division (RND) transporters [14]. The regulation of metal trafficking proteins or their encoding genes is usually modulated by TCSTSs or metalloregulatory proteins. Bacterial TCSTSs are comprised of a membrane-bound histidine kinase (HK) and an intracellular cognate response regulator (RR) protein [15]. Upon reaching an appropriate threshold signal, the bacterial HK undergoes autophosphorylation, which, in turn, transfers the phosphate to its cognate RR protein. Once phosphorylated, the RR undergoes a conformational change which alters

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its binding affinity to specific sequences in the promoter/operator regions of its target genes [16]. As a result, the RR can activate or inhibit the transcription of target genes required for an adaptive response. Another group of regulatory proteins include cytoplasmic metalloregulators, which unlike TCSTSs are comprised of a single protein that can perform dual functions of sensing and responding to metal ions [17–19]. In fact, these proteins are specialized allosteric proteins that can directly bind to a specific or a small number of cognate metal ions [17,18,20]. Upon binding, the protein undergoes a conformational change in the regulatory region allowing it to control the transcription of target genes [18]. The products encoded by these genes can have multiple functions and may include proteins involved in growth, stress tolerance, virulence and metal trafficking within or between cellular compartments [17–19]. The function, mechanism and ion specificity of metalloregulators have been previously reviewed by others [17–18,20]. TCSTSs or cytoplasmic metalloregulators can exist together in bacteria. While they are capable of independent activation and regulation [17–19], intracellular cross-talk between these signaling systems has been noted in some species [21,22].

Of transition metal ions, the homeostasis of Fe has been widely investigated and extensively reviewed [19,23–27]. In this review, we will focus on the relationship between the activity of bacterial TCSTSs and homeostasis of Cu, Mn, Zn, Ni, Ag, Co and Cd.

Copper homeostasis

Cu is an important transition metal required for the growth of most living organisms. Owing to its redox properties, it has a vital role in maintaining biological processes important for cell viability. Cu exists in two oxidation states, Cu⁺ and Cu²⁺, and is utilized by an array of metalloenzymes to catalyze electron transfer reactions. In bacteria, Cu functions as a cofactor for over 30 enzymes, such as superoxide dismutase, cytochrome c oxidase or lysyl oxidase [28,29]. Although required at lower concentrations, accumulation of excess Cu within the cell under aerobic conditions can catalyze the production of hydroxyl radicals via Fenton and Haber–Weiss reactions. The production of reactive oxygen species (ROS) in the presence of Cu is believed to cause cellular damage by reacting with a number of cellular macromolecules such as lipids, proteins and nucleic acids [30].

However, there is little or no direct evidence that shows that Cu-induced ROS generation is the major cause of Cu toxicity. Alternative mechanisms of Cu toxicity in *Escherichia coli* have been suggested where Cu, instead of being involved in oxidative DNA damage, was shown to suppress Fe-mediated oxidative damage [31]. In *E. coli* growth inhibition due to Cu could be reversed by the addition of branched-chain amino acids [32]. The authors proposed that Cu can block the synthesis of branched-chain amino acids by targeting the Fe-S cluster containing dehydratases, where Cu replaces the Fe, rendering them inactive and blocking their activity [32]. However, a similar mechanism was not observed in *Salmonella*, where the addition of exogenous branched-chain amino acids did not revert the Cu-mediated growth inhibition [32]. It was suggested that Cu might have different targets than Fe-S clusters or the bacterium might have evolved multifactorial mechanisms to contend with Cu-induced toxicity [33]. In another study, it was shown that the *E. coli* Cu efflux system CusCFBA (described below) was induced during anaerobic amino acid limiting conditions to protect Fe-S cluster enzymes from endogenous Cu toxicity [34]. Challenges posed by Cu necessitate the activity of complex regulatory networks to maintain Cu homeostasis in the cell. The evolution of divergent mechanisms in various bacterial systems to contend with Cu (and metal ion) toxicity remains a topic that demands further exploration.

To contend with Cu toxicity, bacteria utilize at least one of three principal mechanisms: Cu export across the plasma membrane into the periplasmic space or the extracellular environment; extracellular and/or intracellular Cu sequestration via Cu-binding proteins; Cu oxidation to a less toxic Cu²⁺ state [35]. The mechanisms to maintain Cu homeostasis have been extensively studied in several bacteria including *E. coli*, *Pseudomonas*, *Xanthomonas*, *Enterococcus* and *Bacillus* [36]. In Gram-negative bacteria, excess Cu is either accumulated in the periplasm or is exported out of the cells. In these organisms, the genes contributing to Cu homeostasis are either located on the chromosome or are plasmid born. In many Enterobacteriaceae, such as *E. coli*, Cu stress tolerance is modulated by chromosomal regulons designated *cue* (Cu efflux) and *cus* (Cu sensing), as well as by plasmid-born machinery such as the *pco* system [37]. *E. coli* utilizes cytoplasmic MerR-type regulator CueR, which in

concert with the CusRS TCSTS regulates the expression of target genes involved in Cu homeostasis [21,22,37]. Under aerobic conditions, CueR is activated by elevated intracellular Cu concentrations, which can then directly bind the CueR box in the promoter region of *copA* and *cueO* encoding a P-type ATPase and an oxygen-dependent multi-copper oxidase, respectively [22,37]. The CopA protein helps export excess Cu⁺ from the cytoplasm into the periplasm, where it is oxidized to the less-toxic Cu²⁺ form with the aid of CueO [38]. Under anaerobic conditions, the CusRS TCSTS maintains Cu homeostasis, wherein the CusS sensor kinase is activated by a threshold periplasmic Cu concentration, which then activates its cognate responder protein CusR via phosphorylation [22]. As a result, active CusR regulates transcription of the *cusRS* operon, as well as the adjacent but divergently oriented *cusCFBA* operon by directly binding to the CusR box (AAAATGACAANN TTGTCATTTT) between the *cusC* and *cusR* promoters [39]. The CusCBA (a proton-cation antiporter) and CusF (a Cu chaperone) proteins have also been demonstrated to aid in Cu stress tolerance [40]. Interestingly, no other sequence in the entire genome of *E. coli* was found to contain a CusR box suggesting it binds specifically and only to the intergenic region between the *cusRS* and *cusCFBA* operons [39]. In *Corynebacterium glutamicum*, the *E. coli* homolog of CusRS was identified and characterized as *copRS* TCSTS. CopRS senses extracytoplasmic levels of Cu⁺ concentrations, and induces the set of genes involved in Cu homeostasis and resistance [41]. Like in *E. coli*, CopR specifically binds and regulates the expression of two divergently oriented operons *cg3286-cg3289* and *copR-cg3281*, which encodes for Cu resistance proteins (e.g., putative multicopper oxidase CopO and the putative copper export ATPase CopB) and CopRS TCSTS, respectively [41].

In cyanobacterium *Synechocystis* sp. PCC 6803, CopRS two-component system is known to be essential for copper resistance [42]. CopS was shown to have a high affinity to bind Cu *in vitro*, moreover its localization both in the plasma and thylakoid membrane suggested that CopS can possibly bind and respond to Cu both in the periplasmic and thylakoid lumen in *Synechocystis* [42]. However, further studies are warranted to completely understand the mechanism and conditions involved in Cu binding to CopS under natural conditions. In the presence

of Cu, CopR directly binds and regulates the expression of both *copBAC*, the putative heavy metal efflux-RND copper efflux system, and its own locus (*copMRS* operon) [42].

In *E. coli* two other TCSTSs, CpxAR and YedVW, were shown to be induced in the presence of Cu [39]. CpxAR is involved in membrane stress tolerance; it activates the transcription of CpxP protease for degradation of denatured proteins. Furthermore, a CpxR binding site, a conserved tandem repeat pentanucleotide sequence GTAAA(N)(4–8)GTAAA, was identified in the promoter region of several copper-inducible genes [43]. Knockout mutants of CpxAR were more sensitive to killing under Cu stress suggesting the role of this TCSTS in copper homeostasis in *E. coli* [43]. The function of YedVW is not well characterized; however, YedV kinase has been shown to transfer phosphate not only to YedW but also CusR. The YedVW was not induced by Cu in a CusR-deficient mutant suggesting the induction of YedVW system in a CusR-dependent manner [39]. Although the function of YedVW in Cu homeostasis is not yet characterized, its induction likely results from cross-talk between the CusRS and YedVW systems in the presence of Cu [39].

In *Helicobacter pylori*, a major colonizer of the human gastric mucosa, Cu is imported in a nonspecific manner [44]. Cu ions accumulated in the periplasm are detoxified via the metal export system, *crdAB* and *czcBA*, orthologous to that of the *E. coli* four-component Cu export system CusCFBA and is directly controlled by the CrdRS TCSTS [45]. The expression of *crdA*, encoding the protein most highly expressed under copper stress is directly controlled by the CrdRS TCSTS [45], where CrdR was shown to bind the operator region of the *crdA* promoter. Despite their speculations, the authors did not demonstrate that CrdS can directly sense Cu from the surrounding environment. *H. pylori* mutants deficient in CrdS and CrdR cannot colonize the mouse stomach, indicating a crucial role for Cu homeostasis in the adaptation to the gastric environment [45].

In addition to the utility of chromosomal Cu resistance genes, *E. coli* also utilizes a conjugative plasmid harboring the *pco* gene cluster comprised of *pcoABCDRSE* to contend with Cu stress [46]. These encode the multi-Cu oxidase, PcoA, the outer membrane protein, PcoB, the periplasmic proteins, PcoC and PcoE, and the inner membrane protein PcoD. The *pco* operon

is Cu-inducible and is regulated by a specific *pCoRSTCSTS* encoded by the same operon [46]. Although *cusRS* and *pcoRS* encode homologous Cu-responsive regulatory systems, they do not substitute for one another. The CusRS and PcoRS have different sensitivities or induction profiles to varying Cu concentrations and are comprised of independent copper-responsive regulatory systems in this bacterium [46].

Similar plasmid-encoded regulation of Cu resistance operon, *copABCD*RS, is observed in *Pseudomonas syringae* and is regulated by the corresponding *copRS* TCSTS [47]. CopABCD functions in the binding of copper, while CopR and CopS form a TCSTS system that regulates the expression of *copABCD* [47–49]. In *P. syringae*, multicopper oxidase *copA* and outer membrane protein *copB* are essential to fundamental copper resistance, whereas periplasmic proteins, *copC* and *copD*, are required for full Cu resistance [47–49]. *In vitro* studies suggested the direct binding of CopR to the cop box (a conserved CopR binding region) in the promoter region of the Cu resistance operon [50]. In *Pseudomonas fluorescens* strain TSS, homologous to plasmid-encoded cop operon, however chromosomally located Cu resistance genes *copRSCD* but not *copAB* have been identified [51]. The expression of *copCD* is directly regulated by the two-component responder protein CopR, which autoregulates its expression in response to Cu [51,52]. In addition to Cu resistance, CopRS modulates bacterial growth, biofilm formation and tissue dissemination, thereby suggesting its involvement in the regulation of bacterial virulence determinants in *P. fluorescens* [51].

A link between copper & silver homeostasis

In the periodic table Cu and Ag both belong to Group 11 of the transition metal elements. Similar to Cu, Ag has an antimicrobial effect on bacteria and is widely incorporated into products in medical equipment and public environments [53]. Silver cations (Ag^+) are bactericidal at low concentrations and are used to treat burns, wounds and ulcers. In addition, silver catheters and silver-coated catheters are used for their inhibitory role in microbial growth and biofilm formation [53]. Silver nanoparticles are effective not only against bacterial infections, but also are active against several types of viruses including the human immuno-deficiency virus,

hepatitis B virus, herpes simplex virus, respiratory syncytial virus and monkey pox virus [54]. Because of similar chemical properties of Cu and Ag, bacteria utilize a similar set of genes to cope with their homeostasis and resistance. In *E. coli*, the central role of CusS has been associated with modulating transcription of genes involved in Cu and Ag efflux [21]. CusS is activated in a metal-dependent manner and upregulates the expression of *cusCFBA* that is required for Cu and Ag resistance. In a clinically isolated Ag-resistant strain of *Salmonella*, metal ion resistance was conferred by the plasmid pMGH100, which has nine genes required for Ag resistance that are arranged as three transcriptional units [53]. Genes present within these units encode the SilRS TCSTS, a periplasmic Ag^+ -binding protein (Sile), an Ag^+ efflux pump (a P-type ATPase SilP) and a membrane potential-dependent three-polypeptide cation/proton antiporter (SilCBA). The function of SilP in transporting cytoplasmic Ag directly into the periplasm or across the outer membrane is yet not investigated. However, SilABC forms the three polypeptide RND protein which removes Ag from the cytoplasmic region directly to the outside of the cell without its release into the periplasmic space. The silver-dependent activation of the Sile, SilP and SilCBA is regulated by the SilRS system [53].

Manganese homeostasis

Mn has a vital function in regulation of bacterial metabolism, since it acts as a cofactor for metabolic enzymes such as superoxide dismutase, catalase, pyruvate carboxylase and phosphoenolpyruvate carboxykinase [55,56]. Mn is essential for detoxification of ROS thereby protecting the cells against oxidative stress; also recently, low molecular weight Mn complexes have been shown to reduce oxidative tissue injury and ROS in *in vitro* and *in vivo* biological systems [57,58]. *Bacillus subtilis* utilizes Mn during different stages of its developmental cycle and particularly for efficient sporulation [59].

In *Pseudomonas putida*, a Mn^{2+} -oxidizing bacterium [60], the MnxSR two-component regulatory system is comprised of two sensor kinases, MnxS1 and MnxS2, as well as one responder protein, MnxR. Together MnxS1, MnxS2 and MnxR have a central role in regulating Mn^{2+} oxidation [61]. The oxidation of Mn^{2+} to Mn^{3+} and Mn^{4+} is assumed thermodynamically

favorable as bacteria may derive energy from this reaction. The MnxR regulates Mn^{2+} oxidation; however, in order to be activated, MnxR requires signaling from both cognate sensor kinases since deletion of MnxS1 or MnxS2 results in complete loss of its ability to oxidize Mn^{2+} [61].

Mn together with Ca^{2+} and Cl⁻ act as a catalytic center for the oxygen-evolving photosynthetic machinery in higher plants and cyanobacteria [62]. Cyanobacterial cells take up Mn^{2+} ions using an ABC-type transporter encoded by the *mntCAB* operon: *mntA* encodes an ATP-binding subunit, *mntB* encodes a gene for its hydrophobic subunit and the *mntC* encodes a Mn^{2+} -binding subunit [63]. Two different studies in *Synechocystis* identified a TCSTS comprised of ManS and ManR involved in sensing and regulating the *mntCAB* operon, respectively [64,65]. At high Mn^{2+} concentrations, cell membrane localized ManS is speculated to bind Mn^{2+} and convey the signal to its cognate responder protein, ManR. Once activated, ManR can repress the transcription of the *mntCAB* operon thus inhibiting Mn uptake. On the other hand, under low Mn^{2+} concentrations, ManS is speculated not to bind Mn^{2+} ions and thus ManR remains inactive resulting in derepression of the *mntCAB* operon, facilitating uptake of Mn [64,65].

The effect of Mn in activating and regulating the expression of two-component signaling systems has been observed in several organisms [66–69]. In *Streptomyces reticuli*, a soil microbe, the SenS/SenR system modulates the expression *cpeB*, which encodes a Mn-dependent peroxidase involved in protecting the cells under oxidative stress [66]. In *Mycobacterium tuberculosis*, Mn and Ca are known to activate the TrcRS TCSTS [70]. The TrcRS TCSTS suppresses the expression of *rv1057*, which encodes the only seven-bladed β -propeller protein required for the structural integrity of the cell envelope of *M. tuberculosis* and might be a component of the mycobacterial envelope [69]. Though not much has been published regarding *rv1057*, studies suggest that the Rv1057 protein may function similar to TolB, a β -propeller protein that interacts with peptidoglycan-associated proteins and maintains envelope integrity in Gram-negative bacteria [71]. In *E. coli*, Mn-containing serine/threonine protein phosphatases, PrpA and PrpB regulate the CpxAB TCSTS and the periplasmic protease HtrA/DegP, which help contend with environmental stress tolerance [67,68].

In *Streptococcus mutans*, which is one of the primary causative agents of human dental caries [72], the *sloABCR* operon encodes components of a putative metal uptake system. These genes encode an ATP-binding protein, an integral membrane protein, a solute-binding lipoprotein and a metal-dependent regulator, respectively [73]. The SloABC transports both Fe and Mn, although the SloR represses this system only in response to Mn [73]. The SloR in *S. mutans* is a one-component cytoplasmic metal-dependent regulator, which modulates several physiological functions that include cell adherence, biofilm formation, genetic competence, metal ion homeostasis, oxidative stress tolerance and antibiotic gene regulation [74]. In this organism, SloR-Mn regulates the expression of an orphan responder protein designated GcrR, which acts downstream to tolerate acid stress, an important virulence factor of this cariogenic pathogen [75,76]. The interaction between the metalloregulatory proteins SloR and RR GcrR provides an excellent example of cross-talk between one-component and two-component metal regulatory systems in bacteria. In *S. mutans*, acid tolerance is known to be mediated by several TCSTSs including the CiaHR system that is activated by Ca^{2+} [77]. This system also regulates multiple virulence phenotypes including the ability to produce acid, form biofilm and develop genetic competence [77]. The *ciaRH* is part of the *ciaRHX* operon, where *ciaX* encodes a calcium-sensing signaling peptide that allows the CiaRH system to modulate its operon own expression in response to this cation [77].

Cadmium, zinc & cobalt homeostasis

Bacteria utilize Zn as an enzymatic cofactor and for the integrity of structural elements of the cell. Like other heavy metals, at high concentrations Zn can be highly toxic to the cell. Therefore, intracellular levels of Zn are often tightly regulated by an extensive network of transporters, ligands and transcription factors. In *E. coli*, Zn detoxification is primarily achieved by exporters such as ZntA, a P-type ATPase, ZntB, a cation diffusion facilitator and periplasmic proteins such as Spy or ZraP [78].

In *E. coli*, the BaeSR TCS is one of three extracytoplasmic stress response systems that helps elicit adaptive responses to changing environmental conditions [79]. On sensing transient environments (e.g., stresses posed by indole, tannin, flavonoids, sodium tungstate, or high levels of metal cations), the BaeS is activated and

transduces its signal by catalyzing phosphorylation of the transcription factor BaeR [79]. In *E. coli* and *Salmonella*, BaeR activates the expression of its own operon as well as genes involved in responses to contend with envelope stress, drug resistance and metal resistance [80–82]. In *E. coli*, the BaeSR TCS can sense Fe and Zn in the surrounding environment and, in turn, regulate the expression of genes involved in the formation and modification of membrane structure and function [83]. The BaeSR together with the CpxAR TCSTS coregulate the ZraP and Spy periplasmic chaperones required for envelope stress sensing [84]. Though the function of the periplasmic chaperone, Spy, in Zn detoxification is not known, its induction in the presence of Zn and impaired growth of the knockout mutant in *spy* under Zn stress suggest that it might play a role in Zn detoxification probably by facilitating the folding of the transmembrane or periplasmic transporters involved in Zn export [80]. In *Salmonella typhimurium*, BaeSR was shown to be involved in regulating the expression of multidrug efflux pumps and providing resistance against Cu and Zn toxicity [85].

In *E. coli* another Zn-responsive TCSTS, HydHG TCS system, when under excess Zn concentrations, modulates the expression of the periplasmic Zn-binding protein ZraP to maintain Zn homeostasis [86]. Similarly, in *S. typhimurium*, ZraSR (HydHG) have been shown to modulate the expression of periplasmic protein, ZraP required for regulation of the ZntA transporter of Zn [87]. The periplasmic protein and Zn-dependent chaperone ZraP has an important role in maintaining envelope homeostasis and contributes to the regulation of RR *zraR* of the ZraSR TCSTS [87]. In another study, it was shown that ZraP and ZraSR can be induced by indole. In the *zraP* knockout mutant a significant reduction in *zraR* expression was observed even in the presence of indole [87]. Although it was suggested that ZraP regulated the expression of the ZraSR TCSTS, the fact that both ZraP and ZraSR are induced by indole is noteworthy; since ZraP is a periplasmic chaperone, there is a possibility that it might act as a signal transducer for indole, wherein in the absence of this protein, the signal for ZraR activation is not conveyed resulting in its low expression levels [87]. The same group further showed that regulatory cross-talk occurs between the ZraPSR and BaeSR systems, wherein the loss of BaeR led to the induction of ZraPSR [87].

In *Pseudomonas aeruginosa*, the CzcRS TCS regulates the expression of CzcCBA efflux system, which confers specific resistance against Zn, Cd and Co cations [88]. CzcA functions as a cation–proton antiporter, and CzcB acts as a cation-binding subunit. Both CzcA and CzcB proteins form a membrane-bound protein complex which can catalyze an energy-dependent efflux of Zn, Co and Cd ions [89]. The CzcC provides substrate specificity toward Co and Cd [89]. In the presence of Zn, Cd or Co, the metal-inducible TCSTS, CzcRS, is activated and, in turn, induces the expression of *czcCBA* encoding a metal efflux pump [88]. Further, the CzcRS is involved in cross-talk with the CopRS TCSTS, wherein the CopR regulator required for Cu homeostasis links Cu resistance to Zn tolerance by activating the *czcRS* operon [90]. In *P. aeruginosa*, a putative *cop* box was identified between *czcC* and the *czcR* regulatory gene suggesting that CopR can bind to this region and regulate the transcription of the *czcRS* and *czcCBA* operons [91].

Nickel homeostasis

Ni is required as a structural component of metalloenzymes [92,93]. It is also needed to form a part of the active site of a number of enzymes including peptide deformylase, reductase and ureases, as well as some superoxide dismutases and hydrogenases [92,93]. Like other transition metal ions, at higher concentrations, Ni cations can confer harmful effects such as the generation of free radicals, inhibition of enzyme activity, contribution to DNA damage, developmental defects and cancer [94,95]. *E. coli* utilize Ni to survive under anaerobic growth. In fact, high levels of Ni are used for the optimal activity of Ni/Fe hydrogenases, enzymes involved in H₂ oxidation [96,97]. The NikR transcription factor has been characterized in various bacteria, and is known to repress or activate specific genes in response to Ni availability [98].

Microbial Ni uptake is either accomplished by nonspecific transport systems for divalent cations or by high affinity Ni-specific systems [99,100]. In *E. coli*, an ATP-binding cassette transporter, designated as NikABCDE, serves as the main importer for Ni ions. NikABCDE is comprised of NikA, a periplasmic Ni-binding and Ni-sensing protein; NikB and NikC integral inner membrane proteins; as well as NikD and Nike membrane-associated ATPase proteins [98,101]. The *nikABCDE* operon responds

to the presence of intracellular Ni, oxygen tension and nitrate availability. The transcription of the *nikABCDE* operon is positively regulated by the fumarate nitrate regulatory protein transcription factor in the absence of oxygen [102] and is negatively regulated by the NikR repressor in the presence of high concentrations of Ni ions [103,104]. Another major transcriptional regulator identified for the *nikABCDE* operon is the nitrate responsive NarLX two-component system [105]. In the presence of nitrate, NarX HK phosphorylates the cognate RR NarL, which represses the expression of the *nikABCDE* operon by binding to a distinct operator site from that of NikR [105]. The expression of the operon is tightly regulated by the mechanisms described depending on the presence of stressors involved [98].

In *Synechocystis* species, the *nrsBACD* operon encodes proteins required for Ni resistance [106]. NrsB and NrsA are homologues of the

P. aeruginosa CzcB and CzcA proteins, respectively [106]. Together the NrsA and NrsB form a membrane-bound protein complex that can catalyze Ni efflux by a proton/cation antiport system. The role of NrsC in Ni export is unknown. NrsD, a membrane protein, confers resistance to Ni and also acts as a Ni exporter [106]. Upstream of this operon and in reverse orientation, the NrsRS TCSTS can sense and respond to Ni. Once activated, it modulates expression of the *nrsBACD* operon, whose products regulate Ni homeostasis [106]. When Ni accumulates in the periplasm, the *nrsBACD* operon is activated under the control of NrsSR TCSTS; the accumulation of Ni in the cytosol is sensed by InsR (internal nickel-responsive sensor) [107]. InsR, a CsoR/RcnR like metalloregulator, binds directly at the cryptic transcription start sites within the *nrs* operon which enables independent repression of *nrsD* (of other *nrs* genes) in response to cytosolic Ni ions [107]. In the mutant-deficient

Table 1. Bacterial two-component signal transduction system and metal ions.

Metal Ion	TCSTS	Organism	Operon regulated	RR directly binding to operon	Ref.
Cu	CopRS	<i>Corynebacterium glutamicum</i>	<i>cg3286-cg3289</i> and <i>copR-cg3281</i>	Yes	[41]
		<i>Pseudomonas fluorescens</i>	<i>copRSCD</i>	Yes	[51,52]
		<i>Synechocystis</i>	<i>copBAC, copMRS</i>	Yes	[42]
	CusRS	<i>Escherichia coli</i>	<i>cusCFBA, cusRS</i>	Yes	[21,22]
	CpxAR	<i>E. coli</i>	Specific copper-inducible genes	Yes	[43]
	YedVW	<i>E. coli</i>	Several copper-inducible genes	No	[39]
Plasmid encoded	CrdRS	<i>Helicobacter pylori</i>	<i>crdAB, czcBA, crdRS</i>	Yes	[45]
	pCoRS	<i>E. coli</i>	<i>pcoABCDRS, pcoE</i>	Yes	[46]
Ag	CopRS	<i>Pseudomonas syringae</i>	<i>copRS, copABCD</i>	Yes	[47,50]
	CusRS	<i>E. coli</i>	<i>cusCFBA, cusRS</i>	Yes	[21,22]
Mn	SilRS	<i>Salmonella</i>	<i>silE, silP, silABC</i>	ND	[53]
	MnxSR	<i>Pseudomonas putida</i>	Mn ²⁺ oxidation	ND	[61]
Cd Zn Co	ManSR	<i>Synechocystis</i>	<i>mntCAB</i>	Yes	[64,65]
	BaeSR	<i>E. coli</i>	Several metal resistance genes	–	[79]
		<i>Salmonella typhimurium</i>	Several metal resistance genes	–	[81,85]
	ZraSR	<i>S. typhimurium</i>	<i>zraP</i>	ND	[87]
	HydHG	<i>E. coli</i>	<i>zraP</i>	Yes	[86]
	CzcRS	<i>Pseudomonas aeruginosa</i>	<i>czcRS, czcABC</i>	Yes	[88]
Ni	NrsRS	<i>Synechocystis</i>	<i>nrsBACD</i>	Yes	[106]
	NarLX	<i>E. coli</i>	<i>nikABCDE</i>	Yes	[105]

–: Specific genes only; ND: Not determined; RR: Response regulator; TCSTS: Two-component signal transduction system.

inrS, as a result of Ni-dependent derepression of *nrsD*, an increase in export of Ni from the cytosol to periplasm was observed resulting in enhanced Ni resistance and reduced cytosolic Ni accumulation [107]. The regulation of the *nrsBACD* operon either by cytoplasmic metalloregulatory protein or membrane-bound TCSTS occurs depending on the surplus accumulation of Ni within the cytosol or periplasm, respectively [107]. However, whether the regulatory pathways involve any cross-talk for efficient homeostasis remains a question [107].

Conclusion & future perspective

Despite our enhanced understanding of the role of TCSTSs in bacterial physiology, more studies are required to dissect the direct mechanisms underlying these processes. Bacterial response regulators are well known in modulating metal trafficking and homeostasis by directly binding to the promoter regions of the genes/regions involved (as summarized in **Table 1**); however, the nature of cation-induced activation (i.e., direct or indirect stimulation of the sensor kinases) remains an area of research that deserves greater attention. As discussed earlier, in *Synechocystis*, *in vitro* studies have shown direct binding and activation of the HK by Cu cations [42]. Such studies provide groundwork for more extensive research to understand the biophysical interactions between the TCSTS and metal cations. Emerging research in the field of bacterial metalloregulation reveals the conservation and pairing of TCSTSs with corresponding cation

uptake/efflux systems. Recently, upon analyzing the phylogeny of responder proteins belonging to TCSTSs, patterns of orthology/paralogy between Cu, Cd, Zn and Co efflux proteins, as well as among their regulatory proteins (e.g., CopR, CzcR, CopS, an1d CzcS) was discovered *in situ* [108]. Further, comparative analyses of three-dimensional structures have confirmed a common evolutionary origin for these regulatory proteins *in situ* [108]. Hence, the shared mechanism of activation or function of these systems can be exploited to develop novel therapies against bacterial infections. Bacteria deprived of the genes involved in metal homeostasis or those tied to their regulation tend to have diminished virulence relative to their parent strains. Since metal ion homeostasis and TCSTSs are closely coupled to bacterial survival and virulence mechanisms, understanding the molecular mechanisms underlying these processes can have significant clinical implications in curbing bacterial virulence.

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EXECUTIVE SUMMARY

- Transition metal ion homeostasis is important for bacterial survival. While these cations can be detrimental at high concentrations, they can sometimes serve essential functions in maintaining optimal physiology of these organisms.
- Of cellular components that are involved in metal homeostasis, bacterial two-component signal transduction systems (TCSTSs) have intimate and complex roles in responding, as well as in eliciting cellular responses to metal cation stress.
- Cytoplasmic metalloregulators differ from the TCSTSs, as the former are comprised of a single protein that can perform dual functions of sensing and responding to metal ions, whereas the latter utilizes two different proteins to perform these functions.
- Bacterial TCSTSs accomplish metal ion homeostasis by not only regulating metal export, but also by modulating the production of proteins associated with metal detoxification.
- Many response regulators can directly bind to the promoter regions of their target genes whose products modulate metal trafficking and homeostasis; however, sensing and activation of their cognate histidine kinases by metal cations is still an area which demands more investigative work.
- Bacterial metalloregulation reveals the conservation and pairing of TCSTSs with their corresponding cation uptake/efflux systems.

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